Chapter 14
Pesticides in Soil – Benefits and Limitations to Soil Health

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Summary

Pesticides are important components of many agricultural management systems and their effects on soil and its ability to process them should be included when evaluating soil quality. Pesticides help maintain agricultural productivity by controlling pests, however, management thresholds must be established to minimize potential non-target effects on soil biota and processes. In this chapter, we review: (i) selected examples of pesticide effects on soil biology and ecosystem function; (ii) methodologies to assess these effects; and (iii) conservation management options that may improve the capability of soils to process pesticides, and thereby to function safely and productively. Soil biota typically are resilient to pesticides applied at recommended rates, with only transient disruptions. Conservation management practices that

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increase soil organic matter (OM) and promote accumulation of plant residues on the soil surface help the soil to process many pesticides through sorption and degradation. Our review provides information needed to begin comprehensive coordinated initiatives to establish criteria for assessing and managing pesticide impacts on soil quality.

Introduction

The agricultural use of chemical agents to control pests such as weeds, insects, rodents, nematodes, fungi and bacteria has been practised since the latter part of the 19th century, when Bordeaux spray was first used by Millardet as a fungicide to prevent downy mildew on grapes. During the latter half of the 20th century, pesticide development and use became widespread as improved application and production technologies made it more practical and economical to use pesticides as a management tool. Worldwide pesticide use in 1997 was estimated at 2.58 billion kg (Aspelin and Grube, 1999). Weed control with herbicides was the largest single category of pesticide usage (40%), followed by usage of insecticides (26%) and fungicides (9%).

Integrating pesticide use with other technologies such as improved crop varieties, application formulations and farm equipment, spurred on the Green Revolution and the greatest capacity to produce food and fibre in history. With increased pesticide use, questions on potential effects regarding public health and the environment developed. Concerns arose that pesticides would pollute air, soil and water resources, contaminate the food chain and disrupt ecosystem balance. Environmental consequences might also result from pesticide application at rates higher than recommended, accidental spills or long *in situ* residence time in soil. Given these issues, evaluating soil quality in the agricultural context must also consider pesticide interactions with soil and soil biota.

Various philosophies about soil quality have been proposed and discussed at length elsewhere. A basic definition adopted by the Soil Science Society of America and accepted here (see Schjønning *et al.*, Chapter 1, this volume) considers soil quality as: '... the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation'. Little information is available on relationships between pesticide and soil quality, thus, assessing soil quality with respect to pesticide management necessitates: (i) defining factors that may affect pesticide dissipation or activity; (ii) identifying soil characteristics that may be influenced directly or indirectly by pesticide use; and (iii) evaluating how soil management can mitigate unintended effects of pesticides or enhance intended effects of pesticides. According to Larson and Pierce (1994), soil quality in agricultural settings should be assessed on the basis of the soil's ability to serve as a medium for plant growth (i.e. soil productivity), facilitate water flow in the environment and function as an environmental buffer. All three functions relate to pesticides. For example, high concentrations of pesticides in soil may influence processes such as plant growth and the activity and diversity of biotic populations. The ability of a soil to regulate and partition water flow could determine pesticide residence time in soil or the potential for pesticide movement to non-target areas. The capacity of a soil for pesticide sorption and degradation determines its efficacy for buffering their impact. How effectively a soil processes excess or unused pesticides and mitigates detrimental effects to humans and other species helps to determine its value.

This chapter reviews research on pesticides and soil quality in two sections. The first section reviews recent research concerning pesticide effects on soil components and processes related to soil quality. Numerous studies have evaluated pesticide effects on individual aspects, such as soil biota, biochemical activity and nutrient processing.

Many of these studies only examined initial effects of pesticides in laboratory microcosms, rather than in integrated systems over long periods and at larger scales. Soil is not only a medium for agricultural production, but is often viewed as a filter and processor for xenobiotics; and how a soil is managed can determine its ability to function in this capacity. The second section addresses how conservation management practices: (i) may enhance soil's potential to serve as a medium for facilitating the intended function of an applied pesticide; and (ii) may improve the soil's ability to filter and process pesticides.

Pesticide Effects on Soil Components and Processes

Determining assessment criteria

Assessing the effects of pesticides requires an understanding of which soil components will be influenced by a pesticide and the longevity of these effects. The most sensitive (responsive) components need to be identified and threshold pesticide tolerance concentrations determined. Pesticides have many purposes; therefore establishing general soil quality criteria is difficult, if not impossible. The simplest way to begin the assessment is to categorize pesticides based on similarities in purpose of use and properties. General goals of pesticide use in agricultural systems are the prevention, suppression and eradication of pests that reduce production quality and quantity. Agriculturally important herbicides, insecticides and fungicides are this chapter's focus.

One measure of the productivity of agricultural soils is crop yield, and the ability of a soil to sustain yields is a measure of soil quality. Therefore, beneficial aspects of judicious pesticide use in crop production systems must be considered in a discussion of management criteria when assessing pesticide effects on soil quality. Weeds compete with crops for nutrients, water and sunlight, resulting in reduced yields. Sensitive crop species might be used to establish thresholds at which herbicides are impacting growth and production. Similarly, insects and disease reduce both yield and quality of crop production, and sensitive or beneficial populations might be used in those assessments. It is critical to establish the *minimum* quantity of pesticide necessary to achieve sustainable crop production goals with negligible adverse effects to the soil ecosytem and environment, i.e. the management threshold. The strategy, therefore, would be to adopt management measures (e.g. reduced tillage, site-specific application) that minimize potential non-target effects while achieving the needed productivity.

Exposure to a substance to which an organism is not adapted will probably cause some effect, with certain organisms being inherently more sensitive. Distinction must be made between toxicity to target organisms (pests) and non-target organisms. Target toxicity is the dose required to harm or kill pests of interest. Non-target toxicity is a measure of the degree to which a pesticide can harm or injure beneficial organisms and is usually assessed on a limited number of representative species. Several standard parameters to interpret toxicity have been established, e.g. LD_{50} (lethal dose), LC_{50} (lethal concentration), EC_{50} (effective concentration), that are based on the dose or concentration of a substance that affects or kills 50% of a test population. Parameters such as LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration) provide information on concentrations that result in adverse effects not found in control populations. Whether or not pesticide EC_{50} or LC_{50} values can be used in assessments of soil quality needs systematic, comprehensive evaluation. A simplistic approach for obtaining an estimate of potential toxicity would extract a pesticide from soil and determine whether its concentration falls within published EC_{50}/LC_{50} ranges for key indicator species that inhabit the soil. Measuring pesticide concentrations in soil, however, does not give sufficient information to determine impacts on the total soil ecosystem, i.e. bioavailability.

Limited information exists on standard testing protocols for evaluating lethal or sublethal toxicity in pesticide-treated soil. In aquatic systems, sediment quality assessments have received much attention because contaminated sediment can readily impact water quality (Suter, 1993; Ingersoll, 1995). Partitioning of sediment contaminants between sorbed and interstitial water phases occurs until equilibrium is established. This assumes that sensitive aquatic organisms are more susceptible to solution-phase contaminants than sorbed contaminants. Thus, indices of contaminant availability can be obtained from sediment sorption partition coefficients. One weakness of this approach is that some organisms ingest contaminated sediment particles, thus assimilating the associated pesticide. There are similarities between sediment and soil, and it has been suggested that similar concepts be applied to assess potential toxicity in soil (Suter, 1993). Distinct differences between soil and sediment present some complications in directly relating sediment evaluations to soil. For example, sediments are usually saturated, whereas soils undergo wetting and drying cycles. During drier periods, soil pore space is exposed and pesticides in the vapour phase could impact organisms. Alternatively, pesticides sorbed to dry soil may be more tightly retained, and thus less bioavailable than during moist periods.

Edwards (2002) reviewed some of the currently available methods for assessing effects of pesticides in soil, including: single-species laboratory tests, multispecies tests, integrated soil microcosms and terrestrial model ecosystems. Although much effort has been directed towards harmonizing tests and criteria for assessing non-target effects of pesticides, there are still greatly disparate testing criteria among various countries. Regulatory agencies worldwide have developed a series of diagnostic tests to assess non-target effects, e.g. USEPA (1996) and OECD (2001), for use during registration of agrochemicals. Bioindicators in these tests include plants, earthworms and components of the soil and aquatic biotic community.

Establishing the link from pesticide effects on one or more indicator species to soil ecosystem health is more complicated. Factors such as soil resilience to adverse conditions, pesticide longevity, or indirect effects become important in this assessment. Many pesticides dissipate rapidly from soil. Others may accumulate, thus becoming more concentrated in soil with repeated use; for example, DDT is a classic case of a long-lived, immobile pesticide. How do we assess long-term impact of these pesticides on the soil ecosystem? When a pesticide indirectly inhibits a biotic process, what is the effect on the total soil ecosystem?

The soil ecosystem is considered relatively resilient to short-term stress and capable of recovery from various perturbations, including pesticide stress. Work by Domsch *et al.* (1983) compared the effect of natural stressors (temperature and drought) on soil biological activities to the effects of pesticides in terms of magnitude and duration. As summarized in Fig. 14.1, these studies show that soil can be inhibited as much as 90% of a given parameter, but if recovery is within pre-stress levels, this effect may be considered tolerable to the soil ecosystem.

