



Eric Lichtfouse
Editor

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Genetic Engineering, Biofertilisation, Soil Quality and Organic Farming

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Editor

Dr. Eric Lichtfouse
INRA-CMSE-PME
17 rue Sully
21000 Dijon
France
Eric.Lichtfouse@dijon.inra.fr

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Chapter 1

Avidin and Plant Biotechnology to Control Pests

Harry Martin, Elisabeth P.J. Burgess, Michal Masarik, Karl J. Kramer, Miroslava Beklova, Vojtech Adam, and Rene Kizek

Abstract The urgency of the global food crisis, coupled with the environmental impact of global warming and fuel shortages, indicate that transgenic methods may be required to enhance food production and quality. Widely used chemical insecticides, such as phosphine and methyl bromide, are losing their utility either due to insect resistance or to the environmental damage they cause. It is most unlikely that traditional plant-breeding methods for generating insect resistance will deliver the crop improvements required in the available time frame. In this review, we discuss the application of transgenic avidin, a protein naturally occurring in egg-white, for the protection of rice, maize, potato and apple leaf from insect pests. Avidin binds the vitamin biotin with extraordinary affinity (10^{-15} M). Biotin is a water-soluble

H. Martin and E.P.J. Burgess

The Horticulture and Food Research Institute of New Zealand Limited, Mt Albert Research Centre, Private Bag 92169, Auckland, New Zealand

M. Masarik

Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Kamenice 5, CZ-625 00 Brno, Czech Republic

K.J. Kramer

Agricultural Research Service, US Department of Agriculture, Grain Marketing and Production Research Center, KS 66502 Manhattan, USA

M. Beklova

Department of Veterinary Ecology and Environmental Protection, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, CZ-612 42 Brno, Czech Republic

V. Adam and R. Kizek (✉)

Department of Chemistry and Biochemistry, Mendel University of Agriculture and Forestry, Zemedelska 1, CZ-613 00 Brno, Czech Republic

e-mail: kizek@sci.muni.cz

V. Adam

Department of Animal Nutrition and Forage Production, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemedelska 1, CZ-613 00 Brno, Czech Republic

vitamin required for normal cellular metabolism and growth. The presence of avidin in the diet of insect pests is lethal since biotin is unavailable to them. The use of streptavidin, a bacterial homologue of avidin, is also described. We discuss the sub-cellular targeting of avidin expression in plants to avoid toxicity to the plant host and we describe the qualities of avidin which make it suitable for crop protection during cultivation and storage. Avidin is stable under normal conditions of crop storage but biodegradable and destroyed by cooking. These combined qualities make it an excellent choice for the protection of crops from insects. Finally, we discuss the modification of the avidin gene to allow expression in plants, the methods for transfection of the gene into plants, and the approaches used to quantify gene expression and avidin function in plant tissues. These methods include: polymerase chain reaction; enzyme-linked immunosorbent assay; polyacrylamide gel-electrophoresis; fluorescence polarisation (FP); capillary electrophoresis; tissue-printing; square-wave voltammetry (SWV) and the measurement of larvae morbidity and mortality.

Keywords Transgenic plants • avidin–biotin technology • agriculture • electrochemical method

Abbreviations

AC	alternating current
AdTSV	adsorptive transfer stripping
CPE	carbon paste electrode
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FP	fluorescence polarization
PCR	polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SWV	square-wave voltammetry

1.1 Introduction

Insect pests cause severe economic damage to agricultural crops. Due to the prospect of climate change and population increase, this problem has become a vitally important research topic. Stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests, 70 species of moths and about 355 species of mites causing quantitative and qualitative losses (Rajendran 2002). The economic hardship caused by insect pests is exacerbated by the fact that the chemical insecticides used to suppress them are declining sharply in utility. Phosphine and methyl bromide are two common fumigants used for stored-product protection. Insect resistance to phosphine is now a global issue (Collins et al. 2003; Nayak and

Collins 2008) and methyl bromide, a broad-spectrum fumigant, has been declared an ozone-depleting substance by the US Environmental Protection Agency and, therefore, is being phased out completely.

Due to the urgency of the crisis in food production and demand, it is most unlikely that traditional plant-breeding methods will deliver the crop improvements required, in the available time frame. Thus, it is evident that transgenic methods may be required to enhance food production and quality. Various transgenic modifications of crops have already been developed to improve the nutritional yield of crops; for example, potatoes have been transgenically modified to increase protein content (Chakraborty et al. 2000), while transgenic rice has been developed with enhanced vitamin A (Ye et al. 2000) and iron content (Lucca et al. 2001; Murray-Kolb et al. 2002). Wheat has been modified transgenically to allow crop production in regions of high salinity and in drought conditions (Abebe et al. 2003). Transgenic modification of papaya (Ferreira et al. 2002) and potatoes (Gao et al. 2000) has been developed, which resists viral and fungal infections, respectively.

Several transgenic crops have been developed specifically to deal with insect pests. The most well-known and widely applied transgenic insecticide is the Bt-cry toxin which is derived from the soil bacterium *Bacillus thuringiensis* (Torres et al. 2009). This toxin has been used transgenically in cotton and maize (Barry et al. 2000) to great effect. In addition, rice has been modified transgenically to express an insecticidal lectin from the snowdrop plant (*Galanthus nivali*) (Nagadhara et al. 2004). Here, we review the transgenic use of the biotin-binding protein avidin to control insect pests in a variety of important crops, including maize, rice, potatoes and apples. Avidin is a natural protein present in the egg-whites of birds and its role is to sequester free biotin. Biotin is an essential dietary component for insects, without which they are unable to grow. Avidin differs from other transgenic insecticidal toxins because it is not directly damaging to tissues, rather it merely withholds an essential nutrient from the insects. Biotin, as a normal dietary constituent, is, therefore, a natural antidote to avidin that, in its denatured (cooked) form, is already a normal component of many people's diets. We discuss the techniques for introducing the avidin gene into plants in ways which avoid toxicity to the host species and we summarise the evidence that avidin can be expressed harmlessly in crops while being lethal only to the insects which feed on these plants. Finally, we review the varied methods for detecting and quantifying avidin expression in crops.

1.2 Avidin as a Tool to Protect Plants Against Pests

1.2.1 *The Physiological Functions and Structures of Biotin and Avidin*

Biotin, vitamin H, or B8 (*cis*-hexahydro-2-oxo-1-H-thieno-[3,4]-imidazoline-4-valeric acid) is a water-soluble vitamin that is required for normal cellular metabolism and growth (Alban et al. 2000; Shellhammer and Meinke 1990) and functions as a

carboxyl carrier in carboxylation, decarboxylation and transcarboxylation reactions. Biotin is a dietary requirement of insects since synthesis occurs only in plants, bacteria and certain fungi. While biotin has traditionally been viewed as an essential cofactor of carboxylases, there have also been long-standing suggestions of a role for biotin in the regulation of gene expression (Dakshinamurti 2005; Hassan and Zempleni 2006; Rodriguez-Melendez and Zempleni 2003; Vilches-Flores and Fernandez-Mejia 2005). Recently, a potential role for gene regulation has been shown by specific biotinylation of histones. All five histone classes extracted from blood mononuclear cells contain biotin (Ballard et al. 2002; Kothapalli et al. 2005; Zempleni 2005). The insect central nervous system was shown to be rich in biotin-containing proteins (Ziegler et al. 1995). The fundamental requirement of biotin for many cellular activities of animals, including insects, therefore, suggests that the sequestration of biotin in the diet of pests would profoundly inhibit pest growth and development.

Avidin is a glycosylated protein, composed of four sub-units with a molecular weight of about 67 kDa. Each sub-unit contains one high-affinity, biotin-binding site with a dissociation constant $K_d = 10^{-15}$ M. This interaction exhibits one of the highest known affinities in nature between a protein and its ligand, and it is employed in various fields, including immunohistochemistry, electron microscopy, DNA hybridisation and biosensor technologies. In nature, avidin occurs as a minor component of bird, reptile, and amphibian egg-white where it protects embryos by ensuring that there is no free biotin in the egg-white. The absence of biotin inhibits the growth of many pathogenic microorganisms. Streptavidin has very similar properties to avidin. Their overall amino acid sequences show a low degree of similarity. Resolution of three-dimensional (3D) structures of avidin and streptavidin shows them to share a high degree of structural homology. Both are tetramers of identical sub-units, which fold into an eight-stranded anti-parallel beta-barrel. The biotin-binding site within each promoter is located in a deep pocket in the core of the barrel displaying both hydrophobic and polar residues for recognition of the tightly bound vitamin and consists of residues of the barrel itself and of a loop of the adjacent sub-unit. Moreover, the binding pocket is partly closed in its outer rim by tryptophan residue 110 of a neighbouring sub-unit. Once bound, biotin is almost completely buried in the protein core, with the exception of the valeryl side-chain carboxylate group, which is exposed to solvent. Hydrogen bonds to residues Alanine 39, Threonine 40, and Serine 75 trigger the formation of a network of hydrogen-bonded water molecules. Two tryptophan residues (Trp 70 and Trp 97) and phenylalanine 79 are in close contact with biotin (Fig. 1.1).

1.2.2 Avidin as a Pesticide in Food

Avidin and streptavidin are also resistant to proteolysis. However, both avidin and streptavidin function is greatly reduced by cooking, rendering the avidin harmless to humans following cooking, in the same way that cooked eggs are not harmful to humans. These combined properties render these proteins ideal for inclusion in foodstuffs as a pesticide. The insecticidal properties of avidin have been known since

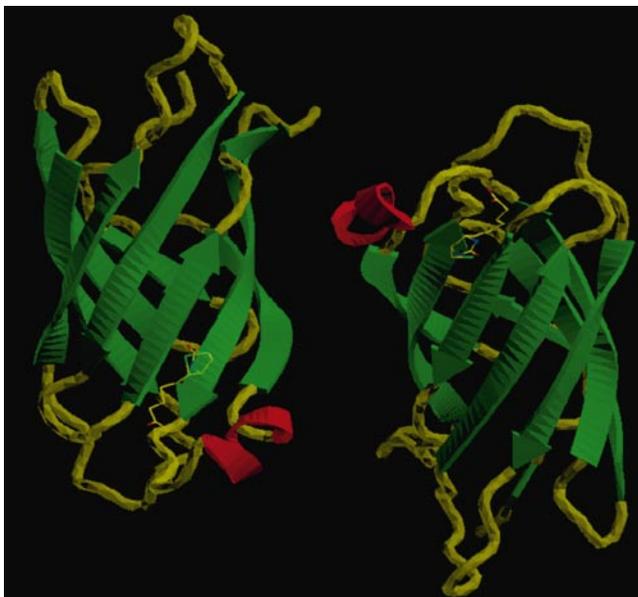


Fig. 1.1 Structural model of avidin and avidin linked to streptavidin (www.cryst.bbk.ac.uk/.../rosano/vbp/avidin.html)

1959, when it was demonstrated that avidin is insecticidal when included in the diet of housefly larvae (Levinson and Bergmann 1959) and subsequently, against a wide range of insects (Table 1.1).

Due to their insecticidal properties, avidin, and the functionally related streptomycetes protein, streptavidin, have been expressed in a variety of agriculturally important plant species, for example, tobacco, apple, maize and rice. Table 1.1 summarises the insecticidal effects of exogenous and transgenically expressed avidin on various insect larvae. A single instance of an insect, which is not susceptible to the presence of avidin in its diet, is the larger grain borer (Kramer et al. 2000), which tolerates high quantities of dietary avidin. Kramer suggests that this might be due to unusually high proteinase activity in the insects' gut digesting the avidin and precluding biotin sequestration. Alternatively, Kramer suggests that the larger grain borer may have a supply of biotin from gut symbionts.

The remarkable safety of transgenic avidin was shown by Kramer (Kramer et al. 2000) who found that mice fed solely on transgenic avidin-maize containing insecticidal quantities of avidin over a 21-day period showed no toxic effects and thrived in the same way as mice fed on control corn-meal. Furthermore, Yoza (Yoza et al. 2005) demonstrated that 97% of avidin functional activity is lost by heat denaturation (i.e. cooking) at 95°C for 5 min. In addition, avidin has the considerable added advantage over conventional insecticides in that, as a component of the stored crop, it is not washed away during processing and continues to act as an insecticide during storage.

Table 1.1 Insecticidal properties of avidin demonstrated in these insect species

Common name	Binomial nomenclature	Avidin source	Reference
Housefly larvae	<i>Musca domestica</i>	Dietary supplement	Benschoter 1967, Levinson and Bergmann 1959
Blowfly larvae	<i>Aldrichina grahami</i>	Dietary supplement	Miura et al. 1967
Merchant grain beetle	<i>Oryzaephilus mercator</i>	Dietary supplement	Saxena and Kaul 1974
Red flour beetle	<i>Tribolium castaneum</i>	Dietary supplement	Morgan et al. 1993
Confused flour beetle	<i>Tribolium confusum</i>		
Sawtoothed grain beetle	<i>Oryzaephilus surinamensis</i>		
Rice weevil	<i>Sitophilus oryzae</i>		
Lesser grain borer	<i>Rhizopertha dominica</i>		
European corn borer	<i>Ostrinia nubilalis</i>		
Indian meal moth	<i>Plodia interpunctella</i>		
Maize weevil	<i>Sitophilus zeamais</i>	Transgenic expression in maize	Kramer et al. 2000
Angoumois grain moth	<i>Sitotroga cerealella</i>		
Lesser grain borer	<i>Rhizopertha dominica</i>		
Saw-toothed grain beetle	<i>Oryzaephilus surinamensis</i>		
Red flour beetle	<i>Tribolium castaneum</i>		
Potato tuber moth	<i>Phthorimaea operculella</i>	Dietary supplement	Markwick et al. 2001
Light-brown apple moth	<i>Epiphyas postvittana</i>		
Green-headed leaf-roller	<i>Planotortrix octo</i>		
Brown-headed leaf-roller	<i>Ctenopseustis obliquana</i>		
Tobacco budworm	<i>Helicoverpa armigera</i>	Transgenic expression in tobacco	Burgess et al. 2002
Oriental leafworm	<i>Spodoptera litura</i>		
Black field cricket nymphs	<i>Teleogryllus commodus</i>	Dietary supplement	Malone et al. 2002
Potato tuber moth	<i>Phthorimaea operculella</i>	Transgenic expression in tobacco and apple	Markwick et al. 2003
Light-brown apple moth	<i>Epiphyas postvittana</i>		
Potato tuber moth	<i>Phthorimaea operculella</i>	Transgenic expression in potato	Meiyalaghan et al. 2005
Confused flour beetle	<i>Tribolium confusum</i>	Transgenic expression in rice	Yoza et al. 2005
Angoumois grain moth	<i>Sitotroga cerealella</i>		

(continued)

Table 1.1 (continued)

Common name	Binomial nomenclature	Avidin source	Reference
Velvet bean caterpillar	<i>Anticarsia gemmatalis</i>	Dietary supplement	Zhu et al. 2005
Beet armyworm	<i>Spodoptera exigua</i>		
Cotton bollworm	<i>Helicoverpa zea</i>		
Colorado potato beetle	<i>Leptinotarsa decemlineata</i>	Dietary supplement	Cooper et al. 2006

Unlike insecticidal chemical sprays, avidin has a minimal effect as an environmental pollutant. The inclusion of avidin in crops raises the possibility of the induction of an allergic response to the protein. However, avidin is known not to be highly allergenic and is absent from the World Health Organization's official list of food allergens, whereas many highly allergenic proteins from common fruit, nuts, corn, and egg-white are present. The official website for the WHO/IUIS Sub-Committee on Allergen Nomenclature is www.allergen.org. This site lists all allergens and isoforms that are recognised by the committee and is updated on a regular basis.

1.2.3 Transgenic Expression of Avidin in Plants

Various strategies have been employed for the transgenic expression of avidin in plants. Hood and co-workers expressed chicken egg-white avidin in maize, achieving an expression level of greater than 2% of aqueous soluble protein extracted from dry seed (Hood et al. 1997), the mature protein localising to the extracellular spaces. The approach taken by Hood et al. was to reverse-translate the chicken egg-white avidin amino acid sequence into DNA using the preferred maize codon usage (Fig. 1.2a). This sequence was inserted into a plasmid, which contained the maize ubiquitin promoter. The avidin-containing plasmid was introduced into an embryonic maize cell line from which plants were cultivated. The aim of Hoods approach was to maximise avidin expression for the commercial production of avidin rather than to use the avidin to protect the maize. Although Hood used the ubiquitin promoter because it is generally thought to be constitutive, the avidin expression was particularly strong in seed and Hood concluded that the ubiquitin promoter has strong seed preference. Hood also noticed that the avidin expressed in this way had profound physiological effects on the plant – the male transformants were sterile. Kramer and co-workers (Kramer et al. 2000) used the same genetic constructs for their study of the insecticidal properties of transgenic avidin-maize.

Yoza et al. (2005) adopted a similar approach to Hood for the expression of avidin in rice except that the glutelin promoter GluB-1r, a seed-specific promoter, was used instead of the ubiquitin promoter on the grounds that a seed-specific promoter will lead to expression of avidin in all seeds whereas male sterility could lead to half of the kernels of avidin-maize containing no avidin. Yoza argues that a

Methods for transformation & detection of the avidin gene

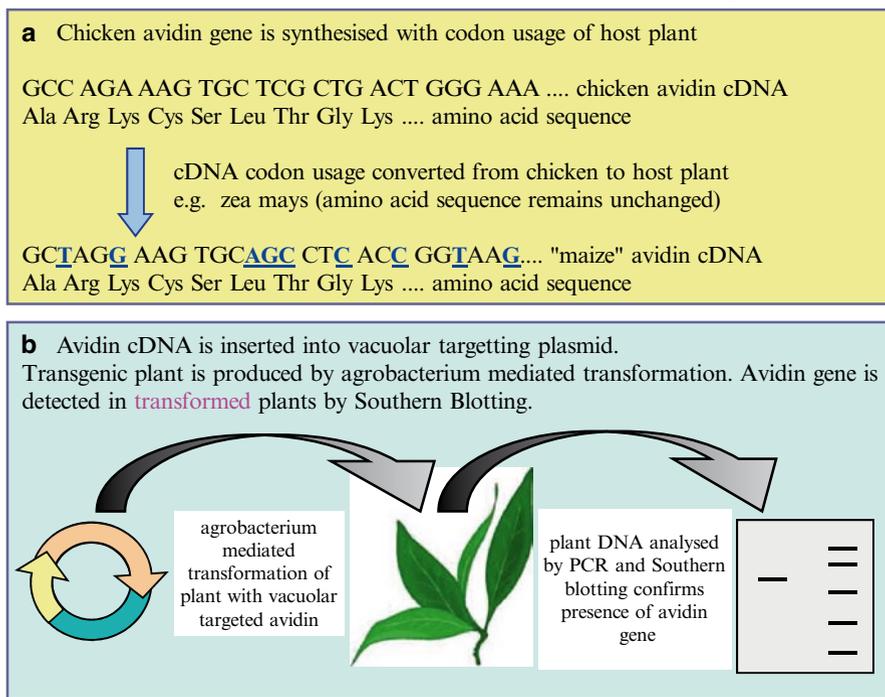


Fig. 1.2 Methods for transformation and detection of the avidin gene. **(a)** Transforming the DNA sequence of chicken avidin into a sequence which will be efficiently expressed in the maize plant. **(b)** The avidin gene is inserted into plants so that the protein avidin is only expressed in plant vacuoles where it does not interfere with the plants' own biotin resources

seed-specific promoter would be more appropriate for the protection of a stored product from pests. In addition, since the Glub-1 promoter is endosperm-specific and is not expressed in pollen, transgenic avidin-rice is fertile. Since rice is self-compatible, male sterility would be a fatal problem.

1.2.4 Targeted Vacuolar Expression of Avidin Reduces Toxicity to Plants

A major function of avidin expression in rice and maize is to protect the seed from pests during storage. In the case of crops such as potato and apples, the pest problem comes during cultivation in the form of damage to foliage, tubers and fruit by the larvae of, for example, the potato tuber moth, the light-brown apple moth or leaf-roller moth. In these cases, targeting avidin expression to seeds would be unproductive and disseminated avidin expression would be more appropriate. Avidin and

the functionally similar protein streptavidin have been transgenically expressed in tobacco and apple (Markwick et al. 2003; Murray et al. 2002). In these studies, the Cauliflower Mosaic Virus promoter was employed which lead to a non-tissue-specific expression of avidin. Male infertility did not occur. However, to prevent toxicity due to sequestration of essential plant biotin, the avidin was targeted to intracellular vacuoles by the use of N-terminal vacuole targeting sequences from potato proteinase inhibitors (Fig. 1.2b). If targeting sequences were not used then avidin expression was lethal (Hood et al. 1997; Murray et al. 2002) since biotin is synthesised in the plant cytoplasm and used in the mitochondrial and chloroplastic compartments (Baldet et al. 1992, 1993). The leaf concentrations of avidin achieved by Murray and Marckwick were approximately 10 μM . This is approximately a 7.5-fold molar excess of avidin over the normal biotin levels in the plant leaf (Murray et al. 2002) and is sufficient to ensure that insect pests feeding on the leaves are killed or never reach reproductive maturity (Fig. 1.3). From a human toxicity aspect, the insecticidal level of avidin in transgenic apple and tobacco leaves is somewhat lower than that of chicken egg-white.

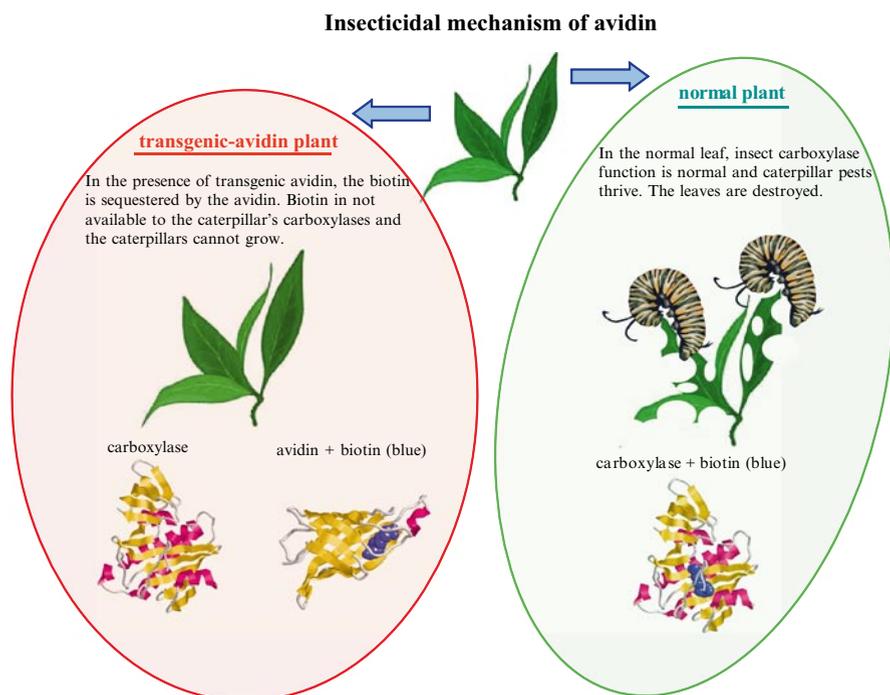


Fig. 1.3 Insecticidal mechanism of avidin. Normal and transgenic-avidin plants develop normally. Transgenic avidin is restricted to vacuoles within the leaf cells and, therefore, cannot interfere with normal leaf biotin function. Avidin is released from the vacuoles and binds the leaf biotin when the caterpillar chews the leaf tissue

1.2.5 Transgenic Avidin in Combination with Other Pesticidal Transgenes

Avidin has been shown to have synergistic effects when used in conjunction with other insect toxins. The *Bacillus thuringiensis*, toxin Bt-Cry3A is active against the Colorado beetle larva (*Leptinotarsa decemlineata*) and has been transgenically expressed in potato, *Solanum tuberosum* (Coombs et al. 2002). In addition, many wild *Solanum* species possess an innate resistance to the Colorado beetle due to the presence of naturally insecticidal leptine glycoalkaloids, expressed only in foliage (Sinden et al. 1986). When potato leaves transgenically expressing Bt-Cry3A are dipped in avidin there is a combined effect of the two insecticides. There is a similar additive effect of combining the natural resistance of the leptine glycoalkaloids with avidin (Cooper et al. 2006). Thus, transgenic expression of avidin is known to be an effective insecticide in isolation and in combination with natural plant insecticides and with other transgenic insecticides.

It is conceivable that transgenic avidin might have adverse effects on the natural predators of insect pests. A study by Burgess (Burgess et al. 2008) in which *Spodoptera litura* (Oriental leafworm) that have been fed avidin, are themselves used as food for *Ctenognathus novaeseelandiae* (Carabid beetles) revealed no evidence of tri-trophic toxicity occurring in the predator. The lack of morbidity in the Carabid beetles related with de-activation and dilution of avidin in the prey of this leafworm. The evidence to date does not support concerns about accumulation of poisonous levels of avidin in the insect food chain.

1.3 Commonly Used Methods to Determine Avidin in Transgenic Plants

1.3.1 Polymerase Chain Reaction (PCR) and Southern Blotting

The successful genomic insertion of the avidin gene in transgenic plants has been confirmed by using the PCR technique (Saiki et al. 1988) and Southern blotting (Southern 1975) in maize (Hood et al. 1999), tobacco (Murray et al. 2002), (Burgess et al. 2002) and rice (Yoza et al. 2005). Southern blotting of restriction enzyme digested plant genomic deoxyribonucleic acid (DNA) revealed three to five copies of avidin gene in several transgenic plants (Hood et al. 1997). However, insertion of the avidin gene into the host plant genomic DNA does not necessarily imply that the avidin protein will be expressed. An essential step for efficient expression of transgenic proteins is that the different codon usage of each host is taken into account. For example, to express chicken avidin protein in maize, Hood (Hood et al. 1997) synthesised an avidin coding DNA sequence that corresponded to efficient codon usage by maize, not chicken (Fig. 1.2b). Various methods are available to confirm expression of avidin protein.

1.3.2 ELISA (*Enzyme-Linked ImmunoSorbent Assay*)

ELISA is the most common and simplest method employed for detecting avidin in transgenic plants. Leaf homogenates containing transgenic avidin are applied to microtitre plates and the avidin in the sample adheres to the microplate surface. After extensive washing, an antibody directed against avidin is added to the plate and following a further washing step, a secondary antibody coupled to an enzyme, such as alkaline-phosphatase or horseradish peroxidase, is added to the plate. The amount of enzyme activity remaining on the washed microplate correlates with the amount of avidin in the original sample. The enzyme activity is usually measured by cleavage of a substrate whose product is coloured or fluorescent. This method, known as indirect ELISA because the enzyme is coupled to the second antibody not the first, has been used extensively to detect transgenic avidin in leaf tissue (Burgess et al. 2002; Christeller et al. 2005; Markwick et al. 2003; Murray et al. 2002; Yoza et al. 2005). A more sensitive variant on this technique, called ‘sandwich’ or ‘capture’ ELISA, was used by Hood (Hood et al. 1997) and Kramer (Kramer et al. 2000) for the measurement of transgenic avidin in maize. In this method, an antibody to avidin is pre-coated onto the ELISA microplate to optimise adsorbing of the transgenic avidin and minimise competition on the microplate for other leaf proteins. That is, the first antibody in the sandwich ELISA concentrates avidin on the microplate surface. Subsequent steps are the same as in the indirect ELISA method.

These antibody-dependent ELISA methods measure avidin protein as an antigen present in a transgenic sample. However, for various reasons, the presence of transgenic avidin protein may not equate to functionally active (i.e. biotin-binding) avidin. Christeller (Christeller and Phung 1998) showed that the level of biotin in apple leaf varies seasonally from 200 to 800 ng biotin per gram of leaf. This equates approximately to a range of 0.8–3.3 μM biotin. Avidin has four biotin-binding sites per molecule and, therefore, a transgenic avidin leaf homogenate containing avidin with less than this level of biotin-binding sites will be saturated with endogenous biotin. ELISA has been used to differentiate the total transgenic leaf avidin from the unbound (functionally active) transgenic leaf avidin (Christeller et al. 2005). In these assays enzyme-labelled antibody to avidin measured total avidin protein while biotin-labelled enzyme detected biotin-binding sites unoccupied by endogenous leaf biotin.

1.3.3 SDS-PAGE (*Sodium Dodecylsulphate Polyacrylamide Gel Electrophoresis*) and Western Blotting

SDS-PAGE is a widely used technique that separates proteins according to their size. Under the influence of an electric field, proteins are electrophoresed through a polyacrylamide gel matrix. Their mobility inversely correlates with their size (Patterson 1994). The binding of the negatively charged detergent SDS to proteins

denatures their unique 3D shape so that they acquire a ellipsoid 3D shape. Also, the SDS confers a strong negative charge on proteins which overrides the normal substantial variation in net charge conferred by amino acid content. Thus, high resolution is achieved because the molecular size of the proteins becomes the only important factor influencing their migration through the gel matrix under the influence of an electric field. For SDS-PAGE, purified protein samples must be applied to the gel since the proteins are usually visualised by non-specific protein stains. The Western-Blotting technique takes SDS-PAGE a step further, since it allows complex mixtures of samples, separated on SDS-PAGE gels, to be analysed by antigen city or even functionality, if non-denaturing conditions are used. Following size-separation on SDS-PAGE, the proteins in the gel are electrophoretically transferred onto a sheet of nitrocellulose or polyvinylidene fluoride, which binds them in position (Fig. 1.4). The membrane can then be probed with antibodies specific for certain proteins, for example, anti-avidin antibodies. In this way, whole leaf homogenates can be analysed to reveal the quantity and molecular size of the transgenic avidin protein they contain. Using non-denaturing conditions allows functional avidin to be visualised and quantified. The avidin is usually visualised using similar reagents to the ELISA procedure except that a chemiluminescent substrate is applied to the membrane whose cleavage results in light emission and the image is captured by camera or on photographic film. Alternatively, the antigenic protein bands on the membrane can be stained using a substrate whose enzymatic product is coloured and insoluble.

Avidin and streptavidin both form a tetramer structure. Each monomer of avidin is 16 kDa in weight. In the case of denaturing SDS-PAGE electrophoresis, when

Methods for detection of avidin protein

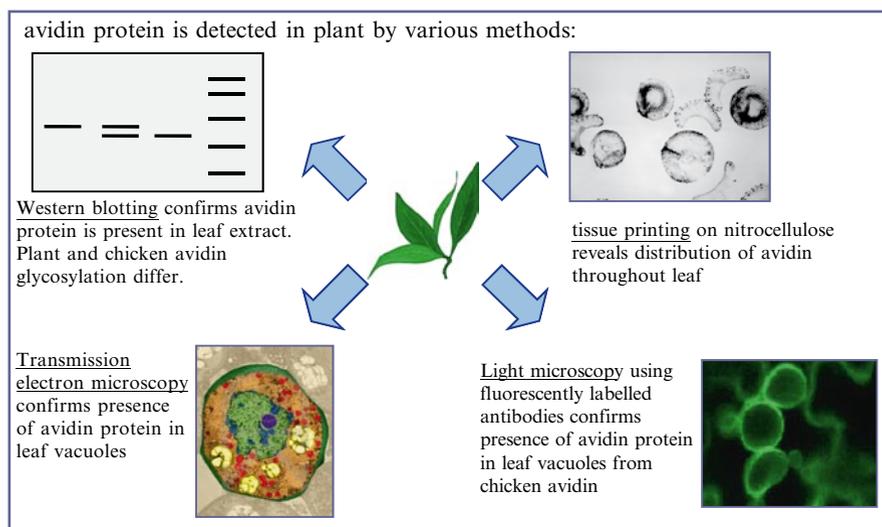


Fig. 1.4 Methods for detecting and analysing avidin protein

samples of avidin were boiled, protein migrated mainly as the monomer (Bayer et al. 1996; Humbert et al. 2005). In comparative stability properties study of avidin and streptavidin, it was determined that, in the absence of biotin, the quaternary structure of streptavidin is more stable than that of avidin (Hytonen et al. 2003; Williams et al. 2003). Biotin stabilises the tetrameric structure of both avidin and streptavidin (Bayer et al. 1996).

The post-translational modification of proteins, including glycosylation, is species- and tissue-dependent. This would imply that chicken avidin expressed in different plant species would have different glycosylation patterns. The variation in glycosylation could potentially affect the avidin's affinity for biotin, and the intracellular location and stability of transgenic avidin. Several studies of transgenic chicken avidin have confirmed that it is glycosylated differently in plants than its natural host species. Using the Western-Blot method Hood (Hood et al. 1997) showed that transgenic chicken avidin synthesised in maize had a molecular weight of 16.6 kDa, 800 Da less than the same gene expressed in its natural host. Treatment of both avidins with N-glycosidase reduced the molecular weights of both proteins to 12.5 kDa confirming that the primary structure of the avidin was identical but that the protein was glycosylated differently when expressed in maize. These results are not surprising since it is well known that glycosylation varies even between closely related species and the glycosylation systems of plants and animals are very different. Even within an individual organism, protein glycosylation is tissue-specific and within a single cell, the glycosylation of an protein is heterogeneous (reviewed by Spiro (2002) and Lis (Lis and Sharon 1993)). Similarly, Murray et al. (2002) showed that while egg-white avidin was fully deglycosylated by treatment with the N-glycosidase F, tobacco-leaf avidin was only partially deglycosylated. A similar application of Western-Blotting by Gatehouse (Gatehouse et al. 2008) showed that chicken avidin and transgenic maize-avidin had clearly different sensitivities to treatment with endoglycosidases F and H. These changes in avidin glycosylation did not cause any noticeable alteration in the affinity of the transgenic protein for biotin.

1.3.4 Semiautomatic Capillary Electrophoresis

Experion is an automated microfluidic electrophoresis system that uses a combination of Caliper Life Sciences innovative LabChip microfluidic separation technology and sensitive fluorescent sample detection. It performs rapid and reproducible analyses of protein, DNA and RNA samples, which allows the analysis of samples within 30 min (Bradova and Matejova 2008). The separation, detection and data analysis are performed within a single platform, so the time-consuming steps in classic electrophoretic methods are minimised. Many types of samples, such as bacterial lysates, protein extracts and chromatography fractions, can be analysed. In addition to the significant shortening of time required, the chip-based method allows both reproducible and accurate sizing and quantification of the proteins. Avidin has been successfully analysed by this technique (Krizkova et al. 2008). The chip electrophoresis

system provides very good reproducibility, simple handling, fast analysis and results comparable with SDS-PAGE (Bradova and Matejova 2008).

1.3.5 Fluorescence Polarisation (FP)

This method exploits the fact that many small fluorescent molecules absorb and emit polarised light in the same plane. For fluors such as fluorescein, Alexa or BODIPY dyes, there is a delay of 4–6 ns between fluorescence excitation and emission. This is a sufficiently long delay for a small molecule the size of biotin to tumble randomly in Brownian motion. However, if the fluorescent molecule is immobilised by binding to a much larger protein molecule then very little movement will occur between excitation and emission. Thus, if biotin is covalently attached to a fluorescent compound such as Alexa-594, then the concentration of avidin or biotin in a sample can be measured from the degree to which the emitted fluorescent light is polarised. A high polarisation signal means that the fluor is bound to avidin, while a low polarisation means that the fluorescent ligand is unbound and, therefore, the avidin concentration in the sample is low. The FP technique is simple, accurate, sensitive and the reagents are inexpensive. FP analysis is often performed in 384 well microplates and is therefore, suitable for high-throughput automated screening. FP was recently applied to the quantification of biotin in normal apple leaves and also used to quantify avidin expression in whole-leaf homogenates from transgenic plants (Martin et al. 2008).

1.3.6 Electrochemical Methods

The strong affinity of avidin for biotin allows biotin binding to be detected electrochemically. Avidin contains a diversity of amino acids in its structure. From an electrochemical point of view, only tyrosine and tryptophan have been found to be electroactive using a variety of electrodes (Brabec and Mornstein 1980a, b; MacDonald and Roscoe 1997). Square-wave voltammetric (SWV) analysis using solid carbon electrodes is very sensitive and yields well-developed signals. However, using a carbon paste electrode (CPE) and base line correction of the data, we can determine well-defined voltammetric signals for both tyrosine and tryptophan at 0.78 and 0.92 V versus Ag/AgCl/3 M KCl, respectively. Electrochemical investigation at carbon electrodes showed that avidin produced oxidation signals due to tyrosine and tryptophan residues.

Square-wave voltammetry at a carbon paste electrode using the adsorptive transfer stripping (AdTS) technique simple is a fast method for determination of avidin (Palecek and Postbieglova 1986). This technique is based on the immobilisation of the analyte in the form of a small drop of solution at the carbon paste electrode, followed by washing and detection steps in a cell containing a supporting electrolyte

(Masarik et al. 2003; Petrlova et al. 2007b; Tomschik et al. 2000). The denatured protein exhibits a fivefold higher response relative to that of the native protein, indicating that there were substantially more aromatic residues exposed to the electrode surface in the denatured state (Masarik et al. 2003). Under optimal experimental conditions, the limit of detection of avidin is in the attomolar range (Petrlova et al. 2007b). Recently, we described an easy-to-use electrochemical technique for the detection of avidin in transgenic plants. Plant extract is added into carbon paste (Fig. 1.5) and avidin present in the extract gives a very distinct signal (Kizek et al. 2005; Masarik et al. 2003; Petrlova et al. 2007a). Krizkova compared electrochemical method with gel electrophoresis and found that methods gave similar results on analysis of transgenic tobacco leaves (Krizkova et al. 2008). Moreover, Fojta et al. and others report on some less common electrochemical methods for the detection of avidin and avidin–biotin interactions (Fojta 2008; Havran 2004; Limoges 1996).

The application of AdTS SWV in conjunction with liquid chromatography, diode array detection and flow injection analysis has allowed the extremely sensitive detection of biotin and avidin in the femtomolar range (Kizek et al. 2005). Moreover, Kizek and his colleagues have proposed an approach to detecting avidin–biotin interaction in transgenic plants (Fig. 1.5). Sugawara and colleagues developed methods for the electrochemical analysis of avidin–biotin interactions using various types of labelled biotins. In particular, they used bisbiotinyl thionine (Sugawara et al. 2004), iminobiotin (Sugawara et al. 2005), *N*-iodoacetyl-*N*-biotinylhexylenediamine (Sugawara et al. 1996a), biotin labelled with Nile Blue A (Sugawara et al. 1996b) and biotin/thionine modified Au electrode (Sugawara et al. 2002).

1.3.7 Tissue Printing

Another commonly applied technique to reveal the large-scale expression pattern of transgenic proteins, including avidin, in plant tissue is tissue printing (Fig. 1.4). Cross sections of plant stems, roots or tubers can simply be pressed against nitrocellulose leaving behind an imprint of the avidin in the tissue. For tissue printing of leaves, the leaves should first be freeze-thawed to break open the cell walls and intracellular organelles before pressing against the nitrocellulose. The tissue-printed nitrocellulose can then be handled like a Western-Blot and probed with biotin-coupled peroxidase to reveal the distribution of avidin expression in the plant sample. This technique has been employed for the detection of transgenic avidin in tobacco (Murray et al. 2002), and maize (Hood et al. 1997).

To determine the sub-cellular localisation of the transgenically expressed avidin, Hood (Hood et al. 1997) performed in situ localisation experiments on thin sections of embedded embryos using anti-avidin primary antibodies and fluorescently labelled secondary antibodies (Fig. 1.4). As expected, Hood et al. observed the avidin being secreted into the cell wall matrix since they had fused the avidin gene to a signal sequence that targeted the protein to the endoplasmic reticulum. In plants, the default

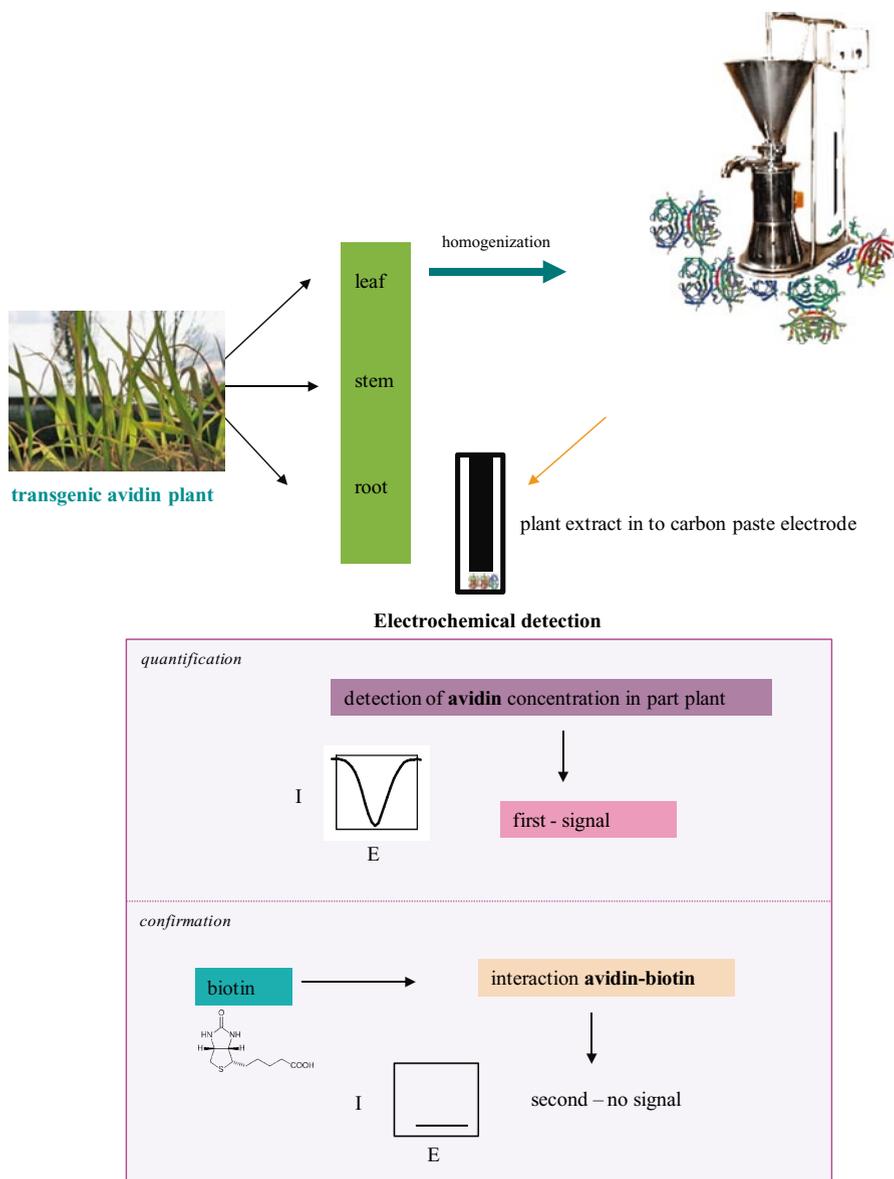


Fig. 1.5 Scheme of fast electrochemical detection of avidin concentration and avidin–biotin interaction in transgenic plants

pathway for proteins in the endoplasmic reticulum is secretion. Using similar fluorescent-light-microscopy methods Murray et al. confirmed vacuolar expression of avidin in tobacco, using a vacuolar targeting signal. Vacuolar expression of avidin in tobacco was also demonstrated by Murray using transmission electron microscopy and gold-labelled antibodies (Fig. 1.4) (Murray et al. 2002).

1.3.8 Mortality and Morbidity Assay

Finally, several researchers have used biological assays, for example, insect mortality, morbidity, development and behaviour to measure the presence of functional avidin in transgenic plants. In these experiments, the larvae of insect pests are placed onto normal or transgenic leaves and their growth and development rates are recorded (Fig. 1.3). These studies are summarised in Section 1.2 and Table 1.1.

The simplest and most direct measure of biological effect on the insect larvae feeding on plants expressing avidin transgenically is mortality: Markwick (Markwick et al. 2003) observed that potato tuber moth larvae fed on transgenic apple and tobacco leaves had a mortality rate of up to 90% within 9 days compared with 100% survival of larvae on normal leaves. By studying the behaviour of the leaf-mining potato tuber moth larvae, Marckwick noted that the insects were unable to detect the insecticidal avidin since they did not leave their leaf mines. There was no evidence of the larvae avoiding the transgenic leaf and seeking alternative food sources. This was an interesting observation because avoidance behaviour had been observed for larvae feeding on leaves containing *B. thuringiensis* toxins (Beuning et al. 2001; Gleave et al. 1998).

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Chapter 2

Cover Crops for Sustainable Agrosystems in the Americas

Johannes M.S. Scholberg, Santiago Dogliotti, Carolina Leoni, Corey M. Cherr, Lincoln Zotarelli, and Walter A.H. Rossing

Abstract Rapid depletion of global fertilizer and fossil fuel reserves, combined with concerns about global warming, have resulted in increased interest in alternative strategies for sustaining agricultural production. Moreover, many farmers are being caught in a vicious spiral of unsustainability related to depletion and degradation of land and water resources, increasing labor and input costs, and decreasing profit margins. To reduce their dependence on external inputs and to enhance inherent soil fertility, farmers, thus, may opt to employ farm-generated renewable resources, including the use of cover crops. However, perceived risks and complexity of cover-crop-based systems may prevent their initial adoption and long-term use. In this review article, we provide a historic perspective on cover-crop use, discuss their current revival in the context of promotion of green technologies, and outline key selection and management considerations for their effective use.

Based on reports in the literature, we conclude that cover crops can contribute to carbon sequestration, especially in no-tillage systems, whereas such benefits may be minimal for frequently tilled sandy soils. Due to the presence of a natural soil cover, they reduce erosion while enhancing the retention and availability of both nutrients

J.M.S. Scholberg (✉) and W.A.H. Rossing
Biological Farming Systems, Wageningen University, Postbox 563, 6700 AN Wageningen,
The Netherlands
e-mail: johannes.scholberg@wur.nl

S. Dogliotti
Facultad de Agronomía, Universidad de la República, Avda. Garzón 780,
Código Postal 12900, Montevideo, Uruguay

C. Leoni
Instituto Nacional de Investigación Agropecuaria - Estación Experimental Las Brujas,
Ruta 48 km 10 Código Postal 90200, Rincón del Colorado – Canelones, Uruguay

C.M. Cherr
Dept. of Plant Sciences, University of California, Davis, CA95616, USA

L. Zotarelli
Agricultural and Biological Engineering Dept, University of Florida, 234 Fraziers-Rogers Hall,
Gainesville, FL32611, USA

and water. Moreover, cover-crop-based systems provide a renewable N source, and can also be instrumental in weed suppression and pest management in organic production systems. Selection of species that provide multiple benefits, design of sound crop rotations, and improved synchronization of nutrient-release patterns and subsequent crop demands, are among the most critical technical factors to enhance the overall performance of cover-crop-based systems. Especially under adverse conditions, use of mixtures with complementary traits enhances their functionality and resilience. Since traditional research and extension approaches tend to be unfit for developing suitable cover-crop-based systems adapted to local production settings, other technology development and transfer approaches are required. The demonstration of direct benefits and active participation of farmers during system design, technology development, and transfer phases, were shown to be critical for effective adaptation and diffusion of cover-crop-based innovations within and across farm boundaries. In conclusion, we would like to state that the implementation of suitable policies providing technical support and financial incentives to farmers, to award them for providing ecological services, is required for more widespread adoption of cover crops.

Keywords Cover crops • green technologies • management • sustainable agro systems • carbon sequestration Americas • pest control • tillage • rotation • weeds • nematode • crimson clover • winter rye • black oats • living mulch • citrus • broccoli • forage • ecological service • adaptation • green manure

Abbreviation

SOM soil organic matter

2.1 Introduction

“Cover crops” are herbaceous plants that alternate commercial crops during fallow periods to provide a favorable soil microclimate, minimize soil degradation, suppress weeds, and enhance inherent soil fertility (Sarrantonio and Gallandt 2003; Sullivan 2003; Anderson et al. 2001; Giller 2001). “Green manures” are cover crops primarily used as a soil amendment and nutrient source for a subsequent crop (Giller 2001). “Living mulches” are cover crops grown simultaneously with commercial crops that provide a living mulch layer throughout the season (Hartwig and Ammon 2002). For the purpose of this review, we will not distinguish among these uses, and use the term cover crop in its broadest context instead.

Historically, cover crops have been effective in closing nutrient cycles and were integral part of food production systems that gave rise to modern agriculture (Drinkwater and Snapp 2007; McNeill and Winiwarter 2004; Pieters 1927). However,

during the second part of the twentieth century, the “contemporary agricultural revolution” resulted in an uncoupling of C and N cycles (Drinkwater and Snapp 2007; Mazoyer and Roudart 2006). As an integral part of the “agricultural revolution” process, the use of inorganic fertilizer greatly increased, since these materials provide growers with a concentrated and custom-designed nutrient source (Smil 2001). The contemporary agricultural revolution, thus, directly contributed to an erosion of traditional techniques for sustaining inherent soil fertility, including the use of cover crops (Baligar and Fageria 2007; Sarrantonio and Gallandt 2003; Gliessman et al. 1981). Farmers throughout the Americas are increasingly being caught in a vicious spiral of unsustainability related to depletion and degradation of land and water resources, increasing labor and input costs, and decreasing profit margins (Cherr et al. 2006b; Dogliotti et al. 2005). In many cases, farmers were forced to enhance family income via intensification, specialization, and production of cash crops, or alternatively, abandon their operations (Dogliotti et al. 2005).

With current concerns related to global warming, rapid depletion of fertilizers and fossil fuel reserves, agriculture is required to provide more diverse ecological services and make more efficient use of natural/renewable resources (van der Ploeg 2008; Cherr et al. 2006b). Within this context, improved integration of the use of cover crops may once again become the cornerstone of sustainable agroecosystems (Baligar and Fageria 2007). However, the development of functional cover-crop-based systems will require a more integrated and system-based approach, rather than reinstating traditional production practices. The scope of this paper is to (i) provide a historic perspective on the use of cover-crop-based systems in agroecosystems; (ii) document specific services and benefits provided by cover crops with special reference to their use in the Americas; (iii) discuss selection procedures for cover crops; (iv) outline key management aspects that facilitate integration and performance of cover crops into agrosystems; (v) discuss potential limitations and challenges during the design and implementation of cover-crop-based systems.

2.2 Historic Perspective

Starting at the cradle of agriculture in southwest Asia, farmers utilized leguminous crops, including peas and lentils, to restore inherent soil fertility and to sustain grain crop production (McNeill and Winiwarter 2004). In England, fallows were replaced by clovers in grain–turnip production systems to improve soil fertility, whereas in the Americas, beans were used for this purpose (Russell 1913). During the early 1800s, continuous population growth and urbanization required the use of more concentrated forms of fertilizer and mined mineral guano deposits to offset declining inherent soil fertility in Western Europe and New World, but this resource was both scarce and relatively expensive (McNeill and Winiwarter 2004). During the 1870s, mucuna (*Mucuna pruriens*) was introduced in Florida as a forage crop and by 1897 it was used by hundreds of citrus growers as an affordable alternative to improve soil fertility while it was also used as a forage crop (Crow et al. 2001; Buckles et al.

1998; Tracey and Coe 1918). *Mucuna* was introduced in Guatemala during the 1920s as a forage source and as a rotational crop for maize-based systems. Its use spontaneously spread and was adopted by farmers in neighboring countries as well (Giller 2001). In Uruguay, vetch (*Vicia villosa* Roth) and oats (*Avena sativa* L.) were introduced as green manures in vineyards around 1960, but due to increased supply of inexpensive fertilizer and lack of suitable cultivation tools, this practice was discontinued (Selaya Garvizu 2000).

Annual winter cover crops were integral part of many North American cropping systems during the first part of the last century (Pieters 1927). However, their use was gradually abandoned due to the availability of inexpensive synthetic fertilizer during the 1950s, which provided growers with concentrated nutrient sources that could be easily managed (Tonitto et al. 2006; Smil 2001). As a result, soil fertility strategies shifted from building SOM and inherent soil fertility via sound crop rotations and supplementary use of (in)organic nutrient sources, to a system dominated by external inputs used to boost labile nutrient pools and crop yields (Drinkwater and Snapp 2007). Moreover, externalities associated with the excessive use of agrochemicals were typically ignored while inherent system's functions and services were gradually being lost (Cherr et al. 2006b). Additionally, the shift toward large-scaled and highly specialized operations diminished inherent diversity and resilience of local agricultural production systems (van der Ploeg 2008; Shennan 2008; Baligar and Fageria 2007; Cherr et al. 2006b).

In terms of awareness of potential negative aspects of industrialized agriculture, the “great dust bowl” occurring in the USA in the 1930s, gave rise to increased emphasis on soil conservation, including the use of cover crops (Hartwig and Ammon 2002). During the 1970s, externalities associated with maintaining large labile nutrient pools became a major concern and practices were proposed to reduce environmental impacts, including the use of cover crops (Drinkwater and Snapp 2007; Mays et al. 2003; Dabney et al. 2001). Although agricultural development resulted in an unprecedented increase in productivity, it also promoted increased specialization and required substantial capital investments, while “real” prices of agricultural commodities dropped by a factor 2–4 between 1950 and 2000. Especially small farmers were not able to adapt to this transition and the majority of them was forced to abandon farming (Mazoyer and Roudart 2006). Moreover, in many developing regions, green revolution technologies were less effective in more adverse, risk-prone, and resource-limited production environments (Shennan 2008; El-Hage Scialabba and Hattam 2008). During the 1960s a modified form of the agricultural revolution occurred in Latin America which involved investments in local infrastructure, access to loans, improved inputs, and price subsidies (Mazoyer and Roudart 2006). However, in Brazil, increased mechanization and intensification of agriculture in hilly regions resulted in rampant erosion and soil degradation, which undermined the inherent production capacity of local production systems (Prado Wildner et al. 2004).

During the 1980s, adoption of cover crops as part of conservation technologies increased exponentially by farmers in southern Brazil (Calegari 2003; Landers 2001). This process has resulted in a gradual reversal of the degradation of the natural production base since farmers were able to partially restored SOM levels and also reduce

their dependence on external inputs. This kind of revolutionary success story inspires confidence in potential role of cover-crop-based technology to reverse the downward spiral of unsustainability that still prevails in many regions. This unprecedented successful expansion of no-tillage technology expansion in this region was clearly driven by farmers who actively engaged in technology development and transfer, and combined with favorable government policies, this greatly facilitated the scaling out process on a more regional scale. On the other hand, the use of no-tillage and/or cover crops by commercial vegetable growers in the SE USA was limited. This was related to the high crop value and risk-averse behavior of conventional producers (Phatak et al. 2002). However, increased concerns related to environmental quality, energy use, and global warming, have resulted in a shift toward resource preservation with an increased focus on sustainability and/or ecological-based (organic) production systems (Shennan 2008; Ngouajio et al. 2003; Hartwig and Ammon 2002; Lu et al. 2000). In summary, although cover crops were abandoned due to green revolution technologies, due to the current interest in green technologies, they are once more becoming the cornerstone of sustainable agrosystems (Baligar and Fageria 2007; Cherr et al. 2006b; Sullivan 2003; Phatak et al. 2002; Shennan 1992).

2.3 Services and Benefits

Regarding the use of cover crops, it is important to distinguish “ecosystem goods” from “ecosystem services” (Shennan 2008). From a producer’s perspective, cultivation of a cover crop may yield direct forage benefits and improved grain yields in integrated systems, while from a policy view its use also provides environmental benefits, e.g., erosion control and clean drinking water. Adoption of cover-crop-based systems tends to be strongly influenced by the perception of different stakeholders of what (direct) benefits cover crops will provide under local conditions and increased awareness of such services is, thus, critical (Anderson et al. 2001). An overview of a number of these direct and indirect services is provided in Fig. 2.1, while specific aspects will be discussed in more detail below.

2.3.1 *Soil Organic Matter*

Maintaining soil organic matter (SOM) is critical for sustaining soil quality and crop productivity, especially in the absence of external inputs (Fageria et al. 2005; Sarrantonio and Gallandt 2003). Cover crops may enhance SOM content in the soil provided that SOM addition rate exceeds SOM breakdown (Calegari 2003; Sullivan 2003). The use of cover crops, the presence of crop residues and SOM, have all been linked to improved soil aggregation and soil structure and enhanced water infiltration, retention, drainage, and soil aeration, thus, reducing runoff and erosion (Sainju et al. 2007; Fageria et al. 2005; Dabney et al. 2001; Miyao and Robins 2001; Creamer

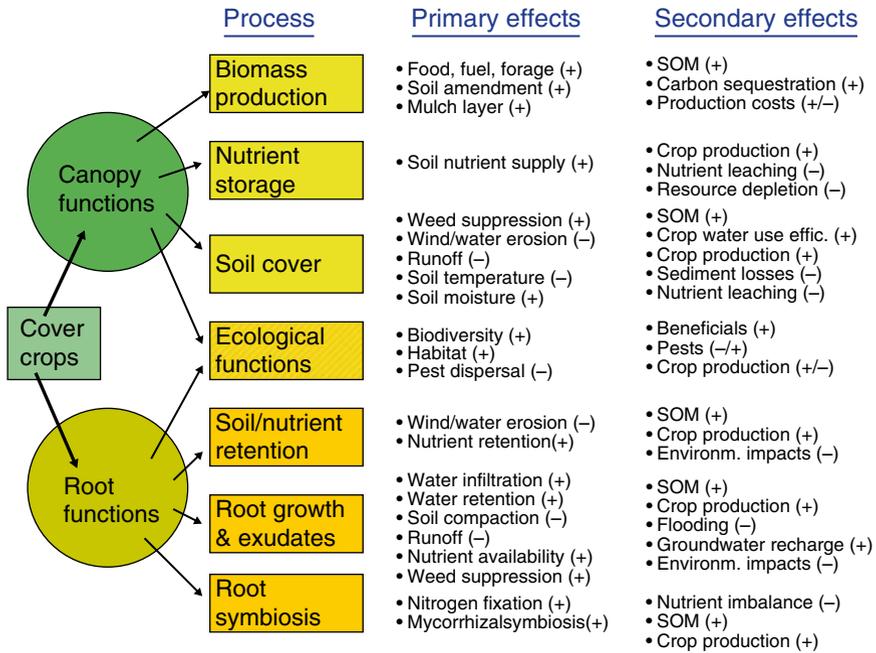


Fig. 2.1 Schematic overview of cover crops and corresponding primary and secondary effects on different agroecological services. SOM: soil organic matter

et al. 1996b; Gulick et al. 1994; Derpsch et al. 1986). Increasing SOM also favors root growth, available water capacity (AWC), effective soil water storage, and potential yield in water-limiting environments (Sustainable Agricultural Network 2007; Fageria et al. 2005; Anderson et al. 2001; Derpsch et al. 1986). Hudson (1994) reviewed historic data sets on the effect of SOM on AWC and showed that AWC was increased by 2.2–3.5% for each percent increase in SOM. Increased SOM also greatly improves cation retention, and combined with complexation and mineralization of nutrients, it, thus, greatly improves crop nutrient availability (Anderson et al. 2001). Cover-crop residues were shown to enhance the benefits of no-tillage on aggregate stability, microbial biomass, SOM, and soil enzymes (Roldan et al. 2003; Zotarelli et al. 2005a, b, 2007; Fageria et al. 2005; Calegari 2003). Amado et al. (2006) emphasized the importance of including leguminous cover crops in no-tillage systems as a strategy for increasing carbon sequestration in tropical and subtropical regions. However, for non-nutrient limited systems and under adverse growth conditions, growing recalcitrant nonleguminous cover crops with a greater biomass production, may be more effective in boosting SOM (Barber and Navarro 1994a).

Overall dry-matter production and nutrient accumulation by cover crops affects their potential to increase SOM. The production capacity of cover crops is dictated by genetic traits, including C3 versus C4 photosynthetic pathways, the ability of roots to form symbiotic associations, canopy characteristics, tissue composition, and growth duration. These traits, in turn, control crop radiation, water and nutrient use efficiencies,

and also provide limitations to how cover-crop-based systems will perform. A review by Cherr et al. (2006b) showed that under optimal conditions, annual cover crop may accumulate up to 4.4–5.6 Mg C ha⁻¹ during a period of 3–5 months.

Overall decomposition of cover-crop residues is affected by (1) amount that is applied; (2) biochemical composition; (3) physical properties as related to crop development stage and/or termination practices; (4) soil texture, temperature, and moisture conditions; (5) soil contact; (6) nutrient availability and fertilizer addition (Balkcon and Reeves 2005; Sullivan 2003; Berkenkamp et al. 2002; Ma et al. 1999; Honeycutt and Potaro 1990; Schomberg et al. 1994). The base temperature for decomposition is assumed to be on the order of -2°C to 0°C, while decomposition rates double when soil temperature increases by 9°C (Yang and Janssen 2002). Decomposition is fastest at high temperatures (30–35°C), adequate moisture (e.g., at field capacity), adequate N tissue levels (e.g. C:N ratios <25), and favorable lignin/N ratios (Cherr et al. 2006b; Quemada et al. 1997). Under hot and humid conditions, decomposition rates may be four to five times greater compared to temperate settings (Lal et al. 2000). Decomposition rates are on the order of 0.2, 0.05 and 0.0095 day⁻¹ for glucose- versus cellulose- versus lignin-based carbon pools (Quemada et al. 1997). Stems, which contain less N and more lignin and cellulose, thus, may decompose up to five times slower compared to leaves (Cherr et al. 2006b).

The fraction remaining after 1 year (the effective SOM addition rate) may be relatively small (e.g. <0.1–0.4) depending on pedo-climatic conditions and residue properties (Yang and Janssen 2000). Compared to the more stable soil C-pool, the addition of cover-crop residues to the stable SOM pool, thus, may be relatively small, since a soil with 1% SOM contains 24 Mg C ha⁻¹ in just the upper 0–30 cm. Additionally, as discussed above, only a small fraction of the C from cover crops may be converted to effective SOM, whereas most of it is lost during the decomposition process.

In Brazil soil C-enrichment, even under no-tillage, was only 10% of the C-addition rate (Metay et al. 2007). In California, the use of cover-crop-based no-tillage tomato system on a clay loam soil cover crops generated 1.8–2.3 Mg C ha⁻¹ year⁻¹. After a period of 5 years, overall soil-C sequestration was 4.5 Mg C ha⁻¹ compared to 3.8 Mg C ha⁻¹ for standard tillage systems, while noncover-crop systems showed a net loss of 0.1–0.4 Mg C ha⁻¹ (Veenstra et al. 2007). Under these conditions, crop carbon addition rate was more important than tillage management in terms of SOM accumulation, where as in Brazil the opposite may be true (Metay et al. 2007; Amado et al. 2006). Use of leguminous cover crop and/or fertilizers will result in a decrease in C:N value of recently formed SOM compared to monocultures of graminaceous cover crops (Ding et al. 2006). A biculture of hairy vetch (*Vicia villosa* Roth) and rye (*Secale cereale* L.) was more effective in sequestering C compared to cover-crop monocultures while adding fertilizer enhanced overall SOM accumulation (Sainju et al. 2006). In India, continuous use of perennial cover crops in a coconut plantation for 12 years greatly enhanced basal respiration, microbial C and N, reduced C:N ratios of microbial biomass, while SOM values in the upper 20 cm also increased by a factor 2–3 (Dinesh 2004; Dinesh et al. 2006).

Under the hot/humid weather conditions and sandy soils prevailing in Florida, use of annual cover crops in a no-tillage sweet corn production system generated

upto 7.2–9.6 Mg C ha⁻¹ year⁻¹. However, despite these high C addition rates, SOM still declined from 1.4% (year 1) to 1.3% (year 2) which was related to the site previously being under pasture (Cherr 2004). After 4 years of cover-crop-based systems, SOM reached an equilibrium of about 1.2% in cover-crop-based systems, while not adding any crop residues, combined with frequent tillage, resulted in a decline in SOM to 0.8%. Under these conditions, alternating vegetable production systems with semipermanent pastures, which tend to have higher effective C addition rates, may be required to boost SOM values. This is in agreement with reports that sod-forming grass-legume leys are more effective in enhancing SOM compared to the use of annual green manures (Hansen et al. 2005; Sullivan 2003). However, for production settings with more fine-textured soils, which is critical for occlusion (protection) of soil organic matter (Zotarelli et al. 2007), the integrated use of cover crops and no-tillage was shown to increase SOM in annual cropping systems as well (Matus et al. 2008; Roldan et al. 2003; Sanchez et al. 2007; Amado et al. 2006; Luna-Orea and Wagger 1996; Barber and Navarro 1994b).

Since changes in SOM are slow and may be masked by inherent variability, the use of models may be useful. Simulations with models such as NDICEA (Van der Burgt et al. 2006) allow improved assessment of how cover crops may affect SOM trends over time. Using this model it was shown that for traditional vegetable cropping systems in Uruguay, SOM values decreased by 420–700 kg ha⁻¹ year⁻¹. Due to erosion rates of 18–19 Mg ha⁻¹ year⁻¹, total SOM loss amounted to 800–1,170 kg ha⁻¹ year⁻¹ (Selaya Garvizu 2000). In cover-crop-based systems, approximately 4–5 Mg ha⁻¹ year⁻¹ crop residues were added, and SOM levels could be maintained while soil erosion was reduced by 67% (Selaya Garvizu 2000).

2.3.2 *Physical Functions*

Cover crops will modify the microclimate by reducing kinetic energy of rainfall, soil temperature fluctuations, wind speed, and crop damage associated with sand blasting (Fageria et al. 2005; Bravo et al. 2004; Anderson et al. 2001; Dabney et al. 2001; Masiunas 1998). Their canopy and residues will diminish the impact of raindrops, thereby reducing soil crusting and erosion, while their stems and root system also provides a physical barrier that can prevent sheet erosion and gully formation (Sarrantonio and Gallandt 2003; Masiunas 1998; Derpsch et al. 1986). Their use on sloping lands can provide a viable and labor-efficient alternative to the development of stone embankments and terracing (Bunch 1996). Erosion control (as shown in Fig. 2.2), thus, is one of the core services that cover crops provide (Prado Wildner et al. 2004). Use of leguminous live mulches, thus, may reduce runoff and soil erosion by 50% and 97%, respectively (Hartwig and Ammon 2002). Cover crops, such as deep-rooted radish, can penetrate compacted subsoil layers and prior root channels can enhance soil water infiltration, root penetration, soil water-holding capacity, and thus, crop water use efficiency of subsequent crops (Weil and Kremen 2007; Sarrantonio and Gallandt 2003; Giller 2001). Cover crops provide a structural habitat



Fig. 2.2 Field demonstration of use of cover crop (*left*) to reduce soil degradation (*right*) in Uruguay

for both beneficial insects and birds, and by reducing light levels and soil temperature fluctuations at the soil surface, they also reduce weed germination (Bottenberg et al. 1997; Bugg and Waddington 1994). Moreover, cover-crop residues conserve soil moisture and favor beneficial fungi producing glomalin that, in turn, enhances the formation of stable soil aggregates, water percolation, and retention (Sustainable Agricultural Network 2007; Altieri 2002).

2.3.3 Soil Fertility

Cover crops and their residues accumulate and/or retain nutrients either by symbiotic N fixation, uptake during growth, or immobilization after crop senescence. Thereby, they enhance nutrient retention and recycling and reduce the risk of potential nutrient-leaching losses by functioning as a “catch crop” (Cherr et al. 2006b; Dabney et al. 2001). Cover-crop-derived nutrients are typically released gradually over time, which may reduce the risk of toxicity, leaching, and thus, may enhance nutrient efficiency compared to use of highly soluble inorganic fertilizers (Cherr et al. 2006b). In many cases, actual yield benefits exceed those expected based merely on cover-crop-derived nutrients, which may be related to cover crops providing a much broader array of ecological services compared to the exclusive use of synthetic fertilizer (Bhardwaj 2006). However, utilization of N released by cover crops can also be poor if nutrient release is not synchronized with the crop demand of a subsequent crop (Baijukya et al. 2006).

Leguminous crops provide supplementary nitrogen via symbiotic N fixation and their relatively low C:N ratio also increases mineralization which reduces the risk of N deficiency for subsequent and/or companion crops (Sanchez et al. 2007;

Cherr et al. 2006b; Schroth et al. 2001). Use of leguminous cover crops, thus, provide an on-farm renewable form of N, thereby, reducing energy cost associated with production and transport of fertilizers (Cherr et al. 2006b). In organic systems, use of leguminous cover crops also offset P accumulation and potential environmental risks associated with the excessive use of animal manures (Cherr et al. 2006b), while the use of a soil-building cover crop may also be required to meet certification requirements (Delate et al. 2003). Under favorable conditions, cover crops may accumulate substantial amounts of N (150–328 kg N ha⁻¹), with 31–93% of this N being derived from biological N fixation (partially) offsetting N removal via harvesting of commercial crops (Cherr et al. 2006b; Giller 2001). Giller (2001) provided an overview of reported N accumulation and fraction of N derived via symbiotic N fixation for commonly used tropical legumes. Similar information for other cover crops and/or nutrients may be obtained elsewhere (Cherr et al. 2006b; Fageria et al. 2005; Calegari 2003).

Roots of cover crops also exude organic compounds which can enhance soil microbial activity, mycorrhizal activity, soil structure, and nutrient availability (Pegoraro et al. 2005; Calegari 2003; Dabney et al. 2001). Prolonged use of grass-clover mixture or annual cover crops, in combination with no-tillage, may reduce runoff and erosion, thereby reducing loss of fertile top soil and SOM, which in turn, can further enhance soil water retention and nutrient use efficiency (Sanchez et al. 2007; Bunch 1996). Deep-rooted types such as rye (*Secale cereal* L.) and sunn hemp (*Crotalaria juncea* L) can effectively scavenge nutrients from deep soil layers and render them more readily available for subsequent crops (Wang et al. 2006; Fageria et al. 2005; Calegari 2003; Sullivan 2003). Fast-growing and deep-rooting cover crops such as winter rye, radish, and brassicas, deplete labile residual N pools and are very effective in retaining nutrients (Vidal and Lopez 2005; Isse et al. 1999; Dabney et al. 2001; Wyland et al. 1996). In Maryland, brassicas depleted residual soil N up to a soil depth of 180 cm and took up more N compared to rye (Weil and Kremen 2007). Leguminous cover crops have low C:N ratios and can release large amounts of N instantaneously and their use, thus, may result in excessive N-leaching especially on sandy soils (Avila 2006; Sainju et al. 2006).

2.3.4 Pest Management

2.3.4.1 Soil Ecology

In balanced ecosystems, pests are internally managed by natural enemies while management practices should be geared toward favoring beneficial organisms rather than eradicating pests (Sustainable Agriculture Network 2007) and promoting disease-suppression mechanisms (van Bruggen and Semenov 2000). During the past decades, there has been increased concern in pesticide use in agriculture, especially in intensively managed vegetable crops (Abdul-Baki et al. 2004; Masiunas 1998). Effective use of cover crops may reduce herbicide use and cost associated with soil

fumigation (Abdul-Baki et al. 2004; Carrera et al. 2004) and they can reduce potential leaching of both nutrients and pesticides (Masiunas 1998; Wyland et al. 1996). Moreover, cover-crop-based systems enhance ecological diversity, productivity, and stability of agrosystems as well (Shenann 2008; Cherr et al. 2006b). Several cultural practices such as crop rotation, cover crops, mulches, composts, and animal manures affect SOM, disease suppressiveness of soils, and thus, minimize both the incidence and severity of soil-borne diseases.

2.3.4.2 Diseases

Cover crops suppress diseases by interfering with disease cycle phases such as dispersal, host infection, disease development, propagation, population buildup, and survival of the pathogen, in a number of ways. The presence of cover-crop mulches minimizes pathogen dispersal via splashing, water runoff, and/or wind-borne processes (Cantonwine et al. 2007; Everts 2002; Ntahimpera et al. 1998; Ristaino et al. 1997). Organic residues also reduce the incidence and severity of soil-borne diseases by inducing inherent soil suppressiveness, while excessive use of inorganic fertilizers may cause nutrient imbalances and lower pest resistance (Altieri and Nicholls 2003). When selecting cover crops, information on how effective these crops are in hosting or suppressing pathogens is needed (Abawi and Widmer 2000). The host–nonhost–tillage system interaction aspect should, thus, be considered carefully (Colbach et al. 1997). Saprophytic pathogens survive on cover-crop residues and this effect is also greatly affected by tillage. In some cases, the cover crops can be a host for the pathogen but will not develop any disease symptoms itself. *Fusarium oxysporum* f.sp. *phaseoli* may prevail in a leguminous cover crops when rotated with beans (Dhingra and Coelho Netto 2001). When cover crops are not properly decomposed, pathogen population such as *Phytium* spp. increase, causing severe epidemics (Manici et al. 2004). Incorporation of crop residues greatly affect soil microbial populations and can increase pathogen inhibitory activity as was shown for Phytophthora root rot in alfalfa, Verticillium wilt in potato and *Rhizoctonia solani* root rot by changes in resident *Streptomyces* spp. community (Mazzola 2004; Wiggins and Kinkel 2005a, b). Incorporation of crop residues with or without tarping can provide soil disinfestation via biochemical mechanisms (Blok et al. 2000; Gamliel et al. 2000). For example, breakdown of Brassica residues containing glucosinolates resulted in the formation of bio-toxins, including isothiocyanates, that provide (partial) control of diseases, weeds, and parasitic nematodes (Weil and Kremen 2007). Cover crops also promote disease suppressiveness by favoring certain groups of the resident soil microbial community, as related to the interactive effects of root exudates and root affinity of different crops on beneficial organisms (van Elsas et al. 2002; Mazzola 2004).

However, in some cases, cover crops increase disease incidence as was shown for pathogens with a wide host range such as *Sclerotium rolfsii* (Gilsanz et al. 2004; Jenkins and Averre 1986; Taylor and Rodríguez-Kábana 1999b, Widmer et al.; 2002). The use of cover-crop residues for mulching enhanced disease suppression of *Sclerotinia sclerotiorum* – beans pathosystem (Ferraz et al. 1999), while in a

no-tillage small grain system, they promoted *Rhizoctonia solani* (Chung et al. 1988). Brassica species were quite effective in controlling Sclerotinia diseases in lettuce, whereas oats and broad beans did not provide any disease control (Chung et al. 1988). Verticillium wilt incidence in potato was reduced when potato was grown after corn or sudangrass compared to planting it after rape or winter peas (Davis et al. 1996). Cover-crop-based systems (leguminous vs brassicas) had no effect on the *Fusarium* incidence in processing tomatoes in California compared to control systems. Although they showed yield benefits compared to noncover crop controls, yields were still lower compared to the use of Metham which was more effective in controlling *Fusarium* (Miyao et al. 2006).

2.3.4.3 Insects

Cover crops and their residues also reduce insect pest populations as was reported for a range of insects, including aphids, beetles, caterpillars, leafhoppers, moths, and thrips (detailed reviews are provided by Sarrantonio and Gallandt 2003; Masiunas 1998). This is related to changes in biophysical soil conditions, formation of protective niches for beneficial organisms, release of allelochemicals, and changes in soil ecology (Tillman et al. 2004; Sarrantonio and Gallandt 2003). Cover crops also increase biodiversity by creating more favorable conditions for free-living bacterivores and fungivores, and other predators. Combined with reduced proliferation of pests, the presence of cover crops (residues) hampers dispersal of visual and olfactory clues emitted by host crops, thus, resulting in more effective insect pest suppression as well (Tillman et al. 2004; Masiunas 1998). However, in other cases, cover crops provide a shelter for insect pests as well (Masiunas 1998).

2.3.4.4 Nematodes

Reduction of nematodes by cover crops is well-documented (Abawi and Widmer 2000; Taylor and Rodríguez-Kábana 1999a; Widmer et al. 2002). Several grassy and leguminous cover crops, including *Crotalaria*, *Mucuna*, and *Tagetes* species, were shown to be nonhost or a suppressor of selected parasitic nematodes (Wang et al. 2007; Crow et al. 2001; McSorley 2001). Crop rotations, including such species, disrupt the life cycle of parasitic nematodes and reduce the risk of breakdown of inbred nematode resistance of commercial crops (McSorley 2001). In some cases, nematode-suppression action is related to the beneficial effects of cover-crop residues on predatory nematodes and nematode-trapping fungi (Cherr et al. 2006b). However, no cover crop will function as a nonhost for all parasitic nematodes, while in several cases, cover crops were shown to favor the growth of parasitic nematodes as well (Isaac et al. 2007; Sanchez et al. 2007; Crow et al. 2001; Cherr et al. 2006b). Reports on crops being a host versus nonhost may conflict at times, which can be related to differences in pedo-climatic conditions, nematode races, and cover-crop cultivars and thus, the results may need to be verified for local production settings.

2.3.4.5 Weeds

In organic systems, cost-effective weed control is the foremost production factor hampering successful transition (Kruidhof 2008; Linares et al. 2008). Well-designed cover-crop systems can reduce herbicide use and labor use for weed control, and cover crops, thus, afford farmers with a cost-effective strategy for weed control, which is a key deciding factor in their adaptive use (Gutierrez Rojas et al. 2004; Anderson et al. 2001; Neill and Lee 2001; Teasdale and Abdul-Baki 1998; Bunch 1996; Barber and Navarro 1994a). Cover crops suppress weeds via resource competition, niche disruption, and release of phytotoxins from both root exudates and decomposing residues, thereby minimizing seed banks, and the germination, growth, and reproduction of weeds (Kruidhof 2008; Moonen and Barberi 2006; Fageria et al. 2005; Sarrantonio and Gallandt 2003). Their effectiveness in suppressing weeds is affected by plant density, initial growth rate, aboveground biomass, leaf area duration, persistence of residues, and time of planting of a subsequent crop (Kruidhof 2008; Linares et al. 2008; Sarrantonio and Gallandt 2003; Cassini 2004; Dabney et al. 2001).

Although cover crops greatly reduced weed growth in conventional vegetable-cropping systems, in some cases applying herbicide may still be required to minimize the risk of yield reductions (Teasdale and Abdul-Baki 1998). Annual cover crops such as mucuna may also be used to control perennial weeds, provided that they effectively shade out these weeds just prior to weeds starting replenishing their storage organs (e.g., rhizomes) with assimilates (Teasdale et al. 2007). Repeated use of annual cover crops combined with no-tillage in organic systems did not control grassy weeds (Treadwell et al. 2007). Their continuous use can also result in a shift toward perennial weeds which can be addressed by alternating crop systems with pastures (M. Altieri, 2008). Use of cover-crop mixtures with complementary canopy characteristics (e.g., rye and clover as shown in Fig. 2.3) and differential root traits (e.g., fibrous vs deep tap roots) will provide superior cover-crop performance and thus, more effective weed control (Linares et al. 2008; Drinkwater and Snapp 2007; Masiunas 1998). Use of a “cover crop weed index” (ratio of aboveground dry weights of cover crops and weeds) was shown to be a useful tool for assessing weed-suppression capacity of cover crops (Linares et al. 2008).

Mowing in orchards can provide more effective weed control when combined with a grassy vegetation compared to its use with annual legumes (Matheis and Victoria Filho 2005). Use of mowed leguminous live mulches in an organic wheat system reduced weed growth by 65–86% but grain yields were only a fraction of those in weed-free controls possibly due to resource competition between cover crops and the wheat crop (Hiltbrunner et al. 2007). Leguminous cover crops may have a competitive edge on weeds under N-limiting conditions, whereas the use of repeating mowing may be effective to control taller weeds for more fertile production sites.

Weed suppression by cover-crop residues is related to the effective soil coverage which may be sustained for 30–75 days (as shown in Fig. 2.4). This depends on



Fig. 2.3 Prostrate growth and flat-leaf angle of crimson clover complements more erect growth characteristics of winter rye (*left*) and black oats (*right*) while these cereal crops also have higher initial growth and are thus more effective in retaining residual soil nutrients on sandy soils in Florida



Fig. 2.4 Use of mowed cowpea residue as a mulch in a subsequent no-tillage broccoli crop (*left*) and forage radish as a live mulch in an organic citrus orchard (*right*) in Florida

decomposition as related to residue amount and biochemical properties, rainfall, soil temperature, and weed pressure/vigor (Teasdale et al. 2004; Ruffo and Bollero 2003; Masiunas 1998; Creamer et al. 1996). Incorporation of residues reduces their weed-suppression capacity due to increased light levels, transfer of dormant seeds to the soil surface, and also results in increased breakdown and dilution of allelochemicals (Masiunas 1998). Rye and barley residues were effective in suppressing broadleaf weeds, while hairy vetch residues enhanced weed growth (Creamer et al. 1996),

which may be related to their releasing nutrients (Teasdale et al. 2007), while rye was less effective in suppressing grassy weeds (Masiunas 1998).

2.3.5 Food and Forage Production

Although food and forage production may not be the main purpose of cover-crop use, some systems may also provide products for human consumption, grazing, and/or to produce fodder as was reported for, example, *Canavalia ensiformis*, *Dilochis lablab*, *Avena strigosa*, *Vicia villosa* (Nyende and Delve 2004; Pieri et al. 2002; Anderson et al. 2001). Examples of cover crops suited for human consumption include *Cajanus cajan* (pigeon pea), *Dolichos lablab*, and cowpea (*Vigna cinsensis*). Integrating livestock components into cover-crop-based systems can improve bio-economic efficiencies, profits, and human health. Potential applications may include the use of cover crops to regenerate degraded pasture land, improvement of the animal diet, and enhancement of the intensification of small-scale farming systems (Anderson et al. 2001). Ironically, mucuna may have been introduced in Central America to be used as forage crop for mules employed in banana plantations (Anderson et al. 2001). In such integrated systems, (leguminous) cover crops may also provide (high-quality) forages, but unless manures are internally recycled, this may reduce soil improvement services and yield benefits provided by the cover crops (Anderson et al. 2001). As an example, cattle grazing of mucuna prior to corn planting reduced its effectiveness in suppressing weeds and improving corn yields (Bernardino-Hernandez et al. 2006).

2.3.6 Economic Benefits

In terms of conventional economics, key considerations are seed and labor costs which tend to account for the largest cost factors of cover-crop-based systems (Sullivan 2003; Lu et al. 2000). The seed costs of leguminous crops are twice as high as small grains, but residues of grains have high C:N ratios and may require additional N application of 25–35 kg N ha⁻¹ to reduce the risk of N immobilization which may offset potential seed cost savings (Sustainable Agriculture Network 2007). In South Georgia, self-reseeding systems of crimson clover were developed in rotation with cotton. In this case, the absence of additional tillage and seed costs combined with the automatic senescence of the cover crop prior to the maturation of the cotton crop resulted in cost-effective systems (Cherr et al. 2006b; Dabney et al. 2001).

Several studies documented significant yield benefits derived from the use of cover crops (Sanchez et al. 2007; Avila 2006; Cherr et al. 2006b, c; Fontanetti et al. 2006; Abdul-Baki et al. 2004; Neill and Lee 2001; Derpsch and Florentin 1992). These yield increases may be related to N benefits, improved soil structure and water

retention, and reduced incidence of pests. In addition to yield benefits, cover crops may also enhance crop quality as is the case of the use of winter rye interplanted with melon that protects the young fruits from sand blasting and scarring. Combined with reduced fertilizer and pesticide costs, this may offset the additional seed and cultivation costs of cover-crop-based systems (Weil and Kremen 2007; Bergtold et al. 2005; Fageria et al. 2005; Sullivan 2003). In addition to reducing fertilizer costs on poor or compacted soils, the use of cover crop (residues) may also enhance aeration and intrinsic yield potential and/or reduce crop risk under water-limited conditions (Sustainable Agriculture Network 2007; Villarreal-Romero et al. 2006; Bergtold et al. 2005; Schroth et al. 2001; Masiunas 1998). Lu et al. (2000) reviewed several studies and commented that many studies only looked at relatively short production cycles. Typically, there were no or only small significant differences in terms of yield benefits. However, in a number of cases, cover-crop-based systems showed appreciable yield fluctuations and higher labor and fuel costs. It was stated that many systems and technologies were still being developed and system performance/yields were either inconsistent or suboptimal. System design and adaptation, thus, may take several years and a number of design and evaluation cycles may be required. This is evident from research by Abdul-Baki during the past decades focusing on developing an integrated technology package, including no-tillage, mixed cover crops, mechanical termination of cover-crop residues, and use of cover-crop-mulched vegetable systems. After initial system design and development in Maryland, this system was perfected, adapted, and successfully used for different crops, regions, and production systems (Abdul-Baki et al. 1996, 1999, 2004; Carrera et al. 2004, 2005, 2007; Teasdale and Abdul-Baki 1998; Wang et al. 2005).

However, many leguminous cover-crop systems may not provide adequate N to meet crop demand and supplemental N fertilizer is still required to reduce the risk of yield reductions of subsequent commercial crops (Cherr et al. 2007; Lu et al. 2000). This is confirmed by a meta-analysis of cropping systems in temperate regions, which showed that legume-fertilized systems had 10% lower yields compared to N-fertilizer systems unless N accumulation in cover-crop residues exceeded 110 kg N ha⁻¹ (Tonitto et al. 2006). A similar study in North America showed that the use of grasses did not affect subsequent maize yields; legumes increased these yields by 37% compared to nonfertilized control systems but yield benefits decreased as N-fertilizer rate increased (Miguez and Bollero 2005). Based on past experiences, limited use of cover crops in high-value commodities was often related to the low cost of inorganic fertilizers. Moreover, most conventional but also some organic nutrient sources have relatively constant and predictable nutrient content and release patterns, while for cover crops, both the nutrient accumulation potential and release patterns tend to be highly variable. Due to this added level of complexity, the integration of cover crops in conventional systems requires farmers to become better managers to ensure optimal system performance (Shennan 2008; Cherr et al. 2006b). However, the exponential (800%) increase in fossil fuel prices between 1998 and 2008 resulted in increases of N- and P-fertilizer prices of 226% and 307%. This unprecedented increase in energy and fertilizer prices, along with the rapid depletion of mineral nutrient reserves, underlines the need for alternative nutrient sources (Wilke and Snapp 2008).

2.3.7 *Ecological Services*

In the past, externalities and actual replacement costs of nonrenewable resources were not included in production costs. Moreover, in many countries, including India and Mexico, fertilizers are greatly subsidized to improve national food security (Cherr et al. 2006b). This undermines the viability of green technologies, more sustainable development options, and puts a heavy burden on local economies. In terms of ecological services, cover-crop-based systems greatly reduce sediment losses associated with erosion, which are the main agricultural pollutants that also reduce the inherent production capacity of agroecosystems, especially in regions such as Brazil and Uruguay (Dogliotti et al. 2004; Prado Wildner et al. 2004; Dabney et al. 2001). Although cover crops should be an integral part of organic production systems, commercial organic growers may still, to a large extent, depend on animal manures, waste products of other sectors, and allowable synthetic compounds. Pursuing an “input substitution” approach hampers the closing of energy and nutrient cycles, and is in contrast with the farm-based integrated organic approach (Cherr et al. 2006b).

For conventional systems, use of cover-crop-derived mulches may reduce the need for plastic mulches and or soil fumigants (Abdul-Baki et al. 1996, 2004). Replacing a bare fallow with cover crops may also enhance nutrient retention and reduce N-leaching by upto 70% (Wyland et al. 1996). A meta-analysis of cropping-system studies showed that nitrate-leaching in legume-based systems was 40% lower compared to conventional systems (Tonitto et al. 2006). However, late planting and slow initial growth will hamper the effectiveness of cover crops in retaining residual soil nutrients (Mays et al. 2003). Poor system design and/or lack of synchronization result in inefficient N use and poor yields (Cherr 2004; Avila 2006). Therefore, for cover-crop-based systems to be ecologically sound and economically viable, development of integrated systems that provide multiple benefits to offset potential risks and investment costs is essential (Cherr et al. 2006b). In the USA, the Natural Resource Conservation Services (NRCS) awards growers for the environmental services associated with cover-crop use (Bergtold et al. 2005). However, improved assessment of true fertilizer costs will be required and farmers growing cover crops should also receive carbon credits as well (Sainju et al. 2006).

In summary, steady-state SOM values and C-addition rates required to sustain SOM will vary widely depending on pedo-climatic conditions and actual management practices. Models may provide an effective tool to assess potential benefits of cover crops in enhancing SOM (Dogliotti et al. 2005; Lal et al. 2000). Although cover crops can enhance inherent soil fertility and improve profits, inadequate management skills, poor system design, and lack of synchronization will greatly reduce such benefits. Especially in organic systems, cover crops can provide cost-effective weed suppression while in conventional systems, the use of cover crops may not be viable unless they provide multiple benefits and farmers are being awarded for ecological capital generated by growing cover crops.

2.4 Selection

The selection of cover crops is based on pedo-climatic conditions, the set of services required, current crop rotation schemes, and alternative management options (Sustainable Agriculture Network 2007; Cherr et al. 2006b; Anderson et al. 2001). An example of steps taken during the screening process of a large number of cover crops (mixes) in Ohio was discussed by Creamer et al. (1997). Although cover crops provide a myriad of services, the “perfect” cover crop simply does not exist. Consequently, priorities among a set of critical services that cover crop (mixture) should offer need to be determined first. These may include (i) providing nitrogen; (ii) retaining/recycling nutrients and soil moisture; (iii) reducing soil degradation/erosion; (iv) sustaining/increasing SOM levels; (v) reducing the incidence of pests; and (vi) providing products and income (Sustainable Agricultural Network 2007; Cherr et al. 2006b). First, a detailed analysis of the current crop management system on a field level, including crop rotations, duration of commercial crops, inter-crop/fallow period, tillage systems, along with an assesment of potential risk of pests and diseases of commercial crops, is required. Some additional practical selection and screening considerations include the following:

- Adaptation to drought, flooding, low pH, nutrient limitations, and shading (live mulch)
- Combining species with complementary growth cycles, canopy traits, and root functionality
- Lack of adverse traits
 - Unfavorable residue properties (e.g., excessively high C:N ratio, coarse, and recalcitrant residues hampering seed bed preparation, allelopathetic properties that hamper initial germination, and growth of subsequent commercial crops)
 - Competition with cash crops for light, land, water, nutrients, labor, and capital
 - Weediness and/or excessive vigor/regrowth after mowing or mechanical killing
 - Ability to promote (host) pests and diseases
- Availability of affordable seeds, suitable equipment, techniques, and information to ensure optimal cover-crop growth, termination, and overall system performance

Following these steps, an initial assesment may be made of perceived benefits and risks which may be used for ranking potential cover-crop (mixture) candidates and/or cultivars; this typically will be based on expert knowledge since no actual data may be available. The next step will be to provide an assessment of the actual services being rendered by such systems (either via field measurements/observation or using computer simulations) to further refine the crop rotation design and cover-crop management (Altieri et al. 2008; Cherr et al. 2006b). In practice, this may be a process of “trial and error” to properly integrate all relevant information as related to local pedo-climatic conditions into the decision-making process. The development of management practices and a suitable site-specific cover-crop-based cropping system that are relevant within the local context, thus, may require several years

and a number of experimental learning cycles, while cover-crop-based systems may continue to evolve over time as well. Some of the most pertinent aspects of cover-crop selection will be discussed in more detail below. The use of expert systems, such as GreenCover (Cherr et al. unpublished; <http://lyra.ifas.ufl.edu/GreenCover>) and ROTAT (Dogliotti et al. 2003), may facilitate the first selection step of designing suitable cover crops.

2.4.1 Adaptation

Adaptation may include day length, temperature, radiation, rainfall, soil, pests, and crop duration aspects. Cover crops can be grouped as being adapted to “cold/temperate” versus “warm/tropical” growth environments (Anderson et al. 2001). The first type may survive a freeze upto -10°C while their growth may be hampered under hot conditions ($>25\text{--}30^{\circ}\text{C}$). Leguminous species within this group include *Lupinus*, *Trifolium*, and *Vicia* species and they grow well in temperate climates, during the winter season in subtropical climates, or in the tropical highlands (Cherr et al. 2006b; Giller 2001). The second group does not tolerate freezes ($<-2^{\circ}\text{C}$) but may thrive under hot ($>35^{\circ}\text{C}$) conditions. Some of the key leguminous species within this group include the genus *Canavalia* (e.g., Jack bean *C. ensiformis*), *Crotalaria* (e.g., sunn hemp *C. Juncea*), and *Mucuna* (e.g., velvet bean). Tropical species may also be more easily grown during the summer months as one moves toward the subtropics or even throughout the year (tropical regions). Both temperature and day length affect crop development and growth duration. Use of simple phenology models facilitates the selection of suitable species for different production environments, which can be particularly important in hillside environments (Keatinge et al. 1998). Over-sowing cover crops into existing crops (e.g., maize) requires the selection of species that are adapted to low initial light regimes (Anderson et al. 2001).

Adaptation to local soil conditions, as related to soil drainage, texture, pH, and presence of compatible rhizobia strain for leguminous crops, is critical (Cherr et al. 2006b; Giller 2001). On soils with adequate moisture storage capacities, cover crops may be grown during the dry season, while in other cases, the growth may be limited by rainfall since adequate soil moisture is required during initial growth. Crop water requirements of cover crops depend on crop type and growth duration, but in many cases, cumulative water use may be comparable to that of commercial crops, and in water-limited systems, cover crops may deplete residual soil moisture reserves as well and may have to be killed prematurely (Cherr et al. 2006b). Especially, leguminous crops may be poorly adapted to either extremely acidic or alkaline soils or poorly drained soils (Cherr et al. 2006b; Giller 2001). When introducing new non-promiscuous leguminous types, the presence of suitable inoculum is critical, since poor nodulation hampers crop growth and N accumulation (Giller 2001). Leguminous crops, although adapted to N-limiting conditions, may have appreciable needs for other nutrients (including K, P, Mo), while due to their slow initial growth, they are not very efficient in utilizing residual soil nutrients.

2.4.2 Vigor and Reproduction

Initial growth of small-seeded cover crops, e.g., clovers, may not be as vigorous compared to larger-seeded types which have more reserves and can be planted deeper, especially when rainfall during initial growth is erratic (Cherr et al. 2006b). Self-seeding types, e.g., crimson clover, may provide an ample seed bank and thus, germination may be triggered automatically when conditions are favorable (Cherr et al. 2006b). However, reseeding types may also become a potential pest themselves, especially when they are hard- and/or large-seeded types. In this case, timely mowing prior to seed set may be required although the original planted crop may become a dormant seed bank in itself unless it is stratified in an appropriate manner. Cover crops such as sunn hemp over time may become rather tall (>3 m) with very thick and recalcitrant stems that may pose serious problems in subsequent vegetable crops, since they can hamper bed formation. In this case, repeated mowing may be required (N. Roe, personal communication). Other cover crops may have a viny and rather aggressive growth habit, e.g., cowpea and velvet bean, that can interfere with commercial crops when used as green mulch as was reported in citrus (Linares et al. 2008).

2.4.3 Functionality and Performance

In many hilly regions in Latin America, cover crops are an integral component of no-tillage systems, since they can reduce soil erosion, labor, and herbicide costs, and can also increase yields (Prado Wildner et al. 2004). In organic systems, they can be a critical component of integrated weed management strategies (Linares et al. 2008). The actual performance of cover crops depends on system design, inherent soil fertility, pedo-climatic conditions, management (including the use of well-adapted species), and crop duration (Cherr et al. 2006b; Giller 2001). Although potential cover-crop production may be highest in warm and high rainfall environments, SOM breakdown and potential nutrient losses under such conditions also tend to be much greater, and thus, net benefits may be actually lower compared to more temperate climates. Information on adaption, growth, and performance may be obtained from the literature (Baligar and Fageria 2007; Sustainable Agriculture Network 2007; Cherr et al. 2006b). Even within cover-crop species, there may be appreciable differences in specific traits that can greatly affect their adaptation and functionality as related to specific production settings (e.g., cold and drought tolerance; shoot:root ratio) as was shown for hairy vetch (Wilke and Snapp 2008). Use of cover-crop mixes with complementary traits may enhance the functionality, productivity, resilience, and adaptability of cover-crop-based systems and thus, facilitate more efficient resource use capture under adverse conditions (Malézieux et al. 2009; Altieri et al. 2008; Linares et al. 2008; Drinkwater and Snapp 2007; Weil and Kremen 2007; Teasdale et al. 2004; Dabney et al. 2001; Creamer et al. 1997). Moreover, a combination

of several species may provide the benefits of different included species within a single year (Calegari 2003), whereas no single cover-crop species consistently performs superior across different years and field sections (Linares et al. 2008; Carrera et al. 2005).

Typically, cover crops are not irrigated nor are they being fertilized. The growth of cover crops may be superior on more fine-textured soils since these soils often have higher SOM values, inherent soil fertility, and better water and nutrient-retention capacities. This may result in a positive feedback mechanism that, in turn, can further boost cover-crop performance over time (Cherr et al. 2006b). However, on very heavy soils, limited drainage may also result in poor aeration and increased incidence of diseases, thus, resulting in poor stands and suboptimal cover-crop performance. In organic tomato production systems in California, mixtures of grasses with leguminous cover crops accumulated more biomass but less N, whereas their residues had higher C:N ratios which delayed mineralization (Madden et al. 2004). On very sandy soils, low inherent soil fertility, among other factors, may limit growth of the cover crops, whereas nutrients accumulated in its residue may be also readily lost due to leaching prior to the peak nutrient demand of a subsequent commercial crop (Cherr et al. 2007). As a result, in adverse production environments, the growth and the benefits that cover crops provide may be limited and integrated soil fertility management practices may be required to enhance overall system performance (Tiftonell 2008; Giller 2001). In summary, a design of an appropriate cover-crop system based on key desired ecological functions, is critical for system performance. The use of expert knowledge and computer-based evaluation tools can facilitate initial screening, while optimal system design may require numerous design cycles to tailor systems to local management conditions.

2.5 Management

2.5.1 Rotation

Developing suitable crop rotation schemes is critical for enhancing systems performance. The design of both spatial and temporal crop arrangements on a farm level will be based on meeting a set of grower-defined production objectives along with adhering to site-specific phyto-sanitary guidelines. Growers typically allocate cover crops to underutilized temporal and/or spatial components of their cropping system, e.g., fallow period or row middles, which constrain their use. The growth season of cover crops is, thus, defined by the cropping season of commercial crops which, in turn, is dictated by rainfall or temperature patterns. Although it requires special equipment, undersowing of a cover crop in an existing crop may be desirable, since it facilitates more efficient resource use while reducing potential nutrient losses and erosion risks (Hartwig and Ammon 2002; Sullivan 2003). In the southern USA, cover crops such as sorghum, sudan grass, or sunn hemp may be grown during times

when it is too hot to grow commercial crops as is the case in Florida (Avila 2006). In the case of more complex arable cropping systems, the use of software tools to explore such options to generate viable alternatives greatly facilitates the design process (Bachinger and Zander 2007; Dogliotti et al. 2003).

2.5.2 *Biomass Production and Residue Quality*

Most cover crops follow a “logistic” or “expo-linear” growth pattern, so after an initial “lag-phase” prior to canopy closure, biomass accumulation rates tend to be relatively constant before leveling off toward crop maturation (Kruidhof 2008; Yin et al. 2003). Although there is a multitude of information on cover-crop performance in terms of biomass and N accumulation at maturity, narrow windows of opportunity for planting commercial crops may require cover crops to be killed prematurely (Cherr et al. 2006b). In this case, simple linear equations, thus, may be developed to estimate the amount of residues as a function of crop yield (Steiner et al. 1996). Alternatively, degree day-based models may be used to predict biomass and N accumulation of cover crops as a function of accumulated temperature units (Schomberg et al. 2007, Cherr et al. 2006c).