Several of the soil biotic parameters selected for a discussion of pesticide effects in this chapter include microbial populations, fauna, microbial biomass, enzyme activity, nitrogen fixation and mycorrhizas. Other factors more indirectly influenced by pesticides include nutrient availability and carbon turnover. Although evaluation of most of these parameters was not designed to assess soil quality, a review of individual pesticide effects gives an indication of which parameters are potential candidates for use in these assessments.

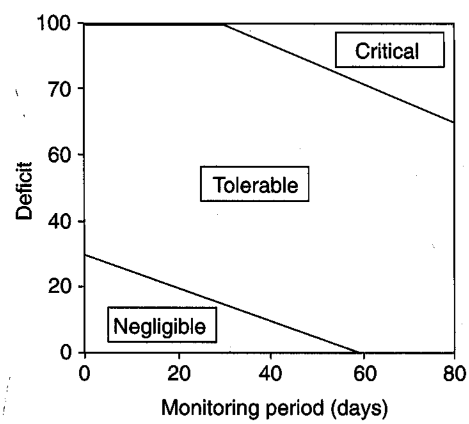

Fig. 14.1. Relationship between monitoring period and deficit vs. the ecological significance of injuries (adapted from Domsch *et al.*, 1983).

Soil microbial populations

Most modern pesticides were developed to target specific pests and even individual enzymes of a particular pest. However, effects on non-target soil microbial populations have been observed. Specific enzymes associated with these non-target microorganisms may be sensitive to pesticides. For instance, the mode of action for sulphonylurea herbicides is inhibition of the enzyme acetolactate synthetase, a component of the biosynthetic pathway of branched amino acids (leucine and isoleucine). This pathway is present in many bacteria; for example, certain pseudomonads are inhibited by sulphonylurea herbicides (Boldt and Jacobsen, 1998).

The effects of several pesticides on microorganisms in studies ranging from *in vitro* culture studies to field assessments are summarized in Table 14.1. *In vitro* studies may be useful for determining mechanisms of inhibition or toxicity for specific pesticides; however, these effects may be reduced or not observed in field soils where sorption and degradation affect pesticide bioavailability. When considering the effects of specific pesticides on soil microorganisms, caution must be used to discern effects of the active ingredient versus the formulated product. In this respect, effects of long-term normal field applications of two 2,4-D formulations were assessed on soil microbial populations (Narain Rai, 1992). Twenty-five weeks after application, total bacteria, fungi and actinomycete populations were reduced over 50% by applying 2,4-D as the *iso*-octyl ester versus the dimethylamine salt. Since herbicides are used to kill vegetation, a microbial response might be attributed to either direct toxic effects of the compound or to indirect effects such as vegetation loss limiting carbon substrate to soil microflora. Nevertheless, numerous studies observed no significant negative effects of normal herbicide applications on culturable soil microorganisms, e.g. Seifert *et al.* (2001).

Fungicides and insecticides may have a greater effect on soil microbial populations than herbicides. Application of the fungicide captan at normal field application rates (2–10 kg/ha) significantly reduced numbers of culturable fungi in four soils over a 30-day study, with a clear dose-dependent response

Table 14.1. Non-target effects of pesticides on soil microorganisms.

Pesticide	Organism	Effects	Reference
2,4-D-*iso*-octyl ester (H)[a]	Culturable soil bacteria, fungi and actinomycetes	Reduced soil populations	Narain Rai (1992)
Bromopropylate (I)[a]	*Azospirillum brasilense*	No effect on growth or N_2 fixation	Gomez *et al.* (1998)
Captan (F)[a]	Culturable soil bacteria, fungi and actinomycetes	Reduced soil populations	Martinez-Toledo *et al.* (1998)
Diazinon (I)	*Azospirillum brasilense*	No effect on growth or N_2 fixation	Gomez *et al.* (1999)
Fenamiphos (I)	Algae and cyanobacteria	None	Megharaj *et al.* (1999)
Fenpropimorph (F)	Actinomycetes, *Pseudomonas* sp., active fungi	Active fungi reduced, no effect on others	Thirup *et al.* (2001)
Glyphosate (H)	*Bradyrhizobium japonicum*	Inhibition, death	Moorman *et al.* (1992)
Imazaquin (H)	Culturable soil bacteria and fungi	No effect	Seifert *et al.* (2001)
Metsulphuron methyl (H)	*Pseudomonas* sp.	Growth inhibition	Boldt and Jacobsen (1998)
Methidathion (I)	*Azospirillum brasilense*	Reduced nitrogen fixation and ATP content	Gomez *et al.* (1998)
Simazine (H)	*Azotobacter chroococcum*	No effect on growth, high concentrations increased N_2 fixation	Martinez-Toledo *et al.* (1991)

[a]F, fungicide; H, herbicide; I, Insecticide.

and up to 80–90% inhibition at the highest rates (Martinez-Toledo *et al.*, 1998). In the same study, however, estimates of culturable bacteria increased up to fourfold at the higher captan rate, possibly because bacteria used captan as a carbon source. Alternatively, death of certain soil fungal populations and reduced competition for nutrients may have been a factor for enhanced bacterial populations resulting from captan application. Bjørnlund *et al.* (2000) found that rates of the fungicide fenpropimorph of 10 and 100× field application recommendations reduced fluorescein diacetate (FDA)-active fungal hyphae, and fungi tolerant to fenpropimorph were selected at the highest application rate. Thirup *et al.* (2001) determined that fenpropimorph inhibited FDA-active fungal hyphae during the first 10 days after application, but had no effect on heterotrophic bacteria, fluorescent pseudomonads and actinomycetes.

Johnsen *et al.* (2001) proposed that assessments of pesticide effects should address microbial community structure and diversity. One approach is to use cultural-dependent approaches such as a substrate utilization assay. The effect of various concentrations of the herbicide glyphosate on substrate utilization by bacterial communities was studied using Biolog GN plates (Busse *et al.*, 2001). Glyphosate (25 mM) reduced overall growth on all substrates in bacterial suspensions from three soils, and species richness was reduced with increasing glyphosate concentration. Analysis of specific microbial components, e.g. fatty acid methyl esters and respiratory quinones, may give insight as to community shifts based on broad taxonomic groups and relative biomass. The effects of the insecticides fenitrothion and chlorothalonil, the herbicides linuron and simazine and the fumigant chloropicrin, applied at 10× field application rates were evaluated on soil microbial community structure using respiratory quinone profiles (Katayama *et al.*, 2001). Chlorothalonil reduced initial total quinones (20%) and profile diversity (18%), but these parameters recovered within 3 days, indicating an immediate loss in biomass of certain microflora. Only the fumigant chloropicrin elicited a long-term change in total quinones (40–50%) and quinone diversity (17%) over the 28-day study.

Modern molecular approaches, e.g. denaturing gradient gel electrophoresis (DGGE), thermal gradient gel electrophoresis (TGGE), amplified ribosomal DNA (rDNA) restriction analysis (ARDRA) and other polymerase chain reaction (PCR) techniques, offer great promise in analysing pesticide effects on soil bacterial community composition. DNA-based tools are especially useful since typically 90–99% of the soil bacterial population are not culturable on artificial media (Tiedje and Zhou, 1996). A combination of DGGE and ARDRA was used to assess effects of the herbicides propanil and prometryne on the bacterial community structure in soil and soil slurries (Crecchio *et al.*, 2001). Propanil dissipated rapidly and did not affect soil microbial composition, except in slurries. Prometryne was more persistent and unique bands were evident in DGGE analysis, representing possible herbicide-degrading microbes.

Engelen *et al.* (1998) evaluated the use of Biolog and TGGE compared to standard assays such as substrate-induced respiration (SIR), dehydrogenase activity and carbon and nitrogen mineralization to assess the effects of the herbicides dinoseb (field application rate) and metamitron (10× field rate) in paraffin oil. Dinoseb had the greatest effect in reducing SIR and dehydrogenase activity, and immediately increasing nitrogen mineralization, even though applied at a lower rate. Metamitron had minimal effects on these parameters. Substrate utilization studies demonstrated that dinoseb also had the greatest effect in reducing the use of several substrates. TGGE analysis of ribosomal DNA extracted from soil after 8 weeks indicated that dinoseb increased the abundance of DNA fragments similar to *Nitrosomonas* and *Xanthomonas* species, whereas other DNA fragments were reduced or not observed.

El Fantroussi *et al.* (1999) used Biolog and DGGE to assess the effects of phenylurea herbicides on microbial community structure in soil enrichment cultures. These studies demonstrated that both community structure and microbial metabolic potential were influenced by herbicide exposure. Both diuron and linuron (15 and 25 mg/l, respectively) caused the demise of one bacterial species for 5 months. Microbial community analysis may provide new information on the effects of pesticides on community structure and diversity, but interpretation of these changes on soil productivity and health has not been established. Overall, with most pesticides studied, deleterious effects on microbial populations were typically transient and below the tolerable level described by Domsch *et al.* (1983), especially when recommended application rates were used.

Pesticide-degrading microbial populations

It is well documented that with certain pesticides, repeated applications can promote microbial populations capable of selectively degrading that pesticide (e.g. Racke and Coats, 1990). The capability of a soil for accelerated degradation might limit the use of that pesticide or related pesticides to control a particular pest. Accelerated degradation has been demonstrated with the thiocarbamate families of insecticides and herbicides, and the phenoxyacetic acid herbicides (Alexander, 1994). El Fantroussi *et al.* (1999) showed that previous application history had no effect on diuron degradation, moderately enhanced chlorotoluron degradation and strongly increased linuron degradation in soil enrichment cultures. Gennari *et al.* (1998) found that previous acifluorfen application enhanced its degradation.

Microbial biomass

Measurement of total soil microbial biomass (typically measured as carbon or nitrogen in biomass) is an extremely useful tool for interpreting soil biological quality. Specific soil microflora constituents can be ascertained based on abundance of specific cellular components. Microbial biomass measurements overcome some limitations of assessing populations considering factors such as culturable versus non-culturable, or if the population is present in dormant propagules such as spores or other resting structures. Microbial biomass is a standardized component of ecotoxicity assessment in OECD guidelines for pesticide registration (Anderson *et al.*, 1992), but is not required in USEPA assessments (see USEPA, 1996).

Glyphosate use within North and South America has increased dramatically with use of transgenic herbicide-resistant crops, thus effects on soil ecological processes are of concern. Haney *et al.* (2000) observed no effect of glyphosate on soil microbial biomass 3 days after application. More recent studies (Haney *et al.*, 2002), however, demonstrated that field application rates of the isopropylamine glyphosate salt increased microbial biomass carbon (17%) and microbial biomass nitrogen (76%) in nine soils 14 days after treatment. Hart and Brookes (1996) showed that glyphosate increased microbial biomass carbon (16%) 56 days after an autumn field application. In other field studies, no long-term effect of glyphosate on microbial biomass was observed in three California pine plantations (Busse *et al.*, 2001). Low-dose herbicides are being introduced to replace herbicides typically used at 10–100× greater quantities. In field trials, normal and tenfold field application rates of rimsulfuron had no effect on biomass carbon. However, under laboratory conditions, doses 10 and 100× field application rates elicited reduced soil biomass (Perruci and Scarponi, 1996).

Soil fauna

Soil fauna (e.g. earthworms, nematodes, microarthropods, protozoans) are important in organic matter (OM) transformations and soil structure formation, and are useful bio-indicators to study xenobiotic ecotoxicity in soil. Toxicity of a given pesticide to earthworms is a component of standardized ecotoxicity tests in pesticide registration by both the OECD (2001) and USEPA (1996). Field application rates of common fungicides, insecticides and nematicides can have deleterious effects on soil earthworms (Edwards and Bohlen, 1992). However, field application rates of two herbicides (2,4-DB, glyphosate) and the insecticide dimethoate did not affect survival and growth of several earthworm species (Dalby *et al.*, 1995).

Analysis of microarthropod and nematode communities in soils has been suggested as a tool for assessing soil quality. Interactions of tillage intensity and pesticides on diversity of microarthropod communities were evaluated at three sites over a 6-month period (Cortet *et al.*, 2002a). These studies indicated that greater microarthropod diversity was maintained under minimum tillage compared to deep tillage, but effects attributed to herbicides were negligible. Litterbag protocols assessed the effects of herbicides (atrazine, alachlor) and insecticides (fipronil and carbofuran, 0.2 and 0.6 kg/ha) on microarthropods (Cortet *et al.*, 2002b). Fiprinol had the greatest toxicity to microarthropods, significantly reducing numbers of *Oribatida* (about 50% after 61 and 102 days). Minimal effects of the other pesticides tested were observed. Martikainen *et al.* (1998) observed that dimethoate insecticide (tenfold field rate) reduced microarthropod populations with the greatest effect on the collembolans, but acari were reduced only when both benomyl fungicide (tenfold field rate) and dimethoate were applied. Although populations of collembolans recovered, species composition changed. Neither pesticide, alone or combined, had an effect on nematodes and enchytraeids.

Effects of long-term benomyl application in a tall grass prairie were assessed on nematode populations (Smith *et al.*, 2000). Benomyl had no significant effect on herbivores, but significantly reduced certain fungal feeders (Tylenchidae) by 13% and predatory nematodes (Dorylaimidae) by 33%. Protozoa may also be affected by various soil-applied herbicides. For example, Ekelund (1999) demonstrated that various groups of protozoa are affected by field application rates of the fungicide fenpropimorph.

Soil enzymatic activity

Metabolic capabilities of soils are often characterized by quantifying the activities of various hydrolytic and oxidoreductive enzymes. Pesticide effects on soil enzymatic activity were extensively reviewed by Schäffer (1993). Soil enzymatic activities are useful indicators of soil health and have been used to determine whether adverse effects of a management practice affect soil biochemical functions. A dehydrogenase enzyme assay is used to assess pesticide soil toxicity in German pesticide registration (Anderson *et al.*, 1992).

Enzyme status in soil, extracellular and bound to clay or humic acids, or as a component of viable organisms, can determine how pesticides affect enzymatic activities. Some pesticides may inactivate an enzyme by competitive binding at an active site, thus blocking the enzyme from acting on the test substrate. Other enzymes, e.g. dehydrogenase, measure oxidative electron transfer during carbon substrate utilization and are reflective of total biotic activity in soil.

Pesticide effects on enzyme activities depend on soil conditions and the pesticide application rate. Omar and Abdel-Sater (2001) observed that bromoxynil (herbicide) and profenophos (insecticide) inhibited cellulase activity when applied at 1 and 5× field application rates. Bromoxynil inhibited cellulase activity by over 30% and 20% after 4 and 10 weeks, respectively. Bromoxynil and profenophos transiently increased acid phosphatase activity at field rates but reduced activity by about 30% at the higher rate for 6 weeks. In a series of studies evaluating sulphonylurea herbicide effects on enzyme activities, conflicting results were observed. Sulphonylurea herbicides applied at normal field rates had no detrimental effects on dehydrogenase or fluorescein diacetate (FDA) hydrolytic activity (Perucci *et al.*, 1999), but FDA activity was reduced in other cases (Perucci *et al.*, 2000). Some negative effects on these enzymes were observed at rimsulfuron rates 10–100× higher than field rates, but effects were only slight and transitory (Perucci *et al.*, 1999).

Several soil enzymes play a role in facilitating soil nutrient availability. Urease is an important enzyme in nitrogen transformations. Urea is often applied as a nitrogen source, and urease hydrolyses urea to ammonium. Substituted phenylurea (monuron, diuron, linuron) herbicides were shown to inhibit urea hydrolysis (10–30%) in soil (Cervelli *et al.*, 1976). These herbicides behaved both as competitive and noncompetitive inhibitors of soil urease activity.

Nitrogen fixation

Although agriculture depends heavily on fertilizer nitrogen, nitrogen fixation by free-living, associative and symbiotic bacteria is the major input of usable nitrogen into global ecosystems. Under USEPA guidelines for pesticide registration, detailed protocols are given to assess pesticide effects on symbiotic nitrogen fixation (see USEPA, 1996).

Several studies have addressed the effects of specific pesticides on non-symbiotic nitrogen fixation. Effects of several insecticides on *Azospirillium brasilense* were evaluated *in vitro* (Gomez *et al.*, 1998, 1999). Bromopropylate and diazinon had no effect on microbial growth or nitrogen fixation, but methidathion and profenophos reduced N_2 fixation in synthetic media. The fungicide captan (3.5–10 kg/ha) significantly reduced populations of aerobic nitrogen-fixing bacteria and nitrogen-fixation activity (40–80%) in four soils over a 30-day incubation with inhibition being highly dose-dependent (Martinez-Toledo *et al.*, 1998).

Symbiotic nitrogen fixation by legumes can provide 30–70% of the nitrogen requirement for a crop. Fungicides and herbicides may either directly affect colonization of rhizobial symbionts, or indirectly influence performance of either the plant or the nitrogen-fixing bacteria *in planta* (Moorman, 1989). Fungicides to control seedling diseases have potential for inhibiting rhizobial inoculant establishment. Commercial application rates of carboxin, captan, pentachloronitrobenzene and thiram to seed reduced the survival of *Bradyrhizobium japonicum* and *Rhizobium phaseoli* (Curly and Burton, 1975; Graham *et al.*, 1980).

Glyphosate-resistant transgenic crops have revolutionized weed control and the acceptance of conservation management practices in North America. The basis of resistance is insertion of an insensitive 5-enolpyruvylshikimate-3-phosphate synthetase (EPSP) gene from an *Agrobacterium* strain allowing expression of a functional shikimic acid pathway, hence glyphosate tolerance. The soybean symbiont *B. japonicum*, however, possesses glyphosate-sensitive EPSP, thus *B. japonicum* growth is inhibited by glyphosate, ultimately resulting in death at concentrations exceeding 5 mM (Moorman *et al.*, 1992; Hernandez *et al.*, 1999). Nodule mass accumulation of glyphosate-resistant soybeans was significantly but inconsistently inhibited by field rates of glyphosate under greenhouse conditions (Reddy *et al.*, 2000; King *et al.*, 2001) and reduced by 21–28% in a 2-year field study (Reddy and Zablotowicz, 2003). Hernandez *et al.* (1999) demonstrated 10–30% inhibition of *B. japonicum* nitrogen fixation by glyphosate under laboratory conditions using bacteroids from glyphosate-treated plants or by treating bacteroids with glyphosate. Inhibition of nitrogen-fixation activity corresponded to glyphosate sensitivity of the *B. japonicum* strain *in vitro*. Nitrogen fixation in glyphosate-resistant soybeans was most severe under moisture stress, and reductions in soybean yield due to glyphosate application were observed in the field during drought (King *et al.*, 2001). The magnitude of inhibition of nitrogen fixation in soybeans due to glyphosate application has not been critically ascertained under field conditions. However, even a small reduction in nitrogen-fixation potential may have long-term effects on sustainable soil nitrogen pools, considering the widespread adoption of glyphosate-resistant technology.