The carbon content of most plant material is relatively constant over time with values being on the order of 40–44% (Avila 2006; Dinesh et al. 2006). Overall plant N concentration typically follows an exponential decay curve over time (“N dilution curve”) and final N tissue concentration is, thus, a function of crop type, crop age, and N supply (Lemaire and Gaston 1997). In terms of N accumulation and subsequent N release of cover crops, based on data outlined by Cherr et al. (2006b) calculated N concentrations for temperate versus tropical legumes are on the order of 1.9–3.6% and 2.6–4.8% compared to 0.7–2.5% for nonleguminous crops which translates to corresponding C:N ranges of 8–15, 11–21, and 16–57, respectively. Calegari (2003) provided a detailed overview on the mineral composition and C:N ratio of different cover crops grown in Brazil. Such information provides an insight into the overall nutrient supply capacity of cover-crop residues, though values may differ on the basis of local soil fertility regimes. As cover crops mature, there is a gradual shift toward both structural and reproductive parts (Cherr et al. 2006c). With aging, both the leaf fraction and the N content of leaves and stems decrease, whereas more recalcitrant compounds and seed proteins may accumulate (Cherr 2004; Cherr et al. 2006b; Lemaire and Gaston 1997). Increasing plant density will result in early canopy closure, higher initial biomass accumulation rates, and dry matter allocation to less recalcitrant and high-N plant parts, while excessive high plant densities may reduce growth due to crowding (Cherr et al. 2006b). Repeated mowing for sod-forming or indeterminate cover crops can delay the shift toward more recalcitrant plant parts, enhance N content, and increase total biomass production (Cherr et al. 2006b; Snapp and Borden 2005). Planting density, time of “mowing” or “killing” cover crops, thus, affect both residue quantity and quality, and may be used to manipulate system dynamics.

2.5.3 Cover-Crop Termination and Residue Management

At the end of the fallow season, cover crops may be killed by herbicide, mowing, flaming, or by a crimper (Sustainable Agricultural Network 2007; Calegari 2003; Sullivan 2003; Lu et al. 2000; Masiunas 1998). Mowing may result in the formation of a compact mulch layer, that in turn, may help to conserve soil moisture and reduce soil erosion (Fig. 2.4). Rolled residues decompose slower compared to the use of mowing or herbicides, while the residue layer also tends to persist longer, and provides more effective long-term soil erosion control (Lu et al. 2000). Timing of mowing, as related to cover-crop development stage, is critical in term of maximizing biomass and N accumulation while reducing the risk that cover crops regrow or set seed and thereby interfere with a subsequent commercial crop (Prado Wildner et al. 2004; Sullivan 2003). The optimal time of residue killing is also related to cover crops' main function. If soil conservation and SOM buildup are priorities, older and more lignified residues may be preferable. However, delaying killing may hamper the effectiveness of rollers/crimpers, whereas residues are also more likely to interfere with planting equipment, while the resulting augmented C:N ratio can also increase the risk of initial N immobilization. Mowing and use of herbicide, on the other hand, will increase residue decomposition and subsequent mineralization (Snapp and Borden 2005). Many farmers may opt to delay planting after residue kill to reduce the risk of transmittance of herbivores feeding on residues invading the new crop, to ensure adequate settling of residues which facilitates planting operations, and to prevent the negative effects of allelopathetic compounds on the emerging crop (Prado Wildner et al. 2004). Alternatively, placement of seeds below the residue layer can reduce the risk of potential allelopathetic substances hampering initial growth (Altieri et al. 2008).

2.5.4 Tillage

Soil incorporation of cover crops enhances soil residue contact and also buffers its moisture content which tends to speed up decomposition, while surface applied residue may have a greater capacity for N immobilization (Cherr et al. 2006b). Surface application of residues also favors saprophytic decomposition by fungi, whereas bacterial decomposition is prevailing more for incorporated residues and repeated tillage tends to greatly enhance mineralization (Lal et al. 2000). Leaving mulch residues of cowpea, used as a cover crop in a lettuce production system, was much more effective in suppressing weeds compared to tilling in residues but it also reduced lettuce yields by 20% (Ngouajio et al. 2003). Use of no-tillage may reduce labor costs, energy use, and potential erosion while increasing carbon sequestration, biodiversity, and soil moisture conservation (Triplett and Dick 2008; Peigné et al. 2007; Giller 2001). In Brazil, it was demonstrated that the integrated use of cover crops with no-tillage is critical for enhancing/sustaining SOM (Calegari 2003). These techniques are complementary work and work synergistically while the use

of conventional tillage will promote rapid breakdown of SOM which may partially offset cover-crop benefits (Phatak et al. 2002). However, in organic production systems, no-tillage may result in increased incidence of grassy and perennial weeds, while for poorly drained/structured soils and under excessive wet soil conditions, its use may have unfavorable effects on soil tilth, crop growth, incidence of plant pathogens, and it may also increase the risk of N immobilization (Peigné et al. 2007). Although no-tillage and the presence of crop residues near the surface may reduce soil evaporation, it can promote root proliferation near the soil surface, thus, rendering subsequent commercial crops more vulnerable to prolonged drought stress (Cherr et al. 2006a).

2.5.5 *Synchronization*

Residue decomposition rates depend on both crop composition management and pedo-climatic conditions (Snapp and Borden 2005). Release patterns tend to be highly variable both in space and time. The release of readily available crop nutrients from cover-crop residues, thus, may not coincide with peak nutrient requirements of a subsequent crop (poor synchrony). This problem is evident from the large number of studies reviewed by Sarrantonio and Gallandt (2003) in which nutrient release was either premature or too late. Residue C:N values will, to a large degree, determine initial decomposition rates together with factors such as the content of water-soluble and intermediate available carbon compounds in the residue (Ma et al. 1999). Nitrogen allocation to root systems may be on the order of 7–32% and 20–25% of its N may be released to the soil prior to crop senescence (Cherr et al. 2006b). Moreover, under hot and humid conditions, nutrient release from low C:N residue materials may be premature and N-leaching losses can be very high (Cherr et al. 2007; Giller 2001). However, under cold and/or dry conditions, use of more recalcitrant residues, and N-limited conditions, will delay initial release and net N immobilization may hamper initial growth of commercial crops (Cherr et al. 2006b; Sarrantonio and Gallandt 2003). However, better synchronization requires improved understanding of residue decomposition and net mineralization. However, since these processes are affected by a large number of biotic, pedo-climatic, and management factors, appropriate use of decomposition models may be required to provide a better insight on how interactions among management factors come into play. These model tools may then be integrated into decision-support tools for farm managers/advisors, which was the rationale for developing the NDICEA model (van der Burgt et al. 2006). Thus, such tools can be effectively used to improve the synchronization of nutrient-release patterns with crop demand which should facilitate the successful integration of cover crops in conventional systems. Based on predictions of such models, management options such as use of different spatial and/or temporal crop arrangements, use of cover-crop mixtures to modify initial C:N ratios, time and method of killing, and method of incorporation, among others may be used to enhance synchronization (Weil and Kremen 2007; Cherr et al. 2006b; Balkcon and

Reeves 2005; Sullivan 2003). As an example, using a biculture of rye and vetch and modifying seed-mixture ratios can facilitate improved synchronization. Increasing the vetch:rye ratio will speed up initial mineralization, reduce the risk of initial immobilization, but may increase potential N-leaching risks (Kuo and Sainju 1997; Teasdale and Abdul-Baki 1998). In summary, it is evident that poor synchronization favors inefficiencies and increases potential nutrient losses. This is one of the key factors deterring conventional farmers from adopting cover-crop-based systems. Use of cover-crop mixes, improved timing of mowing and/or incorporation, along with use of decision-support tools such as NDICEA are some of the key options to enhance synchronization.

2.6 Limitations and Challenges

2.6.1 *Information and Technology Transfer*

Although cover crops provide a myriad of services, their adaptation by conventional farmers typically has been slow (Sarrantonio and Gallandt 2003). In Brazil, they were introduced during the 1970s, but wide-scale adoption took several decades (Prado Wildner et al. 2004). Some potential challenges may include: additional production costs (in terms of land, labor, and inputs), the complexity of cover-crop-based systems, the lack of pertinent information and suitable technology transfer methods, the uncertainty of release patterns from cover-crop residues, and lack of secure land tenure (Singer and Nusser 2007; Cherr et al. 2006b; Nyende and Delve 2004; Sarrantonio and Gallandt 2003; Lu et al. 2000). This additional level of complexity, combined with lack of information on suitable management practices, along with the perceived risks associated with cover-crop-based systems, prevents growers from adopting cover-crop-based systems (Shennan 2008; Sarrantonio and Gallandt 2003).

Regarding information on cover crops, a search of the CAB citation index for “cover crops” clearly indicated an increased interest in cover crops during the past decades. The annual number of papers on this topic decreased from 74 (1961–1970) to 37 (1971–1980), but then increased again from 56 (1981–1990) to 160 (1991–2000), and then to 221 (2001–2007). Despite this impressive increase in publication numbers, producers still cite lack of useful information about cover crops as one of the greatest barrier to their use (Singer and Nusser 2007). Although, during the first half of the last century, most farmers routinely used cover crops, this traditional knowledge base has been gradually lost. Even within research and extension faculty, there was a complete erosion of knowledge and experience as faculty members with a more traditional farm background retired. Moreover, during the past decades, academic interest has shifted toward genetic engineering technology, typically resulting in the recruitment of scientists lacking basic agronomic knowledge. Furthermore, most conventional farmers are not in a position to take the economic risk associated with experimentation and exploration of suitable cover-crop technologies and thus,

increasingly depend on external information sources (Weil and Kremen 2007; Cherr et al. 2006b). Therefore, lack of appropriate information and technology transfer approaches still continues to be among the key factors hampering the adoption of cover-crop-based systems (Bunch 2000).

The traditional “top-down” approach used by research and development institutes to provide technical solutions to farmers in the absence of a thorough understanding of local socioeconomic conditions and agroecosystems appears to be especially ineffective for cover-crop-based systems (Anderson et al. 2001). Establishing “innovation groups,” a technology development and exchange structure in which farmers play a key role and/or “farmer-to-farmer” training networks, in which innovative farmers assume an active role as educators, may be more appropriate for propagating cover-crop-based technologies (Anderson et al. 2001; Horlings 1998). Since most university programs are still poorly equipped to address the specific needs of organic farmers, this producer group may still be forced to engage in some on-farm experimentation with cover-crop-based systems, especially since this group appears to benefit greatly from the use of cover crops (Linares et al. 2008).

2.6.2 Resource Management

The growth and nutrient accumulation among cover-crop-based systems may vary greatly between fields and years, while subsequent nutrient-release patterns are also affected by a great number of pedo-climatic and management factors. Limited knowledge of these processes on a field scale will result in poor synchronization between nutrient release by cover crops and subsequent crop demand of commercial crops, thereby increasing the risk of inefficient N use and poor system performance (Cherr et al. 2006b). Although simulation models could harness some of this complexity, most of these models were developed for scientists and are difficult to implement, whereas models for informed decision-making and improved management of cover crops require a combination of a sound scientific basis with practice-oriented model design (van der Burgt et al. 2006).

In terms of combining cover crops with no-tillage systems, although such systems provide multiple benefits, there are also several additional challenges. Cover crops grown as live mulches or ineffective crop-kill of annual cover crops, such as ryegrass or vetch, can result in cover crops competing with cash crops which may reduce yields (Hiltbrunner et al. 2007; Teasdale et al. 2007; Madden et al. 2004). Residues of cover crops can hamper soil cultivation and initial germination (due to inconsistent seed cover), delay planting operations (since residues need some time to decompose/die), harbor pests and diseases, decrease initial crop growth (due to N immobilization, release of growth inhibiting compounds, or crop competition), and/or reduce soil temperatures (Peigné et al. 2007; Teasdale et al. 2007; Weil and Kremen 2007; Avila 2006; Cherr et al. 2006b; Masiunas 1998). However, in sweet maize a reduction in initial plant stands in no-till rye–vetch cover-crop-based systems was offset by improved growth and yields were still higher compared to bare

soil control (Carrera et al. 2004). But for vegetable crops, the use of cover crops delayed crop maturation and/or reduced both initial crop growth and final yield of subsequent crops (Avila 2006; Sarrantonio and Gallandt 2003; Abdul-Baki et al. 1996, 1999; Creamer et al. 1996). Especially for high-value commodities such as vegetables where precocity may translate into significant price premiums, such effects may have a strong negative impact on profitability (Avila 2006; Creamer et al. 1996). Only after researchers became aware of these issues and the system was redesigned (e.g., by using strip-till) this problem could be addressed (Phatak et al. 2002). Such adaptive learning and innovation cycles should be an integral part of training programs to enhance the efficiency of technology transfer (Douthwaite et al. 2002).

Under water-limiting conditions, use of cover crops will also deplete residual soil moisture levels and thereby, can reduce yields of subsequent crops (Sustainable Agricultural Network 2007). Use of winter cover crops in semiarid conditions in California, reduced soil water storage by 65–74 mm, thereby, impacting the preirrigation needs of subsequent crops and/or performance of subsequent annual crops (Michell et al. 1999). In perennial systems (e.g., vineyards), perennial cover crops were shown to have both higher root densities and deeper root systems, thus, resulting in more pronounced soil water depletion but either one affects spatial and temporal water supply. Grapevines may adapt its rooting pattern to minimize water stress, while supplemental irrigation mainly benefits cover crops (Celette et al. 2008). Although cover crops may provide a time-released source of N which is often perceived to be more efficient compared to inorganic N, poor synchronization will result in high potential N losses and thereby, greatly reduce efficiencies from residue-derived N and may also increase the risk of N-leaching (Cherr et al. 2007).

2.6.3 *Socioeconomic Constraints*

Local perceptions and political priorities can greatly hamper the adoption of cover-crop-based systems. In many cases, local politicians, researchers, and extension staff continue to favor green-revolution-based technologies and are reluctant to invest in traditional legume-based cropping systems (Anderson et al. 2001). In other cases, the perceived complexity and risk associated with the management of cover-crop-based systems may not offset direct benefits. Weil and Kremen (2007) reported that in Maryland, cover crops were only grown on 20–25% of the agricultural land during winter fallow despite farmers receiving \$50–\$100 subsidies for growing such crops. Therefore, unless cover crops provide multiple benefits and services and such advantages are also considered, the use of cover crops may not be cost-effective (Avila et al. 2006a, b; Cherr et al. 2006b; Abdul-Baki et al. 2004). Moreover, since cover-crop-based systems often require several years to evolve and provide the maximum benefits, their use is only viable if land tenure is secure (Neill and Lee 2001). Growers, despite their inherent desire to provide good stewardship of local land resources, face the reality of economic survival and thus, may not be in a position to provide certain environmental services unless they will also generate tangible

and direct benefits (Weil and Kremen 2007). Moreover, researchers engaging in interdisciplinary and participatory research may face appreciable risks and logistic challenges. Many institutes are moving toward more fundamental research and more practical geared research such as cover-crop management may be implemented by extension service and the linkage with research may be poor. Therefore, a critical re-assessment of both research and extension services and increased support for green technologies will be critical. In many cases, the involvement of farmers and local communities in structuring problem definitions and designing sustainable solutions should be enhanced. Furthermore, additional government and corporate support is needed for developing green technologies, especially when market mechanisms are not yet in place to provide incentives for innovations geared toward enhancing sustainability. In summary, awarding researchers for developing and improving green technologies and farmers for providing ecological services will be critical to offset some of the perceived risks associated with engaging in cover-crop-based systems.

2.7 Conclusion

Based on our comprehensive review of the literature, it was shown that there is a pronounced revival of cover crops during the past few decades. However, most of these studies document cover-crop performance for specific pedo-climatic conditions, and there is need for a more system-based approach. Moreover, it is also critical to place potential performance of cover crops in the context of production goals as related to existing system structure and management skills. Although cover crops can contribute to carbon sequestration, such benefits are only significant when soil tillage is minimized. Selection and use of cover crops is mainly based on tradition, perceived benefits, seed availability, seed costs, and technical support. The time of planting and termination of cover crops, as related to planting of commercial crops, are essential to biomass production and nutrient accumulation, while poor synchronization readily offsets potential yield or environmental benefits. The use of decision-support tools such as NDICEA seems desirable to provide a better insight into C and N dynamics in cover-crop-based systems. Despite the numerous benefits of cover crops, the widespread use of cover crops is currently still mainly confined to their integration into conservation tillage practices of conventional agricultural systems in regions prone to soil erosion. In contrast, in organic systems, the use of conservation tillage is still in its infancy. In this case, providing cost-effective weed control and restoring nutrient imbalances associated with the excessive use of animal manures, are among the most critical factors governing their use. We conclude that cover-crop-based systems are most likely to be used when they provide multiple benefits, which is especially important in the absence of significant yield benefits and/or relatively low opportunity costs of chemical fertilizers. Moreover, the use of cover-crop mixes is highly desirable, since this favors system performance under unfavorable/unpredictable growing conditions. Since cover crops have a central function in organic production systems, organic growers provide a critical role to preserve

traditional knowledge and to also generate technical innovations required to address current challenges that may benefit conventional growers as well. Furthermore, technological innovations, via government- and corporate-sponsored research, are essential to further improve and promote green technologies such as cover crops.

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Chapter 3

Cover Crops in Agrosystems: Innovations and Applications

Johannes M.S. Scholberg, Santiago Dogliotti, Lincoln Zotarelli,
Corey M. Cherr, Carolina Leoni, and Walter A.H. Rossing

Abstract Cover crops can reduce the dependence of farmers on agrochemicals while enhancing overall agrosystem's performance. However, the inherent complexity of cover-crop-based systems hampers their adoption by conventional farmers. Therefore, special management skills and alternative research and technology transfer approaches may be required to facilitate their adoptive use by conventional farmers. We propose that development and adoption of suitable cover-crop-based production systems may require the use of an "innovation framework" that includes (1) identification of system constraints, (2) analysis of system behavior, (3) exploration of alternative systems, and (4) system design and selection. We describe case studies from four regions of the Americas (Florida, USA; Paraná and Santa Catarina, Brazil; and Canelones, Uruguay) that illustrate the relationships between this innovation framework and the development and adoption of cover-crop-based production systems. Where successful, development and adoption of such systems appear to relate to a number of attributes including (1) active involvement by farmers in

J.M.S. Scholberg (✉) and W.A.H. Rossing
Biological Farming Systems, Wageningen University, Post Box 563, 6700 AN,
Wageningen, The Netherlands
e-mail: johannes.scholberg@wur.nl

S. Dogliotti
Facultad de Agronomía, Universidad de la República, Avda. Garzón 780, Código Postal 12900,
Montevideo, Uruguay

L. Zotarelli
Agricultural and Biological Engineering Department, University of Florida, 234 Fraziers-Rogers
Hall, Gainesville FL32611, USA

C.M. Cherr
Department of Plant Sciences and Graduate Group in Ecology, University of California,
Davis, CA95616, USA

C. Leoni
Instituto Nacional de Investigación Agropecuaria – Estación Experimental Las Brujas,
Ruta 48 km 10 Código Postal 90200, Rincón del Colorado – Canelones, Uruguay

research and dissemination programs; (2) integration of cover crops into production systems without net loss of land or labor resources; (3) informing farmers of the (direct) benefits of cover crop use; (4) provision of multiple benefits by cover crops, (5) sufficient access to information, inputs, and technologies required for cover crop use; and (6) provision of skills and experience necessary to manage cover crops effectively. Where these attributes are absent and failure to innovate has prevented development and adoption of cover-crop-based systems, policy initiatives to reward farmers for ecological services provided by cover crops may be required.

Keywords Cover crops • green technologies • system analysis • innovation • adoption • sustainability • Americas • green manure • living mulch

Abbreviation

SOM soil organic matter

3.1 Introduction

Cover crops are extensively used to provide a wide array of services (Scholberg et al. 2009). In this review, we do not distinguish between specific applications such as their use for enhancing soil fertility, e.g., green manures, and cultivation techniques by which cover crops are grown simultaneously with commercial crops, e.g., live mulches. We therefore use the term “cover crop” in its broadest context instead.

Historically, cover crops have been an integral part of agricultural production systems (Scholberg et al. 2009). Technological innovations have greatly enhanced agricultural productivity over the last 60 years, but have also eroded many traditional techniques used to sustain inherent soil fertility – including the use of cover crops (Altieri 2002). During this period, many farmers throughout Latin America were caught in cycles of unsustainability related to overexploitation or pollution of water resources, soil erosion, loss of inherent soil fertility, increasing impacts of weeds and pests on crop yields, decreasing agricultural commodity values, and increases in external input prices. Local producers often responded to decreasing family income by intensifying their production (Dogliotti et al. 2005). Typically, this resulted in a shift toward cash crops, increased use of marginal lands, and greater dependence on external capital and labor and production inputs. This process favored further marginalization of local production systems (van der Ploeg 2008; Dogliotti et al. 2005; Cherr et al. 2006b) and saw many farmers and their families leave agricultural production and rural areas altogether. Increased global demand of crops for animal feed concentrates and biofuels has further intensified pressure on land resources (Corral et al. 2008). Although such production systems

may generate local income and employment, these short-term economic benefits do not offset the loss of the long-term agricultural production capacity and human capital of local agricultural communities. Moreover, with current concerns about food security, global warming, and demands for a broader range of agricultural services, unsustainable resource exploitation is highly undesirable. Therefore, there is a need for more sustainable production options and more effective use of local or renewable resources (van der Ploeg 2008; Cherr et al. 2006b). Within this context, cover crops may once again become a cornerstone of sustainable agricultural systems (Scholberg et al. 2009). However, the complexity of cover-crop-based systems combined with the need to maintain reliably high crop yields requires the use of system analysis tools and active engagement of end-users (Shennan 2008; Cherr et al. 2006b). Involvement of the main stakeholders is particularly important, since any intentional change in production systems is always a result of changes in human conduct and therefore requires an individual and collective learning process (Leeuwis 1999). Moreover, solutions to complex problems do not come as “instant technology packages.” Rather, they need to be designed within its context of application with the direct involvement of farmers at all stages of the process, from diagnosis to dissemination (Leeuwis 1999; Masera et al. 2000). This is the only way to ensure relevance, applicability, and adoption of such innovations. Thus, technological innovations such as improved use of cover crops must be explored more efficiently, while farmers must be allowed to more effectively contribute to technology development and transfer, thereby fostering successful and sustainable development (Rossing et al. 2007). Thus, the scope of this chapter is to

1. Provide a conceptual framework for innovation of cover-crop-based systems
2. Contextualize the components of this innovation framework as related to current cover-crop research and development strategies, with emphasis on system analysis tools
3. Describe innovation and technology transfer processed of cover-crop systems in several regions in the Americas

3.2 A Framework for Innovation of Cover-Crop-Based Systems

As biological organisms, cover crops interact with many aspects of a cropping system and its environment. The use of reductionist approaches, small-plot studies, and short-term research is common in agricultural science but may be poorly suited for development and evaluation of suitable cover-crop management strategies (Cherr et al. 2006b). Despite the large number of research and review publications centered on cover crops over the past decade, a conceptual framework and a systems’ perspective that critically evaluates cover crops is lacking. Systems research uses an interdisciplinary approach to design and analyze agroecosystems functioning at different spatiotemporal and qualitative scales (Malézieux et al.

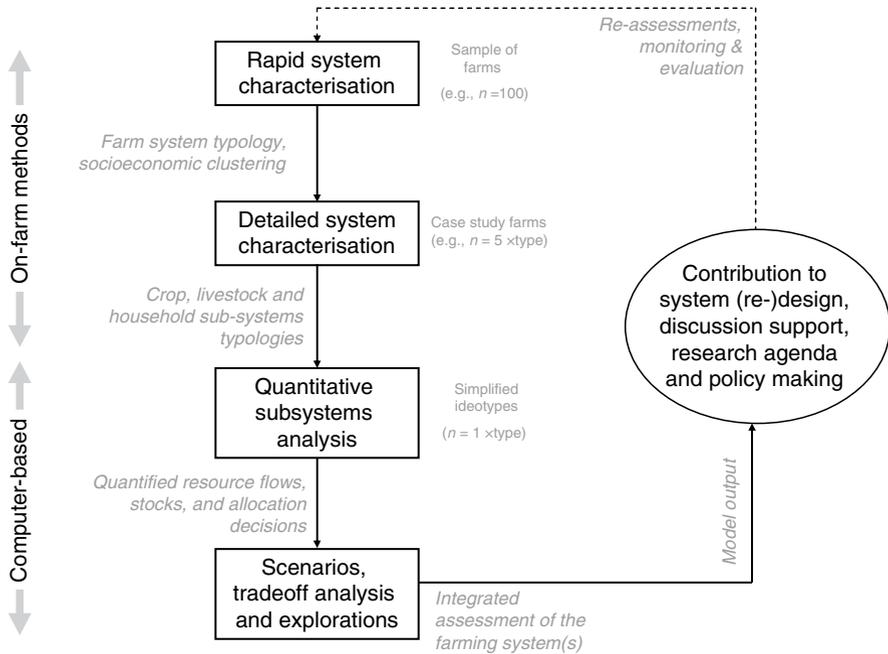


Fig. 3.1 Consecutive steps during farm analysis showing the complementation of both participatory and computer-based exploration and optimization approaches (Tittonell 2008)

2009; Shennan 2008; Drinkwater 2002). The underlying premise of system analysis and design is the use of cyclic knowledge development requiring active involvement from all key stakeholders. Ideally, this framework complements conventional research approaches, as shown in Fig. 3.1 (Tittonell 2008; Rossing et al. 2007). It includes the following:

1. Characterization and diagnosis of constraints: Description of the biophysical and socioeconomic system and defining constraints
2. Analysis of system behavior: explanation of the system behavior in terms of the constraints. The design of such a research may involve an initial assessment of native agricultural systems and models of ecological interactions (Shennan 2008; Altieri and Nicholls 2004)
3. Exploration of alternative systems: For example, generating different crop rotations and management practices either in reality or via simulation
4. Design of alternative system: Development or utilization of more efficient, profitable, or sustainable systems (i.e., via the use of trade-off analysis, model simulations, and optimization; see Fig. 3.1)

We emphasize that each component of the innovation framework is a process rather than an event, and there may be much overlap among the components. For example, integrating system analysis and design with applied field research and

modeling techniques allows improved assessment of system constraints, explanation of behavior, and exploration of alternatives (Tittonell 2008). Within this context, field studies and data collection should be structured in such a manner that they can be used for calibration of simulation models and verification of model performance (Hasegawa et al. 1999). Such models, in turn, can then be used to explore more sustainable development options (Selaya Garvizu 2000).

We also explore the fourth component (design or selection of a system) by using the vehicle of technology development, adaptation, and transfer – which obviously overlaps the other three components. Nonetheless, we believe that the innovation framework we are proposing is a powerful approach for the development of viable cover-crop-based production systems.

3.2.1 Characterization and Diagnosis of Constraints

We organize the constraints on cover crop systems into three broad categories: biophysical, socioeconomic, and information and technology constraints. The categories are not necessarily mutually exclusive. We also briefly examine solutions to some of these constraints, which involve further steps in the innovation framework we are suggesting.

3.2.1.1 Biophysical Constraints

Although the use of cover crops is perceived to enhance production and provide a myriad of services, their adaptation by conventional farmers in North America has been slow (Sarrantonio and Gallandt 2003). The key issue may include the additional cost (in terms of land, labor, and inputs), the complexity of cover-crop-based systems, the lack of pertinent information, and the uncertainty of release patterns from cover-crops residues, and the lack of secure land tenure (Cherr et al. 2006b; Sarrantonio and Gallandt 2003; Lu et al. 2000). Since cover-crop-based systems depend on biological and ecological processes, this makes their management more complex when compared with the use of synthetic fertilizers. Most farmers are poorly equipped to take the economic risk associated with experimentation and exploration of suitable cover-crops technologies (Weil and Kremen 2007; Cherr et al. 2006b).

For example, severe yield reduction can occur when increased plant competition results from live mulch or incompletely killed cover crops (Hiltbrunner et al. 2007; Teasdale et al. 2007; Madden et al. 2004). Without adequate precautions, cover-crop residue may also interfere with soil cultivation, reduce subsequent crop seed germination (owing to poor soil–seed contact), delay planting operations (since residues need some time to decompose/die), harbor pests and diseases that attack subsequent crops (if the cover crop and subsequent crop host the same pests or diseases), decrease initial crop growth or delay crop maturity (owing to N immobilization,

allelopathy, plant competition, or reduced soil temperatures), or reduce soil water availability (Peigné et al. 2007; Sustainable Agricultural Network 2007; Teasdale et al. 2007; Weil and Kremen 2007; Avila 2006; Cherr et al. 2006b; Sarrantonio and Gallandt 2003; Abdul-Baki et al. 1996, 1999; Masiunas 1998; Creamer et al. 1996). Poor synchronization of N release from decomposing cover crops and N uptake by subsequent crops will also result in high N losses (Cherr 2004).

However, if researchers become aware of these issues, then it is often possible to address them through redesign of the cover-crop system. In an example with vegetable crops, use of cover crops combined with zero-tillage reduced initial growth of vegetable crops and prevented growers from targeting the most profitable market windows. Once growers and researchers communicated about the problem, they identified a solution through the use of strip tillage. Such adaptive learning and innovation cycles are critical and therefore will be discussed in more detail in a subsequent section.

3.2.1.2 Socioeconomic Constraints

Technological development has often been perceived as a task of research institutions that subsequently transfer solutions to farmers. Unfortunately, this “top-down” approach frequently fails because it does not adequately include local socioeconomic and environmental conditions in the process of development (Anderson et al. 2001). On-station researcher-managed studies favor highly controlled conditions and research that may not be relevant to growers unless they are actively involved during the design of studies. Several alternative approaches for developing regions have been outlined (e.g., Altieri 2002; Anderson et al. 2001; Giller 2001).

Karlen et al. (2007) outlined and discussed different institutional arrangements (management models) for sharing resources and responsibilities between farmers and researchers. On-farm research often appears risky and costly to participating growers – especially when experimental treatments conflict with growers’ production objectives. Grower intervention in such situations can lead to lack of adequate experimental control (Karlen et al. 2007). From the researcher’s perspective, results of such on-farm studies may be site- or farm-specific with confounding sources of variation (Shennan 2008). However, on-farm studies also tend to be more realistic in terms of scale (field vs. plot), management practices used, and actual production constraints, while they also allow development of chronosequences (e.g., comparing system dynamic at different system development stages) within a relatively short period of time (Drinkwater 2002).

A workshop elucidating opinions of key stakeholders involved in the transfer and adoption of cover-crop-based systems in Latin America indicated that the key factor controlling adaptation of cover crops were nontechnical and include poor seed availability of annual cover crops (Anderson et al. 2001). In Northern Honduras, adaptation of *Mucuna* spp. as a cover crop in maize systems was abandoned by farmers if land tenure was not secure (Neill and Lee 2001). Additional hindrances for improved integration of cover crops in existing cropping systems

may include local perceptions and policies (e.g., local research and extension staff favoring conventional high-input-based technologies to risk-averse small producer rather than fostering traditional legume-based mixed cropping systems (Anderson et al. 2001)). In many cases, researchers and policy-makers may promote their own political agenda (e.g., reducing environmental impacts, minimizing external inputs, and enhancing sustainability) instead of addressing end-user needs. Weil and Kremen (2007) reported that in Maryland, cover crops were only grown on 20–25% of the agricultural land during winter fallow despite farmers receiving \$50–100 subsidies for growing such crops. Despite their inherent desire to provide good stewardship of local land resources, farmers face the reality of economic survival and may not be in a position to provide environmental services without tangible, direct benefits (Weil and Kremen 2007). Unless farmers are aware of these direct benefits of the use of cover crops, they may be reluctant to integrate them into their existing cropping systems. In many cases, the use of cover crops may not be cost-effective unless they provide multiple benefits and services (Avila et al. 2006a, b; Cherr et al. 2006b; Abdul-Baki et al. 2004).

3.2.1.3 Information and Technology Constraints

Constraints on cover crop use in Latin America include the lack of communication among different stakeholders (Anderson et al. 2001). In the Mid-Western USA, farmers indicated the greatest obstacle to development and adoption of cover-crop technology was lack of basic information (Singer and Nusser 2007). Armed with basic information about selection, management, and services of potential cover crops, many farmers might independently test and evaluate these species. Interestingly, much research has been conducted in these areas in North America. A search of the ISI Web of Knowledge for journal publications including “cover crop” or “green manure” or “living mulch” within the topic found over 10,000 manuscripts between 1923 and 2007. Over 61% of these manuscripts were recent (published since 1990) and most seem to be focused on North American production systems. Despite such an impressive increase in publication numbers, North American producers still cite lack of information about green manure and cover crops as one of the greatest barriers to their use (Singer and Nusser 2007). This indicates that information is not lacking, but that it is not transferred effectively.

Historically, most international agricultural research was commodity-based with the main focus being on increasing yields via intensification; use of interdisciplinary and participatory research approaches was limited (Altieri 2002). Over time, research has become more “integrated” and “holistic.” This may be related to increased integration of ecological approaches into mainstream agricultural research (Delate 2002) and the disillusion of green-revolution-based technologies to enhance the livelihoods of farmers in more marginal production settings (Bunch 2000). Current advances in system ecology may be thus used to design and test cropping systems with enhanced plant diversity to improve the functioning of agroecosystems rather than reinstating traditional crop rotations (Drinkwater and

Snapp 2007). However, considering the North American example, this evolution in research will almost certainly not lift constraints to cover-crop use unless producer's involvement is improved as well.

3.2.2 *System Analysis*

Effective research on cover crops inherently requires a system focus and use of long-term studies (Shennan 2008; Cherr et al 2006a). In the current academic climate, implementing this on a field scale may be challenging; extramural funding opportunities for applied long-term farming systems research are limited and within research institutions there exists a growing demand for scientists to generate information and publications quickly and focus more on fundamental research. As a result, most cover-crop publications focus on single system aspects including end-of-season biomass or N accumulation for specific production settings and final yields of subsequent crops. There are some research examples where the relationship between cover-crop growth and environmental conditions were captured (Cherr et al 2006b, Schomberg et al. 2007). When environmental conditions are known or can be predicted, models may be used for assessing cover-crop growth and subsequent decomposition, N release, and long-term impacts to other production systems. Likewise, such models can be applied for system analysis and design, by using field studies for development and calibration of these models (Stoorvogel et al. 2004). Within this context, the use of validated simulation tools will allow of extrapolation of results to other production settings or future scenarios. By utilizing on-farm data to develop and extrapolate such models, researchers therefore can more effectively identify benefits and constraints of cover-crop-based systems.

Integration of cover crops requires modification of the existing crop rotation schemes and design of the suitable alternative rotations (Selaya Garvizu 2000). Although this may be accomplished by trial and error, this is time-consuming, costly, and risky (van der Burgt et al. 2006; Keatinge et al. 1998). The need for quantitative assessment of complex systems across different production environments thus justifies the use of simulation models to integrate processes at a field scale in a more cost-effective manner (Sommer et al. 2007; Stoorvogel et al. 2004; Lu et al. 2000). The use of such models may provide a better insight into both short-term dynamics and long-term system behavior. This can facilitate an improved understanding of processes that are either difficult or costly to measure at different spatial and temporal scales such as long-term effects of cover crop residue management on erosion, production, profits, N leaching, and soil quality (Sommer et al. 2007; Dabney et al. 2001; Lu et al. 2000; Selaya Garvizu 2000).

Lu et al. (2000) used the EPIC model to compare the use of conventional, cover-crop-based, and manure-based corn–soybean systems for a period of 60 years. The authors showed that the use of cover crops could greatly reduce external fertilizer requirements and environmental risk, while gross margins were reduced only by 10%. These approaches may also be used to rapidly design viable alternative crop rotation

schemes (Bachinger and Zander 2007; Dogliotti et al. 2003) or alternative production systems (Tiftonnell 2008; Dogliotti et al. 2005). Such models may range from simple integration of user knowledge and expertise to complex mechanistic models (Stoorvogel et al. 2004). Alternatively, models may focus on either tactical topics (e.g., with a focus on in-season management decisions) or strategic topics (e.g., design of long-term crop rotation or design and evaluation of alternative farming systems).

In terms of cover-crops systems, short-term decomposition dynamics of soil-applied cover-crop residues are typically included in models such as CERES-N, DAISY, NDICEA, and STICS (van der Burgt et al. 2006; Scopel et al. 2004; Berkenkamp, et al. 2002; Gabrielle et al. 2002; Quemada et al. 1997). However, surface-applied residues, which are a key aspect of no-tillage systems, tend to decompose slower owing to poor contact with soil microbes, prevalence of fungal decomposers, and drier conditions, while surface-applied residues also feature greater and more prolonged N immobilization (Schomberg et al. 1994). Thus, most crop growth models may not (accurately) model decomposition of surface-applied residues, which hampers their use to assess long-term effects of residue management or no tillage systems on soil quality and soil erosion (Sommer et al. 2007; Scopel et al. 2004; Schomberg and Cabrera 2001; Steiner et al. 1996). This limitation was overcome by developing surface decomposition modules or modifying decomposition parameters (Scopel et al. 2004; Quemada et al. 1997).

Since the Brundtland report, sustainable development has become integral part of the global policy agenda (Speelman et al. 2007). Within this context, when designing and managing cover-crops systems, operational tools are needed to evaluate their benefits in terms of enhancing sustainability of local natural resource management (NRM) systems within a larger socioenvironmental context (Lopez-Ridaura et al. 2002). This requires a conceptual framework that is participatory, comprehensive, meaningful, and practical, and MESMIS was developed to provide such a tool. This approach uses a cyclic process to aggregate and integrate economic, environmental, and social indicators, and it has been extensively used throughout Latin America (Speelman et al. 2007). The NRM systems are characterized in terms of key attributes (e.g., productivity), critical points are identified (e.g., poor adaptation of cover crops), and corresponding diagnostic criteria (e.g., ability to adapt new technology) developed, which are then translated into specific indicators (e.g., area in which cover crops are being used) that are readily available on a farm scale. The resulting information is then integrated by combining both qualitative and quantitative techniques with a multicriteria analysis (Lopez-Ridaura et al. 2002). Although the MESMIS has greatly facilitated participatory sustainability assessment, it does not allow for long-term system assessment, while the involvement of end-users was also often limited. Further modifications may thus be required so that it can be more effectively used for the exploration of alternative management systems and system optimization as well (Speelman et al. 2007). Moreover, use of simulation models may also facilitate trade-off analysis of different production components such as labor costs, profits, soil erosion, and environmental risk (Dogliotti et al. 2005; Stoorvogel et al. 2004; Lu et al. 2000).

3.2.3 Exploration of Alternative Systems

Model selection/development and application should fit into a larger system analysis framework as shown in Figs. 3.1 and 3.2. However, most existing models aim to enhance scientific understanding, whereas the use of such models for informed decision-making and improved management of cover crops requires a combination of sound scientific basis with practice-oriented model design (van der Burgt et al. 2006). Ideally, model development and application should be inspired by insights provided by farmers (e.g., participatory modeling). Examples of how models may be used in this fashion for the exploration, and design of more sustainable cover-crops-based vegetable production systems in Uruguay will be discussed in more detail later.

The use of the NDICEA model for exploration of more sustainable production practices for vegetable cropping systems in southern Uruguay demonstrated that cover crops could be effective in maintaining and/or enhancing SOM content while reducing external N-fertilizer requirements. However, these benefits differed between soil types (Selaya Garvizu 2000). This work was extended and model-based explorative land use studies were implemented to evaluate a much larger number of potential production systems, thereby providing a strategic support base for re-orientation of local vegetable production systems (Dogliotti et al. 2004). First, the ROTAT system (Dogliotti et al. 2003), a tool that was previously developed for generating crop rotation based on user-selected agronomic criteria, was used to assess all possible crop rotations. One proposed technical intervention was the introduction of cover crops and integrate pastures into vegetable cropping systems to reduce soil erosion and increase SOM. Key input and output parameters, including soil erosion, SOM and nutrient balances, environmental impacts, labor use, and economic performance were assessed by different quantitative standard methods using a target-oriented approach. This work generated a large number of

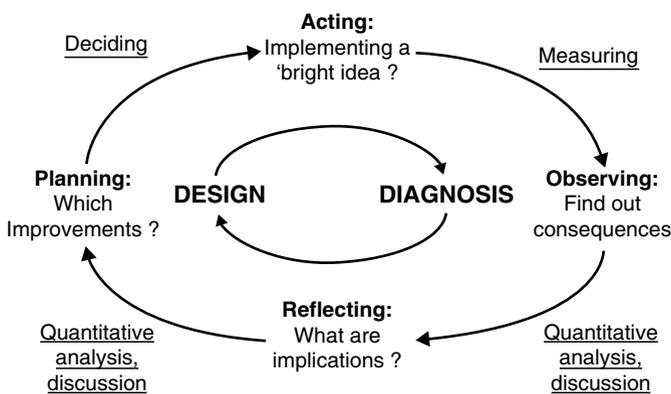


Fig. 3.2 Key aspects (diagnosis vs. design), system development steps (observing, reflecting, planning, and acting), and system develop actions (measuring, analysis/discussion, and deciding/selecting) during experiential learning cycle (Rossing et al. 2007)

alternative production systems, and across these systems, the use of cover crops reduces soil erosion on the average by 45–50% (Dogliotti et al. 2004). By using a mixed linear programming model (Farm Images), production activities could be allocated to production fields differing in soil quality in such a manner that production constraints were met, socioeconomic benefits were maximized, while soil degradation and environmental impacts were minimized. The model was then used to redesign seven local farms, and results showed that erosion may be reduced by 200–400%, the decline in SOM may be reversed, and when compared with the current situation, farm income could be improved for six out of seven farms (Dogliotti et al. 2005). Based on this work, it was concluded that using cover crops during the intercrop period and decreasing the area under vegetable production provide a more sustainable and profitable development option when compared with the current farmer's practice of increased intensification (Dogliotti et al. 2005). This work was then extended to a large number of farm types (based on farm size, soil quality, and supply of labor, irrigation, mechanization using a similar approach to assess the impact of resource endowment on development options and strategic farm design). An example of this approach for assessing the benefits of cover crops on reducing soil erosion and improving SOM content is shown in Fig. 3.3. Finally, it was also shown that farm resource endowment may limit sustainable development options, while reducing environmental impacts is quite likely to reduce family income as well (Dogliotti et al. 2006).

In terms of active farm participation, the FARMSCAPE approach (Carberry et al. 2002) outlines strategies for integrating participatory action research with simulation model approaches. One key finding was that it is critical to first establish the credibility of such models by linking them with on-farm studies and farmers' experiences. Moreover, active participation of pilot farmers was required and simulation tools needed to be flexible so that they can be adapted to specific on-farm management conditions. Via interactive dialogues between farmers and researchers, farmers were able to explore their production system and design alternative management practices similar to the "learning from experience," while this approach can greatly reduce the cost and risk associated with "trying new things" (van der Burgt et al. 2006; Carberry et al. 2002). However, assessing overall ecosystem functioning and services using simulation models remains difficult because of the inherent complexity of biophysical and human dimensions of these systems combined with the ecological and economic processes that control them, and the lack of site-specific data (Sommer et al. 2007). Alternative and more pragmatic approaches may thus be required as well, including the development of sustainability indicators such as MESMIS as discussed earlier.

Another instance of a design tool for cover-crop-based systems includes GreenCover (Cherr et al. unpublished; <http://lyra.ifas.ufl.edu/GreenCover>). This expert system is based on a systematic approach and aims to render information about cover-crops-based systems more relevant, accessible, and organized for potential users by (1) distilling basic "rules" about successful use of cover crops from published studies; (2) applying these rules to farm-specific environment, management, and goals; and (3) using the application of the rules to identify potentially

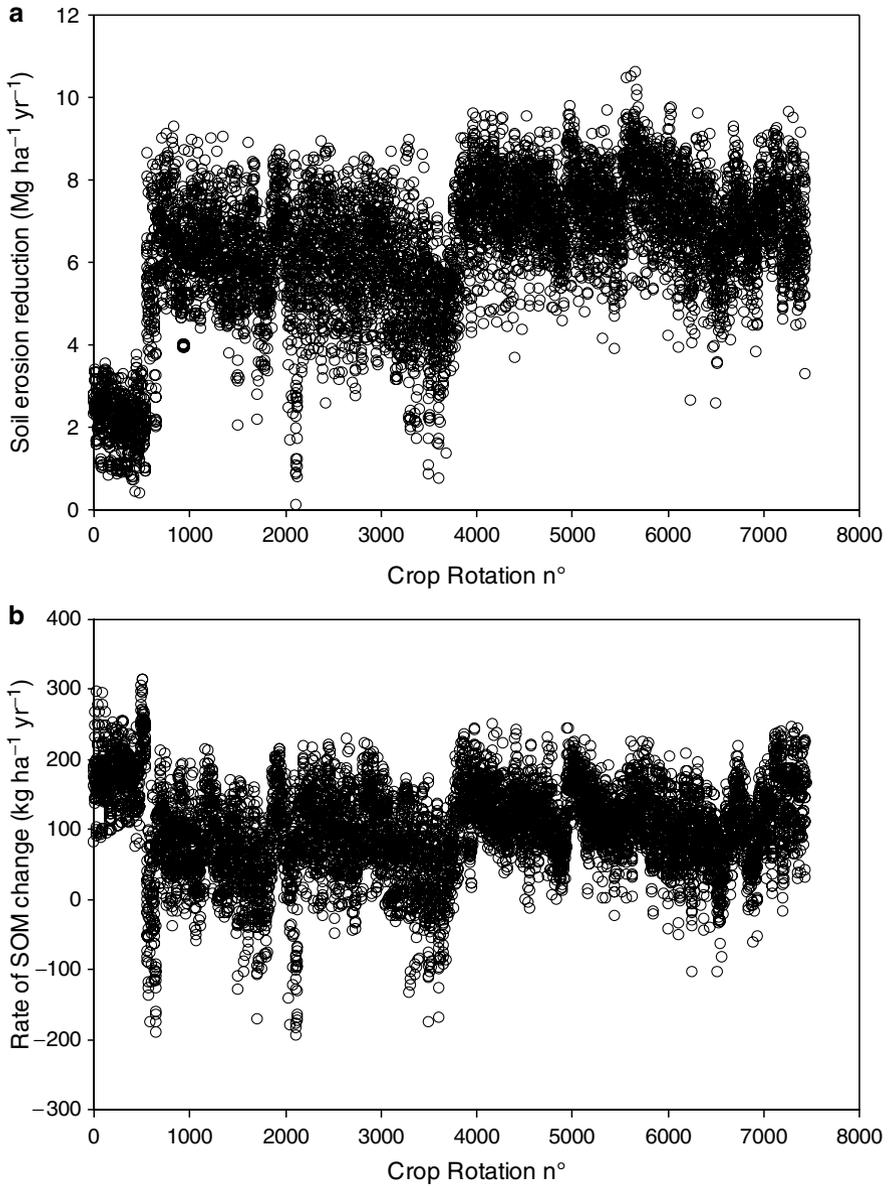


Fig. 3.3 Example of use of simulation models to explore potential benefits of including cover crops during the fallow period on reducing annual soil erosion (**a**) and improving soil organic matter content (**b**) for 7447 different crop rotation schemes in southern Uruguay. Overall soil erosion values were 13.2 versus 6.9 Mg ha⁻¹ year⁻¹ for conventional versus cover-crop-based systems, whereas corresponding values for soil organic matter (SOM) changes were -223 versus 100 kg SOM ha⁻¹ year⁻¹ (Modified from Dogliotti et al. 2006)

suitable cover-crop species from a database containing characteristics of roughly 50 species or species mixtures. In this tool, the user is provided with a list of the species and/or species mixtures as well as links to online management information sources. This kind of approach can be termed as an “information-access tool.” It allows users to interactively explore how changes in management or targeted cover-crop services affect the selection process of cover crops.

3.2.4 Design or Selection of a System

Here, we emphasize modes of cover-crop technology development, adaptation, and transfer as examples of system design or selection. A more detailed discussion on cover-crop management is presented elsewhere (Scholberg et al. 2009). As mentioned earlier, this can also provide insights into the other components of the innovation framework already described.

3.2.4.1 Technology Development and Adaptation

The process of technical innovation of agroecosystems includes elements of continuous generation of “novelties” (Roep and Wiskerke 2004). These may include different constellations of evolutionary variations of native management techniques, local adaptation/simplification of imported high technology, and more revolutionary or external innovations (Douthwaite et al. 2002; Bunch 2000). Innovations can be simple, e.g., new cover-crops species, or complex, e.g., complete technology package including alternative rotations, new varieties, and equipment.

It is critical to first test a “promising technology,” which may be imported from a different production environment on a limited field scale under controlled conditions (e.g., on-station initial screening and development). This may be followed by on-farm testing and further adaptation of the technology in close collaboration with local stakeholders prior to wide-scale promotion of such a technology (Giller 2001). As an example, zero tillage may be perceived as a revolutionary technology that aims to enhance soil ecological functioning and minimize soil degradation of arable cropping systems (Triplett and Dick 2008). Initial adoption of zero tillage after its development in the 1950s was slow and only after a suitable “basket of technology” was developed, e.g., development of special planters, suitable herbicide programs, and accumulation of local expertise. Transfer of cover-crop-based zero tillage systems to other systems that also aimed to minimize the use of herbicides (e.g., organic systems) required development of special roller equipment as well (Creamer and Dabney 2002; Kornecki et al. 2004).

During the adaption process (innovation cycle), close interactions occur between developers (innovative farmers/engineers/researchers), novelties (technical innovations), facilitators (extension workers or pilot farmers), and end-users (farmers). During this initial innovation cycle, developers elucidate farmers’ expert knowledge to design a suitable set of technological innovations (“best bet” technology),

which is then adopted and implemented by pilot farmers on a field scale (“plausible promise”), as discussed by Douthwaite et al. (2002). This may imply further refinement of technological innovations due to prevailing pedo-climatic conditions, farmer’s knowledge and management practices, and socioeconomic factors (Nyende and Delve 2004). During the overall innovation process, there is a gradual transfer of participation and ownership of the innovation from the developer to the adopter who in time becomes the main driving force behind technology transfer (Douthwaite et al. 2002; Neill and Lee 2001).

The key to successful integration of cover crops in zero-tillage systems was the development of appropriate equipment for seeding crops (Triplett and Dick 2008). Such planters needed to be heavier and may also contain row cleaners to push aside crop residues and spoked closing wheels to ensure optimal soil structure and seed–soil contact along with the use of stronger and adjustable pressure springs to ensure a constant seeding depth (Sustainable Agricultural Network 2007). However, this “best bet” technology needed to be further adapted to include strip till (“plausible promise”) for vegetable crops to prevent delays in crop development and thus ensure that growers can benefit from favorable market windows (Phatak et al. 2002). As an example of scaling out, the use of cover crops is often closely linked to zero tillage (Landers 2001), which was developed in the USA during the 1950s and introduced in Brazil during the 1970s (Triplett and Dick 2008). However, it only became more widely adopted in the 1980s. Currently, it is not only commonly used in the USA but also spread to Brazil, Argentina, and Australia (Triplett and Dick 2008). Another example of effective scaling-out of cover crops includes the widespread success and adaptation of mucuna-based maize production systems in Honduras. This process was driven by a spontaneous farmer-to-farmer diffusion-based dissemination. This mechanism for technology transfer was shown to be much more effective than the traditional extension model of technical assistance in different regions (Landers 2001; Neill and Lee 2001).

3.2.4.2 Approaches for Technology Transfer

In practice, promising technical interventions for enhancing the livelihood of farmers and the sustainability of agriculture are often not effectively adopted by farmers (Nyende and Delve 2004; Tarawali et al. 2002). As a result, especially resource-poor farmers often did not benefit from most technological innovations in the past, since they were typically neither appropriate nor affordable (Bunch 2000). Furthermore, traditional approaches for research and technology transfer tend to be reductionist (Drinkwater 2002), lack a “total system” approach (Phatak et al. 2002), and thus are poorly suited for cover-crop-based systems (Cherr et al. 2006b). Moreover, such systems should be designed based on specific biophysical conditions, while technological innovations should also be appropriate within the local socioeconomic context (Cherr et al. 2006b; Douthwaite et al. 2003). Thus, limited adoption of technical innovations may be related to (i) lack of farm-tested appropriate and cost-effective technology; (ii) timing conflicts with the existing operations; (iii)

lack of tangible/direct benefits and/or multiple services; (iv) limited access to resources (including capital and seeds); (v) poor matching of interventions with farmers' priorities; (vi) lack of active participation of farmers during technology development, adaptation, and transfer; (vii) lack of suitable policies and legislation to provide a broader societal support network (Morse and McNamara 2003; Nyende and Delve 2004; Tarawali et al. 2002; Landers 2001). These adaptation factors may vary greatly among regions; for example, the integration of cover crops in some systems (e.g., Brazil) has been successful on a regional scale (Calegari 2003; Landers 2001), while their adaptation in other regions (e.g., SE USA) lagged behind (Phatak et al. 2002). Moreover, technologies should be linked to local traditional knowledge, practices, and experience. Technological innovations thus need to be appropriate within the local context while direct involvement of farmer's at all critical development and adoption stages appears to be critical (Leeuwis 1999). Furthermore, active participation of early adopters during the refinement and dissemination of cover crops systems tends to greatly enhance technology transfer efficiency (Tarawali et al. 2002).

A large number of alternative approaches to conventional research and extension approaches have been proposed and are being used including (i) farming systems research and extension (Weil and Kremen 2007), (ii) farmer participatory research (Giller 2001, Bentley 1994), (iii) campesino-to-campesino approach (Anderson et al. 2001), (iv) prototyping (Vereijken 1997), (v) prototyping combined with model-oriented approach (Bouma et al. 1998), and (vi) co-innovation (Rossing et al. 2007). The first approach aimed to use a more "holistic" and interdisciplinary team approach to facilitate improved understanding of local farming systems and constraints, thereby facilitating the design of more appropriate development options (Douthwaite et al. 2003). However, this method is often rather descriptive and also does not effectively use technological tools including simulation models (Stoorvogel et al. 2004). The second method recognizes that farmers have valuable experience-based knowledge that complements science-based research approaches and that farmers can also be instrumental in structuring both research objectives and suitable technical innovations (Cardoso et al. 2001). Moreover, active involvement of farmers is critical, since any intentional change requires awareness while change in human conduct is also rooted in both individual and collective learning processes (Leeuwis 1999). Fostering active involvement will induce empowerment, which in turn further enhances technical innovation (Cardoso et al. 2001). Although this sounds appealing, its implementation may be challenging owing to social, cultural, and intellectual barriers between farmers and researchers. Moreover, for this method to be successful, a long-term commitment is required from both parties involved (Bentley 1994), which is exemplified by successful participatory projects (Altieri et al. 2008; Cardoso et al. 2001).

The "campesino-to-campesino" approach in Latin America dates back to the 1970s. It has its roots in the popular education movement, and it includes "reflection-action-reflection" elements and emphasizes local empowerment, which is implemented by transferring the control of the development process to the local community. Locally selected farmers (campesinos) also assume leadership, are

actively involved in experimentation, coordinate the promotion and transfer of technical innovations, and at times may be paid part time for their contributions (Anderson et al. 2001). However, this approach requires an appropriate social environment as was the case in, e.g., Nicaragua. In other regions (e.g., Florida), commercial farmers may perceive their technological innovations as a tool to provide them with a competitive edge and may be reluctant to share intrinsic knowledge on such innovations.

Prototyping involves close interaction with farmers to define/rank objectives and to select the corresponding parameters that can be readily quantified (diagnosis and analysis phase). These parameters are then integrated using multiobjective methods to develop a conceptual design (prototype) of an alternative production system (design phase). Subsequently, this “prototype” is implemented, tested, and refined on a field scale in collaboration with selected pilot farmers (rediagnosis and/or redesign phase), before being disseminated to a larger group of farmers (Vereijken 1997). One limitation of this approach is that only a few production systems can be tested in the field (Dogliotti et al. 2004). Stoorvogel et al. (2004) combined the prototyping approach with a model-oriented system analysis approach. However, the active contribution of farmers appeared to be limited (e.g., top-down approach) and the basis for sustainability assessment rather narrow when compared with, e.g., MESMIS (Lopez-Ridaura et al. 2002).

The co-innovation approach is based on the premises that development is a “social” rather than a “technical” process (Douthwaite et al. 2003) and that technology development occurs through a continuous evolving experimental learning and selection process by farmers (Douthwaite et al. 2002). However, use of a system approach to foster systemic innovation rather than incremental change is also critical to revolutionize the technology transfer process. Moreover, the use of an interdisciplinary approach combined with effective use of simulation models may greatly facilitate the selection of suitable development options (Rossing et al. 2007). Full integration of all these components (co-innovation) thus seems to provide a powerful tool for fostering technology development, system design while also enhancing the efficiency of technology transfer and adaptation. Active participation of farmers during the problem identification phase (e.g., development of “problem trees,” as shown in Fig. 3.4) and “fine-tuning” of technical interventions (e.g., during the exploration and design phase) aim to structure solutions that are appropriate within the local context (Anderson et al. 2001). Moreover, use of the “impact pathways” approach, which involves a frequent self-reflection and monitoring of the mutual learning process and development trajectory, allows both researchers and end-users to carefully monitor how development tracks and corresponding impacts evolve over time (Douthwaite et al. 2003).

An example of key aspects of the integration of a system analysis method used in the co-innovation approach will be illustrated based on an Uruguay case study. In this case, the decline in sustainability of local vegetable systems could not be reversed by simple adjustments of single production components or using standard technological innovation packages. Instead, a redesign of the farm systems as a whole was required. However, such a redesign of farm systems at the strategic level

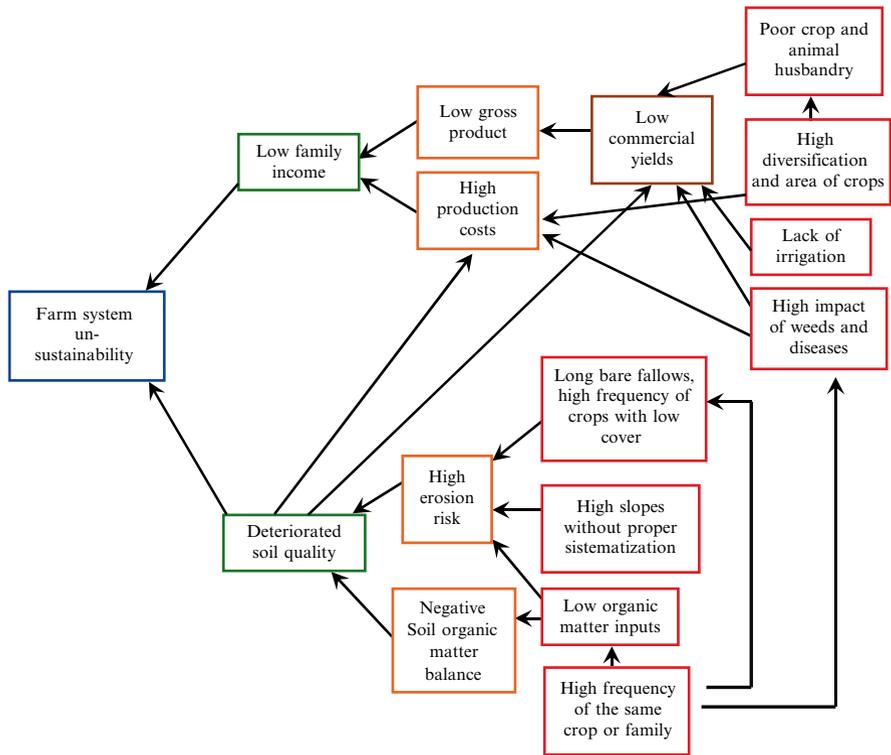


Fig. 3.4 Problem tree, which serves as an initial system diagnosis tool, as identified by a commercial vegetable producer in Uruguay. This diagram exemplifies potential benefits of cover crops to enhance crop diversity, suppress weeds, improve soil cover, and inherent soil fertility of erosion-prone intensive vegetable production systems

could only be achieved by a participatory, interdisciplinary systems approach. Field surveys showed that none of the farmers used cover crops as a standard practice during the intercrop periods and only 27% of the farmers had ever grown a cover crop. Most of the farmers used a tillage fallow during the 3–8 month period in between crops. Only 40% of the farmers intentionally tried not to grow the same crop in the same field next season, while 88% of the farmers did not follow an intentional succession of two specific crops (Dogliotti et al., 2003). Moreover, the maximum time horizon for planning the use of a particular field was less than 1 year for 80% of the farmers (Klerx 2002). The added costs of growing cover crops accounted for just a fraction of total production cost of vegetables and this extra cost was also readily offset by reduced fertilizer cost and increased crop yields (Dogliotti et al. 2005). The lack of machinery for mowing and incorporating large amounts cover crops residues was perceived to be a constraint by some farmers. But the main limitation for adoption appeared to be the short time horizon of planning of farms’ fields use and the lack of defined crop successions or rotations. This survey thus revealed that allocation of crops to fields is rather an “operational”

or “tactical” decision than a “strategic” one, and despite the promising results of cover-crops-based systems in experimental stations and farmers’ fields, their use was not adopted by farmers in the region. The use of simulation-models and expert systems (e.g., ROTAT, Dogliotti et al. 2003) facilitated the exploration of cover-crop-based crop rotation systems that were appropriate within the local context. These initial explorations were then modified based on discussions with local producers, and their feedback was used to “fine-tune” system design prior to on-farm implementation of these systems.

3.2.4.3 Sustainability of Technology Adoption

In addition to inducing change and improvements, technological innovation should also aim to harness long-term sustainable development. Although farmers may be enticed to adopt innovations based on perceived short-term benefits, it may be more difficult to assess how such innovation meets the stability, resilience, and reliability criteria listed by Lopez-Ridaura et al. (2002). Assessing the medium to long-term effects of innovations on agroecosystem functioning is difficult and time-consuming (Drinkwater 2002) and may require use of simulation models (Stoorvogel et al. 2004). Increased management complexity and greater perceived risk may hamper adoption and long-term use of ecology-based systems (Shennan 2008), which can hamper both short-term adoption and long-term use of cover-crops-based systems. In Honduras, extensive adoption of mucuna-based corns systems was abandoned by many farmers within a few years due to changes in land-tenure, invasion of an obnoxious weed, and extreme weather conditions (Neill and Lee 2001). Although simulation models may not capture all potential contributing factors, they may facilitate improved risk assessment for different scenarios. This may be especially important in the context of current trends in climate change and more frequent occurrence of erratic and extreme weather and rainfall patterns (Stoorvogel et al. 2004). Finally, it was also argued that broadening the global genetic base of cover crops proposed for development options needs to be considered (in order to minimize the risk of build up of pests as was the case of *Leacaeana psyllid*). Therefore, diversification of the proposed innovations and developed options will be critical for long-term sustainability of cover-crop-based systems (Anderson et al. 2001). However, preservation and improved integration of traditional knowledge on cover crop practices will be critical as well to prevent an erosion of a collective heritage that took thousands of years to evolve (Altieri 2002).

3.3 Innovations in Cover-Crop-Based Systems in Case Study Regions

Below, we provide a brief historic perspective on key factors related to innovation in cover-crop-based systems in four regions of the Americas (Florida, USA; Paraná and Santa Catarina, Brazil; and Canelones, Uruguay). Special emphasis is placed

on the components of the innovation framework discussed in the previous section: (1) characterization and diagnosis of constraints, (2) analysis of system behavior, (3) exploration of alternative systems, and (4) design of more sustainable production systems. In most cases, we also outline key factors affecting technology transfer and adoption within the context of local socioeconomic conditions and prevailing management practices.

3.3.1 Florida

3.3.1.1 Biophysical Production Environment

The study region (North Central Florida) is located in the Southeastern U.S. (29°25' N and 82°10' W). The average temperature is 19°C, and frosts may occur between November and March. Average annual rainfall is 1,200 mm with 52% of this rainfall occurring from June to September. With an area of 2.5 million ha and a total revenue of \$7.8 billion, agriculture is a key component of Florida's economy (NASS 2007). The statewide average farm size is 99 ha and citrus (251,568 ha), sugarcane (163,968 ha), hay production (105,263 ha), vegetable crops (179,800 ha), peanuts (130,000 ha), and cotton (103,000) are some of the key agricultural crops. Their corresponding contributions to statewide farm revenues were 21.1, 5.5, 1.4, 24.0, 0.9, and 0.4%. In comparison, ornamental crop and livestock operations contributed 12.6% and 18.7% to statewide farm revenues, respectively (NASS 2007). The dominant soil types in the study region include excessively drained sandy soils (>95% sand) containing only 1–2% soil organic matter and soils typically have poor water and nutrient retention capacities (Cherr et al. 2006c; Zotarelli et al. 2007a, b). Most vegetable crops are produced using raised beds covered with plastic mulch in combination with drip irrigation (Zotarelli et al. 2008a, b).

3.3.1.2 Characterization and Diagnosis of Constraints

Within the US, Florida is the largest producer of citrus, tomatoes, sweet corn, watermelon, and snap bean and the second largest producer of bell peppers, cucumbers, and strawberries (NASS 2007). Current concerns about global warming and environmental quality issues will require growers to make more efficient use of water and nutrients and reduce inorganic fertilizer use (Cherr et al. 2006c; Zotarelli et al. 2008a, b). Historically, Florida has greatly depended on the use of fumigants to control weeds, pathogens, nematodes, and insects, and it is one of the largest users of methyl bromide. Future restrictions on the use of methyl bromide may undermine the viability of vegetable production in this region because the cost-effectiveness of alternatives to this fumigant remains an issue (Abdul-Baki et al. 2004). Increased globalization and lifting of trade barriers have also resulted in increased competition with other production regions (e.g., Brazil and Mexico), which have lower labor cost and less restrictive environmental regulations.

Steep increases in fertilizer, fuel, and labor costs along with citrus canker, and citrus greening disease epidemics are among the main concerns for citrus growers in the region. Since inherent soil fertility is poor and potential nutrient losses are appreciable, conventional growers mainly depend on chemical fertilizers (Zotarelli et al. 2008a, b). Organic growers often use external nutrient sources that are expensive, and their use may be restricted by food safety or certification issues (for example, animal manure). For organic growers, effective weed control is one of the key factors hampering successful transition, and cover crops may thus provide them with a cost-effective option to manage weeds (Linares et al. 2008). In our experience, the presence of coarse sandy soils hampered build up of SOM and effective inoculation of leguminous winter cover crops, and supplemental K-fertilizer was required to enhance cover crops performance. Warm-season cover crops, on the other hand, generally thrived on these sandy soils and are readily colonized by native rhizobium species (Linares et al. 2008).

3.3.1.3 System Analysis and Exploration of Alternative Systems

Use of Cover Crops for Weed Suppression in Orchard Systems

Organic vegetable growers in Florida tend to use a weed fallow during the hot and humid summer months, since high pest and disease pressures prevent the cultivation of most commercial crops. However, this practice may also favor build-up of weeds (Collins et al. 2007), while effective weed control remains a key concern of most organic growers (Ngouajio et al. 2003). Therefore, the use of summer cover crops such as sunn hemp (*Crotalaria juncea*) and cowpea (*Vigna unguiculata*) may provide growers with an option to improve inherent soil fertility, prevent the build-up of weed seedbank, and suppress noxious weeds such as yellow nutsedge (*Cyperus esculentus*) and Pigweed (*Amaranthus hybridus*). Greenhouse studies showed that sunn hemp provided relatively poor weed control during initial growth when compared with a more compact crop such as cowpea (Collins et al. 2007). However, field studies showed that sunn hemp was most effective in suppressing weeds toward the end of the growing season, which may be related to its slow initial growth (Linares et al. 2008). Thus, effective weed suppression in annual Florida organic systems may require use of cover crops with complementary growth and canopy characteristics.

Cover crops have been used extensively in perennial production systems throughout the world – especially tree-crop and shrub-crop production systems (Anderson et al. 2001). Some of the main issues of their use are related to effective weed control, uniform and compact growth, adequate erosion control, provision/retention of nutrients, and potential competition for water under water-limiting conditions. Effective use of cover crops may reduce establishment (e.g., fertilizer) cost and/or provide financial returns (e.g., forages and pulses) during the tree establishment period (Anderson et al. 2001). In terms of perennial production systems in the subtropical and tropical regions, the following warm-temperature adapted perennial and annual species may be viable candidates: perennial peanut (*Arachis pinto* and *A. glabrata*), *Canavalia*

spp., pigeonpea (*Cajanus cajan*), *Crotalaria* spp., indigos (*Idigofera* spp.), velvetbean (*Mucuna* spp.), and *Vigna* spp. (Linares et al. 2008; Anderson et al. 2001). However, use of *A. pinto* is not feasible in North Florida due to winter freezes. In this region, winter annuals most commonly used in perennial systems include winter rye, vetch, black oats (*Avena strigosa*), crimson clover (*Trifolium incarnatum*), lupin, and forage radish (*Raphanus sativus*). We tested each of these species for weed control potential in an organic citrus production system in North Florida.

Of the warm-temperature cover-crop species we tested, sunn hemp (*Crotalaria juncea*) was the most prolific cover crop. It generated 5.3–12.6 Mg ha⁻¹ when compared with 5.9–9.5 Mg ha⁻¹ for hairy indigo, 3.7–7.6 Mg ha⁻¹ for pigeon pea, 2.4–5.1 Mg ha⁻¹ for cowpea (*Vigna unguiculata*), and 1.0–2.8 Mg ha⁻¹ for velvetbean (*Mucuna pruriens*). Both sunn hemp and cowpea were most effective in suppressing weeds and reduced weed biomass by 83–97% (Linares et al. 2008). Pigeon pea did not provide effective weed control; similar observations were made with citrus in Bolivia (Anderson et al. 2001) and Brazil (Matheis and Victoria Filho 2005). We used both “bushy” and “vining” *Mucuna* types, but neither performed well under our conditions; this may have been related to the poor water retention capacity of our sandy soils. This is in contrast with studies in Central America where velvetbean grew more vigorously and provided effective weed suppression (Neill and Lee 2001). Although we tested jackbean (*Canavalia ensiformis*) on a small plot basis and this crop appeared to be well adapted to sandy soils, lack of access to seed sources prevented detailed assessment of its performance. In other regions, this crop is a prolific biomass producer, grows well under adverse conditions, is used for forage production as well, and is also suitable for intercropping (Nyende and Delve 2004; Anderson et al. 2001).

In terms of winter cover crops, the best performing species were winter rye (3.2–6.0 Mg ha⁻¹), forage radish (3.2–4.3 Mg ha⁻¹), crimson clover (1.7–5.0 Mg ha⁻¹), and black oats (1.3–3.6 Mg ha⁻¹). Use of cover-crop mixtures (one or more species) greatly enhanced biomass production (3.6–8.0 Mg ha⁻¹). Crop performance and weed suppression by winter leguminous cover crops were erratic during the first years, which were related to the poor adoption to sandy soils, but over time, their performance improved. Rye and radish were more effective in suppressing weeds while mixes of these two cover crops reduced weed biomass to less than 2% of that in controls and thus may provide a very effective weed control (Linares et al. 2008).

The initial growth of perennial peanut (*A. glabrata*) in this system was very slow and its initial weed suppression ability was poor. Repeated mowing improved the performance of this species over time, and it may provide a valuable forage for additional farm income. However, its growth and weed suppression during the first 3 years of study was clearly inferior to the annual cover crops tested.

Annual Cover Crops as a Green Manure in Vegetable Crops

Historically, crops such as velvet bean were used as a green manure until about the 1930s. After this time, the availability of cheap inorganic fertilizers reduced their attractiveness as N sources (Buckles et al. 1998). Currently, cover crops in Florida

vegetable cropping systems are usually incorporated via tillage. However, since tillage enhances soil mineralization, this may offset carbon sequestration and soil quality benefits (Phatak et al. 2002). Conventional production systems for crops such as tomato and pepper include the use of black mulch, which serves to facilitate fumigation, suppress weeds, reduce evaporation, leaching, increase soil temperatures and initial growth, and prevent soil contact of harvestable products (Carrera et al. 2007). However, its use involves energy, economic and environmental costs for production, and purchase and disposal, while their use also can enhance run-off of pesticides (Abdul-Baki et al. 1996, 1999). Experiments conducted by Abdul-Baki et al. (2004) in South Florida demonstrated that cover-crop-based systems (e.g., growing cowpea or velvet bean) had similar marketable tomato yields when compared with the use of mulch and methyl bromide, while production cost could be reduced by \$1,544 ha⁻¹. On-farm demonstration trials by Avila (2006c) in South Florida showed that use of sunn hemp-based systems could offset marketing risk of conventional tomato systems by increasing yields and reducing use of herbicide and external fertilizer inputs. These findings were similar to those of previous studies in which systems based on cover crops and zero-tillage improved soil quality and nutrient retention while reducing agrochemicals, external input use, production costs, environmental impacts, and soil erosion (Abdul-Baki et al. 1996, 2002, 2004).

Another field study was conducted in north central Florida to assess the benefits of a reduced-tillage cover-crop-based system for vegetable crops between 2001 and 2005. This study included different combinations of both summer and winter cover crops [sunn hemp, rye (*Secale cereale*), lupin (*Lupinus angustifolus*), and vetch (*Vicia* spp.); Avila et al. 2006a, b; Cherr 2004]. Overall biomass and N accumulation of summer cover crops were on the order of 8.0–12.2 Mg ha⁻¹ and 146–172 kg N ha⁻¹ whereas production of leguminous winter cover crops was much lower (2.0–4.0 Mg ha⁻¹ and 51–104 kg N ha⁻¹) (Cherr et al. 2006c). However, in the warm and humid climate, most of the N from winter-killed sunn hemp was released quickly, and growth of subsequent cover crops and economic crops was too slow to effectively utilize it (Cherr 2004; Cherr et al. 2006a, c). The use of a vetch and rye biculture allowed uptake of this N and also resulted in improved winter-cover-crop growth and N accumulation (7.2 Mg ha⁻¹ and 135 kg N ha⁻¹; Avila et al. 2006a, b). Changing rye and vetch proportions in this mixture greatly affected the C:N ratio of the cover-crop residue (e.g., 69 for pure rye system, 26 for 67% rye–33% vetch system, and 14 for pure vetch system). Although total biomass was greatest for mixed systems, N accumulation was greatest for pure vetch systems.

In terms of yield benefits to subsequent crops, cover-crops-based systems provided clear yield benefits for sweet corn, broccoli, and watermelon (Cherr et al. 2007; Avila et al. 2006a, b). However, unlike studies at more northern locations (Bhardwaj 2006; Carrera et al., 2007; Burkett et al., 1997), the cover-crops-based systems in Florida only provided limited yield benefits and inorganic N-fertilizer savings. Although the cover-crop-based systems provided N-benefits on the order of 60–70 kg N ha⁻¹, enhanced early economic crop growth, and N accumulation, these systems were still out-yielded by conventional controls receiving 267 kg N ha⁻¹

(Cherr et al. 2007). Generally, the low soil fertility combined with the poor nutrient retention capacity of Florida soils does not support top production levels unless substantial amounts of supplemental nutrients are supplied throughout the growing season. This is related to the prevailing sandy soils that hamper efficient nutrient retention and build-up of SOM even in the absence of tillage. Although cover-crop-based systems provide substantial amounts of both C and N, the enhancement of the inherent long-term nutrient supply capacity of the system appears to be limited, since SOM is poorly protected and nutrients released by residues are prone to leaching. Therefore, the system is poorly buffered, and thus pools are exhausted rapidly prior to the development of an extensive root system of the commercial crop. Pasture systems thus may be more effective in improving inherent soil fertility when compared with annual cover crops. Moreover, maize crops may be particularly unsuited to cover-crop-based systems in these conditions because its capacity for N uptake during early growth is limited. Detailed ^{15}N studies (Zotarelli unpublished data) showed that N-uptake efficiency of sweet maize was only 14% for soil nitrate present at planting when compared with 48% for N released 1 month after planting.

3.3.1.4 Technology Adoption

The adoption of cover-crop-based systems in Florida by farmers is limited and mainly confined to organic producers. Conventional growers may opt to use sorghum Sudan grass (*Sorghum bicolor* var. Sudan grass) during summer fallow and winter rye as a soil cover in commercial vegetable cropping systems. Although cover crops may be perceived as an environmentally sound management option, their use can interfere with the standard management practices in conventional systems in this region. In an on-farm study, it was observed that full-grown sunn hemp was very tall (>2 m), and the thick-stemmed plants produced a recalcitrant residue layer. During subsequent bed formation of tomatoes, this material hampered bed formation and thereby reduced the effectiveness of fumigation and subsequent weed suppression since the residue caused tearing of the plastic mulch. Based on suggestions of the participating grower, use of repeated mowing resulted in a less coarse residue material and acceptable biomass benefits, which underlines the importance of active farm participation during technology development.

In Florida, the absence of incentives, lack of appropriate recommendations, and suitable equipment may hamper widespread adoption of cover-crop-based systems in vegetable cropping systems. Since the use of zero- or reduced-tillage on sandy soils in Florida is limited and there is a lack of suitable planters, the risk of poor initial crop establishment and yield reductions of subsequent commercial crops also increases. These factors may further hamper the use of cover-crops-based conservation systems in the region. In contrast, 58–64% of the farmers in neighboring Alabama use reduced- or zero-tillage for crops such as cotton and maize (Bergtold et al., 2005). Even in North Florida, there may be producers within subregions or

niche markets for whom cover-crop use is more feasible. On the heavier soils in the northwest Florida panhandle, there is more of a tradition to integrate reduced tillage into conventional operations while leguminous winter cover crop also tend to perform better on these soils. Moreover, proximity to Alabama and Georgia may provide opportunities for farmers in this subregion to successfully adapt cover crops systems developed in these neighboring states as well. Both positive and negative incentives (price premiums and regulatory requirements, respectively) may also encourage organic growers in Florida to use cover-crop-based systems. In this case, lack of technical information by traditional local extension approaches and different pedo-climatic conditions from other key organic production regions may force growers to engage themselves with on-farm experimentation and technology development of cover-crop-based systems. So, it appears that lack of incentives and suitable technologies continues to hamper the adoption of cover-crop-based in conventional production systems. While in organic systems, where cover crops can provide a much broader array of services, the lack of viable alternatives justifies development of cover-crop-based systems.

3.3.2 Brazil

3.3.2.1 Biophysical Production Environment

The study region (Paraná) is located in the Southern region of Brazil between latitudes 22°29'S and 26°42'S and longitudes 48°02'W and 54°37'W. Paraná is located in the tropical and subtropical transition zone. The climate is humid-subtropical with hot summers and drought periods no longer than three to four weeks. The mean annual precipitation ranges between 1,400 and 2,000 mm. Most rain occurs during the summer (October–March). Almost 40% of Paraná's area consists of soils derived from basalt beds with heavy clay and fertile soils. In this region, agricultural cropping systems mainly include annual crops such as soybean, wheat, cassava, sugarcane, cotton, and coffee. In the northwest, soils derived from sandstones dominate, and in this region beef cattle and orange production are of greater importance. The agricultural acreage in Paraná amounts to 17.6 million hectare, of which 4.0 and 2.7 million hectare have been planted with soybean and maize, respectively. Nationally, Paraná is the largest producer of beans, maize, and wheat; and second in soybeans, cassava, and sugar cane; third in tobacco; and fourth in coffee. In 2007, Paraná grain production represented 22% of the national production (IBGE 2008). The grain production in 2006 was 11.9 million tons of soybean, 14.3 million tons of maize; 1.9 million tons of wheat; 0.7 million tons of beans. Key factors such as climate and soil have made it possible to produce a wide variety of crops. However, the success of agriculture in Paraná was possible due to efforts of the state research and extension agencies to implement long-term watershed-based soil and water conservation programs including a combination of zero tillage and cover-crop-based crop rotations.

3.3.2.2 Characterization and Diagnosis of Constraints

Intensive agriculture in Paraná started upon colonization during the early 1900s and the state was the main coffee producer for several decades. The area planted with coffee reached 1.8 million ha by 1975. At that time, however, a severe frost decimated coffee and most of the coffee fields were converted to mechanized annual cropping systems and pastures. Additional land was converted to arable land as well as more people moved into the area. Soybean–wheat-based crop rotations became the dominant cropping system during the 1970s and 1980s. These cropping systems featured burning of crop residues followed by tillage with heavy disc harrows and moldboard plows. Soil surface disaggregation, reduced soil water infiltration, soil crusting, and soil compaction led to severe erosion problems (10–40 Mg soil erosion ha⁻¹ year⁻¹) and a steep decline in inherent soil fertility (Calegari 2003; Derpsch et al. 1986). Initially, terracing and planting along contour lines was promoted to minimize further erosion. However, during the early 1970s, zero tillage systems were also introduced in Paraná (Calegari 2003; Landers 2001). During the early 1990s, the acreage under zero tillage in Brazil reached 1 million hectare.

However, the adoption of zero tillage systems intensified other problems such as weeds and pests, and also exacerbated soil compaction, while it also posed problems associated with thatch layer accumulation. The development of soil management and cropping systems strategies, including the use of cover crops, thus became important research topics to improve the sustainability of local agriculture production systems in Paraná (Calegari 2003). In particular, research showed that diversification of crop rotations under zero tillage increased the average yield of soybean and maize and lowered fuel, fertilizer, pesticides, and labor requirements (Muzilli 2006; Calegari 2003). Additional benefits such as increase in soil carbon stock and cation exchange capacity, greater soil water infiltration and soil aggregation, and reduction of runoff have been frequently reported in the literature (Triplett and Dick 2008; Zotarelli et al. 2005a, b, 2007a; Sisti et al. 2004; Calegari 2003; Sa et al. 2001; Six et al. 2000; Boddey et al. 1997; Derpsch et al. 1986).

3.3.2.3 System Analysis and Exploration of Alternative Systems

Weed suppression by cover crops has provided a critical component in the successful adaptation of zero tillage systems in Paraná by cutting herbicide use and weed control costs by up to 25–42% (Teasdale et al. 2007; Derpsch 1998). Use of species, e.g., oats, rye, radish, lupin, and sunn hemp, that can be killed mechanically may further reduce or eliminate herbicide use but some manual weeding during the growth season may still be required (Teasdale et al. 2007). Selection of cover crops is based on local availability of affordable seeds, their effectiveness in providing soil cover and suppressing weeds, and to supply nutrients to a subsequent cash crop. Recommended cover crops in Paraná include oats (*Avena* spp.), white radish (*R. sativus*), pigeon pea, mucuna, vetches, lupins, lablab (*Lablab purpureus*), sunflower (*Helianthus annuus*), pearl millet (*Pennisetum glaucum*), and pastures (see also Calegari 2003).

Table 3.1 Examples of crop rotation systems recommended to Paraná (Embrapa 2006)

State region	Year 1	Year 2	Year 3	Year 4	Year 5	Percent of soybean
North/West	OA/MA	CA/SO	PM+M/SO	WH/SO	–	75
North/West/Central	LU/MA	OA/SO	WH/SO	–	–	66
Southeast	VE/MA	WH/SO	OA/MA	WH/SO	BA/SO	60–80

BA = barley; CA = canola; LU = lupin; M = mucuna; MA = maize; OA=oats; PM = pearl millet; SO = soybean; VE= vetch; WH = wheat

Soybean is the most important cash crop that is also grown most frequently (60–80% of rotations). Table 3.1 provides a brief description of standard recommended crop rotation for zero tillage systems in Paraná for different production regions. Crop rotation design is based on (1) species characteristics (legume vs. gramineae), (2) residue quality and quantity, and (3) occurrence of diseases and nematodes. In terms of fertility management, biological nitrogen fixation (BNF) plays an important role in the improvement of sustainability of local cropping systems. Soybean accumulates large quantities of N, 80% of which is generally supplied by BNF in rhizobium-inoculated varieties (Alves et al. 2006; Zotarelli 2000, 2005). However, owing to the high amount of N removed with the harvested product, relatively little of the N is left in the field (Alves et al. 2002). Under zero tillage conditions, the inclusion of winter legume cover crops such as lupin or vetch every 3–4 years in the crop rotation thus is critical to maintain SOM and inherent soil fertility and to minimize runoff and erosion via enhanced crop water infiltration and soil and nutrient retention. This has been shown to greatly enhance the yields and sustainability of local cropping systems (Derpsch et al. 1986). Well-managed zero tillage/cover-crop-based systems can reduce erosion by 95% (Prado Wildner et al. 2004). On-farm studies in North Paraná showed that zero tillage increased soybean and wheat yields by 34% and 14%, respectively; whereas corresponding additional yield benefits associated with integration of cover crops in crop rotations were 19% and 6% (Calegari et al. 1998).

Recent experiments in this region showed that lupin accumulated up to 10 Mg ha⁻¹ of dry biomass with N accumulation around 250 kg ha⁻¹. The BNF contribution for lupin was approximately 70%, which translates to an input of approximately 175 kg ha⁻¹ of external N being added. Lupin-based maize systems receiving no other N inputs yielded 47% more when compared with maize following oats receiving typical fertilizer rates of 80 kg N ha⁻¹ (Zotarelli 2005). Integrating zero-tillage with winter cover crops also increased soil C accumulation (Sisti et al. 2004) via stabilization of aggregate-associated C (Denef et al. 2007; Zotarelli et al. 2007a). However, as soybean is the main cash crop, use of certain legume cover crops that host soybean diseases must be restricted [such as pigeon pea and lupin cover crops that also host stem canker (*Phomopsis phaseoli*)]. These problems may be solved by changing crop sequence within rotations and/or by using resistant soybean cultivars. Other challenges with cover crops include insufficient mulch layer formation

or reduced emergence of subsequent economic crops when an adequate mulch layer is sufficient to provide other benefits. Again, these problems can be solved by relatively simple changes in management, such as lengthening the interval between the killing of the cover crop and maize planting and use of relatively recalcitrant cover crops (small grains or cover-crop mixtures including small grains).

3.3.2.4 Technology Adoption

One of the key contributing factors to the success of zero tillage systems was the diversification of crop rotations including the use of cover crops. More than 25 million hectare have been cultivated under zero tillage in Brazil in 2006 and, in the same year around 95% of grain crop land was under zero tillage in Paraná. Rapid expansion of cover crop and no till systems was greatly facilitated by participatory farming system approaches. These approaches gave farmers a central role during the problem identification, structuring of solutions and aimed also to strengthen linkages between researchers and extension workers (Sempeho et al. 2000). In general, farmer-to-farmer demonstration and dissemination approaches were the most effective. For larger farmers, both the private sector and experts from nongovernmental organizations (NGOs) also contributed to this process, whereas for smaller farmers, state extension agencies played a more important role (Landers 2001). However, the main boost in adoption occurred when production costs of zero tillage systems were less than those of conventional tillage, suitable recommendations were in place and the method was also effectively integrated in standard teaching and extension programs (Landers 2001). Other factors affecting adoption included: creating awareness of clear incentives for adoption of cover crop and zero tillage systems; active contribution of pilot farmers that championed zero tillage and adapted such systems to local conditions; presence of effective farm organizations; and access to subsidies or credit permitting farmers to invest in technology (Pieri et al. 2002; Sempeho et al. 2000). Moreover, local supply networks for affordable seeds, tools, equipment, and local knowledge were also critical to sustain the continuous development as they promoted local self-reliance ensured long-term sustainability of the effort (Pieri et al. 2002; Landers 2001).

3.3.2.5 Innovations in Cover-Crop-Based Conservation Tillage Systems in Santa Catarina

Santa Catarina is a hilly region in southern Brazil with heavy soils and high annual rainfall (1,200–2,370 mm), and 40–80% of the agricultural land is prone to medium to severe erosion (Prado Wildner et al., 2004). Similar to southern Uruguay, this region features relatively small-scale family-based intensive crop production systems. Some of the key crops include maize, beans, potato, and tobacco as well as intensively managed vegetable systems such as onion, garlic, tomato, cauliflower, pepper, and beets (Prado Wildner et al., 2004). Although there has been a trend of

increased intensification and a rural exodus of farm workers, the hilly topography has hampered development of large-scale mechanized agriculture. During the 1960s, the use of terracing was promoted to stem soil erosion, but (as in Paraná) neither did this address the real problem (e.g., lack of soil cover) nor did it fit local needs, so adoption was poor. During the 1970s, increased mechanization resulted in extensive and devastating soil erosion.

Technical assistance to solve these problems was provided by neighboring institutes in Paraná state. Local extension agents initiated cover-crops-based zero tillage systems on pilot farms in 1978. To ensure availability of cover crops, farmers were provided with small quantities of common vetch (*Vicia sativa*), but they were required to multiply this seed locally. Availability of suitable seeds in Santa Catarina greatly varies depending on the species, year, and region. Relatively, few farmers specialize in the production of cover crops seeds, and in some years, seeds of leguminous cover crops may still not be available; therefore, this remains one of the main constraining factors for adoption of cover-crop-based systems. Despite this constraint, farmland in cover-crop-based zero tillage systems in the region increased from 5% in 1987 to 44% in 1997. This rapid expansion was related to a number of factors: (1) farmer-driven technology, (2) development of a variety of equipment by local entrepreneurs tailored to the specific needs of different farm management types and distribution of this equipment by larger agro-industrial companies, (3) reduced labor requirements from mechanization, which enhanced the livelihood of local farmers, (4) presence of an effective local agricultural research and extension network, (5) government abandoning subsidies for use of agrochemicals during the 1980s, (6) strong presence of family-based farming systems with secure land tenure, and (7) presence of NGOs that helped structure local education and research programs (Prado Wildner et al., 2004). Reported yield of local cover-crop-based maize systems were 30% higher when compared with the conventional systems. The use of cover crops combined with reduced tillage in this region was capable of increasing both SOM and fertilizer-use efficiency and lowering operational costs, but has increased herbicide requirements (Amado et al. 2006; Prado Wildner et al., 2004).

Onion production expanded greatly during the 1970s and 1980s in the Upper Itajai River Valley of the Santa Catarina region. The use of mechanical tillage on steep slope combined with fine textured soils in onion cropping systems that have sparse canopies and add very little residues resulted in pronounced soil erosion (Prado Wildner et al., 2004). Reduced-tillage systems were introduced to combat this problem and were adopted by 60–70% of the farms. Black oat, oilseed radish, and/or vetch are used as cover crops and these are rotated with onions, although onions still must receive supplemental N applications to minimize the risk of N-immobilization. Maize is frequently grown following onion and benefits from residual soil nutrients. In some cases, maize may be intercropped with mucuna, while *Canavalia* and *Crotalaria* species also can be effectively grown as summer cover crops, but farmers usually prefer intercropping with edible beans during this time. Over the years, these systems evolved and were also adapted by local organic farmers. However, in these systems, farmers opted to use a mix of different cover

crops (e.g., a rye, radish, vetch mixtures) to enhance the functionality and performance of the cover-crops system (Altieri et al. 2008). The use of cover crops allowed the development of innovative organic reduced-tillage systems and reduced weed growth by more than 90% and thus provided farmers with a cost-effective weed management option (Altieri et al. 2008). On-farm studies in this region have used systems developed by local farmers based on native knowledge and innovations. The main role of researchers has been to provide suggestions and to make benefits of locally developed systems more explicit to a broader (international) audience. The success story of cover-crop-based systems in Brazil is closely linked to their integrative use in conservation tillage systems. Such initiative may serve as a development framework for other regions and systems with similar conditions, including both conventional and organically managed vegetable production systems in Uruguay.

3.3.3 Uruguay

3.3.3.1 Biophysical Environment

The study region (Canelones) is a hilly region located in Southern Uruguay (34°25' S and 56°15' E). The average annual temperature is 16°C (10°C in July to 23°C in January), and light frosts may occur between June and September. Average annual rainfall is 1,100 mm and water deficits tend to occur between October and March, while water surplus may be observed between May and August. Clay and silty clay loam soils prevail and SOM content for native undisturbed soils may range between 4.5% and 6.5% but may decline to 1–3% under continuous cultivation of conventional agricultural systems. Soil erosion due to intense rainfall events may result in soil losses of 9–15 Mg ha⁻¹ year⁻¹. Soil degradation has resulted in soil crusting, reduced aeration, infiltration, and water retention capacity. More than 70% of the farms are smaller than 20 ha and vegetable production is the main source of income for 27% of growers. The main vegetable crops grown in the area include squash, carrot, onion, garlic, potato, sweet potato and sweet maize, and tomato.

3.3.3.2 Characterization and Diagnosis of Constraints

The Uruguayan vegetable production sector has been facing a cycle of increased production intensity and input prices, falling commodity prices, and depletion of natural resources. Between 1990 and 1998, vegetable production increased by 24%, crop yields increased by 29% while cropped area decreased by 9% (DIEA-PREDEG 1999). Simultaneously, inflation corrected prices of vegetable products between 1992 and 2001 decreased by 34% (CAMM 2002) and an additional 15% between 2001 and 2004 (CAMM 2005). Southern Uruguay has the highest concentration of small or family farms (farms where most of the labor is provided by family members).

Around 88% of the farms with vegetable production as main source of income are family farms (Tommasino and Bruno 2005). Between 1990 and 2000, the number of these vegetable farms decreased by 20% (DIEA 2001). Those farms remaining in business had to increase production and product quality, while reducing product prices to maintain family income.

The strategy followed by most farmers was to intensify and specialize their production systems. The average vegetable cropped area per farm in southern Uruguay increased, while the average total area per vegetable farm stayed approximately the same. The average number of crops per farm also decreased. The observed increase in crop yields was attained via increased use of irrigation, external inputs (fertilizers, biocides, and energy), and higher quality seeds (Aldabe 2005). However, this strategy intensified the pressure on the already deteriorated soils and limited farm resources. Only 27% of the farmers may at times use cover crops, while 90% of the farmers depend exclusively on chemical fertilizers (Klerkx 2002). Increasing crop area and narrowing crop types without an adequate planning has often interfered with farm operations and caused inefficient use of production resources, increased dependence on external inputs, and greater environmental impacts. Consequently, farm incomes are inadequate to cover basic family needs, to maintain farm infrastructure and preserve the natural resource base.

When farmers in Canelón Grande were asked what they perceived to be the main environmental problems, the most common responses were global climate (39%), pollution by residues of agrochemical products (15%), and problems with pests and diseases (11%). Only 9% indicated soil erosion as their main environmental problem (Klerkx 2002). However, 88% of the interviewed farmers were aware of the occurrence of soil erosion on their own farms. The use of terracing and maintaining a rough soil surface were practices that farmers typically perceived to be effective in controlling erosion, while only 8% mentioned the use of cover crops or the importance of maintaining adequate vegetation cover (Klerkx 2002). Lack of farmer knowledge about the benefits of cover crops, therefore, appeared to be a significant constraint to their use, thus hampering development and adoption of cover-crop-based systems in this region.

3.3.3.3 System Analysis and Exploration of Alternative Systems

During the 1990s, several experiments were conducted on experimental stations and commercial farms in South Uruguay to investigate the effects of cover crops and organic amendments on vegetable crop yields and soil quality. When compared with conventional management, these experiments showed significant increases in vegetable crop yields after cover crops and animal manure applications. In crops such as potato, sweet potato, onion, carrot, garlic, and sweet pepper, yield increases ranged from 9% to 65% after summer or winter green manures when compared with fallow (Docampo and Garcia, 1999; Garcia and Reyes, 1999; Gilsanz et al. 2004). Winter cover crops tested included oats, black oats, wheat (*Triticum aestivum*), and peas (*Vicia* spp.) in pure stands or in mixtures; summer cover crops were maize, sorghum (*Sorghum bicolor*), foxtail millet (*Setaria italica*), mucuna, cowpea, and *Crotolaria* species. Aboveground biomass production ranged from 3.5 to 11 Mg

DM ha⁻¹ and 3 to 19 Mg DM ha⁻¹ for winter and summer cover crops, respectively (Peñalva and Calegari 2000; Docampo and Garcia, 1999; Garcia and Reyes, 1999; Gilsanz et al. 2004).

Dogliotti et al. (2005) showed that erosion control support practices such as terracing are not adequate to decrease soil erosion below the tolerance limits in vegetable farms in South Uruguay. However, inclusion of cover crops during the intercrop periods and alternation of horticultural crops with pastures do have the potential to reduce soil erosion by a factor of 2–4 while reversing SOM losses, since SOM values increased with 130–280 kg ha⁻¹ year⁻¹ (Dogliotti et al. 2005). In 2005, a project was initiated by a local team of scientists to develop sustainable vegetable farming systems in six farms in the region. The study was extended to 16 conventional and organic farms in 2007. On each farm, the development process involved a continuous cycle of diagnostic-design-implementation-evaluation components, and initial results were used during a subsequent design and testing cycle as well.

In mixed farming systems, the use of perennial rye grass and red clover (*Lolium perenne* and *Trifolium pratense*) mixtures or alfalfa (*Medicago sativa*) can be a viable production option since it can provide a source of high-quality forage while also enhancing SOM (Selayu Garvizu 2000). Use of cover crops that include a small grain species was considered preferable, since they produce greater amounts of more recalcitrant residues and may be more effective in improving SOM and minimizing erosion. Selection of annual cover crops was based on seed costs, local seed availability, and familiarity to farmers. Based on this, suggested species including black oats (*Avena strigosa*), foxtail millet (*Setaria italica*), oat (*Avena sativa*), sudan grass (*Sorghum × drummondii*), and wheat (*Triticum aestivum* L.) were integrated into the existing vegetable crop rotations. Above-ground biomass accumulation by these cover crops ranged from 4.4 to 7.7 Mg ha⁻¹. Where these cover crops were combined with additions of chicken manure, SOM content increased from roughly 2.1% to 2.7% within the first 2 years of the study (Rietberg 2008). Long-term (40 years) assessment of the cropping system performance using the ROTSOM model [based on the approach for modeling outlined by Yang and Janssen (2000)] SOM values upto 3.5% may be attained, depending on the cropping system, while in the absence of organic amendments, SOM declined to steady-state values around 1.7–1.8% (Rietberg 2008). Although the progress of the expansion of cover-crop-based systems in Uruguay still lags behind by that in Brazil, the proven benefits of such systems and the lack of cost-effective alternatives seem to create a situation that will favor their future use.

3.3.4 Interpretive Summary of Case Studies

In general, the innovation of successful cover-crop-based systems has been relatively successful in Paraná and in Santa Catarina, but relatively unsuccessful in Florida. Attributes that appear to have facilitated the innovation processes in Paraná and Santa Catarina include:

1. Active involvement by farmers in research and dissemination programs
2. Integration of cover crops into production systems without net loss of land or labor resources
3. Informing farmers of the (direct) benefits of cover-crop use
4. Provision of multiple benefits by cover crops
5. Sufficient access to information, inputs, and technologies required for cover-crop use
6. Provision of skills and experience necessary to manage cover crops effectively

In the case of Florida, many of these attributes have been absent. Unlike Florida, in Brazil, suitable cover-crops-based systems for small farms have been developed and successfully implemented in both row crops and vegetable production systems, zero-tillage equipment is readily available, and these technologies are fully integrated into standard production systems (Prado Wildner et al., 2004; Calegari 2003; Landers 2001). Moreover, as indicated before, Florida farmers tend to be more individualistic, may also develop their own technologies to develop a competitive edge, and may not be willing to share these with other farmers. In this region, innovation in cover-crop-based production systems may thus be required to reward of farmers for ecological services provided by cover crops. The growth of certified organic production in Florida and the USA in general may provide a successful example of such a reward. In this case, the US federal government created a labeling and certification standard that provided a reliable market “niche.” Within this market, consumers and producers have allowed to set price premiums that adequately reward producers for organic practices. However, provided that energy and fertilizer prices continue to rise, there may be a direct economic incentive for use of cover crops by conventional farmers as well, provided they will have access to suitable information and cost-effective technologies that can be integrated into their existing systems.