Mycorrhizas

Symbiosis of mycorrhizal fungi with plants is another mutualistic plant–microbial association. Mycorrhizal roots have an altered morphology that enhances nutrient and water uptake. Fumigants used as nematicides and fungicides can profoundly influence mycorrhizal establishment. O'Bannon and Nemec (1978) showed that chloropicrin and methyl bromide completely inhibited *Glomus mossae* and *G. fasiculatum* on citrus, whereas citrus infection by these fungi was not affected by either 1,3-dichloropropene or ethylene dibromide. Cotton mycorrhizal infection was stimulated by nematicides 1,3-dichloropropane and dibromochloropropane in soils highly productive for cotton (Bird *et al.*, 1974).

Herbicide effects on mycorrhizal infection of citrus were evaluated in greenhouse and field studies (Nemec and Tucker, 1983). Bromacil, diuron or trifluralin had no effect on citrus growth or *Glomus etunicatum* chlamydospores in soil. Only high rates of simazine reduced citrus infection by *G. etunicatum*, indicating minor effects of herbicides on mycorrhizal symbiosis. Benomyl reduced mycorrhizal colonization more than 75% in 7-year field trials on a tallgrass prairie, indicating that long-term fungicide application may drastically alter beneficial plant–microbial interactions with significant implications for ecosystem sustainability (Smith *et al.*, 2000).

Carbon and nitrogen mineralization

Assessing pesticide effects on carbon and nitrogen mineralization is a standardized component of testing pesticides for non-target effects in the registration process by the USEPA (1996) and the OECD (2001). Understanding the effects on both processes is important in understanding pesticide interactions in soil and their role in supporting plant growth and overall ecosystem health (Edwards, 2002).

Chen *et al.* (2001) compared the effects of benomyl, captan and chlorothalonil on soil-nitrogen dynamics in laboratory incubations with or without additions of organic materials. Both nitrogen mineralization and nitrification rates were influenced by all fungicides, with captan eliciting the greatest influence on mineralization rates. Captan increased soil NH_4-N, whereas benomyl or chlorothalonil had little impact. Martinez-Toledo *et al.* (1998) showed that captan (2–10 kg/ha) inhibited nitrifying bacteria in four soils (50–90%) during a 30-day study. Applying bensulfuron at normal field rates had no effect on nitrification (Gigliotti *et al.*, 1998), whereas cinosulfuron transiently inhibited nitrification after 1 week, but had no effect at 4 weeks (Allievi and Gigliotti, 2001).

Examining endogenous respiration by monitoring cumulative CO_2 evolution in pesticide-treated soil is one approach to evaluating pesticide effects on mineralization. Under USEPA pesticide registration guidelines, pesticide effects on soil respiration are determined 5 and 28 days after application. Alternatively, short-term monitoring of the respiration of exogenous added substrates, e.g. substrate-induced respiration (SIR) as described by Anderson and Domsch (1978), measures active microbial biomass and can provide relative bacterial and fungal biomass when used with appropriate inhibitors. Assessments using SIR are a component of certain OECD guidelines, e.g. Germany (Anderson *et al.*, 1992). SIR techniques were used in studies by Engelen *et al.* (1998) to demonstrate that dinoseb had a greater effect on microbial activity than metamitron.

Altering soil conditions may influence the proportion of microorganisms that are sensitive to pesticides. In one study, when soils were amended with soybean residue, respiration in metribuzin-treated soil was lower than in soil without metribuzin (Locke and Harper, 1991a). However, in soils without soybean residue, respiration did not differ between metribuzin-treated and non-treated soils.

Selection of tests for assessing pesticide effects

A review of pesticide effects on various organisms and processes in soil provides an indication of how pesticides may affect soil quality. Standard tests of a soil's quality need to be simple, relatively inexpensive and repeatable. We propose several assays that fit these criteria (Table 14.2). Overall, diagnostic tests that assess pesticide effects on microbial biomass, SIR, dehydrogenase activity and nitrogen mineralization, are easy to perform and provide essential information on overall soil microbial activity. DNA-based microbial community analysis can ascertain minute changes in diversity, but does not consider physiological function in soil. Toxicity of pesticides to soil fauna such as earthworms can provide information on a group of organisms involved in processes such as OM decomposition, soil structure formation, and also on

Table 14.2. Proposed criteria for assessing pesticide effects on soil quality and ability of soil to process pesticides or buffer effects.

Criterion	Method
Pesticide effects	
Microbial biomass	Chloroform fumigation
	Substrate-induced respiration
Soil enzyme activity	Dehydrogenase
	Fluorescein diacetate hydrolysis
Soil fauna	Earthworm survival
Mineralizable N	Ammonification
	Nitrification
Processing ability	
Texture	
Sorption	Batch
Soil organic matter	
Pesticide degradation	2,4-D ring or carboxy label mineralization
pH	
Microbial biomass	Chloroform fumigation
	Substrate-induced respiration
Soil enzyme activity	Dehydrogenase
	Fluorescein diacetate hydrolysis

the possible entry of pesticides into the food chain of terrestrial species. An integrated soil microcosm approach (Burrows and Edwards, 2002) assessing microbial activity, nematodes, earthworms, plant productivity and carbon and nitrogen transformations in one system offers much promise.

Conservation Management Practices that Influence Soil Quality Factors Related to Pesticides

The impact of individual conservation management practices on soil quality will determine the long-term profitability and sustainability of a given management system. Understanding interactions among biological, chemical and physical soil properties that relate to pesticide bioavailability and dissipation is necessary to identify appropriate soil management strategies to maintain or improve soil health. Mechanisms associated with pesticide persistence in soil include degradation, sorption, movement and volatilization, and the extent to which farm managers can influence these mechanisms will determine the persistence of pesticide residues and their effects. Key soil factors related to pesticide dissipation that can be influenced by management include plant residue accumulation, quantity and character of soil OM, and soil microflora, chemistry and structure (Locke and Bryson, 1997).

Pesticide transport to groundwater or surface water bodies has major implications relevant to soil quality. International concerns about potential pesticide contamination of drinking water and impacts on human health and the environment have led to increased government regulation. The US Food Quality Protection Act of 1996 (FQPA) provides for major changes in pesticide regulation with respect to assessments of non-occupational exposure from drinking water, residential and dietary sources. FQPA gives the USEPA more latitude in promoting programmes such as Integrated Pest Management and use of alternative management practices that may reduce the risk of pesticide exposure. Similar initiatives are being adopted in the European Economic Community and elsewhere.

These regulatory trends, along with a depressed world agricultural market, have motivated efforts to develop low-cost, environmentally compatible management systems for agriculture. Conservation management strategies that substantially improve soil characteristics while improving the environment include reduced tillage, the use of cover crops or organic amendments, and practices of crop and herbicide rotations (Locke *et al.*, 2002a). These conservation measures are gaining more widespread acceptance as farmers recognize the benefits that accrue from not only preserving soil, but also improving it.

Soil characteristics influenced by conservation management

Reducing tillage quantity and intensity modifies most factors that could potentially affect pesticide persistence and bioavailability in soil (Locke and Bryson, 1997). For many cropping systems, when soil disturbance is reduced or eliminated, a narrow organic soil layer develops at the surface (e.g. Doran, 1980; Edwards *et al.*, 1992). Reduced tillage imparts several changes in the soil surface that gradually diminish with increasing depth (Rhoton, 2000). A crust containing mixtures of soil and plant residues usually develops at the soil surface, forming a protective seal conserving soil moisture and promoting pesticide degradation. However, soil crust formation may enhance pesticide sorption to clays and plant materials, thus minimizing bioavailability. Both the size and diversity of biotic populations increase in the soil surface under reduced tillage (Doran, 1980; Reddy *et al.*, 1995a; Lupwayi *et al.*, 1998; Cortet *et al.*, 2002a), but as organic substrate declines with soil depth, biotic activity diminishes (Zablotowicz *et al.*, 2000). Reduced tillage alters soil structural properties. Increased microbial activity and soil OM promote the formation of macroaggregates, cemented together with microbial exudates (Locke and Bryson, 1997). Conservation tillage soils develop more defined and stable structures, resulting from increased proportions of water-stable organo-clay microaggregates (Rhoton, 2000) and the OM trapped within microaggregates (Bossuyt *et al.*, 2002). Under reduced tillage, there is greater potential for preferential macropore flow in soils because of tunnels formed by faunal activity and voids formed from *in situ* plant residue decomposition.

Cover crops provide erosion control, green manures and weed inhibition. Organic amendments are used as mulch for weed control or moisture retention, for adding nutrients and for accelerated degradation of xenobiotics. Examples of organic amendments are animal manures, plant residues or manufacturing by-products or waste. Rotation of crops may increase soil OM, but this effect is dependent on the cropping sequence (Edwards *et al.*, 1992). If a crop produces abundant biomass (e.g. maize, sorghum), resulting changes in soil OM may be significant. Cover crop residues and organic amendments also stimulate soil biota and enhance soil OM (Reddy *et al.*, 1997a,b; Wardle *et al.*, 1999; Locke *et al.*, 2002b). Microbial activities and populations in hairy vetch and ryegrass cover crop residues were six- and 100-fold greater, respectively, than in underlying soil (Zablotowicz *et al.*, 1998a), whereas ryegrass and poultry litter amendments enhanced soil bacterial and fungal populations (Wagner *et al.*, 1995).

Pesticide dissipation and conservation management practices

Pesticides dissipate from soil via several processes, including degradation, sorption or binding, leaching, movement in surface runoff or volatilization. These processes are interrelated, making it difficult to assess each independently. We will address individual processes in a logical sequence, each building on the next, in the order: (i) pesticide sorption; (ii) pesticide mobility; (iii) initial pesticide transformation; and (iv) transformation of pesticide metabolites, and sequestration, binding and bioavailability of pesticide metabolites or residues.

Pesticide sorption

Pesticide sorption in soil is a dissipation mechanism that is significantly influenced by soil OM (Locke and Bryson, 1997). Pesticide sorption kinetics are often described as biphasic, characterized by the pesticide achieving rapid initial equilibrium distribution into soil components followed by a more gradual sorption phase (e.g. Locke, 1992). The rapid initial phase is believed to involve more accessible, exposed sites on the surface of soil particles and microaggregates. The more gradual phase follows as the pesticide permeates the soil complex to more restricted sorption sites. Pesticide desorption from these restricted sites is usually a slow process resulting from release from physical entrapment within the soil matrix, slow diffusion from restricted sites along tortuous pathways, or greater affinity for soil components than for the bulk solution (Locke, 1992). Within the soil matrix, an increased density of functional sorption sites could lessen the attraction of pesticides for more aqueous phases.

Bulk, electronic and hydrophobic functional areas within pesticide structures drive reactions between pesticides and sorbents (Reddy and Locke, 1994). In turn, soil OM contains numerous sites that react with pesticide functional groups. For many pesticides, sorption and soil carbon content are positively correlated (e.g. Locke *et al.*, 2002c). Since quantity and composition of soil OM play such an important role in pesticide sorption to soil, management strategies that alter characteristics and levels of soil organic components should be considered in pesticide management. As reviewed above, management practices such as reduced tillage, cover crops and animal waste applications can positively influence soil OM characteristics and distribution in soil.

Several studies have assessed herbicide sorption in soils where management practices increased soil OM (e.g. Locke, 1992; Locke *et al.*, 1995; Reddy *et al.*, 1997a; Gaston and Locke, 2000). A consistent trend for many herbicides (alachlor, cyanazine, fluometuron, acifluorfen) was that sorption was greater in surface soils managed through reduced tillage, with a cover crop, or where an organic amendment had been made. This phenomenon was attributed to greater quantities of soil OM accumulation. Comparisons of sorption to soil at lower depths where soil OM was equivalent between tillage treatments indicated little difference (Gaston and Locke, 2000; Zablotowicz *et al.*, 2000).

Plant residue characteristics can affect pesticide sorption, and crop residue management practices can influence pesticide sorbent properties. Some soil OM constituents have a greater affinity for pesticides than do others. Greater proportions of soil OM as humic material were important for oxyfluorfen and metribuzin sorption (Stearman *et al.*, 1989). Reduced-tillage soil had a greater proportion of soil carbon as humin, whereas tilled soil had more humic and fulvic acids. Age or weathered condition of plant residues in soil, or on the soil surface, can also affect pesticide sorption. In comparisons of herbicide sorption to plant residues in various stages of decomposition, more herbicide was sorbed on aged material (Dao, 1991; Reddy *et al.*, 1995b).

Pesticide mobility

Three primary processes are involved in the transfer of pesticides within and from soil: (i) movement through pores or matrices within the soil profile; (ii) transport in surface runoff; and (iii) volatilization. Mobility of a pesticide in soil is closely linked to its sorption affinity for soil components. The stronger the affinity for soil and its constituents, the less likely it will detach and move.

Avenues for pesticide movement through soil include both macropore and micropore flow. Macropore flow involves large pore sizes and consists of channels among soil aggregates, cracks caused by shrink–swell processes, voids left by decayed roots, or tunnels created by fauna (Locke and Bryson, 1997). Macropore flow is important for drainage when soil is saturated. Since large hydraulic conductivities are associated with macropore flow, dissolved pesticides moving through macropores have little interaction with reactive pore surfaces, thus facilitating rapid pesticide leaching to greater soil depths, i.e. preferential flow. Aggregation and channel development in reduced tillage soils potentially provide paths for enhanced pesticide movement when flow is saturated, but results from field studies have been mixed (Hall *et al.*, 1991; Isensee and Sadeghi, 1995). It appears that the most important factors determining pesticide leaching via preferential flow are timing, amount and intensity of rainfall following pesticide application (Isensee and Sadeghi, 1995).

Micropore flow involves inter- and intraparticle diffusion through small pores. Rate of pesticide diffusion through micropores is dependent on the affinity of the pesticide for sorbents on micropore surfaces and moisture gradients within the soil. Interparticle movement is micropore flow between soil particles, whereas intraparticle diffusion is flow within particle matrices and clay lattices. The more interaction a pesticide has with active sorption sites, the greater the likelihood of inhibiting further movement through the profile. Thus, higher concentrations of OM in the surface layer of reduced tillage soils should increase sorption and inhibit downward and lateral movement.

Surface runoff is the movement of water, sediment and chemicals over the soil surface. Runoff occurs when saturated soil cannot take in any more water during precipitation and rainfall is too intense for soil to accommodate water entry. Pesticide movement in runoff occurs when pesticides applied to foliage or plant residues are washed off during precipitation or pesticides sorbed to soil particles move with sediment or are desorbed into runoff water. Plant residues on the soil surface generally reduce volume of runoff, hence lessening pesticide loss. Pesticide applied to the surface of reduced tillage soils may be sorbed by the plant residues or the soil OM layer, thus further movement into the soil may be inhibited.

In soil, a pesticide can either degrade *in situ* by microbial or chemical processes, volatilize or photodegrade, but as long as pesticide residues remain at the surface, they are vulnerable to loss in runoff. The affinity of individual pesticides for soil constituents will determine the extent to which this occurs. The longer pesticides remain in soil or on plant debris, the less likely that they will desorb, because they transfer to restricted sites during the more gradual phase of sorption. Moderate precipitation shortly after pesticide application may be sufficient to move a pesticide into the soil, but perhaps not to the extent of leaching via channelling. When cover crops or fallow-season weeds provide dense foliage covering the soil surface, pesticides applied to soil will be intercepted and retained to some degree (Reddy and Locke, 1996). The quality or condition of the plant residues (Dao, 1991; Reddy *et al.*, 1995b, 1997a) may determine the degree to which pesticides are retained in plant debris or are removed as leachate or runoff.

Volatilization is a major mechanism for pesticide dissipation. Factors regulating pesticide volatilization include soil moisture content, temperature, physicochemical pesticide and formulation properties and pesticide-soil attractions. Pesticide volatilization from soil involves a two-step processes: (i) the evaporation of pesticide molecules; and (ii) dispersion of the resulting vapour into the atmosphere (Taylor and Spencer, 1990). During evaporation, pesticide transforms from a liquid or solid phase to a gas. Further pesticide dissipation in the gas phase depends on air turbulence and diffusion. Reduced-tillage soils may be more insulated from extremes in temperatures, and wetter because of the surface plant residues. Cooler temperatures and lower evaporation rates may also decrease the potential for pesticide loss by volatilization (Gish *et al.*, 1995).

Initial pesticide transformation

Initial modification to pesticide structure can occur via chemical or biological activity. The transformed compound (metabolite) is typically a product of hydrolysis, reductive or oxidative processes and may retain the basic structure of the parent compound, but possess altered biological and physicochemical properties. Hydrolysis is an important initial reaction of pesticide metabolism, since many pesticides have susceptible moieties (e.g. amides, carbamates and ester linkages). Many soil microorganisms produce hydrolytic enzymes, but hydrolysis also results from chemical processes, including low redox potential, soil acidity and interactions with soil OM. Nitroaromatic and aromatic pesticides (dinitroaniline and nitrodiphenylether herbicides; the fungicide pentachloronitrobenzene (PCNB); the insecticide parathion) are all susceptible to microbial metabolism by oxidative and reductive processes, depending on the characteristics of microbial populations, chemical structure and soil environmental factors (Zablotowicz *et al.*, 1998b). Other microbial enzymes (halidohydrolases, dehydrohalogenases and glutathione *S*-transferases) cleave the halogen–carbon bond present in many pesticides. Many pesticides, such as carbamates, chloroacetamides, phenylureas and triazines, additionally contain *N*-alkyl groups, making them susceptible to microbially mediated *N*-dealkylation.