In Canelones, the innovation of cover-crop-based systems remains in an early development stage. In this region, experiences in Paraná and Santa Catarina may provide appropriate development models for implementation of cover-crop-based systems. However, use of system analysis tools such as ROTAT may actually be critical to speed up to technology development and adaptation process since they can provide a systematic structure to streamline the exploration of viable cover-crop-based alternatives to the existing conventional rotations. In this manner, land use options could be evaluated rather effectively, and a limited number of viable alternatives were then further refined during the on-farm testing and development stage. Farmer involvement and participation during system design and development of suitable management options varied from proactive to more passive assimilation of new technologies. Similar to Paraná and Santa Catarina, farmers who joined the project during its inception stage played a critical role during the technology adaptation and transfer processes, and their contributions seem to be invaluable to enhance the regional impact and momentum of technological innovations. Currently, pilot farmers have assumed ownership of new technologies and provided leadership during field demonstrations.

3.4 Conclusion

It is concluded that cover crops can contribute to resource conservation and may provide a viable production option for resource-limited production systems, provided they fit into underutilized niches in the existing agroecosystems. Based on experiences with functional networks within local farm communities (e.g., campesino-to-campesino system), efficient technology transfer of cover-crop-based systems may occur spontaneously with a minimum requirement of external intervention and/or support structures. This development model can foster local development in regions where traditional local social networks favor such an approach. However, in other regions, more extensive interventions may be needed. In this case, the use of co-innovation approach may provide a viable option since it integrates both “science-based intervention” with “farm-based” technology adaptation mechanisms. In this manner, current systems characteristics, challenges and constraints can be mapped out more effectively and models are being used to explore and design desirable development tracks. The use of simulation models to harness some of the complexity of agroecosystems is particularly relevant for cover-crop-based systems. Such an approach may greatly facilitate system design (e.g., development of suitable rotations), assessment of both short-term dynamics (e.g., nutrient synchronization) and long-term impacts (e.g., SOM trends as effected by erosion), and exploration of different development scenarios, e.g., system performance under different climate change scenarios.

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Chapter 4

Improving Bioavailability of Phosphate Rock for Organic Farming

**Anthony C. Edwards, Robin L. Walker, Phillip Maskell,
Christine A. Watson, Robert M. Rees, Elizabeth A. Stockdale,
and Oliver G.G. Knox**

Abstract The sustainable use of nutrients in agricultural food production represents a major emphasis for international research, and evidence that clearly demonstrates the imbalance between nutrient inputs and outputs exists. Nutrient surpluses exist and are most commonly associated with intensive livestock production and present a particular range of environmentally related issues. Nutrient deficiency can also develop, and organically managed systems highlight the difficulties that are involved in maintaining agronomically acceptable concentrations of soil phosphorus (P). A restricted range of P-containing sources, often having poor solubility, exacerbate these difficulties, and obvious benefits would arise if the availability could be “naturally” enhanced. Slow rates of phosphate rock (PR) solubilization under prevailing soil conditions reduce the general agronomic usefulness and potential benefits that any direct applications might provide. Being able to improve rates of dissolution through some control of the solubilization process would offer widespread potential advantages, particularly with respect to better matching patterns of P supply with crop demand. A variety of pre and postapplication opportunities exist to improve the solubility of rock phosphate. Some of these have particular relevance to organic agriculture where phosphate rock represents an important and acceptable “external” source of P. A range of post-application, farm management practices that include green manures and rotations using crops with favorable traits that improve P utilization have been successfully employed. Here, we emphasise pre-application techniques, especially the co-composting of phosphate rock with

A.C. Edwards (✉)
Nether Backhill, Ardallie, Peterhead, AB42 5BQ, UK
e-mail: t.edwards25@btinternet.com; t.edwards@macaulay.ac.uk

R.L. Walker and C.A. Watson
SAC, Craibstone Estate, Bucksburn Aberdeen, AB21 9YA, UK

P. Maskell, R.M. Rees, and O.G.G. Knox
SAC, West Mains Road, Edinburgh, EH9 3JG, UK

E.A. Stockdale
Newcastle University, NE1 7RU, UK

various organic by-product materials that include livestock manures and residual vegetable matter. A range of laboratory incubations have demonstrated the underlying mechanisms involved with solubilization. The significance of microbially induced production of organic acids and acidity during composting is particularly important in this respect. While co-composting with phosphate rock offers a great potential that could be developed for use at the individual farm scale, the key controlling factors and underlying mechanisms are far from being fully understood. A possible time sequence of reactions that might be envisaged include an initial production of protons and organic acids leading to the mineralogical dissolution and release of Ca and P, followed finally by an extended period during aging of the compost where secondary reactions appear to influence the form of P. The consequences of composting conditions and individual processes on immediate and longer-term bioavailability of P once field applied are still poorly defined.

Keywords Phosphate rock • composting • sustainability • nutrient use efficiency

Abbreviations

AM	arbuscular mycorrhizal
FYM	farmyard manure
K	potassium
N	nitrogen
P	phosphorus
PSM	phosphate solubilizing micro-organisms
SB	sugar beet

4.1 Introduction

There is a global requirement for increased food production, which must be achieved while also minimizing potential environmental impacts. Maintaining a balanced supply of the major plant nutrients is a central requirement of sustainable resource management, and maximizing nutrient use efficiency can help achieve this objective (Topp et al. 2007). The sales of agricultural products from farms are inevitably associated with nutrient export that must be replaced through some combination of fertilizer and recycled by-products, for example, manures and crop residues, biological fixation and atmospheric deposition in the case of nitrogen (N), and geochemical release through mineral weathering and dissolution in the case of phosphorus (P) and potassium (K). An imbalance between nutrient offtake and inputs is a common feature of many global agricultural systems (Smaling et al. 1999). Imbalances can take the form of large accumulated surpluses for N and P in intensive livestock (Domburg et al. 2000) and peri-urban (Khai et al. 2007) systems

compared to deficits associated with less intensive and some organically managed agricultural systems (Watson et al. 2002). Links between the build-up of nutrient surpluses in terrestrial systems and an environmental impact, such as nutrient enrichment and eutrophication of aquatic ecosystems, are commonly assumed. Individual nutrient imbalances can give rise to a general inability to fully utilize other soil nutrients efficiently giving rise to an increased risk of loss and environmental impact.

Increasing costs together with a greater general awareness of the energy and resource issues associated with the manufacture and use of fertilizer is shifting the emphasis towards a more sustainable use of nutrients (Kumar and Singh 2001). This has been given particular international emphasis as a result of the recent oil price increase. Placing a greater reliance on the recycling of organic waste is not straightforward. The variability in composition and uncertainty in nutrient bioavailability of recycled materials are important aspects making it difficult to balance inputs with removals for individual elements. The synchronization of nutrient supply and crop demand can also be more of a challenge when using nutrients sources with low solubility. The extent to which imbalances develop varies as a consequence of site-specific management and farm-related factors that include the type of material available for recycling together with farm enterprise and soil type. For example, systems that utilize manure for meeting crop N demand have the tendency to develop a P surplus (Nelson and Janke 2007) while legumes and associated biological fixation contribute only N.

The narrower range of acceptable materials, and therefore general flexibility to manage nutrient availability, means these types of issues are particularly relevant for organic systems. Recent evidence of a declining trend suggests that maintaining an adequate soil P status is especially difficult. For example, soil sampled from five Norwegian organic dairy farms on two occasions (minimum 6 years apart) has demonstrated a general decline in P status (Løes and Øgaard 2001). While in this example most soils still retained an adequate agronomic P status, a negative P balance suggests that an external source of P will be required sometime in the future. Similar concerns over low soil P status have been raised for organically managed Ohio dairy and arable farms (Martin et al. 2007) and negative P balances for Swiss organic farms (Oehl et al. 2002). Increasing the practical options for improving the balance and availability of soil P is considered a priority within an organic management context while also having a general relevance to most agricultural systems (Stockdale et al. 2006).

The general acceptability of phosphate rock (PR) for organic agriculture makes it an obvious choice for common use. However, a major disadvantage associated with direct use of phosphate rock is the limited range of situations where the combination of prevailing cropping systems and soil properties offer conditions that allow dissolution rates to match short-term crop P demand. It is evident that a need exists to be able to better manage phosphate rock dissolution and subsequent availability of P. An increasing range of management options that offer the potential to enhance the within field solubilization of phosphate rock avoiding the need for energy-intensive industrial processes involved in the production of soluble

phosphate fertilizers are being explored. Here we review processes of solubilization relevant to organic agriculture giving particular emphasis to the co-composting of phosphate rock with various organic materials. Where possible some of the underlying mechanisms responsible are explored and areas requiring further investigation highlighted.

4.2 Direct Application of Phosphate Rock

Potential deficits of P in organically managed systems can be offset through the use of materials acceptable within the organic standards (EEC 1976; 2007) which include a range of recycled composted vegetable matter and manures together with phosphate rock. Recommended application rates for phosphate rock are generally poorly defined. Typically large phosphate rock applications are used, often equivalent to three or more times expected annual crop removal (Scholefield et al. 1999) potentially increasing the environment risk of P loss occurring during surface soil erosion events. Poor water solubility of most phosphate rock also represents a major agronomic disadvantage in the short-term for many crops grown either on soils with low P status or for more P-demanding crops, such as potatoes. Comparisons of the agronomic effectiveness between direct applications of phosphate rock and triple super phosphate (TSP) have been more favorable where soil conditions favor phosphate rock dissolution, such as, temperate grasslands. A long-term comparison of large single applications of phosphate rock and TSP resulted in significantly greater herbage dry matter yields with the former; although when smaller annual amounts were compared TSP was superior to phosphate rock (Scholefield et al. 1999).

Compiling a database of agronomic effectiveness for individual phosphate rock sources has advantages (Szilas et al. 2007) which (i) make data accessible, (ii) permit a combined interpretation and allow drawing up conclusions with a wide scope and relevance, (iii) form a basis for assessing the suitability or otherwise of phosphate rock in different agroecological zones, and (iv) the database represents a valuable tool in the determination of research needs regarding utilization and the development of recommendation systems and would be useful to do within the present context.

The direct application of phosphate rock is generally successfully used where (i) local sources represent an economically viable option, a situation often found in developing countries (Nishanth and Biswas 2008), (ii) properties of soil-cropping systems offer conditions favorable for dissolution of phosphate rock. Poor mineral solubility is a common property associated with many types of phosphate rock and particularly in soils with a pH greater than 5.5–6 (Khasawneh and Doll 1978). The three most important soil-related factors that influence the rate of dissolution of phosphate rock in soil are pH, P status, and Ca status (Robinson and Syers 1991). Sources of phosphate rock differ widely in mineralogy and composition that influences their dissolution patterns. For example, while the total P contents of various

Table 4.1 A comparison of the total phosphorus (percent P) contents of various phosphate rock sources (source of data include Schnug et al. 2006 and FAO 2004). The proportion (%) of total P that is citric acid soluble is also shown

Fertilizer	Total P (%)	Proportion (%) of total P extractable using citric acid	Reference
Tilemsi ^a (Africa)	12.2	29.7	Truong et al. 1978
Hahotoe ^a (Africa)	15.5	19.1	Truong et al. 1978
Gafsa ^a (Africa)	13.2	37.8	Truong et al. 1978
North Carolina (USA) ^a	13.0	15.8	FAO 2004
Central Florida (USA) ^a	14.2	8.5	FAO 2004
Araxa (Brazil) ^b	16.2	3.5	FAO 2004

^a Sedimentary

^b Igneous

phosphate rocks might be similar (Table 4.1) the proportion which is citric acid soluble varies widely (<5% to more than >35% of the total P). Various methods have been used to compare the relative effectiveness of individual phosphate rocks (Chien et al. 1990). One popular approach has been to group according to the degree to which the phosphate component of apatite has been substituted by carbonate. Kpombekou and Tabatabai (2003) compared dissolution properties of 12 phosphate rocks that had been grouped into low (Hahotoe, Kodjari, Parc W, Tahoua), medium (Central Florida, North Florida, Khourigba, Tilemsi Valley) and high (Gafsa, Minjingu, N. Carolina, Sechura) reactivity. The degree of substitution by carbonate has important implications for certain mechanisms described in later sections, which directly influence the rate of dissolution.

4.3 Improving P Utilization from Phosphate Rock

Opportunities exist to improve utilization efficiency of phosphate rock through some combination of optimizing the conditions to increase phosphate rock dissolution rates, reduce the capacity of soil to fix/immobilize P or select crop traits that increase uptake/utilization efficiency of P. Here the emphasis is placed upon the first two aspects (dissolution rate and reduced fixation/immobilization) and an operational definition which enables those improvements to solubility that take place during either pre- or post-field application stages to be made (Fig. 4.1).

One of the most common and widespread pre-treatments is a simple physical grinding to reduce the particle size (Kanabo and Gilkes 1988; Watkinson 1994) and increase surface area of rock phosphate, which can improve relative effectiveness by up to three times (Lim et al. 2003). Van Straaten (2002) and Kpombekou and Tabatabai (2003) listed several alternatives that have been used to increase P availability of phosphate rocks: (i) incorporation with various additives

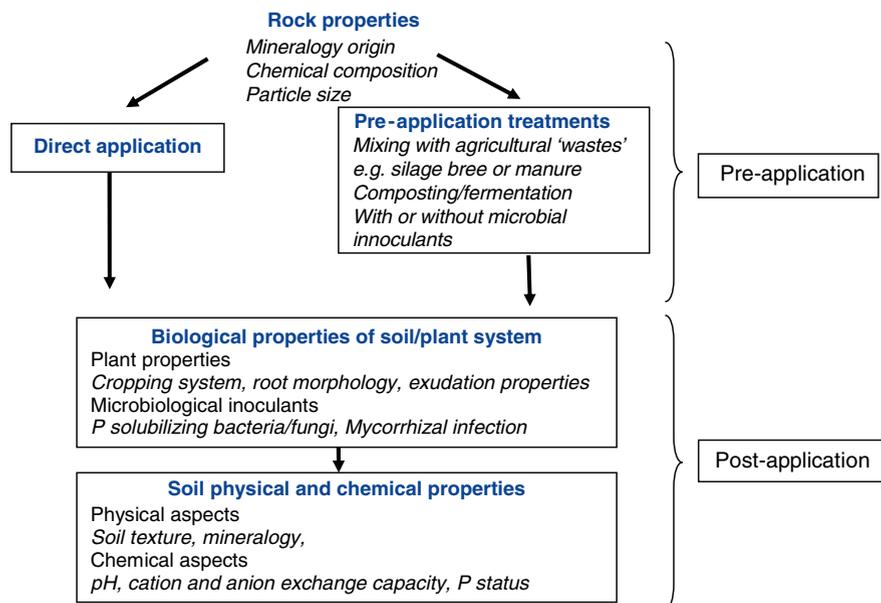


Fig. 4.1 Schematic plan of the factors significantly influencing the dissolution, reactivity, and uptake of P derived from phosphate rock (PR). A major distinction is made between those factors which are likely to apply to either pre- or post-application situations

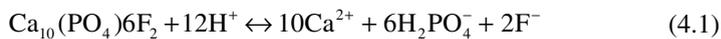
(e.g., Evans et al. 2006), (ii) partial acidulation of phosphate rock (e.g., Chien and Menon 1995), (iii) compaction of rock phosphate with water-soluble P fertilizers (e.g., Kpomblekou and Tabatabai 1994) and, (iv) microbial methods.

The biologically mediated options for enhancing the agronomic effectiveness of phosphate rock have been recently summarized (FAO 2004) as (i) composting organic wastes with phosphate rock (phospho-composts); (ii) inoculation of seeds or seedlings with phosphorus-solubilizing microorganisms (fungi, bacteria, and actinobacteria); and (iii) the inclusion in the cropping system of crop genotypes that exhibit favorable root attributes (in terms of exudate production and soil exploration (Gahoonia and Nielsen 2004)), recently reviewed in White and Hammond (2008).

The current focus upon exploring opportunities for improving the solubility of phosphate rock within organically managed systems means options (i) and (iv) listed above (often in combination) appear particularly relevant. Improved dissolution rates have been achieved by manipulating conditions during pre-application treatments, such as co-composting, which utilizes readily available organic materials together with specific microbial inoculants. Typically these biologically mediated decomposition processes provide the necessary conditions that enhance dissolution rates. A comparatively simple example described by Stamford et al. (2007) involved the incubation of phosphate rock with elemental sulfur. Mixing phosphate rock and sulfur inoculated with *Acidithiobacillus* produced biofertilizers in field furrows. The requirement for *Acidithiobacillus* to be added in combination

with sulfur to produce the necessary acidity resulted in six times the quantity of P solubilized than phosphate rock alone or phosphate rock plus sulfur (Stamford et al. 2007). There are also reports of the direct feeding of phosphate rock to livestock, although no advantage in terms of solubilizing P was apparent from supplementing feed for steers with phosphate rock (Odongo et al. 2007).

The enrichment of organic waste products with minerals is of general interest in the development of sustainable farming. The co-application with on-farm organic materials such as farmyard manure (FYM) and crop residues has frequently been employed in developing countries and recently reviewed by Aery et al. (2006). The incubation of phosphate rock with various types of organic materials and their decomposition products offers potential for “low technology” widely adoptable solutions. There is also an added advantage of being able to incorporate minerals in addition to phosphate rock, such as mica to specifically increase the K content of composts (Nishanth and Biswas 2008). Dissolution rates and release patterns of P and K between the two mineral components differed. The range of processes that contribute to the modified conditions that favor the solubilization of phosphate rock are essentially similar within both pre- and post-application stages. Dissolution rates of most sedimentary phosphate rock can be improved through the combined act of increasing the supply of protons (H^+) and the continuous removal of the reaction products of dissolution (e.g., Ca and P, Equation 4.1) from the dissolution zone (Khasawneh and Doll 1978).



Raising soil cation exchange capacity will increase the ability to remove Ca and can be achieved through application of organic amendments (Nying and Robinson 2006) and adopting management practices that favor the build-up of soil organic matter. The dissolution of calcareous material in the phosphate rock appears to follow two stages, an initial fast rate followed by a second slower stage (Sengul et al. 2006). An increase in the availability of soil P has also been attributed to the addition of organic matter with possible mechanisms include (i) competition for P adsorption sites; (ii) dissolution of adsorbents; and (iii) changes in the surface charge on adsorbents (Iyamuremye et al. 1996). The addition of specific organic acids were demonstrated to decrease soil P adsorption in the order tricarboxylic acid > dicarboxylic acid > monocarboxylic acid (Bolan et al. 1994). Although short-lived in soils, their continual production makes the presence of these acids important (Jones 1998).

Chemically induced changes in the rhizosphere that maximize P uptake through influencing bioavailability of soil inorganic P have been reviewed by Hinsinger (2001) and vary considerably with (i) plant species, (ii) plant nutritional status, and (iii) ambient soil conditions. Kpombrekou and Tabatabai (2003) suggested that “results from direct additions of phosphate rock to soil have been controversial”... ‘while only a limited amount of literature exists on chemical ways to increase P availability of phosphate rocks; on the other hand, biological means to increase available P of phosphate rocks are even more limited.’

The solubilization of phosphatic compounds by naturally abundant phosphorus solubilizing microbes (PSM) appears to be a common attribute under *in vitro* conditions; the performance of PSM *in situ* has been contradictory. The underlying principle of the microbially mediated processing of natural phosphates is the production of organic acids that attack and dissolve the phosphates, converting the P to a bioavailable form. Organic acids (including succinic, citric, and formic) have been used for the industrial beneficiation (refinement) of phosphate rock through the selective removal of accessory minerals such as carbonates (Ashraf et al. 2005). Ivanova et al. (2006) reported the optimization of the industrial process reacting Tunisian phosphorite solubilization with citric, oxalic, and gluconic acids in relation to the following main factors; the acid concentration, reaction time, ratio of solid/liquid phases, and natural phosphate fraction. The variability in the performance is restricting the large-scale direct application of PSM in sustainable agriculture and has been reviewed under a wide range of agro-ecological conditions by Khan et al. (2007). Potential technical solutions include those where conditions are optimized through biotechnological advances, such as selective screening for P solubilizing activity (e.g., Harris et al. 2006), and molecular techniques including genetic modification (Rodriguez et al. 2006). Commercially available products include Jumpstart™ that contains *Penicillium bilaiane*, in which excretion of H⁺ and production of organic acid anions reduce Ca²⁺ activity in solution through complexation. Importantly this treatment can contribute to a short-term solution for P deficiency; it does lead to depletion of the soil P reserve and therefore does not replace the need for some external source of P (Takeda and Knight 2006).

4.3.1 Composting

The objective of most pre-application incubations is to provide conditions that favor the production of acidity and/or chelators of cations (Ca, Al or Fe) (Banik and Dey 1982). Quantifying the individual significance of either mechanism is difficult although some partial insight was gained by Reyes et al. (2001) using a UV-induced mutant of *Penicillium rugulosum*, which had a greatly reduced capability to solubilize phosphate rock as it lacked the capability of secreting organic acids. There is a combined role for organic acids and acidity that are produced during the incubation; the actual significance is highly sensitive to the composition of phosphate rock used. Using closed laboratory incubation systems Kpombekou and Tabatabai (2003) compared a range of organic acids, mono-carboxylic acids (glycolic, pyruvic and salicylic), di-carboxylic acids (oxalic, malonic, fumaric, and tartaric), and tri-carboxylic acids (*cis*-aconitic and citric) to solubilize P from 12 phosphate rocks. Generally the oxalic was most effective, but interestingly this was not the situation for high reactive phosphate rock. Average amounts of P released by all organic acids were 65.5, 55.1, and 11.1 mmol kg⁻¹ for low, medium, and high reactivity phosphate rocks respectively. There was a negative correlation with equilibrium pH and a positive one with Ca released. The following trend, from strongest

to weakest: citrate>oxalate >tartarate>malate>HCl has been suggested by Johnston (1959) and Johnston and Miller (1959). Struthers and Seiling (1960) found citric, oxalic, butyric, malonic, and lactic acid to be effective in increasing P availability. Importantly, many of these laboratory-based incubation systems because they are physically isolated, differ from what might be expected under more open and dynamic field conditions. The dynamic situation where mixed organic acids are continuously produced and utilized resulting in highly variable concentrations is difficult to mimic in the laboratory. It has been suggested that currently unidentified P-solubilizing compound(s) (molecular weight > 500 Da) may be responsible for the partial P solubilization (Chuang et al. 2007).

Singh and Amberger (1998) reported the presence of glycolic, oxaloacetic, succinic, fumaric, malic, tartaric, and citric acids in a water extract of a wheat-straw based compost. These authors made the important observation that initial (up to 30 days) organic acid concentrations were very high and resulted in greater rates of phosphate rock solubilization; this was followed by a rapid decline, reaching negligible amounts after 120 days of composting. The importance of a balanced general nutrient availability was also demonstrated; addition of N increased the production of all the listed organic acids and therefore the overall effectiveness of dissolution. It is clear therefore that the production of reactive organic acids can be high, but their general persistence is largely dependent on the type and properties of organic composted material together with its anaerobic decomposition state (Estaun et al. 1985; Gotoh and Onikura 1971). In mature compost many of these organic acids are likely to be present only in trace amounts. Several of these acids are also phyto-toxic and immature compost may be detrimental to germinating seeds, seedlings, and young plants (De Vleeschauwer et al. 1981). Sundberg and Jönsson (2005) studied the composting process and conditions under which production of organic acids, mainly lactic and acetic acid, are frequently produced during initial microbial degradation of food waste, in a process that reduces the pH to 4–5. This acid-producing process has been observed during storage and collection of waste (Eklind et al. 1997) and during the initial phase of batch composting (Day et al. 1998). During successful composting, the acids are decomposed and pH increases (Day et al. 1998). Bangar et al. (1985) reported the capability of Na-humate to solubilize Mussoorie, a sedimentary phosphate rock, and their significance as chelating agents during composting. Similarly, Satisha and Devarajan (2005) demonstrated the significant role of humic and fulvic acids for chelating Ca and retaining P during composting of a sugarcane residue with Mussoorie phosphate rock.

Some of the reason for the conflicting findings may be explained by differences in phosphate rock properties. For example, Minjingu phosphate rock (Ikerra et al. 2006) or Busumbu phosphate rock (Savini et al. 2006) mixed with a similar Tithonia-based green manure, showed different results. While the former phosphate rock showed a positive dissolution effect of the combination no enhanced effectiveness was observed in the latter case. Poor dissolution rates and limited subsequent plant P uptake from Busumbu phosphate rock may have been related to its high Fe content. Some evidence of a selective action of organic acids on individual

phosphate rock types exists and was well demonstrated by Reyes et al. (2001) who suggested a difference in action between citric and gluconic acids released by mutant strains of *P. rugulosum* with individual fungi showing a phosphate rock type preference for growth. Similarly, Chuang et al. (2007) demonstrated differences in organic acid effectiveness between various types of phosphate rock: gluconic acid was predominantly produced in the presence of Ca–P, whereas oxalic acid predominated with Fe–P and Al–P associated phosphate rock. General differences in the complexing capabilities of organic acids was reported by Hue et al. (1986) who found that the Al³⁺ detoxifying capacities of organic acids (and by inference Al³⁺ chelating ability) were correlated with the relative positions of hydroxyl and carboxylic groups on their main carbon chain. Many effective chelators of Al³⁺ had hydroxyl groups adjacent to carboxylic groups (i.e., α -hydroxy acid structures), positions that favored the formation of stable 5-bond ring structures with Al³⁺. Gluconic acid has a hydroxyl acid structure and is able to chelate Al³⁺ and to a lesser extent, Ca²⁺ and Fe³⁺.

4.4 Composting Conditions

The type of organic acids produced during the composting process represents a potentially important attribute that can be used to enhance phosphate rock solubility. There is scope to modify the composting process through some combination of altering the composting/fermentation conditions and/or addition of specific microbial inocula. In reality, manipulating conditions in a controlled and reproducible way in order to regulate decomposition reactions can prove difficult. The chemical composition or quality of plant residues, as an important regulator of the decomposition system, controls the production of P-solubilizing compounds (Oladeji et al. 2006). The inocula that have been used vary widely (see Table 4.2) but the general mechanism involved appears to be related to organic acid production. Many isolates are selected from soil and therefore may not be adapted to composting conditions. Five strains that were isolated from various composted materials (including farm waste and rice straw), *Enterobacter cloacae* EB 27, *Serratia marcescens* EB 67, *Serratia* sp. EB 75, *Pseudomonas* sp. CDB 35, and *Pseudomonas* sp. BWB 21, showed gluconic acid production and solubilized phosphate rock when added to a broth (Hameeda et al. 2006). The mechanism seemed to include a reduction in pH and a direct correlation between production of gluconic acid and phosphate rock dissolution. Zayed and Abdel-Motaal (2005) demonstrated the benefits of using a phosphate-dissolving fungal strain (*A. niger*) in addition to a cellulose-degrading one (*Trichoderma viride*) added in combination to a mixture of sugarcane residue (one of the largest agro-industrial byproducts in Egypt) and farmyard manure (FYM) that not only improved the fermentation process but also compost quality and the solubilization of phosphate rock measured subsequently using a pot experiment. Acidic conditions (pH 4–5) at the end of the experiment were obtained in all piles receiving *A. niger* and there was a correlation between the amounts of soluble

Table 4.2 A summary of the literature and experimental conditions employed to test the potential for improving availability of phosphate rock (PR) prior to any field application

Reference	Amendments	Country	Exp. details	Phosphate rock type/source	Particle size	Test crop	Inocula
	Livestock wastes						
Alloush 2003	Cattle manure	USA	Linc, p	NC and Syrian		Switch grass	Penicillium spp
Zayed and Abdel-Motaal 2005	Cattle manure plus SB	Egypt	Lp	100 g kg ⁻¹ , w/w		Broad beans	<i>Aspergillus niger</i> or <i>Tricho-derma viride</i>
Agyin-Birikorang et al. 2007	Poultry manure	West Africa	p	Togo PR		Maize	
Mahimairaja et al. 1995	Composted with poultry manure		Linc				
Bangar et al. 1985	Farm wastes, cattle dung, soil, and well decomposed compost	India	Linc	Mussourie RP (8.1 %P)	Included CaCO ₃		
	Crop Residues						
Zayed and Abdel-Motaal 2005	Rice straw and compost, fermentation for 106 days, turned every 15 days	Egypt	Lp,	ni added at 100 g kg ⁻¹ (dw)		Cowpea	<i>A. niger</i> and <i>T. viride</i>
Caravaca et al. 2004	SB	Spain	F	Morocco (12.8% P)	<1 mm	Sorghum	<i>A. niger</i> and <i>Glomus</i> sps to field
Rodriguez et al. 1999	SB/Fermentation (10–30 days)	Spain	Lp			Alfalfa	<i>A. niger</i> NB2
Medina et al. 2006	SB/Fermentation (20 days)	Spain		Morocco (12.8% P)	<1 mm	<i>T. repens</i>	<i>Glomus</i>

(continued)

Table 4.2 (continued)

Reference	Amendments	Country	Exp. details	Phosphate rock type/source	Particle size	Test crop	Inocula
Vassilev et al., 2006a	Four Agri wastes/ Fermentation	Spain	Lp	Morocco (12.8% P)	<1 mm		<i>A. niger</i>
Vassilev et al. 1996	SB	Spain	Lp			<i>T. repens</i>	<i>A. niger</i>
Caravaca et al. 2005	SB	Spain	F	Morocco (12.8% P)	<1 mm	Shrubs	<i>A. niger</i>
Vassilev et al. 2006b	SB/Fermentation	Spain					<i>A. niger</i>
Medina et al. 2007	SB/Fermentation	Spain		Morocco (12.8% P)	<1 mm	<i>T. repens</i>	<i>A. niger</i> plus AM
Biswas and Narayanasamy 2006	Rice straw + urea (0.25 kg N) and fresh cow dung (5 kg) per 100 kg straw	India		Four different PR types 7–10% P		Mungbean	<i>A. awamori</i> .
Singh and Amberger 1998	Composting N, mollases, PR			Sedimentary PR (high CaCO ₃)			
Kumar and Singh 2001	Vermicompost	India		Mussourie			<i>Pseudomonas striata</i>
Banik and Dey 1982	FYM + P, rice + PR	India	Linc	Ca ₃ PO ₄			<i>A. candidus</i> , <i>B. firmus</i> B-7651

Laboratory – L; Laboratory incubation – Linc; pot experiment – p; field experiment – F; ni – no information; SB – sugar beet; NC – North Carolina; dw – dry weight

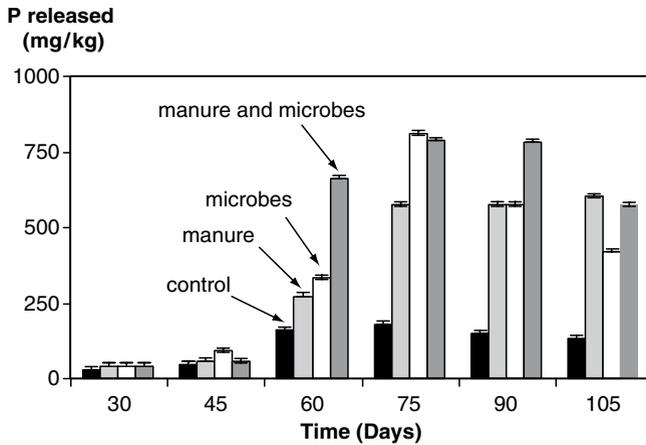


Fig. 4.2 Phosphorus (water extractable) released from sugarcane residue (bagasse) mixed with and without farmyard manure (FYM) that had been enriched with phosphate rock and composted for 105 days (data from Zayed and Abdel-Motaal 2005). After 30 days samples were collected and analyzed after 15-day intervals. Standard deviations are shown and the treatments are: Without any treatment (*black filling*), Treated with FYM (*light gray*), Treated with *A. niger* and *T. viride* (*open*), Treated with *A. niger*, *T. viride*, and FYM (*dark gray*)

P and the reduction in pH values in the compost piles. There may be various practical and cost implications associated with using inocula.

The complex series of reactions that can be involved during co-compositing together with some of the potential difficulties that can arise from interpolation of research findings are highlighted in Fig. 4.2. A phosphate rock/sugar residue mixture was incubated in the presence or absence of FYM and a mixed inocula (Zayed and Abdel-Motaal 2005). The results appear to demonstrate a straightforward response to additions of FYM and/or the inocula although it is extremely difficult to actually quantify the relative contribution of “solubilized” P that derives from either the FYM or the phosphate rock. The complexity of mechanisms that operate during these incubations is also clearly demonstrated by the commonly reported feature that a peak in soluble P occurs, which in this example occurred around day 75. The following decline in water-soluble P suggests secondary reactions perhaps involving a change in chemical form to organic or polyphosphates.

4.5 Forms of P Present in Compost

There has been little direct study on the composition of P that had been solubilized during composting. This is despite the potential importance that chemical form might have upon subsequent bioavailability and reactivity within

soil. It could be postulated that a wide range of P forms might be produced, from simple orthophosphate ions to polyphosphates and a wide range of organic P-containing compounds. Some information could be gained from the few detailed studies made on manure (e.g., Leinweber et al. 1997). Reddy (2007) who applied low-grade phosphate rock to the litter of soybean showed that approximately 71–92% of the total solubilized P was converted to organic P. While changes in C and N forms have been reported, evidence for changing forms of P during the compost period is more circumstantial. For example, Bangar et al. (1985) described some changes in P during decomposition. An increase in Total P content over time was proportional to the loss in organic matter during decomposition. Water soluble P significantly increased when composting was done without phosphate rock, but decreased during composting with phosphate rock. The P soluble in citric acid increased significantly during initial composting with phosphate rock but after 60 days citric acid soluble P decreased. Various possible reasons might explain this observation which include some form of precipitation/sorption reaction of soluble P with phosphate rock components (e.g., Singh et al. 1980) while Mishra et al. (1984) observed that initially there was an increase in soluble P, which later converted to di- and tricalcium phosphates that were citric acid soluble when *Aspergillus awamori* was grown in a medium with phosphate rock as the only source of P. In vitro studies with *A. awamori* also revealed that after a certain period of incubation citric acid soluble P also decreased and was converted into a citric acid insoluble apatite form (Biswas and Narayanasamy 2006). Goenadi and Siswanto (2000) also reported an increase in citric acid soluble, but not water soluble P during an incubation of Moroccan phosphate rock with *A. niger*.

4.6 Evidence of Utilization

Microbially mediated solubilization of insoluble phosphates through release of organic acids is often combined with the production of other metabolites, which take part in biological control against soil-borne phytopathogens. The increase in plant growth may therefore be due to the release of certain plant growth promoting substances (Kucey et al. 1989). In vitro studies show the potential of P-solubilizing microorganisms for the simultaneous synthesis and release of pathogen-suppressing metabolites; mainly siderophores, phytohormones, and lytic enzymes (Vassilev et al. 2006a). Studies including dual inoculation with arbuscular mycorrhizal (AM) fungi and other P-solubilizing microorganisms (Vassilev et al. 2006b) can be expected as the combinations of two such partners with complementary mechanisms might increase overall biocontrol and plant-growth-promoting efficacy, thus providing an environmentally safe alternative to chemicals. The simultaneous application of Rhizobium and PSM (Perveen et al. 2002) and PSM and AM fungi (Zaidi et al. 2003) has been shown to stimulate plant growth more than inoculation of each microorganism alone especially under P-deficient soil conditions.

4.7 Future Research Emphasis

The co-composting of phosphate rock with various organic materials offers a cheap, low technology and therefore widely applicable method of improving P solubility and bioavailability of P over both short- and long-term timescales. Published work on the agronomic effectiveness of phospho-composts are scarce (FAO 2004) and further research is recommended (e.g., Davis and Abbott 2006). Co-composting with phosphate rock could be developed for use at the individual farm scale, but for this to become a widely adopted practice some clear protocol is required. Understanding the range of mechanisms involved and optimizing the composting conditions to maximize processes that result in solubilizing phosphate rock is necessary. Despite an increasing number of studies that describe the enhanced solubilization of phosphate rock actual mechanisms involved have not been fully explained. Interpretation and extrapolation of research findings are being hampered by a general lack of background information coupled with a lack of any standardized experimental methodology. On some occasions even the source of phosphate rock actually used in the experiments is not reported. The change in P solubility that occurs with time during co-composting operations has occasionally been monitored, but this has really only highlighted the complexity of the reactions actually involved. Initial increases in concentrations of soluble P appear to be followed by a decline. This suggests that a series of secondary reactions may be involved in modifying the chemical form of solubilized P as composting proceeds. The effects of any subsequent storage stages or consequences for short- or long-term bioavailability after field application are poorly quantified.

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Chapter 5

Mixed Cropping and Suppression of Soilborne Diseases

Gerbert A. Hiddink, Aad J. Termorshuizen, and Ariena H.C. van Bruggen

Abstract Soilborne pathogens are difficult to manage, especially since the use of methyl bromide has been phased out in most countries. Resistance against many soilborne pathogens is hardly available and fungicides are effective only to a limited extent. In organic agriculture, many problems related to soilborne pathogens are avoided by applying wide rotations, but still some polyphagous soilborne pathogens can be highly problematic, especially since most chemical crop protectants are not allowed. In addition, wide rotations are often economically unprofitable. Therefore, alternative practices to manage soilborne pathogens are needed. In this review, the occurrence of soilborne pathogens in three types of cropping systems are evaluated: (i) continuous cultivation of single crops in monoculture, (ii) crop rotation, and (iii) mixed cropping, i.e., cultivation of multiple crops in the same field at the same time. Both continuous cropping and crop rotation have been investigated extensively. Therefore, in this chapter we focus on mixed-cropping systems in relation to soilborne pathogens, their potential to suppress soilborne diseases, and the mechanisms underlying disease suppression. In general, mixed cropping is practiced to optimize nutrient uptake, control soil erosion, suppress the epidemic spread of airborne pathogens, and improve crop yields per unit of area. While mixed cropping has received attention for its effects on airborne pests and pathogens, the effects on soilborne pathogens are poorly known. In 30 out of 36 publications, mixed cropping showed a significant reduction in soilborne disease and in six, no or a positive effect on disease incidence or severity was found. Diseases caused by splash-dispersed pathogens were less severe in mixed-cropping systems in ten out of 15 studies. The magnitude

G.A. Hiddink (✉)

Enza Zaden, Seed Operations BV, Haling 1e, 1602 DB Enkhuizen, The Netherlands
e-mail: g.hiddink@enzazaden.nl

A.J. Termorshuizen

Blgg, Nieuwe Kanaal 7-f, 6709 PA Wageningen, The Netherlands

A.H.C. van Bruggen

Plant Pathology Department, IFAS, University of Florida, 1453 Fifield Hall,
Gainesville, FL, USA

of disease reduction in mixed compared to single crops varied, from a 63% reduction to a 100% increase in disease. Host dilution appeared to be the most important mechanism of disease suppression for both soilborne and splash-dispersed pathogens (12 and five cases, respectively). Although the use of mixed cropping for soilborne disease suppression is still in its infancy, the wide range of biological effects and interactions observed holds promise for further optimization and management of soilborne diseases, for example, by selecting plant species and cultivars that provide an optimal combination of root architectures.

Keywords Mixed cropping • intercropping • soilborne pathogens • crop rotation • microclimate • monocropping • multiple cropping • disease management • allelopathy • ISR (induced systemic resistance) • SAR (systemic acquired resistance) • microbial antagonism

5.1 Introduction

During the past decades, intensified mechanization and the use of synthetic fertilizers and crop protectants have substantially increased agricultural yields. However, these practices also resulted in an array of adverse environmental side effects, including soil erosion, water pollution, eutrophication, and reduced innate soil fertility (Gliessman 2001). Acquisition of capital-intensive and crop-specific machinery further narrowed rotations. Although these negative side effects of intensive agriculture counteract the initial increase in food production per unit of area (Matson et al. 1997), ultimately they may lead to a decline in total food production because of land becoming unproductive due to soil erosion and pollution. On the other hand, increasing demands for agricultural products can be met only when high yields per unit of area are achieved, especially when productive land is falling short (Hill 2007). Therefore, it is necessary to find more sustainable ways of cultivating crops without sacrificing on the yield.

Narrow rotations of cash crops have resulted in a high incidence of soilborne diseases (Garrett and Cox 2006). Although genetic resistance and effective pesticides are insufficiently available, many soilborne pathogens, such as *Gaeumannomyces graminis* var. *graminis*, can be managed by wide crop rotations (Werker and Gilligan 1990) and other cultural measures (Cook 2001). However, wide crop rotations are, from an economic point of view, undesirable in areas where arable land is limited. Soil fumigants can be highly effective, especially for the control of nematodes, but they have a strong negative impact on non-target organisms and therefore their use is discouraged or prohibited (Martin 2003; Schneider et al. 2003). Methyl bromide, the most common soil fumigant for decades, was added to the list adopted by the Montreal protocol in 1997 and will be banned completely in 2015 (Gullino et al. 2003, Liu et al. 2007). Most soil fumigants are

costly and generally too expensive for low-value crops like cereals or for use by subsistence farmers in the developing countries. The application of methods specifically designed to control soilborne pathogens, such as biological soil disinfestation, soil solarization, and flooding, is also often too costly, so they are applicable only to capital-intensive crops (Blok et al. 2000).

While mixed cropping has received attention for its effects on airborne pests (Björkman et al. 2008; Bukovinszky et al. 2004; Risch et al. 1983) and pathogens (Mundt 2002a; Wolfe 1985), the effects on soilborne pathogens barely have attracted attention. In this review, we evaluate how cropping systems and in particular mixed cropping can affect soilborne pathogens. We first define the different types of cropping systems and specifically continuous single-crop cultivation (monoculture), crop rotation (i.e., change of crop diversity in time), and mixed cropping (i.e., any type of growing multiple crops in the same field at the same time). Then we will in short assess and discuss how these cropping systems can affect the dynamics of soilborne diseases. The effects of mixed cropping on soilborne and splash-dispersed fungal and bacterial pathogens will be discussed as well as the mechanisms underlying disease suppression by mixed cropping. We end this review with recommendations and options for the use of mixed cropping that may contribute to improving the sustainability of agricultural production.

5.2 Design of Cropping Systems to Manage Soilborne Diseases

In modern agriculture, cultivation of single crops in a rotation is the most common cropping system for a vast range of crop species worldwide. If properly designed, crop rotation is the most efficient (cultural) practice to reduce the incidence and severity of soilborne diseases (Cook and Veseth 1991). However, crop rotation is not always practiced. In highly mechanized productions, continuous cultivation of the same single crop is regularly practiced, whereas in areas where mechanization, artificial fertilizers, and crop protectants are too costly, diverse forms of mixed cropping are encountered regularly. Disease suppression related to crop rotation and continuous single-crop production has been extensively investigated (Mazzola 2002; Schneider 1982; Weller et al. 2002). However, the effects of mixed cropping on soilborne pathogens have received considerably less attention. Where in literature effects of mixed cropping on soilborne pathogens are reported, they often appear just as a co-observation in studies on crop productivity. The main reasons why the effects on soilborne pathogens have received little attention are the inconspicuous nature of soilborne diseases (Cook 2001), the aspecific disease symptoms, and the inherent difficulty of designing experiments with mixed-cropping systems. A typical example of a disease with aspecific symptoms is Potato Early Dying (Rowe et al. 1987), caused by *Verticillium dahliae*, which is often erroneously held for drought stress. Furthermore, disease can go unnoticed for some time as is the case for spinach wilt caused by *Verticillium dahliae*, which

induces symptoms only after bolting so that disease is not observed in fresh produce (duToit et al. 2005).

5.2.1 *Successive Cultivation of a Single Crop*

Continuous cultivation of the same single crop in the same field is practiced in areas where the number of crops that can be grown is agronomically and economically limited (Cook 2001). Under these conditions, mechanization makes cultivation more economically feasible but at the same time hinders the adoption of a more diversified crop rotation. In continuous crop cultivation, inoculum densities of soilborne pathogens increase without exception and a certain degree of damage has to be accepted (Shipton 1975). Some cultural measures including reduced tillage can enhance the survival of certain pathogens (Meynard et al. 2003; Pankhurst et al. 2002). Regular tillage can lead to burial of inoculum of *Pseudocercospora herpotrichoides* and limit disease progress in the following season (Colbach and Meynard 1995). On the other hand, reduced tillage and direct drilling resulted in suppression of *Gaeumannomyces graminis* var. *graminis* (Pankhurst et al. 2002) because of increased soil organic carbon concentrations and consequently higher microbial activity compared to conventional tillage. Also stimulation of microbial activity through organic amendments can reduce pathogen inoculum or activity (Hoitink and Boehm 1999).

For certain pathosystems, natural disease suppression is known to be induced during continuous cultivation (Schneider 1982; Weller et al. 2002), e.g., *Gaeumannomyces graminis* in wheat and barley (Gerlagh 1968; Raaijmakers and Weller 1998; Weller et al. 2002), *Rhizoctonia solani* in sugar beet (Hyakumachi and Ui in Sturz and Christie 2003), *Streptomyces scabies* in potato (Menzies 1959), and *Fusarium oxysporum* f. sp. *melonis* in melon (Alabouvette 1999). Induction of disease suppression can take multiple years and generally it is lost after growing other crops (Shipton 1975). The mechanisms involved have been studied extensively and are linked to the microbial community in soil or the rhizosphere. The best-known mechanisms include antibiotic production (e.g., by strains of *Pseudomonas fluorescens*), competition by closely related non-pathogenic strains (e.g., competition for carbon by nonpathogenic *Fusarium oxysporum*), and parasitism (e.g., by *Trichoderma* spp.) (Weller et al. 2002). For these types of disease suppression to develop and to sustain, both the pathogen and a susceptible host plant need to be present and a certain level of damage has to be accepted. Overall, adequate disease suppression in continuous monocropping systems can be induced in several pathogen–crop combinations. However, other pathogens on the same crop can become problematic. Moreover, the unpredictable time span needed for induction of specific disease suppression and the inflexibility of the cropping system, result in limited applicability of this system for soilborne disease management.

5.2.2 Crop Rotation

Crop rotation is the practice of growing crops on the same field sequentially in time. Crop rotation is commonly practiced to avoid the buildup of soilborne pathogens (Cook and Veseth 1991), to maintain a balanced soil fertility, and to avoid intensive soil tillage before planting root crops (Termorshuizen 2001). The beneficial effect of crop rotation against many soilborne pathogens is due to their limited host range (Krupinsky et al. 2002). The host-dependent reproduction of most pathogens (Garrett and Cox 2006) limits inoculum buildup and viability of the inoculum present diminishes in time when nonhosts are grown (Cook 2001). Alternations of dicotyledonous with monocotyledonous crops are effective in limiting the inoculum levels of the majority of soilborne plant pathogens (Agrios 1997). Alternation with hosts that do not support inoculum production can be a measure to reduce the amount of pathogen inoculum. For example, sugar beet is a host to *Verticillium dahliae*, but hardly contributes to inoculum buildup, as microsclerotia have not yet been produced at the time when roots are harvested (A.J. Termorshuizen, personal observation).

Green manure or cover crops cultivated in wintertime can be part of the crop rotation. The main reason to grow a green manure crop is to protect soil from erosion and to prevent leaching of mineralized nitrogen. In narrow rotations with a high pressure of soilborne pathogens, the choice of the optimal green manure crop can be a challenge. For example, to reduce nitrate leaching in sandy soils in wintertime in the Netherlands, it is now obligatory to grow a green manure crop following maize cultivation. Due to the late harvest of maize, the choice of green manure crops is usually limited to a grass or winter cereal, which to a great extent resembles maize with respect to its host status for nematodes. The single option farmers have is to harvest their maize earlier, so that they can still sow mustard. Several green manures are known for their capacity to reduce diseases caused by soilborne pathogens. Incorporation of several *Brassica* species has been shown to reduce disease incidence caused by *Rhizoctonia solani*, *Phytophthora erythroseptica*, *Pythium ultimum*, *Sclerotinia sclerotiorum*, or *Fusarium sambucinum* in potato (Larkin and Griffin 2007). The underlying mechanism involves the production of toxic volatiles during decomposition of the cruciferous organic matter. Marigold (*Tagetes* spp.) is grown as a green manure to specifically suppress *Pratylenchus penetrans* (Kimpinski et al. 2000), which is likely due to toxic plant exudates.

The effective length of crop rotation as a method to manage specific soilborne pathogens depends on the survival of the pathogen. For example, the resting spores of *Spongospora subterranea*, the causal agent of powdery scab of potato, can survive for many years in the absence of a host (Jeger et al. 1996), while the survival of *Gaeumannomyces graminis* is limited to only a few years at most (Gerlagh 1968). Crop rotation is therefore not suitable to manage powdery scab, but it can be a valuable measure to manage take-all disease caused by *G. graminis* (Cook 2001). For various other soilborne pathogens, e.g., *Verticillium dahliae*, *Rhizoctonia*

solani, root knot nematodes (*Meloidogyne* spp.) and root lesion nematodes (*Pratylenchus* spp.), the design of a proper rotation can be difficult because these pathogens are capable of infecting and/or surviving on multiple hosts.

Crop rotation is a flexible disease management system that is capable of reducing disease losses caused by many soilborne pathogens. However, the need for rotating high-value crops with lower-value crops and the relatively high risk of losing a complete crop make this system often less attractive to farmers.

5.2.3 Mixed-Cropping Systems

Mixed cropping is defined as the cultivation of a mixture of two (or more) crops together in the same field (Trenbath 1976; Willey 1979). There are various types of mixed cropping (Geno and Geno 2001; Vandermeer 1990), each of which may affect soilborne pathogens differently (Table 5.1, Fig. 5.1). Mixed-cropping systems can be characterized according to the degree to which roots of different crop species interact, which is determined not only by the mixed-cropping system but also by the root architecture of each of the crops in the mixture (de Kroon 2007; Weaver 1926).

We define here mixed cropping *sensu stricto* as the practice of growing multiple crops simultaneously without a specific spatial structure. This way of cropping is used frequently in slash-and-burn fallow agriculture or ley farming with multilines or species mixtures (e.g., broadcast-sown grass-clover mixes). In a mixed setting, distances between hosts are generally greater than when grown as single crops and disease will spread more slowly (host dilution). Also allelopathy (Natarajan et al. 1985), microclimate change (Luthra and Vasudeva 1940), root camouflage (Gilbert et al. 1994), and microbial antagonism have been proposed as potential mechanisms underlying the disease suppression induced by mixed cropping (Abadie et al. 1998; Soleimani et al. 1996).

Strip mixed cropping is the “strip-wise simultaneous cultivation of multiple crops in rows, wide enough to permit independent cultivation but still sufficiently narrow to interact agronomically” (quoted from: Vandermeer 1990) (Fig. 5.2). Typically, the width of the strips is adapted to the size of the machinery to be used. Since the crops co-occur on a narrow strip, belowground interactions between the different crop species occur relatively infrequently and therefore the effects on soilborne pathogens are considered to be minor.

Relay mixed cropping is the simultaneous cultivation of multiple crops during only part of their field period. The second crop is planted at the time when the first crop reaches its reproductive stage but has not yet been harvested. When root systems of both crops overlap sufficiently, disease-suppressive effects due to allelopathy, microbial antagonism, or physical separation between pathogen and host may occur. Because of the time gap between sowing of both crops (strip), tillage between rows of the standing crop can affect pathogen establishment and spread by burial of inoculum (Colbach and Meynard 1995; Meynard et al. 2003).

Table 5.1 Mixed-cropping systems (Geno and Geno 2001; Vandermeer 1990) and theoretical disease-reducing mechanisms

Name	Possible disease-reducing mechanisms			Mechanization grade	
	Sowing layout	Diversity	Soilborne pathogens		
Strip cropping	Sown in more than one row of the same crop next to each other	Diversity between species	Airborne pathogens	Planting time	Fully mechanized
			Soilborne pathogens		
Relay cropping	Crops sown widespread or in rows	Diversity between species	Airborne pathogens	Planting time	Fully mechanized
			Soilborne pathogens		
Row mixed cropping	A row of one crop is at both sides accompanied by a row of the other	Diversity between species	Airborne pathogens	Planting time	No mechanization to fully mechanized
			Soilborne pathogens		
Mixed cropping	One crop sown in rows or widespread, the other widespread	Diversity between species	Airborne pathogens	Planting time	No mechanization to fully mechanized
			Soilborne pathogens		

(continued)

Table 5.1 (continued)

Name	Sowing layout	Diversity	Possible disease-reducing mechanisms		Planting time	Mechanization grade
			Airborne pathogens	Soilborne pathogens		
Multiline cropping	Completely random widespread or in rows	Diversity within species	- Reduced genetic susceptibility	- Reduced genetic susceptibility	Same planting time	Fully mechanized
Multistorey cropping	Crops grown widespread or in rows but having different dimensions (height, volume, and size)	Diversity between height levels	- Barrier effect/spore trapping - Microclimate (induction of disease) - ISR ^a	- Barrier effect (splash dispersal) - Microclimate (inoculum reduction and induction of disease) - Reduced chemotaxis - Allelopathy - Antagonists/competition	At the same time	No mechanization to fully mechanized
Natural ecosystems	Completely random, no predetermined layout	Diversity within and between species	- Absence of host - Reduced genetic susceptibility - ISR ^a - Barrier affect/spore trapping - Microclimate (induction of disease)	- Absence of host - Reduced genetic susceptibility - Barrier effect (splash dispersal) - Distance effect - Reduced chemotaxis - Allelopathy - ISR ^a - Microclimate (inoculum reduction and induction of disease) - Antagonists/competition	Can be any time	No mechanization

^a Induced systemic resistance

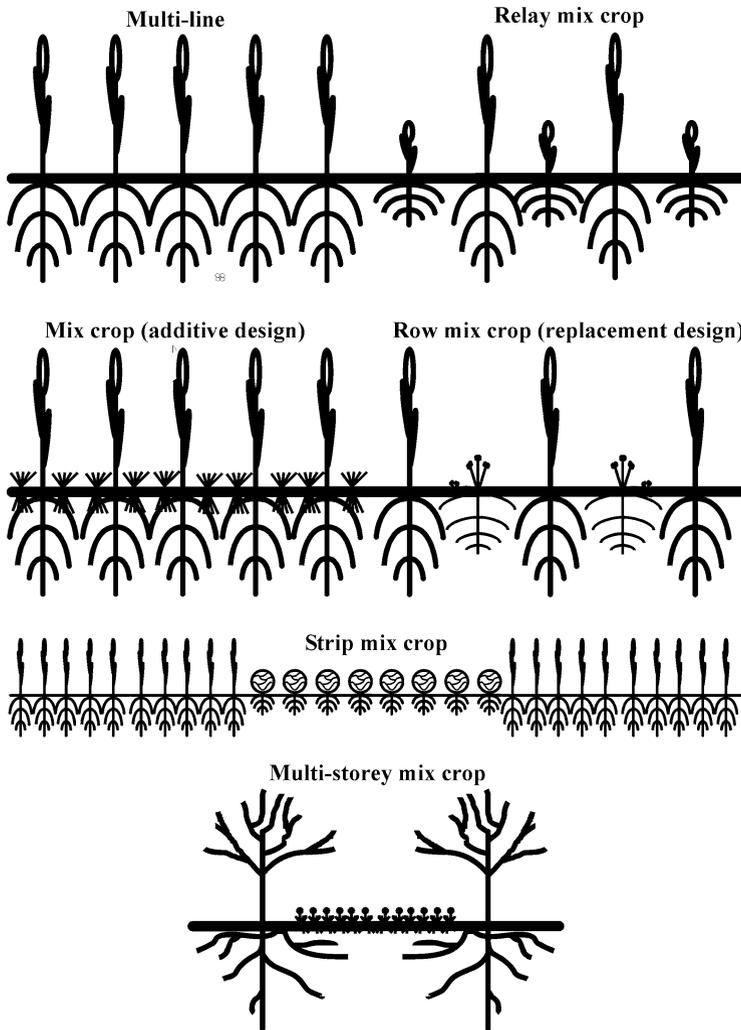


Fig. 5.1 Mixed-cropping systems

Row mixed cropping is defined as the production of multiple crops alternately planted in rows. It can be done in an additive design, where both crops are sown at their single densities (Fig. 5.3) or in a replacement design, where one crop is replaced by the other (Fig. 5.4). Irrespective of plant density, disease can spread within rows like in single-culture cropping systems, but between rows the alternate crop(s) can act as a barrier (Michel et al. 1997). Here, host dilution (replacement design), allelopathy, root camouflage, and microbial antagonism may play a role in disease suppression.



Fig. 5.2 Strip mix crop (Photo courtesy of Tim McCabe 1999, USDA-NRCS)



Fig. 5.3 Mixed crop, Brussels sprouts–barley, additive design (Photo: G.A. Hiddink)



Fig. 5.4 Mixed crop, triticale–clover, replacement design (Photo: G.A. Hiddink)

Multistorey mixed cropping (Fig. 5.5) is the cultivation of tall perennials combined with shorter biannual or annual crops and is practiced in orchards, tree nurseries, and agroforestry. The area between the rows is used to grow a cover crop to suppress weeds, fix nitrogen, reduce nutrient leaching, and increase the productive surface area. Allelopathy is a possible mechanism of disease suppression, but also roots can act as a physical barrier for pathogen spread, root camouflage, and microbial antagonism.

Natural vegetation consists mostly of multiple species and can be considered to be closely related to (zero-tillage) mixed cropping. The disease-suppressive mechanisms that operate in natural ecosystems are probably comparable to the mixed cropping or multistorey mixed-cropping system.

As may be clear from the definitions of the different types of mixed cropping, mixed cropping can have many appearances and characteristics. These characteristics often determine if soilborne diseases can be suppressed and what mechanisms for suppression can be held responsible for this disease suppression.



Fig. 5.5 Multistorey mix crop (Photo courtesy of: Gary Kramer 2001, USDA-NRCS)

5.3 Disease Reduction in Mixed-Cropping Systems

In 30 out of the 36 studies where the fate of soilborne pathogens was investigated in mixed-cropping systems, soilborne disease was significantly reduced in the mixtures. In the remaining six studies, there was no or a negative effect of mixed cropping on disease suppression (Table 5.2). In ten cases, a positive effect was reported for splash-dispersed pathogens against five with no or negative effects (Table 5.2). The most investigated crop appeared to be wheat, where in nine out of 15 cases (wheat as main crop) disease was reduced in the mixture. Clover was most important as secondary crop in six mixtures with a disease reduction in five of those mixtures. In the following sections, we will discuss the most important proposed disease-suppressive mechanisms and try to explain how they could be operational in mixed-cropping systems.

Table 5.2 Effects of mixed cropping on soilborne pathogens

Nr	Type	Pathogen	Pathogen type ^a	Main crop	Second crop	Effect in mixture	Effect magnitude relative to sole crop	Proposed mechanism	Reference
1	Mixed crop ^b	Pythium irregulare	sb	Alfalfa	Wimmera ryegrass	Reduced infection rate	13–44%	Host dilution	Burdon and Chilvers 1976
2	Mixed crop ^b	Rhizoctonia cerealis, Fusarium spp.	sb	Barley	Oats	Reduced disease incidence	<i>R. cerealis</i> : 6%; <i>Fusarium</i> spp.: 23%	Host dilution/ physical barrier	Vilich-Meller 1992
3	Mixed crop ^b	Fusarium spp.	sb	Barley	Wheat	Reduced disease incidence	50%	Physical barrier/ host dilution	Vilich-Meller 1992
4	Mixed crop ^c	Fusarium spp., Phoma spp., Cercospora spp., and black leafhopper	sb and insect	Oat	Berseem clover	Improved plant health	12%	No mechanisms mentioned	Holland and Brummer 1999
5	Mixed crop ^b	Rhizoctonia solani	sb	Radish	Mustard	Reduced disease progress	12% and 38% (fraction mustard in mix resp 25% and 50%)	Host dilution	Orten et al. 2005
6	Mixed crop ^b	Ralstonia solanacearum	sb	Tomato	Cowpea	Reduced wilt	16%	Physical barrier	Michel et al. 1997
7	Mixed crop ^b	Ralstonia solanacearum	sb	Tomato	Soybean	No significant reduction in wilt	–	Physical barrier	Michel et al. 1997
8	Mixed crop ^b	Ralstonia solanacearum	sb	Tomato	Welsh onion	No wilt reduction	–	No barrier present at transplanting	Michel et al. 1997

(continued)

Table 5.2 (continued)

Nr	Type	Pathogen	Pathogen type ^a	Main crop	Second crop	Effect in mixture	Effect magnitude relative to sole crop	Proposed mechanism	Reference
9	Mixed crop ^b	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	sb	Triticale	White clover	Reduced disease severity after 5 successive cycles	1–1.8 point ^d	Changed microbial community structure	
10	Mixed crop ^b	<i>Fusarium oxysporum</i> f. sp. <i>niveum</i>	sb	Watermelon	Rice	Reduced wilt	67%	Allelopathy of root exudates on <i>Fusarium</i> spores	Ren et al. 2007
11	Mixed crop ^c	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	sb	Wheat	Clover	No significant effect on yield		–	Zogg 1963
12	Mixed crop ^b	<i>Rhizoctonia cerealis</i>	sb	Wheat	Barley	Reduced disease incidence	5–30%, Depending on the previous crop	Host dilution	Vilich 1993
13	Mixed crop ^b	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	sb	Wheat	Barley	Reduced disease severity	10–35%, depending on the previous crop	Host dilution	Vilich 1993
14	Mixed crop ^c	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	sb	Wheat	Clover	Reduced disease rating in bioassay	42% (Avg of 2 years)	Reduced survival of the pathogen due to increased nitrogen uptake	Garrett and Mann 1948
15	Mixed crop ^c	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	sb	Wheat	Grasses	Reduced disease severity and incidence in bioassay	4–34%, Depending on grass species cultivated	Host root dilution or direct suppression effect	Gutteridge et al. 2006

16	Mixed crop ^c	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	sb	Wheat	Trefoil	Reduced root infection	25%	Increased densities of <i>Pseudomonas fluorescens</i>	Lennartsson 1988
17	Row mix crop	<i>Sclerotinia sclerotiorum</i>	sb	Bean	Maize	Increased disease incidence and severity	1.8 (mono) vs 2.0 (mix) ^d	- Not mentioned	Van Rheenen et al. 1981
18	Row mix crop	<i>Phoma exigua</i> var. <i>diversispora</i>	sb	Bean	Maize	Reduced disease incidence and severity	3.0 (mono) versus 2.6 (mix) ^d	- Not mentioned	Van Rheenen et al. 1981
19	Row mix crop	<i>Colletotrichum lindemuthianum</i>	sb	Bean	Maize	Reduced disease incidence and severity	1.0 (mono) vs 0.8 (mix) ^d	- Not mentioned	Van Rheenen et al. 1981
20	Row mix crop	<i>Fusarium oxysporum</i> f. sp. <i>laganariae</i>	sb	Bottle gourd	Chinese chive	Reduced disease incidence	73%	Stimulation of antagonists	Arie et al. 1987
21	Row mix crop	<i>Fusarium oxysporum</i> f. sp. <i>laganariae</i>	sb	Bottle gourd	Welsh onion	Reduced disease incidence	60%	Stimulation of antagonists	Arie et al. 1987
22	Row mix crop	<i>Erwinia carotovora</i> ssp. <i>carotovora</i>	sb	Chinese cabbage	Wheat	No effect	-	-	Toshio 1999
23	Row mix crop	<i>Fusarium oxysporum</i> f. sp. <i>ciceris</i>	sb	Chicken pea	Linseed	Reduced disease incidence	18 % disease incidence in mixture ^e	-	Agrawal et al. 2002
24	Row mix crop	<i>Macrophomina phaseoli</i> and <i>Rhizoctonia solani</i>	sb	Cotton	Sorghum and moth	Reduced mortality	65%	Decreased soil temperature	Luthra and Vasudeva 1940
25	Row mix crop	<i>Sclerotium cepivorum</i>	sb	Garlic	Ethiopian mustard	Reduced disease incidence	Present in mono-crops, absent in mixed crops	Release of glucosinolates (biofumigation)	Zewde et al. 2007

(continued)

Table 5.2 (continued)

Nr	Type	Pathogen	Pathogen type ^a	Main crop	Second crop	Effect in mixture	Effect magnitude relative to sole crop	Proposed mechanism	Reference
26	Row mix crop	<i>Ralstonia solanacearum</i>	sb	Potato	Maize	Reduced wilt	2.0 (NS) and 8.2% at low and high density of the monocrop, resp.	Spatial arrangement; host dilution	Autrique and Pots 1987
27	Row mix crop	<i>Ralstonia solanacearum</i>	sb	Potato	Haricot beans	Reduced wilt	3.5 (NS) and 9.7% at low and high density of the monocrop resp.	Spatial arrangement; host dilution	Autrique and Pots 1987
28	Row mix crop	<i>Fusarium udum</i>	sb	Pigeon pea	Sorghum	Reduced wilt incidence	30%	Delayed germination of spores due to Sorghum root exudates (allelopathy)	Natarajan et al. 1985
29	Row mix crop	<i>Macrophomina phaseoli</i>	sb	Sorghum	Pigeon pea or cow pea	Increased inoculum density	100% Increase	Doubling of host	Singh et al. 1990
30	Row mix crop	<i>Ralstonia solanacearum</i>	sb	Tomato	Chinese chive	Reduced wilt incidence	Approx. 60%	Allelopathic reduction of pathogen	Yu 1999
31	Multilines	<i>Rhizoctonia solani</i>	sb	Sugar beet	Sugar beet	Reduced crown and root rot	No data	Host dilution	Halloin and Johnson 2000

32	Multilines	<i>Phytophthora sojae</i>	sb	Soja	Soja	Effect depending on cultivar susceptibility	Monoculture of resistant cultivar: 5% lower yield in multiline cropping (NS); monoculture of susceptible cultivar: 14% higher yield in multiline cropping	Compensation of yield by resistant or tolerant variety	Wilcox and St. Martin 1998
33	Multilines	<i>Helminthosporium victoriae</i>	sb	Oats	Oats	Reduction in disease incidence	23%	Buffering effect of resistant plants (host dilution)	Ayanru and Browning 1977
34	Multilines	<i>Wheat mosaic virus</i> (vectored by <i>Polymyxa graminis</i>)	sb	Wheat	Wheat	Reduced virus disease incidence symptoms	32%	Host dilution with the unsusceptible host	Hariri et al. 2001
35	Multilines	<i>Cephalosporium gramineum</i>	sb	Wheat	Wheat	No reduction of disease incidence as measured by presence of whiteheads	–	–	Mundt 2002b
36	Multistorey crop	<i>Fusarium</i> spp.	sb	Palm tree	Tropical kudzu	Increased half-life time of flax plants in bioassays	20–40%	Increased competition by non-pathogenic fusaria	Abadie et al. 1998
37	Mixed crop ^b	<i>Pseudocercospora herpotrichoides</i>	Splash	Barley	Oats	Reduced disease incidence	10%	Host dilution/physical barrier	Vilich-Meller 1992a

(continued)

Table 5.2 (continued)

Nr	Type	Pathogen	Pathogen type ^a	Main crop	Second crop	Effect in mixture	Effect magnitude relative to sole crop	Proposed mechanism	Reference
38	Mixed crop ^b	<i>Pseudocercospora herpotrichoides</i>	Splash	Barley	Wheat	Reduced disease incidence	50%	Physical barrier/host dilution	Vilich-Meller 1992a
39	Mixed crop ^b	<i>Pseudocercospora herpotrichoides</i>	Splash	Wheat	Barley	No effect	–	–	Vilich 1993
40	Mixed crop ^c	<i>Pseudocercospora herpotrichoides</i>	Splash	Wheat	Clover	Reduced spore dispersal	Spore dispersal 50%	Physical barrier, reduction of inoculum by increased decomposition (active microbial biomass?)	Soleimani et al. 1996
41	Mixed crop	<i>Septoria tritici</i>	Splash	Wheat	Clover	Reduced number of lesions per flag leaf	Approx. 50%	Sieving effect clover	Bannon and Cooke 1998
42	Row mix crop	<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i>	Splash	Bean	Maize	Increased disease severity	20–24%	Favorable microclimate in mixed crop	Mabagala and Saeftler 1992
43	Row mix crop	<i>Phytophthora capsici</i>	Splash	Pepper	Wheat	Reduced disease incidence or severity when sown in stubble	2.5–43%	Reduction of inoculum dispersal	Ristaino et al. 1997

44	Row mix crop	<i>Colletotrichum acutatum</i>	Splash	Strawberry	Sudan grass	Reduced spread of <i>C. acutatum</i> spores	19–49% Less spores depending on rain and crop density	Reduction of dispersal	Niaimpera et al. 1998
45	Row mix crop	<i>Diplocarpon earlianum</i>	Splash	Strawberry	Grass	Reduced spread of diplocarpon spores	–	Reduction of dispersal	Newhouse and Dana 1989
46	Multilines	<i>Rhynchosporium secalis</i>	Splash	Barley	Barley	No effect	–	–	Abbott et al. 2000
47	Multilines	<i>Pseudocercospora herpotrichoides</i>	Splash	Wheat	Wheat	No effect	–	–	Saur and Mille 1997
48	Multilines	<i>Rhynchosporium secalis</i>	Splash	Barley	Barley	Reduced disease severity	Up to 50% depending on mixture composition	Host dilution, morphological factors influencing dispersal	Newton et al. 1997
49	Multilines	<i>Mycosphaerella graminicola</i>	Splash	Wheat	Wheat	Reduced disease severity	17%	Host dilution	Mundt et al. 1995
50	Multilines	<i>Pseudocercospora herpotrichoides</i>	Splash	Wheat	Wheat	No disease reduction	–	Host dilution	Mundt et al. 1995
51	Multilines	<i>Mycosphaerella graminicola</i>	Splash	Wheat	Wheat	Contradictory results	–	–	Cowger and Mundt 2002

^a Sb is soilborne, splash is splash-dispersed pathogen

^b Crops completely widespread sown, at least not sown in rows

^c One crop sown in rows, other crop broadcast sown

^d Disease scores on a scale from 1 (no disease) to 5 (crop completely destroyed)

^e No data from incidence in single crop

5.3.1 Host Dilution

In most studies that report a reduction in soilborne diseases or pathogens in mixed-cropping systems, host dilution is assumed to play a crucial role (Table 5.3). The magnitude of disease reduction is variable but can be as much as 50% (Table 5.2). Host dilution is also regarded as the dominant disease-reducing mechanism for airborne pathogens in mixed-cropping systems (Mundt 2002a). The effect of host dilution will likely be a reduction in disease incidence rather than disease severity on infected plants (Burdon and Chilvers 1982). Host dilution might have direct (an effect on the pathogen itself) as well as indirect effects (influencing other factors than the pathogen) on disease suppression in mixed crops. An increased inter-host distance reduces the spread of pathogens. In *Pythium* garden cress experiments, a distance of 6 cm or more prevented disease spread (Burdon and Chilvers 1975). Similarly, spread of *Rhizoctonia* damping-off in radish–mustard mixtures decreased with increasing densities of the nonhost mustard plants and spread halted at host densities below a threshold density (Otten et al. 2005). When the distance between host plants becomes shorter than the threshold distance, pathogen expansion can become invasive. The threshold distance is affected by the availability of nutrient resources and interactions with competing microbial communities. These thresholds can be determined based on the percolation theory developed in physics (Bailey et al. 2000). Based on this theory Bailey et al. (2000) calculated the probability of invasive spread of *Rhizoctonia solani* in microcosms with hosts at varying distances. This, however, is only applicable for pathogens that are able to bridge the gaps between hosts from a nutrient base.

At increasing densities of susceptible roots, disease spread may accelerate if secondary root infections occur as can be the case for *G. graminis* (Bailey and Gilligan 2000) and *R. solani* (Otten et al. 2005). Such secondary infections likely

Table 5.3 Disease-reducing mechanisms in mixed-cropping systems for soilborne and splash-dispersed pathogens described in the literature

Mechanism	Soilborne pathogens	Splash-dispersed pathogens	Total
Host dilution	12	5	17
Allelopathy (including biofumigation)	4	0	4
Antagonists	5	0	5
Inoculum reduction	2	0	2
Unfavorable microclimate	1	1 ^a	1
Compensation (yield)	1	0	1
Physical barrier	0	5	5
Not mentioned	5	0	5
Total positive effects	30	10	40
Negative or no effects	6	5	11
Total	36	15	51

^aBoth physical barrier and unfavorable microclimate are mentioned for disease suppression, in totals therefore only taken up once (as physical barrier)

occur at a lower rate because of larger inter-root distances in mixed-crop systems. For pathogens with a wide host range such as *R. solani*, slightly or moderately susceptible plants may also serve as nutrient source without expressing striking disease symptoms (Otten et al. 2005), thus reducing the host dilution effect. The intensity of root intermingling in mixed cropping may be an important determinant for the interference processes (Kroon 2007) and the level of disease suppression may therefore be determined by the crops or cultivars grown and their root architectures. In contrast to pathogens capable of bridging the gaps between host plants by transporting nutrients from a substrate base, host dilution has hardly an effect on pathogens without this capacity, such as powdery scab (*Spongospora subterranea*), Verticillium wilt, and clubroot (*Plasmodiophora brassicae*).

For splash-dispersed pathogens in mixed cropping, the host dilution effect is comparable to that of airborne pathogens, influencing disease incidence more than disease severity. The non-host crop simply acts as a physical barrier, thus reducing disease spread as has been shown for *Pseudocercospora herpotrichoides*, the causal agent of eyespot in cereals (Villich-Meller 1992). The barrier function can reduce the impact of raindrops thus reducing dispersal, and it can intercept splashing spores that would reach a host plant under conditions of monoculture (Ntahimpera et al. 1998; Soleimani et al. 1996).

5.3.2 Allelopathy

Allelopathy is defined as any biochemical interaction among plants, including those mediated by microorganisms, resulting in either detrimental or beneficial effects on the interacting plants (Wu et al. 2001). In four studies, allelopathy was suggested to play a role in disease suppression in mixed cropping (Table 5.2). When watermelon was intercropped with rice, allelopathic substances from rice roots reduced production and germination of conidia of *Fusarium oxysporum* f. sp. *melonis*, leading to a 67% reduction in wilt (Ren et al. 2007). The allelopathic exudates only reduced *Fusarium* conidial density in the rhizosphere and not in bulk soil indicating a limited diffusion. Delayed germination of spores of *F. udum*, causing wilt in pigeon pea, has been attributed to allelopathic substances exuded from sorghum roots (Natarajan et al. 1985). To be effective in inhibiting rhizosphere-inhabiting pathogens, allelopathic substances should be present at sufficiently high concentrations in the micro sites where the pathogen is located, and roots of mixed crops should be in close proximity.

An interesting question is whether allelopathy causes death of the pathogen propagules (Ren et al. 2007) or only delays germination (Natarajan et al. 1985). In the latter case, the effect would resemble fungistasis, which is the general phenomenon of restriction of germination and growth of fungal propagules in soil (Lockwood 1977). A high level of soil fungistasis is often assumed to be accompanied by a high level of general disease suppression (Hornby 1983; Janvier et al. 2007; Lockwood 1977). Fungistasis can however also be regarded as a mechanism of delayed

activity if conditions are unfavorable for the pathogen, which is also the case if non-lethal allelopathic substances are formed temporarily. The effect can be detrimental, but beneficial to the pathogen as germination in absence of a host plant is, generally, not a desirable trait for pathogens. Roots of non-hosts can sometimes stimulate the germination of the survival propagules of the pathogen (Mol and van Riessen 1995) leading to a decline in the inoculum density. In relay mixed crops, this premature germination might have a disease-suppressive effect, especially in combination with inoculum burial and enhanced microbial antagonism.

Biofumigation has been proposed as a mechanism to suppress soilborne pathogens when *Brassica* species are used in mixed-cropping systems (Hauggaard-Nielsen and Jensen 2005; Kirkegaard and Sarwar 1998). However, with the exception of the work by Zewde et al. (2007), convincing field data are not yet available. This is in contrast with studies on the biofumigation potential of *Brassica* crop residues (Kirkegaard and Sarwar 1998, Smolinska et al. 2003), which showed disease suppression for various soilborne pathogens especially in controlled greenhouse experiments.

5.3.3 Microbial Antagonists

In five of the cropping systems listed in Table 5.2, enhanced antagonistic populations were proposed as a main mechanism for disease reduction in mixed-cropping systems. In three cases, pseudomonads and probably antibiotics were involved. For example, wheat root infection by *G. graminis* var. *tritici* was reduced by 25% in wheat-trefoil (*Medicago lupulina*) mixes (Lennartsson 1988). Maximum reduction (73%) in fusarium wilt was reached when bottle gourd was mixed with Chinese chive because of stimulation of *Pseudomonas gladioli* populations on the Chinese chive roots (Arie et al. 1987). Also, increased occupation of available niches by non-pathogenic *Fusaria* was held responsible for increased disease suppression in oil-palm–legume mixed cropping (Abadie et al. 1998). The build up of populations of antagonistic microorganisms has been studied mostly in single-crop systems. It seems that the natural build up of antagonists to levels where they are effective takes place mostly as a result of selection or coevolution, i.e., continuous cultivation of the same single crop in the presence of the pathogen (Schneider 1982; Weller et al. 2002). Nevertheless, also in these agro-ecosystems the fate of the same, but introduced antagonistic microorganisms is often inconsistent (Whipps 2001). Rhizosphere microbial communities, including pathogens, antagonists, and plant-growth-promoting bacteria are crop- and cultivar-specific (Germida and Siciliano 2001; Smith et al. 1999) and it might be worthwhile to investigate if these communities can be manipulated by the choice of cultivars in a mixed-crop setting. Crop- or cultivar-specific resistance against races of pathogens is widely known and often applied in mixed crops (Mundt 2002a). Mazzola and Gu (2002) used wheat to stimulate the natural antagonistic populations of fluorescent pseudomonads, which led to control of apple replant disease. The rhizospheres of old wheat cultivars

were less aggressively colonized by fluorescent pseudomonads than those of modern ones (Germida and Siciliano 2001). Among tomato lines, genetic differences correlated with *Pythium* suppression by *Bacillus cereus* and growth of this biocontrol agent on seeds (Smith et al. 1999). Also legumes may stimulate and support antagonistic *Rhizobium* bacteria in the rhizosphere (Dakora 2003; Simpfendorfer et al. 1999), which might result in increased pathogen suppression in mixed crops. When growing white clover together with triticale, take-all disease was reduced (Hiddink et al. 2004; Hiddink 2008), although the exact disease-suppressive mechanism remains elusive.

In mixed crops, increased plant diversity leads to more diverse root exudates and consequently to a more diverse rhizosphere-inhabiting microbial community (Kowalchuk et al. 2002; Westover et al. 1997). Rhizospheres of mixed crops support different bacterial and fungal microbial communities compared to the corresponding single-crop rhizospheres (Hiddink et al. 2004; Song et al. 2007). On the other hand, the effect of mixed cropping on the bulk soil microbial community has not been shown (Hiddink et al. 2005a; Kowalchuk et al. 2002). In a more biodiverse setting, the likelihood to encounter microorganisms with antagonistic properties is higher, but at the same time their densities are expected to be lower under these conditions. However, if a higher biodiversity would mean a higher diversity in functions, a higher rate of consumption of root exudates could be expected, which relates to the root camouflage concept proposed by Gilbert et al. (1994). Although increased microbiological diversity is often referred to as an important indicator for soil health (Doran and Zeiss 2000; Mäder et al. 2002; Van Elsas et al. 2002), with respect to disease suppression, its effects can be both positive (more consumption of root exudates, more antagonists) and negative (potentially effective antagonists suffer more from competition and fail to establish and be active).

For bulk soil, an increased bacteria diversity is sometimes related to increased disease suppression. Hiddink et al. (2005a) reported that higher diversity indices for bulk soil bacteria were correlated with a lower disease severity. Suppression of corky root of tomato, caused by *Pyrenochaeta lycopersici*, was related to a more diverse actinomycete community in bulk soil (Workneh and van Bruggen 1994). Although mixed cropping could increase rhizosphere microbial diversity at intensive intermingling of different roots, the effect on bulk soil biodiversity seems limited (Hiddink et al. 2005a).

Discussing the effect of microbial diversity on disease suppression is complicated since proper methods to quantify diversity are still under development. Cultivation-based approaches do not take into account the non-culturable species, whereas cultivation-independent approaches such as analysis by Denaturing Gradient Gel Electrophoresis (DGGE) underestimate the microbial diversity in soil as only the most abundant species (approximately 0.1–1% of the microorganisms present) are detected (Muyzer et al. 1993). One may assume, however, that the abundant species will also harbor species that contribute to competition for nutrients and space. Another challenge is linking microbial diversity to ecological function (Hiddink et al. 2005a; Nannipieri et al. 2003). The degree of functional redundancy (with respect to disease suppression) could perhaps be regarded as a reliable

measure for disease suppression, but how this redundancy could be measured is as yet unclear (Giller et al. 1997; Nannipieri et al. 2003). This could explain why a high biodiversity can be considered a desirable trait, but until indicators quantifying functional redundancy have developed this topic will remain largely speculative.

There clearly is a contradiction between desiring a high functional diversity on the one hand and a high establishment of a given antagonist on the other hand. In soils with a high microbial diversity, a low conduciveness for establishment and growth of an introduced antagonist or pathogen is to be expected. If disease suppression would be controlled by a single antagonist, a high microbial diversity would then be an undesirable trait of soils. This is in line with the observation that establishment of pseudomonads in organic soils (which showed a higher microbial diversity) is more limited than in conventional soils (Hiddink et al. 2005b).

5.3.4 Microclimate

Mixed cropping generally changes the microclimate. Higher soil coverage leads to lower soil temperatures which have been associated with lower disease incidence of *Macrophomina phaseolina* and *Rhizoctonia solani* in cotton–sorghum mixtures (Luthra and Vasudeva 1940). The lower level of disease severity of the splash-dispersed *Pseudocercospora herpotrichoides* in wheat–clover systems was attributed to a higher decomposition rate of organic material that serves as a base for survival of the pathogen spores (Soleimani et al. 1996). However, increased moisture content in the mixed crop could have increased soilborne pathogens such as *Pythium* spp., which can survive and disperse more easily in moist soils. Likewise, airborne diseases such as halo blight caused by *Pseudomonas syringae* pv. *phaseolicola* could be more severe in mixed bean/maize than in a single bean crop (Mabagala and Saettler 1992).

5.3.5 Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (SAR)

Mixed cropping can bring about ISR (induced by non-pathogenic microorganisms) or SAR (stress inducers like water stress, salinity, allelopathic substances, or pathogens) if one crop creates the right condition for ISR/SAR inducers for which the alternate crop is sensitive (Hamerschmidt et al. 2001). Both ISR and SAR can be interpreted as a form of increased generalized resistance in response to an external stress (Agrios 1997). The response starts from a localized point and can spread throughout the whole plant as a result of signal transduction. Induced resistance could be due to direct effects of stress-inducing root exudates or indirect effects via root-exudate-affected microbial populations (Kloepper et al. 1992). ISR has been mentioned as a mechanism for reduction of several airborne pathogens such as

powdery mildew in barley cultivar mixtures (Chin and Wolfe 1984). However, neither ISR nor SAR have been suggested to play a role in suppression of soilborne pathogens in mixed crops (Table 5.2), probably because of difficulties to prove this experimentally.

5.3.6 *Nutrients and Disease Development*

Nutrients can affect disease development above and belowground (Walters and Bingham 2007). In mixed crops, uptake of nitrogen from undersown clover reduced take-all disease severity in barley (Garrett and Mann 1948). Not only the amount but also the form of nitrogen is important. Exudation of ammonium from clover roots (Paynel and Cliquet 2003) may lead to a reduction in the rhizosphere pH in cereal roots, thereby influencing the antagonistic microbial population and decreasing infection by *G. graminis* (Sarniquet et al. 1992; Smiley 1978). Also, availability of several other elements such as potassium, phosphorus, sulfur, and silicon will influence disease development directly or indirectly (e.g., Walters and Bingham 2007) in mixed crops but are not further discussed in this review.

5.4 **Similarities and Differences Between Disease-Suppressive Mechanisms in the Different Cropping Systems**

All three cropping systems, continuous monocropping, crop rotation, and mixed cropping, can contribute to the management of certain soilborne pathogens. Crop rotation is the most commonly applied method to manage soilborne pathogens. However, while rotation schemes can reduce specific soilborne pathogens, for several other, more generalist pathogens, crop rotation is not necessarily a proper solution. Also, wide crop rotations can be undesirable from an economic point of view. Continuous cultivation of the same crop can result in a persistent decline of a pathogen, as is the case for take-all disease of cereal crops. Continuous cultivation of the same crop has not been “invented” as a management tool for soilborne pathogens per se, but induction of disease suppression is a complementary benefit in situations where no options other than continuous cultivation of single crops are available. This specific suppression usually is only active against a single pathogen leaving opportunities for other soilborne pathogens to develop and cause disease. Mixed cropping has been practiced for ages in all sorts of combinations, although not specifically designed for suppression of soilborne pathogens, but rather as an insurance against crop failures and soil erosion.

In all three types of cropping systems, multiple disease-reducing mechanisms are active, but mixed cropping offers the most diverse form of disease suppression because root systems of different crop species interact. In mixed cropping systems,

the most important disease-reducing mechanism appears to be host dilution. The magnitude of this effect depends on the planting density, the type of mixed cropping, and root architecture of the crops grown. Competition will affect the distribution of roots in mixed crops (de Kroon 2007; reviewed by Hauggaard-Nielsen and Jensen 2005). Allelopathic effects, nutrient concentrations, and water flow will determine how the roots interact and the diversity of (microbial) interactions in the rhizosphere (Bowen and Rovira 1976). Furthermore, as long as host species are mix-cropped with non-hosts in lower densities, host dilution will inevitably lead to a reduction in the number of diseased plants per area.

Other factors that result in disease suppression, such as allelopathy and antagonism induced by the non-host crop, depend on characteristics of all crops present in the mix. Biofumigation using *Brassica* species in mixed cultivation has received attention recently, but its effectiveness is still limited (Hiddink et al. 2005a). Breeding for *Brassica* species exhibiting higher glucosinolate contents is an option to increase their effectiveness (Matthiessen and Kirkegaard 2006). More effective suppression can be expected from legumes, which can excrete allelopathic root exudates and support potentially antagonistic microorganisms, besides fixing nitrogen (Dakora 2003). Also the use of specific crops and cultivars that support antagonistic microorganisms (Mazzola and Gu 2002; Smith et al. 1999) can be a valuable tool to create mixtures that actively suppress soilborne pathogens.

5.5 Practical Feasibility of Mixed Cropping

Although it is clear that mixed cropping can reduce soilborne diseases, it also has an inherent weakness: the presence of multiple crop species may bring about a greater variety of soilborne pathogens albeit likely at lower densities for each of the crops. An important question is whether and how mixed crops should be rotated and what the choice of rotation crops in time should be. When rotated, mixtures of wheat or barley containing oats resulted in lower disease levels in the crops the following year than mixtures of barley and wheat (Vilich 1993). An additional question that should be addressed is: Does mixed cropping of two crops continuously for two (or more) years lead to less disease than growing those same two crops in rotation? It is surprising that, to the best of our knowledge, no answer to this question is available in the literature. The answer to this question can be complex, as was shown by Hiddink (2008). In this study, take-all disease was lower during three consecutive years in a triticale–white clover field compared to single-cropping triticale. However, in the fourth year, *Fusarium* infected white clover and reduced its stand, which in turn caused an increase in take-all in triticale in the mixture to a disease level above that obtained in the single-cropped triticale. Soilborne pathogens with broad host ranges or long-term survival structures are likely to be less suppressed in mixed crops grown repeatedly. If pathogens like *Fusarium* in clover (Hiddink 2008) are not actively suppressed by the co-occurring crop, inoculum will continue to build up and rotating the crops in the mixture would have been a better tool to suppress the pathogens. To manage mixed crops for the

suppression of soilborne diseases requires advanced skills of the farmer and knowledge of the pathogens that might cause diseases in both mixed-crop components. It can be more labor-intensive and not suitable for mechanized production of all crops. Certain crops are not suitable to grow in mixed crops because of their weak competitiveness. The degree of intercrop competition is decisive whether a certain combination can be grown. Thus, although club root, caused by *Plasmodiophora brassicae*, was reduced in a barley–Brussels sprouts mixed crop, yield of Brussels sprouts was reduced by nearly 50% because of competition by barley (Hiddink 2008). However, often an overall yield increase is observed in mixed crops. This effect is generally expressed as the Land Equivalent Ratio (LER) (Vandermeer 1990). The LER is the sum of the yields of both components per unit of land area combined divided by the area of land needed to obtain the same yields when both components are grown as single crops (Vandermeer 1990). Mixed crops have been grown for ages, because of their yield stability and mixed cropping is still practiced for this reason in tropical regions (Vandermeer 1990). Co-occurring crops compensate for failure of one of the crops due to soil and airborne pathogens, weeds, temperature-, and water stress (Vandermeer 1990). This kind of growth compensation is an important reason for mixed cropping.

Overall, we conclude that it is interesting to consider mixed cropping where land-use efficiency and yield assurance are important reasons for practicing mixed cropping. However, application of mixed crops as tools for soilborne pathogen management is still in its infancy and not yet reliable enough.

5.6 Conclusion

In spite of the frequently observed disease or pathogen suppression (40 out of 51 observations) in mixed cropping, this system will not be a panacea for combating soilborne plant pathogens. However, in some cases it can contribute substantially to the management of soilborne pathogens. Design of mixed-cropping systems as a tool for suppressing plant pathogens is still in its infancy compared to continuous monocropping and crop rotation. The available literature is limited and scattered. In this literature review we showed that the most frequently observed disease-suppressive mechanism is host dilution (17 times for soilborne and splash-dispersed pathogens combined). Likely, however, multiple factors affect the extent of disease suppression. We think that much can be done to optimize the disease-suppressive effects based on allelopathy and antagonism. Although we focused on effects of mixed cropping on soilborne pathogens, other benefits should also be considered when evaluating mixed cropping. Reduction in plant pests and weeds has been reported widely (Baumann et al. 2001; Bukovinszky 2004). Reduced growth of one crop results in lower competition and can increase the production of the accompanying crop and thus increase overall yield stability per unit of area. This could be especially useful when no direct control measures such as pesticides are available. Another important benefit of mixed cropping is the higher potential yield per unit of area of cultivated land. This would reduce the plant production acreage needed to produce a certain amount thus using

the available production factors more efficiently and reducing nutrient leaching, water runoff, and soil erosion per unit of yield. More production per area of land also means that competing claims for land needed for the production of human food and animal feed and for the production of bio-fuels can be relieved to some extent if they can be grown on the same area of land at the same time.

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Chapter 6

Decreasing Nitrate Leaching in Vegetable Crops with Better N Management

F. Agostini, F. Tei, M. Silgram, M. Farneselli, P. Benincasa, and M.F. Aller

Abstract The relatively low cost of fertiliser and the increasing demand and competition for cheap food have encouraged the over-fertilisation of field vegetables over the past few decades. However, more recent scientific and public concern over eutrophication of water and the accumulation of nitrates in vegetables for human consumption requires a more effective use of nitrogen fertilisers in a more sustainable manner, which minimises the potential risk of negative effects on the environment and human health. In this review, we present the current state of the art in knowledge of N dynamic in vegetable crops and the latest advances in nutrient management, which could be used to mitigate nitrate losses from vegetables fields to the wider environment. Findings are based on published data and personal communications with researchers and consultants across Europe. Areas of research where further work is required are identified and described. A conclusive chapter reports on the economic and environmental impact of technology transfer of improved nitrogen management in three south European states and in the Netherlands.

Keywords Vegetable crops • nitrate leaching • nutrient management • soil and water pollution • Decision Support System • Integrated Crop Management • soil N • chlorophyll meter • fertigation • slow release fertiliser • nitrification inhibitor • intercropping • mulch • cover crop

F. Agostini (✉)

Department of Energy and Technology, SLU, Uppsala Ulls Väg 30A 756 51, Sweden
e-mail: Francesco.agostini@et.slu.se

F. Tei, M. Farneselli, and P. Benincasa

Department of Agricultural and Environmental Sciences, University of Perugia, Borgo XX giugno 74, 06121 Perugia, Italy

M. Silgram and M.F. Aller

ADAS UK Ltd., Wergs Road Wolverhampton, WV6 8TQ, UK

6.1 Introduction

Nitrogen fertilisation is a conventional practice in the management of field vegetables to ensure a good yield and quality of the marketable product (Bianco 1990). However, the amount of nitrogen fertiliser applied may often exceed the actual crop demand, taking account of other sources of plant-available nitrogen such as the soil, decomposing residues, and applied manures and slurries. This occurs because fertiliser costs are relatively modest compared to the price of the crop product, and the negative environmental effects of supra-optimal application rates of nitrogen fertiliser are often not immediately obvious. In recent years, there has been scientific and public concern about the relationship between land management practices and the enrichment of freshwaters and groundwater (Greenwood 1990; Meinardi et al. 1995; European Commission 1998, 1999; Neeteson and Carton 2001; Tilman et al. 2001; Ramos et al. 2002) and nitrate accumulation in edible portions of vegetables (Maynard et al. 1976).

In order to protect the environment and human health (Cantor 1997; Barret et al. 1998), several organisations have set $\text{NO}_3\text{-N}$ concentration limits for drinkable water: the World Health Organization and the European Union impose limits of $11.3 \text{ mg NO}_3\text{-N L}^{-1}$, which is equivalent to $50 \text{ mg NO}_3 \text{ L}^{-1}$ (European Commission 1998), while the US Environmental Protection Agency (1989) and Health Canada (Health Canada 1996) set the limit at $10 \text{ mg NO}_3\text{-N L}^{-1}$ (equivalent to $43 \text{ mg NO}_3 \text{ L}^{-1}$). Moreover, the European Commission has promulgated several directives (CEC 1991; European Commission 1998, 1999) concerning the protection of waters against pollution caused by nitrates from agricultural sources, in order to respect the above-mentioned limits and minimise the risk of excess nutrient loss to rivers promoting eutrophic status in freshwater and coastal environments. As an overall consequence, ecologically sound fertilisation strategies for field vegetable production (Greenwood 1990; Greenwood and Neeteson 1992; Hochmuth 1992; Neeteson 1995; Rahn 2002; Hartz 2003; Remie et al. 2003; Bertschinger 2004) can allow a significant reduction in both environmental and health risks associated with vegetable production.

The nitrogen (N) fertiliser consumption in the world in 2005–2006 was estimated about 98 Mt, of which 15% was used to support the growing of fruit and vegetables (Heffer 2008). In the EU-15 states, N fertiliser consumption in fruit and vegetables was about 720,000 t (Heffer 2008), and in field vegetables, it was only nearly 0.3 million tonnes (FAO 2000). Typically, the potential nitrate-leaching losses from land growing vegetable crops exceed that from arable cropped soils (Goulding 2000), as a result of the combination of the short crop growth cycle, relatively high N fertiliser requirements, the high water requirements by vegetable crops, which are often partly provided via irrigation (Greenwood et al. 1989), and the nitrogenous nature of vegetable crop residues (e.g. peas), which can mineralise rapidly and lead to increased nitrate leaching in the months following harvest (e.g. Silgram 2005). In addition, lighter sandy textured soils, which are more prone to leaching losses, represent often some of the main production areas of vegetable crops in several European countries.

Land management practices can affect the N fate and the availability of potentially leachable $\text{NO}_3\text{-N}$ (Li et al. 2007), since there is a direct relationship between large $\text{NO}_3\text{-N}$ losses and inefficient fertilisation and irrigation management. The nitrate not captured by plant roots can move in drainage waters promoted by rainfall and/or irrigation because nitrate has a weak negative charge and is not strongly adsorbed to soil particles. The downward movement of NO_3 through the soil profile occurs when significant irrigation water is applied, or under European conditions primarily in autumn and spring when precipitation exceeds evapotranspiration and the soil is at field capacity (Belanger et al. 2003; Kraft and Stites 2003).

In most agricultural areas, drainage represents the main cause of off-site transport of $\text{NO}_3\text{-N}$ (Randall and Goss 2001). In some regions, irrigation or intense precipitation events on sloping landscapes can represent the main mechanism of $\text{NO}_3\text{-N}$ loss in surface run-off to water bodies, especially for soils with low permeability (Bjorneberg et al. 2002). In particular, in Mediterranean countries, the relatively dry growing season during spring and summer creates a relatively low risk of drainage (and hence nitrate leaching) from vegetables. However, the relatively high amount of mineral nitrogen left in soil and/or residues after the harvest of some crops, such as sweet peppers, tomatoes and lettuce, coupled with intense rainfall in the autumn–winter period, which can far exceed the soil infiltration rate, can present a high risk of nitrate losses to groundwaters (Tei et al. 1999).

Moreover, with excessive or poorly timed irrigation, readily available N sources such as ammonium nitrate will be readily leached and present a potential hazard for the environment as the ammonium is rapidly nitrified, and the drainage and/or run-off caused by the intense irrigation application will promote $\text{NO}_3\text{-N}$ loss. Some ammonium and organic-N compounds do also leach from agricultural soils, but in intensively managed systems, their contribution to total loss is typically relatively small (except where livestock manures or slurries have been applied).

Leaching losses can be extremely variable depending on the intensity and distribution of rainfall, on the amount and location of soil and fertiliser N in the profile, on soil physical properties that influence the efficiency with which N is displaced in the percolating water and on plant root distribution. In general, there is a positive relationship between fertiliser N applied and nitrate-leaching losses, given sufficient drainage volume (Fig. 6.1). There is also strong evidence that encouraging farmers to reduce fertiliser N inputs can reduce losses of nitrate, leaving the soil root zone – although due to the transit time of percolating water, it can take many years before this impact may be detected in reduced nitrate concentrations in groundwaters (e.g. Silgram et al. 2004).

At the same time, the irrigation management also influences the amount of nitrate leached and taken up by the crops (Karaman et al. 2005) because of the effects on the width and depth of root distribution in soils. Indeed, leaching of nitrate–N from the root zone depends on the drainage of water out of this zone (Knox and Moody 1991, Zhang et al. 2005; Li et al. 2007) and water-use efficiency (Shaffer and Delgado 2002; Delgado et al. 2006).

Not only do nitrogen losses from agriculture relate to nitrate leaching but they also include gaseous losses as nitrous oxide and ammonia, which are both pollutants

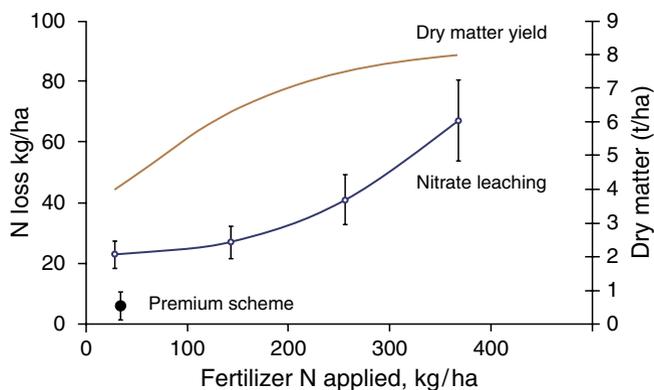


Fig. 6.1 Example of N losses by leaching and yield as dry matter versus amount of applied N fertilised to grass (Modified from Lord et al. 1999)

linked to climate change and acidification (Neeteson and Carton 2001). Therefore, a holistic approach that broadens concerns over nitrate leaching to include the management of nitrogen in the soil–plant system (accounting for N in crop residues and biomass) must also take into consideration the extent of the impact of gaseous losses related to agricultural practices. If practices lead to increased gaseous N emissions by buffering against nitrate losses from fields to water bodies, the environmental pollution risk has only been shifted from one point of impact or ‘receptor’ (water) to another (air). Recent studies on fertiliser management have highlighted the danger of this so-called pollution-swapping between nitrate leaching and ammonia loss in fruit production as a function of the type of nitrogen fertilisers and the application schedule (Cantarella et al. 2003, Stevens and Quinton 2009).

So in order to achieve more sustainable nitrogen management, the research activity should be focused on determining the most effective N-fertilisation systems by investigating the whole dynamic of N in the soil, plant, water and atmosphere.

The aim of this review is to present some of the current advances in nutrient management applied to vegetable production, to highlight the effective application of such methods within the EU as tools to reduce nitrate leaching and to identify areas of research and technology transfer where further work is required.

6.2 Fertiliser Management in Vegetable Crops

Efficient fertiliser management requires adequate tools such as an integrated approach to plant nutrition, while further work is needed to optimise the use of high-tech irrigation–fertilization systems (Battilani et al. 2003). The main consideration, which must be kept in mind in planning measures to limit nitrate leaching, is that only a small proportion of the nitrogen applied to land is actually utilised by plants, in the cases about 40–45% and an even smaller proportion is contained in

the commercially harvested material (Davies 2000). Sound N-fertiliser management, which is necessary to avoid excessive nitrate concentrations both in vegetables and in drainage (and hence drinking) water, requires the farmer to judge the balance between processes that contribute nitrogen to the soil for crop uptake and growth (inputs) and processes that remove mineral nitrogen from the plant root zone (outputs). Since nitrogen in the soil is in a continuous state of flux between organic and mineral pools, these pools, fluxes and losses need to be considered across the whole crop-management cycle. Once all the elements in the N balance have been assessed, the optimum N rate can be evaluated as a result of the difference between inputs and outputs that occur for a specific crop, location, soil and climate situation. Proper N management also requires careful management of other technical aspects, such as the timing and method of application and the choice of the fertiliser to apply (slow or fast release).

However, studies carried out on the impact of good practices in the USA suggest that only a minority of growers may follow fertiliser-management programmes, and empirical criteria are still often preferred by farmers over objective-monitoring methods (Hartz 2003). It is well known that the ‘general’ assessment of nitrogen in the soil–water–plant–air system is not an issue, but the extreme variability due to local conditions makes its practical management a demanding challenge in agriculture (Owen et al. 2003). Some countries, i.e. UK farming press and magazines (<http://www.fwi.co.uk>; <http://www.farmersguardian.com>) produced recommendations to the industry adjusted every spring based on that specific winter’s data on soil mineral nitrogen (SMN) levels and over-winter drainage volumes.

6.2.1 N Balance

Burns (2006) defined that the amount of N taken up by a crop (U_N) is equal to the sum of that recovered from the fertiliser (U_F) and from the soil (U_S) as in the following equation:

$$U_N = U_F + U_S + f_F \cdot N_F + f_S \cdot N_S \quad (6.1)$$

where N_F and N_S are the amounts of N available to the crop from fertiliser and soil, respectively and f_F and f_S are the corresponding average recovery factors for the two types of N supply. Since the amount of N from natural source is not often sufficient to meet crops needs, the remainder must be applied as fertiliser. Burns (2006) also defined the optimum rate of N fertiliser as the minimum amount needed to achieve the required response. At the plant’s optimum N-fertiliser rate (N_{Fopt}), U_N becomes equivalent to the total N demand of the crop (T_N), so

$$T_N = f_F \cdot N_{Fopt} + f_S \cdot N_S \quad (6.2)$$

where N_{Fopt} is also referred to as the N-fertiliser requirement of the crop.

A detailed N balance should take into account many inputs (mineral soil N available at planting, N from the mineralisation of crop residues and indigenous soil organic matter, irrigation and precipitation, and fertilisation) and outputs (N plant uptake, N immobilisation, denitrification, volatilisation and leaching). Since N is vulnerable to a complex variety of processes brought about by the mediating effects of weather on soil microbes, changing physical and chemical soil properties, cultural practices and the effect of preceding crops, the optimum N-fertiliser rate often varies quite considerably from site to site and from year to year (Goodlass et al. 1997). As a consequence, the reliability of N-fertiliser recommendations depends on the accuracy in the estimation of the inputs and outputs of the N balance. In some situations, this has been assisted by sampling soil cores to 90 cm depth and analysing for soil mineral nitrogen levels within the soil in autumn or spring to guide cost-effective fertiliser recommendation strategies (Burns 2006).

However, the evaluation of the optimal N rate is peculiar in vegetables because not only the yield but also other aspects such as fruit size and quality must be considered in the crop nutrient requirement concept (Olson and Simonne 2006). In fact, the concept of economic optimum yields is particularly important for vegetables because a certain amount of nutrients might produce a moderate amount of biomass, but produce negligible marketable product because of small fruit size. Farmers really need to consider the economic optimum fertiliser rate, which will be lower than the plants' optimum rate and depends on the relationship between fertiliser prices and yield price. Burns (2006) pointed out that the commonest methods to measure N requirements are based on maximising marketable or economic yields, but the former has the advantage to be independent of the price of the produce, which can vary. Furthermore, as the value of most vegetable crops far outweighs the cost of fertiliser, there is usually little difference between the two optima.

For all these reasons, in order to improve N management in a sustainable agricultural scenario, an accurate analysis of all the parameters of N balance must be done.

6.2.1.1 Total Crop N Demand

Total N demand is defined as the minimum amount of N a crop must accumulate in its tissues for optimum growth. Total crop N demand depends mainly on its total biomass since the relationship between the critical N concentration, i.e. the minimum N concentration required for maximum plant growth, %Nc (as defined by Greenwood et al. 1990) and the above-ground plant dry weight (DW, t ha⁻¹) is similar within C3 species¹ (Greenwood et al. 1990; Lemaire and Gastal 1997). Nevertheless, every species has its own N-dilution curve according to its own histological, morphological and ecophysiological characteristics, so species-specific critical N-dilution curves have been determined, for example, for potato (Greenwood et al. 1990), cabbage (Riley and Guttormsen 1999), processing tomato (Tei et al. 2002) and lettuce

¹(%Nc = 4.8 DW^{-0.34} as an average relationship for C3 species) and C4 (%Nc = 3.6 DW^{-0.34})

(Tei et al. 2003). Information on total crop N demand are often summarised and generalised in look-up tables based on past reliable agronomic experiments, for practical use of the farmers and technicians (see e.g. MAFF 2000). Models are also used in some cases, and European Union (EU) research efforts have included attempts to develop and test predictive models of fertiliser N requirements in European vegetable systems (Battilani et al. 2003; Karaman et al. 2005).

6.2.1.2 N Supply from Soil

The N supply from soil is the net result of inputs such as mineral soil N available at planting N_{\min} ; N mineralized from soil organic matter N_m ; N released from residues of previous crop N_R ; and outputs, such as mineral N losses due to immobilisation N_i ; denitrification N_D ; volatilisation N_V ; and leaching N_L .

Mineral soil N available at planting depends mainly on the previous crop and its management, cultural practices such as applied N-fertiliser rate and irrigation, prevailing weather conditions (rainfall, temperature) and can be easily measured by laboratory analysis or quick field tests by using ion-specific electrodes (Sibley 2008). For example, in field research carried out in Central Italy (Tei et al. 1999) at optimum fertiliser rates, the mineral N remaining in the soil after harvest of lettuce, processing tomato and sweet pepper was 90–101, 73–89 and 223 kg/ha, respectively.

Soil organic matter mineralisation is a microbially mediated process and in most cultivated soils ranges from around 0.6 to 0.9 kg N ha⁻¹ day⁻¹ during the growing season. The amount of N from mineralisation process is usually estimated by look-up tables (CRPV-RER 2007) or empirical function (Rühlmann 1999), both based on soil organic matter content and soil physical characteristics (i.e. soil texture). Effects such as cultivation method and timing can have a mediating effect on the mineralisation process as microbes are brought into contact with fresh, previously unavailable substrate (Silgram and Shepherd 1999).

Crop residues and green manures represent the highest potential source of N for vegetable cropping system with the exception of chemical fertilizer (Rahn et al. 1992, 1993; Müller and Thorup-Kristensen 2001; Thorup-Kristensen 1994; Thorup-Kristensen and Nielsen 1998, 2003). For instance, brassica residues can contain up to 250 kg N ha⁻¹, which is more than equivalent to the total N demand of many vegetable crops (Burns et al. 1997), sweet pepper up to 130 kg ha⁻¹ and processing tomato about 100 kg ha⁻¹ (Tei et al. 1999, 2002). Guerette et al. (2000) reported that vegetables crops leave behind more mineral nitrogen for the next crop than cereal crops. A wide range of residues quality factors have been found to be correlated with N release (Harrison and Silgram 1998); these include the C/N ratio (Giller and Cadisch 1997; Bending et al. 1998; Bending and Turner 1999), N content (Janzen and Kucey 1988; Vigil and Kissel 1991), lignin content (Frankenberger and Abdelmagid 1985; De Neve et al. 1994; Giller and Cadisch 1997) and lignin-to-N ratio (Vigil and Kissel 1991). The C/N ratio is easy to calculate and is a highly reliable indicator of the nitrogen mineralization from organic compounds. Tremblay et al. (2003) summarised mean values of potential N released as affected by the residues from the previous crop

in order to develop a more practical approach for N-fertiliser management in vegetable systems. However, although the mineralisation of organic nitrogen into mineral N forms available to plants or for leaching has been widely studied, it is a complicated process and results are difficult to predict with confidence.

Recent land use, cultivations and fertilisation history should be taken into account when evaluating the risks of nitrate leaching through their effect on mineralisation, nitrification and hence on the magnitude of the pool of N available for plant uptake or leaching (Neeteson and Carton 2001). For example, long-term monitoring of a field receiving pig slurry applications indicated an enhanced nitrate leaching over 10 years after the applications had ceased (Mantovani et al. 2005). This is an indication that a large, highly labile pool of organic and mineral N had been established over many years and this should be taken into account by reducing future fertiliser N recommendations. In a similar manner, the ploughing up of rotational or long-term grass for vegetable production can release large quantities of mineral N as soil micro-organisms are brought into contact with fresh, previously unavailable substrate (Silgram and Shepherd 1999), and this effect can last for several years after the original cultivation event took place (Silgram 2005). Despite attempts to adjust fertiliser applications to match crop requirements, some rotation systems are at inherently greater risk of nitrate leaching than others due to the release of nitrate from the mineralisation of crop residues which can be difficult to predict and may not be synchronised with the N demands of the subsequent crop. For example, late-harvested crops such as sugar beet leaf tops may mineralise rapidly and may either leach nitrate that same winter when the land is bare, or alternatively may contribute to leaching risk the following winter (termed a 'grandfather effect', Lord and Mitchell 1998). Neeteson and Carton (2001) reported that residual soil nitrogen after the application of the recommended amount of nitrogen is relatively low in Brussels sprouts, white cabbage and onions (20–75 kg N ha⁻¹), but for spinach, leeks and cauliflower the residual (i.e. post-harvest) soil nitrogen can reach values as high as 200 kg N ha⁻¹. In contrast, the incorporation of carbon-rich residues (e.g. wheat straw) has well-known abatement effects against nitrate leaching by temporarily stimulating net N immobilisation (N_p). However these effects are transient, can be subtle (Silgram and Chambers 2002; Agostini and Scholefield 2005) and may be antagonistic (Garnier et al. 2003). A practical application of the effect has been tested in field vegetables (*Brassica napus*) to control nitrate leaching from plant residues: several biodegradable materials rich in carbon including straw and paper mill by-products were added to the soil or composted with the crop residues before application, and both treatments induced a decrease in nitrogen lost as leached and as nitrous oxide (Rahn et al. 2003).

Denitrification losses (N_p) in arable soils are important only when heavy rainfall occurs after a recent N-fertiliser application, but in that case no more than 15–20 kg N ha⁻¹ are denitrified per major rainfall event. In practice, both denitrification and volatilisation losses are usually deemed negligible in an N balance for a vegetable crop and so they can be omitted as occurs in calculations carried out by the Organisation for Economic Co-operation and Development or OECD.

Leaching can occur at any time during the growing season in relation to the pattern and intensity of rainfall and the frequency, intensity and method of irrigation applications, the amount and distribution of N within the soil profile, the biochemical and physical soil properties, and the depth and the architecture of roots, i.e. all factors that influence the soil solution movement below the root zone. For example, the amount and chemical characteristics of the clay-sized fraction in the soil will influence the adsorption of ammonium and consequently nitrate availability, creating a potential retardation mechanism for mineral N leaching; however this delay can only be effectively exploited if deep-rooted plants can subsequently recover the N (Suprayago et al. 2002). In general, most of the N leached during the growing season originates from N_s rather than from N_F because the former tends to be more uniformly distributed to depth, and is more readily displaced from the lower parts of the rooting zone (Burns 1976). While the dynamics of soil N transformation and movement have been comprehensively studied, predicting the net effect of the interplay of immobilization, denitrification, N cycling and leaching processes on the soil–plant system is complex and still deemed problematic (Hartz 2003), especially in a predictive context.

6.2.1.3 N Supply from Irrigation and Rainfall

Nitrogen concentrations in irrigation water can be significant, depending on its source, and particularly in areas with high livestock density. Land also receives wet and dry atmospheric N deposition derived from nitrogen oxides generated by the use of fossil fuels from individual or industrial users (Scudlark et al. 1998; Cape et al. 2004), although the relative importance of industrial inputs varies greatly on a regional and national basis.

6.2.1.4 N Recovery

Greenwood et al. (1989) defined the apparent recovery (REC) of fertilizer N by the crop as

$$REC = (U_F - U_0) / N_F \quad (6.3)$$

where N_F = fertiliser-N rate; U_F = N uptake when N_F is applied; U_0 = N uptake when no fertiliser is applied. *REC* corresponds to f_F in equations (6.1) and (6.2).

The same authors showed that in vegetables the relationship between N-fertiliser rates and N uptake decreased linearly according to the following general equation:

$$REC = REC_0 - bN_F \quad (6.4)$$

where REC_0 = the fitted value of *REC* with an infinitely small amount of fertiliser N; $(-b)$ = the gradient of *REC* against N_F .

Table 6.1 Typical apparent recoveries from yield expressed as Dry Matter (DM) at optimum N-fertiliser rates for a range of field vegetable crops (D.J. Greenwood, personal communication in Burns 2006)

Crop	Yield (t/ha DM)	Uptake (kg/ha)	Percent of Recovery	Response value
Carrots	10.7	193	49	19
Leeks	13.7	268	35	108
Lettuce	2.0	53	7	68
Onion (bulb)	5.1	120	28	214
Radish	1.0	35	14	13
Red beet	11.3	298	34	162
Spinach	1.7	87	11	190
Summer cabbage	7.0	211	85	210
Swede	8.8	356	39	28
Turnip	7.7	309	54	24

The relationship (6.4) is species-specific (Greenwood et al. 1989; Jones and Schwab 1993; Karitonas 2003; Tei et al. 1999, 2000, 2002, Burns 2006) because it depends on the efficiency with which plants extract N from the soil due to differences in root functioning and architecture (Thorup-Kristensen and Sørensen 1999; Thorup-Kristensen and Van der Boogard 1999), but it is also affected by soil conditions, weather conditions, agronomic practices and fertiliser application methods. Although it was a rough estimation of the N-recovery efficiency of a crop, the knowledge of REC value for a species (Table 6.1) is useful for the determination of the optimum N-fertiliser requirements and gives clear information on the proportion of the optimum N-fertiliser not taken up by the crop and so at risk of leaching (Burns 2006).

The recovery factor for soil N (f_s in equations 6.1 and 6.2) is usually estimated from the uptake of N when no fertiliser is applied (U_0 in equation 6.3), although it is a rough estimation because there is an interaction between N-fertiliser rate, available N from soil and recovery factor for soil N (i.e. in general all crops are more efficient at recovering N when the N-fertiliser rate is relatively small) (Burns 2006).

Instead of the apparent recovery, some authors introduce the concept of a 'safety margin' (Tremblay et al. 2003) that is an amount of additional nitrogen to be present in the soil to safeguard the crop from nitrogen shortages that could occur if only the amount of nitrogen required for uptake were present in the soil. In fact below a critical concentration of soil nitrogen, represented by the safety margin (Table 6.2), a plant's efficiency at extracting soil nitrogen is diminished and so the safety margin allows the plant to extract its full quotient of nitrogen from the soil. Crops that have small, shallow roots with few root hairs (leeks and onions) are inefficient at extracting nitrogen, so the safety margin provided must be relatively large. Conversely, plants with long, deep, extensive root systems are more likely to extract soil nitrogen in its different form, so a smaller safety margin can be assumed.

Table 6.2 Mineral nitrogen safety margin required up until harvest (Tremblay et al. 2003)

Mineral nitrogen safety margin required up until harvest (kg/ha)		
<30	30–60	60–90
Carrots (planted late)	Broccoli (harvested in fall)	Cauliflower
Brussels sprouts	Beans	Broccoli (harvested in summer)
Cabbage (planted late)	Chinese cabbage	Leeks
	Iceberg lettuce	Spinach
	Endive	
	Curly kale	
	Kohlrabi	
	Cabbage (planted early)	
	Garden lettuce	
	Carrots (planted early)	
	Radicchio	
	Radishes	
	Beets	
	Celery	

6.2.1.5 Perspectives

Nutrient budgets are being used increasingly by farmers and policy makers at farm and country scales either to increase the understanding of nutrient cycling, as performance indicators and awareness raisers for improved nutrient management and environmental policy, or as regulating policy instruments to enforce a certain nutrient management policy in practice (De Walle and Sevenster 1998).

However, some uncertainties are associated with the budgeting approach due to wrong combination of N type, N source and N-application frequency, which should be taken into consideration for proper uses of the N balance (Oenema et al. 2003). Tests on irrigated crops in high-intensity agricultural regions between the French Alps and the Rhone valley were carried out for 3 years and showed how more than 30% of the applied nitrogen was lost due to irrational timing and unnecessarily high dosages (Normand et al. 1997). Further work is needed to educate farm managers to better exploit the pool of mineralised nitrogen already present in the soil, and consider nitrate leaching losses as ‘lost fertiliser’ (= money) in the context of farm profitability.

Given the limited efficiency of fertiliser use by crops, and the associated residual N available for leaching after harvest, a further consideration is that some authors of N cycle studies comment that they cannot realistically envisage annual reductions of more than 20 kg N ha⁻¹ in open field farming. This implies that regions with low drainage will risk breaking the Nitrates Directive limit on nitrate concentration in surface and groundwaters (e.g. Silgram et al. 2003), and in some areas the only practical solution may be the change from intensively managed and fertilised horticultural systems to a lower input and more extensive land used based on pasture land (Goulding 2000).

So several methods for estimating the different components of the N balance and for relating them to the N requirements have been developed by researchers and used by technicians and farmers although they differ in terms of feasibility, reliability and accuracy.

6.3 Methodologies and Strategies for Improved N Fertilisation

Several methods are available to estimate the N-fertiliser requirements of vegetable crops. The most simple and practical methods, spread among farmers but entirely empirical, are based on *experience and observations*. The *experience method* considers the average N rate applied in the past, which was associated with good yields for a specific local condition, then reduces or increases fertiliser N rates in light of empirical observations such as a large quantity of crop residues, a dry or wet previous winter, later planting, and yields below average, applying traditional ‘rules of thumb’. The *observation method* judges the nitrogen requirement of a crop by the use of diverse ‘diagnostic tools’ (Tremblay et al. 2003) such as plant colour, non-fertilised window (an adjustment of the N fertilisation by comparison of unfertilised plot within the crop used as a soil N availability indicator) or indicator plants (fast-growing plants that have a deep rooting system and a strong ability to extract nutrients from the soil, for example radishes, grown on a small non-fertilised section of the field).

Look-up tables are widely used throughout the world and their complexity varies in relation to the required information, such as previous crop, crop residues, soil texture and depth, average rainfall, to be used for estimating soil N availability at planting and during the crop growth (see e.g. MAFF 2000). Burns (2006) pointed out that ‘the advantage of this method is that it is relatively simple and makes use of accumulated wisdom built up from response data for a wide range of crops grown on different soils over many years, but recent evidence suggests that this approach may not be as reliable as others where N_{\min} is measured directly (Goodlass et al. 1997)’.

The possibility to keep monitoring measurements of soil and plant N ongoing during the period of crop growth is pivotal to the sustainable management of vegetable crop production, where large spatial variability in soil and plant nutrient status is a well-known issue that tends to lead to over-irrigation and over-fertilisation as farmers ‘play safe’ (De Tourdonnet et al. 2001), due to the variability in N supply and because economics dictate extra ‘contingency’ fertiliser is less costly than the (potential risk of) lost yield. However, the recent sharp increase in fertiliser prices could help to limit the risk of supra-optimal fertiliser applications.

6.3.1 Methods Based on Soil Mineral N Content

Soil analyses aim to characterise the soil nitrogen status or to predict its availability during the crop growth phase (Dachler 2001). Several tests are available to determine

Table 6.3 Rooting depth of some vegetable crops (Scharpf 1991a)

0–30 cm	30–60 cm	60–90 cm
Kohlrabi	Beans	Asparagus
Lettuce, leaf	Broccoli	Brussels sprouts
Lettuce, iceberg	Cabbage, early	Cabbage, late
Peas	Cauliflower	Cereals
Radish	Celery	Corn
Spinach	Endive	Rape
	Leek	
	Potato	

N requirements and their reliability depends on many variables. N_{min} and KNS (*Kulturbegleitende N_{min} Sollwerte*) are two methods for developing fertilizer recommendations based on measurements of soil mineral nitrogen.

In the N_{min} method (Wehrmann and Scharpf 1986), the N-fertiliser requirement of the crop (N_{rate}) is estimated as $N_{rate} = N_{target} - N_{min}$. N_{target} is a specific target level of nitrogen that must be available for maximum growth and yield to occur (Feller and Fink 2002); the target value is determined experimentally and takes into account both nitrogen already in the soil and nitrogen supplied by the application of fertilisers. N_{min} is determined from soil samples collected early in the field season, just before seeding or transplanting, taken to a depth of 0.3, 0.6 or 0.9 m depending on the root depth of the crop (Table 6.3). The method makes no adjustment for N mineralised during growth.

The KNS (*Kulturbegleitende N_{min} Sollwerte*) method, instead of just one target value, uses target values that differ throughout the season (Lorenz et al. 1989), so the KNS method recommends nitrogen to apply at planting and as top-dress or side-dress applications during the growing season.

Goodlass et al. (1997) found that the recommendations from an N_{min} method were marginally closer to experimental estimates of the N-fertiliser requirement of the crop based on maximum yields than most other methods tested. However, some other researchers have found the N_{min} method less robust (e.g. Neeteson 1989).

The soil tests can be done at the laboratory or by a quick test using several tools (e.g. Nitratecheck 404, Mercoquant, Cardy meter), but their reliability is limited by the representativeness of the field sampling procedure, since the spatial distribution of nitrate in soils is not homogeneous. Moreover, the samples must be chilled quickly to prevent any changes in nitrate content while awaiting analysis, as poor protocols for sample storage and transit can lead to large additional releases of mineral N which can render results meaningless.

However, the measurement of the magnitude of the soil mineral nitrogen (SMN) pool accessible to plant roots is not very reliable when there are periods with high rainfall during the growing season, or under high temperatures, or in soils with high organic matter contents (Wehrmann and Scharpf 1986). Stoniness is also a factor, as laboratory results in milligrams per kilogram need to be converted to kilograms per hectare to a given sample depth using an assumed bulk density for the soil. Bulk density values vary with soil texture and organic matter content, and stone content

will reduce this soil bulk density. Application or incorporation of high amounts of slurry or green manure and crop residues can also produce misleadingly high values (Dachler 2001). The accuracy of soil N content and load indicators should also be verified (Makowsky et al. 2005). An attempt to improve the use of SMN data is the creation of spatial statistic maps as has been tested within the Nitrogen Sustainable Management Programme in Agriculture (PGDA) implemented in Wallonia, Belgium (Curtois et al. 2005). A nitrogen balance based even on single local determination (i.e. before the growing season) can differ by 10–20 kg ha⁻¹ or more from a balance calculated from empirical tables. However, direct analysis of soil sampled at farm level presents the problem of correct sampling methodology and appropriate storage. It is easy to understand how the required facilities and technical skills to correctly sample, handle, store and analyse soil samples are seldom available to vegetable producers. Therefore, direct determination in the field of soil and plant nitrogen through sensors can be a more practical and cost-effective methodology (Dachler 2001).

The different methods to predict nitrogen availability and their modifications look not only to the current state of mineral N in the soil but also to the soil mineralisable N pools, which are more stable than mineral nitrogen. Several soil analytical techniques have been developed and modified (Table 6.4) for this purpose, and the joint use of this methodology with the measuring of mineral nitrogen could provide a better evaluation of the soil nitrogen supply to the crop (Dachler 2001). However, in soils with high organic matter content or treated with organic matter and crop residues, the estimation of N_{\min} is a problematic task. The nitrogen released from humus during the vegetable growing period is affected by environmental events, soil characteristics and cropping practices, and therefore the N_{\min} target values can vary spatially and must be measured at a local level (Tremblay et al. 2003). However, even with soil analysis results, farmers do not always translate knowledge of adequate nutrient supply in the soil into a lower input of fertilisers as suggested by findings in Finnish vegetable production (Salo et al. 2001).

An alternative method to complement conventional soil analysis, where several determinations are required over a long period, is the use of electrical conductivity (EC) measurements carried out on the soil solution by probes based on Time Domain Reflectometry (TDR) (De Neve et al. 1999) or Frequency Domain Reflectometry

Table 6.4 Determination method for available nitrogen (Adapted from Dachler 2001)

Method for determination of available soil N	Year of development
Hot-water-soluble N	1976
Organic soil substances	1978
Electro-ultrafiltration extractable N	1979
Free organic N	1982
Soluble organic N	1987
Water-soluble organic substances	1988
Anaerobic incubation, organic N	1988
N-rich non-humic substances	1990
Potential mineralisable nitrogen	1993

(ThetaProbe). The use of TDR probes allows the estimation of available soil nitrogen and immobilised N in non-saline soils (De Neve and Hofmann 2001), and further calibration and development of specific TDR instruments could extend the use of this method, which has so far been limited to experimental work on study farms. The time and the frequency of a reflected TDR wave in the soil depends on its water content and on the quality and quantities of the ions in it, while mathematical models can be built to use the quality of the reflected wave to calculate the concentration of such ions (Krishnapillai and Sri Ranjan 2009, Souza et al. 2006). However, an extended comparison between chemical and EC based N measurements has proved how only the first one can give a constant reliable assessment of N soil (Baumgarten 2006), and factors such as soil texture, organic matter and stone content can influence the accuracy of results.

6.3.2 *Methods Based on Evaluation of the Crop Nutritional Status*

Various measurements can be made to determine nitrogen-fertiliser requirements based on plant tissue nitrogen content. The use of these methods in vegetable production systems is deemed particular relevant in the ‘dynamic optimisation of N supply’, i.e. a method of N management based on periodic monitoring of nitrogen content in vegetables during their growth.

As with soil sampling, plant analysis must be conducted carefully, because also the nitrate concentration in plants is heterogeneous (Mills and Jones 1996; Lorenz and Tyler 2007). Standard laboratory analysis involves analysing the most recently matured leaf of the plant for an array of nutrients based on dried plant parts. The resulting analyses can be compared against published ranges for the specific crop (Mills and Jones 1996; Lemaire and Gastal 1997; Gastal and Lemaire 2002; Tei et al. 2002, 2003) to determine if the crop is at sub-optimal, optimal or at ‘luxury’ (i.e. supra-optimal) levels of uptake. Standard laboratory analysis can result in very accurate measurements (Mills and Jones 1996), and therefore it can represent one of the most accurate methods of estimating plant N status. However, this procedure is time consuming for most diagnostic situations in the field (Lemaire 2008); especially if a rapid crop N status evaluation is required to adjust the N recommendation rate in a dynamic N-management system. Thus, quick tests like *sap test* or *chlorophyll readings* have been developed and are increasingly used operationally (Matthaus and Gysi 2001, Simonne and Hochmuth 2006; Farneselli et al. 2007a, b).

The ‘*sap test*’ measures the $\text{NO}_3\text{-N}$ present in xylem and phloem sap plus the apoplastic, cytosolic and vacuolar water on the leaves; thus, it results a direct measure of current N supply. Once absorbed by roots, nitrogen is transported to the leaves where it is transformed and incorporated into living material. Thus, nitrate concentrations in the aerial part of the plant provide a good indication of the adequacy of N applied to the crop. In particular, nitrate in the leaf petioles seems to give the best indication of crop nutritional status because it is more sensitive to

fluctuations in N availability than the sap extracted by leaf blades. Nitrate content in sap can be measured by different tools which, in general, are highly correlated with results from conventional laboratory analysis (Errebhi et al. 1998; Coulombe et al. 1999; Hartz et al. 2000). The most common are: Merkoquant test strips, which react to the $\text{NO}_3\text{-N}$ content by producing a colour, the intensity of which varies directly with the concentration; an ion-specific electrode, as Horiba-Cardy Meter, which reads directly the $\text{NO}_3\text{-N}$ concentration in the sap. Several plant sap quick test kits have been calibrated for N in many crops including vegetables (Coltman 1987; Vitosh and Silvia 1994; Delgado and Follet 1998; Errebhi et al. 1998; Coulombe et al. 1999; Taber 2001; Jimenez et al. 2006; Farneselli et al. 2006a; Erdal et al. 2007). Even if the sap test procedure is markedly affected by many factors (Paschold and Scheunemann 1989; Vitosh and Silvia 1996; Farneselli et al. 2006a), when carefully undertaken it can be a reliable tool for monitoring the crop nutritional status of many vegetable crops (such as processing tomato) and with results consistent with the critical N-curve method (Tei et al. 2002) for the most important time period in fertiliser management (Farneselli et al. 2007a).

The *chlorophyll meter readings*, such as SPAD-502 meter by Minolta, is another common quick test (Hoel 2003; Swiader and Moore 2002; Sexton and Carroll 2002; Arregui et al. 2006). It detects differences in leaf nitrate content by measuring the light transmittance through leaves. The device is simple to use and, since it estimates the nitrate content in the tissue of intact and growing leaves, it is not destructive and does not require the preparation of any chemical samples for analysis. SPAD readings are an accurate method to evaluate the crop nutritional status because the chlorophyll content is usually highly correlated with the nitrogen level and yield, but, at the same time, it is affected by several factors such as cultivar, environmental conditions, plant growth stage, pests and diseases (Piekielek and Fox 1992; Gianquinto et al. 2006). The SPAD meter is therefore best used together with other crop and meteorological monitoring tools. The reliability of the SPAD method has been tested with good results on several crops including corn (Piekielek and Fox 1992), cereals (Arregui et al. 2006), potato (Gianquinto et al. 2003; Olivier et al. 2006), tomato (Gianquinto et al. 2006, Farneselli et al. 2007a), pumpkins (Swiader and Moore 2002) and beets (Sexton and Carroll 2002).

These approaches helped to implement good practices in vegetable N management in several Canadian states (Westerveld et al. 2003). Tremblay et al. (2003), in their guide to vegetable nitrogen fertilisation, give a very good judgement on the use of these field devices, but consider them as a complement to more conventional soil analysis. Others (Neurkirchen and Lammel 2002; Schroder et al. 2000) judged such methods – if calibrated according to different varieties and environments – to be so precise as to be able to provide all the necessary laboratory determinations for plants and soils. However, most crop indicators seem to be more effective at diagnosing N deficiencies rather than N excesses, with the exception of the sap test (Radersma and van Evert 2005). The main benefit of the new methods tested is mostly as a ‘field troubleshooter’ for identifying low nitrogen status situations – in contrast, their use for reducing over-fertilisation could be more problematic. Indeed, a review of the available quick test tools including test strips and SPAD meters, Hartz (2003) highlighted this method’s limitations due to the high

equipment cost and demanding calibration process, and the sometimes weak relationship between apparent plant N status and the real plant N demand.

Nevertheless, if the scope of the recommendation is limited to fertiliser top-dressings of nitrogen during vegetable growth, then sap nitrogen content has proved to be cheap, more accurate in characterising spatial variability (i.e. in broccoli CV = 9% against CV = 29% where CV is coefficient of variability), faster, and less weather-dependent compared to conventional N_{\min} determination (Matthaus and Gysi 2001). An improvement on the crop indicators approach can be achieved using ‘crop windows’, which are field plots where the crop is kept at maximal N status, if the difference between the crop indicator index between the window crop and the field crop is large, an additional application amount can be calculated for the field crop (Radersma and van Evert. 2005, Wiesler et al. 2002). Such reference plots should also set accordingly the growth stage when each diagnostic system is planned to be used (Tremblay and Belec 2005).

Another contemporary approach, which is currently being studied in France and in Italy for tomatoes, melon, aubergine and other uncovered field vegetables, is the “Index of Nitrogen Nutrition” (INN), which is the ratio of the percentage N content in the plant to the critical percentage N content at which the plant stops growing. Its development implies the identification of a part of the plant in which the N content is representative of the whole plant N status, and the development of a specific function linking N dose applied and plant growth. This methodology is applicable only if a diagnostic instrument is developed and calibrated for plant N testing in the field (Le Bot et al 2001; Dumoulin et al. 2002a, b).

The choice of the best method of assessing N requirement using soil or plant analyses depends on the crop and soil type. While researchers agree that adjusting fertiliser recommendations according to soil mineral nitrogen test is a good practice especially in high N situations (Goodlass et al. 1997; Hartz 2003; Burns 2006), there is no consensus on the best method for monitoring dynamic crop nutritional status during the growing season. For evaluating crop nutrient status, results from different studies have lead to different conclusions: certain crops appear to be assessed accurately using the sap test, while others do not show as strong a correlation between sap nitrate content and crop nitrogen supply and are therefore better managed using soil nitrate testing. Research carried out in lettuce and broccoli concluded that there was a higher accuracy associated with soil testing compared to sap testing (Coulombe et al. 1999; Hartz et al. 2000); while in potato, fertiliser cost and leaching losses were reduced based on the sap test. However, other researchers have found that the status of many others vegetables crops such as potato, cabbage, carrots, onion and tomato was accurately assessed using the sap test (University of Minnesota 1996; Westerveld et al. 2003; Farneselli et al. 2007a).

6.4 Nutrient Modelling and System Analysis

Concerns in recent decades over the loss of nutrients from agriculture to water bodies have had to balance the commercial pressure for yield maximisation against environmental policy agendas including water quality legislation, climate change targets

and environmental sustainability. The resulting investigations have led to a 'holistic' new approach to nitrogen fertilisation in vegetable production at farm and regional scale (Huffman et al. 2001). Although some general recommendations can be derived from experimental results for specific case study scenarios, the variability in soils, climate, hydrology and management means that it is not possible to provide a fully exhaustive range of fertiliser parameters for all the species of vegetable produced in the EU on all the different soils (Goulding 2000).

Mechanistic simulation models have been developed representing system processes at different levels of detail in order to simulate, test and explore the interactions in soil–plant systems for crop growth and nutrient uptake (Le Bot et al. 1998; Marcelis et al. 1998). In general, simulation models are intended for researchers in order to study the nitrogen interaction in the plant–soil system, by supplying data sets collected from experiment with data on local meteorological conditions. With the application of those models, researchers are able to evaluate which parameters are important in the nitrogen balance and may be modified to determine which factors are critical in the nitrogen balance. Since simulation models usually need accurate information on several eco-physiological parameters they are generally unsuitable for providing practical advice to farmers and technical advisory services (Grignani and Zavattaro 2000) unless they are embedded in user-friendly computer-based Decision Support Systems (DSS) for use in commercial practice (Battilani and Fereres 1999).

Exploring system dynamics and responses using simulation models is clearly less labour-intensive and more flexible than field-based experimental work (Whitmore 1996) but very few N models are based specifically on vegetable studies. One widely used software packages is WELL_N (Greenwood et al. 1987; Rahn et al. 1996) that since its release has been used widely by large sectors of the UK field vegetable industry (Burns 2006). It was developed by Greenwood et al. (1987) to present the response of winter wheat to N fertilizer and was later extended to include the simulation of growth of 25 vegetables and major arable crops and the release of N from crop residues. The DSS WELL-N uses an embedded simulation model of crop N response (N_ABLE) simulation model (Greenwood et al. 1996), which includes a complete crop rotation, and is able to evaluate the effects of different soil management strategies on nitrate leaching from intensive vegetables rotations. Other examples of DSS are N-Expert (Fink and Scharpf 1992; Stenger et al. 1999), Irriguide (Bailey and Spackman 1996; Silgram et al. 2007), Conseil-Champs (<http://www.agrigestion.ca>) and Agri-Champs (<http://www.lavoieagricole.ca>). Battilani et al. (2003) also developed a simple tool-model (FERTIRRIGERE) for managing water and nutrient supply in drip-irrigated processing tomatoes.

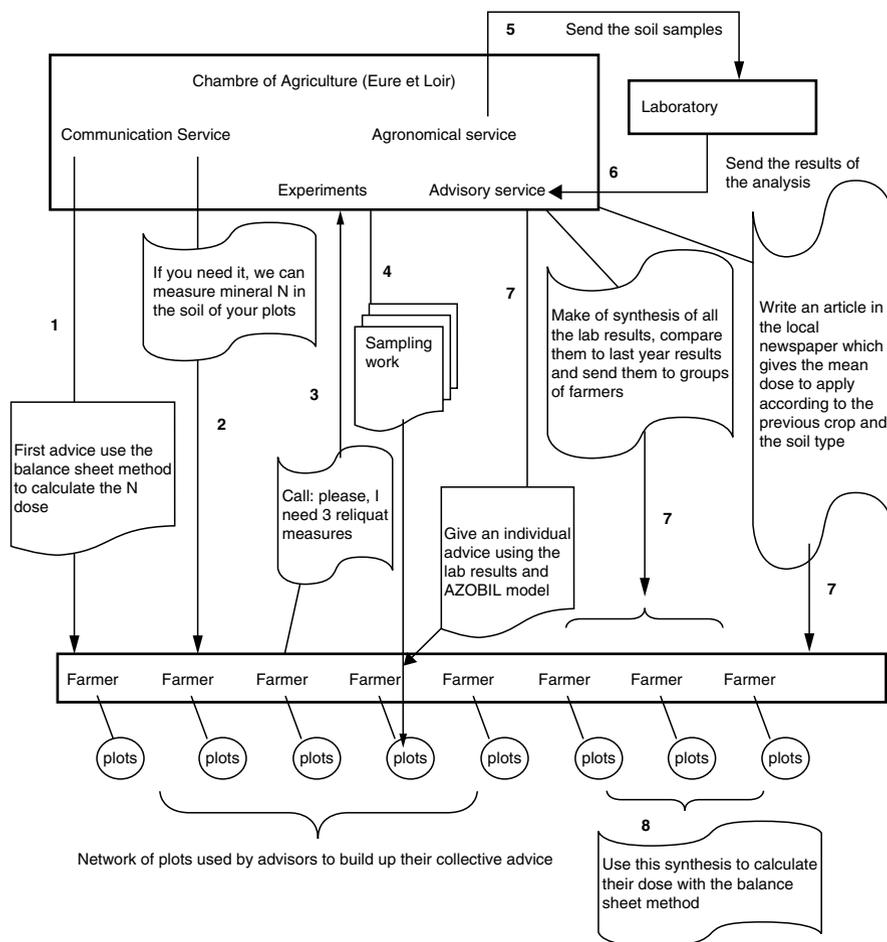
Catchment-scale assessments based on models using spatial data on soil, weather and crops are needed for planning the reorganisation of (and scenarios for) changes in agricultural activity in a more sustainable way. Such methods can estimate the potential for nitrate leaching over a large area from different production and nitrogen-management systems by linking simulation models, soil and climate data and geographical information systems (Hoffmann and Johnsson 1999; Lilburn

and Web 2002). The end results are tools that allow judgments of the potential impacts of ‘good practice’ (Huffman et al. 2001; Haberlandt et al. 2002), and help identify ‘hot spots’ at high risk of nutrient pollution due to a combination of land use, soils, climate and hydrological conditions. Areas posing a high risk of diffuse pollution from agriculture (due to the combination of land use, soil, management, climate, slope, location, etc.) can then be targeted in a focused, spatially defined manner either within the EC Nitrates Directive (within Nitrate Vulnerable Zones (NVZs)) or the EC Water Framework Directive (within River Basin Management Plans). Member States are already developing such approaches, at national or regional level, for some or all crop types (e.g. Italy - project for Soil Quality for Sustainable Agriculture and Forestry, M. Pagliai, personal communication; UK – ‘MAGPIE’, Lord and Anthony 2000, in UK same approach is followed for P management also, PSYCHIC, Davison et al. 2008; Collins et al. 2007). Ideally, the models should provide also a ‘cost curve’ analysis of the required costs and benefits associated with different mitigation measures in a range of farm systems involved (Anthony et al. 2005).

However, the use of mechanistic models at field/farm level is often hampered by the lack of localised data or the required level of competence (Grignani and Zavattaro 2000). With limited relevant calibration datasets, it is not surprising that in many cases, Decision Support Systems (DSS) at farm level related to vegetable production tend to underestimate nitrate leaching (Uhte 1995). However, this does not imply that the systems approach is not highly valuable in terms of its potential for improving the sustainability of horticulture (Rabbinge and Rossing 2000; Visser de et al. 2005). At regional scale, advanced statistical methods (such as fuzzy statistics: Bardossy et al. 2003) and research techniques (e.g. linear programming, neural networking or genetic algorithms: Gary 2003) can provide the required data and expert knowledge to fully exploit the potential associated with different modelling approaches. For simulation exercises at this scale, a smaller (e.g. 2 × 2 km) grid and the use of more detailed datasets are always advisable (Borgensen et al. 2005).

Similar exercises have been carried out also at smaller scales (100–200 km²) on vegetable production in the Valencia region in Spain (De Paz and Ramos 2002). Their results, supported by the application of spatial and multivariate analyses, helped to define critical patterns in soils and climate, which were then used to limit N fertilisation according to crop demand to minimise the risk of leaching associated with periods of greatest drainage. From the farmers’ point of view, nitrate leached out of the soil root zone represents money wasted on ‘lost’ fertiliser.

These kinds of projects can also generate information for developing farm-level databases to identify agro-ecological indicators, which can evaluate the sustainability of different elements in vegetable production systems (Mempel and Meyer 2002). For instance, the ‘Indigo method’, developed in France to analyse vineyards and fruit production (Gary 2003), allows the linking and ranking of each factor in the cropping system in relation to a set of environmental parameters. Each user can then select a minimal number of variables to monitor in a specific strategy such as nitrate-leaching reduction, pesticide limitation etc.



1 Different phases of the decision process

Fig. 6.2 Interaction between technical consultants and vegetable farmers using a Decision Support System (Meynard et al. 2002)

However, the implementation of DSSs over large areas cannot be effective without a well-connected network including farmers, technical advisory services, and local authorities. Meynard et al. (2002) described this interaction between farmers and advisory services using a DSS to generate guidelines in crop management (Fig. 6.2). However, care is required to prevent the quality of the information supplied losing detail and integrity during the communication process from farmer or farm adviser to modeller, which could result in misleading recommendations being produced. The use of such DSS tools is necessarily limited to the range of typical situations (crop, soil, climate, hydrology) for which they were originally developed. Realisation of the

potential advantages of using DSSs to guide more sustainable vegetable management and production systems is dependent on (i) appropriate tools to allow upscaling from field to regional level, (ii) appropriate parameters at different spatial scales and (iii) specific procedures to promote dialogue and help disseminate the resulting information and advice back to farmers.

At a farm level, a careful monitoring of nitrogen status in soils and crops at a local level coupled with simulation models of water and N cycling in the plant–soil system can be translated into targeted crop management based on the spatial variability of agronomic characteristics using geographic positioning system (GPS) instruments linked to geographic information system (GIS) references. This methodology of ‘precision farming’ is becoming more widely used in open field vegetables and fruit orchards (Van Alphen and Stoorvogel 2000, Smit et al. 2000). For example, in Sweden, this precision farming technique is being used to characterise within-field variability in fertiliser N requirements, water status, or pest/disease risk in vegetable systems, where it has proved to be cost-effective (H. Sandin, August 2006). Although such methods can prove cost-effective, such approaches require high technological input in terms of equipment and training of the operator, which means that this is not a practical option in some situations. Some researchers have suggested coupling biophysical simulations with economic modelling at the planning stage to identify the most profitable management of N inputs (Smit et al. 2000). Carrying out such an economic optimisation, Smit et al. (2000) found the use of precision agricultural systems was highly cost-effective for N input management in ware potato in the Netherlands, and concluded that in precision farming the best economic return was reached when applying good agricultural practices.

6.5 Agronomic Options in N-Fertilizer Management

There is a broad recognition of the need to improve the adoption of best management irrigation and fertiliser management practices in vegetable growing. Since $\text{NO}_3\text{-N}$ is mobile and relatively unreactive (Rajput and Patel 2006) and, therefore, susceptible to movement through diffusion and mass transport in the soil water, water management is inevitably linked to N management. Careful timely applications of N fertiliser and irrigation water can limit the amount of nitrate leaching below the root zone (Drost and Koeing 2001), such as occurs with well-managed fertigation techniques. Once the optimum N rate is applied, a suitable evaluation of plant nutritional status during the growing season is necessary to make adjustments accounting for N availability (Coltman 1987; Smith and Loneragan 1997; Simonne and Hochmuth 2006). Other key aspects of N fertilization and irrigation management which must be correctly evaluated to improve N management include rate, application timing and method and type of fertiliser (Neeraja et al. 1999). For example, field experiments carried out for 3 years on irrigated crops in high intensity agricultural regions between the French Alps and the Rhone valley showed that more than 30% of the applied nitrogen was lost due to inappropriate timings, which

were not synchronised with crop N demand and comprised unnecessarily high dosages (Normand et al. 1997).

Since the relationship between N applied and nitrate leaching is non-linear, with nitrate leaching increasing sharply once optimal N application rates are approached, nitrate leaching could be disproportionately reduced for a relatively modest reduction in N application rates. If farmers were somehow compensated for the resulting lower yield, a possibility could be modifying the Common Agricultural Policy to include a grant-type payment for lower impact agriculture, then this approach could be the solution for high-risk land uses such as vegetable production systems (Tremblay et al. 2003). However, this is unlikely to be compatible with the 'polluter pays principle' underpinning EC environmental legislation.

Compared to other agricultural land uses, the growing of vegetable crops are associated with amongst the highest soil mineral nitrogen values in the spring (e.g. Silgram 2005). The mineralisation of N from these residues can proceed rapidly (especially under warm Mediterranean conditions in the spring) thereby making it difficult to capture this N using cover crops except if rainfall is limited during the growing season (Kraft and Stites 2003). Possible solutions include considering low or zero fertiliser input systems (i.e. organic land management), soil-less systems (hydroponics), or reversion to low impact vegetable crops to compensate for the decreased yield due to low fertiliser inputs (Kraft and Stites 2003). There is also the relatively new idea of accepting a limited reduction of yield through a sub-optimal fertiliser regime, with the reduction varying as a function of crop type. Such sub-optimal applications may also promote a higher concentration of sugar and vitamin C in the harvested material, which may have implications for market prices with traders (such as supermarkets and food manufacturers).

Where the nitrate leaching risk is high post-harvest, then the irrigation and fertilisation management have limited potential as control tools (by improving fertiliser use efficiency through placement, timing, rooting, or variety), with alternative solutions involving modifications to the crop rotation and/or *inter-cropping with deep rooted crops providing a potential solution to reduce the N available for leaching (Sidat et al. 2000).

6.5.1 Localised Fertilisation

Placement of N fertilisers close to the plant can play an important role to help prevent or minimise the risk of nitrate leaching, especially in vegetable crops which are usually grown in rows, by increasing N fertiliser recovery. This localised placement of N is particularly efficient in reducing leaching risks at the beginning of the growing season (i.e. starter fertiliser technique) as when plants are small, roots exploit a very limited soil volume and the N uptake is slow. The use of starter fertiliser, in comparison with conventional N application timings, promotes both faster and higher root and top growth, increasing yield and reducing N losses (Costigan 1988; Ma and Kalb 2006; Osborne 2006). This placement of soluble nutrients close to the

seed is especially important in cold, wet soil in which nutrient availability and root growth are generally reduced. Localised fertiliser placement can also be performed by banding fertiliser on the crop rows. I.G. Burns (personal communication) suggested to restrict first applications to a narrow band and to apply a second application as top dressing at the normal rate. For example, in cauliflower, roots expanded laterally to exploit about half the row width within 4–8 weeks of planting and crops planted with the optimum rate of base dressing recovered most of the applied N within 8 weeks. Such banded fertiliser approaches can be effectively used for cauliflower, onion, lettuce and potato. However, the most effective technique to synchronize as much as possible N uptake with N availability is fertigation.

6.5.2 *Fertigation Techniques*

Fertigation methods tend to increase the nitrogen use efficiency (NUE) while N losses to the environment are minimised, maintaining a balance between food production and environmental quality (Farneselli 2008). Since micro-irrigation has emerged as an appropriate water-saving technique especially for row crops, and applying fertiliser in the water via drip irrigation can be a more efficient fertiliser management practice, the fertigation technique is becoming very common on vegetable crop systems. The advantages of the fertigation over broadcast method of fertilizer applications are emphasized by several researchers (Phene 1999; Singandhupe et al. 2003; Mohammad 2004).

The high water- and N-use efficiency of fertigation (which represent the major benefits of this technique) are due to rate splitting according to the crop requirement at any growth phase and due to the localised placement of fertiliser close to the roots. As a consequence, fertigation can reduce the risks of nitrate leaching, surface evaporation and deep percolation without any decrease of yield and quality in produce (Battilani 2001, 2006; Singandhupe et al. 2003; Hebbar et al. 2004; Janat 2004; Battilani and Solimando 2006). Several studies conducted on different crops (Li et al. 2004) showed an increase in yield of crops grown with fertigation techniques compared to conventional ones: Singandhupe et al. (2003) recorded a 3.7–12.5% increase in yield and 31–37% decrease in water consumption for tomato grown with drip irrigation compared to furrow irrigation systems; while Hebbar et al. (2004) recorded a tomato fruit yield 19% higher in drip irrigation compared to furrow irrigation. Nevertheless, fertigation is often managed empirically, both for irrigation and mineral nutrition aspects, so that its advantages are not fully exploited, and mismanagement of fertigation can lead to nitrate contamination of surface waters, groundwaters and soils (Battilani 2001).

Achieving maximum fertigation efficiency requires knowledge of crop-specific water and nutrient requirements at any site throughout the growth cycle (Tei et al. 2002) and attention to the timing of water and N delivery to meet (but not overwhelm) crop needs. At a given water and nutrient supply, fertigation frequency affects water volume and N rate per application, and thus soil moisture and nutrient

concentration in the rhizosphere between irrigations, with consequent changes in crop growth, N uptake and yield (Cook and Sanders 1991; Locascio and Smajstrla 1995; Silber et al. 2003). As a consequence, the careful management of irrigation and/or fertigation frequency is one of the major management variables affecting fertigation efficiency. High fertigation frequency is often advocated in the technical literature (Bar-Yosef and Sagiv 1982) because it keeps soil moisture and nutrient concentration constant near the root zone, so that nutrient diffusion in the soil is easy (Silber et al. 2003). At the same time, water movement is mainly controlled by capillary forces instead of gravitational ones (Phene 1999) with consequent leaching reduction. Moreover, high fertigation frequency makes it possible to more precisely modulate the concentration of the nutrient solution in the irrigated root zone according to crop needs at any growth stage (Bravdo 2003).

Some authors (Cook and Sanders 1991; Locascio and Smajstrla 1995; Silber et al. 2003) have found that for processing tomato, a daily or weekly fertigation significantly increased yield compared to less frequent fertigation; although differences between daily and weekly intervals were not significant even on a sandy soil. The authors hypothesised that yield limitation at low fertigation frequency is mainly the result of nutrient deficiency rather than water deficiency. However, crops are able to counteract small, short-lived nutrient concentration variations, and therefore plants do not necessarily show nutrient stress. Moreover, some studies have demonstrated that if a little stress is given, root penetration increases and the yield may increase with reduction in the cost of irrigation (Dalvi et al. 1999).

There is a need to evaluate lower-fertigation frequency in greater detail, because there is limited evidence of the benefit of higher-frequency fertigation. This is because frequent fertigation regimes are not easy to manage and increase water waste due to both evaporation from the constantly wet soil surface and the large portion of the irrigation cycle used for system charge and flush (Simonne et al. 2005). Previous research conducted by Li et al. (2003, 2004) observed that the water distribution pattern is affected by several variables with consequences on the root growth and N leaching. The emitter discharge rate and the application rates of water and nitrogen affect the wetting pattern and solute movement; in particular an increase in the water application rate allows greater water distribution in a vertical direction for a given volume applied (Farneselli et al. 2008). The fertigation–irrigation frequency may also affect biomass accumulation and partitioning because a different water and nutrient availability in the root zone can affect plant water and nutritional status with possible consequences on root growth and shoot/root ratio, leaf assimilation and transpiration, canopy architecture, light absorption and distribution inside the canopy (Hebbar et al. 2004). Results from experiments carried out in Central Italy in processing tomato have suggested that high fertigation–irrigation frequencies increased the above-ground crop dry matter (DM) accumulation and N uptake only when N supply was very high and exceeded crop critical requirements (i.e. for luxury N consumption) while for optimal and sub-optimal crop N status it had no effect (Farneselli et al. 2007b). In contrast to patterns of biomass and N accumulation, the size ratio between the different parts of the plant did not change with the fertigation frequency. Moreover fertigation frequency can affect the timing of ripening and/or fruit quality (breaks, rottenness, size and size uniformity, nutritional

parameters) (Hebbar et al. 2004; Colla et al. 2001; Erdal et al. 2007). However, the strategy of controlling nitrate leaching based on split fertiliser applications and careful irrigation management may only have a low impact on nitrate-demanding crops with shallow-rooting systems (i.e. potato, faba bean) especially under heavy unpredictable rainfall (Andrasky and Bundy 1999). In the slightly more strict regime applied in the USA, where the nitrate ($\text{NO}_3\text{-N}$) limit in agricultural groundwater is 43 mg L^{-1} against the 50 mg L^{-1} applied in the EU, the control of nitrate leaching through the management of irrigation and fertilisers has proved a complete failure (Kraft and Stites 2003).

6.5.3 *Slow-Release Fertilisers*

The use of slow-release fertilisers serves the same purpose as split applications, providing nitrogen more slowly as the plant requires it (Li 2003; Khah 2003). This kind of fertiliser has the benefit of saving time, since all fertiliser can be applied in a single dressing at the beginning of the season, although it also has some notable disadvantages such as the need for special application equipment and the more expensive product compared to conventional fertilisers (Jin 1996; Schaller 2000; Khah 2003; Prasad et al. 2004) with N release not always coinciding with crop N requirements (Peltonen 1994). Moreover, the use of organic fertiliser (Heeb 2005; Herencia 2007; Pavlou 2007) or fertiliser with the appropriate nitrate–N/ammonium–N ratio or nitrification inhibitors could also be a valuable strategy for improving N-fertiliser management (Narayan 2002).

6.5.4 *Nitrification Inhibitors*

The use of new nitrification inhibitors 3,4-Dimethylpirazole phosphate (DMPP) has also been considered in addition to urea (Pasda et al. 2001). Linaje et al. (2005) in central Spain measured a reduction of 50% of N leaching with the application of DMPP to broccoli. Mantovani et al. (2005) obtained similar results by adding DMPP to pig slurry. This approach could be considered as an alternative to calendar-linked applications of manure (e.g. in the context of restrictions on the timing of manure applications imposed by the EC Nitrates Directive), thus avoiding the costly need for storage facilities.

6.5.5 *Intercropping*

The aim of an intercropping system is to increase the crop root density, and this approach is most successful when implemented using 'compatible' species, which have different peak times of N uptake and different rooting depths (Baumann et al. 2003). When implemented in this manner there need not be significant effects on overall yields.

One species may exploit available nitrogen, which is not accessible or required by the other crop. For example, testing different intercropping systems with faba bean undersown with brassicas such as oil radish (*Raphanus sativus* var. *oleiformis*) or white mustard (*Sinapis alba*) proved more efficient than ryegrass and cereal which reduced the faba grain yield (Justus and Kopke 1995). The depth of the rooting zone will give an indication of potentially viable intercrop combinations in vegetable systems (see Table 6.3). Paschold et al. (2003) carried out research in intensive vegetable production systems in Germany which provided evidence of the potential for intercropping in vegetable production in Europe to serve as an effective tool for controlling nitrate leaching. These authors reported that the growth of oil radish (*Raphanus sativus* var. *oleiformis*) between asparagus ridges was a useful technique for reducing nitrate leaching after the growing season of asparagus had ended (rather than leaving the soil bare over winter). The N_{\min} residual in the soil (0–90 cm depth) decrease in average from 250 kg ha⁻¹ to 150 kg ha⁻¹, with an average increase in asparagus yield of 1.2 t ha⁻¹. A further element of a mixed-intercropping system is the creation of a green cover, which covers the soil surface otherwise unoccupied by growing plants and thereby achieves the same effect as mulching. This is an established feature of the management of some vegetable fields and fruit orchards, which is carried out using inert materials such as polyethylene.

6.5.6 Mulching

Sweeney et al. (1987) worked in an open field growing tomato with overhead irrigation and mulching with polyethylene. This system reduced water drained from the soil and enabled nitrogen uptake to reach 53% of the applied amount, with 42% of N applied remaining in the soil and 5% lost as leached nitrate. Similar results have been reported for the growth of pepper (Romic et al. 2003).

6.5.7 Cover Crops

Many researchers have pointed out the feasibility of using autumn crop covers to manage the nitrogen husbandry for the succeeding cash crop, prevent the nitrogen leaching and improve the soil characteristics especially by increasing the soil organic matter (Harrison and Silgram 1998; Thorup-Kristensen et al. 2003; Macdonald et al. 2005). As broadly accepted, the phrase ‘catch crop’ is used when dealing with cover crops that are grown to catch available nitrogen in the soil and thereby minimising nitrate-leaching losses, while the term ‘green manure’ is used when dealing with cover crops that are grown mainly to improve the nutrition of the subsequent crops (Tosti 2008). A good catch crop (e.g. cereals and crucifers) should have an early sowing date (Thorup-Kristensen and Pedersen 2006), a

prompt germination and fast growth rate at both above and below-ground levels (Thorup-Kristensen 2001), and a deep root apparatus (Kristensen and Thorup-Kristensen 2004). Green manures for supplying N are usually leguminous species able to accumulate considerable amount of nitrogen: in a Mediterranean environment like central Italy, values ranging from 150 to 250 kg ha⁻¹ for annual clover (Campiglia et al. 2005) with maximum values of more than 300 kg ha⁻¹ (Benincasa et al. 2004) for faba bean and hairy vetch green manures. In southern Italy nitrogen supply of 45 and 165 kg ha⁻¹ are reported for vetch and cow pea respectively, while faba bean supply was between 72 and 193 kg N ha⁻¹ (Fagnano et al. 2005; De Luca et al. 2006; Sulas et al. 2007). The net contribution in terms of nitrogen input to the system (i.e. the nitrogen derived from atmosphere) was estimated 70–80% of the total nitrogen supplied by legumes (Seddaiu et al. 2007; Sulas et al. 2007).

Recent research found that it is possible to modulate N supply and release from green manures to a subsequent crop by mixing grass and legumes (Boldrini et al. 2006; Tosti et al. 2008) and that the unit cost of nitrogen from green manures is much lower if compared to nitrogen from organic fertilisers (Chaves et al. 2006; Guiducci et al. 2004). However, because the N release will depend on the C/N ratio in residuals and the mineralisation rate, experimental results can be contradictory (Harrison and Silgram 1998). The use of mixtures of hairy vetch (*Vicia villosa* Roth.) and barley (*Hordeum vulgare* L.) with high proportion of vetch (>50%), for example, allowed an optimal N nutritional status of processing tomato without promoting luxury N consumption (Tosti et al. 2008).

The use of cover crops or catch crops is limited by farmers' reluctance to adopt voluntarily a practice which demands extra time associated with establishment and destruction, possible extra seed costs, and the risk of encouraging the persistence of weeds, pests, or diseases which may interfere with the growth and yield of the next main crop (Tremblay et al. 2003). Only some form of incentive scheme or their compulsory use as a requirement under Code of Good Agriculture Practice would assure their more widespread adoption by farmers (Vos and Putten 2004; Vos et al. 2005).

6.5.8 *Cultivar Nitrogen Efficiency*

For nitrogen, it has been noted that differences in nitrogen efficiency occur at the crop level and also in some cases at the cultivar level. N-efficient crops and cultivars are characterised by deep rooting depths (with enhance N uptake efficiency) and high utilisation efficiency. Schenk (2006) stated that 'nutrient use efficiency is a potential tool for sustainable vegetable production in the field. Some breeders are going down this avenue and are selecting cultivars under nutrient limiting conditions. The development of nutrient efficient cultivars is a challenge for horticultural science not only with a view to reducing the flow of nutrients into natural compartments of the environment but also taking into consideration production conditions in countries where access to fertilisers is limited'.

6.6 N-Leaching Assessment

The quantification of nitrate leaching from soils to water has specific difficulties (Kucke and Kleeberg 1997). A rapid and reliable estimation of $\text{NO}_3\text{-N}$ moving below the root zone is crucial to reducing the risk of nitrate leaching (Aveline and Guichard 2005; Makowsky et al. 2005). Since water movement in the soil and $\text{NO}_3\text{-N}$ concentrations in the soil solution are strictly linked, both these phenomena have to be investigated. Several different approaches could be adopted to assess N leaching. Load may be determined directly by soil sample analysis or by collecting leachate from drainage lysimeters. Mathematical simulation models have become also useful tools in assessing and understanding the movement of fertilisers through soil into groundwater (Shaffer et al. 1991; Jabro et al. 1994; Bailey and Spackman 1996; Karaman et al. 2005; Silgram et al. 2007).

Monitoring the $\text{NO}_3\text{-N}$ concentrations in the soil solution by suction cup lysimeters placed at different depths, is also another method to assess nitrate leaching below the root zone. This method seems to be particularly useful when the measurements of nitrate-N concentration are used to calculate the N leached by integrating them with estimates of drainage volume between successive samplings, or by changes in soil moisture readings taken simultaneously using soil moisture probes (Moreno et al. 1996; Vazquez et al. 2005, 2006; Farneselli et al. 2007b). The accuracy of the resulting load assessment greatly depends on the hydraulic conductivity of the soil and the evapo-transpiration of the crop. The nitrate concentration component is affected mainly by the accuracy in sampling the soil solution, which is affected by the resident soil nitrogen pools and applied fertilisers or manures. The different sampling methods of the soil solution may sample the nitrate from the two sources in different proportions, and may sample different pore sizes of soil water, and therefore results are most reliable when incorporated into long-term monitoring programmes with replication (Kerft and Zuber 1978; Lord et al. 2007). However, due to difficulties in maintaining good hydraulic contact between the soil and the ceramic (or similar) material, suction cups often do not operate well in chalk soils where water is held very tightly in the smallest pores. Another method of nitrate-leaching assessment could be to calculate the load by multiplying the $\text{NO}_3\text{-N}$ concentration in soil samples by the wetted soil volume (Farneselli et al. 2006b, 2007b). Results produced using this method can be useful in drip irrigation systems, where knowledge of the wetted zone volume can be gained by visualising soil water movement using soluble blue dye (German-Heins and Flury 2000; Simonne et al. 2003, 2005, 2006; Farneselli et al. 2006b, 2007b).

6.7 Research and Technology Transfer in European Union: Case Studies

EU States have applied the Nitrates Directive by developing research frameworks, funding specific research projects, and developing consulting committees, which have produced documents to help advising farmers on agriculture practices with

more sustainable environmental impacts. Some specific documents have been designed to address nitrogen and phosphorus management issues, whereas in others instances, case studies and initiatives have been more holistic and have focused on the management of a given crop or crop group. Several examples are given below, focusing on Mediterranean countries with specific case studies.

6.7.1 Italy

In Italy field vegetables in 2005 were grown in about 470,000 ha with a total production of about 13 million tonnes. Organic vegetable production was about 12,000 ha (i.e. about 1% of total land in organic cultivation) (Pimpini et al. 2005). Greenhouse production was about 34,000 ha with a total production of 1.5 million tonnes (mainly *Solanaceae*, *Cucurbitaceae*, lettuce and strawberry). Total value of vegetable production was about €6.6 billion. Fresh markets represent the main destination of vegetables, but minimally processed vegetables show the highest rate of increase (about +17% year⁻¹). Vegetable production is widespread in all the regions even if production from the south are mainly destined for the fresh market while those from the north mostly go to processing (except Puglia region in the south that is the lead area for processing tomato with about 30% of national production).

Peculiar characteristics of vegetable production in Italy are the small farm size (c. 1.7 ha) with two to three crops per year in a wide range of crop combinations (e.g. pepper–fennel–spinach; early potato–tomato–fennel; tomato–French bean–cauliflower; peas–beans–spinach; carrots/peas–chicory; tomato/zucchini–fennel/salads; potato–eggplant) and products destined for the fresh market. Large farms are not frequent, with cropping systems usually simpler and oriented around the food industry (e.g. processing tomato; spinach or peas for frozen food), well-mechanised and with use of external manpower.

Due to the Mediterranean climate, spring–summer vegetables are always irrigated, often using saline or partially saline waters. This has pushed towards the more widespread use of low-pressure irrigation systems, which also produce little or no risk of leaching.

According to a study from the Istituto Sperimentale per l'Orticoltura (Research Institute for Horticulture), published as an integration of the PANDA framework, Italian vegetable production is a strange dichotomy: horticulture is the most highly productive agricultural sector (on a gross income basis) after beef, but it is also associated with the smallest average farm investment in terms of land use.

A large framework project (Produzione Agricola Nella Difesa dell'Ambiente, PANDA) has been carried out since 1996 in Italy to develop environmentally sustainable agricultural technologies. The whole framework deals with soil resilience, pollution from agriculture, and pollution from non-agricultural sources. The PANDA project comprised three elements (Environmental vulnerability, Field trials, Analytical systems), which did not explicitly cover vegetable production, but which included related technical management practices. Great importance was paid

to soil protection, which was judged as the most critical environmental factor in the Mediterranean area. Among the aims of PANDA was an inventory of areas vulnerable to inputs of nitrogen and other nutrients from agriculture as well the design of a Code of Good Agriculture Practice ('Codice di buona pratica agricola') for Italy according to the framework given in the Nitrates Directive. Research projects were undertaken on irrigation and fertiliser management with special attention to nitrogen and phosphorus inputs from organic sources including biomass and livestock effluents (Mastrorilli 1999). The field experiments were focused mainly on cereals, or mixed cereal and dairy/beef systems, and in smaller scale on peach and citrus fruit systems. Considerable emphasis was given to modelling studies for several different example crops (Francavigli and Benedetti 1995) and at a larger scale for regional assessment of pollution from agriculture (Coccatto and Di Luzio 1996; Boatto et al. 1996). The Good Agricultural Practices designed within the PANDA framework was adopted by the Italian government (DM 19/04/99) as a general framework for the rules designed at regional level for each crop. In Italy, each regional government is responsible for the application of environmental and agricultural EU Directives. Concerning the inputs of nitrogen, the code gave very general background information and proposed accounting for the nitrogen already present in the soil or returned in crop residues when calculating the N requirement for the next crop. The code does not detail sampling methodologies or specific analyses, or the use of DSS at farm level. For some open field vegetable crops, the suggested amounts of nitrogen input (kilograms per hectare) for standard expected yields (tonne per hectare) are provided in Table 6.5.

A national advisory system on vegetable fertilisation does not exist, but instead there is an advisory service at a local level through farmer associations and local governments. The local network provides the farmers with recommended amounts of nutrient inputs for each growth stage using results from monitoring trials. The codes for Integrated Production applied by each Regional Government often include Nutrient Balance Systems (NBS) for calculation of the fertiliser crop

Table 6.5 Suggested N inputs based on standard yields for different vegetable crops in Italy (http://www.politicheagricole.it/norme/mezzitec/19990419_DM.htm)

Species	N-fertiliser		Species	N-fertiliser	
	requirement	Target yield		requirement	Target yield
	kg N ha ⁻¹	t ha ⁻¹		kg N ha ⁻¹	t ha ⁻¹
Garlic	120	12	Asparagus	180	5
Carrots	150	40	Artichokes	200	15
Onions	120	30	Cabbage	200	30
Rape	120	25	Broccoli	150	15
Cucumber	150	60	Melon	120	35
Watermelon	100	50	Sweet pepper	180	50
Strawberry	150	20	Tomatoes	160	60
Aubergine	200	40	Courgette	200	30

requirements. However, several researchers consider that a more 'scientific' approach is needed with a monitoring network for soil mineral nitrogen (N_{\min}) and the use of DSS within the local advisory services due to the high variability of Italian soils and climates.

Extensive programmes of research and monitoring on nitrogen management in vegetable crops, mainly in open fields, have been undertaken since 1990 by several universities in Italy via EU (e.g. LIFE) and national (e.g. COFIN, PRIN, FISIR) framework projects. These research programmes have studied the effect of N-fertiliser rate, fertilisation methods and N-fertiliser source (i.e. mineral, organic and green manures) on growth and N uptake of the most important vegetables (i.e. processing tomato, lettuce, sweet pepper, aubergine, potato) to provide parameters needed to model growth and N uptake in vegetables, and indices to evaluate the nutritional condition of the crop, and the environmental risks associated with different cropping systems. Within this context, in June 2004, an ISHS international meeting 'Towards ecologically sound fertilisation strategies for field vegetable production' was organised by the Department of Agricultural and Environmental Sciences, University of Perugia (proceedings published in *Acta Horticulturae* 700, 2006) with about 100 participants from 26 countries throughout the world and 50 scientific contributions. In the conclusion of this symposium, it was noted that the development of sound fertilisation strategies has to take into account the needs and suggestions of, researchers, policy makers, farmers and consumers who have to interact with each other.

In Italy, fertigation is becoming the standard method of nutrient applications to vegetables in order to increase fertiliser-use efficiency and limit the risk of diffuse pollution via run-off and leaching. This technique is applied on about 70% of the open field production area. However, if the high nitrate content in irrigation water is not adequately taken into account in the calculation of N-fertiliser crop requirements, then this can lead to an over-fertilisation of vegetable crops. For example, in the South Lazio region, nitrate levels in water tables at 10 m depth can easily fluctuate between 50 and 300 mg L⁻¹ (V. Magnifico, personal communication).

6.7.2 *Spain*

The highly differentiated climate present within Spain, coupled with large differences in soil types, results in high spatial variability in nitrogen-fertiliser requirements and use. Considering scientific literature and statistical data, in Spain only around 35% of the total N applied is effectively used by crops, which is much less than the global average efficiency of around 50% (Soler-Rovira et al. 2005).

The Autonomous Communities (Spanish local governments) are the main authorities responsible for implementing the Nitrates Directive (91/676/EEC) including the associated codes of Good Agricultural Practices relevant for their areas. However, the responsibility to carry out and implement agricultural and environmental

research is shared between the Spanish government, Autonomous Communities and universities, sometimes with the collaboration of private companies.

In order to group all researchers and projects about nitrogen in agriculture and to properly disseminate their results, in 2002 the Spanish government, many Spanish universities, and Autonomous Communities Research Centre created the Network of Efficient Use of Nitrogen in Agriculture (RUENA), (Red del Uso Eficiente del Nitrogeno en la Agricultura). The aims of the network are (i) to provide a forum for all people investigating the efficient use of nitrogen fertilizer in agricultural systems and (ii) to create a 'round table' to support the development of consensus and consistent recommendations concerning the management of nitrogen fertilizer applied to crops. The RUENA network is involved in the development of all relevant European, National and Regional legislations on nitrogen in agriculture, including those concerning fruit and vegetable crops, and includes researchers and institutions specifically involved in such area of study. A specific website (<http://www.ruena.csic.es>) provides information on the current and past projects, publications, and contact details for the thematic area of nitrogen use in agriculture.

Before RUENA, Spain had developed some national research projects on the correct use of nitrogen in agriculture such as the 'Dynamic of nutrients and improvement of fertilisation techniques in citrus trees' (1993–1996); the 'Monitoring of nitrate contamination in aquifers in Jarama river basin' (1992–1995); 'The efficient use of water and nitrogen in horticultural crops in the open air by application of plastic padding and fertigation' (1998–2001) and 'The application of pig slurry to olive crops' (1998–2000). Under the RUENA umbrella, there are currently several framework research programmes relevant to the use and misuse of nutrients in agriculture, including some dealing with the environmental impact of vegetables and fruit crops.

Within the RUENA framework projects, investigations identify optimum nitrogen-fertiliser rates and timings to obtain optimal yields and harvest quality, and to limit potential risks of nitrate leaching to water bodies. The main aspects include studies on the spatial and temporal distribution of nitrogen fertilisers applied to crops, the methods of quantifying nitrogen demand, the role of crop rotations in N requirements and the use of models to predict fertiliser nitrogen requirements. However, current activities are focused on maize crops, which have the highest rate of nitrate leaching due to the common practices of applying nitrogen at a rate 2 or 2.5 higher than the recommended amount. In this same framework, the Agricultural Research Technologic Institute of Calaluña (IRTA) jointly with Fundació Mas Badia (Estació Experimental Agrícola) and regional governments have developed the 'Programme to improve nitrogen fertiliser use in agriculture in Baix Empordà (Cataluña)' (Plan Pilot per la Millora de la Fertilització Nitrogenada a L'agricultura del Baix Empordà). The research objective is the identification of optimal nitrogen rates, maintaining high crop yield and quality, but minimising the negative effect to the environment (F.D. Olivé, personal communication). Recently, this programme has been extended to cover horticultural crops and fruit trees.

Several investigations were carried out on plant demand for nitrogen, optimal timing for nitrogen-fertiliser applications, the most efficient use of irrigation

systems in nitrogen application to crops and nitrogen supply in the soil. The design and development of a software family to manage sustainable irrigation systems (or ADOR: ‘una familia de programas de ordenador para la gestión y la planificación del uso del agua de riego y sus implicaciones medioambientales’; <http://web.eead.csic.es/oficinaregante/ador>) have been carried out within this framework since 2001 by researchers from Estación Experimental de Aula Dei (CSIC), technical personnel from Aragon Regional Government, and Aragon farmers, funded by the Spanish Government. The ADOR software helps farmers to manage irrigation systems by planning the irrigation season, and supporting cost analysis evaluating the opportunities to modernise irrigation facilities. A large database was implemented to support the software, which can be used with any irrigation system (surface, drip, sprinkling) and in any water distribution net (canal or piping). No information is available on the dissemination of the software and its use by farmers and its impact on the current practices. However, comments collected by researchers in the field suggest that even when farmers were involved, they did not adopt new practices until there was an economic incentive to do so.

In the main vegetable production areas of Spain (i.e. Aragon, Valencia, Murcia, Extremadura, Andalucía, Aragon, Rioja, Cataluña, Navarra), prior to the introduction of the Nitrates Directive legislation there was a general lack of consideration by farmers about the environmental problems associated with nutrient leaching caused by irrigation and fertilisation. This led to a high level of mineral nitrogen (from 173 to 232 kg ha⁻¹) in the soil profile (0–90 cm depth) and to the subsequent high risk of groundwater contamination by nitrate leaching (Gimenez et al. 2001). The unwillingness of the farmers to comply with this Directive suggests that the situation even after its implementation remains unchanged; however, no monitoring studied has been so far carried out to effectively quantify the real impact of the Nitrates Directive.

More recently, however, several monitoring studies have been implemented to assess the potential and actual contamination caused by nitrogen applications to agricultural soils. The results of those activities have highlighted problems in several horticultural and fruit regions. In the AC of Valencia, Ramos et al. (2002) has shown that around 8% of the Valencia Community population have water supplies with nitrate concentrations above 50 mg L⁻¹. This is confirmed by the studies of the Instituto Valenciano de Investigaciones Agraria (IVIA), which demonstrated that agricultural nitrogen inputs were much higher than the values recommended by research, and that nitrate leaching values were in most cases within the range of 150–300 kg N ha⁻¹. In the Valencia region, GIS/modelling studies (De Paz and Ramos 2001) on a typical 2-year crop rotation (potato-lettuce-onion-cauliflower) showed that the whole open field vegetables area of about 230 km² in the North of Valencia is at high risk of nitrate leaching due to the lack of awareness of farmers on the risks posed by excessive fertiliser N applications. As shown in Table 6.6, the N-fertiliser rates applied to vegetable crops in Valencia are higher than actual N crop requirements. Artichoke, early potato and onion were the three crops with higher leaching rates than other crops. From these crops, nitrate leaching typically varied between 240 and 340 kg N ha⁻¹ depending on the nitrogen-fertiliser treatment,

Table 6.6 Crops and N fertilizer applied and N uptake by crops (kg ha^{-1}) in AC of Valencia (Ramos et al. 2002)

Crop	N fertilizer applied	N crop uptake
	kg N ha^{-1}	kg N ha^{-1}
Artichoke	470 ± 260	130–210
Onion	500 ± 280	110–210
Lettuce	460 ± 210	45–54
Potato	700 ± 450	180–270
Pepper	1030 ± 630	180–270
Tomato	940 ± 245	225–365
Cauliflower	220	40–310

Table 6.7 Maximum values of nitrogen applied in vegetable crops in Andalucia, Spain

Crops	N rates
	kg N t^{-1} of yield
Artichoke	11.5
Asparagus	5.0
Aubergine	11.5
Broad Bean	11.5
Cabbage	11.5
Carrot	5.0
Cauliflower	5.0
Courgette	11.5
Cucumber	2.6
Garlic	6.8
Green beans	11.5
Lettuce	5.0
Melon	3.5
Onion	3.5
Peas	11.5
Pepper	5.0
Potatoes	4.2
Tomato	3.5
Watermelon	3.5

representing about 66–70% of total N input in the onion crop and 38–65% of total N input in the potato crop (Ramos et al. 2002).

Also in Andalucia (Table 6.7) and Navarra (Table 6.8) N crop requirements recommended by the codes for Good Agricultural Practice are by default increased by the farmers who wish to apply additional fertiliser as a safety margin to guarantee high yields.

In Almeria province (Andalucia region), there are approximately 25,000 ha of plastic greenhouses used for intensive vegetable production and which represent a significant potential source of nitrate leaching. Studies carried out on nitrate leaching from greenhouse pepper (Gallardo et al. 2006) showed that fertigation with a reduced

Table 6.8 Optimal N-fertiliser rates recommended for vegetable crops in Navarra, Spain

Crop	Cultural practices	Fertilisation (kg N ha ⁻¹)			No. applications during crop cycle
		Total rate	Before crop	During crop cycle	
Artichoke	First year with manure	160	0	160	2-3
	First year without manure	220	60	160	2-3
	Second year	80	0	80	1-2
Garlic	Sprinkling irrigation	180-200	60	120-140	3
	With manure	140	40	100	2
Aubergine		120-180	0	120-180	2-3
Courgette		145	45	100	2-3
Onion		160	60	100	2-3
Cauliflower		175-200	50	125-150	2-3
Brussels sprouts		180-200	50	130-150	2-3
Asparagus	Without irrigation	100	0	100	1
Spinach	With irrigations	180-200	0	180-200	2-3
	Sprinkling irrigation	140	40	100	3-4
Green pea		30	30	0	0
Melon		160	50	110	2-3
Potato		110	40	70	2
Pepper		100-130	0-30	100	2-3
Leek		150	50	100	2-3
	Mechanical harvest	90-110	40-50	50-60	2
Processing tomato	Manual harvest	190-200	70-80	120	2-3

concentration of N (i.e. 7–9 mmol N L⁻¹ = 168 kg N ha⁻¹) compared to the standard (i.e. 10–12 mmol N L⁻¹ = 194 kg N ha⁻¹) reduced nitrate leaching by 7%. Thompson et al. (2002, 2005) confirmed that in Almeria region the contribution to nitrate leaching to surface water was much higher, in terms of surface area, from open hydroponic systems than from conventional vegetable production in clay soils.

6.7.3 France

The former Agriculture and Environment Ministry of France, now Ministry of Ecology and Sustainable Development, has organised a consulting committee for dealing with pollution from agriculture (CORPEN) since 1984. This committee has been in charge of the Code of Good Practice since 2001, with special attention to N and P losses. CORPEN produces several documents and studies, some of which are focused on vegetable crops.

The French legislation applying the Nitrates Directive demands generally that the fertilisation must be done according to a nitrogen balance which accounts for irrigation water and soil mineral nitrogen (SMN). SMN is calculated differently according to the environmental conditions and crop type. Specifically for vegetable crops, it details different timings and amounts for the application of N fertilisers taking into account the soil organic matter contents, climatic regime and soil characteristics in different regions. CORPEN produced edited tables for the most common vegetables (beans, tomatoes, lettuce, etc.) where the N balance was described for the more common crop rotations used in France.

In some leguminous vegetables (e.g. peas), N fertilisation is largely avoided because the symbiotic N fixation is assumed to supply 75% of the vegetable crops' N requirements in French soils. Also, early sowing to increase the root depth and the use of catch crops has been suggested for vegetables with high N contents in their residues. The implementation of the Nitrates Directive in France is undertaken at Regional and Departmental (county) level after 'contracting' with the professional association of producers and advisers. Results have an extremely variable impact in relation to crops and regions with a little evidence of coordination and consistency at a broader national level. CORPEN has so far limited itself to scientific advice and providing communication between the different stakeholders.

Vegetable crops of primary interest for controlling nitrate leaching are tomatoes, lettuce, strawberry and melon in the South East; cauliflowers in Brittany, carrot in Normandy, Brittany and the southern part of Bordeaux (where sandy soils once used for intensive corn crops have been converted to high-quality carrot production). Soil-less and soil-based systems in glasshouses are widespread in the South West in the Pyrenees region.

In contrast to Italy, France has a strong national network of support and technical advice to the producers based on professional associations and local authorities. This allows an easier transfer of knowledge from main research institutions

(Universities and INRA) to farmers (mainly the units based at Rennes, Avignon and Montpellier). For fruit and vegetables this role is played by the CTIFL (<http://www.ctifl.fr>) jointly with regional research station and local agriculture associations (Chambres d'agriculture). This organisation offers support in designing sustainable fertilisation and rotation plans, and publishes fertilisation tables for the main vegetable and fruit production systems. However the efficacy of this knowledge transfer depends mainly on the dialogue between advisory services and producers' associations and this is extremely variable between different regions and departments.

In France, help to the horticultural sector is equivalent to only 5% of farm income compared to 50% of farm income for a farmer involved in grain production. This difference makes the regional associations more sensitive to the requirements of the Nitrates Directive. Many producers are too preoccupied with the introduction of a pest management initiative required under other EC Directives to have the time and will to tackle fertilisation issues. For example, in the processing tomato production area around Avignon, most farmers still apply 300 kg N ha⁻¹ when uptake is only 120–150 kg N ha⁻¹, largely because they perceive it as too complicated and potentially risky to switch to new, reduced N-input management. (P. Robin, personal communication). However, an alternative example is the intensive production of cauliflower in Brittany, which is traditionally based on widespread use of pig slurry. After long-standing pressure from the authorities and with the support of INRA in Rennes, producer associations suspended N applications for 5 years and are now slowly introducing a more rational approach to fertilisation based on N balance.

In the South West, only the more 'enlightened' producers in open field vegetables tend to use N-balance systems and some simple Decision Support System tools. In the 'Midi', the main area for melon, lettuce and chicory production, 80% of farmers use a sap test and 20% use an N-balance system for determining N-fertiliser crop requirements.

At national level, the original methodology promoted by CORPEN (before the Nitrates Directive) included a programme to monitor and advise farmers on their fertiliser management ('Fertimiueux'), which was on a voluntary basis and mainly tackled grain production. In the south-west of Normandy region in 2000, partially in response to the Nitrates Directive, an integrated management programme (i.e. 30% reduction of fertiliser rates, crop rotations with less vegetables and at least 30% of cereals, establishment of hedgerows) was introduced to reduce eutrophication of the coastal area: results showed a decrease of about 30% in nitrate concentrations in groundwaters (P. Robin, personal communication). Many technical advisers and researchers consider this scheme a good demonstration of a practical approach for the effective implementation of the Nitrates Directive. CTIFL and INRA are continuing similar trials on fertilisation management for the main vegetable crops, mainly tomatoes, cauliflowers and melon, in the Midi region and in Brittany.

The design of a sustainable N management for melon production has been the aim of joint research between INRA in Avignon and CTIFL-Balandran: a diagnostic

method (PILazo-melon) for N requirements based on petiole sap test has been successfully tested across a wide range of soil and climate in France on different varieties of melon (Le Bot, personal communication; Dumoulin et al. 2002a, b).

6.7.4 *The Netherlands*

In most parts of North and Central Europe, national advisory systems are all based on N_{\min} target values (Scharpf 1991b; Rahn et al. 2001). This, however, does not avoid the risk that amounts of nitrogen applied may exceed requirements, because of either the limitations of the method or the unwillingness of farmers to strictly adhere to the advice provided. The determination of soil mineral nitrogen often takes place in the autumn; although the long time lag between this sampling and the period of highest N demand can generate errors in estimating fertiliser requirements (Paschold et al. 2001) it can also provide a useful measure of the N potentially available for leaching following harvest of the previous crop. Improved systems (e.g. KNS, Nitrogen Balance System) which account for the N_{\min} level through the growing season, still sometimes overestimates the crop demand and requires large amounts of soil analysis and careful fertiliser management, which may result in a less user-friendly solution for the farmer. However, a similar approach in the USA (pre-side dressing nitrate test [PSNT]) seems capable of equivalent or better results by only measuring soil nitrate in the top 30 cm of soil just before the application (Hartz 2003). Those methodologies could be greatly improved if coupled with models specifically developed for vegetable crops, accounting for the potential N losses during the season as a function of weather conditions (EU_Rotate_N project newsletter 2003), which is definitely the most unpredictable factor involved (Paschold et al. 2001).

A completely different approach that avoids the use of models and can be an improved 'rule of thumb' for farmers to top-dress crops is the so-called Nil-N-plot system, based on the concept of 'unfertilised windows'. A 2-year test on 12 different vegetable fields in Germany (Weier et al. 2001) showed great differences due to the use of the N_{\min} system. The suggested application rates were from 20% lower to 10% higher than those calculated as a function of the amount of mineral N at the start of the season, but no yield decrease was recorded. This method may be a simpler way to take account of the effect of nitrogen released from crop residues during the growing season without the use of expensive soil analysis or complex mechanistic models, although soil mineral nitrogen testing certainly still retains value in situations where levels are expected to be high (e.g. in fields with a manure/slurry history, of fields following legumes, potatoes, etc.).

In the last 3 years, a worldwide network of researchers, mainly based in Germany and Quebec, have developed a set of recommendations to improve the N-balance approach as a main tool for controlling N leaching. The result of their efforts has been synthesised in a guide to sustainable nitrogen management in fruit and vegetable crops which is published on-line and has been designed to be updated to ensure continued relevance (Owen et al. 2003).

In the Netherlands, a group of regulations has been set up to support implementation of the Nitrates Directive and to consider the need to reduce ammonia emissions from agriculture. The main measure to reduce nitrate leaching is a ban on the spreading of animal manure, and to keep overall control of the nutrient input in agricultural systems, levies have been designed linked to annual surpluses of nitrogen and phosphorus (Neeteson et al. 2003). This system, originally known as the MINAS or MINerals Accounting System, was introduced in the 1998 with the aim to cut down in 5 years the allowable N surplus in grassland from 300 to 180 kg N ha⁻¹ and in arable land from 175 to 100 kg N ha⁻¹; in case of over-surplus, a levy is required of €2.3 kg⁻¹ of nitrogen and €9 kg⁻¹ of phosphate.

However, the system's compatibility with the Nitrates Directive was overestimated and the system was challenged by the EC. Several technical differences in nutrient balance persisted between MINAS and the Nitrates Directive: nutrient balance in the Nitrates Directive was fixed ahead of the crop cycle, in MINAS is calculated instead immediately before the grown season with the nutrient supplies from soil, crop residues, animal manure, atmosphere and biological N₂ fixation accurately estimated; however, the nutrient from manure generated in farm was not explicitly accounted. The system has been updated several times, but eventually was found to be incompatible with the Nitrates Directive and was ultimately closed in 2005.

To test the effect of these policies, a joint project ('Telen met toekomst' or 'Farming with a future') on four experimental farms and 33 commercial ones (where land management was based on the initial results from the experimental units) was established. Two main systems were tested: one, 'economically feasible', where nitrogen was applied according to measurements carried out by the NBS Dutch scheme, and second, 'environmentally desirable', where the nitrogen application was carried out with strategy tailored to the different farms with the aim of cutting down nitrogen inputs. The project also accounts for phosphorus inputs. The main aim was to explore if it was possible for commercial farming to reduce inputs over a 5-year period without a significant decrease in farm income (Neeteson et al. 2001). The overall results reported so far vary greatly between crop types and locations. However, the nitrogen surplus was still much higher than the target of 100 kg N ha⁻¹ in both systems. Even if the project continued into 2005, evidence reviewed so far suggests that decreasing nitrogen inputs in isolation is not sufficient to reduce N inputs to this target value, but this needs to be combined with site-specific management initiatives (e.g. timing, placement, variety) to help increase the nitrogen-use efficiency, even if these actions cause an increase in costs (Van Dijk and Smit 2006, Smit et al. 2005). The nitrogen inputs on the farms under the 'economically feasible' system were higher than the recommendations due to incomplete account of the nitrogen added in organic manures; the decrease in manure applications was also compensated by a slight increase in chemical N to avoid a yield penalty. However, the complete cessation of organic manure applications without replacing with fertiliser under the environmentally feasible system had no effect on yield. Under both the schemes low phosphorus inputs were applied and no yield reduction occurred.

Results from trials in leek fields (Neeteson et al. 2003) showed how operating under an ‘environmentally desirable’ management scenario induced a soil mineral nitrogen reduction of around 50% without any notable yield loss. However, the system used in this case to limit the N input was a fertigation scheme which cost about €1,000 ha⁻¹ more than the classical Nutrient Balance scheme. Further investigations (Radersma et al. 2005) also found that N-crop quick tests were more effective than N-soil quick tests for managing N split application in crop and decreasing N leaching.

6.7.5 *Final Considerations*

The technical impact of the most recent research on N management in fruit and vegetable production systems has been reviewed in relation to the implementation of the Nitrates Directive in some EU states. The state of knowledge in management practices is generally fairly advanced and there are tested methodologies supported by published data which allow a more sustainable horticultural sector without decreases in yield or quality. However, although there is still scope to refine and improve the technologies, the major challenge is disseminating results to farmers and farm advisers and promoting changes in farm management practices that minimise the risk of diffuse pollution from vegetable production systems.

Some issues, such as nitrate concentrations in surface water systems used for irrigation, are increasingly becoming an environmental pollution risk (e.g. in Spain and Italy, Padana valley). Measures such as more widespread use of drip irrigation systems have become more widely applied at field level over the past few years through the broader adoption of advanced technologies, which are more efficient in terms of water use. Other measures have had more limited success, including the farm-scale use of software tools (Decision Support Systems, DSS), the use of regular soil nutrient analyses, and the use of nitrogen probes and sensors.

In all the countries investigated, the farmers have rarely taken into account the suggestions evolving from the latest research, and they often continue to over-fertilise at levels between 20% (Italy, France) and 200% (Spain) above recommended levels. The high irrigation input required by some crops makes this behaviour increasingly dangerous for the environment. This is the case for crop systems such as tomatoes, strawberry in protected systems; aubergine, pepper and lettuce in open fields and citrus trees in Spain where immersion irrigation is still in use. In the case of open field crops in North France and North Italy, specific tests to measure N status in certain crops and the associated crop N requirement are still missing (cauliflower, carrots, cabbage, Brussels sprouts, spinach, onion). Simpler approaches to calculating N balances and N requirements, which may include soil mineral nitrogen testing, are still not as widely used as they could be to help estimate crop N requirements more accurately in these high-residue situations. The amount of leaching from vegetables crops varies greatly across the countries reviewed. For example, glass-house tomatoes in south France can leach up to 1,000 kg N ha⁻¹ (Le Bot et al. 2001).

Although leaching is much less from similar systems in south and central Italy, it is still sufficient to contribute to nitrate levels in water tables fluctuating from 50 to 300 mg l⁻¹ (V Magnifico, personal communication). The evidence reviewed suggests that open field vegetable crops in southern Europe can leach 100–300 kg N ha⁻¹ year⁻¹ from the soil root zone towards groundwaters depending on the soil, precipitation, irrigation, and management factors.

6.8 Conclusion

This review has described advances in N management to reduce nitrate leaching applied to vegetable production and their effective application. Areas of research where further investigations are required have been recognised such as (i) relation between crop residues and following crop management, (ii) prediction of the net effect of the different components of the soil N cycle on the soil-plant system, (iii) farmers' perception of N leaching as monetary loss, (iv) creation of spatial statistical maps of soil mineral nitrogen, (v) relation between N plant status and N plant effective demand.

Current research in nitrogen management aims to design the most effective N-fertiliser management systems that are able to produce a profitable and high-quality yield together with a more sustainable environmental 'footprint'. This research combines investigations on nitrogen soil dynamic and its use by crops, focusing on understanding the merits of alternative methods, tailored to each crop, climate and soil, for assessing (i) the effective plant N requirement; (ii) the soil availability of N which the plant roots can access; (iii) the associated losses, mainly due to nitrate leaching, which can be particularly high in field vegetable crops where irrigation is required. This fundamental research is currently leading to sophisticated technical solutions such as (i) innovative measurement instrumentations and methods, (ii) computerised tools for management and simulation of 'if then' scenarios, (iii) new crop-management systems. However, these advances are not always implemented by farmers at the scale required to produce an effective and lasting impact on the environment. The proper N budget, which all these techniques allow, implies an increase of available data from local datasets, whose realisation is demanding in terms of time and finance. The empirical approaches are generally still preferred because they can be more reliable for specific local conditions when detailed data are missing. Moreover, some tools (the so-called decision support systems) are generally not sufficiently user-friendly for farmers; they have been designed for farm advisers and agronomists, who are professionally qualified to choose from the large number of available techniques and methods and interpolate their results using their own experience tailored to specific regional conditions.

Recent advances in agronomy such as improved irrigation timing schemes, localisation of fertiliser applications in time and space and the combination of these elements in fertigation schemes where a crop calibration frequency is a key point all appear effective for decreasing N leaching without yield losses. In some specific

cases, the use of slow-release fertilisers or nitrification inhibitors have also yielded encouraging results, but their more widespread use is difficult to generalise. In many cases, other more classic agronomical methods such as catch crops, mulching and intercropping that are unappealing for conventional farming due to the increased input of time and resources needed can be considered when the higher value of yield can justify the increased inputs as occur sometime in organic farming.

Finally, it must be noted that there is a natural limit on our ability to minimise nitrate leaching, which is governed by plant physiology, soil characteristics and weather conditions; even with the most advanced cultural tools a sustainable but still profitable management of field vegetable is not always within reach and so the only option left can be a land use different from vegetable crop.

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Chapter 7

Manure Spills and Remediation Methods to Improve Water Quality

Shalamar D. Armstrong, Douglas R. Smith, Phillip R. Owens, Brad Joern, and Candiss Williams

Abstract Within the last 2 decades the transition in livestock production technology and intensity has resulted in an increase in annual livestock production and a drastic decrease in the number of livestock operations. Consequently, the susceptibility of current livestock operations to experience manure spills is far greater relative to livestock farms 20 years ago, due to increased herd size per farm. Therefore, manure spills in agricultural communities have become a pervasive issue and have led to the catastrophic contributions of nutrients and pathogens to surface and groundwaters, human health issues, and large fish kills. Furthermore, the current remediation methods for manure spills that reach surface waters focus on mitigating contaminants in the water column and give no attention to the manure-exposed ditch sediments that remain in the fluvial system and continue to impair the water column. Therefore, this chapter addresses the causes, environmental impacts, and current and alternative remediation methods for manure spills in agricultural streams. Geographic data suggest that the location of animal-feeding operations and the occurrence of manure spills were highly correlated with the location of tile-drained agriculture fields. In addition, at least 14% of reported manure spills were separately attributed to the failure in waste storage equipment and over-application of manure in the states of Iowa and Ontario, Canada. Evaluations of the downstream impacts of manure spills have reported ammonia, total phosphorus, and total N concentrations that were at least 28 times the average upstream concentrations before the spill occurred. Studies have also determined that the current manure spill remediation method results in soluble phosphorus and nitrogen concentrations significantly greater than the Environmental Protection Agency total phosphorus nutrient critical limit, 24 h after

S.D. Armstrong (✉), P.R. Owens, B. Joern, and C. Williams
Agronomy Department, Purdue University, 915 West State St., West Lafayette,
IN 47907-2054, USA
e-mail: sarmstro@purdue.edu

S.D. Armstrong and D.R. Smith
USDA-ARS, National Soil Erosion Research Laboratory, 275 South Russell St.,
West Lafayette, IN 47907, USA

the plume of the spill has passed. However, supplemental treatment of manure exposed sediments resulted in at least a 50% decrease in the soluble phosphorus concentrations which was in compliance with the phosphorus nutrient criteria.

Keywords Manure spills • manure spill remediation methods • alum • ammonium • phosphorus • sediments

7.1 Introduction

Surface and groundwater degradation from agricultural losses of nitrogen and phosphorus are global environmental issues. Throughout the world, the consequence of nitrogen and phosphorus losses from agricultural fields to enriched waterways is realized in hypoxia zones such as the Gulf of Mexico (Alexander et al. 2008), the Black Sea (Tolmazin 1985), and the Baltic Sea (Rabalais et al., 1999). Thus, the US Environmental Protecting Agency has identified agricultural drainage, both surface and subsurface, as the primary source of nutrient losses to freshwater systems in the USA (USEPA 1995). Environmental studies have also found that manure spills are a major source of nutrient loading to agriculture streams and that the use of livestock manure in agricultural practices has contributed to 15% of the nitrogen loading in the Mississippi River drainage basin that discharges into the Gulf of Mexico (Hoorman et al. 2005; Ribauda et al. 2003). Therefore, this chapter focuses on the causes, impacts, and current and alternative remediation methods of manure spills.