The effects of conservation management on initial pesticide degradation, i.e. disappearance of parent pesticide, have been evaluated with varied results. Levanon *et al.* (1994) observed an enhanced rate of atrazine degradation in reduced-tillage soil, whereas more rapid degradation of bentazon (Gaston *et al.*, 1996a) and acifluorfen (Gaston and Locke,

2000) occurred in conventional-tillage soil relative to no-tillage soil. The enhanced metolachlor degradation in soil from an untilled vegetative buffer area as compared to degradation in soil from an adjacent tilled field, was attributable to higher microbial activities (Staddon *et al.*, 2001). Fluometuron degraded more rapidly in surface conventional-tillage soil even though microbial activities were higher in no-tillage soil (Zablotowicz *et al.*, 2000). Increased fluometuron degradation in conventional tillage resulted from lessened sorption (greater vulnerability to degradation) and better distribution of plant residues and soil due to mixing during tillage. Wagner *et al.* (1996) found that some soils with a history of bentazon use were better able to degrade it under no-tillage than conventional-tillage management, whereas in other soils there was negligible tillage effect. Still other research showed little or no effect of tillage on initial pesticide degradation, e.g. alachlor (Locke *et al.*, 1996), metribuzin (Locke and Harper, 1991b), fluometuron (Locke *et al.*, 1995) and chlorimuron (Reddy *et al.*, 1995a).

Pesticides applied to live cover crops or decomposing plant residues may be intercepted and retained by the plant tissue, which may also delay pesticide degradation. Zablotowicz *et al.* (1998a) determined that although desiccated cover-crop residues were more biologically active than associated underlying soils, 2,4-D mineralization was slower in plant residues than in surface soil. Fluometuron degradation in ryegrass residues was as rapid as in soil, but required high moisture conditions. Sorption to cover-crop material may provide a degree of protection from degradation. Eventually, pesticides retained by plant residues will either degrade in place or elute to soil below. However, soils associated with cover crops can provide an environment conducive to pesticide metabolism. Enhanced 2,4-D degradation in surface soil from a cereal rye cover crop as compared to fallow soil was attributed to elevated populations of 2,4-D degraders in the cover-crop soil (Bottomley *et al.*, 1999). Laboratory results, however, indicated that the half-life of fluometuron in surface soil from cover-crop areas was approximately 22 days longer than that from areas without a cover crop (Brown *et al.*, 1994).

Amending soil with plant materials or manure can accelerate pesticide degradation via a process called biostimulation, used to detoxify contaminated soils (Zablotowicz *et al.*, 1998b). Adding poultry litter, maize meal or ryegrass to soil enhanced cyanazine and fluometuron degradation (Wagner and Zablotowicz, 1997a), an effect attributed to greater microbial activity in amended soils. Fluometuron degradation was stimulated in soils amended with rice, hairy vetch or ryegrass in another study (Wagner and Zablotowicz, 1997b). In contrast to other studies, metribuzin degradation did not differ between soil amended and not amended with soybean plant residues, although microbial respiration was higher (Locke and Harper, 1991a).

How important are soil characteristics and management to the *initial* degradation of a pesticide? For many pesticides applied to soil, the initial chemical structure may be necessary for it to function successfully as a pesticide, and metabolites may exhibit less or no activity. Ideally, if pesticide efficiency is to be achieved, a balance must be struck between the factors of pesticide bioactivity, sorption and vulnerability to degradation. If pesticides can function as intended under conservation management systems for the same duration as in conventional systems, then the lack of difference in the initial degradation of the parent pesticide structure is a positive outcome for environmental quality.

Transformation and binding of pesticide metabolites or residues

Although generalized conclusions cannot be made about initial pesticide degradation in conservation-tillage soils, distinct trends were observed concerning the dynamics of pesticide metabolites in these systems. Pesticide metabolites possess a myriad of characteristics, and their fate is ultimately determined by metabolite physicochemical properties and soil conditions. Metabolites that are mobile or readily vulnerable to degradation may be transient, whereas less mobile pesticides may have a long residence in soil. Some metabolites are more polar than the parent pesticide, e.g. sulphonic and oxanilic acids associated with chloroacetamide metabolism.

The characteristics of metabolites found in soil may help to explain how soil management influenced pesticide transformation. In several studies, conventional-tillage soils tended to have a larger proportion of applied pesticide accumulated as polar metabolites than reduced-tillage soils. For example, in two of three soils, recovery of one polar metabolite was greater in conventional tillage than in no-tillage soils, 63 days after chlorimuron application (Reddy *et al.*, 1995a). More polar metabolites of metolachlor accumulated in a tilled-field soil than in an untilled vegetative buffer soil (Staddon *et al.*, 2001). Alachlor metabolites oxanilic and sulphonic acids were present in greater quantities in conventional-tillage soil than in no-tillage soil (Locke *et al.*, 1996). Polar metribuzin metabolites were more abundant in conventional-tillage and non-amended soils than in no-tillage soil or soil amended with plant residues (Locke and Harper, 1991a,b). These studies suggest that greater accumulation of polar metabolites in conventional-tillage soils resulted from lower microbial degradation activity, compared with reduced-tillage soils. However, in soils amended with ryegrass residues, more hydroxycyanazine was recovered than in non-amended soil (Wagner and Zablotowicz, 1997a). Greater proportions of polar metabolites in conventional-tillage soils may have implications relevant to subsequent movement of pesticide residues. Some polar metabolites are more easily desorbed into the aqueous solution phase (e.g. cyanazine amide, chloroacid cyanazine; Reddy *et al.*, 1997b), thus making them more vulnerable to loss in runoff or through leaching. Other polar metabolites, such as hydroxycyanazine acid or hydroxyatrazine have greater affinity for soil humic components, making them less mobile (Lerch *et al.*, 1997; Reddy *et al.*, 1997b).

Some metabolites are more non-polar than their parent pesticides, resulting in potential for greater sorption to soil (e.g. aminoacifluorfen; Locke *et al.*, 1997). More non-polar metolachlor metabolites were extracted from untilled vegetative buffer soil than from tilled soil after 13 days of incubation (Staddon *et al.*, 2001). Lower quantities of non-polar alachlor metabolites were extracted from either conventional- or no-tillage soils than polar metabolites, probably because they were more tightly sorbed (Locke *et al.*, 1996).

Mineralization is the endpoint of pesticide metabolism, where pesticide carbon is completely degraded to CO_2. Pesticide residues that are mineralized would therefore have to be labile, accessible to microorganisms and have low recalcitrance. The impact of management on mineralization can vary with each pesticide, and no clear pattern emerges. Mineralization of alachlor residues in no-tillage soil was greater than in conventional-tillage soil, perhaps corresponding to the decline in polar metabolites (Locke *et al.*, 1996). In another study, greater initial mineralization of atrazine residues was attributed to higher microbial activity in no-tillage soils (Levanon *et al.*, 1994). However, lower mineralization of metribuzin and chlorimuron residues was observed in no-tillage or soybean-residue-amended soils (Locke and Harper, 1991a,b; Reddy *et al.*, 1995a).

The proportion of a pesticide that cannot be extracted from soil by solvents is termed non-extractable or bound pesticide residue. It is extremely difficult to identify non-extractable pesticide residues, but possible components include: (i) strongly sorbed parent pesticide; (ii) strongly sorbed metabolites or fragments of the degraded pesticide molecule; (iii) pesticides or metabolites polymerized into humic material via oxidative coupling reactions catalysed by various enzymes; and (iv) physical sequestration of pesticides or metabolites. Physical sequestration occurs when pesticide residues become entrapped within soil microaggregates or within organo-clay matrices or lattices from which they are removed only with great difficulty. In undisturbed soil, pockets of aggregates may be protected from weathering and remain entrapped. Channels of water move around rather than through these aggregates, and thereby reduce the potential for release of bound pesticide residues. If pesticide residues are sequestered within soil aggregates, release may occur if the soil is disrupted, as with tillage.

In several pesticide fate studies involving soils from conservation-managed areas, we observed that surface soils from reduced tillage systems contained a greater proportion of applied pesticide as non-extractable residues, e.g. metribuzin (Locke and Harper, 1991a,b), alachlor (Locke *et al.*, 1996), sulfentrazone (Reddy and Locke, 1998) and metolachlor (Staddon *et al.*, 2001), but there are exceptions, e.g. fluometuron (Zablotowicz *et al.*, 2000), bentazon (Gaston *et al.*, 1996a,b; Wagner *et al.*, 1996) and chlorimuron (Reddy *et al.*, 1995b). In some cases, the process is reversible; for example formerly non-extractable hydroxyatrazine residues can be extracted, e.g. after liming soils (Kells *et al.*, 1980).

Sorbed or bound pesticides or metabolites may lose substantial activity and toxicity, i.e. become less bioavailable (Alexander, 1994). For example, sorption of herbicides to soil may reduce toxicity to weeds. Gaston *et al.* (2001) observed a positive correlation between soil OM content and weed control activity of fluometuron, implying that as soil OM increases, herbicide bioactivity decreases. Crop residues can intercept herbicide applied to the soil surface (e.g. Locke *et al.*, 2002b), perhaps reducing its bioavailability for controlling weeds. Reduced weed control with a cover crop, however, might be offset by the cover crop inhibiting weed growth by shading or allelopathy (Locke *et al.*, 2002a). If sorption of a pesticide prevents or hinders degradation, it could have prolonged *in situ* residence. The balance between maintaining sufficient bioavailability for adequate weed management and reducing herbicide longevity in soil becomes critical when crops are rotated. If crop rotation is practised, it is necessary that herbicides applied for weed control in the first crop not interfere with management and production of the subsequent crop.