In the USA, between the years 1982 and 1997, the number of livestock per feeding operation increased by 10%, while the number of feeding operations decreased by 50% (Gollehon et al. 2001; Fig. 7.1). Between the years 1980 and 1995, the number of swine farms in the Netherlands decreased by 32%, while swine production increased or remained constant (van der Peet-Schwering et al. 1999). Similarly, in France there was a 25% increase in swine production between the years 1985 and 1995 and the province of Brittany accounted for 55% of swine production within a land area that was only 6% of the total agricultural land of France (Dourmada et al. 1999). This trend is a reflection of the industrialization of livestock production that has increased production efficiency, the quantity of manure produced daily, and the pressure applied on the related manure-management systems (Fig. 7.1). Consequently, the occurrence of manure spills in agricultural communities and the degradation of surface and groundwater have become more prevalent and have led to the contribution of nutrient and pathogen loading to source, surface and groundwaters globally (Burkholder et al. 1997; Mallin 2000; Hoorman et al. 2005). Therefore, nitrogen and phosphorus contamination of surface and groundwaters have been heavily associated with intensive livestock production, whether gradually through feedlot runoff events and leaching of waste lagoons, or catastrophically through animal waste spills. For example, animal feeding operations have contributed to the impairment of 50% of the lakes and 20% of the rivers in the USA (USEPA 2003).

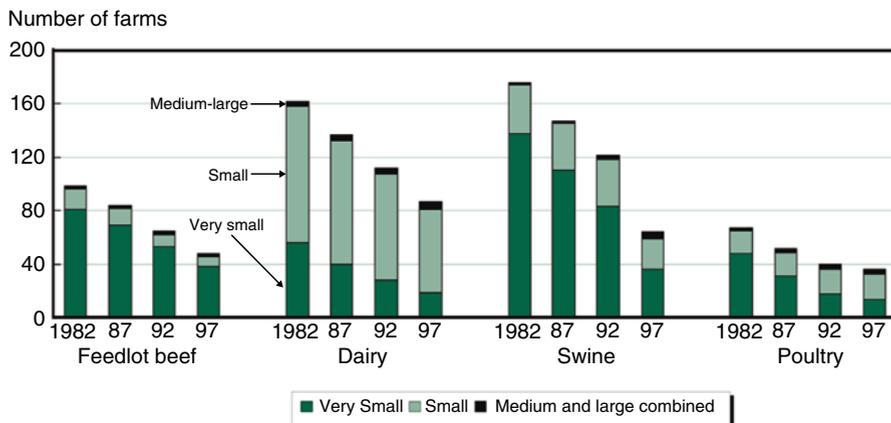


Fig. 7.1 The results of an assessment of confined animal farms by species and size within the years 1982–1997. The findings clearly illustrate the decline in very small and small livestock farms and the emergence of medium-to-large livestock operations (Gollehon et al. 2001)

Nitrogen and phosphorus losses from manure spills pose a significant threat to the health of humans and aquatic ecosystems. Manure spills have been found to result in nitrate contamination of groundwater and source water that leads to methemoglobinemia (Blue-baby syndrome) (Townsend et al. 2003). A survey of nutrient levels in the groundwater of the USA found that 9% of rural wells and 1% of community wells had concentrations of nitrate–N greater than the 10 mg L⁻¹, which is the maximum contaminant level for drinking water (Mueller et al. 1995). They also found that in areas near intensive livestock operations, wells were more likely to be contaminated above 10 mg L⁻¹ nitrate–N. In addition, manure spills from livestock operations have led to contamination of surface water and source water by *Escherichia coli*, *Campylobacter*, and *Cryptosporidium* that resulted in widespread diarrhea, vomiting, fever, and even death (Guan and Holley 2003; Hoxie et al. 1997). Aquatic ecosystems that receive excessive loading of nitrogen and phosphorus could lead to eutrophic conditions, due to nitrogen and phosphorus being nutrient that contribute to eutrophication in freshwater ecosystems (Correll 1998), fish kills from toxic levels of NH₃ and NH₄ (Mallin 2000; De La Torre et al. 2004; Kater et al. 2006).

7.2 Causes of Manure Spills

In addition to the drastic increased herd size per farm, government and state regulations affect the susceptibility of animal-feeding operations to experience a manure spill. The most current regulation that affects confined animal-feeding operations manure-management systems is the ruling made by the Environmental Protection

Table 7.1 Chemical analysis of various animal wastes, suggesting that total phosphorus (TP) is most prominent in swine effluent (Hutchins et al. 2007). TN: total nitrogen

CAFO type	NH ₄ -N (mg L ⁻¹)	TN	TP
Beef feedlot (sl)	33	63	14
Dairy (pl)	84	185	30
Poultry (pl)	656	802	50
Poultry (sl)	289	407	23
Poultry (tl)	58	96	30
Swine sow (tl)	944	1,290	264
Swine finisher (tl)	1,630	2,430	324
Swine nursery (tl)	1,370	2,040	368

Secondary lagoon (sl), primary lagoon (pl), tertiary lagoon (tl)

Agency in 2003 (USEPA 2003). This regulation requires confined animal-feeding operations and large animal-feeding operations to develop and implement a nutrient-management plan in conjunction with applying for a National Pollution Discharge Elimination System permit. The permit specifies how manure is managed and disposed on each qualified livestock operation. However, the pressing issue is that permit holders' nutrient-management plans must comply with the agronomic nutrient requirement of the crops in the fields where manure is applied. Therefore, the volume of manure disposed is restricted to a rate that cannot exceed the nitrogen and phosphorus demand of the receiving agricultural field. For swine producers, phosphorus is the nutrient that results in the greatest limitations of manure application rate, since swine waste contains more phosphorus relative to the phosphorus demand of most crops (Table 7.1).

Contamination of surface water through manure violations often occurs on tile-drained fields where liquid manure is applied (Kinley et al. 2007). The function of tile drainage is to provide a pathway for excess water from poorly drained soils to be removed from agricultural fields. However, tile drainage has become a conduit for nutrients to surface waters when liquid manure is applied in excess (Kinley et al. 2007). This issue is most documented in the Midwestern USA and Canada where agricultural tile drainage is necessary for crop production (Fig. 7.2), and coincidentally, confined animal-feeding operations are prevalent. For example, in the southern portion of Ontario, Canada, over 70% of the agricultural fields are tile drained (Spaling and Smit 1995) and at least 20% of the total area is tile-drained cropland in Midwestern USA such as in Indiana, Ohio, Iowa, and Illinois (USDA 1987). Furthermore, studies have demonstrated that the application rate of manure is the driving factor of phosphorus and nitrogen loss after liquid manure is applied in the presence of tile drainage (Ball et al. 2007; Cook and Baker 2001). It has also been noted that fields that contain macropores and shallow water tables are more susceptible to manure violations after land application (Steenhuis et al. 1994; Stone and Wilson 2006). Macropores such as root channels, worm holes, and natural soil cracks allow liquid manure to bypass the soil matrix to be intercepted by the tile drain that facilitates transport to surface waters (Watson and Luxmoore 1986).

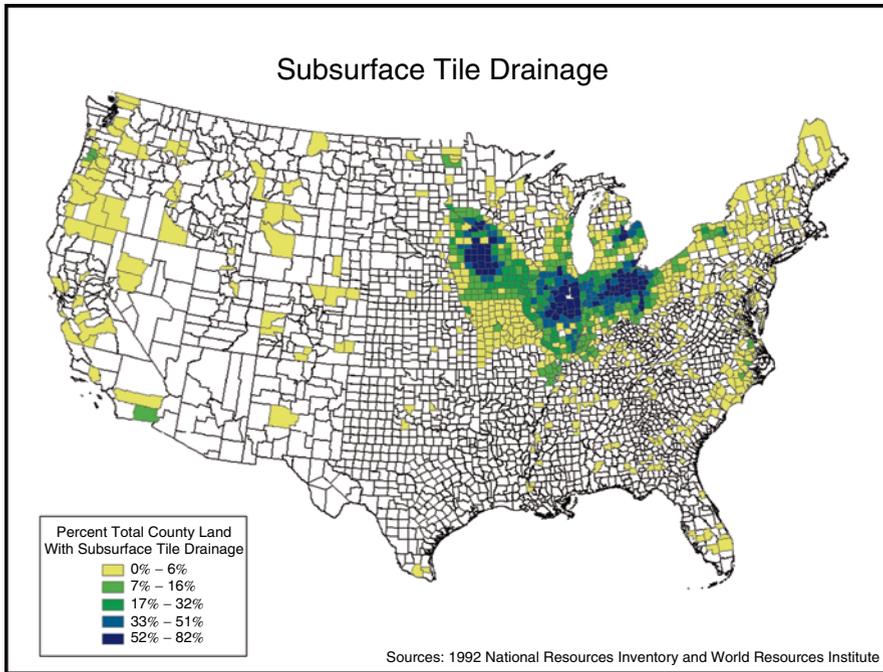


Fig. 7.2 The similarity in subsurface drainage density which is related to the density of swine density in the USA (Sugg 2007)

During a 1-year study Muller et al. (2003) monitored the concentrations of NO_3^- , NH_4^+ , and pH in tile flow in an agricultural field. As a result of a manure application and preferential flow they observed a spike in the load of NH_4^+ (22.0 g $\text{NH}_4\text{-N}$), which resulted in a daily nitrogen load that was 65% greater than before the manure application.

Hoorman et al. (2005) investigated the factors that caused manure violations in the state of Ohio within a 4-year period. They found that between the years of 2000 and 2003 there were 98 manure spills reported. Heavy precipitation after land application of liquid waste accounted for 41 of the 98 manure violations, making it a primary cause of manure violations. The next contributing factor was manure storage mismanagement and equipment failure (e.g., ruptured pipes, holes, and failure of on-site manure transport equipment) that accounted for 33 of the 98 manure violations. According to Osterberg and Wallinga (2004), in Iowa, from 1992 to 2002, 304 manure spills were reported. Both manure storage overflow and equipment failure were responsible for 24% of the spills, runoff from animal feeding operations accounted for 18%, and over-application accounted for 14% (Fig. 7.3). In Southwest Ontario, Canada, 229 manure spills were reported between the years of 1988–1998. Spray irrigation application accounted for 40%, insufficient storage accounted for 16%, and equipment failure was the cause of 14% of the manure spills (Fig. 7.3, Merkel 2004).

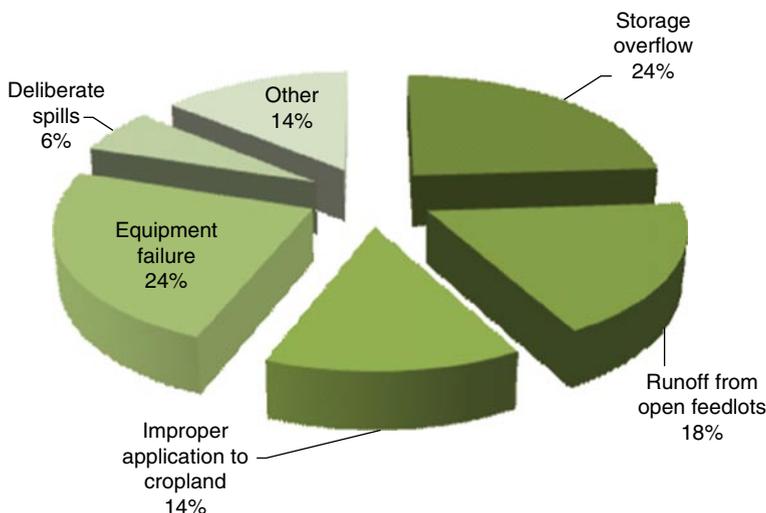


Fig. 7.3 The causes of major manure spill in Iowa, leading swine producing state, between the years 1992 and 2002 (Osterburg and Wallinga 2004)

In February 2008, a manure spill in Quebec, Canada resulted in the release of over 26,400 l of liquid cattle manure that drained into a nearby creek and contaminated a neighboring domestic well. This spill was caused by a broken valve on a pipe that was used to transport manure to a manure storage tank (Johnston 2008). Similarly in February of 2008, a 6 in. pipe on a cattle farm in Walkersville, MD resulted in approximately 21.8 million liters of manure to be pumped into Glade Creek and the contamination of the town's water supply, leaving citizens to boil their drinking water or purchase bottled water for 2 weeks. Bacteria counts in surface water and groundwater were 57 and 20 *E. coli* per 100 ml, and both were significantly higher than the drinking water standard of one bacteria colony forming units per 100 ml (Hauck 2008).

7.3 The Impact of Manure Spills

Unintentional manure spills in the past have impacted both aquatic ecological systems and human health (Novak et al. 2000; Mallin and Cahoon 2003; Mead 2004). Manure spills are large contributors of nutrients and pathogens, which are two of the top three water impairments in the USA, according to the Environmental Protection Agency (USEPA 2000). As mentioned previously, the excess of nutrients and pathogens leads to elevated biochemical oxygen demand (BOD), fish kills, and accelerated eutrophication (USDA 1997; Frey et al. 2000) and manure spills can lead to catastrophic loading of these water contaminants. According to Indiana department of environmental management (1995), agricultural feedlots are one of the possible sources

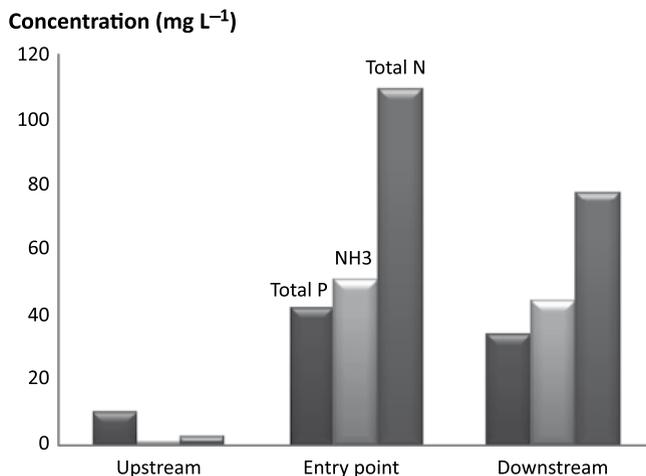


Fig. 7.4 Average concentration of total N, P, and ammonia from upstream, entry point, and downstream sampling location of 97 manure spills (Hoorman et al. 2005)

of *E. coli* contamination, which has been identified as being responsible for over 80% of 6,451 river miles in Indiana being declared unsafe for swimming or human contact. Hoorman et al. (2005) collected upstream, entry point, and downstream water samples during investigations of 97 manure spills (Fig. 7.4). The results of this study suggested that the downstream impacts of manure spills are catastrophic. The average downstream ammonia, total phosphorus, and total nitrogen concentrations after a manure spill were 49, 28, and 68 times greater than the average upstream concentrations, respectively (Fig. 7.4). Contamination of water with bacteria, such as *E. coli* and salmonella, are commonly associated with manure spills and when ingested, bodily illnesses such as hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura may occur (Thu 2002). In addition, nitrate losses from manure spills pose a significant threat to the health of humans through the contamination of groundwater and drinking water (Townsend et al. 2003).

Nitrogen from a manure spill has an immediate impact on fish and benthic organisms through ammonia toxicity. Manure spills contribute excessive concentrations of nitrogen, and cations such as Ca^{2+} and Mg^{2+} that increase pH creating optimum conditions for ammonia toxicity (Poxton 2003). Studies have demonstrated that ammonia toxicity in fish and benthic organisms occur under alkaline conditions where the acid base reaction between OH^- and NH_4^+ produces toxic concentrations of NH_3 (Kater et al. 2006).

In addition, studies have indicated that at $\text{pH} < 8.3$ both NH_4^+ and NH_3 contribute to toxicity (Scholten et al. 2005). Fish naturally excrete metabolic NH_3 concentrated waste from their blood through diffusion to the water column (Kater et al. 2006). However, this diffusion of waste will only occur when the concentration gradient of NH_3 is greater in the blood of the fish relative to the water column. Additionally,

if the NH_3 concentration in water column becomes elevated enough, the concentration gradient could reverse, and NH_3 has the potential to be actively transported into the organism through an exchange with Na^+ on the gills of the fish (Kater et al. 2006). Therefore, after the occurrence of a catastrophic manure spill, the NH_3 excretion of fish is inhibited and toxic levels of NH_3 builds up within the fish, which ultimately leads to severe ammonia toxicity and high fish mortality.

Initial phosphorus loading from a manure spill and phosphorus desorption from manure-exposed sediments in streams and drainage ditches can lead to accelerated algal blooms and enhanced eutrophic conditions in receiving lakes, ponds, and reservoirs. Lakes and ponds receiving elevated phosphorus additions from manure spills could result in explosive algal blooms and the growth of other aquatic plants that eventually cover the water surface. After the death of the algae and aquatic plants, decomposition occurs through microorganisms that consume large fractions of dissolved oxygen (Scholten et al. 2005). This oxygen depletion ultimately leads to reduced oxygen supply for fish and benthic organisms, reduced growth of benthic organism, and fish kills. Moreover, carbon loading in fluvial systems can also result in oxygen depletion due to increased microbial activity and high oxygen consumption by microorganisms.

7.4 Current Manure Spill Remediation Methods

Currently, the recommended emergency response actions for manure spills that contaminate a drainage ditch or streams are (i) to contain and isolate the contaminated area using earthen or temporary dams, (ii) de-water the contained area using pumping equipment, and (iii) redistribute the recovered waste into an alternative storage system or to land-apply the waste in compliance with state regulations (IDEM 2002). However, the major inadequacy of the conventional spill remediation plan is the lack of attention given to the phosphorus-enriched ditch sediments that have been exposed to manure and remain in the fluvial system. Studies have demonstrated that phosphorus and nitrogen desorption from untreated contaminated sediments continue to impair the water column for weeks, after the spill has occurred.

For example, Burkholder et al. (1997) evaluated the impacts of a manure spill from a farm in Onslow, North Carolina, that released 97.5 million liters of swine manure into the surrounding drainage ditches. This spill resulted from heavy precipitation from a hurricane and faulty farm-operator management of manure storage. They found that the average total phosphorus concentration 2 days after the spill was 100 times greater than the total phosphorus average of $0.047 \text{ mg P L}^{-1}$ from the previous 10 months (Table 7.2). Furthermore, with continual sampling of the water column at 5, 14, and 61 days after the spill they observed that total phosphorus concentrations were 7.6, 2.1, and 5.5 times greater than the previous 10-month average, respectively (Burkholder et al. 1997). A possible explanation for elevated total phosphorus concentrations days and weeks after the manure spill had occurred could be that sediment phosphorus concentrations exist in equilibrium with the

Table 7.2 The ability of sediments to act as a phosphorus source for up to 61 days after a manure spill has occurred due to sediment phosphorus desorption (Burkholder et al. 1997)

Time	Phosphorus concentration (mg P L ⁻¹)
Ten months before spill	0.047
Two days after spill	4.79
Five days after spill	0.36
Fourteen days after spill	0.106
Sixty-one days after spill	0.29

phosphorus concentration of the overlying water column within a fluvial system. Therefore, these elevated phosphorus concentrations observed days and weeks after the contamination plume had passed clearly indicate that the sediments became significant sources of phosphorus thereby releasing phosphorus into the water column. In other words, the sediments that contained elevated concentrations of phosphorus, release phosphorus to the subsequent flow with low phosphorus concentrations to maintain equilibrium with the water column.

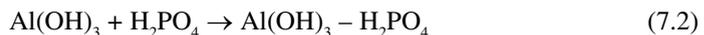
Burkholder et al. (1997) also found that the density of fecal coliform bacteria at 5, 14, and 61 (7.0×10^2 , 71.9×10^4 , and 1.2×10^3 colony-forming units) days after the spill was greater than the state standard of 200 colony-forming units/100 ml. Therefore, this could be evidence that fecal coliform bacteria is surviving for days after the spill and is being redistributed back into the water column.

7.5 Alternative Sediment Amendments

Environmental and waste management scientists have provided vital findings that demonstrated the efficacy of aluminum sulfate (alum) as a treatment to reduce phosphorus availability in manure storage, after land application of manure, in ponds and wetlands, and in phosphorus-enriched sediments that have been contaminated by waste water treatment plants (Ann et al. 1999; Dao et al. 2001; Steinman et al. 2004; Choi and Moore 2008). There are two proposed mechanism in which alum reduces the availability of phosphorus in manure, soil solution, and sediment pore water. The first is shown in equation 7.1 where aluminum disassociates from SO_4^- in solution and forms a coprecipitate with PO_4^- (Moore and Miller 1994).



The second proposed mechanism involves the formation of amorphous aluminum oxide that adsorbs soluble phosphorus from solution (Peak et al. 2002; Hunger et al. 2004).



Moreover, as time after phosphorus adsorption to amorphous aluminum oxide increases the formation of minerals such as varisite ($\text{AlPO}_4 \cdot 2\text{H}_2\text{O}$) and wavellite [$\text{Al}_3(\text{PO}_4)_2(\text{OH})_3 \cdot 5\text{H}_2\text{O}$] may form and persist under acidic conditions.

Sims and Luka-McCafferty (2002) conducted a large-scale on-farm poultry litter study where alum was amended to poultry litter in 97 poultry houses for a 16-month period. They found that alum amendment at a rate $1.0 \text{ kg alum m}^{-2} \text{ flock}^{-1}$ (approximately $0.09 \text{ kg alum per bird}$) decreased the dissolved phosphorus content in manure by 67%. Similar studies have also demonstrated that the addition of alum to poultry litter resulted in a reduction of phosphorus loss via runoff by as much as 52–87% using the following application rates 1:5 ratio of alum to poultry litter (Shreve et al. 1995); applications of 5%, 10%, 15% alum to poultry litter on a weight basis (Delaune et al. 2004); and 10% alum application by weight to poultry litter (Smith et al. 2004). Smith et al. (2005) investigated the effect of alum application on the phosphorus concentration and adsorption properties of sediments from tile-fed drainage ditches in an agricultural watershed in northeast Indiana. They determined that applying alum reduced the extractable phosphorus in sediments by 50–90% and the portioning index by 50% (Fig. 7.5). Haggard et al. (2004) evaluated the use of alum as a chemical amendment to sediments from streams that received a daily influx of phosphorus from a municipal waste water treatment plant's effluent discharge. Results from their study demonstrated that applying alum with CaCO_3 to phosphorus-enriched sediments resulted in a significant reduction in sediment labile phosphorus, equilibrium phosphorus concentrations, and a significantly increased in the phosphorus-buffering capacity of the sediments. The sediment equilibrium concentration is the concentration at which the net phosphorus adsorption and desorption of fluvial sediment is zero

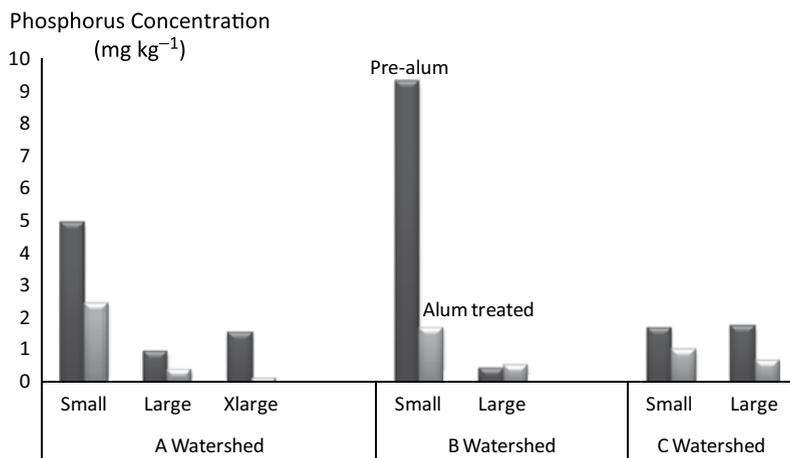


Fig. 7.5 The effect of alum application on the soluble phosphorus concentration of sediments collected from three watersheds within the St. Joseph River Watershed in North East Indiana (Smith et al. 2005)

(Taylor and Kunishi 1971) and the buffering capacity is a measure of the sediments ability to adsorb phosphorus per unit increase in phosphorus water concentration.

The efficacy of the current and an alternative manure spill remediation, where alum was used to reduce soluble phosphorus desorption from sediment following a manure spill were evaluated through a series of manure spills using fluvarium techniques. The manure spills were simulated for 24 h within a stream simulator using sandy and clayey stream bed sediments. The current manure spill remediation method was simulated by draining the contaminated water column, and uncontaminated water was circulated over alum treated and untreated sediments to simulate subsequent flow after the spill has occurred. Results from this study demonstrate that the current manure spill remediation method removes phosphorus from the contaminated water column, but does not adequately remediate manure exposed sediments that remain in the water column. Thus, sediments that received only the current manure spill remediation treatment desorbed soluble phosphorus in the water column to a maximum of 0.22 mg P L^{-1} which was significantly greater than the Environmental Protection Agency nutrient criteria for soluble phosphorus in that region. Furthermore, results suggested that a surface application of alum to clay and sandy sediments following a manure spill decreased phosphorus released from manure-exposed sediments by over 70% and mitigated the soluble phosphorus concentration in the water column below the Environmental Protection Agency nutrient criteria for phosphorus (Author's unpublished data).

Although the effectiveness of alum to reduce the availability of soluble phosphorus in sediments is well-known, the impact of alum on benthic organism is death. Steiman and Ogdahl (2008) studied the ecological effect of using alum as an amendment to reduce the phosphorus concentrations in Spring Lake, Michigan. The alum treatment was applied in 2006; data from an ecological assessment were collected eight months later, and were compared to a control (pretreatment) set of ecological data from the same lake recorded in 2003. In a laboratory experiment they found that the phosphorus flux from untreated sediments in 2003 was 43 times greater relative to sediments collected in 2006 after being treated with alum and that alum treatment reduced the mean pore water phosphorus and significantly reduced the extractable phosphorus. Additionally, they determined that the population of benthic invertebrates declined following alum applications, while Narf (1990) observed an increase in invertebrate density. Smeltzer et al. 1999 observed a decline in sediment invertebrate density 1 year after alum treatment, a recovery to the pretreated levels within 2 years, and a significant increase above pretreatment levels 10 years after the alum treatment.

7.6 Conclusion

Increased livestock production efficiency due to the emergence of new technology and confined animal-feeding operations has severely impacted the surface and source water of agricultural communities. Furthermore, it has been observed that greater

herd sizes per livestock operation have led to enormous volumes of waste produced daily and excessive pressure on waste-management systems to maintain waste storage capacity. As a result, in Ohio, 41% of manure spills that occurred during a 3-year period were attributed to lagoon breaches and excessive precipitation. In Iowa, 48% of manure spills within a 10-year period were attributed to manure storage equipment failure and lagoon breaches, and in Ontario, Canada 40% of manure spills that occurred within a 10-year period were due to over-application of animal waste through spray irrigation.

Data have also suggested that the current remediation plan for manure spills is efficient in removing the nutrient contamination in the water column following a manure spill, but was not effective in remediating the sediment of the fluvial system. Due to astronomical loading of phosphorus and nitrogen during a manure spill benthic sediments initially act as sinks and are saturated. However, when subsequent flow enters the fluvial system after the plume of the spill has passed, the sediment acts as a phosphorus source to water column due to greater phosphorus and nitrogen in the sediment relative to the water column. Studies of the manure spills have demonstrated that the water column total phosphorus 3 months after the passing of the manure spill plume was five times greater than the 10-month average total phosphorus of the water column. Therefore, supplemental treatment is needed to remediate the entire fluvial system following a manure spill. The uses of alum on a small plot and watershed scale to reduce the vulnerability of soluble phosphorus have been effective in reducing phosphorus in runoff by as much as 50%. Moreover, data from a manure spill simulation experiment determined that with a molar application of alum the phosphorus desorption following a manure spill was reduced by at least 50%. Results from the studies in this chapter have raised the awareness of the impact associated with manure spills in agricultural streams and have presented novel, practical, and affordable solutions that can be used to remediate surface and source water following manure spills.

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Chapter 8

Cropping Systems Management, Soil Microbial Communities, and Soil Biological Fertility

Alison G. Nelson and Dean Spaner

Abstract Consumers are demanding more organic products, in part because of concerns over environmental issues in conventional agriculture. Modern, high-input agriculture can cause groundwater contamination, soil erosion, and eutrophication of surface waters. It may be possible to enhance natural nutrient cycling and reduce our dependence on inorganic fertilizers in cropping systems. To do so, we have to manage our cropping systems to encourage diverse soil microbial communities and arbuscular mycorrhizal fungi. This chapter reviews the impacts of cropping management practices on soil microbial diversity and arbuscular mycorrhizal communities. Systems that have reduced tillage, diverse crop rotations or intercrops, low applications of inorganic fertilizers and pesticides, and some organic fertility inputs tend to encourage a large and diverse microbial community with mycorrhizal fungi. Organic systems should strive for minimum tillage and the avoidance of bare soil fallow in rotation. Well-managed conventional systems with minimum tillage and inorganic crop inputs can be as effective as organic systems in encouraging soil biological fertility. Both organic and conventional cropping systems should incorporate intercrops into their systems to encourage diversity within the soil system.

Keywords Diversity • arbuscular mycorrhizal fungi • organic management • conventional management • tillage • crop rotation • fertilizers and pesticides • organic farming • soil biodiversity • tillage • no till • crop rotation

A.G. Nelson and D. Spaner (✉)

Department of Agricultural, Food and Nutritional Science, University of Alberta, 4-10 Agriculture/Forestry Centre, Edmonton, AB, Canada T6G 2P5
e-mail: dean.spaner@ualberta.ca

8.1 Introduction

Consumers are becoming increasingly concerned with food safety, the presence of pesticides and genetically modified organisms in their grain products, and the negative environmental effects of conventional agriculture (Klonsky 2000). This increase in suspicion of industrial food production systems has, in part, translated into increased demand for organic food products. In Canada, there are now over 3,600 organic farms on more than 500,000 ha (Macey 2006). The organic market in Canada has been growing by 15–20% per year since the late 1990s, while the food industry overall has grown 2% per year 1992–2000 (Sahota et al. 2004; Klonsky 2000).

Consumers purchase organic food products because they perceive these foods to have unique attributes and/or superior quality attributes compared with conventional foods (Yiridoe et al. 2005). Modern, high-input cropping systems have created numerous environmental, social, and economic problems, including groundwater contamination, increased farm specialization, exacerbation of crop pest problems, soil erosion, energy dependency, high-input expenses, less farm economic resilience, and eutrophication of surface waters (Soule and Piper 1992; McRae et al. 2000). Organic systems of production are often believed to have lower negative environmental impacts than conventional systems, including maintaining biodiversity within the agroecosystem. However, many of the perceived attributes of organic products cannot be measured, and necessitate faith on the part of the consumer that the desired attributes are present (Ritson and Oughton 2007). Organic systems of production may or may not increase soil biodiversity.

Soil microbes play important roles in agroecosystems. This review is concerned with the microflora in the soil system, which are the smallest organisms in the soil and include bacteria, actinomycetes, fungi, and algae. The soil is a habitat for large numbers of diverse soil microbes. Within a gram of soil there can be thousands of millions of fungi and bacteria; about 95% of the species in the soil still remain unknown (Uphoff et al. 2006). Bacteria and archaea are single-celled microbes and have roles in organic matter decomposition, biological transformation of nutrients, as well as some plant, animal, or other soil microbe symbionts. Fungi are present in many forms in the soil, and have many roles within the soil system. These roles include plant or animal symbionts, organic matter decomposition, soil aggregation, plant and animal pathogens, etc. Actinomycetes are a particular form of prokaryote whose morphology resembles that of fungi; they have roles in soil aggregation, production of antibiotic compounds, organic matter turnover, and nitrogen fixation (Brady and Weil 2002). Algae have roles in the cycling of carbon, nitrogen, and water; stabilizing soil, and forming symbiotic associations with plants (Belnap 2005).

Soil fertility refers to the soil's ability to supply nutrients to crops (Watson et al. 2002). Soil microbes affect soil fertility in many ways, including plant symbioses with arbuscular mycorrhizal fungi and *Rhizobia* bacteria, organic matter turnover, mineral immobilization and dissolution, and soil aggregation (Davis

and Abbott 2006). Managing soil biological fertility may be a key to successful sustainable agricultural systems producing high-quality food products (Lee and Pankhurst 1992).

For environmental and economic reasons, in addition to market demand, improvements in cropping systems and the food products they create must be achieved through improvements in the efficiency of natural nutrient cycling and not through the use of additional inputs (Patriquin 1986; Yeates et al. 1997; Galvez et al. 1995). Soil microbial communities have a large role in nutrient cycling, and can be affected by agricultural management practices. It may be possible and feasible to tailor cropping systems management to encourage diverse microbial communities and specific beneficial microorganisms, and thereby promote efficient nutrient cycling and plant nutrient uptake. This chapter will discuss some of the roles of soil microbial diversity and mycorrhizae in nutrient cycling and plant nutrient uptake, and review the literature on the effects of management practices on soil microbial diversity and mycorrhizal colonization in agricultural systems. We will then examine the impact of combining the reviewed management practices on microbial diversity in organic and conventional cropping systems. We will discuss the feasibility of managing an agroecosystem for soil biodiversity.

8.2 Soil Microbiological Diversity in Agroecosystems

Plants are autotrophs, creating the organic molecules they require for growth and development using elements absorbed mainly from the soil solution (Salisbury and Ross 1992). Plants mainly take up elements in inorganic forms (Schimel and Bennett 2004; Xu et al. 2006). Microbes play a critical role in soil nutrient cycling, decomposing organic matter and mineralizing nutrients into inorganic, plant-available forms (Kennedy and Gewin 1997; Prasad and Power 1997; Stark et al. 2004; Uphoff et al. 2006).

Soil microbial diversity can be defined in terms of structural diversity, referring to the organisms present within the community, and functional diversity, referring to the functions carried out by the community. A population refers to a group of organisms of the same species within an environment, while the community refers to the interacting group of organisms within the environment (Fig. 8.1). Diversity is a measure of the variety of organisms within the community. Soil microbial diversity can impart resistance and resilience to disturbance and stress within agroecosystems (Brussaard et al. 2004, 2007). Soil fungal communities under organic management were reported to be more resistant to environmental disturbance, such as a hurricane (Wu et al. 2007). One requirement of a well-functioning soil is “diversified and abundant populations of soil organisms to mobilize nutrients” (Uphoff et al. 2006). Diverse microbial communities more effectively use complex organic compounds, are more efficient carbon users, and are better able to mobilize nitrogen than less complex microbial communities (Bonkowski and Roy 2005). All of these factors suggest

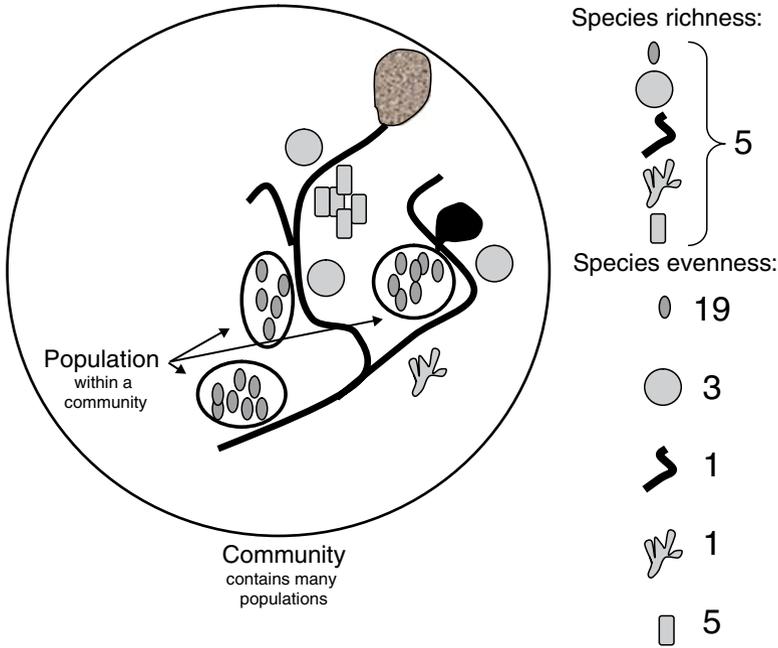


Fig. 8.1 A drawing to show the difference between a population, all one species within a soil system, and a community, a grouping of populations within a soil system. A population is present within a community. Species richness is a measure of how many different species are present within a soil system. Species evenness is a measure of how even the numbers of the various species are within a soil system. Diversity is a measure that incorporates both species richness and evenness

that lowered soil microbial diversity will have negative effects on the efficiency of nutrient cycling in the soil (Bonkowski and Roy 2005). The relationship between soil biological diversity and ecosystem functioning has not been fully elucidated (Anderson 2003; Coleman et al. 1994; Robertson and Grandy 2006), Giller et al. 1997). Also, we do not know the relative importance of soil biological diversity on the integrity and sustainability of a given soil system (Welbaum et al. 2004). However, we do know that at some point in the loss of soil microbial diversity there will be a loss of ecosystem functioning (Coleman et al. 1994; Giller et al. 1997). A change in microbial community structure due to disturbance can result in a reduction of soil functional stability (Griffiths et al. 2004). This means that until we know the functions carried out by specific organisms, maintaining diversity is a way of ensuring ecosystem functionality. Numerous studies have examined the effects of agricultural management practices on soil microbial community and diversity. It may be possible to manage an agroecosystem to increase soil biodiversity and soil biological fertility; however, this is mainly managed indirectly. We influence soil microbial communities by altering crop rotations, crop choice, tillage, and inputs (Brussaard et al. 2007).

8.3 Arbuscular Mycorrhizal Fungi in Agroecosystems

Within the diverse community of soil microbes, arbuscular mycorrhizal fungi play an important role in nutrient cycling and uptake in crop plants. Arbuscular mycorrhizal fungi form, generally, mutualistic associations with the roots of over 80% of known plant species, including wheat and other cereal crops, corn, rice, and legumes (Habte 2006; Rillig 2004). Arbuscular mycorrhizal fungi get their name from the arbuscules, or tree-shaped clusters of hyphae which form within a plant root after infection (Habte 2006). The arbuscules are where nutrient exchange with plants occurs; carbon products from the plant host flow to the fungus, while nutrients taken up by the fungus flow to the plant (Sylvia 2005; Figure 1). Mycorrhiza are important in nutrient uptake for plants, because the fungal hyphae not within the plant root represent increased surface area for absorption of essential plant nutrients, as well as an increase in the soil area explored. Mycorrhiza can take up a number of nutrients, including: nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, copper, and zinc (Al-Karaki et al. 2004; Mohammad et al. 2005; Ryan et al. 2004; Cruz et al. 2004; Mohammad et al. 2003). However, where mycorrhiza are most beneficial is in the uptake of relatively immobile nutrients such as phosphorus, copper, and zinc (Habte 2006). The importance of mycorrhiza in the uptake of immobile nutrients is due to the hyphae accessing nutrients that are not within reach of the plant roots, and because these nutrients do not flow to root surfaces by mass flow (Habte 2006). Increased uptake of phosphorus through mycorrhizal colonization can significantly increase phosphorus concentrations in wheat grains, with the intensity of the effect altered by wheat cultivar, mycorrhizal species, and the soil environment (Al-Karaki et al. 2004) (Fig. 8.2).

In addition to plant nutrient uptake, mycorrhiza can generate a number of other benefits to the soil system and the plant. Some other benefits of mycorrhiza within the soil system are stabilization of soil aggregates, suppression of plant fungal pathogens, reduction of plant parasitic infection by nematodes, protection of plants from drought and saline conditions, and protection of plants from heavy metals (Habte 2006). Mycorrhiza also have an effect on the community structure of other soil microorganisms, by contributing carbon compounds to the soil system as well as influencing soil structure (Hamel 2004; Hamel and Strullu 2006). The benefits of mycorrhiza have resulted in researchers pointing to AMF as critical to the development of sustainable agricultural systems (Douds et al. 1997; Rabatin and Stinner 1989; Hamel 2004), Plenchette et al. 2005).

8.4 Management Practices Affecting Soil Microbiological Diversity

Soil microbes need water, energy (in the form of soil organic matter or plant and animal residues), and essential elements from the soil solution, soil minerals, or soil atmosphere. Physically, the critical controlling factors of microbial diversity are: soil

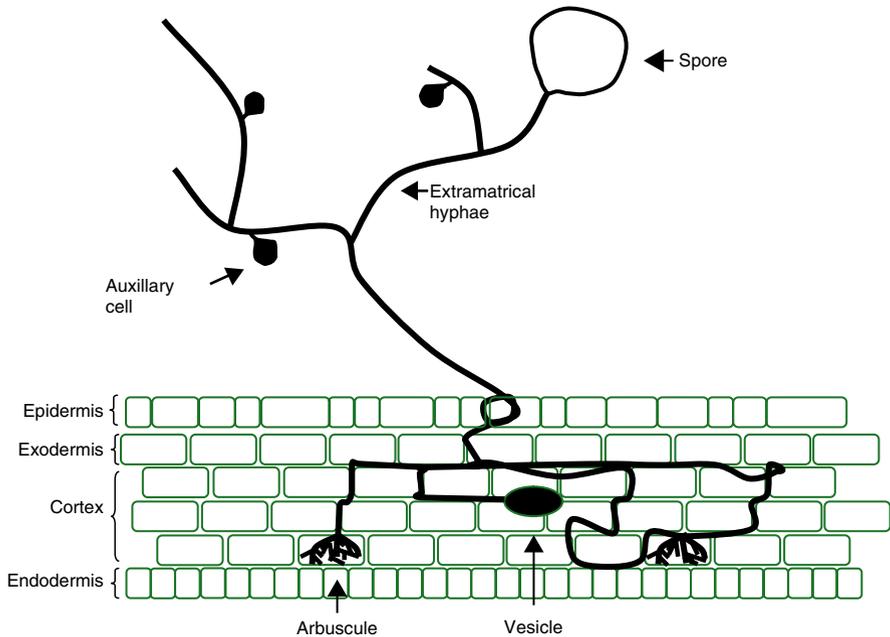


Fig. 8.2 An example of an arbuscular mycorrhizal fungi association with a plant root showing some of the typical structures present. Arbuscules form within the apoplastic space of root cortical cells and are believed to be the site of nutrient exchange between the fungi and plant. Vesicles can form in some species/strains of mycorrhizae, their function is not fully known, but is believed to have a role in storage. Extramatrical hyphae (hyphae extending beyond the root) take up nutrients from the soil. Hyphae can access areas beyond the root depletion zone for some nutrients (hyphae can go about 10mm beyond the root – the P depletion zone is about 1mm around the root). Spores are structures of asexual reproduction. Auxillary cells can also be found on the extramatrical hyphae; their positioning on the hyphae and their shape are species/strain-dependent

organic matter content, the composition of the mineral fraction, and the relative proportions of air and water (Thies and Grossman 2006). Chemically, important factors affecting microbial diversity are: pH, cation- and anion-exchange capacity, mineral content and solubility, buffering capacity, concentration of nutrient elements in the soil, concentration of gases; e.g., oxygen, carbon dioxide, in the soil, soil water content, and salinity or sodicity (Thies and Grossman 2006). While management practices can alter microbial communities directly, for the most part, management practices change microbial communities indirectly by altering a number of the above-named soil properties affecting microbial diversity. Because most microbes are heterotrophic, soil organic matter content and the type and amount of organic materials added to the soil are two critical soil factors affecting microbial diversity (Shannon et al. 2002). Some management practices that have been studied for their effect on soil microbial communities are tillage, crop choice, and rotation practices and chemical use (Fig. 8.3).

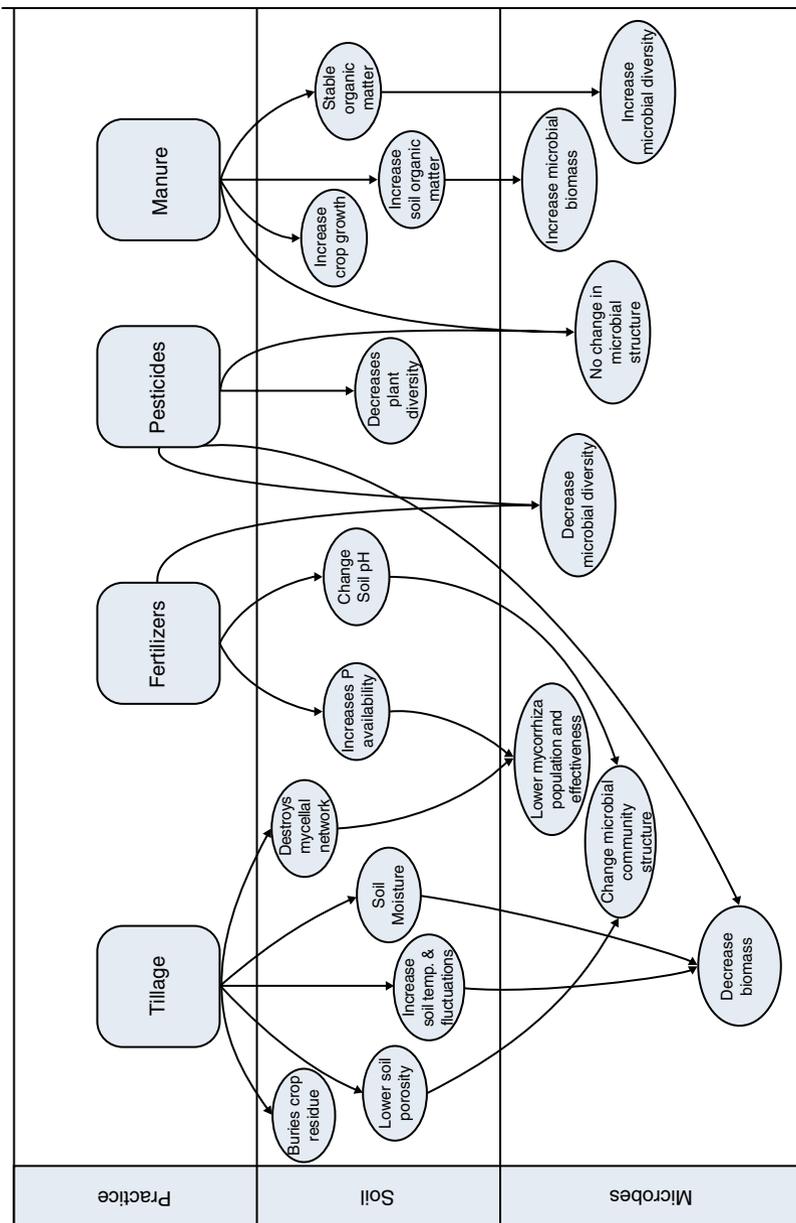


Fig. 8.3 Flow chart of the reviewed management practices and their impact on the soil microbial community. Management practices generally affect the soil microbial community indirectly by altering the microbes' soil habitat

8.4.1 Tillage

Tillage has negative effects on soil structure, breaking aggregates, compacting the soil, and adversely affecting pore size distribution and structure (Huwe and Titi 2003). Tillage also buries crop residue and changes soil water and temperature regimes (Kladivko 2001). In general, lower tillage intensities will have a positive effect on soil microbial communities. Zero tillage systems are characterized as having increased soil moisture and fewer fluctuations in soil temperature than conventional tillage systems, thereby increasing soil microbial populations (Kladivko 2001). Tillage alters the soil microbial community structure, both immediately following, and with increasing time after a tillage event (Calderón et al. 2000; Jackson et al. 2003). Changes in microbial communities due to tillage can be measured 7 years following the cessation of cultivation, with microbes responding to soil conditions taking a long time to change following disturbance (Buckley and Schmidt 2001; Buckley and Schmidt 2003). Increased tillage intensities alter microbial community composition and substrate utilization (Cookson et al. 2008). In Alberta, Canada, tillage was found to decrease soil microbial diversity and evenness (Lupwayi et al. 1998). Microbial activity also decreases with increasing tillage intensity. In comparing zero-till, organic, low-input, continuous corn and grassland systems, soil metabolic activity and nitrogen mineralization were highest in systems of minimal tillage (the zero-till and grassland systems) (Weil et al. 1993). Tillage disturbs the soil biotic community, possibly having a negative effect on the efficiency of nutrient cycling (Werner and Dindal 1990).

Tillage intensity has a large effect on the fungal fraction of the soil microbial community. It is generally believed that zero-tillage systems are fungal dominated, while conventional tillage systems are bacterial dominated (Kladivko 2001). Tillage decreases the fungal component of a soil microbial community for at least 2 weeks following an operation (Jackson et al. 2003). Tillage negatively affects mycorrhiza populations. Mycorrhizal colonization potential of the soil is related more to the presence of fungal hyphae and colonized root pieces than to spore populations (Douds et al. 1997). Thus, tillage has a direct effect on mycorrhizal colonization, as tillage destroys the mycelial network within the soil (Evans and Miller 1990; Boddington and Dodd 2000). Conventional tillage systems have lower levels of mycorrhizal survival and proliferation than zero-tillage systems, thereby reducing the benefits of mycorrhizal associations to plants and soils (Kabir 2005). Tillage can also have negative effects on the sporulation of some AMF species and the distribution of spores through the soil profile (Jansa et al. 2002; Rabatin and Stinner 1989; Abbott and Robson 1991, Boddington and Dodd 2000). In addition to reducing mycorrhiza abundance, differences in mycorrhiza community structure due to tillage system (conventional vs zero) have been observed (Jansa et al. 2002). The reduction in mycorrhiza populations by tillage has been linked to reduced P absorption in crops (Abbott and Robson 1991; Evans and Miller 1990).

8.4.2 Crop Choice and Rotation

Most soil microbes are heterotrophic and thus the type and amount of organic materials added to the soil has a significant impact on microbial community structure (Shannon et al. 2002). Crop rotation is one of the most important tools available to farmers to manage agronomic issues (von Fragstein et al. 2006). Differential effects of plant species on soil microbial communities may be caused by differences in plant material composition and differences in plant root exudates. Root exudates are influenced by environmental and plant factors, including the nutritional status of the plant, so the influences of plant root exudates on microbial communities may be site- and time-specific (Grayston et al. 1998; Koo et al. 2006). Greater crop rotation intensity and diversity can positively affect microbial communities.

Different field crops may or may not have differing effects on soil microbial community and diversity. The rhizosphere of monocropped wheat (*Triticum aestivum* L.) had more bacteria and fungi present than the rhizospheres of forage species such as ryegrass (*Lolium perenne* L.) or bentgrass (*Agrostis capillaries* L.) (Grayston et al. 1998). The differences in the microbial communities of various plant rhizospheres led to differences in carbon source utilization patterns, indicating that plant species affected microbial functional diversity (Grayston et al. 1998). Other studies have reported little to no difference amongst the rhizospheres of various crop species. Microbial diversity has been reported to be similar under wheat, maize (*Zea mays* L.), and faba bean (*Vicia faba* L.) (Song et al. 2007). Of the bacteria associated with red clover (*Trifolium pretense* L.) and potato (*Solanum tuberosum* L.), 73% were of the same species (Sturz et al. 1998).

Crop species may or may not have differential effects on soil microbial communities, depending on environmental and plant factors. However, soil microbial diversity does increase with increased aboveground plant diversity (Garbeva et al. 2006). Intercropping can increase microbial diversity when compared with crops grown in monoculture (Song et al. 2007). Increasing rotational diversity can also increase microbial diversity. A legume green manure–wheat rotation exhibited greater microbial diversity than continuous wheat (Lupwayi et al. 1998). Replacement of the tilled fallow phase of a fallow–wheat rotation with green legume fallow increased soil microbial community biomass, carbon, nitrogen and microbial community, due to an increase in soil organic matter (Biederbeck et al. 2005).

Genetic differences within a crop species also play a role in the structure of the microbial community. Differences have been found in microbial communities associated with different wheat and canola (*Brassica napus* L.) cultivars (Siciliano et al. 1998; Germida and Siciliano 2001). The microbial community structure associated with the wheat cultivar Cadet was altered when a pair of homeologous chromosomes conferring root-rot resistance were substituted from the wheat variety Rescue to Cadet (Neal et al. 1972).

Crop cultivars that have been developed through genetic engineering can have a temporary impact on microbial diversity and community structure that lasts the life

cycle of the plant (Dunfield and Germida 2003, 2004). Plants with transgenes affect soil microbes directly by releasing transgene proteins into the environment as well as indirectly through a change in root exudates (Liu et al. 2005). Lower diversity, or altered structure, in the community of bacteria within the roots of transgenic, glyphosate-tolerant canola cultivars versus non-transgenic or other herbicide-tolerant transgenic cultivars has been reported (Siciliano and Germida 1999). While genetically engineered crops do affect the soil microbial community, these effects (being temporary and dependent on the type of transgene) are likely not as important in comparison to the effect of other management practices like rotation, tillage, and chemical use (Dunfield and Germida 2004).

Crop species and varietal selection can also greatly affect mycorrhiza populations. Plant species from the *Chenopodiaceae* and *Cruciferae* families, including the western Canadian canolas *Brassica napus* L. and *Brassica rapa* L., are not generally colonized by mycorrhiza (Plenchette et al. 1983). About 80% of all plants form mycorrhizal symbiosis, although some species are more dependent on mycorrhiza than others. Mycorrhizal dependency is measured as the percent increase in growth of a plant when colonized by mycorrhiza. Field crops have an average mycorrhizal dependency of 44%, compared to 70% for wild plant species, with a large degree of variation between species within these averages (Tawaraya 2003). Legume species have mycorrhizal dependency values of around 90%, maize has a medium mycorrhizal dependency of about 50%, while modern wheat, oat (*Avena sativa* L.), rye (*Secale cereale* L.), and barley (*Hordeum vulgare* L.) varieties are considered weakly dependent, with values between -13% and 50% (Plenchette et al. 1983; Hetrick et al. 1992; Mosse 1986; Tawaraya 2003). While examining modern wheat cultivars and their ancestors, researchers concluded that mycorrhizal dependency is being bred out of modern wheat varieties, and is a challenge to the optimization of mycorrhizal in cropping systems (Hetrick et al. 1993; Rillig 2004). To successfully manage mycorrhiza populations in agricultural systems there needs to be crop breeding aimed at “mycorrhizal effectiveness” (Hamel 2004). Mycorrhiza can improve plant nutrient status in lower soil nutrient levels, providing benefits to organic and conventional systems, by lowering the fertility input requirements. The loss of mycorrhizal dependency would mean the loss of an important natural advantage to agricultural systems.

Crop rotation plays a large role in determining the mycorrhiza population in the soil and colonization potential. A diversity of host plants can increase the diversity of the mycorrhiza population and increase colonization levels (Rabatin and Stinner 1989; Sattelmacher et al. 1991). Non-mycorrhizal plants in rotation have lower soil mycorrhiza spore populations than mycorrhizal plants, thus lowering the infectivity of the soil in the subsequent year (Douds et al. 1997). Conversely, the presence of a host plant species helps maintain the mycorrhizal inoculum potential of a soil (Kabir 2005; Kabir and Koide 2000). Some crops tend to encourage a larger mycorrhizal community, with greater species richness (Douds and Millner 1999). An overwintering cover crop can maintain AMF populations when no crop is present. Mycorrhizal colonization potential was higher in soil with a hairy vetch (*Vicia villosa* Roth) winter cover crop than in soil without a cover crop (Galvez et al. 1995).

The host plant species can also affect mycorrhizal diversity, with greatest diversity under soybean (*Glycine max* L.) or sunflower (*Helianthus annuus* L.) (Jansa et al. 2002). The specificity of some mycorrhiza to certain crop species in rotation may influence the diversity and infectivity of the mycorrhiza populations in the following crop (Hamel and Strullu 2006).

8.4.3 Chemical Inputs

Agriculture is essentially an extractive system. Nutrients are taken out of fields and exported in the form of grain, crop biomass, and/or animal protein. At some point, nutrients must be added back to both organic and conventional systems to ensure their sustainability. In conventional systems, this mainly takes the form of inorganic fertilizers, while in organic systems fertility inputs include manures and composts. The effect of inorganic and organic fertility amendments on soil microbes has been studied by some researchers.

The addition of manure to a soil increases microbial biomass, and may alter the community structure of soil microbes by increasing soil organic carbon (Frostegård et al. 1997; Fauci and Dick 1994). The type of substrate added (e.g., compost versus fresh plant material) may or may not affect community structure (Drenovsky et al. 2004; Fauci and Dick 1994). The handling of manure prior to application also appears to have an effect. Biodynamic agricultural systems are a form of organic farming that includes metaphysical and spiritual aspects, and prescribes specific compost treatments to be applied to the soil at specific calendar dates. Fließbach and Mäder (2000) reported that microbial communities supplied greater stabilized organic matter in biodynamically composted manure, with a lower metabolic quotient than those supplied uncomposted manure. Comparing traditionally and biodynamically composted manure, soil biological activity was similar but metabolic quotient higher in biodynamic treatments. Thus, researchers hypothesized that biodynamic compost treatments had a more diverse microbial community (Zaller and Köpke 2004). The reasons for the greater performance of biodynamic composts are unclear; however, we believe that if care is taken in the preparation and composting of manure, it should be as good as biodynamic preparations.

Inorganic fertilizers, in comparison to manures and composts, do not directly add organic carbon to the soil, but can alter soil chemistry, specifically soil pH, thereby changing soil microbial habitats (Bünemann et al. 2006; O'Donnell et al. 2001). Soil treated with inorganic fertilizers tend to have lower microbial biomass, as well as a different community structure than soil treated with organic fertility amendments, such as manures or composts (Marschner et al. 2003; O'Donnell et al. 2001; Peacock et al. 2001; Seghers et al. 2003; Suzuki et al. 2005). Up to 10 days following fertility input, there can be a change in community structure; however, these effects generally disappear by 91 days after application (Stark et al. 2007). Following long-term application of organic and inorganic fertilizers, researchers reported an increase in the amount of bacteria present (Marschner et al. 2003).

However, this change in structure was not accompanied by a change in enzyme activity, indicating that ecosystem functioning was not affected by this change in structure. Inorganic fertilizers can also alter microbial communities indirectly through increased plant production. In some cases, the impact of inorganic fertilizers and the change in soil pH have a greater effect on soil microbial community, than organic fertilizers, which tend to increase soil organic matter (Suzuki et al. 2005). An extreme case of an agricultural field polluted by inorganic fertilizers had higher organic carbon, total nitrogen, and C/N ratio, but lower diversity and richness of microbial DNA sequences than fields with no, or normal agrichemical use (Yang et al. 2000).

The application of phosphorus fertilizers can have a positive, neutral, or negative effect on mycorrhizal colonization (Manske 1990; Abbott and Robson 1991; Rabatin and Stinner 1989). The negative effect of phosphorus fertilizers on mycorrhiza populations has been attributed to increased soil levels of available phosphorus (Hamel and Strullu 2006). With high levels of phosphorus, root cell membranes are more stable, reducing root exudates, thereby reducing colonization levels (Habte 2006; Mosse 1986). Lower mycorrhizal colonization levels in wheat have been attributed to the application of superphosphate, a soluble P fertilizer (Ryan et al. 2004).

As well as decreasing mycorrhizal colonization, high levels of available nutrients serve to decrease the relative benefits of mycorrhiza and can actually decrease plant productivity (Aikio and Ruotsalainen 2002; Ryan and Graham 2002; Stewart et al. 2005). The mycorrhizal demand for crop carbon may actually decrease yields in some conventional, high-fertility environments (Ryan and Graham 2002). The relationship between higher phosphorus levels and lower mycorrhizal colonization is specific to plant species and cultivars (Habte 2006). Roughly half of 44 spring wheat varieties grown under high phosphorus conditions exhibited parasitic effects of AMF inoculation, with lower shoot dry weight in inoculated plants (Manske 1990).

Results are less clear when comparing organic and inorganic fertility inputs. Clay soil treated with manure had higher levels of active hyphae than soils treated with inorganic fertilizers (Kabir et al. 1997). Another study reported AMF colonization to be greater under composted manure versus raw manure or inorganic fertilizer (Douds et al. 1997). Mycorrhizal infection was found to decrease with increasing amounts of manure inputs; as well as a smaller mycorrhizal effect on plants when using sterile versus unsterile manure (Brechelt 1990). The decreased effects of mycorrhiza with increasing amounts of applied manure were attributed to greater nutrient availability.

Conventional systems rely (at least in part) on chemical pesticides to control weed, disease, and insect problems in the field. The impact of pesticides on microbial population biomass has been studied, and, in general, when pesticides are applied at recommended rates, there is little to no impact on microbial populations (Fraser et al. 1988; Seghers et al. 2005; Shannon et al. 2002). However, Johnsen et al. (2001) suggested that insufficient studies have been conducted to assess the effect of pesticide use on microbial diversity. We do know that some pesticides can

have a harmful effect on soil microbes, but which pesticides, and the long-term impact of changes to the soil community because of those pesticides is unknown (Bünemann et al. 2006). Herbicides have little effect on the soil community, while some insecticides and fungicides had negative effects on soil microbes (Bünemann et al. 2006). In a case of field-scale pesticide pollution, microbial biomass declined on fields with normal application rates, but pesticide pollution did not appear to alter the diversity of the microbial population (Yang et al. 2000). However, because plant diversity affects soil microbial diversity (discussed in previous section), the use of herbicides may indirectly affect microbial diversity by killing weeds and reducing plant species diversity.

Pesticides can have a negative effect on mycorrhizal communities, lowering colonization and sporulation of certain species; however, these effects seem to be temporary (Gosling et al. 2006). The use of the herbicide diclofop lowered dry weights of wheat inoculated with AMF, possibly due to a decline in colonization (Rejon et al. 1997). Another study reported that AMF colonization levels were not affected by herbicides (Ryan et al. 1994). At recommended rates, most herbicides do not appear to alter mycorrhizal communities (Mosse 1986). Some fungicides can lower mycorrhizal numbers, by directly affecting the fungi (Bünemann et al. 2006). Herbicides may reduce mycorrhizal numbers through a reduction of host weeds (Gosling et al. 2006).

8.5 Organic and Conventional Cropping Systems

The preceding sections examined the effects of management practices individually (see Fig. 8.1). Cropping system comparisons present challenges as reductionist science because many factors must vary in order to ensure proper functioning of the respective systems (Lampkin and Padel 1994). Organic and conventional systems are not defined by a set group of practices; they are an aggregate of a number of management practices dictated by farmer choice and site-specific requirements, rendering generalizations about cropping systems quite difficult (Harrier and Watson 2003). This implies there is no clear definition of the two separate systems, but rather a spectrum of systems, into which all farms would fall (Lampkin and Padel 1994). The International Federation of Organic Movements defines organic agriculture as “a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved” (IFOAM 2008). At its most basic, and common to all organic agricultural systems, organic agriculture is defined by the absence of synthetic pesticides and fertilizers. For this chapter, we consider conventional cropping systems to be all systems of production not including biodynamic and organic. Despite the difficulties in studying and comparing organic and conventional cropping systems, there is value in such studies.

Both organic and conventional cropping systems consist of a number of different management practices (chemical use, tillage, crop rotation, and crop choice) used in combination. Despite the ranges of management practices used on organic and conventional cropping systems, there are a number of factors that are commonly found to differ in the two systems.

Organic systems tend to have higher organic matter, more weeds, lower yields, and lower phosphorus levels than conventional systems (Entz et al. 2001; Pimentel et al. 2005). The absence of inorganic fertilizers and pesticides in organic systems generally leads to greater weed populations, higher tillage intensities, and lower soil nutrient levels. As well, organic systems often have more diverse crop rotations and higher plant diversity within fields than conventional systems. Soil organic matter is an important determinant of microbial populations, serving as a source of energy for microbes, and ultimately an important pool of nitrogen, phosphorus, and sulphur (Stockdale et al. 2002). Organic systems employ a number of practices that serve to increase organic matter content, generally resulting in slightly higher levels of organic matter in organic systems versus conventional systems (Bossio et al. 1998; Drinkwater et al. 1995; Shepherd et al. 2002). Organic management exhibited greater or equal soil organic carbon levels to conventional management in long-term studies (Wander et al. 1994; Fließbach and Mäder 2000). In some cases, organic management has resulted in lower organic matter contents than conventional management (Girvan et al. 2003; Stark et al. 2004). This may be due to lower yields in organic systems returning lower levels of organic matter to the soil. In the case of extensive dryland cropping systems in western Canada that tend to rely on tillage for weed control, it is suspected that these organic systems would have similar, or lower organic matter and organic carbon levels to their conventional counterparts.

8.6 Cropping Systems Management and Microbial Communities

With greater organic matter to provide an energy source for microbes, it is not surprising that a number of studies have reported higher microbial biomass in organic systems than conventional systems (Fließbach et al. 1997; Hole et al. 2005; Mäder et al. 2002; Fließbach and Mäder 2000; Wander et al. 1995). The diversity and structure of soil microbial communities is also important in these systems. Some studies have reported shifts in microbial communities with organic versus conventional management, while others have reported no differences between microbial communities under the two management systems (Bossio et al. 1998; Yeates et al. 1997; Girvan et al. 2003; Wander et al. 1995; Lundquist et al. 1999). Differences are generally expected between microbial communities in organic versus conventional management, and the absence of differences in some studies has been attributed to the greater effects of soil type and time of sampling (Bossio et al. 1998; Girvan et al. 2003; Stark et al. 2004; Wander et al. 1995). A comparison of organic and

conventional pastures reported no difference in soil biological diversity (Parfitt et al. 2005). In this case, the similarity between the two systems may be due to a lack of sufficient management differences, with only fertility regime differing. Additionally, perennial intercrops may have exhibited a greater effect on microbial diversity than the fertility inputs. Differences in soil microbial community structure between organic and conventional systems is not necessarily negative, but indicative that these systems have very different soil conditions and perhaps require different functions from the soil microbes. While studying the effect of moisture stress on organic and conventional soils, Lundquist et al. (1999) reported different community structure in the two soils, but no differences in community response to stress. However, if we are to strive toward a reduced dependence on inorganic fertilizers, we must ensure that the soil microbial community can carry out functional requirements to recycle nutrients efficiently.

Mycorrhizal potential and actual colonization has been reported to be greater in grasslands, organic and low-input systems versus conventional systems (Eason et al. 1999; Entz et al. 2004; Galvez et al. 1995; Mäder et al. 2000, 2002; Oehl et al. 2003, 2004; Sattelmacher et al. 1991; Scullion et al. 1998). This is in large part due to the fact that the application of phosphorus fertilizers, even at low rates, decreases root colonization of mycorrhiza (Clapperton et al. 1997; Mäder et al. 2000; Ryan et al. 1994, 2004). Differential mycorrhizal community structure has been reported, with organic systems maintaining community structures similar to natural systems. Conventional systems tend to have lower species richness, with the associated risk of lower mycorrhizal functioning (Oehl et al. 2003, 2004). Exceptions, of course, have been reported. No differences were reported in diversity of soil fungal communities in organic and conventional systems in Florida (Wu et al. 2007). Eason et al. (1999) reported the percent mycorrhizal infection of roots was one-third greater on organic farms than conventional farms; however, there was a great deal of variation in management practices between the farms, and therefore a great deal of variation in the mycorrhizal infection rates amongst farms (Eason et al. 1999).

Mycorrhizal host plants need not be crop plants. Weeds may host mycorrhiza during rotation phases with non-host crops or during the overwintering period. The presence of weeds may also have a positive effect on soil processes, through the addition of plant residues and root exudates (Werner and Dindal 1990). Weeds present during the crop season may also provide greater plant diversity for the mycorrhiza, as plant diversity is usually positively correlated to a diversity of AMF (Douds et al. 1997; Rabatin and Stinner 1989). Mycorrhizal weed species can increase mycorrhizal diversity and abundance, as well as influence community structure, improving the mycorrhizal potential of soil (Vatovec et al. 2005). (Table 8.1 provides a list of some mycorrhizal and non-mycorrhizal weed species.) One field study maintaining dandelions (*Taraxacum officinale* Weber ex Wigg) as a winter cover crop reported that the weed provided mycorrhizal inoculum potential for a subsequent maize crop, increasing mycorrhizal colonization and phosphorus concentration of the maize (Kabir and Koide 2000). Wheat grown in the presence of a non-mycorrhizal weed, *Chenopodium album*, experienced lowered levels of mycorrhizal colonization, while maize experienced an increase in colonization (Stejskalova 1990).

Table 8.1 Summary of mycorrhizal colonization ability of various weed species (Adapted from Vatovec et al. 2005, source is Vatovec et al. 2005 unless otherwise specified)

Mycorrhizal or non-mycorrhizal?	Common name	Family	Species	Source
Mycorrhizal	Ragweed	<i>Asteraceae</i>	<i>Ambrosia artemisiifolia</i>	
Mycorrhizal	Canada thistle	<i>Asteraceae</i>	<i>Cirsium arvense</i>	
Mycorrhizal	Dandelion	<i>Asteraceae</i>	<i>Taraxacum officinale</i>	Kabir and Koide 2000
Mycorrhizal	Cocklebur	<i>Asteraceae</i>	<i>Xanthium strumarium</i>	
Mycorrhizal	Velvetleaf	<i>Malvaceae</i>	<i>Abutilon theophrasti</i>	
Mycorrhizal	Quackgrass	<i>Poaceae</i>	<i>Agropyron repens</i>	
Mycorrhizal	Giant foxtail	<i>Poaceae</i>	<i>Setaria faberi</i>	
Mycorrhizal	Yellow foxtail	<i>Poaceae</i>	<i>Setaria lutescens</i>	
Mycorrhizal	Nightshade	<i>Solanaceae</i>	<i>Solanum nigrum</i>	
Non-mycorrhizal	Pigweed	<i>Amaranthaceae</i>	<i>Amaranthus retroflexus</i>	
Non-mycorrhizal	Mustard	<i>Brassicaceae</i>	<i>Brassica kaber</i>	
Non-mycorrhizal	Lambsquarters	<i>Chenopodiaceae</i>	<i>Chenopodium album</i>	
Non-mycorrhizal	Smartweed	<i>Polygonaceae</i>	<i>Polygonum lapathifolium</i>	
Non-mycorrhizal	Purslane	<i>Polygonaceae</i>	<i>Portulaca oleracea</i>	
Non-mycorrhizal	Curly dock	<i>Polygonaceae</i>	<i>Rumex crispus</i>	

Mycorrhizal colonization can also increase or decrease the growth of weeds in the field, depending on the soil and species (Vatovec et al. 2005). The presence of mycorrhiza can change the composition of weed communities, selecting for host species. Conversely, the weed community can alter mycorrhiza communities, with diverse weed hosts encouraging increased mycorrhizal diversity (Jordan et al. 2000). Soil taken from fields under organic, transitioning to organic and conventional management had similar mycorrhizal colonization levels of various weed species (Vatovec et al. 2005). The presence of weeds within a crop lowers crop productivity. However, it appears that organic fields may derive some benefits from weed pressure that is ubiquitous within these systems.

8.7 Interactions and the Relative Importance of Management Practices

Rotation and tillage practices interact to alter microbial communities, with previous crop effects greater under zero-tillage management (Lupwayi et al. 1998). Tillage may have a greater effect on soil microbial populations than herbicides (Table 8.2).

Table 8.2 Summary of the management practices and their relative impact on the soil microbial community and mycorrhizal community

Management practices	Effect of management practice on:	
	Microbial community	Mycorrhizal community
Tillage		
Reduced tillage		Positive*
Heavy tillage		Negative
Rotation		
Diverse rotation		Positive
Intercrops		Positive
Fallow in rotation		Negative
Non-mycorrhizal crop in rotation	?	Negative
Transgenic crop in rotation	Negative	?
Crop inputs		
Organic fertility amendments		Positive
Inorganic fertility amendments	Positive or Negative (depends on fertilizer effects organic matter inputs to soil, soil pH, etc.)	
Fungicides	Negative	Negative
Insecticides		Negative
Herbicides		Negative

*Items in bold indicate that the management practice has a large effect on the community in question. Items not in bold have a small effect on the community

In a study comparing zero- and conventional-tillage systems, researchers reported that any effect of increased herbicide use on microbial diversity in a zero-tillage system was overridden by the greater effects of tillage (Lupwayi et al. 1998). Other researchers concluded that fertilizers can affect microbial populations more than pesticides (Yang et al. 2000). The use of transgenic crops in rotation was concluded to be less important to microbial community structure than rotation, tillage, or chemical use (Dunfield and Germida 2004). Still others have suggested that long-term management histories have a greater effect on microbial communities than current practices and crop selection (Buckley and Schmidt 2003). Similarly, it was reported that soil and environmental factors had a greater effect on microbial structure than short-term management practices such as fertility inputs (Stark et al. 2007; Wakelin et al. 2008). An Australian study reported that soil pH was the most important soil characteristic when determining microbial community diversity and function (Wakelin et al. 2008).

While tillage is important in determining mycorrhiza abundance and diversity, other management practices play important roles. Studies have reported host plant species to have a greater effect on mycorrhizal diversity, and cropping system (organic vs conventional) to have a greater effect on mycorrhizal abundance than tillage (Galvez et al. 2001; Jansa et al. 2002). The greater effect of cropping system on mycorrhizal colonization is most likely due to differences in soil phosphorus levels. However, rotation phase has been reported to have a greater effect on infection potential of the mycorrhizal population than the fertility amendment used (Douds et al. 1997).

Site-specific factors play a role in the relative importance of management practices on soil microbial communities, as well as how management practices will impact soil microbes. In general, farming practices that sustain or create soil conditions that are optimal for plant growth will also encourage abundant and diverse soil microbial communities (Thies and Grossman 2006). While site-specific characteristics are important in determining the structure of the soil biological community and how management practices affect that biological community, the general principles of managing for a productive, sustainable system remain the same across ecosystems (Uphoff et al. 2006).

8.8 The Management of Soil Biological Fertility

While certain organisms or functional groups play specific roles in soil nutrient cycling (e.g., Rhizobia bacteria fixing atmospheric nitrogen into ammonia), it is likely impossible to manage the agricultural soil system specifically for all the beneficial organisms and functions desired. It is estimated that, at most, 5% of the soil microorganisms have been identified and their role studied (Anderson 2003; Uphoff et al. 2006). Because the environmental control of soil nutrient release is complicated and because we have a limited ability to predict soil processes, manipulating individual microbial processes affecting soil fertility is not a viable option (De Neve et al. 2004; Robertson and Grandy 2006; Watson et al. 2002). Realistic management strategies to improve biological nutrient cycling must rely on well-established knowledge. To improve biological nutrient cycling, cropping systems can be managed to ensure diverse microbial communities and abundant mycorrhizal populations.

Maintaining soil biological diversity is important to maintain the integrity of the functioning of the soil system. There is some functional redundancy in soil systems; however, our limited understanding of microbial systems makes any theory about functional redundancy speculative (Anderson 2003; Kennedy and Smith 1995). Lowered soil microbial diversity, or a change in soil community structure within an agroecosystem may not have negative impacts on soil biological fertility. However, despite our incomplete understanding of the connections between microbial diversity, ecosystem functioning, and functional redundancy, we do know that at some point in the loss of soil microbial biodiversity there will be a loss of function (Coleman et al. 1994). Diversity in the soil microbial community should be maintained to ensure nutrient cycles and other soil functions continue.

In organic systems, there is already effort expended on improving soil fertility through the creation of diverse soil microbial communities, because these systems rely on biological fertility for the production of crops (Davis and Abbott 2006). Organic systems, with manure and compost fertility inputs, low (or no) fertilizer and pesticide inputs and diverse plant communities, seem fairly well-designed to encourage a healthy and diverse soil microbial community. However, in dryland prairie systems, with extensive farms, tillage is a large component of weed control

of organic systems. Lowered tillage would improve the soil conditions for microbial diversity and mycorrhizal colonization; organic systems should strive for minimum tillage. As well, avoiding bare soil fallow in crop rotations would help to maintain the microbial community, especially the mycorrhizal component (Gosling et al. 2006).

Conventional systems represent a large range and combination of management practices, making generalizations difficult. A well-managed conventional cropping system, with minimum tillage, and low fertilizer inputs should experience similar or identical microbial diversity and mycorrhizal levels as a well-managed organic cropping system. If soil biological fertility is encouraged, through careful management (including reduced tillage, lowered fertility and pesticide inputs, and diverse crop choices), conventional systems should be able to lower fertilizer rates. Lowered fertilizer inputs, especially in reduced tillage systems where mycorrhizal communities can thrive, can offset some of the negative impacts of high-input systems. Both organic and conventional systems should begin to incorporate intercrops into rotations. Aboveground diversity is very important in determining belowground diversity.

8.9 Conclusion

The impact of production practices on soil microbial diversity and mycorrhizal colonization has been studied to varying degrees (Vandermeer et al. 1998). This paper reviewed work in the area of crop management practices and their impact on soil microbial diversity and mycorrhizal communities. We have not reached a point where definitive relationships between production practices and microbial community structure can be defined. All of the management practices discussed in this paper have aspects of site-specificity in their effect on microbial communities, making broad generalizations about the effect of a particular practice on microbial structure difficult. Knowing the ecological principles guiding microbial community structure can help farm managers tailor their cropping practices to a particular set of conditions. In general, lowered fertilizer and pesticide use, diverse crop rotations that include mycorrhizal plant species, reduced tillage systems as well as the use of intercrops and cover crops can maintain or improve indigenous mycorrhizal communities and microbial diversity (Plenchette et al. 2005; Thies and Grossman 2006). Organic cropping systems should strive for minimum tillage systems while conventional systems should work toward lowered reliance on fertilizers to supply crop nutrients. Both organic and conventional cropping systems should begin to grow intercrops to encourage diversity within the soil system.

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Chapter 9

Cyanobacterial Reclamation of Salt-Affected Soil

Nirbhay Kumar Singh and Dolly Wattal Dhar

Abstract Salinity has been an important historical factor which has influenced the life span of agricultural systems. Around 10% of the total cropped land surface is covered with different types of salt-affected soils and the Asian continent accounts for the largest area affected by the salinity of various intensities. Cyanobacteria are capable of not only surviving, but thriving in conditions which are considered to be inhabitable, tolerating desiccation, high temperature, extreme pH and high salinity, illustrating their capacity to acclimatise to extreme environments. Until recently, the responses of cyanobacteria to salinity stresses were poorly documented as compared to heterotrophic bacteria and phototrophic eukaryotic algae. Cyanobacteria can be used to reclaim alkaline soils and fertility can be improved for subsequent cultivation of cereal crops, sugarcane and horticultural crops. Therefore we present here a review on cyanobacterial reclamation of salt-affected soil.

Substantial progress has been made towards better understanding of the physiological mechanisms responsible for salinity tolerance and osmotic adjustment in cyanobacteria. Many researchers throughout the world have worked on probable mechanisms of salt tolerance studies in cyanobacteria. These organisms evolved about 3,000 million years ago and are considered to be the primary colonisers of the inhospitable ecosystems. The physiological aspects for the adaptation of cyanobacteria to high salinities include (a) synthesis and

N.K. Singh (✉)

Department of Microbiology, C.P. College of Agriculture, S.D.A.U., S.K. Nagar,
Gujarat-385506, India
e-mail: nirbhaysingh78@gmail.com

D.W. Dhar

Centre for Conservation and Utilization of Blue Green Algae (CCUBGA),
IARI, New Delhi-110012, India
e-mail: dollywattaldhar@yahoo.com

accumulation of osmoprotective compounds, (b) maintenance of low internal concentrations of inorganic ions and (c) expression of a set of salt-stress proteins. Exposure of cyanobacterial cells to different abiotic stresses resulted in rapid expression of several stress-regulated proteins and modifications in protein synthesis programme. The synthesis of organic solutes like disaccharides (sucrose, trehalose and glucosyl glycerol), quaternary amines (glycine betaine) and free amino acids (glutamine) are well-documented. The protection against alkaline environment is provided by the synthesis of specific fatty acids, sucrose- and osmotic-stress-induced proteins. In cyanobacteria, accumulation of internal osmoticum in the form of inorganic ions and prevention of intracellular Na^+ accumulation by the curtailment of Na influx and by efficient active efflux mechanisms or metabolic adjustments have been investigated in depth. The Na^+ extrusion in cyanobacteria is driven by a Na^+/H^+ antiporter, which is energised by enhanced activity of cytochrome oxidase. The inhibition of sodium ion influx appears to be a major mechanism for the survival of cyanobacteria against salt stress and synthesis of salt-stress proteins have been found in cyanobacteria. These organisms have been recognised as an important agent in the stabilisation of soil surfaces primarily through the production of extracellular polysaccharides which are prominent agents in the process of aggregate formation and increase in soil fertility. Cyanobacterial application results in the enrichment of soil with fixed nitrogen, soil structure improvement and declining trend of pH, electrical conductivity (EC) and Na^+ . The extracellular polysaccharides excreted by cyanobacteria have been reported to be responsible for binding of soil particles, thus, leading to the formation of a tough and entangled superficial structure that improves the stability of soil surface and protects it from erosion. The potential impact of these organisms on agriculture through their use as soil conditioners, plant growth regulators and soil health ameliorators has been well-recognised. Besides bringing about an improvement in the yield of rice, cyanobacteria produce direct and indirect beneficial changes in the physical, chemical and biological properties of soil and soil–water interface in the rice fields, which are of agronomic importance. Certain cyanobacteria have been found not only to grow in saline ecosystems but also improve the physico-chemical properties of the soil by enriching them with carbon, nitrogen and available phosphorus. Flushing of field may not be effective for the reclamation of saline soils and the addition of cyanobacterium inoculum along with the addition of gypsum is required before irrigation to ameliorate saline soils. Nitrogen-fixing cyanobacteria can be used as biological input to improve soil texture, conserve moisture, scavenge the toxic sodium cation from the soil complex and improve the properties of soils. Virtually negligible information exists on the genetics of cyanobacterial halotolerance. The presence of combined nitrogen which effectively curtails sodium accumulation and supports extra nitrogen demand for osmoregulation during salt stress confers considerable salt tolerance on cyanobacteria.

Keywords Cyanobacteria • saline/alkaline • reclamation • technology • crop response • blue-green algae • salt stress

9.1 Introduction

Salt stress is one of the most serious factors limiting the productivity of crops including staple diet in many countries. Around 10% of the total cropped land surface is covered with different types of salt-affected soils (Boyer 1982; Nelson et al. 1998). Such soils are distributed throughout the world including deserts, plains, coastal areas, river valleys, and over-irrigated lands. The Asian continent accounts for the largest area (410 million hectares) affected by salinity of various intensities (Szabolcs 1993). In arid and semi-arid regions of the world, salt-affected soils can be primarily divided into saline and alkaline soils. Salinity and alkalinity are the major problems associated with soil management in arid and semi-arid regions of the world (Szabolcs 1979). Saline soils are the soils that have developed due to the influence of sodium salt (mainly NaCl or Na₂SO₄) whereas alkaline soils have developed mainly due to the influence of Na₂CO₃ and NaHCO₃ (Szabolcs 1993). The saturated pH of saline soil is less than 8.5 and the electrical conductivity is generally more than 4.0 dS m⁻¹ whereas pH of alkaline soil is more than 8.5 and electrical conductivity is less than 4.0 dS m⁻¹ at 25°C. Saline soils are not suitable for crop production although they have high agricultural potential. Salt-affected soils generally contain sufficient neutral soluble salts in the root zone and adversely affect crop growth and production. Soluble salts are predominantly the chlorides and sulphates of sodium, calcium and magnesium of which sodium chloride is the dominant salt (Hashem 2001). Alkaline (sodic) soils have a high pH and exchangeable sodium (ES), measurable amounts of carbonates, and undergo extensive clay dispersion leading to poor hydraulic conductivity and reduced soil aeration resulting in poor crop production in such soils. Different types of factors which cause crop stress under salt-affected areas have been reported in literature (Gupta and Abrol 1990).

Blue-green algae or cyanobacteria have been reported to grow extensively on alkaline or 'usar' soils in India (Singh 1950) and on the saline soils of the USSR (Gollerbach et al. 1956). These are capable of not only surviving, but thriving in conditions which are considered to be inhabitable, tolerating desiccation, high temperatures, extreme pH and high salinity, illustrating their capacity to acclimatise to extreme environments (Stal 2007). Cyanobacteria can be used to reclaim alkaline soils and fertility can be improved for subsequent cultivation of cereals crops, sugarcane and horticultural crops (Singh 1950, 1961; Aziz and Hashem 2003). A large number of researchers throughout the world have worked on probable mechanisms of salt tolerance studies in cyanobacteria and lot of information has been generated (Pandhal et al. 2008). Cyanobacterial reclamation of salt-affected soil is moreover a neglected field and very little work has been done in this context; however, the main thematic areas on which salt studies in cyanobacteria are centred can be grouped into four major classes (Table 9.1).

Table 9.1 Main thematic areas for salt-related studies in cyanobacteria

Thematic areas for salt-related studies in cyanobacteria	References
1. Biochemical- and physiology-based studies	Tang et al. 2007; Wiangnon et al. 2007; Bhadauriya et al. 2007; Abhishek et al. 2006; Ferjani et al. 2003; Hagemann et al. 1994; Marin et al. 2002, 2003; Singh et al. 2002; Kempf and Bremer 1998; Mikkat et al. 1997; Reed et al. 1986; Gabbay-Azaria and Tel-Or 1993; Roberts 2005
2. Salt intake and cell signalling	Elanskaya et al. 2002; Shoumskaya et al. 2005; Buck and Smith 1995; Waditee et al. 2001, 2002; Apte et al. 1987; Reed et al. 1984; Reddy et al. 1989; Hagemann and Marin 1999; Mikkat et al. 1996
3. Gene level responses, e.g. salt-regulated genes, microarrays, mutational analysis of salt tolerance determinants	Billini et al. 2008; Marin et al. 2004; Vinnemeier et al. 1998; Bohnert et al. 2001; Kanesaki et al. 2002; Marin et al. 1998; Miao et al. 2003; Chauhan et al. 1999; Joset et al. 1996; Karandashova et al. 2002
4. Post-genomics	Fulda et al. 1999, 2000, 2006; Hagemann et al. 2001; Hagemann and Erdmann 1994; Pinner et al. 1992; Huang et al. 2006

9.2 Cyanobacterial Distribution in Salt-Affected Ecosystems

Cyanobacteria, both heterocystous and non-heterocystous, evolved about 3,000 million years ago and are considered to be the primary colonisers of inhospitable ecosystems (Fig. 9.1). One of the most unique features of these organisms is their versatile occurrence (Brock 1973). Due to their early evolutionary history, cyanobacteria occur abundantly in a wide range of habitats, including saline soils and coastal swamps (Amsaveni 1995; Komarek 1998; Hoffmann 1989). The predominant genera that are ubiquitous in tropical soils are *Anabaena*, *Aulosira*, *Calothrix*, *Nostoc*, *Plectonema* and *Westiellopsis*, while localised distribution of *Haplosiphon*, *Scytonema* and *Cylindrospermum* has also been reported (Gopaldaswamy et al. 2007). The microbes which have growth optima above pH 9.0 and require salt for growth have been termed as alkalophilic or halophilic and those which have growth optima below pH 9.0 but can survive extended exposure above it and can tolerate high salt concentration have been termed halotolerant (Hoffmann 1989). The occurrence of cyanobacteria in saline/alkaline soil has been described by many workers (Singh 1950, 1961; Ali and Sandhu 1972). Cyanobacteria, particularly belonging to the genera *Anabaena*, *Synechocystis* and *Aphanothece*, have been classified into three groups relating to their salt tolerance: salt sensitive (or stenohaline), moderately halotolerant, and extremely halotolerant (Reed and Stewart 1988; Pandhal et al. 2008). pH is particularly considered an important factor in influencing cyanobacterial distribution and abundance in soil (Sardeshpande and Goyal 1981). These organisms initially appear on land after the first shower of monsoon and prefer neutral to slightly alkaline conditions (Singh 1978). The sequence of cyanobacterial appearance in an alkaline (saline) soil having monsoon type of character indicated their dominance in such soils (Whitton and Potts 2000; Pandey et al. 2005). A large

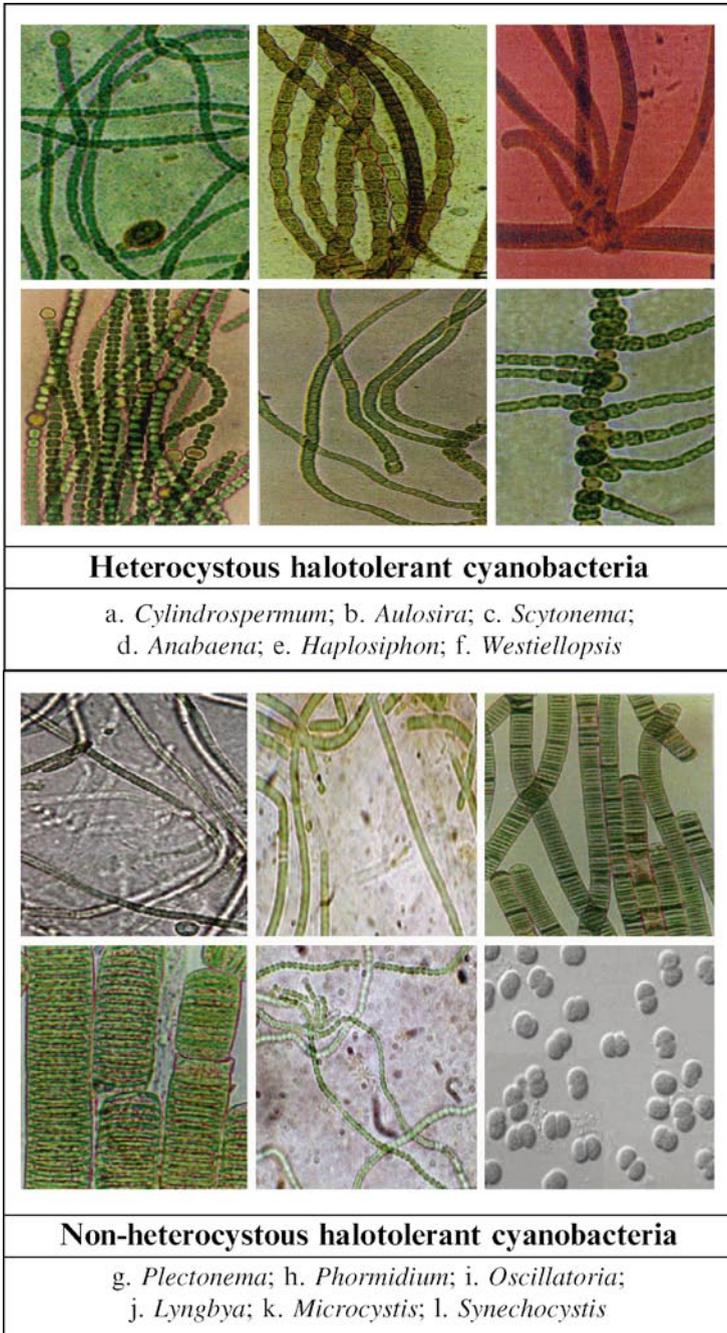


Fig. 9.1 Heterocystous halotolerant cyanobacteria (a) *Cylindrospermum*; (b) *Aulosira*; (c) *Scytonema*; (d) *Anabaena*; (e) *Haplosiphon*; (f) *Westiellopsis*. Non-heterocystous halotolerant cyanobacteria (g) *Plectonema*; (h) *Phormidium*; (i) *Oscillatoria*; (j) *Lyngbya*; (k) *Microcystis*; (l) *Synechocystis*

number of cyanobacterial strains, irrespective of their morphological organisation, ecological distribution and nature of carbon and nitrogen nutrition, have potential to scavenge toxic sodium (Na^+) cation from the soil and subsequently improve soil properties (Kratz and Myers 1955). Cyanobacteria have been found to differ considerably in their ability to resist salt stress (Apte and Thomas 1983).

9.3 Uptake of Na^+ and Ca^{2+} Salts by Cyanobacteria

9.3.1 Na^+ Uptake

Although cyanobacteria are known to require Na^+ for growth (Allen and Arnon 1955) and nitrogen fixation (Apte and Thomas 1980), the exact physiological processes involved in their salt tolerance are not yet known. The ability to absorb Na^+ have been studied in a freshwater cyanobacterium *Anabaena* L-31 and a saline form *Anabaena torulosa* (Apte and Thomas 1974). The kinetics of Na^+ transport in these cyanobacteria as revealed by the use of radiotracer $^{22}\text{Na}^+$ suggested that their Na^+ transport properties are better suited for their survival in such environments.

There have been several reports emphasising the need for Na^+ by cultures of many unicellular non-diazotrophs, e.g. *Chroococcus* sp. (Emerson and Lewis 1942), *Anacystis nidulans* (Kratz and Myers 1955) and *Microcystis aeruginosa* (McLachlan and Gorham 1961) as well as the diazotrophic filamentous cyanobacteria *Anabaena variabilis*, *Nostoc muscorum* (Kratz and Myers 1955) and *Anabaena cylindrica* (Brownell and Nicholas 1967). Allen and Arnon (1955) reported Na^+ requirement by cyanobacteria lies in the range of 1–5 ppm (17–85 μM) whereas Kratz and Myers (1955) found a much higher requirement (680 μM). About 20–25 μM was the minimum level of Na^+ required for detectable N_2 -supported growth of both the freshwater and brackish water cyanobacterium (Apte and Thomas 1984).

In *Anacystis nidulans*, Na^+ transport has been shown to be regulated by an active extrusion of the cation by a proton antiport system (Dewar and Barber 1973; Paschinger 1977). Na^+ influx in *A. torulosa* and *Anabaena* L-31 is probably a passive-carrier-mediated diffusion process while the regulation of Na^+ transport is achieved by an active extrusion of Na^+ . The Na^+ extrusion appears to be mediated by a Na^+ - K^+ ATPase which is distinct from the conventional F1-F0 ATPase (Paschinger 1977; Heefner and Harold 1982). In *A. torulosa*, Na^+ uptake saturates quickly, follows Michaelis-Mentor kinetics and shows a high affinity for Na^+ . *Anabaena* L-31 also shows a Michaelis-Mentor type of rapid uptake but it has a much lower affinity for Na^+ than *A. torulosa*. The difference in K_m is probably in accordance with the metabolic requirement of Na^+ in a brackish water and a freshwater form (Apte and Thomas 1983). Salt-tolerance of *Anabaena torulosa* resembles those of glycophytes rather than halophytes. It is known that organic acids, amino acids or carbohydrates accumulate under stress to build up the osmotic potential (Flowers et al. 1977). High nitrogenase activity favouring enhanced accumulation

of amino acids for better osmoregulation and ability of mat formation and sporulation can contribute to the success of this cyanobacterium in saline environment (Fernandes and Thomas 1982).

9.3.2 Ca^{2+} Uptake

Calcium is required by cyanobacteria for heterocyst differentiation, nitrogen fixation (Chen et al. 1988; Smith et al. 1987), PS-II activity (Baker and Brand 1985; England and Evans 1983; Piccioni and Mauzerall 1978), and for phosphate uptake (Keurson et al. 1984). Ca^{2+} transports in bacteria occur through two routes of cation transport, import and export. Import of Ca^{2+} via a uniporter or a leak is driven by the membrane potential across the membrane. Export of Ca^{2+} occurs against the membrane potential and often against concentration gradient (Rosen 1982). The Ca^{2+} uptake kinetics is influenced by the different concentrations of cation itself, light, ATP, specific inhibitors/uncouplers, and calcium antagonist, agonist, and calmodulin antagonists in the cyanobacterium (Pandey et al. 1996).

The cyanobacterial cells remove Ca^{2+} from the medium in two possible ways, the first involving rapid binding/uptake of metal cation (first 10 min) followed by the slower second phase of 1 h (Pandey et al. 1996). Similar biphasic cation uptake has also been reported in yeast (Norris and Kelly 1977) and cyanobacteria (Khummongkol et al. 1982). A number of experiments reported that cyanobacteria exhibit a concentration-dependent uptake of Ca^{2+} (Shehata and Whitton 1982; Singh 1985).

Ca^{2+} uptake in *Nostoc* is an energy-dependent process (Pandey et al. 1996). Light-dependent Ca^{2+} uptake is similar to those for Cu^{2+} and Hg^{2+} uptake in *Nostoc calcicola* (Pandey and Singh 1993; Verma and Singh 1990), in contrast to cadmium uptake in *Anacystis nidulans* (Singh and Yadav 1985) and Al uptake in *Anacystis nidulans* (Pettersson et al. 1986), where metal uptake was independent of light. The vital role in active ion transport played by PSII-mediated energy generation is reported in the membrane vesicles of *Anabaena variables* (Lockau and Pfeffer 1983).

The deficiency of calcium resulted in disruption of the outer membrane, irreversible reduction in swimming speed, changes in the morphology of the cell surface and appearance of carotenoid-containing subcellular particles in the medium containing motile *Synechococcus* strain (Brahamsha 1996) and in a non-motile freshwater *Synechococcus* species (Resch and Gibson 1983). The addition of calcium restored motility to the level of untreated cells. This effect was specific as no other divalent ion tested could substitute for calcium. The plot of motility restoration was a steep sigmoid, indicating that the binding or effect of calcium is highly cooperative (Tisa et al. 1993; Tisa and Adler 1995). It is thought that this action potential is used to communicate the reversal signal to all the cells in the trichome (Murvanidze and Glagolev 1982). Womack et al. (1989) showed that calcium is required both for gliding (0.1–0.3 mM) and induction (1 mM) of the gliding machinery in myxobacteria.

9.4 Mechanisms of Salt Tolerance in Cyanobacteria

Cyanobacteria are able to tolerate stresses predominant in salt-affected soils such as nutrient deficiency, salinity, drought and temperature up-shift (Apte et al. 1997). Adaptation to salt stress in cyanobacteria consists of at least three phenomena: (a) accumulation of internal osmoticum in the form of inorganic ions (Miller 1976) or organic solutes (Blumwald et al. 1983; Mackay et al. 1983; Reed et al. 1984); (b) contribution of ion transport processes (Apte et al. 1987; Apte and Thomas 1983, 1986; Reed et al. 1985; Reed and Stewart 1985; Thomas and Apte 1984); and (c) metabolic adjustments (Blumwald and Tel-Or 1984; Thomas and Apte 1984). Several workers have pointed out the various mechanisms adopted by cyanobacteria for salt tolerance (Table 9.2).

Table 9.2 Mechanisms adopted for salt tolerance by cyanobacteria

Mechanisms for salt tolerance	References
1. Production of stress-responsive protein	Apte and Bhagwat 1989; Bhagwat and Apte 1989; Iyer et al. 1994; Apte et al. 1998; Hengge-Aronis 1993; Nystrom and Neidhardt 1993; Volker et al. 1994; Huang et al. 2006; Wilkinson and Northcote 1980; Fulda et al. 2000; Kroll et al. 2001; Pandhal et al. 2008
2. Restricted entry of Na ⁺	Roychoudhury et al. 1985; Jha et al. 1987; Kaushik and Nagar 1993; Jha and Kaushik 1988; Hu et al. 2003; Acea et al. 2003; Pandey et al. 2005; Singh 1950; Thomas 1978; Malam et al. 2007; Nisha et al. 2007
3. Na ⁺ Efflux	Paschinger 1977; Heefner and Harold 1982; Apte and Thomas 1983; Ardelean 1966; Thomas and Apte 1984; Krulwich 1983; Waditee et al. 2001, 2002; Padan and Schuldiner 1996; Krulwich and Guffanti 1989; Espie and Kandasamy 1994; Mochizuki-Oda and Oosawa 1985
4. Na ⁺ -dependent K ⁺ Uptake in prokaryotes	Bray 1997; Shinozaki and Yamaguchi-Shinozaki 1997; Bremer and Kramer 2000; Hasegawa et al. 2000; Morbach and Kramer 2002; Reed and Stewart 1985; Dinnbier et al. 1988; Record et al. 1998; Whatmore et al. 1990; Whatmore and Reed 1990; Holtmann et al. 2003; Bakker 1993; Stumpe et al. 1996; Oren 1999; Reed et al. 1985; Marin et al. 1998; Ferjani et al. 2003; Berry et al. 2003; Matsuda et al. 2004; Mikami et al. 2002; Suzuki et al. 2000.
5. Compatible solutes and lipids in salt tolerance	Goel et al. 1997; Mackay et al. 1984; Reed et al. 1986; Joset et al. 1996; Hagemann and Erdmann 1997; Hagemann et al. 1987, 2001; Erdmann et al. 1992; Mikkat and Hagemann 2000; Deshniem et al. 1995, 1997; Ishitani et al. 1995; Nakamura et al. 1997; Hufleijt et al. 1990; Khamutov et al. 1990; Ritter and Yopp 1993; Tasaka et al. 1996; Allakhverdiev et al. 1999, 2000a, b, 2001; Singh et al. 2002; Blumwald et al. 1984; Padan and Schuldiner 1994; Kates et al. 1984; Kamada et al. 1995
6. Enhancement of Cyanobacterial Salt Tolerance by Combined Nitrogen	Apte et al. 1987; Reddy et al. 1989; Cheeseman and Delvin 1985; Suput 1984; Sprott et al. 1984.

9.4.1 Role of Stress-Responsive Proteins

The polypeptides induced in response to high NaCl content is referred to as Ionic Stress Proteins (ISPs) and by heat stress are called Heat Shock Proteins (HSPs). Stress proteins induced both by high NaCl and sucrose but not by heat shock are called Osmotic Stress Proteins (OSPs). There are some commonly induced proteins by heat, salinity as well as osmotic stress and these are called General Stress Proteins (GSPs). Thus, large number of proteins described as ISPs, HSPs or OSPs, etc. actually belong to the category of GSPs (Apte and Bhagwat 1989; Bhagwat and Apte 1989; Iyer et al. 1994). These stress proteins are generally expressed at low levels and for a short period (Apte et al. 1998). Many of the starvation-induced proteins overlap with proteins synthesised under heat shock, high osmolarity, nitrogen and phosphate starvation, exposure to heavy metals and other stresses and interfere with the flow of carbon in the central catabolic pathway of carbon breakdown (Hengge-Aronis 1993; Nystrom and Neidhardt 1993; Volker et al. 1994). Some of the stress-tolerance mechanisms are expressed at all times and are used to encounter some frequently encountered stresses, e.g. low-level constitutive expression of major Heat Shock Proteins (HSPs). On the other hand, most of the adaptive nature of responses remains shutting off under normal conditions of growth and are expressed in a need-based manner (Apte et al. 1998).

Exposure of cyanobacterial cells to different abiotic stresses resulted in rapid expression of several stress-regulated proteins and modifications in protein synthesis programme (Iyer et al. 1994). The expression of almost all the abiotic stress-induced proteins in *Anabaena* appears to be brought about by transcriptional activation of stress-responsive genes and even a mild change in growth conditions significantly alters the protein synthesis pattern in cyanobacteria (Apte et al. 1998).

Many proteins from the plasma membrane associated with salt stress were screened for proteomic changes in the plasma membranes of *Synechocystis* in response to salt (Huang et al. 2006). Increases in phosphate and nitrite/nitrate binding proteins have been hypothesised as a necessity for cells to overcome salt-induced nutrient deficiency, a problem resulting from plasma membrane structural changes (Wilkinson and Northcote 1980). Further salt-induced proteins, thought to play significant roles in stress, included vesicle-inducing protein (Kroll et al. 2001); membrane-bound peptidyl-prolyl isomerase B, which could be involved in maintaining the integrity of proteins in the plasma membrane and CoxB, which plays a role in managing photosynthesis in stressed cells (Fulda et al. 2006).

Nitrate induces Osmotic Stress Proteins expression and this is related to the enhanced osmotolerance exhibited by nitrogen-supplemented *Anabaena* cultures (Iyer et al. 1994). Most salt-related cyanobacterial proteomic studies have utilised the model organism *Synechocystis* sp. PCC6803. At 2–4% w/v NaCl concentrations this organism is reported to respond and adapt to the salt stress through synthesis of general and specific stress proteins, altering the protein composition of extracellular layers, and re-directing control of complex central intermediary pathways (Pandhal et al. 2008).

9.4.2 Restricted Entry of Na⁺

Cyanobacteria respond to high salinity in the soil by restricting the entry of sodium ions and thus, preventing the cell injury by keeping a low internal concentration of Na⁺ (Roychoudhury et al. 1985; Jha et al. 1987). A high level of Na⁺ adsorption in presence of K⁺ over other cations could be a prelude to the greater uptake of sodium, but it remained trapped in the polysaccharides (Kaushik and Nagar 1993). The adsorption of various cations to the polysaccharides may be a purely passive process; although a minor variation in the quantity of cations may be due to the nature of polysaccharides (Jha and Kaushik 1988).

9.4.2.1 Polysaccharides

Cyanobacteria have been recognised as an important agent in the stabilisation of soil surfaces primarily through the production of extracellular polysaccharides, which are prominent agents in the process of aggregate formation and increase in soil fertility (Hu et al. 2003; Acea et al. 2003; Pandey et al. 2005). Salt tolerance of cyanobacteria has a potential biological utility in the reclamation of salt-affected agricultural soils (Singh 1950). The advantage of this approach probably lies in the apparent ability of cyanobacterial polysaccharides to ‘chelate’ considerable amounts of Na⁺ and temporarily immobilise the excess Na⁺. However, the removal of the algal-bound Na⁺ from soil ecosystems is a big challenge (Thomas 1978). There are a large number of N-fixing cyanophyceae that produce extracellular polysaccharides and this division offers potential for the development of soil conditioners and significantly increased productivity (Malam et al. 2007). The amount of polysaccharides produced was reported to increase with increasing NaCl concentration in salt-tolerant *Westiellopsis prolifica* (Jha et al. 1987).

9.4.3 Na⁺ Efflux

An active Na⁺ efflux is driven by a Na⁺/H⁺ antiporter involving a proton translocating ATPase in *Anacystis nidulans* (Paschinger 1977), and resembles that in *Streptococcus faecalis*, where efflux is mediated by a Na⁺-stimulated ATPase which is insensitive to several inhibitors of conventional ATPase (Heefner and Harold 1982). While ATP-driven Na⁺ pump in *S. faecalis* requires only ATP and does not require proton motive force, in *Anabaena* spp., Na⁺ efflux probably requires an H⁺ gradient. Therefore, the ‘Na⁺ pump’ of *Anabaena* spp. can be described as a Na⁺/H⁺ or Na⁺/K⁺ antiporter similar to Na⁺/H⁺ antiporter in *A. nidulans* but involving a ATPase distinct from the conventional one. The energy source for Na⁺ extrusion may be ATP derived from oxidative phosphorylation (Heefner and Harold 1982).

Maintenance of low intracellular Na⁺ concentrations and exclusion of Na⁺ appear to be responsible for the salt tolerance of *Anabaena torulosa*, a brackish

water species (Apte and Thomas 1983). Presence of nitrate or ammonium severely reduced influx and stimulated efflux of Na^+ in *Anabaena* species (Thomas and Apte 1984). A higher respiratory activity was observed in light under salt-stress conditions than in control, which suggests the involvement of respiratory activity in the extrusion of Na^+ ions outside the cell (Ardelean 1966).

Distinct from the enzyme-linked primary transport involving Na^+ and related processes, secondary active transport such as the Na^+/H^+ antiporter, first predicted by Mitchell in 1966, has been since elucidated (Krulwich 1983). The Na^+/H^+ antiporter which catalyses translocation of Na^+ and H^+ in opposite directions may operate in either direction across the cell membrane. Moreover, movement of Na^+ ions coupled to another metabolite in the same direction by the symport mechanism also facilitates substrate transport (Waditee et al. 2002). It is reported that *Anabaena halophytica* contains a Na^+/H^+ antiporter which can confer salt tolerance on the cells (Waditee et al. 2001).

During the generation of a pH gradient across the membrane, an increase in the extracellular pH would lead to a decrease in the pH gradient and consequently the intracellular Na^+ might also decrease. The observed increase of nitrate uptake at increasing extracellular pH might be accounted for by an increase of Na^+ -gradient mediated by Na^+/H^+ antiporter. Indeed, the activity of Na^+/H^+ antiporter in *Anabaena halophytica* has shown to increase with increasing pH (Waditee et al. 2001). A reduction of nitrate uptake was observed in *Anabaena halophytica* in the presence of an inhibitor of Na^+/H^+ antiporter which shows the involvement of Na^+/H^+ antiporter in the uptake of nitrate (Mochizuki-Oda and Oosawa 1985). The role of Na^+/H^+ antiporter in the generation of sodium motive force to power Na^+ /solute symport has also been proposed for the transport of anions across the membranes (Krulwich and Guffanti 1989; Espie and Kandasamy 1994).

9.4.4 Na^+ -Dependent K^+ Uptake in Prokaryotes

The condition of hyperosmolality caused by high salinity or drought constitutes a major challenge to the growth of prokaryotes (Bray 1997; Shinozaki and Yamaguchi-Shinozaki 1997; Bremer and Kramer 2000; Hasegawa et al. 2000; Morbach and Kramer 2002). During the first phase, bacterial cells accumulate additional K^+ from the medium through their K^+ uptake systems and synthesise glutamate concomitantly. Thereby, they increase the ion content of their cytoplasm and counteract plasmolysis brought about by water efflux. During its second phase of the cellular adaptation, cyanobacteria replace this internal potassium glutamate by synthesising high concentrations of glucosylglycerol and sucrose, which are accumulated as compatible solutes in their cytoplasm (Reed and Stewart 1985). Within a few minutes, Na^+ is replaced by K^+ and subsequently, it takes lot of time to replace the high K^+ concentration in the cytoplasm by glucosylglycerol as well as minor amounts of sucrose (Reed et al. 1985).

During its long-term adaptation to high NaCl concentrations, *Synechocystis* sp. PCC 6803 also synthesises and accumulates glucosylglycerol (Marin et al. 1998; Ferjani et al. 2003). Strain PCC 6803 has been predicted to contain at least three

types of K^+ uptake systems; Kdp system, which is probably of minor importance (Berry et al. 2003); a Ktr system (Nakamura et al. 1998), which appears to play a role in salt stress by high NaCl concentrations (Berry et al. 2003) and K^+ channels. It has been reported that in *Synechocystis* sp. PCC 6803, the *ntpJ* gene (*slr1509*) is essential for both the adaptation to high NaCl concentrations and the bicarbonate transport via the SbtA system. SbtA-mediated bicarbonate transport was also dependent on the presence of external Na^+ . It was interpreted that NtpJ functions as a Na^+ efflux system required for the removal of Na^+ from the cells after a hyperosmotic shock with NaCl and/or Na^+ uptake due to Na^+/HCO_3^- symport via SbtA (Matsuda et al. 2004).

Transmembrane ion transport processes play a key role in the adaptation of cells to hyperosmotic conditions. Heterologous expression experiments in *Escherichia coli* show that three *Synechocystis* genes are required for K^+ transport activity. They encode an NAD^+ -binding peripheral membrane protein, an integral membrane protein (belonging to a superfamily of K^+ transporters) and a novel type of *ktr* gene product (Matsuda et al. 2004). The genome of *Synechocystis* sp. PCC 6803 contains 43 genes that encode putative histidine kinases, identified as a sensor of osmotic stress (Mikami et al. 2002; Suzuki et al. 2000).

9.4.5 Role of Compatible Solutes and Lipids in Salt Tolerance

The protection against alkaline environment is also provided by the synthesis of certain fatty acids, sucrose and osmotic-stress-induced proteins (Goel et al. 1997). The inducible synthesis of compatible solutes such as sucrose is synthesised in salt-sensitive strains of cyanobacteria such as *Synechococcus* (Mackay et al. 1984; Reed et al. 1986; Joset et al. 1996; Hagemann and Erdmann 1997); glucosylglycerol is synthesised in strains with intermediary tolerance such as *Synechocystis* sp. PCC 6803 (Hagemann et al. 1987, 2001; Erdmann et al. 1992; Mikkat and Hagemann 2000); glycine betaine is synthesised in salt-tolerant *Synechococcus* sp. PCC 7418 (Mackay et al. 1984; Joset et al. 1996). Direct evidence for the ability of these compatible solutes to protect the cyanobacterial cells may be seen from studies of transgenic systems (Deshnium et al. 1995, 1997; Ishitani et al. 1995; Nakamura et al. 1997).

Many reports have suggested the role of lipids in the protection of cyanobacteria against salt stress (Hufleijt et al. 1990; Khamutov et al. 1990; Ritter and Yopp 1993). When photosynthetic organisms are exposed to salt stress, the fatty acids of membrane lipids are desaturated (Allakhverdiev et al. 2001). Targeted mutagenesis has been used to alter genes for fatty acid desaturases in *Synechocystis* and have produced strains with decreased levels of unsaturated fatty acids in their membrane lipids (Tasaka et al. 1996) as well as decreased tolerance to salt (Allakhverdiev et al. 1999). Their results demonstrated that an increase in the unsaturation of fatty acids in membrane lipids enhanced the tolerance to salt stress of the photosynthetic and Na^+/H^+ antiport systems of *Synechococcus* (Allakhverdiev et al. 2000a, b; Singh et al. 2002).

The unsaturation of fatty acids in membrane lipids might activate the Na^+/H^+ antiport system through enhanced fluidity of the membrane with resultant protection of PSII and PSI activities (Blumwald et al. 1984; Padan and Schuldiner 1994). The activities of several membrane-bound enzymes are known to be affected by changes in membrane fluidity (Kates et al. 1984; Kamada et al. 1995). The unsaturation of fatty acids might stimulate the synthesis of the Na^+/H^+ antiporter(s) and/or H^+ ATPase(s). The increased density in the membrane of these components of the antiport system might result in a decrease in the concentration of Na^+ in the cytosol, which would tend to protect PSII and PSI against NaCl -induced inactivation and to accelerate the recovery of PSII and PSI activities (Allakhverdiev et al. 2001).

9.4.6 Enhancement of Cyanobacterial Salt Tolerance by Combined Nitrogen

Enhanced salt tolerance in the presence of combined nitrogen is suggested by (a) all nitrogen compounds which protect against salt, reduce Na^+ influx; (b) proline and glycine which are ineffective against Na^+ influx, offer no protection against salt stress; (c) the effectiveness of different nitrogen compounds in protecting against salt stress follows the order of efficiency with which they inhibit Na^+ influx; (d) moreover, the relationship between inhibition of Na^+ influx and enhancement of salt tolerance is found to be independent of the inherent ability of cyanobacteria to tolerate NaCl , i.e. not only a salt-sensitive strain becomes salt-tolerant, but tolerance of *A. torulosa* is further enhanced beyond its normal abilities (Apte et al. 1987; Reddy et al. 1989). Provision of nitrate, ammonium, glutamine, glutamate, and aspartate in the medium enhances the salt tolerance of *Anabaena* by two- to threefold (Apte et al. 1987). Presence of NH_4 , NH_3 , or certain amino acids in the medium has been shown to prevent intracellular accumulation of Na^+ or K^+ in certain animal systems (Cheeseman and Delvin 1985; Suput 1984) and microbes (Sprott et al. 1984) usually by stimulating the efflux of the cation (Reddy et al. 1989).

9.5 Influence of Cyanobacterial Application on Salinity Related Soil Properties

Cyanobacterial application results in the enrichment of soil with fixed nitrogen, soil structure improvement and declining trend of pH, electrical conductivity and Na^+ . These changes improved the crop vigour and yield in salt-affected soil (Kaushik et al. 1981; Subhashini and Kaushik 1981; Kaushik and Krishnamurti 1981; Kaushik and Subhashini 1985) (Table 9.3). Enrichment of salt-affected soils with native cyanobacterial isolates, over a period of time improved the soil quality and resulted in the decrease of pH, exchangeable sodium (ES), Na/Ca and overall increase in N, P, organic matter and

Table 9.3 Influence on pH, electrical conductivity (EC) and exchangeable sodium (ES) of sodic soils after 3 years of BGA (blue-green algae) application (initial soil pH = 10, exchangeable Na⁺ = 10.44 meq 100 g⁻¹, CEC = 11.19 meq 100 g⁻¹)

Treatments	After summer paddy of								
	1985			1986			1987		
	pH	EC	ES	pH	EC	ES	pH	EC	ES
Control	9.65	1.93	9.72	9.67	0.6	9.51	9.30	0.64	5.21
BGA	10.45	1.36	5.44	9.20	1.1	4.67	7.71	1.08	1.26
Gypsum	9.35	1.61	4.78	9.13	2.13	5.97	7.7	1.31	1.15
BGA + gypsum	9.30	1.59	4.78	8.73	1.52	2.39	7.66	1.03	0.87

water-holding capacity of soil. This reduced the sodium adsorption ratio, which is an index of alkalinity and improved the hydraulic conductivity of sodic soils (Subhashini and Kaushik 1981; Rai et al. 1998). These results compared favourably to the use of chemical amendments such as gypsum in such soils (Kaushik and Krishnamurti 1981).

9.5.1 Soil pH

Under natural conditions, most of the cyanobacteria grow in neutral to alkaline conditions (Fogg 1956) and sometimes the growth of diazotrophic cyanobacteria in rice fields is limited by low pH (Whitton and Potts 2000). Alkaline soil with high pH and Na⁺ content favour the growth of diazotrophic cyanobacteria with a consequent decrease in pH. Indeed, cyanobacteria are reported to decrease soil pH from 9.2 to 7.5 under natural conditions and pyrite application speed up the process of saline soil reclamation (Singh 1961; Verma and Abrol 1980). The application of *Westiellopsis* decreased the soil pH from 8.05 to 7.71 which was due to combined influence of leaching and release of organic acids through microbial decomposition of amendments (Prabu and Udayasoorian 2007). Such reduction in pH had been reported in laboratory experiments, without any crop (Subhashini and Kaushik 1984) and with rice (Elayarajan 2002).

9.5.2 Electrical Conductivity

The application of cyanobacteria to saline soil reduces the electrical conductivity of saline soil (Kannaiyan et al. 1992). Leaching of salt-affected soil alone cannot result in a substantial reduction in the electrical conductivity. But algalisation with *Westiellopsis* and amendments with gypsum showed progressive decrease in soil electrical conductivity, followed by leaching (Prabu and Udayasoorian 2007). The extracellular polysaccharide production by cyanobacteria offers some temporary

relief by chelating exchangeable cations in soil and thereby decreases the soil electrical conductivity (Apte and Thomas 1997). In salt-affected areas in India, application of cyanobacteria combined with gypsum or sulphur changed the soil pH from alkaline to neutral, reduced exchangeable Na and electrical conductivity, and led to the development of soil aggregates in the long term (Kaushik and Krishnamurti 1981; Kaushik 1989).

9.5.3 Exchangeable Sodium

Reduction in soil exchangeable Na content was recorded with the application of gypsum and *Westiellopsis*. This in turn, exhibited a favourable effect on leaching of Na in soil complex (Rogers and Burns 1994). The extracellular polysaccharide production by cyanobacteria offers some temporary relief by chelation of excess Na⁺ present in soil (Apte and Thomas 1997). Cyanobacteria also reduce sodium ion content of the soil by making calcium ions available through solubilisation of calcium carbonate nodules, possibly by releasing various organic acids, like oxalic, oxaloacetic, lactic and succinic acids (Bhatnagar and Roychoudhury 1992).

9.5.4 Sodium Absorption Ratio and Exchangeable Sodium Percentage

High toxicity due to sodium can be reduced by decreasing the sodium absorption ratio and exchangeable sodium percentage which can be augmented by increasing the proportion of Ca²⁺ and Mg²⁺ to Na⁺ in soil exchange complex by the addition of their salts (Pandey et al. 2005). Application of *Westiellopsis* cultures along with high exchangeable Ca and Mg amendments caused significant changes in soil sodium absorption ratio and exchangeable sodium percentage (Rogers and Burns 1994).

9.6 Improvement of Soil Properties

9.6.1 Soil Structure

The extracellular polysaccharides excreted by cyanobacteria have been reported to be responsible for binding of soil particles leading to the formation of tough and entangled superficial structure that improves the stability of soil surface and protects it from erosion (Rogers and Burns 1994). The mucilaginous sheath of *Aphanothece* sp. formed a grey substratum firmly holding the soil particles together which checked wind- and water-mediated soil erosion, particularly in light and sandy soils (Singh 1961).

Cyanobacterial sheaths and extracellular polysaccharides also play a significant role in water storage due to the hygroscopic properties of polysaccharides. Several workers have reported that the application of cyanobacterial cultures to problem soils improved the soil physical properties with enhanced hydraulic conductivity (Malam et al. 2001a, b) which increased the productivity of rice and rapeseed crop. Enhanced hydraulic conductivity resulted in better root penetration and increased nutrient uptake from nutrient-limiting sodic and saline soils (Fernandez et al. 2000).

9.6.2 Nutrient Content

As early as 1950, cyanobacterial inoculation was reported to build up organic matter in soil (De and Sulaiman 1950). The application of cyanobacteria to saline soil enhances the soil organic carbon content due to the addition of organic matter in the form of algal biomass (Kannaiyan et al. 1992; Apte et al. 1997). Cyanobacterium *Nostoc muscorum* is also reported to increase the organic carbon content of soil (Rogers and Burns 1994). This content increased significantly in the post-harvest soil in response to cyanobacterial application in the paddy field (Hashem 2001). In the *Westiellopsis* inoculated soil at 90 day, the organic matter content was three times greater than that of non-inoculated soil (Prabu and Udayasoorian 2007).

The photosynthetic nitrogen-fixing cyanobacteria are well-equipped to handle deficiency of nitrogen which otherwise decreases the overall tolerance of cyanobacteria to different environmental stresses (Apte et al. 1997; Apte 1992, 1993). The available nitrogen content of soil increased during the incubation of arid environments with *Westiellopsis* sp. (Rogers and Burns 1994; Lange et al. 1994). Both heterocystous and non-heterocystous cyanobacteria have the capability to scavenge available nitrogen (NO_3^- and NH_4^+) and phosphorous from secondary treated sewage effluent and produce massive biomass which can in turn strengthen the soil with valuable nutrients (Singh and Dhar 2006, 2007). Cyanobacteria can also fix a significant amount of atmospheric nitrogen and the amount of nitrogen fixed on average is 30–40 kg N ha⁻¹ crop⁻¹ which in turn can improve soil fertility (Roger and Kulasooriya 1980; Aziz and Hashem 2003; Metting 1990).

Available soil phosphorous and sulphur also increased in response to cyanobacterial application (Hashem 2001). Solubilisation of muscorrie rock phosphate – a source of P_2O_5 and a raw material for fertilizer industry, by nitrogen fixing *Tolypothrix tenuis*, *Scytonema cincinnatum* and *Hapalosiphon fontinalis* was observed under in vitro studies (Roychoudhury and Kaushik 1989). Other cyanobacterial strains namely *Calothrix braunii*, *Tolypothrix ceylonica*, *Scytonema cincinnatum*, *Haplosiphon fontinalis* and *Westiellopsis prolifica* have been reported to solubilise insoluble rock phosphate and make it available to the crop plants (Roychoudhary and Kaushik 1989). Vitamin B₁₂ synthesis and liberation by *Tolypothrix tenuis*, *Nostoc muscorum* and *Haplosiphon frontalis* has also been demonstrated (Misra and Kaushik 1989a). In addition to free amino acids, like serine, arginine, glycine,

aspartic acid, threonine, glutamic acid, etc. strains of *Nostoc* and *Haplosiphon* also produce growth-promoting substances, namely indole-3-acetic acid and indole-3-propionic acid (Misra and Kaushik 1989b).

9.7 Crop Response in Saline–Alkaline and Sodic Soils

The role of cyanobacteria in the sustained fertility of flooded/irrigated rice field soils is well-established (Singh 1961; Venkataraman 1975; Roger 1996). The potential impact of these organisms on agriculture through their use as soil conditioners, plant growth regulators and soil health ameliorators has also been well-recognised (Venkataraman 1975, 1979; Vaishampayan et al. 2001; Whitton 2000). The positive effect of cyanobacterial inoculation often increases with time and only a fraction (2–10%) of nitrogen fixed is immediately available to the crop and the remainder is released following death and decomposition of the algae (Venkataraman 1979). The salt tolerance of cyanobacteria has been exploited with some success in the reclamation of saline and sodic soils (Thomas and Apte 1984). The practice of utilising cyanobacteria as an efficient source of biofertilizer for rice has been advocated and adopted in the tropical countries, where conditions are favourable for their mass multiplication (Venkataraman 1981; Kannaiyan 1990).

Species of *Nostoc*, *Anabaena*, *Tolypothrix*, *Aulosira*, *Cylindrospermum*, *Scytonema*, *Westiellopsis* and several other genera are widespread in tropical rice field soils and are known to contribute significantly to their fertility (Venkataraman 1981; Kaushik 1994). A series of experiments lead to the conclusion that (i) blue-green algae are effective as bioameliorant in saline and sodic soils, (ii) the benefits of blue-green algae can further be enhanced if gypsum at 50% of its required dose is applied along with blue-green algae, (iii) both grain and straw yields are enhanced by the combined application of blue-green algae and gypsum at all the levels (25%, 50%, 75% and 100% of gypsum or blue-green algae alone). Such improved crop yields may be due to reduction of soil pH, exchangeable sodium and electrical conductivity (Kaushik et al. 1981; Subhashini and Kaushik 1981; Kaushik and Krishnamurti 1981) as well as enrichment of soil with fixed nitrogen, increased availability of phosphorous, improved soil aggregation and hydraulic conductivity (Subhashini and Kaushik 1984; Kaushik and Subhashini 1985).

9.8 Crop Yield

It is well-known that besides bringing about an improvement in yield of rice (ranging from 5% to 25%), cyanobacteria produce indirect or direct beneficial changes in the physical, chemical and biological properties of soil and soil–water interface in rice fields, which are of agronomic importance (Mandal et al. 1998). In tropical rice

Table 9.4 Yield (q ha^{-1}) of paddy (var. Pusa 33) and wheat (var. 2393) in salt-affected soils after 2 years of reclamation with different treatments including BGA (blue-green algae)

Treatments	Paddy	
	Grain	Straw
Control	6.15	5.15
BGA	41.0	30.6
Gypsum 100%	42.0	30.40
BGA + Gypsum 100%	45.4	33.2
	Wheat	
BGA	28.50	32.75
90 kg N	32.75	37.75
BGA +90 kg N	35.25	42.25
120 kg N	35.70	41.75
BGA +120 kg N	39.75	52.38
150 kg N	39.25	49.38

fields, biological nitrogen fixation is mainly a cyanobacterial process and part of the nitrogen demand of the rice crop is met through the indigenous populations or through their application as biofertilizers (Mitra 1951). The yields obtained in blue-green-algae-treated plots were observed at par with gypsum ($41\text{--}42 \text{ q ha}^{-1}$), although combined application of both was still better. The yield of wheat recorded after 2 years of reclamation with blue-green algae and/or gypsum, was also better in the treatment with combination of blue-green algae and chemical fertilizer than the fertilizer alone (Kaushik 1994; Table 9.4).

Extracellular products of cyanobacteria counteracted NaCl-induced inhibition on shoot length and increased root dry weight, nullified the salt effect on shoot dry weight (Aziz and Hashem 2004; Rodríguez et al. 2006). Extracellular products from *Scytonema hofmanni* reverted completely or partially many of the NaCl-induced effects on growth and biochemical attributes of rice seedlings. The cyanobacterial extracellular products also contain gibberellin-like substances which may be responsible for the alleviation of adverse effect of salt stress on crop productivity (Rodríguez et al. 2006). Seed priming in *Lupinus termis* with cyanobacterial culture filtrate increased chlorophyll 'a' and 'b', reduced carotenoids content, increased auxin, gibberellic acid and cytokinin content and decreased abscissic acid content (Haroun and Hussein 2003; El-Shahaby 1992).

Water infiltration and soil surface stability are related to cyanobacterial biomass which favourably influences the crop yield (Jeffries et al. 1993a, b). In greenhouse experiments, nitrogen levels in *Sorghum halepense* were higher when the plant was in pots with cyanobacteria than in the pots without cyanobacteria. Dry weight of plants in pots with cyanobacteria was up to four times greater than in pots without cyanobacteria (Harper and Pendleton 1993; Shields and Durrell 1964; Brotherson and Rushforth 1983; Pendleton and Warren 1995).

9.9 Residual Effect of Cyanobacterial Application in Crop Field

Certain cyanobacteria have been found not only to grow in saline ecosystems but also improve the physico-chemical properties of the soil by enriching them with carbon, nitrogen and available phosphorus (Antarikanonda and Amarit 1991). Cyanobacteria fix as much as 28 kg Nha⁻¹year⁻¹ and are important because algalisation may affect plant size, number of tillers, ears, spikelets and filled grains per panicle (Metting 1990). In addition, cyanobacterial sheath material is often coated with negatively charged clay particles which are more nutrient rich than sand (Black 1968), as they bind positively charged macronutrients and prevent them from leaching through the soil profile (Belnap and Gardner 1993). Compounds in the gelatinous sheath material of several cyanobacterial taxa were able to chelate iron, copper, molybdenum, zinc, cobalt, and manganese. Four cyanobacterial genera (*Anabaena*, *Anacystis*, *Lyngbya*, and *Nostoc*) reported to possess this ability, are commonly represented in biological crusts (Lange 1974; Shields and Durrell 1964). It is also possible that the nutrient differences result from thermal effects, as crusted soils are darker and warmer than uncrusted soils; nutrient uptake by vascular plants would occur at a higher rate (Shields and Durrell 1964).

The organic metabolites produced by cyanobacterial growth are released in the extracellular soil environment and their degradation products can accumulate and increase the organic N content, which subsequently maintains the fertility of soil year after year (Roger and Kulasooriya 1980; Ladha and Reddy 1995). Growth of cyanobacteria in soil decreases the C/N ratio due to N₂ fixation (Watanabe et al. 1977). An increase in C/N ratio in the experiment conducted under natural conditions with natural inoculum compared to *Nostoc calcicola* inoculation may be due to the growth of many non-N₂-fixers. Soil properties such as soil structure can be improved via the production of extracellular substances by cyanobacteria (Whitton and Potts 2000).

Polysaccharides produced by cyanobacteria excrete a number of compounds which diffuse around soil particles, glue and hold them together in the form of micro-aggregates (Brotherson and Rushforth 1983; Alexander and Calvo 1990). Well-developed aggregate stability and surface roughness resist soil particle dislodgement by wind (Chepil and Woodruff 1963). Enrichment of saline soils with the indigenous cyanobacterial strains may help in ameliorating the land and making them suitable for obtaining higher yields. Benefits other than nitrogen fixation include solubilisation of phosphorus, improved soil structure and synthesis of growth promoting substances which also has beneficial effect on the succeeding crop and helps in improving the physico-chemical properties of soil and crop yield (Khan et al. 1994; Saxena and Kaushik 1992). A long-term fertility experiment indicated that the use of chemical fertilizers is not sufficient to restore soil fertility and under such conditions, biofertilizers like cyanobacteria could provide essential nutrient and organic matter to the soil (Singh and Bisoyi 1993).

9.10 Technology for Soil Health Improvement

The reclamation of sodic soils involves chemical amendment with gypsum or Fe pyrites and leaching to remove excess salts. Reclamation by biological methods is slower and depends on the incorporation of green manures (Rao and Burns 1990). Saline soils can be reclaimed easily by irrigating the field with good quality of water so as to facilitate leaching of salts. The salts move below the root zone and thus, crops escape the injurious effects of salts. Only flushing of the field may not be effective and the addition of cyanobacterium inoculum along with addition of gypsum is required before irrigation to ameliorate saline soils (Kaushik and Krishnamurti 1981).

Pyrite used as a chemical corrective to reclaim sodic soil lacks complete oxidation and may cause toxicity by releasing ferrous and sulphide ions. However, cyanobacteria growing in soils utilise iron and sulphide for their growth. Nitrogenase induction by sulphide in non-heterocystous cyanobacteria is optimal at high pH (Villbrandt and Stal 1996). The sulphide acts as a reducing agent and its addition to the diazotrophic *Plectonema boryanum* enhanced nitrogenase activity (Kashyap et al. 1996). In microbial mats, iron may participate as iron sulphide (FeS) or pyrite (FeS₂). Iron reacts with oxygen and keeps the partial pressure of oxygen sufficiently low to allow efficient photosynthesis and N₂ fixation by cyanobacteria (Whitton and Potts 2000).

The gypsum requirement for reclaiming the saline soil depends upon the level of Na⁺ present in the soil. The calcium ion (Ca²⁺) present in the gypsum (CaSO₄·2H₂O) replaces the Na⁺ on the cation exchange site and results in the formation of insoluble salt (Na₂SO₄). This can be easily removed from the root zone by impounding sufficient water in the field. The calcium acts as flocculating agent and helps in the formation of soil aggregates and thus, improves the physical and chemical properties of soil for production of cereals and plantation crops (Singh 1961). The results become more satisfactory if gypsum is applied along with good amount of inoculum of halotolerant cyanobacterial strains (Kaushik 2005).

The calcareous saline soil contains huge amounts of carbonate. Addition of pyrite in such soils may result in the formation of sulphurous and sulphuric acid, which may react with carbonate and eventually produce CO₂. The growth of cyanobacteria in such soils increased soluble CO₂ (Singh 1961). The inoculation of HCO₃⁻-resistant mutants of *Nostoc calcicola* in pyrite-treated alkaline soil led to better reclamation results as determined by pH coupled with growth (population) compared to the same treated soil inoculated with the wild type (Pandey et al. 2005).

The area to be ameliorated should be first of all levelled and divided into sub-plots (size 200 m²), bound by thick earthen embankment of 45 cm height and should be puddled properly before flooding. Then algal ameliorant (usually halotolerant nitrogen fixing) should be broadcasted at 20 kg ha⁻¹ (soil based) or 2 kg ha⁻¹ (straw based) in the standing water which should remain stagnant in the field for at least 2 weeks for algal proliferation with a total incubation period of the soil with algae of at least 10 weeks. Lastly the soil should be puddled and transplanted with paddy seedlings with normal required dose of fertilizer (Kaushik 2005).

9.11 Amelioration of Saline Soils Using Halotolerant Nitrogen-Fixing Cyanobacteria

Cyanobacterial reclamation of saline and alkaline soils has been suggested as early as 1950 (Singh 1950). It has been noticed that nitrogen-fixing blue green algae can be used as biological input to improve soil texture, conserve moisture, scavenge the toxic sodium cation from the soil complex and improve the properties of such soils (Subhashini and Kaushik 1981). They have reported that *Tolypothrix ceylonica*, *Haplosiphon intricatus* and two species of *Calothrix* (*C. braunii* and *C. membranacea*) showed absorption of sodium $0.8 \mu\text{g mg}^{-1}$ algal dry weight grown in the medium containing $1.425 \text{ mg Na ml}^{-1}$ (Subhashini and Kaushik 1982).

Systematic work done in IARI (New Delhi, India), has led to the conclusion that (i) incubation of soil with blue-green algae (native/mixture of known blue-green algae) improves the physico-chemical properties of saline and alkaline soil (Kaushik et al. 1981). (ii) *Nostoc*, *Anabaena*, *Calothrix* and *Plectonema* are the common forms of cyanobacteria observed in soils of pH 9.0 and above. (iii) The distribution of nitrogen-fixing cyanobacterial forms seems to be localised. The strains colonising salt-affected soils are mostly similar to those observed in normal paddy field soils; however, those found in highly alkaline soils are different (Subhashini and Kaushik 1982). (iv) These halotolerant strains secrete excessive extracellular polysaccharides in response to salts and thus chelate some of the toxic cations (Roychoudhury et al. 1985). (v) Some of the cations undergo temporary immobilisation as their salts are taken up by the organisms for their own growth. (vi) Some halotolerant nitrogen-fixing cyanobacterial strains liberate organic acids and enzyme alkaline phosphatase which solubilises the insoluble CaCO_3 in soil, and make Ca^{2+} available to replace Na^+ from the soil complex. (vii) Inoculation of soil-based blue-green algae results in improvement of soil aggregation, hydraulic conductivity, organic carbon and total nitrogen of the saline soils (Subhashini and Kaushik 1984). (viii) Some of the forms are also phosphate solubilisers; hence, available content of soil phosphorous increases and lastly (ix) raising of paddy crops after amelioration gives crop yield as good as in normal soils (Kaushik 1994).

9.12 Conclusion

Physical and chemical methods such as addition of gypsum and sulphur, or excessive irrigation used in the reclamation of alkaline soils do not completely remove the soluble salts and exchangeable sodium. These organisms, by virtue of their dual capacity for photosynthesis and N_2 fixation, are capable of contributing to productivity in a variety of agricultural and ecological situations (Fogg et al. 1973). Understanding the salt response in cyanobacteria will make a relevant impact on understanding the detrimental effects of salinity on crops plants (Pandhal et al.

2008). These organisms can be used to reclaim alkaline soils because they form a thick stratum on the surface of the soil during the rainy season and the winter months. The algal material incorporated in the soil conserves organic C and N, and organic P as well as moisture, and converts Na^+ clay to Ca^{2+} clay. Organic matter and N added by cyanobacteria bind the soil particles, and thus improve soil permeability and aeration (Singh 1961). They are capable of solubilising microbial nutrients and dissolving insoluble carbonate nodules through the secretion of oxalic acid (Singh 1961). Improvement of soil aggregation by lowering the pH and electrical conductivity and by increasing the hydraulic conductivity of saline and alkaline soils by cyanobacteria has been well-documented (Kaushik and Subhashini 1985).

Specific metabolic requirement of sodium ($<100 \mu\text{M}$) in cyanobacteria especially under N_2 -fixing conditions has been reported. Sodium does not influence several structural and functional features associated with diazotrophic growth like heterocyst differentiation, synthesis of nitrogenase proteins, transport of molybdenum and protection of nitrogenase from oxygen. However, vital functions like nitrogenase activity, photosynthesis, quality and quantity of proteins, membrane potential and energy status of N_2 -fixing cells are affected by sodium deficiency. A primary effect of Na^+ deficiency is inhibition of uptake and utilisation of phosphate leading to depletion of nucleotide phosphate pools. This in turn results in inhibition of N_2 fixation apparently due to limitation of ATP supply.

Accumulation of K^+ , exclusion of Na^+ and maintenance of low intracellular Na^+ levels, synthesis of carbohydrates, polyols, amino acids and quaternary amines for osmoregulation and other adaptations of metabolism are principal features associated with and contributing to the salt tolerance in cyanobacteria. Extracellular mucopolysaccharides chelate significant amounts of sodium. Intracellular sodium exists as a free cation and is not incorporated into any biomolecule, especially proteins. Na^+ influx in N_2 -fixing *Anabaena* spp. is carrier-mediated and is regulated by the proton-motive force, particularly the membrane potential of cells. Low intracellular concentrations are maintained by active efflux. While the nature of this efflux is uncertain in N_2 -fixing cyanobacteria, in *Anacystis nidulans* it is mediated by Na^+/H^+ antiporter and decreases the efficiency of oxidative phosphorylation.

Virtually negligible information exists on the genetics of cyanobacterial halotolerance. Genetic engineering of these photoautotrophic diazotrophs for enhanced halotolerance and subsequent agricultural exploitation is an attractive area of future research. The presence of combined nitrogen, which effectively curtails sodium accumulation and also supports extra nitrogen demand for osmoregulation during salt stress, confers considerable salt tolerance on cyanobacteria. Exploiting the potential of cyanobacteria for reclamation of saline sodic soils needs more serious efforts than those made in the past.

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Chapter 10

Measuring Environmental Sustainability of Intensive Poultry-Rearing System

Simone Bastianoni, Antonio Boggia, Cesare Castellini, Cinzia Di Stefano, Valentina Niccolucci, Emanuele Novelli, Luisa Paolotti, and Antonio Pizzigallo

Abstract Sustainability of human activities is one of the most important concerns of the European Union. Consequently, the need to assess the level of sustainability achieved both at local and at government level is increasing. This process involves all economic sectors, including agriculture and, in particular, livestock. Until several years ago livestock production systems were mainly focused on production efficiency and qualitative characteristics of meat. However, nowadays rules regarding animal welfare and environmental impact are becoming more and more compulsory and require attention by all the poultry chain. European subsidies are in many cases linked to an environmentally sound behaviour of farms. However, there is still an ongoing discussion regarding the definition of sustainable-agriculture strategic objectives, the criteria to take into account, the actions to develop, and the methodological tools to use for the evaluation. This chapter provides suggestions for improving the environmental evaluation part of a process of sustainability assessment specific for intensive poultry production. The environmental sustainability of an intensive poultry-rearing system is evaluated through the use of three different methods: Emergy Evaluation, Ecological Footprint Analysis and Life Cycle Assessment (LCA). For each of the three methods a review of its application in agriculture, and specifically in poultry breeding, is presented. Through Emergy Evaluation we found that diet is the most important impact factor for the analysed system, accounting for more than 82% of the total emergy flow. Our results obtained from Ecological Footprint Analysis point out that cropland, which is connected

S. Bastianoni, V. Niccolucci, and A. Pizzigallo
Department of Chemical and Biosystems Sciences and Technologies, University of Siena,
Via A. Moro, 2, 53100 Siena, Italy

A. Boggia (✉), C. Di Stefano, E. Novelli, and L. Paolotti
Department of Economics, Appraisal and Food Sciences, University of Perugia,
Borgo XX Giugno, 74, 06121 Perugia, Italy
e-mail: boggia@unipg.it

C. Castellini
Department of Applied Biology, University of Perugia, Borgo XX Giugno, 74,
06121 Perugia, Italy

with chicken diet, is the main land component in the indicator, accounting for 73% of the total. Particularly, the high quantity of maize and soya needed for feed requires much cropland. Finally, using LCA, we found that feed production is the element which contributes the most to the environmental impacts of the system, influencing the impact category 'land use'. As Ecological Footprint, LCA regards the cultivation and the transformation of maize and soya as the processes with the strongest impact. Therefore, although the three methods use specific indicators and methodology, they come to the same conclusions for the system investigated. After applying each method to the poultry system, we propose a comparative analysis between the three methods, based on four different criteria: representativeness, verifiability, reproducibility, comprehensibility. By comparing the methods according to these criteria, we found that each of them shows both positive and negative aspects, strengths and weaknesses, but all of them are effective in representing the environmental features of a given activity, and the results can be used as input in the sustainability assessment process. The choice to use Emergy Evaluation, Ecological Footprint Analysis, or LCA can depend upon the main objective of the assessment process. However, in many cases it is not necessary a choice because the three methods can be used together, and the results can be integrated to build combined indicators, capable to ensure a wide and complete analysis.

Keywords Emergy • ecological footprint • life-cycle assessment • poultry • sustainability

10.1 Introduction

Poultry is one of the major and fastest growing sources of meat, representing over 25% of European meat production in 2007. Because of their nutrient content and relatively low caloric value, egg and poultry products are natural candidates to meet consumer demands of Western countries. Until several years ago, the livestock production systems were mainly focused on production efficiency and qualitative characteristics of meat; however, nowadays rules regarding animal welfare and environmental impact are becoming more and more compulsory and require attention by all the poultry chain. It is widely known that the production of food requires resources such as land, water, materials, and energy, and causes emissions such as greenhouse gases, pesticides, heavy metals, and various other wastes. This is particularly evident for intensive animal production that uses a large amount of world grain (36%) which could be directly used for human nutrition.

However, the rapid evolution of the poultry industry toward intensive production systems has strongly enhanced the efficiency, the growth and the feed conversion of birds, but has reduced the resource use per kilogram produced.

Indeed, a recent UK study on the impact of several animal species showed that poultry resulted as the most environmentally efficient meat comparing resources used in the production of beef, sheep meat, poultry meat, eggs and milk

(Williams et al. 2006). Next comes pork, followed by sheep meat and beef. The efficiency of chicken in converting its feed into meat plays a big part. This efficiency had been achieved through a strong selection of traditional breeding and through better matching of feed to the birds' dietary needs at each stage of their development. The poultry industry of the future needs to meet increasing consumer demand while addressing issues of health, safety, animal welfare and environmental impact. At the same time the increasing relevance of sustainability has initiated a debate on appropriate frameworks and tools that will provide guidance for a measure of sustainability which should capture, address and suggest solutions for a series of issues that affect different stakeholders. However, sustainability assessment is still not a mature framework and several indexes have been developed with different responses.

The agricultural and rural policy of EU has increased the attention to the environment in the last 10 years; however, there is still an ongoing discussion regarding the definition of sustainable-agriculture strategic objectives, the criteria to take into account, the actions to develop, and the methodological tools to use for the evaluation of the same. Sustainability is a multi-dimensional concept: economic, social and environmental aspects must be considered simultaneously. 'Sustainable economic development involves maximizing the net benefits of economic development, subject to maintaining the services and quality of natural resources over time' (Pearce et al. 1988). The Renewed EU Sustainable Development Strategy, published in 2006, encourages development of sustainable indicators to ensure proper assessment of the situation in each challenge, and not only for an overall monitoring of the strategy. In this way, the development of indicators and a proper assessment of sustainability are key issues.

This chapter aims to provide suggestions for improving the environmental evaluation part of a process of sustainability assessment specific for intensive poultry production. In this study environmental sustainability of an intensive poultry-rearing system is evaluated, through the use of three different methods: Emergy Evaluation, Ecological Footprint Analysis and Life Cycle Assessment (LCA). For each of the three methods a review of its application in agriculture, and specifically in poultry breeding, is presented.

After applying each method to the poultry system, we propose a comparative analysis among the three methods, based on four different criteria: representativeness, verifiability, reproducibility, comprehensibility.

10.2 The Intensive Poultry-Rearing System

The farm surface area is 1.5 ha. Part of this area belongs to the animals' buildings (2,585 m² of covered surface), and the remaining surface to firm's road network. The construction materials are mainly steel tubes, bricks, polyvinyl chloride, polyurethane and concrete for the foundations. The shelters are air conditioned to maintain a constant humidity level (65–85%) and the right temperature (17–28°C) in order to maximize the chickens' performances. Feed and drinking systems are completely automatic. Table 10.1 shows the main characteristics of the farm. The

Table 10.1 Main characteristics of poultry-rearing system

Buildings and space allowance	
Total birds per cycle (<i>n</i>)	45.334
Surface area covered (m ²)	2.585
Density (birds/m ² covered surface)	17.5
Productive performance ^a	
Final weight (kg)	2.6
Age at slaughtering (days)	50
Daily weight gain (g/day)	51.2
Cycles of production/year (<i>n</i>)	6
Feed index	2.02
Mortality rate (%)	4
Output after slaughtering (%)	83

^aMean performance considering a female/male ratio = 1

Table 10.2 Diet composition for poultry rearing, from the Ross Breeders–Broiler management manual (Aviagen Technical Team 1999)

Total ingredients	100%
Maize	40.00%
Wheat bran	8.00%
Sorghum	12.00%
Soybean oil	1.00%
Soybean meal	34.00%
Salt	2.00%
Bicalcium phosphate	1.00%
Calcium bicarbonate	1.00%
Additives	0.80%
Coccidiostatic	0.03%
DL-methionine	0.01%

analysis concerns the poultry production of a whole year. Energy and material requirements for poultry were assessed at the end of the growing period without taking into account transport to the slaughtering house, slaughtering, processing of carcasses and distribution.

The accounted animals in a year are 261,120, depurated of mortality rate. The duration of each cycle is 50 days which implies six cycles of production in a year. The genetic strain of birds is ROSS 308. When animals arrive at the farm they are about 40 g, while their mean weight when they leave is 2.6 kg; therefore feed index is 2.02. After the end of every production cycle the rearing buildings are cleaned and sanitised and there is an all-in all-out period of 10 days. All the indicators containing a reference to weight measure units took into account the carcass weight, calculated as 83% of the live weight.

The diet is formulated with common ingredients according to the standard recommendations of Ross Breeders-Broiler management manual (Aviagen Technical Team 1999). Table 10.2 illustrates the diet composition. For each productive cycle

several vaccines and antibiotic treatments are administered. Coccidiostatic molecules are also administered until 10 days before slaughtering age.

10.3 The Methods

10.3.1 Life Cycle Assessment

Life Cycle Assessment (LCA) has been defined by International Standardization Organization (ISO) 14040 of 2006 as a ‘compilation and evaluation of the inputs and outputs and the potential environmental impacts of a product system throughout its life cycle’. It is a method to evaluate the environmental impacts of products, activities and services, based on a ‘cradle-to-grave’ approach. This means that it is based on the identification and quantification of the flows of substances, materials and energy, to and from the techno sphere (which is the set of all human activities) and the environment, during the entire life cycle of the product or activity. The life cycle consists of the following phases: extraction of raw materials, production and assembly of the materials, use, and disposal of the product. Figure 10.1 shows a scheme of the overall structure of an LCA and of the considered elements.

LCA is an iterative method. This means that initial choices and initial requirements can be adapted later when more information becomes available (Goedkoop et al. 2008). Also old data can be replaced with new ones or with more precise data, re-evaluating in this way the earlier actions.

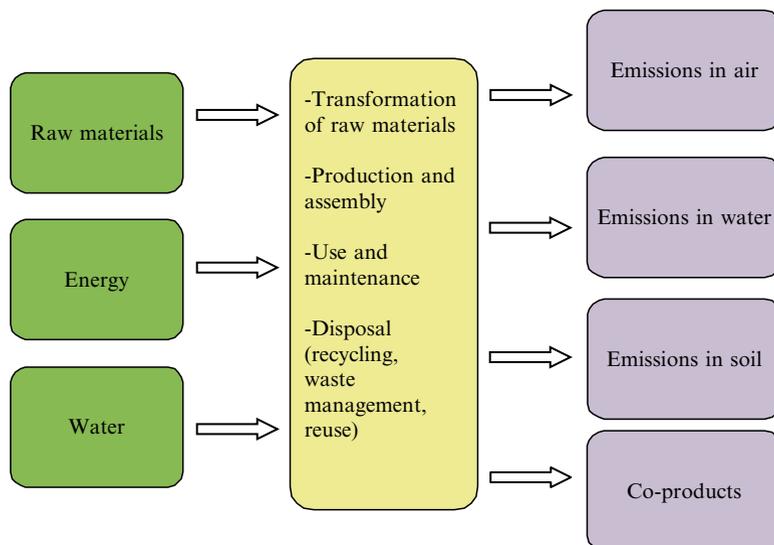


Fig. 10.1 Structure of a Life Cycle Assessment (ISO 14040)

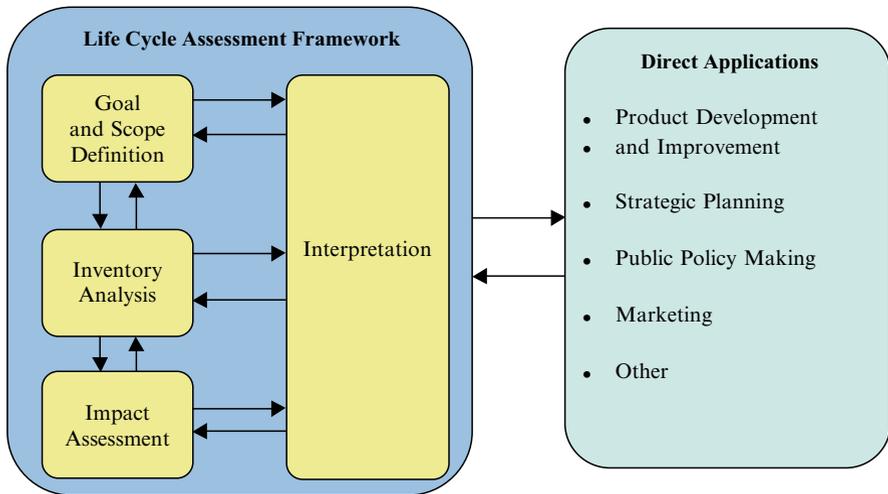


Fig. 10.2 The general methodological framework for Life Cycle Assessment (ISO 14044)

The implementation of LCA products, services, or production processes is developing quickly in all the sectors of economic system. In agriculture, and particularly in animal husbandry, the LCA approach is fundamental to have a complete view of environmental impacts, emissions and resources consumptions which are involved in every step of the productive chain, from the cultivation of crops and their transformation for making feed, to the phase of breeding.

Figure 10.2 shows the main methodological framework of LCA, established by ISO. ISO 14044 sets the requirements for every phase of the LCA. ISO standards contain the elements that should be considered when conducting an LCA, and when communicating the results. They are very important guidelines that provide an international reference on principles, framework and terminology for conducting and reporting LCA studies. The LCA methodology, according to ISO requirements, consists of four main phases enumerated below.

10.3.1.1 Goal and Scope Definition

Defining the goal of the study means determining clearly the reasons for carrying out the study and determining the application, and the intended audiences (Goedkoop et al. 2008). Some LCA studies could serve more than one purpose and the results may be used both internally and externally to the subject conducting the study.

The scope definition describes instead the most important methodological choices, assumptions and limitations made in the study. Initially the Functional Unit or comparison basis must be defined. It describes the primary function(s) fulfilled by a product system, and indicates how much of this function is to be considered in the

intended LCA study. It will be used as a basis for selecting one or more alternative product systems that might provide these function(s) (Guinée et al. 2002). Therefore all the process inputs and outputs will refer to the Functional Unit.

After the Functional Unit, it is necessary to determine the system boundaries intended as the level of tracing of the system; the spatial, temporal, geographical and technological characteristics of the used data; the criteria for the inputs and outputs inclusion; and the level of sophistication of the study.

10.3.1.2 Life Cycle Inventory

This phase consists in collecting all the necessary data, and quantifying the inputs and outputs of the considered production system. Its main result is an inventory table listing the quantified inputs and outputs associated with the Functional Unit. The system under study must be modelled as a complex sequence of unitary operations that communicate among themselves and with the environment through inputs and outputs (Pizzigallo et al. 2008). Two main types of data can be distinguished: the foreground data, which are typically specific data describing a particular production system, and the background data, which relate to general materials, energy, transport, waste management. The first should be determined, if possible, by communicating with data providers and developing questionnaires, while background data can be easily found in databases or the literature.

10.3.1.3 Life Cycle Assessment

This third phase consists in the evaluation of environmental impacts deriving from the data collected in the Inventory. Life cycle impact assessment is defined by ISO as the phase in the LCA aimed at understanding and evaluating the magnitude and significance of the potential environmental impacts of a product system (Goedkoop et al. 2008). Different impact categories and assessment methods can be selected, depending on the goal and the scope of the study. Initially, the results of the Inventory analysis are assigned to relevant impact categories. For example CO₂ and CH₄ emissions are both assigned to the impact category 'global warming', while SO₂ and NH₃ emissions are both assigned to the impact category 'acidification'. The 'baseline' impact categories are: depletion of abiotic resources, impacts of land use, climate change, stratospheric ozone depletion, human toxicity, ecotoxicity (aquatic and terrestrial), photo-oxidant formation, acidification and eutrophication. Moreover, there are 'study-specific' impact categories, which could be included in the LCA study, depending on its goal and scope (Goedkoop et al. 2008).

Once the impact categories are selected and the Inventory results are assigned to them, it is necessary to define the characterisation factors. These factors should reflect the relative contribution of an inventory result to the impact category indicator result. For example, on a time scale of 100 years the contribution of 1 kg CH₄ to global warming is 42 times higher than the emission of 1 kg CO₂. This means

that if the characterisation factor of CO₂ is 1, the characterisation factor of CH₄ is 42. Thus, the impact category indicator result for global warming can be calculated by multiplying the LCI result by the characterisation factor.

After characterisation, the normalisation step can be carried out, as optional step. Normalisation is a procedure needed to show to what extent an impact category contributes to the overall environmental problem. This is done by dividing the impact category indicators by a 'Normal' value. There are different ways to determine the 'Normal' value. The most common procedure is to calculate the impact category indicators for a region during a year, and divide this result by the number of inhabitants in that area. Finally, it will be necessary to determine which phases of the production system contribute the most to the identified impacts.

10.3.1.4 Life Cycle Interpretation

This last phase consists in interpreting the results, and compiling conclusions and recommendations to improve the environmental performances of the studied system.

In the field of animal husbandry several LCA researches have been conducted, especially for cattle and pig production systems. An interesting article of Halberg et al. (2005) compares different environmental assessment tools for the evaluation and improvement of European livestock production systems. Among them, Life Cycle Assessment and Ecological Footprint Analysis are considered.

Another study evaluates the effectiveness of environmental indicators derived from three methods that are widely used in animal production: Input–Output Accounting, Ecological Footprint Analysis and LCA (Thomassen and de Boer 2005). The data used to evaluate the environmental indicators effectiveness were collected from eight organic dairy farms in the Netherlands.

During the past years several LCA studies comparing different milk production systems have been conducted. In a Swedish study an LCA is performed on organic and conventional milk production at farm level in Sweden, focusing especially on concentrate feed production (Cederberg and Mattsson 2000). Other studies on similar topics consider different aspects of the livestock productions systems, i.e. the differences in terms of energy flows, in the production of conventional and organic milk (Grönroos et al. 2006). The study of Haas et al. (2001) applies the LCA methodology to evaluate the impacts caused by three different typologies of pasture: intensive, extensive and organic.

There are also several LCA studies performed in the sector of pig breeding: for example, the research by Basset-Mens and van der Werf (2005) compares three different production systems, while Eriksson et al. (2005) focus on the impact of feed choice in three pig production scenarios. Other studies consider the environmental impacts of different pig production potential scenarios to illustrate environmental benefits and disadvantages integrated in the production systems (Cederberg and Flysjö 2004), or to analyse the implications of uncertainty and variability in the LCA of pig production systems (Basset-Mens et al. 2006).

Only few researches have been conducted in reference to LCA studies in the poultry sector. Bennett et al. (2006) present the results of an LCA applied to an Argentinean conventional production of maize grain, compared with a similar production from a genetically modified variety, showing its impact when fed to broiler chickens. Another study (Ellingsen and Aanonsen 2006) aims to assess the environmental impacts of Norwegian cod fishing and salmon farming, compared with chicken farming.

The study of Pelletier (2008) about the environmental performance in the US broiler poultry sector aims to analyse, through LCA, the macro scale environmental impacts of material and energy inputs and emissions along the US broiler supply chain, as opposed to the most published research regarding the potential environmental impacts of broiler production, which is focused principally only on farm-specific emissions.

10.3.2 Ecological Footprint Analysis

The Ecological Footprint Analysis is a biophysical resources accounting method able to measure the load that a population or a production activity imposes on the ecosphere. The Ecological Footprint is an area-based indicator as it expresses the impact in terms of area (real and virtual) that is effectively required to sustain that population or activity (Rees 1992; Wackernagel and Rees 1996). Formally, the Ecological Footprint of a certain population or a production activity is defined as the area of productive land and water ecosystems required, on a continuous basis, to produce the resources consumed and to assimilate the waste produced, wherever on the earth the relevant land/water may be located and with the prevailing technology (Wackernagel and Rees 1996; Monfreda et al. 2004, Wackernagel and Kitzes 2008, Kitzes et al. 2007). The methodology also proposes a second indicator called Bio-capacity that measures the annual production of biologically provided resources (Wackernagel and Rees 1996).

Both Bio-capacity and Ecological Footprint are expressed in terms of global hectares (gha), or hectares with global average productivity (Kitzes et al. 2007; Galli et al. 2007). It is a normalised unit useful to make a comparison among lands with different productivity (Monfreda et al. 2004).

Six categories of productive areas are usually included in the calculation: crop land, grazing land, fishing grounds, forest area, built-up land and energy land (or carbon footprint, that is the amount of forest land required to capture those carbon dioxide emissions not sequestered by the oceans) (Wackernagel and Rees 1996). Yield factor and Equivalence factor are used to translate these six land types into global hectares (Monfreda et al. 2004). Equivalence factor represents the relative productivity of the six categories of land and water area, while yield factor represents local to global average productivity of the same land category.

The difference between Bio-capacity and Ecological Footprint defines a sort of ecological balance. When Ecological Footprint exceeds the Bio-capacity, the region runs an ecological deficit, which means that a population uses more resources than

annually available. The opposite of ecological deficit is ecological reserve or surplus. The Footprint method is widely used to give a measure of the (un)sustainability of consumption patterns at different scales: regional (see for example Folke et al. 1997; Bagliani et al. 2008), national (see for example Erb 2004; Medved 2006; Moran et al. 2008) and global (Van Vuuren and Bouwman 2005; WWF 2006).

Ecological Footprint has also been analysed as temporal series together with economic indicators such as Gross Domestic Product – GDP (Jorgenson and Burns 2007) and Index of Sustainable Economic Welfare - ISEW (Niccolucci et al. 2007), or incorporated in thermodynamic-based methods (Zhao et al. 2005, Chen and Chen 2006; Nguyen and Yamamoto 2007).

Up-to-date industrial and agricultural Footprint applications are still rare. Studies on cultivation of tomatoes (Wada 1993), conventional versus organic wine farming (Niccolucci et al. 2008), shrimp and tilapia aquaculture (Kautsky et al. 1997) have been carried out to highlight the appropriation of natural capital, the efficiency of natural resource use, and the environmental pressure. Evaluations of the environmental impact of farms (van der Werf et al. 2007) and dairy production (Thomassen and de Boer 2005) as well as assessment of economic and ecological carrying capacity of crops (Cuandra and Björklund 2007) proposed the Footprint jointly with other methods, such as Life Cycle Assessment, Energy Analysis and Economic Cost and Return Estimation.

10.3.3 *Emergy Evaluation*

Solar Energy (from now Emergy) represents the total amount of available solar energy (i.e. exergy), directly or indirectly required to make a product or to support a process; the Emergy of a product is therefore related to the way it is produced. It is expressed in solar energy joule (sej). All process inputs (i), including energy of different types and energy inherent in materials and services, are converted into Emergy by means of a conversion factor called transformity (Tr , Emergy per unit energy, $sej J^{-1}$) and the Emergy flow to a product (Em , sej) is calculated as

$$Em = \sum_i Tr_i E_i \quad (10.1)$$

where E_i is the available energy. A higher transformity means that more Emergy is needed to produce a unit amount of output. (See Equation 10.2, where E_o is the energy of the output (measured), while Tr_o is the transformity of the output (calculated).

$$Tr_o = \frac{Em}{E_o} \quad (10.2)$$

The circularity of Equations 10.1 and 10.2 is avoided since, by definition, transformity of solar energy is $1 sej J^{-1}$. In this way all inputs are converted into the solar

equivalent energy needed to create those energy flows; each flow is multiplied by its transformity and summed, and the result is the amount of total resources (renewable and non-renewable) that have been necessary in order to obtain a product or a process (Equation 10.1). When an input is available in mass unit, instead of Joules, a specific emergy is used, measured in sej g⁻¹.

Emergy analysis obeys a logic of memorization (i.e. emergy is ‘accumulated’ over time and not simply ‘conserved’) and therefore needs its own algebra that was summarised in four main rules by Brown and Herendeen (1996):

1. All emergy sources of a process are assigned to the processes output.
2. By-products from a process have the total emergy assigned to each pathway.
3. When a pathway splits, the emergy is assigned to each ‘leg’ of the split based on its percentage of the total energy flow on the pathway.
4. Emergy cannot be counted twice within a system: (a) emergy in feedbacks cannot be double counted; (b) by-products, when reunited, cannot be added to equal a sum greater than the source emergy from which they were derived.

For an in-depth discussion of this issue and the differences between energy and emergy analyses, see Brown and Herendeen (1996) and Odum (1996). For our purpose it is important to note that in our calculations among solar energy, rain and wind, only the highest of the three contributions to the total emergy flow will be considered, since they are co-products of the same phenomenon, i.e. the sunlight reaching the biosphere (Odum 1996). The baseline of global emergy flow used in this paper is 9.44×10^{24} sej year⁻¹. Emergy analysis separates renewable from non-renewable inputs and local from external inputs. These distinctions allow to define several emergy-based indicators that can provide decision support tools, especially when there are several alternatives (Bastianoni and Marchettini 1996; Brown and McClanahan 1996; Odum 1996; Ulgiati et al. 1995).

Emergy evaluation classifies inputs into different categories (i.e. local renewable, R, local non-renewable, N; and purchased, F). On the basis of these classes, some indicators can be computed in order to assess the sustainability of the use of resources.

The environmental loading ratio (ELR) is the ratio of purchased (F) and non-renewable local emergy (N) to renewable environmental emergy (R). A high value of this ratio indicates a low proportion between the use of non-renewable resources and that of renewable resources, so that environmental cycles are overloaded. The emergy investment ratio (EIR) is the emergy of purchased inputs (F) divided by local emergy, both renewable and non-renewable (N + R). A high level of this index represents a certain fragility of the system because of its dependence on inputs from other economic systems. The emergy flow density (ED) is given by the total emergy flow (R + N + F) supporting a system divided by its area. If this ratio is high, a large quantity of emergy is used in a certain area: this can mean a high stress on the environment and regards the land surface as a limiting factor for future development.

Emergy evaluation is particularly suitable for studies in agriculture, as it is a system in which natural and man-made contributions interact in order to obtain the final product, emphasising the role of ecological inputs that constitute the basic life

support for living beings, for instance, in primary production (Lagerberg and Brown 1999; Brandt-Williams 2002).

In the past, emergy was already applied to several agricultural systems, both for comparative evaluations and simple agricultural systems (see for example Cavalett et al. 2006; Lefroy and Rydberg 2003; Liu and Chen 2007; La Rosa et al. 2008), and in particular to grape or wine productions together with exergy and Life Cycle Assessment (Bastianoni et al. 2003; Pizzigallo et al. 2008). Castellini et al. (2006) have already emphasised the importance of poultry farming production for Italian agriculture.

10.4 Results

10.4.1 Life Cycle Assessment

An LCA of an intensive poultry-rearing system has been carried out, considering data related to the farm for what concerns the breeding phase. The data have been collected through a direct survey of the farm reality. The goal of the LCA was to evaluate the environmental impacts associated to the system. The LCA results are then involved in the comparison with the results of the other two methods, the Emergy Analysis and the Ecological Footprint. The Functional Unit considered in the LCA is 1 kg of poultry meat.

For what concerns the scope definition, in this LCA only the phases of production of raw materials and production of the product ‘poultry meat’ have been taken into account, leaving out the phases related to the product use and disposal. This choice has been made to obtain the same basis of comparison of the methods, as the other two methods do not consider the use and disposal phases, but they only take into account the production phase. In reference to spatial and temporal boundaries, European and Italian production systems, during the most recent years, have been considered as boundaries for the analysis.

With regard to the implementation of the inventory, local data (related to Umbrian reality) have been used where possible, in particular for the processes ‘maize cultivation’, ‘sorghum cultivation’, and ‘soya cultivation’, which represent some of the components of the poultry feed, and also for the processes ‘transformation of maize in feed’, ‘transformation of soya in feed’, and for the overall phase of poultry rearing. The database Ecoinvent from SimaPro 7 software has been used for the other data (Nemecek et al. 2004).

The impact assessment phase has been developed using the method ‘Eco-Indicator 99’ (Goedkoop and Spriensma 2001). It is a method to measure various environmental impacts, and it is based on a damage function approach. The damage function presents the relation between the impact and the damage to human health or to the ecosystem. Impacts can be computed according to 11 different impact categories, or they can also be aggregated into three wider categories (Human Health, Ecosystem Quality, Resources). In our study we present the impact assessment for the 11 impact categories.

Results of impact assessment are already presented in the normalised version. Normalisation consists in dividing the impact category indicators by a ‘normal’ value. As said above, the most common procedure is to determine the impact category indicators for a region during a year and divide this result by the number of inhabitants in that area. Therefore, final results are expressed in Points: the higher the score, the more important is the impact.

The LCA carried out consists of three main phases: cultivation, feed production and breeding. Every phase includes different sub-processes. The cultivation phase involves the cultivation processes of maize, sorghum, soya and grain, which constitute the raw materials of the feed. Every single process includes all the necessary inputs to obtain the cultivated product (seed, fertilizers, pesticides, use of machinery, transport inside and outside of the farm), and the related emissions. Regarding emissions derived from the use of fertilizers, a national manual of emissions has been considered (Bini and Magistro 2002). The second phase investigated consists in the feed production. It includes, for each crop, the transformation process from crop to feed, involving mainly water, energy and fuel consumption. In this case emissions have been evaluated through direct surveys of the firms’ realities. The final product is then obtained by assembling the transformed crops together with other minor components (calcium carbonate, sodium chloride, bi-calcium phosphate, and other chemical organic additives). Finally, in the poultry-breeding phase the main input is the feed, and the other inputs considered are water, fuel and energy consumption, and all the infrastructures materials (steel, aluminum, synthetic rubber, glass, plastic, copper, zinc). The principal emissions related to breeding are also taken into account (ammonia, methane, dinitrogen monoxide) (European Commission 2003). Table 10.3 reports the main emissions for each phase.

Figure 10.3 shows the principal components belonging to the system life cycle. Feed production is the element which contributes the most to the environmental

Table 10.3 Principal emissions in the Life Cycle Assessment study (database Ecoinvent; method of impact assessment Ecoindicator 99)

Substance	Unit	Value	Process mainly contributing	Value in the process
NO _x	(g/FU ^a)	3.8	Feed production	3.0
CO ₂ biogenic	(g/FU)	10.2	Feed production	10.0
CO ₂ fossil	(g/FU)	677.0	Feed production	567.4
CO biogenic	(mg/FU)	95.1	Feed production	88.6
CO fossil	(g/FU)	1.3	Feed production	1.0
Particulates, <2 µm ^b	(mg/FU)	382.0	Feed production	335.6
Particulates, >10 µm	(mg/FU)	387.0	Feed production	328.5
Particulates, 2–10 µm	(mg/FU)	197.0	Feed production	174.7
SO ₂	(g/FU)	2.5	Feed production	2.1
Methane	(mg/FU)	463.0	Breeding phase	463.0
Methane biogenic	(mg/FU)	18.4	Feed production	18.1
Nitrates	(g/FU)	4.3	Feed production	4.1

^a FU = Functional unit

^b µm= Micrometers

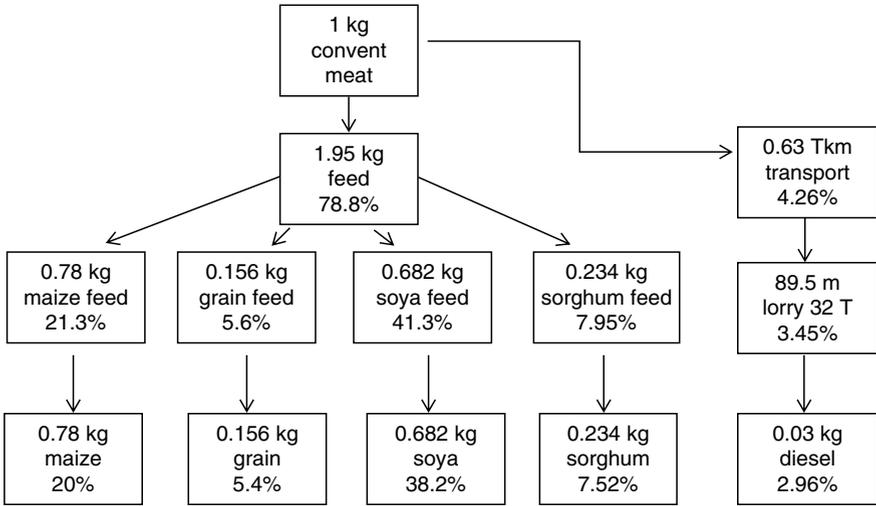


Fig. 10.3 Conventional poultry system life cycle. This figure shows the principal processes involved in the conventional poultry system. For each process the relative quantity (in kg) necessary to produce 1 kg of poultry meat (Functional Unit) and the process contribution to the environmental impact of the system, in percentage, are presented

impacts in the system. In particular the cultivation and then the transformation of maize and soya are the processes with the strongest impact.

With regard to the impacts assessment, Fig. 10.4 reports the analysis conducted with Eco-Indicator 99. The figure shows the normalised impact categories. The category showing the greatest impact is ‘land use’, followed by ‘fossil fuels’ and ‘respiratory inorganics’ categories. The impact assessment carried out for each phase shows that feed production weighs the most on these three impact categories, while the breeding phase influences especially the two categories, ‘acidification and eutrophication’ and ‘respiratory inorganics’ and, to a minor extent, the ‘climate change’ category.

10.4.2 Ecological Footprint

The Ecological Footprint of a product is defined as the sum of the Footprint of all the activities required to create, use, and/or dispose of that product (Global Footprint Network 2009). As suggested by the document ‘Ecological Footprint Standard 2009’ (Global Footprint Network 2009) there are two widely used approaches for calculating the Footprint of a complex finished product: process-based life-cycle assessment and extended input–output life cycle assessment.

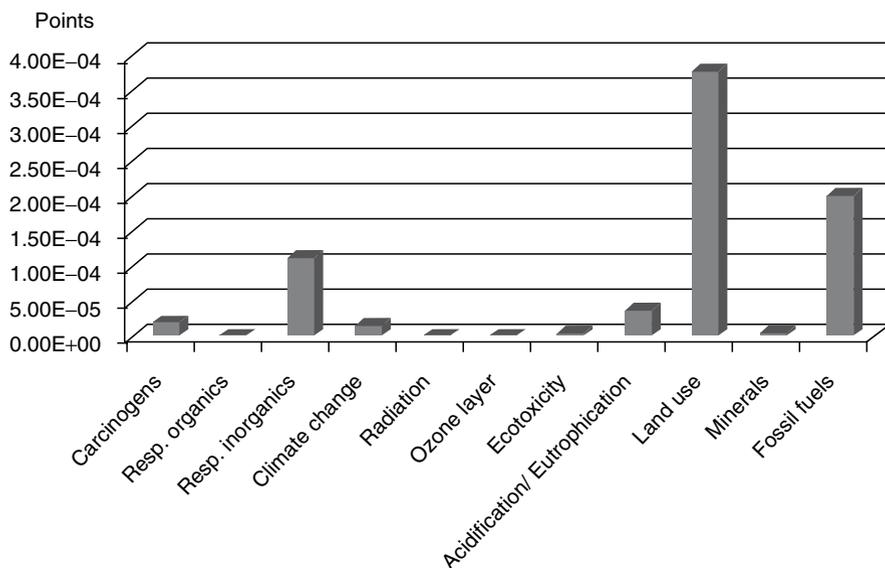


Fig. 10.4 Conventional poultry system impact assessment. The figure shows the environmental impact of the conventional poultry system relative to the 11 different impact categories by the method Eco-Indicator 99. Results are expressed in normalised Points. The higher the score, the more important is the impact. 4.00E-04 refers to 0.0004

In this study a ‘life cycle approach’ is used. All relevant inputs, from cradle to gate (until the animals leave the farm, without taking into account slaughtering processes and retailing), are accounted to give an estimation of environmental impacts. Information is provided directly by the farm and refer to 2008. Table 10.4 reports the inventory of energy and material data (considered on the basis of their lifetime) required to sustain this conventional poultry production.

As first step each input is converted into relative bio-productive areas by means of specific conversion factors as indicated in the footnotes of Table 10.4. When opportune conversion factors are not directly available, energy intensity coefficients are adopted to convert data into energy units. A conversion into emission of CO₂ and then into the area of forest needed for sequestration is then performed. A world-average carbon absorption factor of 0.2071 ha tCO₂⁻¹ is used to translate the emissions into forest land necessary to absorb them (Global Footprint Network 2006).

Furthermore, due to the lack of detailed information on the feed, data for 1–12 input are extracted from ECOINVENT® database (Nemecek et al. 2004). In this way it is possible to know how much carbon dioxide is emitted and how wide are cropland and built-up land necessary to support the production of one functional unit of a given input by considering similar production processes. For example, it was found that the production of 1 kg of maize emits 0.31 kg of CO₂ and requires 0.28 m² of built-up and 1.28 m² of cropland.

Table 10.4 Energy and material data, with relative conversion factor, for conventional poultry production

	Unit	Quantity	Conversion factors			
			Energy land (kg CO ₂ /unit)	Built-up land (m ² /unit)	Crop land (m ² /unit)	
Input						
1	Maize	kg	5.48E+05	0.31 ^a	0.28 ^a	1.28 ^a
2	Wheat bran	kg	1.10E+05	0.20 ^a	0.01 ^a	1.26 ^a
3	Sorghum	kg	1.64E+05	0.20 ^a	0.01 ^a	1.34 ^b
4	Soya meal	kg	4.66E+05	0.50 ^a	0.05 ^a	2.80 ^a
5	Sodium chloride	kg	2.74E+04	0.20 ^a	0.002 ^a	0.00002 ^a
6	Bicalcium phosphate	kg	1.37E+04	0.04 ^a	0.003 ^a	0.0001 ^a
7	Calcium bicarbonate	kg	1.37E+04	0.04 ^a	0.001 ^a	0.0001 ^a
8	Additives	kg	1.10E+04	1.60 ^a	0.003 ^a	0.00003 ^a
9	Cocciostatic	kg	4.52E+02	1.60 ^a	0.003 ^a	0.00003 ^a
10	DL-Methionine	kg	1.37E+02	1.60 ^a	0.003 ^a	0.00003 ^a
11	Drugs and antibiotics	kg	2.67E+02	1.60 ^a	0.003 ^a	0.00003 ^a
12	Disinfectants	kg	2.75E+02	0.40 ^a	0.004 ^a	0.00005 ^a
13	Buildings and shelter ^c					
14	Machinery	t	3.20E-01	2,770 ^a		
15	Steel	t	2.20E-01	2,770 ^a		
16	Plastic	t	2.41E-02	1,700 ^a		
17	Human labour	Work-days	597.50	–	–	–
18	Electricity	kWh	3.08E+04	0.48 ^c		
19	Diesel	l	6.00E+02	2.65 ^d		
20	Liquid petroleum gas	l	2.50E+04	1.69 ^d		
21	Copper	kg	1.81E+00	1.53 ^a	0.72 ^a	0.00076 ^a
22	Water	l	1.94E+06	0.00037 ^f		
23	Buildings and roads	m ²	1.50E+03	–	–	–
Output						
1	Poultry	kg	5.63E+05			

5.48E+05 is for 5.48×10^5 .

^aFrom Ecoinvent database.

^bOur estimation.

^cOur evaluation on Italian electricity system in 2006.

^dIPCC 2006.

^eThis input is the sum of several inputs of different kind. It is not possible to provide a single value for this input or a single conversion factor. All these data are available upon request.

^fChambers et al. 2000.

Human labour contribution is also included by allocating the Footprint of an average Italian citizen (WWF 2006) on the basis of the number of work hours per year. Each kind of land (energy, cropland and built-up) is then normalised into global hectares by means of its equivalence factor obtained from the WWF Living Planet Report (WWF 2006). Finally, the Ecological Footprint for poultry production is given as the sum of all croplands, energy lands and built-up areas.

Results show that the total amount of bio-productive land, or Ecological Footprint, required for the conventional poultry production is 721.60 gha year that means 12.81 gm² year kg⁻¹ of chicken. Comparison with other kind of meat production is not possible due to the lack of specific Footprint literature. However, Gerbens-Leenes and Nonhebel (2005) estimated the land requirement (values are expressed in m² year kg⁻¹) for producing three different types of meat: beef (20.9), pork (8.9) and chicken (7.3).

The ratio of the total Footprint value with respect to Bio-capacity (item 23 in Table 10.4, expressed in gm²) measures how much the overall demand exceeds the local supply of resources. The value calculated for this production is 172. This means a very high dependence on resources imported from outside of the system that generally are not renewable. The lower this ratio, the lower the request of natural capital from outside (or greater is the virtual land-component).

Figures 10.5 and 10.6 show the Footprint results by land and consumption categories, respectively. The main Footprint land component is cropland (73%). This can be related to chickens' diet that requires high quantities of feed, especially

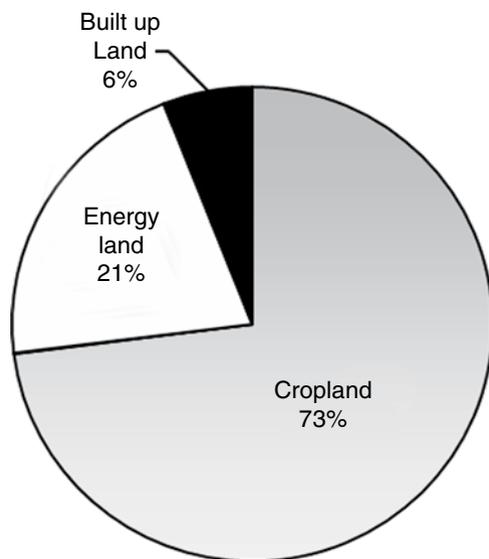


Fig. 10.5 Ecological Footprint for conventional poultry production disaggregated by land categories. The main contribution is due to cropland which is highly needed to cultivate maize and soya meal

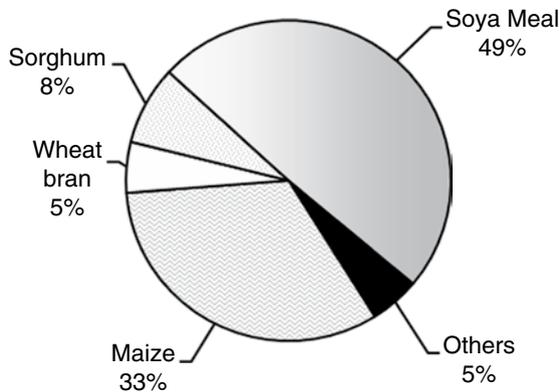


Fig. 10.6 Ecological Footprint for conventional poultry production disaggregated by consumption categories. Main contributions are related to chickens' diet

maize and soya meal, which, in turn, requires wide cropland. Energy land (or the land needed to absorb the carbon dioxide emissions) accounts for 21%, while built-up is just 6%. The other land components are not relevant. These values are quite typical for this kind of product.

When Footprint is considered according to consumption categories, it is possible to detect the contribution of each input. Results show that the 95% of the total Footprint is given by the diet component. In particular, soya meal and maize are Footprint-intensive cultivation. Footprint results agree with those derived from Energy evaluation.

10.4.3 *Emergy Analysis*

All the results are related to the whole system under analysis. Table 10.5 shows the emergy evaluation of the system considered. Moreover, all the inputs to the system are differentiated by their categories, as described in the methods paragraph. Some of the emergy flows listed in the tables are considered only partially renewable, according to the percentage of renewable inputs required for their production.

For all the inputs that determine the diet we have considered their characteristics of renewability/non-renewability. Human labour is also considered partially renewable in emergy evaluation, according to Ulgiati et al. (1994). The diet is the most important factor in the whole emergy evaluation, accounting for more than 82% of the total emergy flow. The percentage of renewability of these inputs is not very high since they come from industrialised agriculture. Conventional poultry production uses techniques that utilise various additives, growth hormones and other chemicals to help produce their chickens faster and larger in size, aiming to be

Table 10.5 Raw inputs and energy evaluation of the poultry production analysed in this study

#	Inputs	Unit	Flow (unit year ⁻¹)	Transformity (sej unit ⁻¹)	Reference ^a	Energy flow (sej year ⁻¹)	Type of resources ^b
1	Solar energy	J	5.83E+13 ^c	1.00E+00	a	5.83E+13	R
2	Rain	g	1.03E+10	8.99E+04	a	9.28E+14	R
3	Wind	J	1.32E+11	1.50E+03	a	1.98E+14	R
4	Geothermal heat	J	4.73E+10	2.55E+04	a	1.20E+15	R
5	Erosion of soil	J	5.01E+11	7.38E+04	b	3.70E+16	N
6	Water	g	2.67E+07	4.74E+07	f	1.27E+15	N
7	Liquefied petroleum gas (LPG)	J	5.84E+11	5.54E+04	d	3.24E+16	F
8	Concrete	g	1.38E+07	1.09E+09	d	1.50E+16	F
9	Bricks	g	3.29E+06	2.21E+09	e	7.26E+15	F
10	Straw for litter	J	4.41E+04	4.30E+03	f	1.90E+08	42 % R 58 % F
11	Steel	g	1.38E+06	4.18E+09	d	5.78E+15	F
12	Copper	g	1.81E+03	6.24E+10	d	1.13E+14	F
13	Plastics	g	4.06E+05	9.86E+09	f	4.01E+15	F
14	Maize	g	5.48E+08	7.82E+08	f	4.28E+17	22% R 78% F
15	Wheat bran	g	1.10E+08	5.41E+09	f	5.93E+17	42% R 58 % F
16	Sorghum	g	1.64E+08	6.92E+08	f	1.14E+17	37 % R 63 % F
17	Soybean oil	g	1.08E+07	1.66E+05	f	1.79E+12	10 % R 90 % F
18	Soy flour	g	4.66E+08	1.82E+09	f	8.47E+17	10 % R 90 % F
19	Salt	g	2.74E+07	1.00E+09	f	2.74E+16	F
20	Bicalcium phosphate	g	1.37E+07	3.90E+09	f	5.34E+16	F
21	Calcium bicarbonate	g	1.37E+07	1.00E+09	f	1.37E+16	F
22	Additives	g	1.10E+07	1.48E+10	b	1.62E+17	F

(continued)

Table 10.5 (continued)

#	Inputs	Unit	Flow (unit year ⁻¹)	Transformity (sej unit ⁻¹)	Reference ^a	Energy flow (sej year ⁻¹)	Type of resources ^b
23	Cocciostatic	g	4.52E+05	1.48E+10	b	6.69E+15	F
24	DL-Methionine	g	1.37E+05	1.48E+10	b	2.03E+15	F
25	Drugs	g	2.67E+05	1.48E+10	b	3.95E+15	F
26	Disinfectants	g	2.75E+05	1.48E+10	b	4.07E+15	F
27	Human labour	J	4.40E+09	7.38E+06	f	3.25E+16	10% R 90% F
28	Electricity	J	1.11E+11	1.24E+05	d	1.38E+16	F
29	Diesel	J	2.06E+10	6.60E+04	d	1.36E+15	F
30	Total energy flow	g	5.63E+08	4.27E+09		2.41E+18	Y

^a References for transformity and specific energy: (a) Odum et al. 2000; (b) Brandt-Williams 2002; (c) Odum 1996; (d) Brown and Arding 1991; (e) Brown and Buranakarn 2003; (f) Castellini et al. 2006.

^b Local renewable input (R), local non-renewable input (N), purchased input (F).

^c 5.83E+13 is for 5.83×10^{13}

Table 10.6 Summary of the main energy-based indexes for the conventional poultry production analysed

Energy index	Expression ^a	Value	Unit
Investment ratio (EIR)	$F/(N + R)$	3.69	–
Environmental loading ratio (ELR)	$(N + F)/R$	4.07	–
Empower density (ED)	$(R + N+ F)/\text{area}$	1.61E+14 ^b	sej ha ⁻¹ ·year ⁻¹

^a Local renewable input (R), local non-renewable input (N), purchased input (F).

^b 1.61E+14 is for 1.61×10^{14} .

competitive in the current market. These inputs reach 10% of the total energy flow supporting the system and are considered as non-renewable.

Energetic resources, such as fuels, electricity and liquid petroleum gas, human labour and buildings materials make up the rest of the inputs since the other natural renewable inputs, such as sun, rain and wind, represent less than 1% of the total.

Table 10.6 shows how the characteristics of renewability and the location of the inputs are reflected in the energy indicators. The investment ratio is quite high, indicating that the emergy acquired from outside the system is 3.69 times higher than the local emergy. The environmental loading ratio (ELR) indicates that the non-renewable resources are more than four times higher than the renewable ones, demonstrating a high concentration of non-renewable inputs in the area, confirmed by the empower density (ED), that can highly impact the environmental characteristics of the area. The impact suggested by this ratio can be located anywhere, since the exploitation of non-renewable resources has an impact per se, while their use implies another impact, the empower density, which is around two orders of magnitude higher than in the case of agricultural or extensive breeding systems; it suggests that the main impact is local. This explains the need of further inputs for the cleaning up and the additional energy, material (and economic!) expenses for the environmental and health safety of the system.

10.5 Discussion

In order to assess the quality of the information that each method provides on sustainability, we adopted the following judgement criteria:

1. *Representativeness*: ability to describe all the features of the observed phenomenon
2. *Verifiability*: possibility to check the information of the model
3. *Reproducibility*: ability to achieve the same results in future time
4. *Comprehensibility*: ability to be easily understandable for people who do not deal with the specific research argument

10.5.1 Representativeness

Representativeness is the most important feature of the four above-mentioned criteria because it corresponds to the link between the object to judge and the way it is represented in the analysis. The objective of the analysis, as stated in the introductory part of the chapter, is to assess the environmental sustainability of a poultry-rearing system by means of three different methods. Generally, sustainability is connected with three main dimensions: economic, environmental and social. In the specific case of the poultry-rearing system we focus particularly on the environmental one.

Two aspects must be analysed to evaluate the state of environmental sustainability of a system: the impact or exploitation of a resource, and the availability of that resource (Bell and Morse 1999). In our specific case the resource corresponds to the environment as a whole. In assessing the ability of the three methods to bring out information on environmental sustainability, we analyse how they reflect the two aspects just mentioned.

In the three assessment methods the impact on the environment is evaluated in different ways. This can be easily noticed by the measure unit employed in each analysis (Table 10.7). LCA has several categories of impact. For each category there are several indicators. Depending on the aspect observed by the indicators (damage to human health, damage to ecosystem or damage to mineral and fossil resources) the measure unit can be Disability Adjusted Life Years (DALY), Potentially Disappeared Fraction of plant species (PDF m² year) or additional energy requirement to compensate lower future ore grade (MJ surplus energy). LCA provides information about direct and indirect effects on human being caused by environmental changes. The direct effects are captured by the categories concerning the impact on human health while the indirect effects by the categories concerning the ecosystem and the mineral and fossil resources. Our results for LCA (Fig. 10.4) show that the main impact categories affected are in ascending order: respiratory inorganics, fossil fuels, land use.

In the Ecological Footprint Analysis the indicator used to describe the impact on the environment is one, the Ecological Footprint. The measure unit is the global hectare (gha). The Ecological Footprint allows understanding which type of land category is mainly used or impacted: crop land, land to absorb greenhouse gas emissions, or built-up land (Fig. 10.5). Thanks to the Ecological Footprint, we found that 9.35 gha of the 12.81 gha of impacted land used to produce 1 kg of poultry in a year belong to the category crop land. Therefore the main human pressure on the ecosystem for the production of poultry meat derives from crop cultivation.

Among the indicators developed by Energy Analysis, the Environmental Loading Ratio is the one focusing more on environmental sustainability. The measure unit is the Solar joule. Our Energy Analysis shows that four trillion solar joule are employed to produce 1 kg of meat. This indicator represents the ratio between resources provided by the economic system (external to the analysed production system and not renewable) and renewable resources, describing in this way how much the system relies on resources exploited in a not-sustainable manner.

Table 10.7 Denomination of indicators and measure units used in the analysis (Goedkoop and Spriensma 2001)

Life Cycle Assessment		Ecological Footprint Analysis		Emergy Analysis	
Categories of the indicators employed for the analysis	Measure unit	Indicators employed for the analysis	Measure unit	Indicators employed for the analysis	Measure unit
Carcinogens	DALY	Ecological Footprint	gha	Free renewable energy (R)	sej
Resp. organics	DALY	Bio-capacity	gha	Free non-renewable energy (N)	sej
Resp. inorganics	DALY			Purchased energy brought by the economic system (F)	sej
Climate change	DALY				
Radiation	DALY				
Ozone layer	DALY				
Ecotoxicity	PDF m ² year				
Acidif./Eutrop.	PDF m ² year				
Land use	PDF m ² year				
Minerals	MJ surplus energy				
Fossil fuels	MJ surplus energy				

DALY: Disability Adjusted Life Years PDF m² year: Potentially Disappeared Fraction of plant species

MJ surplus energy: Additional energy requirement to compensate lower future ore grade

gha: Global hectare

sej: Solar joule

R: local renewable input, N: local non-renewable input, F: purchased input

However, without classifying the type of emergy used, Emergy Analysis is not able to provide significant information about human counteractions to the impact produced.

Therefore, considering these differences in terms of measure unit and type and quantity of indicators used, we can state that the multi-dimensionality of LCA brings out much more information on the impacts than Ecological Footprint or Emergy Analysis, also because it considers the indirect effects on human being caused by environmental changes. The information on the environmental impacts is broader than in the other methods. A common information that all the three

methods convey (Figs. 10.3, 10.6 and Table 10.5) is that the major source of the impacts is the feed for animals.

On the other hand, Ecological Footprint and Emery Analysis have other advantages which LCA does not offer. LCA allows giving judgements on the impacts generated by the poultry production, in relation to a previous state of the environment taken as reference point (Goedkoop and Spriensma 2001). However, a trend from a previous state does not provide any information about the resources availability and LCA analysis is not able to evaluate how much of the consumed resources are still available. Although it is not possible to define precisely a sustainable state (Bell and Morse 1999) we cannot affirm that a production system is environmentally sustainable only considering the dynamism of its impacts.

Instead Ecological Footprint Analysis uses the bio-productive land effectively owned by the breeding system (Bio-capacity) as an indicator of resources availability. The measure unit of this indicator is the global square meter. The mono-dimensionality of the method allows comparing the value of impact with the value of available resource, thus to define if the production system, concerning only the category of the ecosystem exploitation, is sustainable. In our study the ratio between Ecological Footprint and Bio-capacity shows that the production system is not sustainable (Fig. 10.7) because the bio-productivity used by the system is 172 times higher than the bio-productivity really owned.

For what concerns Emery Analysis, as stated above it is possible to classify the type of Emery source used in the system (Fig. 10.8). In our study, 79% of the total amount of emery necessary to produce 1 kg of poultry derives from external and non-renewable factors provided by the economy (F), 1.5% derives from non-renewable factors available in the spatial boundary of the breeding system, and 19.5% derives from renewable factors. Through the Environmental Loading Ratio, we can see that the non-renewable emery is four times higher than the renewable one. As in the Ecological Footprint Analysis, the mono-dimensionality of the method allows to compare resources depletion with resources availability (which in this case can be identified with the rate of renewable factors). Finally the results show that the breeding is not sustainable.

We can conclude that every method gives useful but different information for the representativeness of environmental sustainability in the analysed rearing system. LCA has a micro-focus approach; through its multi-dimensionality it describes in detail how the human well-being is affected, allowing a real intervention on concrete problems and indicating the direction to follow with respect to a previous system state. On the other hand, Ecological Footprint and Emery Analysis consider the availability of natural resources and not only the impact produced. This allows to state if a production system is sustainable from the environmental point of view. However, only one measure unit and dimension is used, leading to a reduced amount of information.

Since LCA is composed of multiple indicators it is possible, as some software allow, to integrate also the indicators concerning Ecological Footprint and Emery Analysis. In this way the information on environmental sustainability could be complete, thanks to the fusion of the three different methods perspectives.

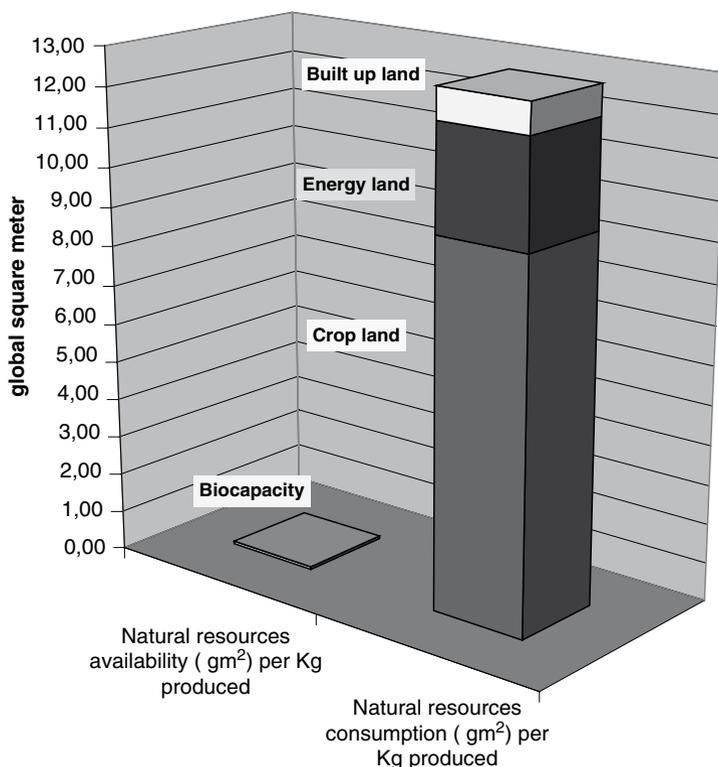


Fig. 10.7 Resources exploitation and resources availability in Ecological Footprint Analysis

The analysis refers to a single case study. Nevertheless, the three methods turn out to be more useful in the environmental sustainability decision-making process when considering the same production system over time, or comparing two production systems that provide the same output.

There are other important information to take into account about representativeness. The three methods can be considered systemic because the researcher has the possibility to set the boundaries (spatial and time limits) of the analysed system (Bell and Morse 1999). A negative aspect concerning Ecological Footprint is the absence of computation of matter and water depletion, unlike Emergy Analysis and LCA, in which these two aspects are taken into account for the final values of their indicators.

A general weakness of LCA method is that often available databases offer data coming from realities which are very different from the one represented in the study. In this case the results are not properly representative of the situation investigated.

The resilience effect can be regarded as the strength of Ecological Footprint. In fact the sub-category of required productive land to absorb carbon dioxide (energy land) includes the environment mitigation of human greenhouse gases production.

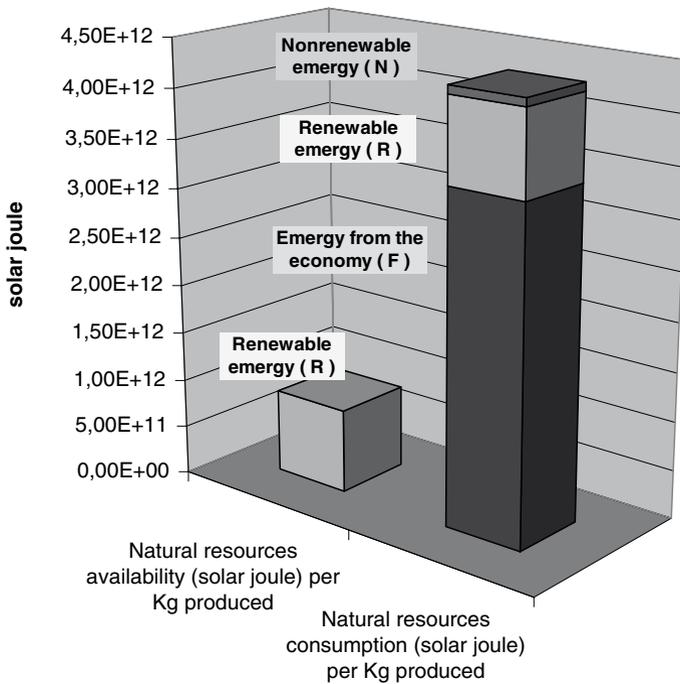


Fig. 10.8 Resources exploitation and resources availability in Energy Analysis

Negative and more relevant aspects of Energy Analysis about representativeness are strictly related to the general validity of the theory. Ayres (in Hau and Bakshi 2004) argues that it is hard to connect a defined value of solar joule to the matter (rocks and minerals) and its several specific states. Also Hammond (2007) raises doubts on the physical validity of Energy.