Selection of tests for assessing pesticide processing ability in soil

Changes in the soil environment caused by increased carbon accumulation have impacts on pesticide fate. Management activities such as adding manures or plant material, using cover crops and reducing tillage promote accumulation of carbon-rich material in the soil surface, resulting in increases in biotic activity and changes to soil structure. Our discussion of effects of conservation soil and crop management on pesticide dissipation provides a background for selecting methods to assess the ability of soils to process pesticides. Several protocols proposed in Table 14.2 should provide an indication as to whether a pesticide is bioavailable (i.e. active or residual) and a given soil's intrinsic ability for pesticide dissipation.

Conclusions

As pesticides are an extremely diverse group of chemicals; it is impossible to thoroughly explore the ramifications of all pesticides on soil and environmental quality in this limited space. We have introduced pesticides and emphasized their importance to crop production, while recognizing environmental and human health concerns. The management threshold for pesticides was defined as the *minimum* quantity of pesticide necessary to achieve sustainable crop-production goals with negligible adverse effects to the soil ecosystem and environment. Microbial and faunal populations are generally tolerant of pesticides, exhibiting only minor transient perturbations when recommended rates are used. Likewise, major biotic processes such as enzyme activity, respiration and carbon and nitrogen transformations are minimally impacted. In many cases, crop and soil management practices that increase soil OM and plant residues impart attributes to soil that can impede pesticide movement and enhance degradation, while not hindering pesticide efficacy.

Satisfactory and generally applicable measures of pesticide effects and management in relationship to soil quality do not exist, although some regulatory organizations have made substantial progress. This chapter provides a background to facilitate the establishment of comprehensive strategies for determining acceptable protocols for these assessments. Questions for discussion include:

- How do we measure or assess soil quality with regard to pesticides?
- How do we determine the most meaningful indicators?
- What can we do to ameliorate negative effects and enhance positive attributes of pesticide use?

- How will we evaluate measured side effects?
- Can we establish management thresholds below which adverse pesticide effects are negligible?

Based on the current state of knowledge, we summarized a group of diagnostic tests that may be performed to assess pesticide impact on soil health and the ability of a soil to process pesticides or limit negative effects. Issues of experimental scale (*in vitro* to watershed) and duration of assessment are challenges. Differences in regulatory approaches and philosophies may preclude establishing international standards, but within regions or nations, efforts to develop strategies can be initiated using the following logical steps.

- Use the information reviewed here as a basis for further debate on pesticide contributions to soil quality issues.
- Establish testing criteria for measuring pesticide impacts on soil quality.
- Develop and adopt standard protocols for cost–benefit analysis and risk management assessments.
- Plan, coordinate and implement a series of multilocation, holistic studies to provide appropriate databases for refinement and modelling.

References

Alexander, M.A. (1994) *Biodegradation and Bioremediation*. Academic Press, San Diego, California, 302 pp.

Allievi, L. and Gigliotti, C. (2001) Response of the bacteria and fungi of two soils to the sulfonylurea herbicide cinosulfuron. *Journal of Environmental Science and Health Part B – Pesticides, Food Contaminants and Agricultural Wastes* 36, 161–175.

Anderson, J.P.E. and Domsch, K.H. (1978) A physiological method for the quantitative measurement of microbial biomass in soil. *Soil Biology and Biochemistry* 10, 215–221.

Anderson, J.P.E., Castle, D., Ehle, H., Eichler, D., Laerman, H.-T., Maas, G. and Malkomes, H.-P. (1992) *Guideline for the Official Testing of Plant Protection Products*, 2nd edn. Part VI, 1-1 Effects on the activity of soil microflora. Biologische Bundesanstalt für Land- und Forstwirtsshaft, Braunschweig, Germany.

Aspelin, A.L. and Grube, A.H. (1999) *Pesticide Industry Sales and Usage: 1996 and 1997 market estimates*. Office of Prevention, Pesticide, and Toxic Substances, United States Environmental Protection Agency, 733-R-99–001.

Bird, L.S., Rich, J.R. and Glover, S.U. (1974) Increased endomycorrhizae of cotton roots in soil treated with nemacides. *Phytopathology* 64, 48–51.

Bjørnlund, L., Ekelund, F., Christensen, S., Jacobsen, C.S., Krogh, P.H. and Johnsen, K. (2000) Interactions between saprophytic fungi, bacteria and protozoa on decomposing wheat roots in soil influenced by the fungicide fenpropimorph (Corbel®): a field study. *Soil Biology and Biochemistry* 32, 967–975.

Boldt, T.S. and Jacobsen, C.S. (1998) Different toxic effects of the sulfonyl urea herbicides metsulfuron methyl, chlorsulfuron, and thifensulfuron methyl on fluorescent pseudomonads isolated from soil. *FEMS Microbiology Letters* 161, 29–35.

Bossuyt, H., Six, J. and Hendrix, P.F. (2002) Aggregate-protected carbon in no-tillage and conventional tillage agroecosystems using carbon-14 labeled plant residue. *Soil Science Society of America Journal* 66, 1965–1973.

Bottomley, P.J., Sawyer, T.E., Boersma, L., Dick, R.P. and Hemphill, D.D. (1999) Winter cover crop enhances 2,4-D mineralization potential of surface and subsurface soil. *Soil Biology and Biochemistry* 31, 849–857.

Brown, B.A., Hayes, R.M., Tyler, D.D. and Mueller, T.C. (1994) Effect of tillage and cover crop on fluometuron adsorption and degradation under controlled conditions. *Weed Science* 44, 171–175.

Burrows, L.A. and Edwards, C.A. (2002) The use of integrated soil microcosms to predict effects of pesticides on soil ecosystems. *European Journal of Soil Biology* 38, 245–249.

Busse, M.D., Ratcliff, A.W., Shestak, C.J. and Powers, R.F. (2001) Glyphosate toxicity and the long-term vegetation control on soil microbial communities. *Soil Biology and Biochemistry* 33, 1777–1789.

Cervelli, S., Nannipieri, P., Giovanni, G. and Perna, A. (1976) Relationship between substituted urea herbicides and soil urease activity. *Weed Research* 16, 365–368.

Chen, S.-K., Edwards, C.A. and Subler, S. (2001) Effects of the fungicides, benomyl, captan, and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. *Soil Biology and Biochemistry* 33, 1971–1980.

Cortet, J., Ronce, D., Poinsot-Balaguer, N., Beaufreton, C., Chabert, A., Viaux, P. and de Fonseca, J.P.C. (2002a) Impacts of different agricultural practices on the biodiversity of microarthropod communities in arable crop systems. *European Journal of Soil Biology* 38, 239–244.

Cortet, J., Gillon, D., Joffre, R., Ourcival, J.-M. and Poinsot-Balaguer, N. (2002b) Effects of pesticides on organic matter recycling and microoarthropods in a maize field: use and discussion of the litterbag methodology. *European Journal of Soil Biology* 38, 261–265.

Crecchio, C., Curci, M., Pizzigallo, M.D.R., Ricciuti, P. and Ruggiero, P. (2001) Molecular approaches to investigate herbicide-induced bacterial changes in soil microcosms. *Biology and Fertility of Soils* 33, 460–466.

Curly, R.L. and Burton, J.C. (1975) Compatability of *Rhizobium japonicum* with chemical seed protectants. *Agronomy Journal* 67, 807–808.

Dalby, P.R., Baker, G.H. and Smith, S.E. (1995) Glyphosate, 2,4-DB and dimethoate: effects on earthworm survival and growth. *Soil Biology and Biochemistry* 27, 1661–1662.

Dao, T.H. (1991) Field decay of wheat straw and its effects on metribuzin and S-ethyl metribuzin sorption and elution from crop residues. *Journal of Environmental Quality* 20, 203–208.

Domsch, K.H., Jagnow, G. and Anderson, T.-H. (1983) An ecological concept for assessing the side effects of agrochemicals on soil microorganisms. *Residue Reviews* 86, 65–105.

Doran, J.W. (1980) Soil microbial and biochemical changes associated with reduced tillage. *Soil Science Society of America Journal* 44, 765- 771.

Edwards, C.A. (2002) Assessing the effects of environmental pollutants on soil organisms, communities, processes, and ecosystems. *European Journal of Soil Biology* 38, 225–231.

Edwards, C.A. and Bohlen, P.J. (1992) The effects of toxic chemicals on earthworms. *Reviews of Environmental Contamination and Toxicology* 125, 23–99.

Edwards, J.H., Wood, C.W., Thurlow, D.L. and Ruf, M.E. (1992) Tillage and crop rotation effects on fertility status of a Hapludult soil. *Soil Science Society of America Journal* 56, 1577–1582.

Ekelund, F. (1999) The impact of the fungicide fenpropimorph (Corbel) on bacteriovorous and fungivorous protozoa in soil. *Journal of Applied Ecology* 36, 233–243.

El Fantroussi, S., Verschuere, L., Verstraete, W. and Top, E.M. (1999) Effect of phenylurea herbicides on soil microbial communities estimated by analysis of 16S rRNA gene fingerprints and community-level physiological profiles. *Applied and Environmental Microbiology* 65, 982–988.

Engelen, B., Meinken, K., von Wintzgerode, F., Heuer, H., Malkomes, H.-P. and Backhaus, H. (1998) Monitoring of a pesticide treatment on bacterial soil communities by metabolic and genetic fingerprinting in addition to conventional testing procedures. *Applied Environmental Microbiology* 64, 2814–2821.