10.5.2 Verifiability

In LCA the verifiability of the model is possible but not for the overall set of the data. In fact foreground data derive from the communication with data providers. As a consequence they are generally obtained from real measurements or surveys. On the contrary, background data derive from databases or literature; hence they could be also assessed values.

Although Ecological Footprint and Bio-capacity are composite indicators based on a mono-dimensional value they can be considered quite verifiable. In fact both are a sum of many productive land categories; hence the values of the latter are measurable with real and existent tools.

The verifiability of Emergy Analysis information is a major problem. The measure unit of the model is mono-dimensional and the solar joule values are necessarily assessed since, at the moment, there are no available tools that can directly measure them.

10.5.3 Reproducibility

Reproducibility of results is one of the advantages of LCA. In fact this assessment method has a consistent set of specific databases which contain a huge amount of information. However, the results of the LCA study are strictly dependent upon the initial assumptions and upon the type of data used, and they can significantly change if using different information from the databases or starting from different assumptions. Moreover, if the complexity and the scope of the LCA study increase, processing time and costs will grow considerably.

Despite the easy computation in Ecological Footprint, the information reproducibility on sustainability pays the consequences of the lack of a specific database. This problem is highlighted by the calculation of the productive land required to absorb CO₂. There is no matter-specific direct conversion factor to assess this value. When considering each evaluated item, first of all it is indispensable to find the amount of greenhouse gas emissions and secondly to get the corresponding productive land to absorb them.

Emergy computation corresponds to a simple product of two factors. The reproducibility of the information raises problems only in reference to the conversion-factor (transformity). In fact, the same transformity was used for many assessed factors of the breeding system because of the lack of appropriate and specific conversion factors.

10.5.4 Comprehensibility

Regarding comprehensibility, unfortunately LCA language is not easily understandable by a 'not expert public'. This is because one of the main outputs of the method is the inventory table, which represents a long series of data; that is, all the set of emissions deriving from the system.

On the contrary, comprehensibility is probably the strongest feature of Ecological Footprint. The indicators language is easily understandable even though specific. Explaining the concept to farmers from whom data have been collected did not seem difficult as happened in the case of other models. This was twice as effective on the survey: first of all because farmers were able to provide more appropriate data to build the indicator, secondly because this reinforced in themselves the awareness of being an active part of the survey team. Therefore, the quantity of available information was higher than usual.

Unlike Ecological Footprint, the language of Emergy Analysis is not quickly comprehensible. People who are not used to deal with this specific subject have difficulties in understanding what a solar joule corresponds to.

Table 10.8 reports the main characteristics of the three different methods and allows to appreciate the differences for each of the above mentioned criteria. Each method presents both positive and negative aspects.

10.6 Conclusion

The appropriate instrument for a multi-dimensional representation of sustainability is a suitable set of indicators that must be an integral part of an assessment methodology. The three methods that we compared in this study provide a solution, since they are able to cover most of the information needs for the environmental dimension of sustainability in agriculture. We have detected several analogies when comparing the methods in terms of results related to the analysed system, that is, the intensive poultry-rearing farm.

Thanks to the Emergy Evaluation we found that for the analysed system the diet is the most important factor in the whole analysis, accounting for more than 82% of the total emergy flow. Our results obtained from Ecological Footprint Analysis point out that crop land, which is connected with chickens' diet, is the main land component, accounting for 73% of the total. The high quantities of maize and soya needed for feed require much crop land. Finally, thanks to the use of LCA, we found that feed production is what contributes the most to the environmental impacts of the system, influencing the impact category 'land use'. Our LCA analysis comes to the same conclusion as Ecological Footprint: the cultivation and the transformation of maize and soya are the processes with the strongest impact.

Finally, in our study both Emergy and LCA pointed out that the percentage of non-renewability of the inputs is high, with respect to the renewable ones. Emergy leads to this conclusion thanks to the Environmental Loading Ratio, while LCA thanks to the use of 'fossil fuels' impact category. Therefore, although the three methods use specific indicators and methodology, they come to the same conclusions for the system investigated.

By comparing the methods according to the four criteria of representativeness, verifiability, reproducibility and comprehensibility, we conclude that each of the three methods shows both positive and negative aspects, strengths and weaknesses, but all of them are effective in representing the environmental features of a given activity; therefore, the results can be used as input in a sustainability assessment process.

The choice to use Emergy Evaluation, Ecological Footprint Analysis, or LCA depends upon the main objective of the assessment process. If we are dealing with a problem of environmental impacts, LCA is a reliable tool to analyse the situation from a multi-dimensional perspective. On the contrary, if we are dealing with a problem of resources availability, Ecological Footprint or Emergy Analyses are

Table 10.8 The three methods positive and negative aspects

	Life Cycle Assessment		Ecological Footprint Analysis		Emergy Analysis	
	Positive aspects	Negative aspects	Positive aspects	Negative aspects	Positive aspects	Negative aspects
	Systemic	No carrying capacity	Systemic.	No matter and water depletion computation	Systemic	No resilience
Representativeness	Many impact categories considered	Data from realities different from the one investigated No resilience	Carrying capacity		Carrying capacity	Uncertainty of basic theory assumptions
Verifiability	Measurable values	Some values necessarily assessed	Resilience		Matter and water computation	Mono-dimensional. Values necessarily assessed.
Reproducibility	Presence of specific databases	Results strictly dependent from the type of data. Complexity of the study implies more costs and time	Easy computation	Absence of specific database	Easy computation	Absence of specific database
Comprehensibility		No easy access language	Easy access language			No easy access language

better ways to evaluate the exploitation level of the analysed resources. However, in many cases it is not necessary a choice because the three methods can be used together, and the results can be integrated to build combined indicators, capable to ensure a wide and complete analysis.

Therefore, all environmental impact indicators used in our study, resulting from the application of the three methods to the case study, constitute a proper set of environmental indicators, to be used for sustainability assessment. So far, there are too few applications of the three methods in agriculture. In particular, in the livestock sector they are really rare, and the situation in poultry breeding is even worse. On the other hand, the need to conduct studies on the relationships between livestock and the environment is widespread throughout the world.

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Chapter 11

Compost Use in Organic Farming

Eva Erhart and Wilfried Hartl

Abstract Organic farming is a sustainable agricultural system that respects and relies on natural ecological systems. Its principles exclude the use of synthetic pesticides and fertilizers. Instead it is based on management practices that sustain soil quality and health. Composting of organic residues and the use of compost in agriculture bring back plant nutrients and organic matter to the soil that otherwise would be lost. Nevertheless, there are some potential risks associated with compost use, such as the accumulation of heavy metals or organic pollutants, which must not be neglected.

Some types of organic farms, such as stockless farms or vegetable farms, have difficulties sustaining soil humus using only organic farming sources. For such farms, using biowaste compost from separately collected organic household waste might be a solution, which in addition helps to close nutrient and organic matter loops of the whole society. Here we compile information on beneficial effects and potential risks associated with compost use and on crop yields and quality, with compost under an organic farming perspective.

The most important benefit of using compost is the increase in soil organic matter (SOM). Under temperate climate conditions, 6–7 t ha⁻¹ year⁻¹ (dry wt.) compost is sufficient to maintain the soil humus level of medium-textured soils; higher rates increase the soil humus content. Regular compost addition enhances soil fauna and soil microbial biomass and stimulates enzyme activity, leading to increased mineralization of organic matter and improved resistance against pests and diseases, both features essential for organic farming. Through the significant increase in the soil's content of organic carbon, compost fertilization may make agricultural soil a carbon sink and thus contribute to the mitigation of the greenhouse effect.

Phosphorus and potassium in compost become nearly completely plant-available within a few years after compost application. The nitrogen-fertilizer value of compost is lower. In the first years of compost application, N mineralization may

E. Erhart (✉) and W. Hartl

Bio Forschung Austria, formerly Ludwig Boltzmann-Institute for Biological Agriculture and Applied Ecology, Rinnboeckstrasse 15, A-1110 Vienna, Austria
e-mail: e.erhart@bioforschung.at

vary from -15% to $+15\%$. Nitrogen recovery in the following years depends on the site- and cultivation-specific mineralization characteristics and will roughly be the same as that of soil organic matter (SOM).

Soil cation exchange capacity (CEC) increases with compost use, improving nutrient availability. Moderate rates of compost of $6\text{--}7\text{ t ha}^{-1}\text{ year}^{-1}$ dry wt. are sufficient to substitute regular soil liming. In the available micronutrient status of the soil, only minor changes are to be expected with high-quality composts. Increasing soil organic matter exerts a substantial influence on soil structure, improving soil physical characteristics such as aggregate stability, bulk density, porosity, available water capacity, and infiltration. Increased available water capacity may protect crops against drought stress.

Plant-disease suppression through compost is well established in container systems. In field systems, the same processes involving the suppression of pathogens by a highly active microflora supported by the supply of appropriate organic matter are likely at work.

When using high-quality composts, such as specified by the EU regulation 2092/91, the risk of heavy metal accumulation in the soil is very low. Nitrogen mineralization from compost takes place relatively slowly and there are virtually no reports of uncontrollable N-leaching. Concentrations of persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), or polychlorinated dibenzodioxins and dibenzofurans (PCDD/F) in high-quality composts usually approach the usual soil background values. Also the overall hygiene and hygiene concerning plant diseases and weeds are not a problem if quality composts produced in a monitored system are used.

Most studies found positive yield effects of biowaste compost. However, the effect of biowaste compost applied at moderate rates usually takes some years to develop. It depends on the factors determining nutrient mineralization from soil and compost and also on crop-related factors such as the nutrient requirements and uptake dynamics of the respective crop rotation. Crops with longer growth periods can make better use of compost. Many vegetable crops respond favorably to compost fertilization, often immediately after the first application. Crop quality is usually not affected by compost fertilization in cereals and slightly positively influenced in vegetable crops.

Keywords Soil humus • nitrogen • phosphorus • potassium • soil structure • heavy metals • organic pollutants • yield • crop quality • compost • organic farming • Cd • Zn • Ni • Pb • Hg • Cu • Cr • PAH • dioxin • CEC • soil pH • soil N • nitrate, P, K • micronutrients • soil aggregate • soil water • plant disease • maize • wheat • barley • potato • tomato broccoli • cabbage • cauliflower • cantaloupe • legume • onion

11.1 Introduction

Organic farmers do not use synthetic fertilizers and pesticides but seek to augment ecological processes that foster plant nutrition and yet conserve soil and water resources. Maintaining and improving the soil's quality and fertility are central to

organic farming (EU regulation 2092/91). Therefore, practices that add organic material are routinely a feature of organically farmed soils to sustain soil humus as the basis of soil's natural fertility.

One of the principles of organic farming is to close the nutrient cycles in the farm. However, there is some controversy among organic farmers about whether the principle of the closed nutrient cycle is to be regarded in a strict sense and external inputs to the farm are to be minimized or whether nutrient cycles may also include the consumers of agricultural products and biowaste compost from separately collected organic household wastes may be returned to an organic farm, thus closing the larger loops of nutrients and organic matter of the whole society. There are some types of organic farms, such as stockless farms or vegetable farms, which find it difficult to sustain the humus content of their soils. In other cases, management before conversion has left a soil organic-matter content too low to ensure soil functions. For such farms, and for farms that are unable to remedy some nutrient deficiency through organic farming sources such as manure or leguminous crops, the use of high-quality biowaste compost might be a viable alternative.

In organic farming, only the following types of compost are allowed:

- Compost from organic material derived from organic farms is permitted without restrictions.

Other composts, such as listed below, may be applied only if adequate nutrition of the crop being rotated or soil conditioning is not possible by the methods listed in the regulation. The need for such composts must be recognized by the inspection body.

- Composts from animal excrements (factory farming origin forbidden)
- Composts from mixtures of vegetable matter
- Composted bark from wood not chemically treated after felling
- Compost from source-separated household waste, produced in a closed and monitored collection system and not exceeding the following heavy metal limits (in mg/kg dry matter): Cd 0.7, Cu 70, Ni 25, Pb 45, Zn 200, Hg 0.4, Cr_{tot} 70, Cr (VI) 0 (EU regulation 2092/91).

The aim of this study is to compile the information on compost use, which is documented in scientific publications, under an organic farming perspective.

11.2 Beneficial Effects of Compost Use

11.2.1 *Soil Organic Matter*

The most important benefit of using compost is the increase in soil organic matter. Most arable soils contain only 2–4% organic matter by weight, yet very little about these soils is not significantly influenced by the organic matter in them. Organic matter provides much of the soil's capacity to store nutrients and water. It plays

a critical role in the formation and stabilization of soil structure, which in turn produces good tilth and drainage and resistance to erosion. It cannot only carry and make available nitrogen, sulfur, and phosphorus but also improve the availability of nearly all nutrients, whether applied as fertilizer or weathered from minerals. It promotes the health of the soil ecosystem and stimulates organisms that cycle carbon and protect plants from disease (Weil and Magdoff 2004).

The concentration of organic matter in soils is primarily related to climate (temperature and precipitation), to soil texture (clay content) and to soil drainage status. Crop rotation and management usually play a smaller, but important role (Shepherd et al. 2002). Organic matter accumulation in soils is maximum when the difference between annual plant productivity and annual decomposition is highest. In cropped soils, organic matter accumulation also depends on the balance between what is exported from the soil and what remains as residues. Low mean annual temperatures tend to slow decomposition much more than productivity. Within limits, more rainfall tends to increase plant growth more than it does decomposition; therefore, soil organic matter tends to be positively correlated with annual precipitation. If environmental factors are similar, finer-textured soils tend to accumulate higher amounts of organic carbon (Weil and Magdoff 2004).

Nearly all the nitrogen and large proportions of the phosphorus and sulfur found in soils occur as constituents of soil organic matter. The soil organic matter serves as both the principal long-term storage medium and as the primary short-term source of these and other nutrients. The nutrients in soil humus are transformed into plant-available forms by microorganisms. Humus exerts important physical effects on the soil. It promotes the formation of a stable aggregate structure, which improves soilwater-holding capacity and aeration. Humus itself also has a high water-holding capacity. Humic substances buffer the pH of the soil. The dark color of the uppermost soil layer, which is due to humic substances, promotes soil warming in spring and thus elongates the growth period. A high humus content allows also moist soil to be tilled without causing compaction (Golueke 1975; Schachtschabel et al. 1998; Stevenson 1982; Weil and Magdoff 2004).

Soil organic matter is subject to biochemical decomposition and transformation and there are different levels of fragmentation, transformation, and biodegradation. Parts of newly added organic material, which are of greater stability, may persist for many years in the soil. Some of the functions of soil humus, however, are not due to the long-term persistence of soil organic matter (SOM), but to its permanent turnover and short-lived metabolic products. The maintenance of a large and active soil microflora, for instance, which mineralizes nitrogen and mobilizes other nutrients, depends on a repeated supply of organic material (Sauerbeck 1992). A relatively small portion of soil organic matter with a half-life measured in months or a few years accounts for most of the biological activity in soil and plays a particularly important role in maintaining soil quality. The active pool of soil organic carbon provides the fuel that drives the soil food web (Weil and Magdoff 2004). A significant increase in the soil's content of organic carbon may make agricultural soil a carbon sink and thus contribute to the mitigation of the greenhouse effect.

The profound effects that organic matter has on almost all soil properties make soil organic matter management on the farm the basis for sustainable agricultural production. In general it is assumed that between 1% and 5% of the soil organic matter, depending on the kind and intensity of soil cultivation, are mineralized annually in temperate climate agroecosystems. In order to maintain the soil's humus level and to refill the active pool of soil organic carbon, which nourishes the soil food web, at least the same amounts of organic matter must be added to the soil every year.

Compost typically has a high organic matter content and organic matter in well-cured compost is highly humified, its C/N ratio being similar to that of soil humus. Compost organic matter contents range between 15% and 50% d.m. (dry matter) and humic acid contents between 15% and 45% o.d.m. (organic d. m.) (Diez and Krauss 1997; Smidt and Tintner 2007; Zethner et al. 2000). Therefore, well-cured compost has a very high humus-reproduction value (VDLUFA 2004; Leithold et al. 1997; Kolbe 2007).

11.2.1.1 Humus Content

Numerous experiments show that compost fertilization regularly leads to a distinctive increase in the humus content of the soil. Such increases were reported from field trials with 1–28 years duration, with annual compost applications ranging from 6 to 90 t ha⁻¹ and situated on a broad range of sites and soil types.

One year after application of 80 t ha⁻¹ compost made from the organic fraction of household waste to an infertile Rensic Leptosol near Madrid, Spain, soil organic carbon was significantly increased (by 9 g kg⁻¹) as compared to the untreated control (Illera et al. 1999).

In 3-year compost field trials situated near Kassel, Germany, biowaste compost was applied at rates of 30 and 100 t ha⁻¹ year⁻¹ (wet wt.), respectively, to a sandy soil and to a loamy silt loess soil. On the sandy soil, soil C_{org} increased from 14.3 to 14.5 and 16.5 g kg⁻¹, respectively. On the loamy silt loess soil, soil C_{org} increased from 11.0 to 13.0 and 17.8 g kg⁻¹, respectively (Stöppler-Zimmer and Petersen 1997).

When 30 t ha⁻¹ (wet wt.) compost from pruning waste and crop residues was applied to each of five crops during a 3-year experiment on a loam soil with a vegetable rotation near Seville, Spain, soil total organic carbon increased from 7.8 to 13.5 g kg⁻¹, while in the treatment with mineral fertilization, C_{org} amounted to 8.5 g kg⁻¹ (Melero et al. 2007).

Timmermann et al. (2003) conducted six field trials with biowaste compost for 5 and 8 years, respectively, on mostly silty loam soils in Baden-Württemberg, Germany, under a maize–winter wheat–winter barley rotation. With annual bio-compost applications the C_{org} content of the soil increased by 1.3 g kg⁻¹ C_{org} per 5 t ha⁻¹ (dry wt.) of compost applied on average.

Clark et al. (1998) investigated the changes in soil quality during the transition from conventional to organic farming on silty loam in Sacramento Valley, USA. The organic

treatment received 4–7 t ha⁻¹ (dry wt.) composted manure every second year and vetch cover-crop residues every fourth year. After 8 years, soil total carbon content in the organic treatment had increased significantly from 9.11 to 10.21 g kg⁻¹, while it remained the same in the conventional treatment.

Biowaste-compost fertilization in organic farming (at average annual rates of 6, 11 and 16 t ha⁻¹ dry wt.) was compared to conventional mineral fertilization and to no fertilization on a silty loam Fluvisol near Vienna, Austria. The crops grown were mainly cereals, with potatoes every fourth year. After 10 years, soil organic carbon content had increased from 19.9 to 20.5–21.7 g kg⁻¹ in the three treatments with increasing rates of biowaste-compost fertilization, while it remained the same with mineral fertilization and decreased to 18.3 g kg⁻¹ without fertilization (Hartl and Erhart 2005), Fig. 11.1.

Diez and Krauss (1997) recorded increases in soil C_{org} content from 14.5 to 16.9 g kg⁻¹ on a loamy loess soil and from 19.2 to 22.2 g kg⁻¹ on a gravelly soil, with an average annual input of 4.4 t ha⁻¹ organic matter (in 14.8 t ha⁻¹ compost dry wt.) in field experiments of 20 years duration, under the humid climatic conditions of Bavaria, Germany. The crop rotation of the experiments included sugar beet/potatoes, winter wheat, and summer barley.

The DOK-experiment in Therwil, Switzerland, compares, among other treatments, fertilization with composted manure at a rate corresponding to 1.4 livestock units in biodynamic farming with conventional mineral fertilization on a sandy loam Luvisol. The crop rotation includes potatoes + green manure, winter wheat + intercrop, cabbage/beets, winter barley, and 2–3 years grass clover. After 21 years of compost fertilization, soil organic carbon had increased by 1% in the biodynamic treatment (using manure compost), while the soils in the organic treatment (with rotted

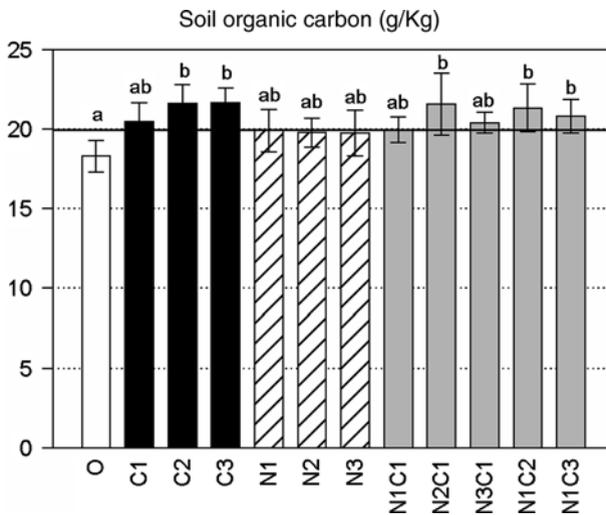


Fig. 11.1 Soil organic-carbon contents (g kg⁻¹) at 0–30 cm depth in spring 2003 (bars) as compared to the initial level in spring 1993 (horizontal line: 20 g/kg). Treatments with the same letters are not significantly different at $P \leq 0.05$ (From Hartl and Erhart 2005. With permission from Wiley-VCH)

manure) and in the conventional treatment with manure had lost 9% and 7% of their C_{org} , respectively. With mineral fertilization 15% and with no fertilization 22% of soil C_{org} were lost (Fliessbach et al. 2007). Although the crop rotation included plenty of green manuring, intercrops, and grass clover, additional compost fertilization distinctly increased soil humus content.

No significant increases in soil humus were found in 3 years' trials with moderate annual inputs (24 and 30 t ha⁻¹ year⁻¹ wet wt., respectively) on a Cambisol (Ebertseder et al. 1997) and on sandy podsoles and gley soils (Boisch 1997), most probably due to the low clay content of the soils and the short duration of the experiments.

From these experimental results it may be concluded that in general for medium-textured soils under temperate climate conditions, around 6–7 t ha⁻¹ year⁻¹ (dry wt.) of compost application are usually sufficient for the maintenance of the soil humus level; higher rates increase the soil humus content.

11.2.1.2 Humus Composition, Soil Microbiology, and Soil Fauna

Besides the effects of compost fertilization on the total humus content of the soil, there are also effects on the composition of soil organic matter. Humus fractionation showed that the humin fraction with compost application was approximately 50% higher than in the other soils of the study (Fliessbach et al. 2000). Microbial biomass C and N as well as their ratios to the total and light fraction C and N pools in the soils of the organic systems were higher. This is interpreted as an enhanced decomposition of the easily available light fraction pool of soil organic matter, which points to a more efficient utilization of organic matter by a large and diverse microbial biomass (Fliessbach and Mäder 2000; Mäder et al. 2002). Composts are very diverse in respect of their feedstocks; they are in different stages of biodegradation and of different biochemical composition, such as their contents of soluble C, cellulose, and lignin. The type and diversity of plants and organic residues added to a soil can influence the type and diversity of organisms that make up the soil community, and vice versa (Weil and Magdoff 2004). Soil microbial populations are also altered through the addition of the compost microflora (Ros et al. 2006).

Regular addition of organic matter (compost) increases soil microbial biomass and stimulates enzyme activity (Fliessbach and Mäder 2000; Lalande et al. 1998; Pascual et al. 1997; Schwaiger and Wieshofer 1996; Serra-Wittling et al. 1995), leading to increased mineralization of organic matter and improved resistance against pests and diseases. By providing an additional food source compost fertilization also enhances earthworm abundance and biomass (Kromp et al. 1996; Mäder et al. 2002; Pfozter and Schüler 1999).

11.2.1.3 Cation Exchange Capacity

Negatively charged soil particles such as clay minerals and humic substances are able to adsorb cations. Adsorbed cations are kept in a status in which they cannot

be leached, but may only enter the soil solution through exchange for other cations. Only after that they may be leached or taken up by plants. This property enables soils to hold nutrients in the soil–plant cycle or at least to delay their being lost into adjacent ecosystems (such as lakes and rivers or groundwater). The total exchangeable cations are referred to as the cation exchange capacity (CEC). Soil CEC is greatly influenced by the input of organic matter. The average CEC of organic matter is $2 \text{ mmol}_c \text{ g}^{-1}$, whereas the CEC of clay is around $0.5 \text{ mmol}_c \text{ g}^{-1}$ and that of silt around $0.1 \text{ mmol}_c \text{ g}^{-1}$ (Schachtschabel et al. 1998).

In an experiment with biowaste-compost fertilization at average annual rates of $15\text{--}39 \text{ t ha}^{-1}$ (wet wt.), the CEC was closely correlated with the humus content of the soil and increased linearly with the amount of organic matter added via compost during 5 years. Compared with the unfertilized control, CEC rose by 3–7% in the compost treatments. In the treatments receiving mineral fertilizer only, the CEC was the same as in the unfertilized control. In a second experiment, with compost application at a total rate of 130 t ha^{-1} compost in different doses and intervals during 6 years, CEC increased by 4–10%, in proportion with the increase in humus content (Hartl and Erhart 2003).

Also Businelli et al. (1996) recorded a significant increase in CEC in a 6-year experiment on a clayey loam soil near Perugia. The same was reported by Frohne (1990) after a single application of 240 t ha^{-1} biowaste compost on a compacted loess Luvisol.

11.2.2 Soil pH

Biowaste-compost pH is usually around 7.5–7.8 (Timmermann et al. 2003; Vogtmann et al. 1993a; Zethner et al. 2000). Numerous field experiments show that compost fertilization increases the soil pH in acidic soils (Alin et al. 1996; Hue et al. 1994; Timmermann et al. 2003) and slightly acidic soils (Alföldi et al. 1993; Diez and Krauss 1997; Ebertseder et al. 1997; Stöppler-Zimmer and Petersen 1997; Timmermann et al. 2003). In neutral to slightly alkaline soils pH is usually unaffected (Diez and Krauss 1997; Erhart et al. 2002; Timmermann et al. 2003).

In summary, the amount of base-forming cations supplied to the soil with the application of moderate doses of compost ($6\text{--}7 \text{ t ha}^{-1} \text{ year}^{-1}$ dry wt.) is sufficient for the maintenance or a slight increase in pH, and therefore can substitute regular soil liming.

11.2.3 Nitrogen

11.2.3.1 N Mineralization

On average, biowaste compost contains $11.5\text{--}16.4 \text{ g kg}^{-1}$ total nitrogen (Timmermann et al. 2003; Vogtmann et al. 1993a; Zethner et al. 2000), which is present mostly in

humus-like organic compounds. More than 90% of total compost N is bound to the organic N pool (Amlinger et al. 2003a). Between 30% and 62% of the total N of bio-waste and yard-waste composts are present in humic acids (Smidt and Tintner 2007).

Therefore, a large portion of the nitrogen present in compost is not readily available to plants, but it can to a certain degree be mineralized and subsequently be taken up by the plant, or immobilized, denitrified, and/or leached. N mineralization from composts is affected by the same factors that affect the N mineralization of organic N in soils. Compost-related factors include the C and N content of the compost, the C/N ratio, the biodegradability of compost C and the compost microflora. The biochemical composition, such as contents of soluble C, cellulose, and lignin, appears to play a crucial role for mineralization (Gagnon and Simard 1999, Mary et al. 1996). For instance, compost organic N derived from vegetal tissues was much more resistant to mineralization than organic N derived from animal wastes (Canali et al. 2003). Site-related factors include soil texture, pH, and climate.

The N-mineralization rate of composts can be determined either in incubation experiments or in pot or field trials. In the latter, nitrogen uptake by the compost-fertilized crop is compared to that of a crop without fertilization or with mineral N fertilization. In incubation experiments using composts from different feedstocks and of varying maturity, N-mineralization rates ranging from -30.3% to $+14.3\%$ of the organic N were recorded (Chodak et al. 2001; Gagnon and Simard 1999, Hadas and Portnoy 1997, Siebert et al. 1998). In general, incubation experiments show that N-mineralization rates of mature composts are higher than of immature composts. In pot studies with compost-amended soil, plant N recovery ranged from 2% to 15% (Hartz and Giannini 1998, Iglesias-Jimenez and Alvarez 1993, Scherer et al. 1996).

In field experiments, mineralization is further influenced by soil-cultivation measures and plant-soil interactions, and so it is important to know this dynamic to adjust the time of the compost application. The N uptake of field crops depends also on the respective crop N requirements and N-uptake dynamics. Therefore, N mineralization calculated from the results of field trials varied from -14% to $+15\%$ (Brandt and Wildhagen 1999; von Fragstein and Schmidt 1999; Gagnon et al. 1997; Hartl and Erhart 2005; Nevens and Reheul 2003). High N-mineralization and N-recovery rates are reported when N-rich, well-biodegradable composts and/or crops with high nutrient demand and a long growth period are used, while immature composts and yard-waste composts with low N content usually show low N-mineralization and N-recovery rates.

Subsequently, the remaining portion of compost N and compost humus is incorporated into soil humus. Therefore, on the longer term, the mineralization rate of this portion will be the same as that of soil organic matter.

A survey of numerous field experiments showed, that nitrogen recovery in the first year after compost application was between 2.6% and 10.7% (Amlinger et al. 2003b). Therefore, around 5% of the compost N may be assumed to be plant-available in the first year. Nitrogen recovery in the following years was dependent on the site- and cultivation-specific mineralization characteristics and was around 2–3% of the compost N applied (Amlinger et al. 2003b).

One strategy therefore might be to apply compost to leguminous cover crops. Legume residues decompose quickly and provide available nitrogen whereas compost decomposes more slowly and contributes more to organic matter buildup. Targeting the application of compost to a legume or mixed legume–grass crop permits the legume to act as an N buffer against the variable or negative N release from composts (Lynch et al. 2004). Incorporating residues with a range of C/N ratios can lead to the timely mineralization of available soil N for crop uptake.

Sanchez et al. (2001) found that nitrogen mineralization (of the same added material) was distinctively higher in a diverse system which had received diverse crop residues plus composted manure than in a conventional corn plus mineral-fertilizer system. Also Drinkwater et al. (1998) showed that the application of relatively diverse residues that differ in terms of biochemical composition can significantly increase soil C while meeting crop N needs. On the other hand, applying compost to leguminous cover crops might also buffer excess nitrogen to reduce the risk of N-leaching (Lynch et al. 2004).

Organic sources of N, such as manures, composts, or legume cover crops, can furnish adequate crop nutrition to full-season crops while maintaining relatively low levels of available N for most of the growing season. On the other hand, N mineralization from organic sources might lag behind in the needs of early short-season crops and might continue in the fall after full-season crops have been harvested. Therefore, when organic sources of fertility are used, additional available N might be needed for early-season crops, and catch crops should be used to prevent excess N-leaching following the growing season (Magdoff and Weil 2004).

Due to the large amounts of organic matter present in compost, significant increases in soil total nitrogen content are quite common with compost fertilization. Such increases were reported from numerous field trials on a broad range of sites and soil types (Alin et al. 1996; Businelli et al. 1996; Cortellini et al. 1996; Diez and Krauss 1997; Hartl and Erhart 2005).

11.2.3.2 Nitrogen-Leaching

For one, the increased mineralization potential which results from the rise in soil total nitrogen content is desired and necessary in organic farming in order to feed the crop plants from the soil resources, but for another, it holds the risk of increased nitrogen-leaching to the groundwater. Several experiments, conducted under varying soil and climate conditions, showed that compost fertilization usually resulted in equal or lower nitrate-leaching losses than corresponding mineral fertilization.

With compost fertilization at rates of 43 and 86 t ha⁻¹, respectively, drainage water nitrate concentrations in the first year were not significantly different from the unfertilized control, which amounted to 5.2 ppm, while with mineral N fertilization at 400 kg ha⁻¹, drainage water nitrate concentrations increased to 41.5 ppm. Maize grain yields with compost were the same as in the control, while they increased significantly with mineral fertilization. In the second year, when neither compost nor mineral fertilizer was applied, NO₃-leaching losses of the mineral fertilized treatments were still 300% of those in the compost-only treatments. Wheat yields in the compost treatments were twice and three times, respectively, as high as in the

control. In the mineral fertilizer treatment wheat yields amounted to 170% of those without mineral fertilizer (Pardini et al. 1993). When five different fertilization regimes were applied to lysimeters filled with sandy soil, total nitrate-leaching losses decreased in the order mineral fertilization, fertilization with manure compost, refuse compost, unfertilized, yard-trimmings compost. Cumulative nitrogen export through the crops decreased in the same order (Leclerc et al. 1995). Small lysimeters were fertilized during 6 years with composts of varying origin (Jakobsen 1996). When the residual effect was tested in the seventh year without fertilization, NO_3 -leaching losses were higher in the lysimeters with compost owing to the decomposition of compost in the soil. After a new fertilization, however, NO_3 -leaching losses were smaller in the lysimeters with compost than in those with mineral fertilization. Dry matter yields of barley in the last experimental year were 50% higher with mineral fertilization than with compost.

Nitrate in groundwater was measured beneath a 3-year field trial with vegetables on a sandy soil over a shallow groundwater table (Maynard 1993). Nitrate concentrations in the groundwater were lower in the compost treatments (with annual rates of 56 and 112 t ha^{-1} , sufficient to provide the fertilizer requirements for intensive vegetable production) than in the control plots, which had received mineral fertilizer. In 3-year field experiments with biowaste compost on podsollic, gley-podsolic, and Luvisol soils in Northern Germany, none of the compost treatments (total application 26 and 42 t compost ha^{-1}) resulted in clear increases in soil water nitrate contents (Boisch et al. 1993). Yields in the compost treatments were not significantly higher than in the unfertilized control in most years (Boisch 1997). Nitrate concentrations in soil water in a field experiment with yard-trimmings compost, manure, manure compost, and mineral fertilization were also similar in all treatments on a Luvisol in Switzerland and the same was true for the yields (Bernier et al. 1995). With biowaste-compost fertilization at 16 and 23 t ha^{-1} year⁻¹, respectively, on average of 11 years, nitrogen-leaching to the groundwater as determined using ceramic suction cups was not increased as compared to mineral fertilization at 41 and 56 kg N ha^{-1} year⁻¹, respectively, in an 11-year crop-rotation experiment on a Molli-gleyic Fluvisol near Vienna, Austria. Even intensive nitrogen mineralization during a 4-month period of bare fallow did not cause pronounced differences between the fertilization treatments (Erhart et al. 2007). The yields did not differ significantly between compost and mineral-fertilizer treatments in most years (Erhart et al. 2005).

The results of these experiments show that normally compost fertilization does not pose a risk for groundwater eutrophication. N mineralization from compost takes place relatively slowly and there are virtually no reports of a sudden, ecologically problematic rise in soluble N pools and uncontrollable N-leaching.

11.2.4 Phosphorus

Phosphorus concentrations in biowaste composts generally range from 2.7 to 4.0 g kg^{-1} (Timmermann et al. 2003; Vogtmann et al. 1993a; Zethner et al. 2000). On the one hand, composts enrich the soil phosphorus status by their direct contribution,

as 20–40% of the P in compost is immediately plant-available (Vogtmann et al. 1993a). Organic P in composts from plant materials is readily decomposed to release ortho-phosphate, which is available to plants (He et al. 2001). But organic matter does not only provide a source of P from mineralization but also can reduce the capacity of acid soils and of soils with a pH > 8 to lock up P by fixation. Organic soil amendments reduce the sorption of P in soils and increase the equilibrium P concentration in the soil solution (Hue et al. 1994; Weil and Magdoff 2004).

Increased soil contents of available P frequently occur after compost application (Businelli et al. 1996, Cortellini et al. 1996, Diez and Krauss 1997, Parkinson et al. 1999). With application of 39 t ha⁻¹ year⁻¹ (wet wt.) biowaste compost, plant-available soil contents of phosphorus were significantly higher than in the unfertilized control. In the treatments which had received 27 and 15 t ha⁻¹ year⁻¹ the soil contents of plant-available phosphorus were in the same range as with 48 kg P₂O₅ ha⁻¹ year⁻¹ in mineral superphosphate or triplephosphate fertilizer. Also plant phosphorus contents showed that the phosphorus supply with compost fertilization was approximately as high as in the mineral fertilizer treatments (Hartl et al. 2003). In the DOK experiment, soluble fractions of phosphorus were lower in the compost treatment than in the conventional treatment, but the flux of phosphorus between the matrix and the soil solution was highest in the system with compost application. Phosphorus flux through the microbial biomass was faster in compost-treated soils, and more phosphorus was bound in the microbial biomass (Mäder et al. 2002; Oehl et al. 2001).

Phosphorus availability is crucial for optimum N fixation by legumes. Green-waste compost, applied on acid soils (pH 5.4) very low in P, provided sufficient P for red clover to achieve optimal nitrogen fixation. The effect of green-waste compost was nearly equivalent to that of triple-superphosphate (Römer et al. 2004).

Most studies found that P in compost became nearly completely plant-available within three vegetation periods after compost application (Amlinger et al. 2006). Therefore, it may be concluded that the total P content of composts can be calculated as a substitute for mineral P fertilization.

11.2.5 Potassium

The concentrations of potassium in composts vary from 8.4 to 12.5 g kg⁻¹ (Timmermann et al. 2003; Vogtmann et al. 1993a; Zethner et al. 2000), depending on different sources of feedstocks. Green-waste compost, for example, often exhibits elevated K contents. However, the composting process may also have a substantial influence on K availability. Due to the high water solubility of K, leaching losses may occur if the compost is exposed to rainfall. Immediate plant availability of K in composts can be more than 85% of the total K content (Vogtmann et al. 1993a) and the remainder is easily mineralizable.

Soil contents of available K typically increase with application of composts made from plant residues (Businelli et al. 1996, Cabrera et al. 1989, Diez and

Krauss 1997, Parkinson et al. 1999). For example, application of 27 and 39 t ha⁻¹ year⁻¹ (wet wt.) of biowaste compost for 5 years significantly increased plant-available soil contents of potassium in a field experiment in Austria, while they remained the same in the control and increased slightly, not significantly with mineral fertilization at rates of 74 kg K₂O ha⁻¹ year⁻¹. Plant potassium contents showed that the potassium supply with compost fertilization was approximately as high as with mineral fertilization (Erhart et al. 2003).

In the DOK experiment, under rather humid conditions, soluble fractions of potassium were lower with manure-compost fertilization than in the conventional treatments (Mäder et al. 2002).

Clear increases in plant-available soil potassium contents were found in field experiments in Baden-Württemberg (Timmermann et al. 2003). Average compost rates of 10 t ha⁻¹ (dry wt.) year⁻¹ were sufficient to counteract or even overcompensate the decrease in soil potassium contents caused by plant uptake and leaching.

From these findings it may be concluded that the total K content of composts can be accounted for in the fertilizer calculation.

11.2.6 Micronutrients

Iron (Fe), Mn, Cu, Zn, B, and Mo are essential elements for crop production and food quality (Marschner 1995). A long-term diet containing low concentrations of Fe, Mn, Cu, and Zn has been reported to cause human malnutrition. The availability of Fe, Cu, and Zn in calcareous soils is generally low, and an external source of these nutrients is needed for improved crop yield and food quality (He et al. 2001).

Addition of organic matter to soil can either decrease or increase metal availability, solubility, and plant uptake. Insoluble organic matter usually forms insoluble organometal complexes or sorbs metal ions, making them less available for plant uptake or leaching. However, many organic amendments have a soluble C component or produce soluble decomposition products, and the soluble organic matter can increase metal solubility by forming soluble organometal complexes. Metals are also released through the biodegradation of organic matter by microorganisms. The influence of soil organic matter on metal mobility can also be modified by the solution pH (Weil and Magdoff 2004). Crops, and even cultivars, differ considerably in their sensitivity to individual trace elements, and within the plant, trace elements are not uniformly distributed among plant tissues (Adriano 1986).

Incorporating a municipal-waste compost at 48 t ha⁻¹ or a municipal-waste biosolids co-compost at 24 t ha⁻¹ into a calcareous limestone soil increased concentrations of soil-extractable metals, but caused no significant changes in tomato and squash (*Cucurbita pepo* L.) fruit concentrations of Cu and Zn compared to an unamended control (Ozores-Hampton et al. 1997).

Leaves of tomato plants grown in soil amended with municipal-waste compost showed decreased Mn and Cu contents compared to leaves from plants grown in

unamended soil in a study by Stilwell (1993). These results were attributed to reduced availability of Mn and Cu in the compost-amended soil due to increases in pH and organic matter content.

In the DOK experiment in Switzerland, amounts of plant-available Mn, Zn, and Cu in the sandy loam Luvisol (measured using $\text{CaCl}_2/\text{DTPA}$ extraction) were not significantly different between the organic and biodynamic treatments receiving rotted and composted manure, respectively, and the mineral fertilizer treatment and the unfertilized control after 26 years (Fischer et al. 2005).

In a long-term fertilization trial in Darmstadt, Germany, plant-available Zn contents (measured using $\text{CaCl}_2/\text{DTPA}$ extraction) of the very sandy soil were significantly higher after 24 years of cattle-manure compost fertilization than with mineral fertilization, while Mn and Cu contents did not differ between the treatments (Fischer et al. 2005).

With increasing rates of biowaste compost (5–20 t ha⁻¹ dry wt.) applied to mostly silty loam soils in Baden-Württemberg, Germany, with a maize–winter wheat–winter barley rotation for 5 and 8 years, mobile Cu concentrations in soil (measured using NH_4NO_3 as extractant) increased slightly and Cu contents of crop products showed a slightly increasing trend. Mobile Zn concentrations in soil (in NH_4NO_3 extract) decreased slightly with increasing compost rates, while Zn contents of crop products were largely unaffected (Timmermann et al. 2003).

Similar trends were reported from a field experiment in Austria where 9.5–25.5 t ha⁻¹ year⁻¹ (wet wt.) of biowaste compost were applied for 10 years to a calcareous Molli-gleyic Fluvisol and compared to mineral and no fertilization. Cu and Zn concentrations measured in soil saturation extract did not differ between the fertilization treatments. Cu concentrations in the potentially bioavailable fraction (measured in LiCl extract) were higher in the medium and high compost treatments than in the unfertilized control (Erhart et al. 2008). As total Cu concentrations in the compost treatments were only slightly, not significantly, increased, this was attributed to the higher soil organic matter concentrations and microbial activity in the compost treatments. Plant Cu uptake was higher with compost fertilization than with no fertilization, even though not in all crops. All Cu concentrations in crops were in the normal range reported in the literature or below that (Bartl et al. 2002). Total soil Zn concentrations were increased with high application rates of compost. In the LiCl extract, Zn was not detectable. Plant-uptake data showed increased Zn concentrations in compost-fertilized oat grains, while spelt and potatoes did not differ from the unfertilized control (Bartl et al. 2002). Manganese uptake by plants was lower with compost fertilization in oats, and about the same in spelt and potatoes. Molybdenum uptake by plants was increased in compost-fertilized spelt and unaffected in oats and potatoes (Bartl et al. 1999).

When 30 and 60 t ha⁻¹ (wet wt.) of compost made from cotton wastes, sewage sludge, and olive-mill waste water (whose chemical characteristics and micronutrient content, however, were similar to those of biowaste compost) were applied to a calcareous, sandy clay loam textured soil in Spain, the Fe and Mn contents in the chard (*Beta vulgaris*) plants grown were higher than in the control which received mineral fertilizer. Cu and Zn contents in chard were unaffected by treatment.

The micronutrient contents of salad and barley, which were grown after chard, were only slightly affected (Cegarra et al. 1996).

In conclusion, with the use of high-quality compost (EU regulation 2092/91), only minor changes in the available micronutrient status of the soil are to be expected. As for crop plants, different species show varying micronutrient-uptake/exclusion patterns and micronutrients are not distributed uniformly between plant roots, stem, leaves, and fruits.

11.2.7 Soil Structure

In agronomic terms, a “good” soil structure is one which shows the following attributes: optimal soil strength and aggregate stability, which offer resistance to structural degradation (capping/crusting, slaking, and erosion, for example); optimal bulk density, which aids root development and contributes to other soil physical parameters such as water and air movement within the soil; optimal water-holding capacity and rate of water infiltration (Shepherd et al. 2002). Crops yield better in well-structured soils: Körschens et al. (1998) suggest a 5–10% benefit of good structure. Of course, root restriction may not necessarily penalize crop productivity, but it will do so if the supply of water and nutrients is inadequate (Shepherd et al. 2002). This is particularly important in organic farming, where deficits in soil structure may not be compensated by mineral fertilization.

It is not the optimum soil structure per se, which is decisive, however, but rather the ability of the soil to withstand structural degradation by the impact of rain, termed aggregate stability (Sekera and Brunner 1943). Increased aggregate stability protects the soil from compaction and erosion. Decreased bulk density and higher porosity improve soil aeration and drainage.

Increasing soil organic matter exerts a substantial influence on soil structure, particularly if – as in the case of compost application – CaO is supplied to the soil at the same time (Martins and Kowald 1988), improving soil physical characteristics like aggregate stability, bulk density, porosity, available water capacity, and infiltration (Giusquiani et al. 1995, Kahle and Belau 1998, Khalilian et al. 2002). To a remarkable degree, increased organic matter can counteract the ill effects of too much clay or too much sand (Weil and Magdoff 2004).

11.2.7.1 Aggregate Stability

As shown by Tisdall and Oades (1982), the water stability of aggregates depends on organic materials. The organic binding agents have been classified into (a) transient, mainly polysaccharides; (b) temporary, roots and fungal hyphae; and (c) persistent, resistant aromatic components associated with polyvalent metal cations, and strongly sorbed polymers. Roots and hyphae stabilize macro-aggregates, defined as >250 µm in diameter. Consequently, macroaggregation is controlled by soil

management, as crop rotation, cover crops, mulches, organic fertilization, and tillage practices influence the growth of plant roots and the oxidation of organic carbon. The water stability of microaggregates (<250 μm in diameter) depends on the persistent organic binding agents, organomineral complexes, and humic acids (Chaney and Swift 1986), and appears to be a characteristic of the soil, independent of management (Tisdall and Oades 1982).

Increasing the soil organic matter content usually increases aggregate stability. Within a limited range of soil organic matter contents, the relationship for a given soil is nearly linear. However, across a wider range of soil organic matter, the relationship between these two variables is likely to be curvilinear, because at very high levels of soil organic matter, additional organic matter has little further effect on soil aggregation (Haynes 2000).

Addition of easily degradable organic material such as green manures leads to a rapid, but short-lived rise in aggregate stability. Addition of compost, in contrast, causes a slow, but long-standing increase in aggregate stability as its organic matter mainly consists of humic substances, which constitute relatively stable binding agents (Haynes and Naidu 1998). Therefore, a combination of green manures and compost application is optimal, because it combines the advantages of both.

Compost application usually influences aggregate stability immediately after a relatively short time (less than 3 years; (Asche et al. 1994; Kahle and Belau 1998; Steffens et al. 1996). With continued compost application, the effect continues also on the longer term (Ebertseder 1997; Martins and Kowald 1988, Petersen and Stöppler-Zimmer 1999; Sahin 1989; Siegrist et al. 1998; Timmermann et al. 2003). Soil bulk density decreases with compost application, although that takes longer than improving aggregate stability (Ebertseder 1997; Lynch et al. 2005; Timmermann et al. 2003).

The maturity of the compost used may impact its effect on aggregate stability. In an agricultural field experiment on loamy silt loess soil situated near Kassel, Germany, mature composts, which had been processed for 3 months had a greater effect on aggregate stability than immature composts, which had been processed for only 12–25 days (Petersen and Stöppler-Zimmer 1999). Heavy silt and clay soils benefit most from improved aggregate stability through compost application (Timmermann et al. 2003).

11.2.7.2 Porosity

Also soil pore volume typically increases with compost application. The proportion of large, continuous vertical coarse pores (>50 μm) is decisive for soil aeration and warming, and thus for root growth, and for soil water infiltration. Soil friability is improved with increasing pore volume of large and medium pores (Wegener and Moll 1997). In the subsoil, the proportion of large, continuous pores correlates with earthworm abundance (Poier and Richter 1992).

The increase in pore volume with compost application was found to be due to a rise in the proportion of coarse pores (Ebertseder 1997; Giusquiani et al. 1995; Martins and Kowald 1988; Sahin 1989) or in the proportion of medium and coarse pores, respectively (Steffens et al. 1996). Giusquiani et al. (1995) reported the

greater porosity in the compost-treated plots to be due to an increase in the amount of elongated pores, which are considered most important both in soil–water–plant relationships and in maintaining good soil structure conditions.

11.2.7.3 Soil Water Availability

The water regime in soils is influenced by soil organic matter in several ways. First, organic matter increases the soil's capacity to hold plant-available water, defined as the difference between the water content at field capacity and that held at the permanent wilting point. It does so both by direct absorption of water and by enhancing the formation and stabilization of aggregates containing an abundance of pores that hold water under moderate tensions (Weil and Magdoff 2004). Hudson (1994) assessed the effect of the soil organic-matter content on the available water content of surface soils of three textural groups. Within each group, as organic matter increased, the volume of water held at field capacity increased at a much greater rate than that held at the permanent wilting point. As a result, highly significant positive correlations were found between organic-matter content and available water capacity. As organic-matter content increased from 0.5% to 3%, the available water capacity of the soil more than doubled (Hudson 1994).

An increase in soil water-holding capacity was observed in many studies with compost use, though it appears to take some time to come into effect. Evanylo and Sherony (2002), for example, did not find an increase in soil water-holding capacity after 2 years of compost application, and the effect was not very pronounced in other short-term trials (Avnimelech and Cohen 1993; Kahle and Belau 1998). In longer compost trials on the contrary, clear increases in water-holding capacity were reported (Giusquiani et al. 1995) (Fig. 11.2).

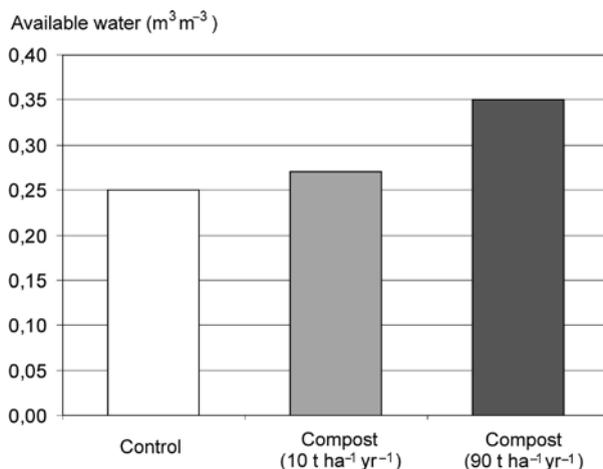


Fig. 11.2 Effects of compost addition on the available water in the surface layer; means of four replications. The linear term of regression (physical parameters : compost rates) was significant at $P \leq 0.01$ (Drawn from data from Giusquiani et al. 1995)

Changes in the soil water regime may also be documented by measuring the soil water content, although this is more difficult because soil water content is also influenced significantly by crop water uptake. Zauner and Stahr (1997) and Lynch et al. (2005) observed higher soil water contents with compost fertilization, while Gagnon et al. (1998) found differences only in summer and only on a sandy loam, not on clayey soil.

Increased available water capacity may protect crops against drought stress. In dryland farming systems, where moisture is normally the most yield-limiting factor, improving soil moisture retention is an important nonnutrient benefit of compost application, which may exceed nutrient benefits (Stukenholtz et al. 2002).

11.2.7.4 Soil Water Infiltration

However, as important as the provision of ample water for plant growth is the capacity of the soil to absorb water as it impacts from rain or irrigation. When, because of structural properties at the soil surface, the rate of water infiltration into the soil surface is lower than the rate of rainfall, a portion of the rain is lost as surface runoff. The effect on the supply of water available for plants growing in that soil is similar to a significant reduction in rainfall (Weil and Magdoff 2004).

Improved soil structure through compost application increases soil water infiltration (Ebertseder 1997), although this also seems to take some time, as the small effects reported from short-term experiments show (Evanylo and Sherony 2002).

There is a close connection between soil infiltration and floods. Increased infiltration cannot influence the number of heavy rain events, but arguably their consequences. As agriculture occupies large areas, it may be supposed that even small changes in soil infiltration rate have significant effects on the number and magnitude of floods (Schnug and Haneklaus 2002).

11.2.8 Plant-Disease Suppression

There are numerous reports of composts suppressing plant diseases caused by *Pythium*, *Phytophthora*, *Rhizoctonia*, *Fusarium*, and *Aphanomyces* spp. and *Sclerotinia sclerotiorum* in growing media (Bruns and Schüler 2002; Erhart and Burian 1997; Hoitink and Fahy 1986; Hoitink et al. 2001; Lievens et al. 2001). Today composts are recognized to be as effective as fungicides for the control of root rots such as *Phytophthora* and *Pythium*. In some cases composts have successfully replaced methyl bromide in the US ornamental plant industry (Hoitink et al. 2001). Plant protection through composts is of particular importance in cultivation systems where the use of fungicides is impossible or not allowed, as in organic production or in production of potted herbs for fresh home consumption (Fuchs 2002; Raviv et al. 1998).

Disease control with compost is attributed to four factors: competition between beneficial organisms and the pathogen, antibiosis, parasitism, and induced systemic

resistance. Two classes of biological control mechanisms known as “general” and “specific” suppression have been described for compost-amended substrates (Hoitink et al. 2001). The “general suppression” phenomenon is related to the total amount of microbiological activity in composts and is known to suppress pathogens such as *Pythium* and *Phytophthora* spp. The second type of suppression, elicited by a specialist group of microorganisms capable of eradicating a certain pathogen, such as *Rhizoctonia solani*, is referred to as “specific” suppression.

The most critical factors for plant-disease suppression are the colonialization of the compost by an appropriate microflora and the decomposition level of the organic fraction in composts (maturity/stability), which affects biological control through supporting adequate activity of biocontrol agents (Hoitink and Boehm 1999). As shown by Stone (2002), the same processes involving active organic matter are likely at work in field systems.

High-rate, single-term amendments of organic matter can generate disease suppression in the first season after amendment. When composted dairy manure solids were applied at 28 and 56 t ha⁻¹ (dry wt.), they reduced the severity of *Pythium* damping-off of cucumber by 30%, of bean root rot by 29% and of corn root rot by 67% 2 months after amendment (Darby et al. 2006). Such high amendment rates, however, cannot be applied year after year for environmental and economic reasons.

Field studies that assess low-rate single-season organic matter amendments report highly variable impacts on disease incidence and yield (Lewis et al. 1992; Lumsden et al. 1983), whereas longer-term studies report more predictable improvements in yield, quality, and disease suppression (Daamen et al. 1989; Workneh et al. 1993).

11.3 Potential Risks Associated with Compost Use

11.3.1 Heavy Metals

The accumulation of heavy metals in soils and crop plants is the most often cited potential risk of compost application. Heavy metal contents in composts vary widely dependent on compost feedstocks. For organic household and yard wastes, source separation, as introduced in Europe, proved to be effective in largely reducing compost heavy metal contents.

In organic farming, there are strict heavy metal limits for biowaste composts to be used. Composts from source-separated household waste must be produced in a closed and monitored collection system and must not exceed the following heavy metal limits (in mg/kg dry matter): Cd 0.7, Cu 70, Ni 25, Pb 45, Zn 200, Hg 0.4, Cr_{tot} 70, Cr (VI) 0 (EU regulation 2092/91).

Key interactive processes in the soil system affecting the partitioning of trace metals between the aqueous, bioavailable, and the solid phase include precipitation, ion exchange, adsorption onto organic matter, oxides and allophanes, and absorption into

biological material. The major factors driving the biogeochemical processes in soils are pH, cation exchange capacity, and redox potential. Soil microorganisms interact with trace metals in various ways which may render them more or less bioavailable. Crops differ considerably not only in their general sensitivity to trace elements, but also in their relative sensitivity to individual trace elements. The uptake of trace elements may vary considerably among cultivars; and within the plant, trace elements are not uniformly distributed among plant tissues (Adriano 1986).

The results of field experiments show that with the use of high-quality biowaste composts, increases in soil heavy metal concentrations are not measurable in the shorter term.

In the experiments of Kluge and Mokry (2000), 7–9 t ha⁻¹ (dry wt.), compost from biowaste and green waste per year were applied for 3 years to six different agricultural sites. The soil total heavy metal concentrations were unaffected by the compost fertilization. In the mobile heavy metal concentrations (measured using NH₄NO₃ as extractant) even a decrease was reported (Kluge and Mokry 2000).

Strumpf et al. (2004) applied 20 and 50 t ha⁻¹ (dry wt.) biowaste compost of urban origin in a single dose to an experimental field, where 12 vegetable species were grown in the following 3 years. Soil total heavy metal concentrations were not affected by the compost application. No difference was found in the heavy metal concentrations of the soil solution (extracted by suction cups) between the compost treatments and the untreated control. Also the heavy metal concentrations in the vegetables grown in the experiment did not differ between the treatments.

In the experiment of Oehmichen et al. (1994), 4–24 t ha⁻¹ compost was applied annually to agricultural crop rotations on two experimental sites for 3 years. Soil heavy metal concentrations at the end of the experiment were not significantly different from the unfertilized control.

No increases in total soil heavy metal concentrations were measurable after 10 experimental years with total applications of 95, 175, and 255 t biowaste compost (wet wt.) ha⁻¹, respectively, to a Molli-gleyic Fluvisol cropped with cereals and potatoes, except for Zn in the treatments with the highest application rate. In the mobile heavy metal fractions measured in soil saturation extract and LiCl extract, no significant increases were detected except for Cu in LiCl extract (Erhart et al. 2008). Plant heavy metal uptake data showed no significant differences in Ni uptake between the fertilization treatments. Pb was not detectable in crops. Cd concentrations in grains of oat and spelt and potato tubers were significantly lower with compost fertilization than with no fertilization. In the potatoes which had received mineral fertilizer, significantly higher Cd concentrations were found, most probably due to the Cd input via superphosphate and triple superphosphate fertilizer (Bartl et al. 2002) (Fig. 11.3). The total Cd loads imported via phosphorus fertilization appear small, but they are much more likely available to biota than the Cd bound in the soil (Sager 1997).

It might be concluded, that with the use of high-quality composts, such as specified by the EU regulation 2092/91, the risk of heavy metal accumulation in the soil is very low. As compost application usually leads to a rise in soil organic matter and, with that, improves the sorption capacity of soils, mobile heavy metal fractions in most cases remain the same or even decrease with compost use.

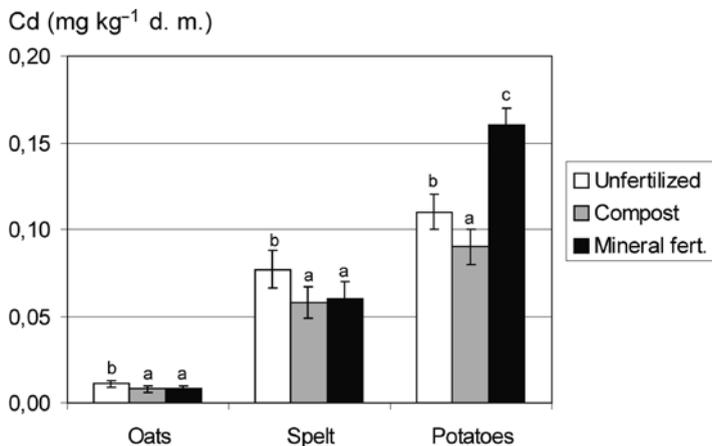


Fig. 11.3 Cd contents (mg kg^{-1} d.m.) in crops fertilized with biowaste compost at $32 \text{ t (wet wt.) ha}^{-1} \text{ year}^{-1}$ for 7 years, as compared to mineral fertilization (71 kg N , 19 kg P , and $68 \text{ kg K ha}^{-1} \text{ year}^{-1}$) and to no fertilization. Treatments with the *same letters* are not significantly different at $P \leq 0.05$ (Drawn from data from Bartl et al. 2002)

11.3.2 Organic Compounds

In high-quality biowaste composts, the contents of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), or polychlorinated dibenzodioxins and dibenzofurans (PCDD/F) and other compounds are low due to the separate collection of the compost feedstock (Brändli et al. 2007a, b; Timmermann et al. 2003; Zethner et al. 2000). Investigations of pesticide residues in compost detected few of the target pesticides. The compounds that were found occurred at low concentrations (Büyüksönmez et al. 2000). The composting process contributes to the degradation of organic compounds by the heat generated and by microbiological and biochemical oxidative processes (Amlinger et al. 2006).

The application of compost to the soil may have varying effects concerning organic pollutants. Due to their high organic-matter content, composts may bind pollutants and thus lower their availability and toxicity. The increased activity of the soil microflora provides improved conditions for biological (oxidative) degradation of pollutants (Amlinger et al. 2006).

Accumulation scenarios of persistent pollutants (PAHs, PCBs, PCDD/F) show that, assuming realistic half-lives in soil, the average input through deposition and compost will not lead to an accumulation in soil (except a slight increase in PAHs in the case of very low soil background values). The input is more than offset by natural degradation (Amlinger et al. 2004). The absence of changes in the soil concentrations of PCBs and PCDD/F was confirmed by measurements in field experiments in Germany (Timmermann et al. 2003).

The pollutant loads are so small, that even with overly high compost rates no measurable increase in soil contents may be expected (Amlinger et al. 2006).

11.3.3 *Hygiene, Plant Diseases, Weeds*

Most plant pathogens are inactivated during proper aerobic composting. For bacterial plant pathogens and nematodes, the majority of fungal plant pathogens, and a number of plant viruses, a compost temperature of 55°C for 21 days is sufficient for ensuring eradication. For *Plasmodiophora brassicae*, the causal agent of clubroot of Brassicas, and *Fusarium oxysporum* f. sp. *lycopersici*, the causal agent of tomato wilt, a compost temperature of at least 65°C for up to 21 days is required for eradication. Several plant viruses, particularly Tobacco Mosaic Virus (TMV) are temperature-tolerant. However, there is evidence that TMV and Tomato Mosaic Virus are degraded over time in compost, even at temperatures below 50°C (Noble and Roberts 2003; Termorshuizen et al. 2005).

Timmermann et al. (2003) found in their examination of numerous composts for the RAL-GZ quality control, that the overall hygiene (e.g., concerning *E. coli*) and hygiene concerning plant diseases of biowaste composts was always warranted, if sufficiently high temperatures (65°C) were attained during at least 7 days in the composting process. The same was true for viable weed seeds and plant parts in the composts. As their analyses showed, quality composts were virtually free of viable weed seeds and plant parts.

In the field experiments in Baden-Württemberg, weed ratings were conducted routinely (at a total of 42 experiment years) and the occurrence of weeds was found to be not increased with biowaste-compost use (Timmermann et al. 2003).

11.4 Crop Yields and Quality with Compost Use

11.4.1 *Agricultural Crops*

The total of all effects of compost use is reflected best in crop yields. The effect of compost fertilization on crop yields depends on the factors determining nutrient mineralization from soil and compost, but also on crop-related factors such as the nutrient requirements and uptake dynamics of the respective crop.

11.4.1.1 Cereals

With cereals, a wide range of yield responses to compost fertilization has been recorded. Nonsignificant wheat yield increases followed the application of 6.9 t ha⁻¹ (dry wt.) biowaste compost on a parabrown soil in Germany (von Fragstein and Schmidt 1999). Also in the experiment of Oehmichen et al. (1995), small and only partly significant yield increases were found. In a trial in Southeast England, however, with municipal solid-waste compost at 50 and 100 t ha⁻¹ (wet wt.) on a loamy clay soil, compost-treated plots produced grain yields comparable to those which

received 75 or 150 kg ha⁻¹ mineral N fertilizer (Rodrigues et al. 1996). On a Gray Luvisolic soil with low inherent soil fertility and relatively poor soil structure in Alberta, Canada, municipal solid-waste compost was applied at rates of 50, 100, and 200 t ha⁻¹ (wet wt.). Wheat yields were 170% and barley yields were 270% of the untreated control in the 50 t ha⁻¹ treatment (Zhang et al. 2000).

In the DOK experiment in Switzerland, winter wheat yields with manure-compost fertilization in organic farming reached 90% of the grain harvest of the conventional system in the third crop rotation period (Mäder et al. 2002).

In a 3-year field experiment on a podsolic soil in Germany, rye yield increased for 5–12%, when 30 and 60 m³ ha⁻¹ (corresponding to approx. 20 and 40 t ha⁻¹ wet wt.) yard-trimmings compost were applied annually (Klasink and Steffens 1996). Oehmichen et al. (1995) reported rye yield increases in the range of 9–15% after annual application of 6–18 t compost (wet wt.) ha⁻¹ on a Luvisol.

While barley yields were significantly higher than the control with 4.1 t ha⁻¹ (dry wt.) biowaste compost on a parabrown soil in Germany (von Fragstein and Schmidt 1999), 150 t ha⁻¹ (wet wt.) of garden-waste compost was necessary to give significantly higher barley yields on a sandy loam soil in Britain (Cook et al. 1998).

In five field experiments in Baden-Württemberg, Germany, with a duration of 5–8 years and a maize–winter wheat–winter barley rotation, average yield increases between 11% and 28% were recorded with biowaste-compost fertilization at 5, 10, and 20 t ha⁻¹ (dry wt.) as compared to the unfertilized control (Timmermann et al. 2003) (Fig. 11.4).

With application of 9–23 t ha⁻¹ year⁻¹ (wet wt.) biowaste compost, yields of cereals and potatoes increased for 7–10% compared to the unfertilized control (average of 10 years). On a fertile Fluvisol under relatively dry climatic conditions, the yield

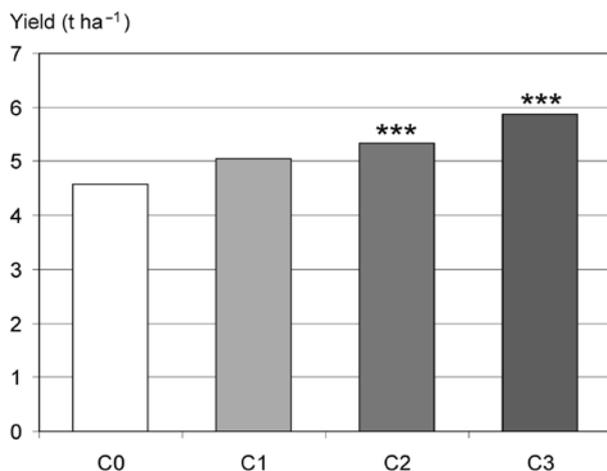


Fig. 11.4 Average yields 1995–2002 of five field experiments with a maize–wheat–barley rotation with biowaste-compost fertilization at 5 t ha⁻¹ year⁻¹ (dry wt.) = C1, 10 t ha⁻¹ year⁻¹ = C2, and 20 t ha⁻¹ year⁻¹ = C3 as compared to no fertilization = C0. Stars indicate statistically significant differences at $P \leq 0.01$ (Drawn from data from Timmermann et al. 2003)

response to the compost applications was very low in the beginning and increased slightly with the duration of the experiment (Erhart et al. 2005).

11.4.1.2 Potatoes

Little influence of compost application (at 43 and 86 t (wet wt.) ha⁻¹, respectively) on potato tuber production was recorded by Volterrani et al. (1996) on a sandy soil in Italy. In the experiment of Klasink and Steffens (1996), potato yield increased for maximally 4%. In the DOK experiment in Switzerland, potato yields with manure-compost fertilization in organic farming were 40% lower than in the conventional system mainly due to low potassium supply and the incidence of *Phytophthora infestans* (Mäder et al. 2002).

In an experiment of Vogtmann et al. (1993b), 80, 20, and 50 t ha⁻¹ (wet wt.) biowaste compost had been applied in subsequent years to a silty loam Luvisol. The potato yield in the compost treatment was significantly higher than in the control, and comparable to that produced with 200 kg N ha⁻¹ mineral fertilizer.

11.4.1.3 Maize

Maize has a very high N requirement, and also a longer growth period than cereals. On a shaly silt loam in Pennsylvania, dairy manure leaf compost was applied at 27 t ha⁻¹ (dry wt.) annually for 3 years. In the first year, maize yields were significantly lower than with mineral fertilization at 146 kg N ha⁻¹, but in the second and third year they were not significantly different (Reider et al. 2000). Parkinson et al. (1999) applied green-waste compost at 15, 30, and 50 t ha⁻¹ (wet wt.) on a silty loam soil in South West England. There was a positive yield response of 1–18% to the application of compost in each of the 3 years.

In a field experiment at Wye, Britain, manure compost at 25 and 50 t ha⁻¹ was compared with inorganic N fertilizer at 50 and 100 kg N ha⁻¹ using two forage maize varieties. While the fresh yield of the late-maturing variety was higher, the yield of the early maturing variety was only about equivalent to that with inorganic fertilizer, showing the positive influence of a longer growth period on the N uptake from compost (De Toledo et al. 1996).

11.4.2 Vegetable Crops

In vegetable production, intensive soil cultivation promotes rapid mineralization of soil organic matter, which may lead to a gradual loss of soil fertility. Most vegetable crops need a soil that is rich in organic matter, well-structured, and with a high water-holding capacity. Therefore many vegetable crops, particularly the highly nutrient-demanding ones, respond favorably to compost fertilization, often already

after the first application. Supplying the total N requirements of vegetables with compost is possible, as several experiments have shown, but as N mineralization from compost is often as low as 5–15% of total N, large amounts of compost are required. In order to achieve high yields, but avoid a buildup of nutrient concentrations in the soil through excessive compost rates, compost could be combined with either legumes or organic fertilizers in which N is more readily available.

11.4.2.1 Solanaceous Crops

For the solanaceous crops, tomatoes (*Lycopersicon esculentum*), peppers (*Capsicum annuum*), and eggplant (*Solanum melongena*), equal to significantly greater yields with compost compared with mineral fertilizer, were reported. For example, in a 3-year trial on two sites (one with sandy soil, one with loamy soil), in which fertilization with chicken-manure compost at 56 and 112 t ha⁻¹, respectively, was compared to mineral fertilization at 146N-64P-121K (kg ha⁻¹; Maynard 1994), and in an experiment with sugarcane filtercake compost at 224 t ha⁻¹, compared to mineral fertilizer at up to 153 kg N ha⁻¹ on a fine sand soil (Stoffella and Graetz 1996).

When a processing tomato–proteic pea–rotation on a silty clay soil in southern Italy was fertilized for 4 years with 7.1 t ha⁻¹ (dry wt.) compost from olive mill residues, sludge, straw, and orange wastes, tomato yields did not differ significantly from those in the treatment receiving 100 kg N ha⁻¹ as ammonium nitrate (Rinaldi et al. 2007).

11.4.2.2 Cruciferous Crops

In the cruciferous crops broccoli, cauliflower (*Brassica oleracea convar. botrytis* var. *italica*, and var. *botrytis*, respectively), kohlrabi (*B. oleracea convar. caulorapa* var. *gongylodes*), and cabbage (*B. oleracea convar. capitata*), improved crop responses with compost fertilization compared to fertilizer-only treatments were recorded, provided crop nutrient demands were satisfied. For example in a study by Roe and Cornforth (1997), who used dairy manure compost at rates of 22, 45, and 90 t ha⁻¹ plus mineral fertilizer at 112 kg ha⁻¹ N to grow autumn broccoli. Broccoli yields were up to twice as high as with mineral fertilizer alone. When biowaste compost was applied at 60 t ha⁻¹ (dry wt.) to kohlrabi, yields were similar to those with 70 kg ha⁻¹ N as mineral fertilizer (Vogtmann and Fricke 1989).

In the chicken-manure compost experiment by Maynard (1994) described above, yield of broccoli and cauliflower from the compost plots equaled the fertilized control at both sites in all 3 years with two exceptions (one higher, one lower). In another experiment with leaf compost at 56 and 112 t ha⁻¹ plus fertilizer at 0, 73N-32P-61K, and 146N-64P-121K (kg ha⁻¹), respectively, broccoli and cauliflower did not obtain optimum yields on the reduced fertilizer plots until the second and third years (Maynard 2000).

11.4.2.3 Cucurbitaceae

When cantaloupe (*Cucumis melo*) was grown with manure compost at 22, 45, and 90 t ha⁻¹, respectively, plus 23N-14P (kg ha⁻¹), cantaloupe yields were up to three times as high as with mineral fertilization only (Roe and Cornforth 1997).

11.4.2.4 Legumes

The response of legumes to compost may differ from that of other crops due to their ability to fix N, but nevertheless they may profit from nutrients other than N and from improved soil conditions. Snap bean (*Phaseolus vulgaris*) seedling emergence and plant survival were increased by the addition of 2.5 cm of leaf compost as a mulch over rows after seeding. Pod yields were equal to significantly higher with compost mulch (Gray and Tawhid, 1995). Baziramakenga and Simard (2001) used a paper residues/poultry-manure compost at 0, 14, 28, and 42 t ha⁻¹ (dry wt.), supplemented or not with mineral fertilizer at 0, 60, 120, and 180 kg P₂O₅-K₂O ha⁻¹. Snap bean yields in the compost treatments increased significantly compared with the untreated control and were similar to those in the mineral-fertilizer treatments.

11.4.2.5 Onions

In a 3-year trial with four cultivars of onions (*Allium cepa*) annual applications of 112 t ha⁻¹ leaf compost plus 146N-66P-121K (kg ha⁻¹) were compared to mineral fertilization only (Maynard and Hill 2000). After 3 years of compost additions, yields of the three Spanish onion cultivars from the compost plots were significantly greater than from unamended plots. Year-to-year variability in yields in response to variable rainfall was significantly lower and percentage of colossal and jumbo-sized onions was greater in compost-amended plots. Repeated compost additions also reduced the incidence of soft rot disease.

11.4.3 Crop Quality

The crop quality of cereals is usually not affected by compost fertilization (Cook et al. 1998, Erhart et al. 2005, von Fragstein and Schmidt 1999, Oehmichen et al. 1995). In potatoes and cabbage, lower concentrations of nitrate and free amino acids with compost than with mineral fertilization were observed (Vogtmann et al. 1993b, Roinila et al. 2003; Erhart et al. 2005). Those lower nitrate concentrations in vegetables are supposedly due not only to lower soil nitrate levels in the compost plots, but also to the slow-release nature of compost. In tomatoes, compost fertilization was reported to yield higher titratable acidity values, higher electrical conductivity

(E.C.), and a somewhat superior sensory quality (Madrid et al. 1998, Vogtmann et al. 1993b).

As a general trend, Vogtmann et al. (1993b) found compost to positively influence food quality, to improve storage performance of vegetables, and to yield a slightly better sensory quality.

11.5 Conclusion

Probably the most important benefit of using compost is the increase in soil organic matter. Numerous experiments show that compost fertilization regularly leads to a distinctive increase in the humus content of the soil. Moderate levels of compost application (around 6–7 t ha⁻¹ year⁻¹ dry wt.) are usually sufficient for the maintenance of the soil humus level. Regular compost addition increases soil fauna and soil microbial biomass and stimulates enzyme activity, leading to increased mineralization of organic matter and improved resistance against pests and diseases, both features essential for organic farming. Composting permits to recycle leftover organic matter, which otherwise would be lost. Through the significant increase in the soil's content of organic carbon, compost fertilization may make agricultural soil a carbon sink and thus contribute to the mitigation of the greenhouse effect.

As phosphorus and potassium in compost become nearly completely plant-available within a few years after compost application, the total P and K content of composts can be accounted for in the fertilizer calculation. The nitrogen-fertilizer value of compost is lower. In the first years of compost application, N mineralization calculated from the results of field trials varied from -14% to +15%. Nitrogen recovery in the following years depends on the site- and cultivation-specific mineralization characteristics and will be roughly the same as that of soil organic matter. Moderate rates of compost are sufficient to substitute regular soil liming.

Increasing soil organic matter exerts a substantial influence on soil structure, improving soil physical characteristics like aggregate stability, bulk density, porosity, available water capacity, and infiltration. Increased aggregate stability protects the soil from compaction and erosion. Decreased bulk density and higher porosity improve soil aeration and drainage. Increased available water capacity may protect crops against drought stress. These effects gradually improve soil fertility. And they improve soil qualities such as soil workability, resistance to erosion, water-holding capacity and soil activity, which are essential for crop production particularly in organic farming, where deficits in soil structure may not be compensated by mineral fertilization. On the medium and long term the soil-improving effects of compost application have at least the same, if not a greater importance than its fertilizer effects.

When using high-quality composts, such as specified by the EU regulation 2092/91, the risk of heavy metal accumulation in the soil is very low. Nitrogen mineralization from compost takes place relatively slowly and there are virtually no reports of a sudden, ecologically problematic rise in soluble N pools and uncontrollable

N-leaching. Therefore, compost fertilization does not pose a risk of groundwater eutrophication.

Concentrations of persistent organic pollutants (PAHs, PCBs, PCDD/F) in high-quality composts usually approach the usual soil background values. Also the overall hygiene and hygiene concerning plant diseases and weeds is not a problem if quality composts produced in a monitored system are used.

Most studies found positive yield effects of biowaste compost. However, the effect of biowaste compost applied at moderate rates usually takes some years to develop. It depends on the factors determining nutrient mineralization from soil and compost, but also on crop-related factors such as the nutrient requirements and uptake dynamics of the respective crop rotation. Crops with longer growth periods can make better use of compost. Many vegetable crops respond favorably to compost fertilization, often already after the first application.

Crop quality is usually not affected by compost fertilization in cereals, and slightly positively influenced in vegetable crops.

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Chapter 12

Beneficial Microorganisms for Sustainable Agriculture

Arshad Javaid

Abstract There was a desperate need for food to recover the economy of the 1950s and 1960s. Farmers all over the world were advised to rely on intensive production methods and synthetic pesticide inputs to increase the productivity. No doubt, these chemical-based agricultural practices substantially increased crop yield. However, indiscriminate use of agrochemicals have contributed significantly to the environmental pollution and adversely affected human and animal health. In addition, the increasing cost of these agrochemicals has continued to lower the farmer's net cash return. The global use of synthetic pesticides at the start of this millennium exceeded 2.5 million tons per year. A growing worldwide concern for these problems has motivated researchers, administrators, and farmers to seek alternatives to chemical-based, conventional agriculture. One such product is effective microorganisms (EM) developed by Japanese scientists. Effective microorganisms are a mixed culture of beneficial and naturally occurring microorganisms, such as species of photosynthetic bacteria (*Rhodospseudomonas palustris* and *Rhodobacter sphaeroides*), lactobacilli (*Lactobacillus plantarum*, *L. casei*, and *Streptococcus lactis*), yeasts (*Saccharomyces* spp.), and Actinomycetes (*Streptomyces* spp.). These beneficial microorganisms improve crop growth and yield by increasing photosynthesis, producing bioactive substances such as hormones and enzymes, controlling soil diseases, and accelerating decomposition of lignin materials in the soil. Experiments conducted on various agricultural crops in different parts of the world have shown good prospects for the practical application of these beneficial microorganisms in improving crop yield and soil fertility. Application of beneficial microorganisms generally improves soil physical and chemical properties and favors the growth and efficiency of symbiotic microorganisms such as nitrogen fixing rhizobia and arbuscular mycorrhizal (AM) fungi. Nonetheless experiences of some researchers revealed that the effect of these microorganisms on crop growth and yield was usually not evident or even negative in the first test crop. However, this adverse effect can be overcome through repeated

A. Javaid (✉)

Institute of Mycology and Plant Pathology, University of the Punjab,
Quaid-e-Azam Campus, Lahore, Pakistan
e-mail: arshadjpk@yahoo.com

applications of these microorganisms. Research on these microorganisms has shown that crop yields tend to increase gradually as subsequent crops are grown. Foliar application of beneficial microorganisms avoids many of the biotic and abiotic factors and constraints of the soil environment, and thus increases the crop growth and yield significantly. Application of beneficial microorganisms also reduces seed bank of weeds in agricultural soils by enhancing the rate of weed seeds germination. There are reports of management of various fungal and bacterial pathogens as well as insect pests due to application of beneficial microorganisms. These microorganisms have shown a great promise in dairy wastewater treatment. They can reduce NH_3 concentration in poultry manure up to 70% possibly by transforming NH_4^+ to NO_3^- . Research conducted so far concludes that benefits of beneficial microorganisms can be best exploited through their repeated applications for few years in combination with organic amendments and applying them as foliar spray. Integrated use of organic matter plus beneficial microorganisms with half mineral NPK can yield equivalent to that of full recommended NPK fertilizers dose. Beneficial microorganisms can also be used for wastewater treatment, pest and disease management, and to reduce the abiotic stresses on crop growth and yield.

Keywords Biofertilizer • effective microorganisms • nature farming • sustainable agriculture

12.1 Introduction

Fertilizers as a source of plant nutrients and pesticides as plant protection measures are being used to increase the crop production. The global use of synthetic pesticides at the start of this millennium exceeded 2.5 million tons per year (Bhanti and Taneja 2007). However, imbalance and frequent use of these agrochemicals have polluted the environment to a great extent. There is a growing concern that food produced under such farm management may not be safe or of good quality. This has shifted the scientific approach toward some alternative measures (Shaxson 2006). In the recent past, some successful efforts have been made to at least partially substitute agrochemicals with natural substances to minimize the bad effects of the former (Kannaiyan 2002). One such effort was made by Dr. Teruo Higa, Professor of Horticulture, University of the Ryukyus, Okinawa, Japan, who conducted pioneering work in advancing the concept of “Effective Microorganisms” (EM) (Higa 1991). EM consists of mixed cultures of beneficial and naturally occurring microorganisms that can be applied as inoculants to increase the microbial diversity of soils and plants. EM is not a substitute for other management practices. It is, however, an added dimension for optimizing our best soil and crop management practices such as crop rotations, use of organic amendments, conservation tillage, crop residue recycling, and biocontrol of pests. If used properly, these beneficial microorganisms can significantly enhance the beneficial effects of these practices (Higa and Wididana 1991).

12.2 Beneficial Microorganism's Cultures

First solution of beneficial microorganisms contained over 80 microbial species from 10 genera, isolated in Japan. However, with time the technology was refined to include predominant populations of lactic acid bacteria and yeast and smaller numbers of photosynthetic bacteria, actinomycetes, and other types of microorganisms. All of these are mutually compatible with one another and can coexist in liquid culture (Higa and Parr 1994). The functions of principle microorganisms in EM are as follows.

12.2.1 Photosynthetic Bacteria

Photosynthetic bacteria include *Rhodospseudomonas palustris* and *Rhodobacter sphaeroides*. These bacteria are a group of independent, self-supporting microbes. These are considered the pivot of EM activity and support the activity of other microorganisms in EM culture. They synthesize useful substances from secretions of plant roots, organic matter, and harmful gases such as hydrogen sulfide, by using sunlight and the heat of soil as sources of energy (Kim et al. 2004). The useful substances produced by these bacteria include amino acids, polysaccharides, nucleic acids, bioactive substances and sugars, all of which promote plant growth and development (Higa 2000). The metabolites developed by these microbes are absorbed directly by plants (Kim and Lee 2000; Ranjith et al. 2007).

12.2.2 Lactic Acid Bacteria

Lactic acid bacteria include *Lactobacillus plantarum*, *L. casei*, and *Streptococcus lactis*. They produce lactic acid from sugars and other carbohydrates produced by photosynthetic bacteria and yeasts (Hussain et al. 2002). Lactic acid is a strong sterilizing compound, suppresses harmful microorganisms such as *Fusarium* and enhances decomposition of organic matter (Higa and Kinjo 1991). These bacteria promote the fermentation and decomposition of materials such as lignin and cellulose, thereby removing the undesirable effect of undecomposed organic matter (Gao et al. 2008; Valerio et al. 2008).

12.2.3 Yeasts

Yeasts include *Saccharomyces cerevisiae*. Yeasts synthesize useful substances required for plant growth from amino acids and sugars secreted by photosynthetic bacteria, organic matter, and plant roots (Higa 2000). The bioactive substances such

as hormones and enzymes produced by yeast promote active cell and root division. The secretions are also useful substrates for other microorganisms in EM culture viz. lactic acid bacteria and actinomycetes (Hussain et al. 2002).

In the beginning, five formulations of beneficial microorganisms were developed starting from EM₁ to EM₅. EM₁ had predominantly filamentous fungi that are heat resistant and hasten the decomposition of organic amendments. It was initially developed for preparing compost quickly but it is not produced any more. EM₂ is used primarily to protect plants from soil-borne pathogens, diseases, and insects. It contains predominantly the species of genus *Streptomyces*, which produces antibiotics that suppress harmful microorganisms. It also contains smaller numbers of photosynthetic bacteria, yeast, and molds. EM₃ is comprised predominantly of photosynthetic bacteria with smaller numbers of yeast and actinomycetes. It enhances the growth, yield and quality of crop, and improves soil physical properties (Anonymous 1995). EM₄ consists mainly of the lactobacilli with smaller number of photosynthetic bacteria, *Streptomyces* spp. and yeast. It increases the availability of nutrients to plants by enhancing the decomposition of organic wastes and residues. It also suppresses the activity of harmful insects and pathogenic microorganisms (Sajjad et al. 2003). EM₅ is prepared by mixing EM₂, EM₃, and EM₄. It is used to suppress pathogens and to ward off harmful insects. It is especially used for cultivating fruit trees and vegetables (Anonymous 1995).

EM is often inoculated to organic matter fermented and the mixture is called EM Bokashi. It can improve the ability of microorganisms to break down organic matter, thereby providing plant nutrients to make better yield and quality (Xu et al. 2000; Yan and Xu 2002).

In Pakistan, EM is being produced at Nature Farming Research Center, Faisalabad and is available in the form of EM-Bioab, EM-Biovet, and EM-Biocontrol. EM-Bioab is used in agricultural crops along with organic manures as a substitute of chemical fertilizers. EM-Biovet is used in livestock and poultry production while EM-Biocontrol is used in crops, vegetables, and orchards for prevention and remedy of diseases and insect pest attack (Hussain et al. 2002).

12.3 Soil Application of Beneficial Microorganisms

12.3.1 Positive Effects on Plant Growth

Experiments conducted throughout Japan as well as in many other countries have shown good prospects for the practical application of beneficial microorganisms. Research has shown that the inoculation of these beneficial microorganisms cultures to the soil/plant ecosystem can improve soil quality, soil health, and yield and quality of crops.

12.3.1.1 Cereal Crops

Most of the research work on beneficial microorganisms technology has been conducted on cereal crops, particularly rice and wheat. Hussain et al. (1999, 2000) conducted a long-term field experiment in Pakistan to determine the agronomic and economic merits of beneficial microorganisms in a rice–wheat cropping system. They found that when NPK fertilizers and organic manures were combined with EM, higher straw and grain yields were obtained as compared to corresponding treatments without EM application for both the test crops. Beneficial microorganisms applied in combination with NPK, green manure, and farmyard manure caused a significant increase in nutrient uptake by the grains and straw of each crop. The average net profit from rice and wheat production using beneficial microorganisms was US\$ 44.9 and 62.35 ha⁻¹, respectively. Corales et al. (1997) studied the effect of beneficial microorganisms in transplanted and wet direct seeded rice. Results showed that the reduced inorganic fertilizers (by 25–50%) in combination with EM or EM Bokashi gave comparatively better results in increasing yield of rice as compared to inorganic fertilizers alone. Xu (2000) conducted a study under glass house to determine the effects of beneficial microorganisms and various organic amendments on the growth, photosynthesis, and yield of sweet corn, compared with chemical fertilizers. Beneficial microorganisms applied with organic fertilizers promoted root growth and activity, and enhanced photosynthetic efficiency and capacity, which resulted in increased grain yield. This was attributed largely to a higher level of nutrient availability facilitated by beneficial microorganisms application over time. Similar experiments conducted in many other countries including China, Japan, Vietnam, Korea, Nepal, and Bangladesh have also revealed increase in crop growth and yield of rice and/or wheat when beneficial microorganisms application was carried out in combination with various organic amendments and different doses of inorganic fertilizers (Lee 1994; Ta and Chanh 1996; Iwaishi 2000; Sherchand 2000; Chowdhury et al. 2002).

12.3.1.2 Vegetable Crops

The demand for organically grown food crops has increased markedly in the recent years as consumers have become more concerned about pesticide residues in the human diet. Consequently, organic crop production system is gaining popularity worldwide. Generally, farmers utilize available crop residues, animal manures and off-farm vegetative materials as organic amendments to supply plant nutrients and maintain soil productivity. However, the yields of food crops grown in these systems are generally low. Use of beneficial microorganisms has been shown to increase the yield and quality of food crops in organic farming systems. Daly and Stewart (1999) investigated the effect of beneficial microorganisms on vegetable crops on organic farms in Canterbury, New Zealand. They found that beneficial microorganisms plus molasses increased the onion yield by 29%, pea yield by 31%, and sweet corn cob weights by 23%. Daiss et al. (2008) reported that Swiss chard

(*Beta vulgaris* L.) treated EM plus EM Bokashi had higher phosphorus and magnesium contents than control plants. Enhanced nutrient utilization efficiencies of the plants in beneficial microorganisms-applied treatments have also been reported in capsicum (*Capsicum annuum* L.) and cowpea (*Vigna unguiculata* L.) by Sangakkara et al. (1998). Sangakkara (1998) suggested that yields of sweet potato (*Ipomoea batatas* L.) and bush bean (*Phaseolus vulgaris* L.) were significantly increased by the application of beneficial microorganisms to traditional organic system in Sri Lanka. Increase in yield due to beneficial microorganisms application has also been reported for certain other vegetables, viz. radish, cabbage, and lettuce (Lee 1994; Naseem 2000).

12.3.1.3 Fruit Crops

Higa (1988) conducted experiments on various horticultural crops and concluded that beneficial microorganisms can increase the yield and quality of fruits. Similarly, Paschoal et al. (1998) have reported an increase in the yield of oranges owing to the application of beneficial microorganisms. Xu et al. (2000) reported that beneficial microorganisms inoculation to both Bokashi and chicken manure increased photosynthesis and fruit yield of tomato plants. Application of beneficial microorganisms also increased vitamin C concentration in fruits from all fertilization treatments. They concluded that both fruit quality and yield could be significantly increased either by inoculation of beneficial microorganisms to the organic fertilizers or application directly to the soil. According to Joo and Lee (1991), with beneficial microorganisms application, yield of citrus increased significantly as compared to traditional method of farming. Fuel cost index was also declined with beneficial microorganisms farming system. Wibisono et al. (1996) obtained a significant effect on plant height, number of shoots, and number of leaves in *Citrus medica* when beneficial microorganisms were applied along with rice straw at 2.5 t ha⁻¹. Tokeshi and Chagas (1997) studied the hormonal effect of beneficial microorganisms on citrus germination. They concluded that beneficial microorganisms have hormonal action as gibberellic acid. The potential of seedling survival and vigo r was measured by emergence speed. There was rapid emergence speed with beneficial microorganisms as compared to control.

12.3.1.4 Other Crops

Application of beneficial microorganisms is also known to enhance crop growth and yield in certain other crops of economic importance such as cotton, coffee, and sugarcane. Khaliq et al. (2006) conducted a field experiment to determine the effects of integrated use of organic and inorganic nutrient sources with and without beneficial microorganisms on growth and yield of cotton. They observed that organic material and beneficial microorganisms did not increase the yield and yield-attributing components significantly but integrated use of both resulted in a 44% increase over control. Integrated use of organic matter plus beneficial

microorganisms with half mineral NPK yielded equivalent to that of full recommended NPK fertilizers dose. Economic analysis suggested the use of half mineral NPK with organic matter and EM saves the mineral N fertilizer by almost 50% compared to a system with only mineral NPK application. Increased yield in sugarcane due to beneficial microorganisms application in combination with various organic materials have been reported. However, the increase in yield over control was not as much pronounced as in other crops (Punyaprueng et al. 1993; Zacharia 1995). Chagas et al. (1997) suggested that coffee plant propagation could be substantially improved by replacing the chemical fertilizers with Bokashi plus EM. Beneficial microorganisms application in combination with cow dung significantly increased the germination and physical growth parameters including shoot and root length and biomass, vigor index, collar diameter, and leaf number of *Albizia saman*, a medium-to-large-sized tree native to Central America, West Indies, and Guyana and is widely distributed in the tropical forests of Asia (Khan et al. 2006).

12.3.2 Negative or No Effects on Plant Growth

Majority of the scientists who are engaged in promoting EM technology have no doubt that plant growth is just as good or better and the quality of plant products is superior to conventional farming (Daly and Stewart 1999; Hussain et al. 1999; Yamada and Xu 2000; Iwaishi 2000; Khaliq et al. 2006; Khan et al. 2006). In contrast to that, experience of some workers revealed that the effect of beneficial microorganisms on crop growth and yield was usually not evident or even negative particularly in the first test crop. Rashid et al. (1993) noted that beneficial microorganisms applied along with farmyard manure did not improve wheat grain yield and N uptake in wheat over farmyard manure alone and standard dose of chemical fertilizers. Similarly, Yousaf et al. (1993) did not find any significant effect on crop growth in maize when beneficial microorganisms were applied along with different soil amendments such as farmyard manure, poultry manure, sewage sludge, and NPK fertilizers. Bajwa et al. (1998a) noted an inhibitory effect of EM application on plant growth in *Brassica campestris* L. In another study, Bajwa et al. (1999a) observed similar effect of beneficial microorganisms application on root and shoot biomass production in *Trifolium alexandrinum* L. Priyadi et al. (2005) conducted a field experiment to elucidate the effect of chicken manure and beneficial microorganisms on the yield of corn and chemical and microbial properties of two types of acidic wetland soils. The results showed that the interaction between soil types and chicken manure application affected the corn yield, while beneficial microorganisms had no effect. Bajwa et al. (1995a) showed that application of beneficial microorganisms in heat-sterilized soil induced a significant positive effect on crop growth and yield in wheat while adversely affected crop growth in non-sterilized soil, indicating that microorganisms in EM solution had to face a competition with soil indigenous microflora. However, in a recent study, Javaid et al. (2008) reported negative or no effects of EM application on yield of wheat both in heat-sterilized and unsterilized soils.

It has been found that in beneficial microorganisms treated soils, generally crop yields tend to increase gradually as subsequent crops are grown. Sangakkara and Higa (1994a) studied the effect of beneficial microorganisms application on growth and yield of eggplant (*Solanum melongena* L.), capsicum (*Capsicum annum* L.), and tomato (*Lycopersicon esculentum* L.) for two seasons. During the first season, the effect of beneficial microorganisms application was insignificant while in the second season significant effect of these microorganisms for increasing crop growth and yield was evident. Sangakkara et al. (1998) conducted a 3-year study to evaluate the efficiency of beneficial microorganisms and organic matter on crop growth and nutrient uptake in capsicum and cowpea (*Vigna unguiculata* L.). They reported that the effect of beneficial microorganisms application was pronounced in the second and third year as compared to the first one. Similar responses of crop growth to beneficial microorganisms application have also been reported in wheat (Javaid et al. 2000a), *Phaseolus vulgaris* L. (Javaid et al. 2002) and pea (Javaid and Bajwa 2002). According to Kinjo et al. (2000) the lack of consistency in results of the experiments regarding beneficial microorganisms application may be due to variable cultural conditions employed in previous studies. They tested this hypothesis by applying beneficial microorganisms and chemical fertilizers in soils with different cultural practices. One soil was collected from an organic farm and the other from a conventional farm. They observed useful effects of beneficial microorganisms on yield of radish in soil collected from organic farm but these effects were not evident in the soil collected from conventional farm. Imai and Higa (1994) stated that the observed decline in crop yields can often be attributed to the fact that soils, where conventional farming is practiced, have become disease-inducing or putrefactive soils from long-term use of pesticides and chemical fertilizers. Consequently, it takes time to establish a disease-suppressive or zymogenic soil. Until this conversion process is completed, it is virtually impossible to exceed crop yields that were obtained with conventional farming methods.

12.3.3 Effect on Soil Properties

Studies have shown that beneficial microorganisms significantly improved certain physical and chemical properties of the soil. Higa (1989) found that the application of beneficial microorganisms to soil increased NO_3^- concentration from 4.5 to 5.1 mg 100 g⁻¹ dry soil. Zhao (1998) noted that application of beneficial microorganisms significantly increased the available nutrients, organic matter, and total nitrogen and lowered the C:N ratio in the soil. Paschoal et al. (1998) showed that beneficial microorganisms application in *Citrus* agro-ecosystem significantly increased soil organic matter content, level of some macronutrients including Ca, Mg, and K; soil cation exchange capacity, and lowered soil base saturation. Park (1993) reported that application of manure and beneficial microorganisms improved the topsoil by reducing bulk density and dispersion ratio thus made the soil less compact and more resistant to erosion. Improvements in soil physical properties like bulk density

and hydraulic conductivity due to beneficial microorganisms application has also been reported by Hussain et al. (1994). Lee (1994) reported the increased levels of available P_2O_5 , Ca, and Mg in the soil due to EM solution and EM plus compost application. Beneficial microorganisms application is known to enhance nodulation and nitrogen fixation efficacy of soil rhizobia (*Rhizobium/Bradyrhizobium*) in leguminous crops (Sangakkara and Higa 1994b; Javaid et al. 2000b; Yan and Xu 2002; Javaid 2006). Similarly, beneficial microorganisms application also stimulated development and functioning of other soil-borne symbiotic microorganisms such as arbuscular mycorrhizal fungi and thereby enhance soil fertility and plant nutrient acquisition (Javaid et al. 1995; Mridha et al. 1997; Bajwa et al. 1999b).

12.4 Foliar Application of Beneficial Microorganisms

Foliar application of beneficial microorganisms avoids many of the biotic and abiotic factors and constraints of the soil environment. Farmers of Indonesia have learnt to use beneficial microorganisms on vegetable crops much like a foliar fertilizer, akin to the foliar application of micronutrients. On-farm tests and demonstrations have shown that foliar applications of beneficial microorganisms can increase the growth and yield of vegetable crops in a relatively short time, even though no organic amendment is added to the soil (Widdiana and Higa (1998)). These authors conducted a field study to determine the effects of foliar-applied beneficial microorganisms on the production of garlic, onion, tomato, and watermelon, compared with the recommended application of chemical fertilizers. Foliar solutions of beneficial microorganisms at a concentration of 0.1%, 0.5%, and 1% were applied at 1- and 2-week intervals. Most vegetable yields were generally higher with foliar-applied beneficial microorganisms compared with the chemical fertilizer control. The highest yield of garlic was obtained with beneficial microorganisms at 0.1% applied at 1-week intervals, and was 12.5% greater than the fertilized control. The highest yield of onion and tomato resulted from weekly applications of beneficial microorganisms at 1%. Yield for these two crops were 11.5% and 19.5% higher than the fertilized control. However, there was no significant increase in watermelon yield from foliar application of beneficial microorganisms at any dilution level.

Xiaohou et al. (2001) conducted various studies in China to investigate the effect of foliar application of beneficial microorganisms on yield and quality of various crops. He reported that in field trials, sprinkling of 0.1% beneficial microorganisms solution improved the quality and enhanced yields of tea, cabbage, and sugar corn by 25%, 14%, and 12.5%, respectively. Yousaf et al. (2000) investigated the effect of seed treatment and foliar application of beneficial microorganisms on growth and yield of two varieties of groundnut (*Arachis hypogaea* L.). They recorded an 18% and 17% increase in yield in varieties CG-2261 and CGV-86550 due to seed treatment, and 58.1% and 58.3% increase due to combined application of seed treatment plus foliar application over control, respectively.

The type of soil amendment also affects the performance of foliar applied beneficial microorganisms. However, the mechanism is not known so far. Javaid (2006) compared the effect of foliar and soil application of beneficial microorganisms on growth and yield of pea (*Pisum sativum* L.) in soils amended with NPK fertilizers, farmyard manure, and green manure. The results showed that soil and soil plus foliar application of beneficial microorganisms either exhibited insignificant effect or suppressed the plant growth and yield. However, foliar application alone significantly enhanced shoot biomass by 70% in NPK treated soil. Similarly, foliar application of beneficial microorganisms significantly increased the number and biomass of pods by 157% and 266%, and 126% and 145% in NPK fertilizers and green manure amended soils, respectively.

12.5 Effect of Beneficial Microorganisms on Symbiotic Microorganisms

12.5.1 N_2 -Fixing Rhizobia

Legumes are unique among crop plants in that they are capable of contributing a limiting resource to the agroecosystem by fixing N_2 . The history of crop husbandry is replete with examples of yield enhancement of a non-legume crop by legumes grown either in rotation (Voss and Shrader 1984) or as multicrops (Heichel and Henjum 1991). Historically, these management practices were the mainstay of N replacement in cropping systems until the advent of economical commercial N fertilizers (Heichel and Barnes 1984). Soil bacteria belonging to the family *Rhizobiaceae* and falling in the genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium*, and *Azorhizobium* are capable of forming nodules on leguminous plants (Wei et al. 2008). The species of *Azorhizobium* form nodules on the stems of tropical legumes *Sesbania* and *Aeschynomene* while species of other genera form nodules on roots of leguminous plants. While colonizing the nodules, the bacteria develop into N_2 -fixing bacteroids providing the host plant with NH_4^+ as nitrogen source. In return, the plant supplies the bacteroids with vital organic compounds. During the establishment of active symbiosis, a well-coordinated exchange of molecular signaling between the legume host and bacterial partner occurs leading ultimately to the formation of nodules (Lakshminarayana and Sharma 1994).

Application of beneficial microorganisms is known to have variable effects on the development of nodulation and nitrogen fixation in legumes. It caused a significant reduction in nodule number but increased the size and biomass of nodules in *Trifolium alexandrinum* (Bajwa et al. 1999a). In a similar experiment Javaid et al. (2000c) noted a significant increase in nodulation in *Vigna radiata* due to beneficial microorganisms application. Javaid et al. (2002) have reported similar effects of long-term beneficial microorganisms application and organic manures on nodulation

in *Phaseolus vulgaris* L. Sangakkara and Higa (1994b) studied the effect of EM on nodulation parameters of vegetable beans (*Phaseolus vulgaris*) and mungbean [*Vigna radiata* (L.) Wilczek] in soils with low and high population of rhizobia. Application of beneficial microorganisms significantly increased the most probable number counts of bacteria in the soils. The greatest change was observed in soil with low inherent microbial populations. Nodulation and nitrogenase activity, characteristics of both the legumes, were also significantly enhanced by beneficial microorganisms application especially when grown in nutrient-depleted soil. Addition of fertilizer decreased the process of biological nitrogen fixation. However, this adverse impact was reduced with beneficial microorganisms application. Application of EM Bokashi significantly increased both the nodule numbers per plant and fresh weight per nodule in peanut (Yan and Xu 2002). Recently Javaid (2006) conducted a pot experiment to evaluate the efficacy of foliar and soil application of EM on nodulation in pea in soil amended with NPK fertilizers, farmyard manure, and *Trifolium* green manure. Results indicated that at flowering stage, beneficial microorganisms application depressed the nodulation and maximum number of nodules were recorded in uninoculated control in all the three soil amendments. Difference between control and microbial inoculated treatments was more pronounced in NPK and farmyard manure amendments than in green manure amendment. At maturity, foliar spray of effective microorganisms significantly enhanced the number of nodules in NPK fertilizers amendment. A similar but insignificant increase in the number of nodules was also recorded due to foliar spray in green manure amendment. Soil and soil plus foliar application of effective microorganisms depressed nodulation in all the three soil amendment systems at this growth stage. Effect of foliar spray and soil application of effective microorganisms on nodules biomass in different soil amendment systems was generally similar to that of nodules number. This variation in nodulation could be attributed to various factors including the soil physical and chemical properties, soil amendment, cropping and agricultural practices history, soil indigenous rhizobial and other microbes population, environmental conditions of the area, and concentration of beneficial microorganisms.

12.5.2 Arbuscular Mycorrhizal Fungi

The fungi that are probably most abundant in agricultural soils are arbuscular mycorrhizal (AM) that account for 5–50% of the biomass of soil (Olsson et al. 1999). These fungi are multifunctional in ecosystems. Colonization of roots by AM fungi has been shown to improve growth and productivity of several field crops (Javaid et al. 1994; Kapoor et al. 2004; Cavagnaro et al. 2006; Chen et al. 2007) by increasing nutrient element uptake (Al-Karaki 2002; Pasqualini et al. 2007); enhanced tolerance to various biotic (Khaosaad et al. 2007) and abiotic stress factors (Arriagada et al. 2007); and improving physical, chemical, and biological properties of soil (Rillig and Mummey 2006).

Few studies, mostly by our research group, have been conducted to assess the effect of beneficial microorganisms application on mycorrhizal colonization, and effect of dual inoculation of beneficial microorganisms and mycorrhizae on crop growth and yield of test species. Variable results were obtained in these interactive studies. The variation in response of crop growth, yield, and mycorrhizal colonization to beneficial microorganisms application or co-inoculation of beneficial microorganisms and mycorrhizae was generally associated with the nature of the test species, soil amendment, and history of beneficial microorganisms application. Bajwa and Jilani (1994) studied the interaction of beneficial microorganisms and mycorrhizal fungi in sterilized pot soil and found that beneficial microorganisms significantly enhanced the mycorrhizal colonization in maize and the combined inoculation resulted in significantly increased crop growth and yield. Bajwa et al. (2002) found that beneficial microorganisms application increased maize growth in both farmyard and green manure amended soils while mycorrhizal colonization was favored by beneficial microorganisms only in farmyard manure amended soil. Similarly Bajwa et al. (1995b) assessed the usefulness of dual inoculation of beneficial microorganisms and two mycorrhizal species viz. *Glomus mosseae* and *G. fasciculatum* in improving growth and yield of tomato. They noted a significantly greater shoot dry biomass and fruit yield in *G. mosseae* plus beneficial microorganisms and *G. mosseae* plus *G. fasciculatum* plus beneficial microorganisms as compared to respective sole mycorrhizal treatments. In another experiment, Bajwa et al. (1995c) studied the effect of beneficial microorganisms application on crop growth, mycorrhizal colonization, and nutrient uptake in soybean by introducing extra-mycorrhizal spores. They reported that indigenous mycorrhizal flora of field soil did not respond positively to beneficial microorganisms application and mycorrhizal infection failed to develop properly with subsequent adverse effects on host plant growth. However, beneficial microorganisms significantly supported externally introduced mycorrhizal inoculum and marked influence was observed on crop growth and nutrient uptake. Javaid et al. (1995) showed that application of beneficial microorganisms in unsterilized field soil enhanced mycorrhizal colonization in the roots of pea, resulting in increased growth, yield, nodulation, and nitrogen nutrition in host plant. Similarly, Bajwa et al. (1998b) observed a significant increase in root and shoot growth, and shoot P and N content in chickpea due to co-inoculation of beneficial microorganisms and mycorrhiza. Beneficial microorganisms application also favored mycorrhizal development in root cortex of host chickpea plants. In another study, the authors also noted similar effects of beneficial microorganisms and mycorrhizae under allelopathic stress caused by aqueous leaf extract of *Syzygium cumini* (L.) Skeels (Bajwa et al. 1999b). Javaid et al. (1999) conducted a field study to evaluate the effectiveness of beneficial microorganisms application on mycorrhizal colonization and subsequent growth and yield in sunflower, at two growth stages viz. 40 and 70 days after sowing. In 40-day-old plants, beneficial microorganisms supported mycorrhizal association, which resulted in a parallel increase in number and biomass of leaves as well as stem length while stem biomass remained unaffected. However, beneficial microorganisms application failed to induce any remarkable change in extent of mycorrhizal colonization at 70 days

growth stage. However, the number of arbuscules was enhanced by beneficial microorganisms application at this growth stage that resulted in a parallel increase in vegetative growth and yield of the host plant. By contrast, Bajwa et al. (1999a) while studying the effect of beneficial microorganisms on mycorrhizal colonization, nodulation, and crop growth in *Trifolium alexandrium* L., in soils amended with farmyard manure and green manure, noted that EM significantly enhanced mycorrhizal colonization but exhibited an inhibitory effect on crop growth. Javaid et al. (2000b) conducted pot experiment in farmyard and green manure amended soils with two different histories of beneficial microorganisms application using *Vigna mungo* as test species. They observed a better response of crop growth, nodulation, and mycorrhizal colonization to beneficial microorganisms application in soil 1 where beneficial microorganisms application was started 6 months prior than the soil 2. In a similar experiment, Javaid et al. (2000c) noted a significant increase in mycorrhizal colonization in *Vigna radiata* due to beneficial microorganisms application. In another study, Javaid et al. (2000a) assessed the effects of long-term application of beneficial microorganisms and organic manures on mycorrhizal colonization, crop growth, and yield of wheat in soils with three different histories of beneficial microorganisms application. Beneficial microorganisms proved more effective in increasing mycorrhizal colonization and yield in wheat in soil with oldest history of application of these microorganisms. Similarly, Javaid et al. (2002) have reported similar effects of long-term application of beneficial microorganisms and organic manures on crop growth and mycorrhizal colonization in *Phaseolus vulgaris* L. Mridha et al. (1997) reported that dual inoculation of arbuscular mycorrhizae and EM resulted in significant better plant growth in *Sesbania rostrata* as compared to either beneficial microorganisms or mycorrhizal inoculation. Furthermore, beneficial microorganisms application enhanced percentage root colonization and mycorrhizal spores.

12.6 Pest Management with Beneficial Microorganisms

12.6.1 Weed Management

Weeds compete with crops for nutrients, available moisture, space and sunlight, which results in yield reduction. Weeds also deteriorate the quality of farm products and hence reduce the market value. Since weeds are present in all food crop systems irrespective of the intensity of the crop management (Schroeder et al. 1993), thus their control is vital to achieving high yields. Weeds have traditionally been controlled by manual and cultural methods. With the development of synthetic agrochemicals in the 1940s, the reliance on chemicals to obtain weed-free cropping systems increased. However, the indiscriminate use of these synthetic chemicals has led to pollution problems in most agricultural systems, and more importantly the development of herbicide-resistant and problematic weed species (Chhokar et al. 2008; Doole and Pannell 2008). Modern biological agricultural systems do

not permit the use of synthetic herbicides for weed control because of their potential to become environmental pollutants and harmful residues in the food chain. There is a growing interest in nonchemical weed control methods worldwide.

Application of beneficial microorganisms is known to stimulate seed germination and early growth of food crops (Sangakkara and Higa 1994a) and can create a more favorable root surface-rhizosphere environment for crop plants that improves plant growth and protection (Sangakkara 1996). It is likely that these documented beneficial effects of beneficial microorganisms on crop plants would also be extended to weeds, and could enhance weed seed germination, early growth and development, and their level of infestation. Consequently, there is considerable interest in whether beneficial microorganisms through this process, over time, could reduce soil weed seed-bank. Maramble and Sangakkara (1998) conducted a study to determine the effect of beneficial microorganisms on weed population and weed growth grown with organic amendments during the dry season of 3 consecutive years in Sri Lanka. The application of organic amendments alone suppressed weed growth, although the variation among the years was insignificant. Beneficial microorganisms applied with organic amendments enhanced weed growth during the first year which then declined significantly during the succeeding years. In a similar study, Maramble et al. (1996a) investigated the influence of beneficial microorganisms and organic matter on weed populations of two annual food legumes in consecutive wet and dry season over 2 years. The lowest weed populations and crop yields were obtained from plots not receiving beneficial microorganisms or chemical fertilizers. Application of beneficial microorganisms especially with organic matter having a low C:N ratio enhanced weed populations in the first year. The impact was more pronounced in dry than in wet season. In the second year, beneficial microorganisms increased crop yields and reduced weed populations and biomass significantly. The authors suggested that long-term studies are required to get more benefits of beneficial microorganisms as an alternative method for controlling weeds. Maramble et al. (1996b) reported that application of beneficial microorganisms significantly increased tuber germination and subsequent growth of purple nutsedge (*Cyperus rotundus* L.) plants. However, beneficial microorganisms significantly lowered the number of tubers and tuber biomass at the time of flowering. The authors suggested the poor tuber formation in beneficial microorganisms treated nutsedge plants during the first season would reduce the weed infestation during the following season.

12.6.2 Control of Fungal Pathogens

Different species of turfgrass are widely used worldwide for golf courses, athletic fields, and landscaping. *Sclerotinia homoeocarpa* (Lib.) Korf & Dumont, causal agent of dollar spot disease is considered the most prevalent turfgrass pathogen in North America, Central America, Australia, New Zealand, and Europe. Fungicides are a major input for controlling this disease. To evaluate effective alternative approach,

Kremer et al. (2000) conducted in vitro laboratory bioassays to determine the effects of beneficial microorganisms on growth and development of *S. homoeocarpa*. The results showed that beneficial microorganisms amendment in potato dextrose agar medium at 1.0% and 4.0% significantly inhibited hyphal growth of *S. homoeocarpa*. Following in vitro bioassays, greenhouse study was conducted to investigate the effect of beneficial microorganisms on disease development by *S. homoeocarpa* in turfgrass and turf quality. They found that beneficial microorganisms treated compost treatment had significantly less disease than the standard golf green substrate. According to Tokeshi et al. (1998) soils treated beneficial microorganisms were found to be suppressive to the soil-borne plant pathogen *Sclerotinia sclerotiorum*. Similarly Jonglaekha et al. (1995) reported that root rot in strawberry, caused by *Rhizoctonia fragariae*, can be considerably controlled either by mixing beneficial microorganisms compost in the soil or applying beneficial microorganisms solution for 4–6 times at weekly intervals. Application of beneficial microorganisms also reduced the incidence of wilt disease of potato (Jonglaekha et al. 1993). Encouraging results have also been recorded in the management of anthracnose of sweet potato caused by *Colletotrichum gloeosporioides*, suppressing populations of soil-borne phytopathogenic fungus *Phytophthora cinnamomi*, and control of black sigatoka disease of bananas caused by *Mycosphaerella fijiensis* (Tokeshi and Chagas 1996; Aryantha and Guest 1997; Elango et al. 1997). Control of fungal pathogens may be attributed to the activity of lactic acid bacteria in the beneficial microorganisms mixture that produce lactic acid, a strong sterilizing compound (Higa and Kinjo 1991; Higa 2000).

12.6.3 Control of Bacterial Pathogens

Few studies have been conducted to investigate the effect of beneficial microorganisms on bacterial diseases. Castro et al. (1996a) conducted an in vitro study and found that beneficial microorganisms inhibited the growth of *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas solanacearum*. They extended the study to evaluate the potential of these microorganisms for control of *X. campestris* pv. *vesicatoria* in sweet pepper (*Capsicum annum* cv. *margareth*) under field conditions and obtained promising results in the management of the disease (Castro et al. 1996b). The suppressive effects of beneficial microorganisms have also been reported against bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Myint et al. 1996).

12.6.4 Control of Insect Pests

Very little work has been carried out regarding the effectiveness of beneficial microorganisms against insect pests. Nasiruddin and Karim (1996) conducted a

field trial to test the efficacy of one formulation of beneficial microorganisms (EM₅) in reducing the damages caused by the red pumpkin beetle (*Aulacophora foveicollis*) and the melon fly (*Bactrocera cucurbitae*) in cucurbitaceous vegetable crops. EM₅ reduced the beetle infestation by 38% and melon fly infestation by 28.3–35.8% over the untreated control. Chemical insecticides showed more than 80% reduction of infestation of the two insect pests. Pickleworm (*Diaphania nitidalis*) is a serious pest of cucumber and other vegetables of the Cucurbitaceae family. The conventional control of this pest calls for excessive use of synthetic pesticides which pollute both product and environment. Wood et al. (1997) reported that incidence of disease and damage by the pickleworm was significantly reduced by foliar applications of beneficial microorganisms fermented plant extracts in combination with EM₅.

12.7 Role of Beneficial Microorganisms under Abiotic Stresses

Few reports are available in the literature regarding the role of beneficial microorganisms under abiotic stress factors such as allelopathy, acidity, and salinity. Bajwa et al. (1999a) reported that application of beneficial microorganisms significantly reduced the adverse impact of aqueous leaf extract of an allelopathic tree *Syzygium cumini* (L.) Skeels on plant growth, yield, and shoot nitrogen content of chickpea (*Cicer arietinum* L.). The efficacy of beneficial microorganisms was further enhanced by dual inoculation with arbuscular mycorrhizal fungi.

Pairintra and Pakdee (1994) suggested that beneficial microorganisms treated compost can be used as an efficient soil amendment in ameliorating a slightly saline soil. Aluminum toxicity is considered to be the most important growth limiting factor in many acid soils in Malaysia, especially those having pH levels below 5.0. Anuar et al. (1997) conducted a field experiment to evaluate the use of beneficial microorganisms in reducing the aluminum toxicity of an acid soil, its effect on the yield of sweet potato and selected chemical changes of the soil. The results showed that exchangeable aluminum was reduced with the application of beneficial microorganisms. Furthermore, these microorganisms increased the yield of sweet potato and also influenced the chemical characteristics of the acid soil.

12.8 Treatment of Dairy Wastewater with Beneficial Microorganisms

Wastewater originated from dairy operations may contain certain human pathogens including *Escherichia coli*. In addition, excess nutrients present in dairy wastewater can also pollute surface and ground waters. Beneficial microorganisms and duckweed have shown a great promise in dairy wastewater treatment. According to

Rashid and West (2006) combined application of beneficial microorganisms and duckweed growth significantly reduced the ammonium nitrogen, total phosphorus, total suspended solids, and biological oxygen demand after 3 months and is a very efficient way of dairy wastewater treatment. Li and Ni (2000) reported 42–70% reduction in NH_3 concentration in poultry manure when beneficial microorganisms were added to both drinking water and feed. The mechanism involved suggests that as EM is a mixed culture of many species of microorganisms, some of which can transform NH_4^+ to NO_3^- , thereby decreasing the potential for N-fraction.

12.9 Conclusion

Extensive studies carried out in various countries of Asia Pacific Region have shown that beneficial microorganisms enhance the growth, yield, and quality of various agricultural and horticultural crops possibly through rapid decomposition of organic matter, production of biogenic substances, improved soil quality, and enhanced growth and efficacy of symbiotic. However, the affectivity of beneficial microorganisms varies with soil type, source and amount of soil nutrients, and test crop species. The negative or no effects of beneficial microorganisms as reported by some workers can be overcome through repeated applications of these microorganisms. For the best exploitation of advantages of beneficial microorganisms, they should be used in combination with organic matter plus half dose of NPK fertilizers. Furthermore, they should be used as foliar spray as this practice avoids many of the biotic and abiotic factors and constraints of the soil environment.

Most of the studies with beneficial microorganisms limited to their effects on crop growth and yield. However, in other areas of agriculture such as management of pests like weeds and insects, and control of plant pathogens including bacteria and fungi, only few studies have been undertaken. These few studies, however, exhibited very encouraging results in the management of pests and diseases with beneficial microorganisms. Similarly, few studies conducted so far to manage the environmental problems like treatment of wastewater and poultry manure revealed that the benefits of beneficial microorganisms can also be exploited in these areas. However, more intensive and systematic studies are required in these areas to provide a better understanding of the usefulness of beneficial microorganisms technology in various farming systems and environmental issues to provide safe food products to the consumers.

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Chapter 13

Foliar Fertilization for Sustainable Crop Production

Seshadri Kannan¹

Abstract Plants require inorganic nutrients in addition to carbon dioxide and water for growth and production. Nutrients are present in soil, but get depleted unless supplied through fertilization. Soil feeding is the normal practice, but has limitations with respect to its availability to the plants. The elements such as phosphorus, potassium, and most of the micronutrients are fixed in the soil complex, while the more soluble nutrients such as nitrogen are easily leached down the soil. What is lost through leaching reaches the aquifer and pollutes the groundwater. For instance nitrates and phosphates can be harmful to humans. With increasing costs of fossil fuel, which provides the raw materials for fertilizer manufacture, there is a need to find innovations in fertilizer usage techniques. Foliar application is one such technique. Here I review the extensive work that has been carried out on the effectiveness of foliar-applied nutrients, the mechanisms of foliar absorption, and transport. The leaf components such as the cuticular membranes, the trichomes, the cuticular pores, ectoteichodes, their properties, and their role in the nutrient transport into the plant leaf are reviewed. Cuticles are permeable to nutrient ions present in aqueous forms and have distinct structures like pores. But it is not known if these pores facilitate easy entry into the leaf cells. The trichomes increase the amount transported into the leaf by providing more area for absorption. The cuticles have two types of lipophilic substances, the cutin and the cuticular wax, which influence the permeability of nutrient ions to varying degrees.

It is clear that nutrients reach the leaf cells, after penetrating the cuticle, and are further transported to other parts through plasmadesmata. Some micronutrients are not as freely mobile as the major nutrient elements such as N, P, or K. The age

¹Retired. Present address: 38 South Mada St., Thiruvanniyur, Chennai 600041.
This review marks the 82nd birthday on 28 December.

S. Kannan (✉)
Biology and Agriculture Division, Bhabha Atomic Research Centre,
400085 Mumbai, India
e-mail: seshkann@yahoo.co.in

of the leaf and the pH of the spray liquid are important for foliar absorption. The absence of plasmadesmastic connections between the guard cells and the epidermal cells is also important. One element Cl has been found to be transported from the applied leaf to other parts rapidly, showing it is freely mobile. This should be true for many anions.

The concept of limiting factors and the law of the maximum proposed by Wallace group are useful in raising the yield plateau, and when soil supply poses the “limiting” factor, foliar feeding will help increase the crop yield. Modern technique of sprinkler irrigation system can be exploited to supply the nutrient elements in the irrigation water, which will be economical in foliar fertilization. Foliar nutrition is very practical to correct micronutrient deficiencies, which are very important for maximizing the yield. Crop breeders could also help evolve cultivars, which give good response to foliar feeding.

Keywords Critical growth stage • cuticle • eutrophication • inorganic nutrients • leaf uptake • sprinkler irrigation

13.1 Introduction

The fact that food production has to be increased over the coming few decades to feed the growing population needs no emphasis. The population is predicted to reach nine billion in 2030, and feeding them is the greatest challenge for scientists (Gomiero et al. 2008), and the agricultural land is dwindling in area, one main reason being the large-scale urbanization, while the existing arable land is getting impoverished in nutrients owing to continuous cropping. The environmental impact of intensive agriculture and the adverse effects of climate change also threaten food security in many parts of the world and there is an urgent need to develop more innovative agricultural technologies, which would help preserve the agro-ecosystems, and to deal with the reduced availability of fossil fuels, which are presently used for fertilizer production. One of the methods is to increase the nutrient-use efficiency of crops. Besides the nutrients, the other most important input is carbon dioxide that is derived from the atmosphere. In order to improve crops' ability to convert the atmospheric carbon dioxide into food, plant engineers directed their attention to RuBisCo (Ribulose-1, 5-bisphosphate carboxylase/oxygenase), which catalyzes the photosynthetic process, and yet is considered a very inefficient enzyme (Mann 1999). While other efficient forms of catalysts found in red algae could not be yet harnessed for higher plants, the geneticists have but to stay with RuBisCo at least for some time. The tools that helped double the food production since 1960 have lost their edge, putting an end to the productivity increases. Efforts to engineer the crop plants to get over the yield plateau and push yields to a new level of a projected 40% increase in global demand for rice, wheat, and maize by the end of next decade are needed. While on the one side, plant breeders could concentrate on the development

of new cultivars through genetic engineering, they also fear that farmers may not have enough water to grow the new crops or may be forced to use more fertilizers especially when less fertile lands have to be brought under cultivation. This recourse will undoubtedly poison the ecosystems and permanently damage the soils. Furthermore, the geneticists have hitherto made selection or evolved high-yielding cultivars, which nevertheless are more responsive to higher fertilizer input, adequate water, and the right environment for efficient photosynthesis. But water and nutrients do impose new constraints for higher yields. Interestingly, it is not true that given all these inputs, the yield increase will reach without any limitations. The old and well-founded concept of “limiting factors” explains how various inputs influence the yield, and how the yield plateau could be raised by providing the input that imposes a limit, and crop production can be increased (Wallace and Wallace 2003).

There has been a growing tendency with the farmers to “over-fertilize” when they do not obtain good response to a particular input, for example, fertilizers. This leads to an excessive loss of fertilizers from the soil to the aquifers or these inorganic elements get accumulated in the crop production and finally into the biological cycle causing health hazards. Due to that the amount of nitrogen lost in the runoff is very high, raising the nitrate levels in the water system. Such a situation has arisen in the Gulf of Mexico, and the high nitrate levels in the sea are traced to agriculture. In California where half of the country’s vegetables are grown, there are about 120 water sources classified as containing “excessive nutrients.” The drinking water in a community in Salinas Valley is banned because of high nitrate levels in the water system. Nitrate-N concentrations in groundwater in Japan have increased greatly in the last 2 decades (Kumazawa 2002). With the use of controlled-release fertilizers, nitrogen-use efficiency was increased considerably, bringing down the level of nitrate-N in the groundwater. Increasing levels of atmospheric gases, especially carbon dioxide as a result of burning fossil fuels, not only cause health hazards but also affect crop yield. Contrarily, there is one school which believes and has produced evidence that this increase actually will enhance crop production instead of reduction, provided other inputs such as water and nutrients are increased proportionately. In the coming sections these will be discussed as also the different views on the inputs required for increasing crop production.

Advances in an understanding of plant nutrition, development of slow-release fertilizers and soluble nutrients, and improvement in soil- and tissue-testing methods have all contributed to the increase in the yield and quality of crops. Future developments will have to focus on fertilization in an increasingly competitive global economy. Foliar application of nutrients has been in practice over 6 decades with the objectives to provide the required nutrients most effectively and in a few cases economically for crops that have larger leaf area. Several papers appeared on the field experiments on foliar sprays of nutrients especially N, P, and K on crops from the work at Rothamsted experiment station during the 1950s. The great response for foliar absorption by apple leaves led to an extensive usage of urea sprays on fruit trees, pineapples, and vegetable crops in many countries.

There was a renewed interest in foliar nutrition in the 1960s when radioisotopes became available to trace the movement of nutrient elements within the plant as also in the understanding of the mechanisms of leaf uptake, and the means to increase its effectiveness. Now sprinkler irrigation has become a common practice in field crops and with the advent of computers and electronic devices, the supply of water could be regulated with great precision. Taking advantage of the sprinkler irrigation supplying nutrients too could be included in the spray liquid and nutrient supply could also be regulated using computer technology. Fertilization through the soil serves the most convenient means and will continue to be so in the future too. However, for the aforesaid reasons like over-fertilization, and eutrophication, other methods of feeding need serious consideration. Foliar feeding is one method which can meet the requirement as a supplement to soil feeding in many cases. Bi and Scagel (2007) recommend foliar N application for raising nursery plants. While this can correct the N deficiency in the early growth stages, it can decrease the amount of total N necessary and minimize N runoff. Supplementing a traditional N-fertilization program with foliar applications gives growers more management options. One of these is the timing of foliar application which is based on the specific goals of production, and the benefits which are desired.

In a recent review, Fernández and Eichert (2009) state that foliar fertilization is an agricultural practice of increasing importance in practical terms. In theory, application of nutrient sprays may indeed be an environmentally friendly fertilization method since the nutrients are directly delivered to the plant in limited amounts, thereby helping to reduce the environmental impact associated with soil fertilization. However, response to foliar sprays is often variable and not reproducible due to the existing lack of knowledge of many factors related to the penetration of the leaf-applied solution. It is the objective of this review to examine what has been so far studied and outline the prospects of this technology.

13.1.1 Ability of the Leaf to Absorb Nutrients

The fact that leaves as well as other aboveground parts are capable of absorbing nutrients has been known for over a century (Gris 1844). The acquisition of S, N, Mg, and Cu from the industrial gases via the rains known as “wet deposition” and “Occult precipitation” was also recorded in England (Dollard et al. 1983). Scientists found iron and sulfur compounds which were released into the atmosphere during the ore-smelting activity, and absorbed by the leaves of trees and transported to other parts. The absorption of atmospheric NH_3 and SO_2 by plant leaves is also known (Aneja et al. 1986). While the uptake of N and S serve as nutrients, the absorption of pollutants like lead and cadmium released from the burning of fossil fuel is harmful. The most recent report shows how fast an element can enter the leaf and become toxic more quickly than if supplied through the roots. It relates to very rapid absorption of boron from boron-containing water by vegetable leaves, while boron through soil took a long time to reach the plant parts (Ben-Gal 2007).

There was an interesting study regarding the question which forms of N, i.e., NH_4 or NO_3 is taken up, as also whether the roots or the fronds of the aquatic plant *Landoltia punctata* are capable of greater absorption (Fang et al. 2007). It was found that both fronds and roots absorbed both forms of N, and the overall capacity of roots and the fronds to take up ions was similar. The above findings provide enough evidence about the capacity of the leaf to take up nutrients.

13.1.2 Foliar Supply of Micronutrients: Influence of Growth Substances

One area where foliar application is most effective is in the control of micronutrient deficiencies. Foliar application was practiced for the supply of B, Cu, Mg, Mn, and Zn in many crops for timely control. Swietlik and Faust (1984) have summarized the results of the experiments with micronutrient sprays on fruit trees. There have been many reports which appeared over several years. Some of the recent findings are given here (Table 13.1).

Certain additives and growth substances increased the effectiveness of foliar uptake. Studies were made with radioisotopes and growth regulators on various plants and a few nutrient elements and some of these are presented here (Table 13.2).

A large number of studies were made to measure the rate of mobility of foliar-applied nutrients, and radioisotopes were widely employed for the experiments. Basically, the nutrient element supplied to the leaf would move in either direction, i.e., toward the tip or base of the leaf. While the root-absorbed nutrients would reach the leaf and other aboveground parts through the xylem under the transpiration pull, the foliar-applied elements can move out of the leaf or be transported to the base of the plant. To describe a few, there were three experiments conducted by

Table 13.1 Foliar application of micronutrients in different crops

Nutrient elements	Crops	References
Iron	Sorghum, vegetables, ornamental plants, fruit trees	Ritter 1980
Manganese	Fruit trees, vegetables, soybean	Gettier et al. 1985; Young 1983
Manganese	Lupin	Hannam et al. 1984
Zinc	Apple, grapevine	Peryea 2006
Zinc	Pistachio and walnut fruit crops	Zhang and Brown 1999, Swietlik 2002
Boron	Tomato, turnip	Williams et al. 1983
Copper	Citrus, onion	Young 1983
Molybdenum	Poinsettia	Cox 1992
Calcium ^a	Tomato, strawberry	Drake and Bramlage 1983
	Blueberry	Stückrath et al. 2008

^aIs not classified as a micronutrient

Table 13.2 Foliar uptake of nutrients and influence of growth substances

Element	Plants	Uptake	Authors
N, P,+K with With Dropp/TIBA/Pix. (see text)	Milk thistle	Yield increased	Geneva et al. 2008
⁵⁹ Fe+ABA/kinetin Zn-chelate + GA ₃	Bean	Increased absorption Washington Navel Orange in Egypt	Kannan 1986 Eman et al. 2007

GA = gibberellic acid, ABA = abscisic acid

scientists. In one experiment, the movement from the site of application to the rest of the leaf was followed. Ringoet et al. (1971) developed a technique to measure the rate of movement of ⁴⁵Ca placed on the middle of an oat leaf by in vivo counting with β -sensitive semiconductor detectors. They found that Ca migrated in the acropetal direction at low concentrations, but moved down to the base of the leaf at concentrations above 0.02 M. They concluded that Ca was fairly mobile and the downward transport was through the phloem while it moved upward in the xylem. There are some nutrient elements for which stable isotopes were not available and a new technique was developed for tracking the movement of boron (Chamel and Eloy 1983). They used ¹¹B/¹²B and measured the ratio of the two in plant samples with a highly sensitive spark-source mass spectrometry and microanalytical method using a laser-probe mass spectrograph. They found that the largest fraction of foliar-applied B remained in the applied leaf itself. However, it entered the leaf surface and remained bound as polysaccharide complexes. It was concluded that B is partially mobile, like Fe, Mn, or Zn. Relative mobility of various elements in the leaves was reported in several studies (Kannan 1990).

Thus there is enough evidence that leaf is capable of absorbing inorganic nutrients supplied in aqueous forms and then transporting to both the shoots as also the roots. Elements like Ca were found in the xylem and phloem too.

13.2 Responses to Foliar Supply of Nutrients – Studies with N, P, and K

13.2.1 Response to N

The application of nutrients like N, P, and K to the leaf during the autumn, i.e., prior to the leaf fall, is considered beneficial to fruit trees on the hypothesis that these will be remobilized during the spring growth from the stored organs. Oland (1960) was the first to report that urea sprayed to apple trees prior to leaf fall was most effective to supply N for use in spring growth. This is good for another reason in

that high concentrations of urea can be given with the least damage. The effectiveness of spraying foliage of 1-year-old nectarine trees with urea to provide nitrogen to augment the seasonal internal cycling of N was examined (Tagliavini et al. 1998). The tree was sprayed with a 2% urea solution labeled with ^{15}N just before leaf senescence. Remobilization of both labeled and unlabeled N for leaf growth the following spring was quantified. During leaf senescence, the majority of ^{15}N was withdrawn from the leaves into the shoot and roots. About 38–46% of ^{15}N in the trees was recovered in the new growth. However, more N taken up by the roots was mobilized for leaf growth in the spring than was withdrawn from the senescent leaves receiving N through sprays. They concluded that the foliar-supplied N had to be supplemented by soil application for new growth. In the case of apple trees, 3% urea sprays given twice a week increased the whole-plant N content which also enhanced the utilization of reserve carbohydrates for new growth (Cheng et al. 1999).

Nitrogen management for orchard trees is very important and the growers are keen on increasing productivity and fruit quality without affecting the environment. They find foliar sprays of N as an alternative to soil feeding. Experiments have been carried out to ascertain the time for foliar sprays suitable for greatest response. Using ^{15}N labeled $\text{Ca}(\text{NO}_3)_2$, the uptake, partition of N, and its remobilization in pear (*Pyrus communis* L) after 1 year of foliar application were assessed (Quartieri et al. 2002). The young trees were divided into three groups, viz., (A) received 3 g of labeled N from mid-March to mid-June, trees of group (B) received 3 g of labeled N from late June to fruit harvest (August 20). Both A and B also received unlabeled N at 3 g/tree from late June to fruit harvest and from mid-March to mid-June respectively. A third set (C) received N at 6 g/tree throughout the season. Fruits and leaves were analyzed for N and were found to contain similar amounts of N derived from remobilization of stored N and from spring uptake (March–June, treatment A); only about 10% of N was derived from N taken up after June (B). Although abscised leaves contained ten times higher amounts of N taken up early (A) than late (B) treatments, similar amounts of labeled N were recovered in the whole tree framework, in winter in trees of group A and B. Remobilization of N in the following spring accounted for 23–24% of the labeled N in the tree, regardless of the timing of N uptake. They found that a limited amount of N given before fruit harvest did not increase the fruit N content, but increased its storage in the roots during winter, which was remobilized the following spring. Similar findings were also recorded by Dong et al. (2002) from experiments with urea sprays on apple trees.

Improvement of the grain protein in high-yielding cereals has been the most important goal in recent times because of the high premium it fetches the farmers, and studies have clearly shown that foliar sprays of N increased in grain protein in wheat. Optimal timing for N sprays on wheat was examined (Bly and Woodard 2003) and the results showed that postpollination foliar N gave the highest grain protein. Likewise there was significant increase in total grain N and protein content from the postflowering sprays of urea-ammonium nitrate (Wuest and Cassman 1992; Woolfolk et al. 2002).

The absorption and utilization of N and its influence on the carbon metabolism in *Ricinus communis* L. were examined (Peuke et al. 1998). N was taken up more readily from $(\text{NH}_4)_2\text{SO}_4$ than from KNO_3 , and more evenly distributed between the shoot and the root while nitrate-N was transported largely to the roots. The sprays increased both organic and inorganic particles on the surface of the leaf thus increasing its wettability and absorption. Furthermore, the presence of the nutrient particles on the leaf surface and in the stomata indicated that the entry of nutrients in the thin water films entered through the stomata and the cuticles.

Various factors influence the absorption by the leaf and the leaf age and form of nutrients are important in influencing the uptake. Urea-N has been employed for such studies. However, for comparison between N from urea and N from KNO_3 leaves of citrus were dipped into a solution of 11.2 g N/l and the uptake was measured for several hours (Lea-cox and Syvertsen 1995). It was found that uptake of N per unit leaf area was 1.6- to sixfold greater for 2-month old leaves than for older leaves. In another experiment, it was found that 24% and 54% of applied N were taken up after 1 and 48 h respectively from N-urea and under similar durations, only 3% and 8% of N from KNO_3 were taken up. Urea increased the leaf N much more than the KNO_3 form of N.

Though urea sprays have been used for several crops over many years, this could not supply the entire N requirement of the crop. Nevertheless, experiments were conducted to study if foliar-applied urea can be sufficient to increase the fruit growth and yield of peach [*Prunus persica* L. Batsch (Peach Group)] cultivar, Early Maycrest (Johnson et al. 2001). In a 3-year experiment, the authors compared a total foliar urea to an equivalent amount of N through the soil. Though foliar treatment supplied adequate amounts of N to the various organs viz., the roots, shoots, and fruit buds, the mean fruit weights were lower than those fertilized through the soil. However, when a 50:50 combination treatment of soil-applied N in late summer with foliar-applied N in October was given, the fruit yields and fruit weights equaled the soil-fertilized ones. It was concluded that some soil-application of N is necessary for optimum fruit growth and this is needed in order to have good root proliferation which would in turn facilitate an increased soil uptake. This combination of fertilization also reduces excessive vegetative growth and offers as a viable alternative for maintaining tree productivity and reducing soil pollution at the same time.

Foliar supply of urea to citrus trees for N fertilization has been particularly useful in reducing groundwater pollution with nitrates. The seasonal absorption characteristics of three urea compounds, viz., triazone-urea, liquid urea, and spray grade urea by citrus leaves were examined (Bondada et al. 2001). Factors like the age and the N status of the leaves influenced the rate of absorption. In the field grown plants, leaf N was increased equally in all the three formulations of N. Young leaves from 1-week to 1-month old, absorbed greater amounts of N than the older leaves (3–6 months). With the increasing epicuticular wax concentrations, ^{15}N uptake was decreased. Triazone-urea increased the N concentration more than urea sprays. In the plants grown in the greenhouse, ^{15}N absorption was greater through abaxial leaf surfaces than through adaxial surfaces, in general. Applying foliar ^{15}N -urea during night (2,000–2,200 h) resulted in greater absorption of N than in the mornings

(0800–1,000 h) or afternoons (1,200–1,400 h). Triazone-urea acted as a slow-release N source that could be exploited for foliar application to be effective over an extended period of time.

Foliar supply of nutrients assumes importance under various conditions, important one being the inability of the roots to absorb the soil nutrients. Such a situation prevails when the plant roots are submerged under water or when the plants are grown in hypoxic nutrient solutions, causing marked inhibition of uptake of major nutrient elements by the roots. Under hypoxic conditions in particular, the reserve metabolic energy of root cells is considerably reduced. The foliar application is an accepted practice to supply nutrients to shoots under such conditions. Nitrogen, phosphorus and potassium in soluble forms are readily absorbed by aerial plant parts, often much more efficiently than from supplementary soil treatments (Xie and Zhang 2004). Foliar sprays of nitrogen fertilizers caused appreciable yield improvement in waterlogged cotton (Hodgson 1982) and rape (Zhou et al. 1997). Although application of fertilizers to waterlogged soil has been found to improve plant growth, the excess water in the soil causes dilution of the nutrients in the root medium. In addition, waterlogging reduces the ability of plant roots to take up and transport mineral nutrients to the shoot (Ashraf and Rehman 1999; Malik et al. 2001). Foliar spraying of nutrients serves as an efficient way to improve the nutrient status of the waterlogged plants. Pang et al. (2007) studied the waterlogging effects on nutrient uptake from foliar sprays in six barley cultivars with contrasting tolerance to waterlogging. It was found that the adverse effects of waterlogging were greatly reduced by the foliar sprays of nutrients, in all cultivars in general. The sprayed plants had better shoot and root growth and reduced leaf senescence. Auxin was found to be accumulated at the shoot base of the sprayed plants. It is explained that the better growth was primarily due to increase in the synthesis of growth-promoting substances like auxin. Many of the issues relating to foliar fertilization by plants have been reviewed by Wojcik (2004). He emphasizes that this mode should be included in the integrated plant production scheme because it is environment-friendly with prospects of the increasing crop productivity and good-quality yields. Foliar feeding is profitable for perennial fruit crops with deep-rooting systems since soil surface application of most fertilizers is slow in response. One such condition prevails in Australia where passion fruit is cultivated. Passion fruit (*Passiflora edulis* Sims) is cultivated extensively in Australia and the growers face the problem of fertilizing the plants through soil since the shorter photoperiod in winter reduces the vegetative growth and the low temperature prevents soil N uptake. Commercial growers resort to foliar sprays of urea during winter for providing adequate N (Menzel et al. 1986).

Foliar supply is effective under conditions of decreased nutrient availability in soil, dry topsoil, and decreased root activity during the reproductive stage. Ca sprays are beneficial to increase the calcium content of fruits and cereal grain. For nutrients which are phloem-mobile this method is particularly more effective. Recently, foliar application of some products, like seaweed extracts, hydrolyzed proteins, and amino acids is being popularized. But their influence on crop production is not fully established. Wojcik has presented the findings of several workers, on the

mechanisms of penetration of nutrient ions through cuticles, entry into the leaf cells through the plasma membrane, and the factors influencing the foliar absorption.

13.2.2 Response to N, P, K, Ca

Foliar sprays could be given for supplying not only N but also other major nutrients. The results of a few experiments are given here. Early experiments on response to foliar application were reported in USA and England. Barley, Brussels sprout, French bean, tomato, and sugar-beet plants grown in soil in pots and sprayed with nutrient solutions containing nitrogen, phosphorus, potassium, and a spreader, had higher nutrient content and dry weights than control plants sprayed with water and spreader only. Increase in nutrient content occurred with high or low levels of nutrient supply to the roots and was approximately proportional to the concentration of spray and to the frequency of spraying. There was no difference in N uptake in sugar beet, from ammonium sulfate, calcium nitrate, or urea in equivalent concentrations. Furthermore, nutrient uptake from solutions sprayed on leaves influenced root uptake of nutrients (Thorne 1954).

Foliar sprays of N and P on wheat were given at different stages of the crop and the response with respect to the soil water content was also examined (Alston 1979). Nitrogen sprays given after ear emergence, as a supplement to soil application of ammonium sulfate resulted in increased grain yield when the soil moisture was adequate. When phosphorus was also included in the spray liquid containing N the yield was increased much higher. Liquid foliar fertilizer (“Agroleaf” Scotts Co., OH, USA contains NPK at 20:20:20) at 0.3% was sprayed twice a week on pea plants (Hristozkova et al. 2006) which increased the nitrate reductase and glutamine synthetase in the shoots especially when Mo was supplied.

The effectiveness of foliar sprays at certain critical growth period has been discussed (Kannan 1990). Experimental results with maize to find out whether foliar N, P, K, and S at the grain development stage would be beneficial were not supportive of the hypothesis (Below et al. 1984). Soybean is classified as a “self-destructive” crop requiring large amounts of N during the “seed-fill” period. Experiments with foliar sprays of N, P, K, and S during the R-5 and R-7 stages of soybean showed positive results (Syverud et al. 1980). Several studies have been made recently on the effectiveness of foliar sprays at specific growth periods. One such study was to find out the effectiveness of foliar sprays of nutrients for increasing the duration of the flowering stage of milk thistle (*Silbum marianum* L.) so that seed yield and the silymarin content could be increased (Geneva et al. 2008) Soil and foliar application of fertilizers and growth regulators together were compared and it was found that yields could be increased when nutrients and the growth substances Pix and Regalis were given as foliar sprays, while soil application of nutrients with Pix alone and not Regalis, produced greater yields. Pix is mepiquat chloride, BASF; and Regalis is Prohexadione-Ca, BASF. However, plant growth regulators with either soil or foliar application increased the total silymarin content equally.

In another experiment, Stancheva et al. (2008) examined the effects of Agroleaf, (1) “Agroleaf total” (N:P:K = 20:20:20 + microelements), twice during the vegetative growth stage on 20-days interval until the rosette phase; (2) “Agroleaf with high P” (N:P:K = 12:52:5 + microelements), before the blooming stage; and (3) “Agroleaf with high K” (N:P:K = 15:10:31 + microelements), after the blooming stage. Their study included the effects of growth regulator thidazuron on foliar versus soil fertilization, on the seed yield and silymarin content. Combined application of the fertilizers with thidazuron affected the growth, accumulation of nutrients (N, P, and K), nitrate reductase activity, reducing sugars and free amino acids content significantly. These changes were associated with altered flowering rate, enhanced seed ripening, and increased yield. Treatment of milk thistle plants with thidazuron in combination with foliar fertilizer increased seed yield due to an increase in the number of lateral stems, the number of flower heads and the seed fresh weight per flower head. Silymarin content in the seeds was also positively increased by using thidazuron. These results show that foliar fertilization is beneficial, when given at a given growth stage.

An interesting case relates to K application to cotton. K deficiency is widespread across the US cotton Belt over several years and it is noticed in the latter half of the season in a wide range of soils and amongst many cultivars. This deficiency is largely a result of increased use of N and also the introduction of high-yielding and faster-fruited cultivars, which need large amounts of K and the roots do not cope with this demand. Potassium deficiency is also due to a low K status of many soils. Cotton is more sensitive to low K availability than most other major field crops. Foliar application of K is very effective for countering late-season K deficiency quickly and efficiently. Significant yield increases from foliar-applied K were obtained in 40% of field trials with an average increase of about 75 kg lint/ha. KNO_3 was preferred to K_2SO_4 . Cotton plants respond very well for K sprays in Egypt. Three to four foliar sprays of K to be given during the first 5 weeks of boll development at 7–10 days intervals starting at the commencement of flowering are recommended. K given as KNO_3 or K_2SO_4 was equally effective (Eid et al. 1997). A comparative study of foliar versus soil application of K to “French” prune trees was made (Southwick et al. 1996). Their trials over 3 years revealed that four sprays of KNO_3 (20–22 L/tree were as effective as single annual soil application of KNO_3 at 1.4–2.3 kg/tree. It was also found that foliar KNO_3 sprays given four times throughout the growing season corrected incipient K deficiency and gave same or higher yields than soil application.

K requirement has increased recently in many regions because of periodic drought conditions resulting in soil compaction and poor soil availability. Soil K availability is very much reduced in the states of Missouri, Illinois, and Kansas, where the subsoils are highly clayey. Lesser amount of K was applied as fertilizer by the farmers, due to low commodity prices. Nelson et al. (2005) studied the response to foliar-applied K at several growth stages and the cost-effectiveness, for soybean in claypan soils. K as K_2SO_4 was given as a pre-plant soil at 140, 280, and 560 kg ha⁻¹, or foliar supply at 9, 18, and 36 kg ha⁻¹ at V4, R1-R2, and R3-R4 stages of development. The results showed significant increase in grain yield from 727 to 834 kg ha⁻¹ when K spray was given at 36 kg ha⁻¹ at the V4 and R1-R2 stage, but

when given at the R3-R4 stage, grain yield increased but not as high as at V4 or R1-R2. It was also revealed that foliar K could only be a supplemental option when the climatic and soil conditions are unfavorable to root uptake.

Muskmelon (*Cucumis melo* L. *Reticulatus* group) responds very well to foliar supply of K (Lester et al. 2005). In this crop, the fruit sugar content is directly related to K-mediated phloem transport of sucrose into the fruit. During the muskmelon fruit growth, soil fertilization was inadequate due to poor root absorption capacity. Under such conditions K supplement as potassium metalosate (24% K) through foliar sprays was very effective in improving fruit quality. Their conclusion was that carefully timed foliar K nutrition can alleviate the K deficiency effects on fruit quality and marketability. Furthermore, there were many additional benefits from the foliar spray of K. Fruits from plants, given the foliar K matured 2 days earlier, were firmer with higher K, soluble solids, total sugars, vitamin C, and beta carotene than those of control plants.

Calcium sprays are very effective on improving the quality of fruits (Kadir 2004). Trees (apple *Malus domestica* graft) were given one to eight sprays at 8.971 kg ha⁻¹. More than six sprays improved fruit quality, increase in fruit weight, size, appearance, redness, and less scald incidents. The ratio of soluble solids to titratable acidity was also increased. Fruit skin redness was the most significant effect and related to a linear increase in amount of Ca in fruit and leaf tissues. There was also an increase in K, Mg, P, and N in the fruit.

13.2.3 Response to N, P, K with Micronutrients

Foliar application of N, P, and K can include micronutrients also with good results on yield and quality of crops. The effects of foliar application of N-P-K mixtures with or without S, B, Fe, and Zn at V5-V8 stages on the oil and protein content in soybean were examined (Haq and Mallarino 2005). But the results were not consistent. In one trial increase in the oil and protein content was obtained, but in another, it resulted in decrease in protein content. By this combination of major and minor elements, there was no saving in the cost, which is likely to accrue when herbicide is included with the nutrients.

There are specific instances where a single major nutrient is combined with a micronutrient and again the results were not supportive of this combination. The effects of N and B sprays together were examined on soybean yield in the soybean production region of Mid-Atlantic Coastal Plain (Freeborn et al. 2001). In their experiments a few factors were examined, viz., the effects of (i) application rate and reproductive stage timing of N or B on seed yield; and (ii) cultivar, row spacing, or planting date on the response to N and B application at R3 stage. N was supplied to the soil at 0, 14, 28, 56, 84, 112, or 168 kg ha⁻¹. B was given to the foliage at 0, 0.14, 0.28, or 0.56 kg ha⁻¹ to either R3 or R5 stage. It was found that though the N and B concentrations in the leaf tissues were above the minimum required, maximum yield was not influenced by N or B. The results were disappointing, since foliar B was not needed when the soil supply was adequate for higher yields.

In contrast to the results with N and B, the sprays of both K and B were beneficial. In Arkansas, research showed that the K requirement of the fast-fruited and high-yielding cultivars of cotton far exceeded the uptake capacity of the plants from the soil. Furthermore, the root activity of these cultivars actually decreases during flowering and boll development. Various K compounds viz., KNO_3 , K_2SO_4 , $\text{K}_2\text{S}_2\text{O}_8$, and KCl, and buffers for the spray solution, including addition of B were evaluated (Howard et al. 1998). In another study, KNO_3 , or K_2SO_4 solution, unbuffered, or buffered to pH 6 and 4 were sprayed at 4.1 kg K ha^{-1} . A third study had combination of soil-applied and foliar-applied B and K. Foliar sprays were given as 93.5 L ha^{-1} with water at early flower or 2 weeks after flowering and repeated four times at fortnightly intervals. Yields from the 4 K sources were 10% higher than the control or unbuffered solution. Addition of surfactant (ethoxylated alkylaryl phosphate esters) to KNO_3 gave 5% more yields. Foliar application of $0.11 \text{ kg B ha}^{-1}$ plus 4.1 kg K ha^{-1} increased the yield by 13%. Foliar K solution either buffered or combined with B was a relatively inexpensive way to increase cotton yield. These treatments in various combinations brought about returns eight to ten times the cost of the chemicals used.

13.2.4 Response to Micronutrients

In Egypt, surveys were undertaken to identify crop nutritional problems during 1977–1995 and it showed that the crops in general suffered from micronutrient deficiencies and responded very well to foliar supply of the nutrients resulting in higher yields (Fawzi and El-Fouly 1998). The results of experiments with foliar application of nutrients in cotton in Egypt and other countries were presented in an interregional symposium (Oosterhuis 1997).

Iron is one of important micronutrients and foliar sprays of iron as chelates are effective in correcting chlorosis. In a recent study, the interaction of FeHEDTA given along with post-emergence broadleaf herbicides was examined (Franzen et al. 2003). Three herbicides, *acifluorfen*, *imazamox*, and *lactofen* were applied with or without FeHEDTA. At one location, Fe amendment lowered the yields with *acifluorfen* and *lactofen*. But yields were higher with Fe in the *imazamox* combination, although weed control was less effective. Therefore, the combination with herbicides is not recommended, and the chelate alone is beneficial.

Recently there has been some renewed interest in foliar application of Zn especially in view of increasing yield when given with bacterial fertilizers. Ebrahim and Aly (2004) studied the effects of Zn foliar application along with soil bio-fertilization on wheat (*Triticum aestivum* cv. Sakha 155) plants grown for 70 days in greenhouse under controlled conditions. Zn sprays were at 0, 25, 50, 100, 200 mg L^{-1} as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and the soil was inoculated with *Azotobacter chroococcum* (Ar) and/or *Azospirillum brasilense* (Am) isolates. All test attributes namely, mineral content, photosynthesis, metabolites, and dry matter accumulation, were enhanced by Zn at 25 and 50 mg L^{-1} , but at higher levels of 100 and 200 mg L^{-1}

these were reduced. The bio-fertilizers influenced the growth attributes, especially more so when Ar and Am, than either Ar or Am were used, with the higher levels of Zn, N, Mg, Mn, carbohydrates, and total soluble proteins were also increased in the shoot. These treatments enhanced $\text{Chl}_a + \text{Chl}_b$, photosynthetic activity, and IAA concentration in the shoot. Spraying a by-product of olive oil on rice *Oryza sativa* cv. Puntal increased the concentration of Fe, Cu, Zn, and Mn as well as the chlorophyll content, the grain yield, and grain protein content. The sprays enhanced the uptake of N and K by the plants from the soil (Tejada and Gonzalez 2004). This was because the by-product was not only rich in humic substances but also in both macro and micronutrients.

A combination of two or more micronutrients in the spray has also been beneficial. In a pot trial, one-year-old apple trees were given a foliar spray containing MnSO_4 , ZnO, "Solubar," copper oxichloride and urea at rates respectively, of 1.0, 0.5, 1.0, 0.25, and 2.5 g L⁻¹ water, and soil-applied fritted trace elements (FTE). The carrier used (FTE-504Fe®) contained the micronutrients Mn, Zn, Cu, and boron, and mixed with the growing medium of sand before planting, and was given at 0, 100, and 200 g m⁻³ of sand. Where foliar sprays were given with soil FTE at 100 g m⁻³, the total dry weight of the tree and that of the rootstock were significantly increased by 16% and 29% respectively over the control (Wooldridge 2002).

Response to foliar sprays of nutrient elements given at an appropriate time has been recorded in a few crops. B and Zn sprays given during the "prebloom" stage in apple yielded a very good crop (Stover et al. 1999). The treatments were given on the basis of a hypothesis that the sprays may accelerate recovery of the vascular tissue damaged by the cold winter. The following were the treatments: (1) sprays of B (22.8 mM), (2) Zn-EDTA (0.75 mM), (3) B + Zn-EDTA, (4) Zn-EDTA + urea (59.4 mM), (5) B + Zn-EDTA, (6) B, Zn-EDTA and urea. The treatments 1–5 were given at prebloom at 0.5 in. green stage, and the sixth one at the pink stage. In all treatments with B and Zn sprays, the yield was higher by 22–35% than the control. The authors attributed the high yield to greater retention of flower buds which would have abscised before anthesis but for the treatments.

There has been limited study on the spraying of elements which are not nutrients per se. One such is titanium. The effect of foliar sprays of titanium (Ti) on vigor, fruiting, and quality and the storage life of apple (*Malus domestica Borkh*) was examined (Wojcik and Klamkowski 2004). The experiment was carried out in 2000–2001 on mature "Szampion" apple trees. The trees were sprayed with TiCl_4 solution at the rate of 2.5 g ha⁻¹: (1) before blooming at the stage of green and pink bud; (2) during blooming, at the beginning of flowering and the petal fall; (3) after blooming, 1 and 3 weeks after petal fall; and (4) before fruit picking, 4 and 2 weeks before commercial harvest. However, the sprays did not affect the vigor, fruit set, yield, and appearance. But it increased the leaf Ti, when given 30, 60, and 90 days after full bloom. Ti sprays given before harvest and 90 days after bloom enhanced Ti content in the leaf and fruit tissues. They did not get the desired anticipated effect of Ti spray under conditions of optimum nutrition. When Ca was included in the spray with Ti^{+4} ascorbate, the quality of plum was improved (Alcaez-Lopez et al. 2004).

These authors sprayed soluble calcium (Ca) on plum trees in combination with two bio-activators containing Ti^{+4} ascorbate alone or with marine algae extract, and examined the commercial quality of fruits, with respect to their resistance to post-harvest handling damage. They found that all the treatments containing Ti increased tree development and fruit size. At harvest, the fruits from the Ti-sprayed trees showed increased resistance to compression and penetration, but decreased the weight loss during post-harvest storage. Furthermore, the external red color was improved and the color parameters remained more stable during storage than in the control. Ti significantly increased Ca, Fe, Cu, and Zn concentrations in both the peel and the flesh. It was concluded that Ti facilitated greater absorption and translocation of minerals and the assimilation processes. Both the experiments gave different effects of Ti sprays, which was due to the fruit trees tested, i.e., one is apple and the other is plum.

13.2.5 Varietal Differences in Response to Foliar Application

The response of crop plants to foliar supply of nutrients has been different amongst crop species as well as cultivars within the same species and has been well-documented (for review see Kannan 1990). Here only a few are described. The differences in poinsettia for Mo deficiency stress within cultivars were observed (Cox 1992). Of the six cultivars studied, “Gross Supjibi” “Peace Regal Velvet” and “Peace Noel” showed no deficiency symptoms when grown in minus-Mo nutrient medium and thus are Mo deficiency-stress-tolerant. Similarly, there were varietal differences in soybean for Zn-deficiency tolerance and they also responded to foliar Zn application differently (Rose et al. 1981). Soybean crop was sprayed with $ZnSO_4$ before flowering and at one site, Narrabri, a single spray at 4 kg ha^{-1} gave 13% yield increase while at another two sites Trangie and Breeza, two sprays increased the yield by 57% and 208% respectively. One of the varieties, Forrest was the most responsive to the sprays. Results further revealed the differential sensitivity to Zn deficiency and there was good response in crops even though they did not show any deficiency symptoms.

The results of work described in this section provide evidence that the foliar supply is effective in crop yield and also smaller amounts of nutrients can be given instead of large amounts given to soil. A few of the conclusions are that N, P, and K foliar sprays are effective especially when given at the appropriate time. Urea is transported and stored when given before the onset of fall in fruit trees. Foliar application of N as post-pollination sprays of urea gave very good grain yield and highest grain protein in cereals. However, it is not possible to supply the entire N requirement of a crop through sprays. A 50:50 combination of soil and foliar fertilization has been found to maintain tree productivity as also reduce soil pollution. Age of the leaf is important; the younger leaves are capable of greater absorption than a mature leaf. Results of foliar sprays of N, P, K, and S during the grain development stage in soybean have been not uniform. Excessive soil supply of N induces deficiency of

elements like K as obtained in cotton. Significant yield increases were obtained from foliar sprays of K in such conditions. In many regions where there is periodic drought which reduces K availability in the soil, foliar K has given significant increase in grain yield in soybean. Sprays are effective in crops grown in clay soils where K is bound by the clay complex. The case of muskmelon is interesting. During the fruit growth soil fertilization of K was inadequate and K supplement through foliar sprays was very effective in improving fruit quality. Foliar spraying of micronutrients has been very effective in correcting deficiency, especially in Fe. There are differences amongst crop cultivars in their response to foliar application of micronutrients.

13.3 Mechanisms of Foliar Absorption of Inorganic Nutrients

13.3.1 Leaf Components and Foliar Uptake: The Cuticle

The outer surface of the leaf is covered by a cuticle which envelopes the entire leaf including the stomatal pores, epidermal hairs, or trichomes. In the periods of the 1960s, not much was understood on the nature of cuticle, and also in relation to its function for solute transport. Much later, Wattendorff and Holloway (1980) studied the structure of the cuticle of *Agave americana* L. and identified six layers: first, the epicuticular wax, then the cuticle proper embedded within an external and an internal layer, and also an exterior and an interior cell wall. But none of these layers have distinct identities, since their boundaries merged with one another. The properties of the cuticle are important for the nutrient uptake by the leaves. The formation and development of the cuticle are continuous with the growth of the leaf or fruit. The property of the cuticle is modified during ontogenesis. The cuticular wax of the adaxial surface of apple leaves was analyzed for the chemical composition, micromorphology, and hydrophobicity just as the leaf unfolded (Bringe et al. 2006). With the increasing age of the leaf, the hydrophobicity of the adaxial leaf surface decreased significantly. The contact angle that is made between the leaf surface and the solute droplets also decreased, thus facilitating greater absorption of solutes placed on the leaf. It was also seen that the amount of apolar cuticular wax per unit area was lower in the old leaf, $0.9 \mu\text{g cm}^{-2}$, while the young ones had higher amounts ($1.5 \mu\text{g cm}^{-2}$). This also indicates that older leaf will be less resistant to solute absorption assuming that more wax would offer greater resistance to solute entry. The authors have obtained evidence for the first time that the epicuticular wax contained tocopherol.

13.3.2 The Trichomes

The cuticular surface has structures referred to as “trichomes” which are uni- or multicellular projections of different shapes. Trichomes and stomata occur between ordinary epidermal cells which are also covered by cuticle.

The thickness of the cuticular membrane varies with its location. It is thinner over the periclinal walls and thicker between the anticlinal walls of the epidermal cells. The cuticle undergoes changes in shape and structure and the deposition of epicuticular wax continues with the growth of the organs (Miller 1986). Furthermore, the cuticle remains hydrophilic in the early growth, but becomes hydrophobic with maturity, and there is no further change when the growth ceases. The permeability of the cuticle to solutes also varies with the development of these properties. The presence of an inner cuticle which forms a uniform layer on the inner periclinal walls bordering substomatal cavities was observed (Pesacreta and Hasenstein 1999). This cuticle is continuous with the external one through the stomatal pores. "Abaxial internal cuticle" refers to the cuticle surrounding the substomatal cavities of the abaxial epidermis. Their observations showed that the internal and external cuticles formed a continuous hydrophobic envelope around the epidermis excepting the regions where it is connected to the underlying mesophyll parenchyma cells and also over the midvein. The authors claim this as the first report on the existence of an extensive internal cuticle. The physiological role of the internal cuticle may perhaps be to prevent water loss. They also found that the epidermal cells secreted cuticular material on their anticlinal and periclinal walls and the noncuticularized region of the wall could function as the region for transmitting information on the water potential of the leaf. In species with large islands of epidermal cells covered by the internal cuticle, information to reach the guard cells would take a longer time. Leaf internal cuticle has not previously been studied in detail, and yet its existence has profound implications for the path of water movement. The cuticle also plays an important physiological role during the development of fruit from the time of fertilization. While it prevents water loss, it provides the barrier for any infection by microorganisms. The development of the cuticle follows the same pattern as that of the leaf. Dominguez et al. (2008) studied the tomato fruit cuticle at the microscopic level during its growth and ripening and recorded the differences in cuticle thickness and composition from the time of epidermal differentiation, changes in the distribution of the lipid, pectin, and cellulose within, appearance of pegs and cuticular invaginations, the thickness of the cuticle, and the polysaccharide components. The amount of cuticle per surface area increased with the fruit development, reached its maximum in about 15 days after anthesis, and then remained constant till the fruit ripening. There was also loss of polysaccharides from the cuticle, beginning with the ripening of the fruit till the development of red color. The chemical composition of the cuticle would greatly influence its permeability to solutes.

The cuticular composition plays an important role in its permeability properties. However, it is redundant to discuss the cuticular composition studied by several workers recently. A brief mention is made here. Wen et al. (2006) examined the leaves of *Taxus baccata* L., which comprised needles covered with tubular epicuticular waxes which varied in diameters and lengths. The cuticular wax was a mixture of long chain fatty acids, phenyl esters, alkanes, and tocopherols. While the epicuticular layer had aldehydes and alkanes, the intracuticular wax had higher amounts of cyclic constituents. These could help explain the differences in the rates of permeability of polar and nonpolar solutes. The formation of the tubular crystals

on the cuticles as a spontaneous physico-chemical process may be relevant with respect to the establishment of gradients between the epi- and intracuticular wax layers and local phase separation of solutes.

Several features of the trichomes have been recorded (Grauke et al. 1987). The bulbous base of the trichome extends over the entire thickness of the epidermal cells, with the basal accessory cells bulging around the trichome. The cuticle development at the base of the trichomes is less than in the rest of the regions of the trichomes, and greater absorption of solutes is likely through these basal regions, a feature favorable for better foliar uptake by the leaves. It is found that in the leaf of *Cannabis*, new initials of trichomes arise regularly, maintaining a nearly constant density. The presence of four morphologically and ontogenetically different glandular and non-glandular trichome types and a bristle hair type have been established recently (Kolb and Müller 2004). They found that all these four types secreted lipids, flavones, and terpenes and some cell wall components. The changes in the trichomes during leaf development, especially the number of trichomes and the composition of the exudates have also been investigated (Valkama et al. 2004). Density of both glandular and non-glandular trichomes decreased drastically with leaf expansion, although their numbers per leaf remained constant, showing that the final number in a mature leaf is established early in time. However, the functional role of trichomes are likely most important at the early stages of leaf development. Perhaps this property may explain the differences in foliar uptake of different ages of the plant leaves.

13.3.3 Pores and Ectoteichodes

The existence of wax-exuding pores and channels in the cuticle was postulated long ago and even the existence of anastomosing microchannels for the extrusion of wax. The presence of pores and canals in the dewaxed leaf stem and fruit cuticles was revealed through photomicrographs (Miller 1986). The transcuticular canals are found oriented perpendicular to the outer and inner membrane surfaces and terminate as discrete pores, in the adaxial leaf cuticles of *Hoya carmosa*. The presence of giant pores in the cuticles has been recently identified in certain plants found in Australian Wet Tropics (Carpenter et al. 2007). The pores are large in diameter and ubiquitous, not previously recorded in leaf cuticles. Such structures have been found in *Eidothea zoexylocarya*, about 1 μm in diameter that extend perpendicular to most of the way through the cuticle from inside. They also observed that these occurred on both sides of the leaf, but were significantly absent in the cuticle associated with stomatal complexes on the abaxial surface. The pores on both the abaxial and adaxial inner cuticular surfaces were present on all specimens of *E. zoexylocarya*, although their positions could not be discerned on the outer cuticle surface using SEM. The pores were abundant ($1.2 \times 10^5 \text{ mm}^{-2}$) in the cuticle associated with the normal epidermal cells. On the abaxial surface these were absent from the cuticle associated with the guard cells and the subsidiary cells, as also in the cuticle overlying the cell immediately

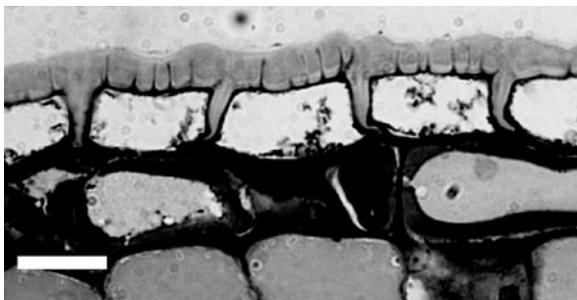


Fig. 13.1 Adaxial transverse leaf section of *Eidothea zoexylocarya* showing the pores extending perpendicularly down through the cuticle (Reproduced with the kind permission of the authors (Carpenter et al. 2007) and the editor American Journal of Botany)

lateral to the subsidiary cells. In the surface view through the light microscope, the pores appeared as canals penetrating across the cuticle. Transverse view of the cuticle showed the pores reaching nearly all the way through the 4–8- μm thick cuticles from the inside. Pore diameters were nearly uniform in diameter, and were mostly perpendicular to the leaf surface. The conductance of the astomatous cuticular leaf surface was measured to find if they are more leaky to water vapor, and it was however very low, suggesting no role in the conductance of water vapor. Their studies did not provide any role for these cuticular pores, either in the transport of water or solutes, however (Fig. 13.1).

Structures in the cell wall of wheat leaves, named “Ectoteichodes” were identified and considered as pathways for solute transport across the cell walls, and the permeability of ions through the cuticle is also considered to be facilitated by these structures which were visible microscopically (Franke 1971). Ectodesmata or ectoteichodes were demonstrated in epidermal walls of mesophytic plant species using methods of fixation with Gilson solution (Schönherr and Bukovac 1970). Whole leaves or leaf segments were fixed for 12 h at 38°C and the epidermis was stripped off, washed in 30% ethanol to remove HgCl_2 , treated with potassium iodide for 5–10 min, and then stained with pyoktanin and the tissue was then examined microscopically. After this procedure, ectodesmata appeared as dark bands from the cuticle toward the protoplast of the epidermal cells. If potassium iodide and pyoktanin were omitted from the treatment, ectodesmata appeared crystalline and birefringent, in polarized light. Cuticles over anticlinal walls form plenty of ectodesmata, while they are rarely found over periclinal walls. The effect of removing waxes on the distribution pattern of ectodesmata indicated that waxy domains are impermeable to HgCl_2 even though it dissolved in benzene. Perhaps crystalline wax domains are impermeable to HgCl_2 while amorphous lipids are not. This would imply that ectodesmata are formed in the cell wall wherever cuticular waxes are in the amorphous state and crystalline waxes are scarce. The authors are not fully convinced that ectoteichodes are discrete entities in the cuticles but could form under the influence of some treatments.

13.3.4 Cuticular Permeability of Nutrients

Perhaps the most important function of the cuticle is to protect the plant from desiccation by preventing the loss of moisture from the leaf surface. However, it is not impermeable to water and water is lost to some extent through cuticular transpiration. The sorption and retention of water is an important property of the cuticle. Chamel et al. (2006) measured the sorption of water by cuticular membranes isolated from leaves over a wide range of relative humidity using a “magnetic suspension microbalance.” The sorption isotherms were not linear but increased more rapidly at higher values of relative humidity. The water content was measured for a few species at 80–99% and it ranged from 1.1% to 7.7% of the dehydrated weight. The sorption did not decrease even after extracting the soluble cuticular lipids from the cuticle. This may partially explain how the humidity favors foliar absorption of aqueous solutions.

The permeability properties have been studied with the help of radioisotopes using cuticular membranes isolated enzymically from the leaves and fruits in several laboratories in the early 1960s and later in the 1980s. Penetration studies and ion-binding properties of enzymically isolated cuticular membranes were carried out using special apparatus and radioisotopes in the 1960s. Cuticles from the adaxial surfaces of grapefruit leaves were isolated (Orbovic et al. 2001) and the movement of ^{14}C -labeled urea was measured for several hours, using a “dose diffusion” system developed by them. It was found that within the first 4–6 h of application, the rate of penetration increased with the increase in temperatures from 19°C upto 28°C, and other factors, viz., relative humidity, cuticle thickness, and the contact angle of the droplets placed on the cuticle surface all influenced the penetration. The same group (Bondada et al. 2006) made further studies on penetration of ^{14}C -labeled urea from the upper cuticular surface to the inner side and it was found to follow asymptotic curve, with an initial lag phase of about 10 min, and a quasi-linear phase reaching a rate of 2% h⁻¹, then a plateau at 144 h. The total amount penetrated was 35%, and the rate decreased with the thickness of the cuticle. They found the epicuticular wax appeared as platelets which increased with the leaf age. Furthermore, dewaxing the cuticles increased the rates of penetration of urea with the maximum of 64%. The findings on the citrus leaf cuticles are important with respect to determining the time of foliar fertilization.

The permeability of the astomatous cuticular membranes of *Populus canescens* leaves to nutrient ions has been investigated in detail (Schönherr and Schreiber 2004). These leaves have trichomes when very young, but these are shed with the growth of leaves to full size. Cuticles enzymically isolated from the adaxial surfaces of fully expanded leaves were used. The ionized Ca salts with anhydrous molecular weights ranging from 111 to 755 g mol⁻¹ were employed. The penetration was found to be a first-order process with the rate constants (k) decreasing exponentially with mol. wt. They concluded that there were differences between the diffusion of large ionic species through the aqueous pores (polar pathway) and that for neutral solutes diffusing through cutin and waxes (lipophylic pathway), and recommended the formulation of large solutes as ionic species for effective sprays.

Further research on the polar paths of diffusion of ions revealed that ions penetrated cuticles via water-filled pores and the cuticle covering the stomata and trichomes served as preferential sites for ion entry (Schreiber 2005). They have given a future direction of research in entry of solutes through cuticles. The chemical nature of these polar domains have yet to be characterized, as it is important in agriculture, since polar diffusion is the most important and faster route for the entry into the leaf and later translocation of the nutrients. The study relating to cuticular entry has great significance now more than ever before, since many compounds which are used for developing the transgenic plants are ionic, and should be suitable for penetrating through the cuticle. Plants in the field are in several places exposed to acid precipitation with the pH of the pollutant reaching to values of 4 and below. However, the leaf tissues are affected only when the leaf cuticle permits the entry of the ions.

The information about the cuticles and their permeability properties as revealed by several studies made earlier have been summarized recently (Kersters 2006). There have been major advances on the quantification of cuticular permeability to water and its dependence on leaf temperature. The roles of epicuticular and intracuticular waxes as also the aqueous pores have been examined. But the differences in permeability properties of cuticles amongst the plants remain a big challenge. The water transport in lipophilic pathways and the aqueous pores depend on the humidity and temperature. Cuticles are considered to act as solution-diffusion membranes for the entry of water. Kersters concludes that many ecophysiological relevant questions remain unanswered. Some of these are: What influences the composition and formation of different forms wax, and how does it affect the cuticular permeability? "What is the temperature dependence of 'P' in situ? How large is lateral variability in P at the cellular and subcellular levels, and how does it affect stomatal behavior?" A new term is introduced as P which is the cuticular permeability to water characterized by the variable permeance (P), and is the ratio of water flow rate density to driving force, the latter being expressed as a concentration difference.

Schönherr and his group had carried out extensive research on the permeability properties of cuticles over 3 decades. In one such study, the penetration of ^{54}Ca labeled CaCl_2 by the upper and lower surfaces of apple, pear, bean, and corn leaves was measured using the leaf disks of these plants (Schlegel and Schönherr 2002). Very significant observations were made. It was found that the penetration of ions was slower across astomatous leaf surface than through the stomatous one, and the half-time for penetration through the latter ranged from 0.5 to 9 h. This and other findings led them to conclude that initial entry of solutes was through stomata, but further penetration was across the cuticle serving as the major pathway. The appearance of black silver precipitates in the cuticle present inside and over the guard cells, as also in the trichomes and the cuticle surrounding their base, proved that the ionic species essentially entered into the leaf via the same route, i.e., the cuticle present all over the leaf. Schönherr (2002) examined permeability of salts of Ca and K which have hydration shells and found that both cations and anions entered the cuticles in equivalent amounts by diffusion through the aqueous pores and is influenced by humidity and the hygroscopicity of the salts. The point of deliquescence of the salts is important for the diffusion. Nitrates and chlorides of Ca and Mg,

and K_2CO_3 have greater permeability but not salts like nitrates and phosphates of K. The driving force for the diffusion is the concentration difference across the cuticular membrane and most of the nutrient salts excepting $MgCl_2$ have high aqueous solubility and thus have greater permeability. Humidity is very important which increases the solubility and therefore spraying is most effective if given in the evenings.

Schreiber and Kerstiens (2006) described the work of Schönherr and his group and about the papers presented in a week-long symposium held near Izmir in Turkey on the occasion of his 65th birthday and retirement in March 2005. Former students and colleagues are now working in Germany, Switzerland, and the UK, on various aspects of transport across cutinized and suberized barriers present in the cuticles. "One person who has immensely improved our understanding of such transport barriers is Professor Jörg Schönherr. Since the 1970s he has been investigating the transport properties of cutinized and suberized barriers at the plant/air interface from 1970s. His group focused initially on ecophysiological questions, dealing with water permeability, and then turned on to the ecotoxicological and agronomic questions of the uptake of xenobiotics and agrochemicals into leaves. In the last few years, Schönherr's contribution to our knowledge on the cuticular components, and their permeability to agrochemicals have been remarkable and have been acknowledged by several workers, particularly Schreiber and Kerstiens. Schönherr's research group have been investigating the transport across the cutinized and suberized barriers at the plant-air interface from 1970s and have greatly enhanced our understanding of foliar uptake of polar compounds, viz., ions and hydrophilic organic molecules state Schreiber and Kerstiens. It has been shown that the cuticle over the trichomes and guard cells differs in structure and permeability over the rest of that covering the leaf. Ions are lipid-insoluble and therefore enter through an aqueous pathway present in the cuticles. Aqueous pores are largely present in the cuticular ledges, at the base of trichomes, and also in the cuticles present over anticlinal walls. Permeability of cuticles to ions depends on humidity and reaches the maximum at 100% humidity. Wetting agents increased the rates of penetration, indicating that the pore openings are surrounded by waxes. The pores in cuticular ledges of *Helxine soleirolii* allowed the entry of high molecular weight compounds, viz., berberine sulfate (MW 769 g mol⁻¹). And finally, Schreiber and Kerstiens suggest a method for the future quantification of changes in leaf or stem surface barrier properties of transgenic plants with modified cutin and/or wax biosynthesis. The set of papers presented in the meeting and published in the journal of botany documents the advances in understanding barrier properties of cutinized and suberized barriers of plants that have been achieved within the last couple of decades. Recently the biochemistry of cuticular wax formation by the epidermal cells mainly studied in *Arabidopsis thaliana* and complemented with those obtained from other plant species, has been reviewed by Samuels et al. (2008). The plant cuticle is described as a hydrophobic layer covering the epidermis and forming a continuous seal over the outer walls of the epidermal pavement, guard, and trichome cells. The ultrastructure of the cuticle varies widely among plant species, organ types, and the developmental states. It ranges from a procuticle on emerging organs to a mature one that gets completed after tissue expansion has ceased. All cuticles fall

into two types of highly lipophilic substances, viz., (1) cutin and (2) cuticular wax. Because of the covalent linkages between its monomers, cutin offers resistance to mechanical damage, for example, the leaf itself. The cuticular wax is monomeric and can be extracted by organic solvents. Its main function is to prevent nonstomatal water loss, one of the key adaptations in the evolution of land plants.

This chapter discusses leaf components such as cuticular membranes, trichomes, cuticular pores, the presence of Ectoteichodes, and their properties and their role in nutrient transport into the plant leaf. Cuticles are permeable to nutrient ions present in aqueous forms and it is not known if the cuticular pores facilitate easy entry into the leaf cells. The trichomes increase the amount transported into the leaf by providing more area for absorption. All cuticles have two types of lipophilic substances, the cutin and the cuticular wax, which influence the permeability of the cuticles to nutrient ions to varying degrees.

13.4 Pathways of Nutrients Following Foliar Uptake

Foliar absorption of inorganic nutrients involves a number of steps. The ions after passing through the cuticular membranes gain entry into the leaf cells. This process is mediated by energy and is similar to the absorption by root cells. Many micronutrients which are easily supplied to the plants through foliage for correction of the deficiency, behave differently with regard to the absorption compared to the major nutrients like N, P, and K. Some are easily mobile and others less so. Zinc comes under the less freely mobile element. The uptake pattern of Zn was measured using ^{65}Zn , in both intact and detached leaves of pistachio and walnut (Zhang and Brown 1999). They found that the mature leaves of pistachio and walnut retained 8% and 12% of the amount applied on the leaves and about half of these moved into the leaves and translocated out of the applied area. There were differences in the absorption amongst the age of the leaves especially in pistachio, in which the immature (young) leaves absorbed more than the older ones. Such differences were not obtained in walnut leaves. The uptake was higher at pH 3.5 and decreased with rise in pH in pistachio, while no significant difference was noticed with walnut. Furthermore, the uptake was not influenced either by light or by the metabolic inhibitors, suggesting that the process is a passive one. These are significant findings on the mechanism of foliar uptake of Zn.

13.4.1 *Transport of Nutrients in and out of the Leaf*

After passing through the cuticular membranes, the nutrient ions enter the epidermal cells and the mesophylls beneath. The space between the leaf cells forms a continuum, thus providing the “free space” or apoplast. The free space is relatively small and varies for different plant leaves. This accounts for about 3–5% of the total volume in the leaf tissue of wheat (Crowdy and Tanton 1970). The apoplastic concentrations of K and Ca in the regions adjoining the guard cells of *Commelina*

communis were high, ranging from 50 to 75 mol m⁻³ for K⁺ and 0.05–4 mol.m⁻³ for Ca²⁺ (DeSilva et al. 2006). These two cations play an important role and contribute significantly to the intracellular signaling and response of the guard cells toward the changes in stomatal aperture.

The ions after passing through the cuticular membranes gain entry into the leaf cells. This process is mediated by energy and is similar to the absorption by root cells. It is necessary to understand therefore the steps involved in the foliar uptake of nutrient elements which are reviewed in the following sections. While the major nutrient elements are taken up readily and transported to other parts, the micronutrients behave differently. Some are easily mobile and others less so. Zinc comes under the less freely mobile element. The uptake pattern of Zn was measured using ⁶⁵Zn, in both intact and detached leaves of pistachio and walnut (Zhang and Brown 1999). They found that the mature leaves of pistachio and walnut retained 8% and 12% of the amount applied on the leaves and about half of these moved into the leaves and translocated out of the applied area. There were differences in the absorption amongst the age of the leaves and also plant species. In pistachio, the immature (young) leaves absorbed more than the older ones. But such differences were not obtained in walnut leaves. The uptake was higher at pH 3.5 and decreased with rise in pH in pistachio, while it was not influenced either by light or by the metabolic inhibitors, suggesting that the process is a passive one. However, no significant difference was noticed with walnut with respect to the pH.

The nutrients take two routes to reach the vascular tissues for transport to other parts and one is the apoplast as mentioned earlier. The second route is the transport through the symplast, which takes place between the cells through the cytoplasmic continuum. The mechanisms of apoplastic and symplastic transport of ions are not fully understood, and our knowledge is largely a conjecture and assumed to be similar to the transport of assimilates from the leaf cells. Structures like “plasmatabule” in the transfer cells of leaf minor veins of *Pisum sativum* L. have been associated with the transfer of solutes between the leaf cells and then to vascular systems (Harris and Chaffey 1985). Plasmatabules were found in the transfer cells and the specific distribution of the plasmatabules reflected further membrane amplification within the transfer cells for the movement of solute from apoplast to symplast. Plasmatabules appeared as tubular evaginations of the plasmalemma and are found in the regions of high solute flux between apoplast and symplast (Chaffey and Harris 1985). Franceschi and Giaquinta (1983) also identified a highly specialized layer, called the paraveinal mesophyll (PVM) in the leaves of some legumes. The PVM is a one-cell layer forming a network in the phloem region, and are non-photosynthetic tissues interspersed between the palisade and spongy mesophylls. It is positioned in such a way that the photosynthates produced passed through this region before reaching the phloem.

The cell-to-cell communication in leaves was studied using fluorescent probes, e.g., 6-carboxyfluorescein and Lucifer yellow CH, which do not pass through plasmalemma and are thus ideal to reveal the nonplasmatic connections (Erwee et al. 1985). The dye moved freely in the epidermal cells of *Commelina cyanea*, showing that they are symplastically linked, as are those between the mesophylls cells.

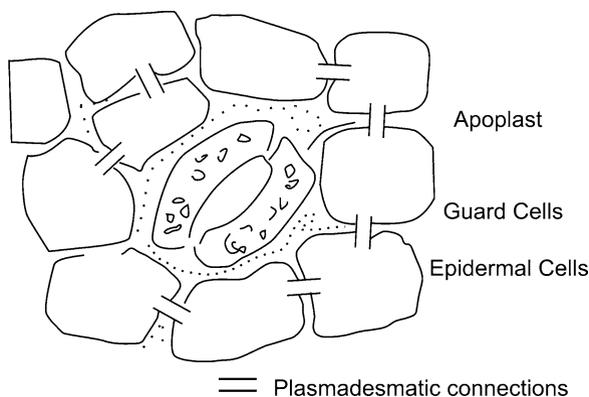


Fig. 13.2 Diagrammatic presentation of the epidermal cells with stomata, guard cells, and the plasmadesmats between them and guard cells. Thus, ions like K absorbed by guard cells move between the guard cells but not into the epidermal cells. Note the lack of symplastic connections between the epidermal cells (Drawn after Erwee et al. 1985)

But injection of the dye into the epidermal cells of the leaves of *Vicia faba* and *Antheaphora pubescens* showed poor symplastic connection between the guard cells and epidermal cells. Mesophyll cells are well linked by plasmadesmata (Ringoet et al. 1971). Erwee et al. (1985) made an important finding that the guard cells themselves are isolated from cell-to-cell communication with the epidermal cells in the leaves. Thus, ions such as K absorbed by guard cells move between the guard cells, but not the epidermal cells, due to the lack of symplastic connections (Fig. 13.2).

Similar study on the movement of Lucifer Yellow introduced into the leaf was followed by fluorescence microscopy (Farrar et al. 1992). They found that dye transfer from mesophyll cells into the parenchymatous bundle sheath (PBS) and from PBS to mesophyll sheath occurred readily. In another experiment when fluorescent peptides and dextrans were injected into the cytoplasm and cortical endomembrane network of the epidermal cells of *Nicotiana tabacum* and *Torenia fournieri*, the endomembrane network was similar to the endoplasmic reticulum and no cell-to-cell movement of dextrans injected into the cytoplasm was obtained (Cantrill et al. 1999). Thus it was concluded that the endoplasmic reticulum acted as a pathway for intercellular communication through the desmotubule and plasmadesmata. In another experiment, “thin cell layer explants” of tobacco were cultured to produce adventitious vegetative shoots and a fluorescein isothiocyanate-labeled peptide (F(Glu)3 MW 799) was microinjected on the epidermal cells to assess the permeability of the symplast during the adventitious shoot regeneration. A period of increased symplastic movement of the dye was detected and was greater in the regenerating layers than in the nonregenerating TCLs. This led to the conclusion that the symplastic linkage was reinitiated with the first line of cell divisions (Cantrill et al. 2001). From the point of view of the ion movement within the leaf tissues, symplastic transport is established.

13.4.2 *Transport of Foliar Applied Nutrients to Other Plant Parts*

The nutrients reach the apoplasts after penetrating the cuticular membranes of the leaf surface and are absorbed by the leaf cells. The apoplasts and the symplasts are common to the ions absorbed either from the foliar sprays or by the roots and transported to the leaf in the normal course. It is therefore likely that the apoplast plays a major role in the various intercellular processes, viz., water as well as nutrient distribution. There have been a few studies on the long-distance transport of nutrients from the sprayed leaves to other parts. The transport of micronutrients, viz., Fe, Mn, and Zn is generally much slower than N, P, or K. These in chelated forms are transported more readily to other parts. Bukovac and Wittwer (1957) used radioisotopes and classified the rates of mobility of the elements in the following order: Freely mobile K, Na, P, Cl, and S; partially mobile are Fe, Mn, Zn, Cu, and Mo; and immobile are Ca and Mg. Ca is very important for the development of fruits. However, it is absorbed directly by the fruits, and foliar spraying on the plants to cover the fruit surfaces is the best method for correcting Ca deficiency. There have been two significant contributions to transport of foliar-applied nutrients. One is the movement of anion ^{36}Cl which was applied to a leaf of *Tradescantia viridis* and transport to the leaves above and below as well as the stem portions between the leaves was followed (Penot and Gallou 1977). They found large amount transported to the new leaf (bud leaf No.1) and to the stem No.1. However, larger amount was still found in the applied leaf. The data showed that the anion is rapidly transported more or less evenly to all leaves and stem above and below the applied leaf and also to the root, over a period of 3 h (Fig. 13.3). Another finding relates to the transport of relatively less mobile micronutrient Zn and very little is known about its transport from leaves to other plant organs. A few forms of Zn for suitability to provide enough Zn were examined in wheat *Triticum aestivum* (Haslett et al. 2001). ^{65}Zn was applied to either leaves or to the root and the transport was followed. The forms were ZnO, ZnSO₄, Zn chelates. Foliar supply of Zn in general increased its content in shoot. It was translocated to leaves above and below the treated leaf as well as to the toot tips. Their experiment by girdling the stem proved that Zn was transported from the leaves to the roots via the phloem.

The most important nutrient given as spray is N which has the advantage of great mobility in plants. The findings of Okano et al. (1983, 1984) are significant. The translocation of ^{13}C and ^{15}N from the spray was very similar. N was transferred very rapidly in the first few hours and continued for 8 days. Both ^{13}C and ^{15}N moved together in the bulk stream of the phloem of the rice plant. N transport was examined by Tatsumi and Kono (1981). They found that 25% of N applied was translocated from the upper leaf to the roots. The study with fruit trees by Zilkah et al. (1987) revealed that foliar applied urea moved basipetally from the current flush of leaves to the developing fruits which acted as the “sink” for N.

From the foregoing discussion it is clear that nutrients reach the leaf cells, after penetrating the cuticle, and are further transported from them to other parts through

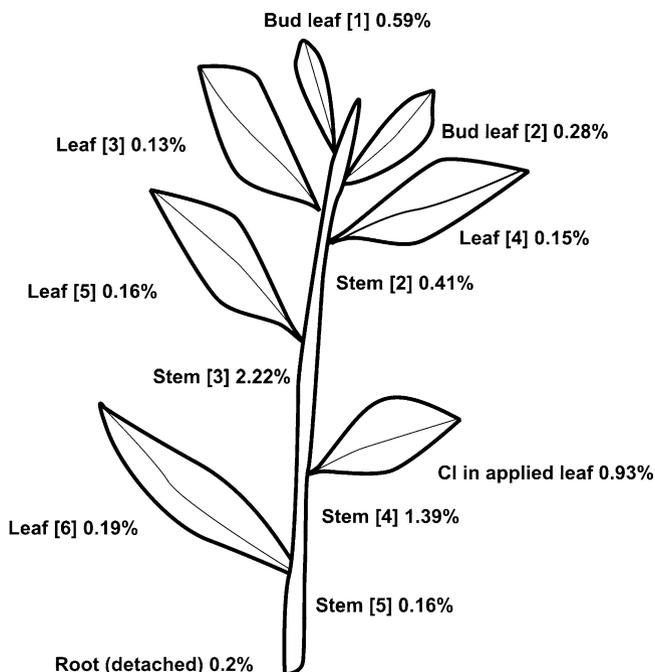


Fig. 13.3 Acropetal transport of ^{36}Cl from the supplied leaf to other leaves above and below in *Tradescantia viridis*. The amount of Cl found after 3 h of application, in the leaves are given as % (Redrawn after Penot and Gallou 1977)

plasmadesmata. Some micronutrients are not as freely mobile as the major nutrient elements like N, P, or K. The age of the leaf and the pH of the spray liquid are important for foliar absorption. Significant is the absence of plasmadesmatic connections between the guard cells and the epidermal cells. One element Cl has been found to be transported from the applied leaf to other parts rapidly, showing it is freely mobile. This should be true to many anions.

13.5 Foliar Nutrition and the Law of the Maximum

The resources needed for raising the food production are good soil, adequate water, and fertilizers. The land is becoming increasingly limited and all efforts should be made to raise the yield levels on the existing agricultural lands. To meet the food demands, the average yield of all cereals must be increased by 80%. Furthermore, using the currently available technologies, yields can be doubled in the Indian subcontinent, Latin America, and East European countries, and by 100–200% in sub-Saharan Africa, if three conditions, viz., political stability, entrepreneurial freedom, and all the production inputs are guaranteed. More food can be produced if new lands, i.e., lands that are hitherto considered unfit for supporting crops, are brought under plough.

Wallace and Wallace (2003) have discussed various methods for increasing crop production, and introduced the concept of “The Law of the Maximum” for closing the crop-yield gap. They have extrapolated both the concepts of the Liebig’s Law of the Minimum and Mitscherlich’s law of the Minimum and discussed them in his small book. “The greatest response for any one input is obtained when very few limits remain. Input factors interact to multiply the value of the other when other limiting factors are corrected. Limiting factors are multiple, and their interactions, which can be graphed to give Multiple Action Yield Fraction plots, determine final yields. The MAYF may be used to maximize specific aspects of crop production, such as per unit of land or per unit of irrigation water.” They have listed salient points needed for consideration to close the gap to achieve high yields: Increasing the MAYF by adding N, P, and K may be difficult because the Sufficiently Values of each may already be near 1.00. Attention to non-NPK factors is probably necessary for further improvement. Some non-NPK factors are quantity of soil, water, soil availability; many aspects of crop management are other possible limitations. The Law of the Maximum also requires attention to non-NPK factors. In this respect, foliar supply of a nutrient which imposes the limit to yield rise, becomes relevant. For example, as the yield at one stage reaches the plateau, it implies some nutrient is needed at that growth stage or fruiting stage. Supplying that by the roots will not reach the shoot for use in fruiting. Citing a case of cotton, K sprays at boll formation promptly respond to the timely need, and the yield is increased. Similarly, during the “seed-filling” period of soybean crop, N supply through sprays has been effective. Autumn sprays of N for fruit trees provide the nutrient when the new flush in spring season begins. Soil supply in these cases is less effective. This causes the yield to rise above the plateau. An interesting case is with regard to the increase in the atmospheric carbon dioxide, which happens with the burning of fossil fuel. This can be viewed as an excess of one nutrient, making some essential nutrient elements insufficient. It was found recently that supplying NO in the gaseous form increases crop yield and quality especially when atmospheric CO₂ is increased. Jin et al. (2009) grew spinach plant (*Spinacia oleracea* cv. Huangjia) in closed growth chamber to investigate the effects of gaseous NO application on vegetable production in greenhouses. Treatment of low concentration of NO gas (ambient atmosphere with 200 nL L⁻¹ NO gas) significantly increased the shoot biomass of the soil-cultivated plants as compared with the control treatment (ambient atmosphere). In addition, the NO treatment also increased the photosynthetic rate of leaves, indicating that the enhancement of photosynthesis is an important reason leading to more biomass accumulation induced by NO gas. Furthermore, the NO treatment decreased the nitrate concentration and increased the amount of soluble sugar, protein, antioxidants like vitamin C, glutathione, and flavonoids, and ferric-reducing antioxidant power (FRAP) in shoots of the plants grown in soil, suggesting that the gaseous NO treatment can not only increase vegetable production but also improve vegetable quality. In addition, the effects of the combined application of NO and CO₂ (NO 200 nL L⁻¹ and CO₂ 800 μL L⁻¹) on shoot biomass was even greater than the effects of elevated CO₂ (CO₂ 800 μL L⁻¹) or the NO treatment alone, implying that gaseous NO treatment can be used in CO₂-elevated greenhouses as an effective strategy in improving vegetable production.

13.6 Foliar Nutrition Through Sprinkler Irrigation

The development of drip-irrigation technology in 1960s opened the doors for the greatest advancement in fertilizer management especially for vegetables. Early work for example, in Israel (Bar-Yosef 1977) and later in other countries formed some of the basis for the water-management technology that is used today. Drip irrigation led to improvements in water application efficiencies and also reduced the amount of water used for many vegetables by 50–70% (Clark 1992). Furthermore, the reduction in water application has positive implications for nutrient efficiencies, especially N since it is closely related to irrigation efficiency in sandy soil production areas (Hochmuth 2000). Fertilizer materials could be injected into the drip irrigation system, a process referred to as “fertigation.” Soluble nutrients such as N and K are supplied through drip irrigation by injecting small amounts and are also regulated according to the seasonal crop requirement. Schedules for such injections were developed in Florida and other southern states (Cook and Sanders 1991). Water conservation technology has also been developed using many advanced technologies and remote monitoring with computers. One recent advancement is described below.

Writing on the ARS role to help conserve the vital resource for agriculture, Evans (2007) has presented a new system called “Wireless Watering.” (Fig. 13.4). He states



Fig. 13.4 Wireless Watering: The picture shows in-field sensing stations monitor soil moisture and soil and air temperatures. All in-field data are sent wirelessly to the base station for determining the precise timing for irrigation. The base station communicates back and forth with the mobile irrigation cart and the grower, who controls the station. This picture is included in this review to propose that nutrients could be given through the sprinkler water (Credit: Robert Evans, USDA-ARS Northern Plains Agricultural Research Laboratory, 1500 North Central Ave., m Sidney, MT 433–5038. Picture from Agricultural Research July 2007 page 6)

that worldwide, irrigation is the largest single consumer of freshwater, using up to 60% of this precious resource, and most of this is for growing food, animal feed, fiber, and fuel crops. Evans and Kim, who designed and integrated the wireless components, are still evaluating the full benefit of this system on the water saving, and also conserving fertilizers. This will help reduce the pollution of the groundwater and soil. They further state that if irrigation farming is to be profitable in future, such kind of innovations is important. Evans says: "Before, we were focused on how much productivity we could extract from a unit of land, now it is time to start thinking about how much we can wring from each unit of water consumed during the production process." Evans' idea can be further extended for the fertilizer application through foliar sprays.

13.7 Conclusion

The results of several studies discussed above reveal that foliar feeding is a practical method to provide nutrients to plants. However, there are limitations. The leaf area needed to accept the foliar sprays is the first requirement. Crops like sugarcane, plantation crops, and fruit trees satisfy this requirement. The absorptive capacity and effective transport from the leaf to other parts are important. Major nutrients could be given as sprays for providing the crops during "critical" periods like "grain filling" when the nutrients are needed the most for grain development. In such cases, soil application would take a longer time to reach the "sink" regions.

Soil fertilization often results in over-fertilization. As an example, increased nitrate content in the soil frequently leads to undesirable changes in the vegetation composition, since higher content in the edible plant parts is dangerous to human health. Foliar sprays are very effective in crops grown on degraded soil. Urea given as sprays to alfalfa grown in a freshly exposed coal mine spoil have given high yields. These reports have been discussed in this review.

Ecosystems receiving more nitrogen than is required by the plants are known as "nitrogen-saturated." Such a situation contributes to both inorganic and organic nitrogen to freshwater. The chemical forms of N also cause serious concern with regard to the eutrophication. This is also the case with increased levels of phosphorus. Phosphates are less soluble than nitrates, and high amounts of phosphates inhibit the uptake of other elements. Foliar feeding of agricultural crops is now practicable and is practiced for N and most of the micronutrients like Fe and Zn which are given for correction of the deficiency as it is manifested late in the plant growth. As mentioned earlier, foliar sprays cannot substitute soil fertilization in all cases. But it can supplement soil application, and can be introduced in sprinkler irrigation system.

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