Gaston, L.A. and Locke, M.A. (2000) Acifluorfen sorption, degradation, and mobility in a Mississippi Delta soil. *Soil Science Society of America Journal* 64, 112–121.

Gaston, L.A., Locke, M.A. and Zablotowicz, R.M. (1996a) Sorption and degradation of bentazon in conventional- and no-till Dundee soil. *Journal of Environmental Quality* 25, 120–126.

Gaston, L.A., Locke, M.A., Wagner, S.C., Zablotowicz, R.M. and Reddy, K.N. (1996b) Sorption of bentazon and degradation products in two Mississippi soils. *Weed Science* 44, 678–682.

Gaston, L.A., Locke, M.A., Zablotowicz, R.M. and Reddy, K.N. (2001) Spatial variability of soil properties and weed populations in the Mississippi Delta. *Soil Science Society of America Journal* 65, 449–459.

Gennari, M., Fournier, J.C., Dughera, R. and Negre, M. (1998) A study on the mineralization of C-14 acifluorfen in soil: comparison between treated and untreated soils. *Fresenius Environmental Bulletin* 7, 338–344.

Gigliotti, C., Allievi, L., Salardi, C., Ferrari, F. and Farini, A. (1998) Microbial ecotoxicity and persistence in soil of the herbicide bensulfuron-methyl. *Journal of Environmental Science and Health Part B – Pesticides, Food Contaminants and Agricultural Wastes* 33, 381–398.

Gish, T.J., Sadeghi, A. and Wienhold, B.J. (1995) Volatilization of alachlor and atrazine as influenced by surface litter. *Chemosphere* 31, 2971–2982.

Gómez, F., Salmeron, V., Rodelas, B., Martinez-Toledo, M.V. and Gonzalez-Lopez, J. (1998) Response of *Azospirillum brasilense* to the pesticides bromopropylate and methidathion on chemically defined media and dialyzed-soil media. *Ecotoxicology* 7, 43–47.

Gomez, F., Martinez-Toledo, M.V., Salmeron, V., Rodelas, B. and Gonzalez-Lopez, J. (1999) Influence of the insecticides profenofos and diazinon on the microbial activities of *Azospirillum brasilense*. *Chemosphere* 39, 945–957.

Graham, P.H., Ocambo, G., Ruiz, L.D. and Duque, A. (1980) Survival of *Rhizobium phaseoli* in contact with chemical seed protectants. *Agronomy Journal* 72, 625–627.

Hall, J.K., Mumma, R.O. and Watts, D.W. (1991) Leaching and runoff losses of herbicides in a tilled and untilled field. *Agricultural Ecosystem and Environment* 37, 303–314.

Haney, R.L., Senseman, S.A., Hons, F.M. and Zuberer, D.A. (2000) Effect of glyphosate on soil microbial activity and biomass. *Weed Science* 48, 89–93.

Haney, R.L., Senseman, S.A. and Hons, F.M. (2002) Effect of Roundup Ultra on microbial activity and biomass from selected soils. *Journal of Environmental Quality* 31, 730–735.

Hart, M.R. and Brookes, P.C. (1996) Soil microbial biomass and mineralization of soil organic matter after 19 years of cumulative application of pesticides. *Soil Biology and Biochemistry* 28, 1641–1649.

Hernandez, A., Garcia-Plazzola, J.I. and Becerril, J.M. (1999) Glyphosate effects on phenolic metabolism of nodulated soybean (*Glycine max* L.Merr.) *Journal of Agricultural and Food Chemistry* 47, 2920–2925.

Ingersoll, C.G. (1995) Sediment tests. In: Rand, G.M. (ed.) *Fundamentals of Aquatic Toxicology.* 2nd edn. Taylor and Francis, Washington, DC, pp. 231–255.

Isensee, A.R. and Sadeghi, A.M. (1995) Long-term effect of tillage and rainfall on herbicide leaching to shallow groundwater. *Chemosphere* 30, 671–685.

Johnsen, K., Jacobsen, C.S., Torsvik, V. and Sorensen, J. (2001) Pesticide effects on bacterial diversity in agricultural soils – a review. *Biology and Fertility of Soils* 33, 443–453.

Katayama, A., Funassaka, K. and Fujie, K. (2001) Changes in respiratory quinone profile of a soil treated with pesticides. *Biology and Fertility of Soils* 33, 454–459.

Kells, J.J., Rieck, C.E., Blevins, R.L. and Muir, W.M. (1980) Atrazine dissipation as affected by surface pH and tillage. *Weed Science* 28, 101–104.

King, A.C., Purcell, L.C. and Vories, E.D. (2001) Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to glyphosate applications. *Agronomy Journal* 93, 179–186.

Larson, W.E. and Pierce, F.J. (1994) The dynamics of soil quality as a measure of sustainable management. In: Doran, J.W. (ed.) *Defining Soil Quality for a Sustainable Environment. Soil Science Society of America Special Publication* No. 35, Madison, Wisconsin, pp. 37–51.

Lerch, R.N., Thurman, E.M. and Kruger, E.L. (1997) Mixed-mode sorption of hydroxylated atrazine degradation products in soil: a mechanism for bound residue. *Environmental Science and Technology* 31, 1539–1546.

Levanon, D., Meisinger, J.J., Codling, E.E. and Starr, J.L. (1994) Impact of tillage on microbial activity and the fate of pesticides in the upper soil. *Water Air and Soil Pollution* 72, 179–189.

Locke, M.A. (1992) Sorption–desorption kinetics of alachlor in surface soil from two soybean tillage systems. *Journal of Environmental Quality* 21, 558–566.

Locke, M.A. and Bryson, C.T. (1997) Herbicide–soil interactions in reduced tillage and plant residue management systems. *Weed Science* 45, 307–320.

Locke, M.A. and Harper, S.S. (1991a) Metribuzin degradation in soil: I Effects of soybean residue, metribuzin level, and soil depth. *Pesticide Science* 31, 221–237.

Locke, M.A. and Harper, S.S. (1991b) Metribuzin degradation in soil: II Effects of tillage. *Pesticide Science* 31, 239–247.

Locke, M.A., Zablotowicz, R.M. and Gaston, L.A. (1995) Fluometuron interactions in crop residue-managed soils. In: Kingery, W.L. and Buehring, N. (eds) *Conservation Farming: a Focus on Water Quality.* Mississippi Agriculture Forestry Experiment Station Special Bulletin 88–7, pp. 55–58.

Locke, M.A., Gaston, L.A. and Zablotowicz, R.M. (1996) Alachlor biotransformation and sorption in soil from two soybean tillage systems. *Journal of Agricultural and Food Chemistry* 44, 1128–1134.

Locke, M.A., Gaston, L.A. and Zablotowicz, R.M. (1997) Acifluorfen sorption and sorption kinetics in soil. *Journal of Agricultural and Food Chemistry* 45, 286–293.

Locke, M.A., Reddy, K.N. and Zablotowicz, R.M. (2002a) Weed management in conservation production systems. *Weed Biology and Management* 2, 123–132.

Locke, M.A., Zablotowicz, R.M., Steinriede, R.W. and Dabney, S.M. (2002b) Conservation management practices in Mississippi Delta agriculture: implications for crop production and environmental quality. In: van Santen, E. (ed.) *Making Conservation Tillage Conventional: Building a Future on 25 Years of Research.* Alabama Agricultural Experiment Station and Auburn University Special Report No. 1, pp. 320–326.

Locke, M.A., Zablotowicz, R.M. and Gaston, L.A. (2002c) Environmental fate of fluometuron in a Mississippi Delta lake watershed. In: Arthur, E.L., Clay, V.C. and Barefoot, A.C. (eds) *Terrestrial Field Dissipation Studies: Purpose, Design, and Interpretation.* American Chemical Society Symposium Series 842, American Chemical Society, Washington, DC, pp. 206–225.

Lupwayi, N.Z., Rice, W.A. and Clayton, G.W. (1998) Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. *Soil Biology and Biochemistry* 13, 1733–1741.

Martikainen, E., Haimi, J. and Ahtianen, J. (1998) Effects of dimethoate and benomyl on soil organisms and soil processes – a microcosm study. *Applied Soil Ecology* 9, 381–387.

Martinez-Toledo, M.V., Salmeron, V. and Gonzalez-Lopez, J. (1991) Effect of simazine on the biological activity of *Azotobacter chroococcum*. *Soil Science* 151, 459–467.

Martinez-Toledo, M.V., Salmeron, V., Rodelas, B., Pozo, C. and Gonzalez-Lopez, J. (1998) Effects of the fungicide captan on some functional groups of soil microflora. *Applied Soil Ecology* 7, 245–255.

Megharaj, M., Singelton, I., Kookana, R. and Naidu, R. (1999) Persistence and effects of fenamiphos on native algal populations and enzymatic activities in soil. *Soil Biology and Biochemistry* 31, 1549–1553.

Moorman, T.B. (1989) A review of pesticide effects on microorganisms and soil fertility. *Journal of Production Agriculture* 2, 14–22.

Moorman, T.B., Becerrill, J.M., Lydon, J.M. and Duke, S.O. (1992) Production of hydroxybenzoic acids by *Bradyrhizobium japonicum* strains after treatment with glyphosate. *Journal of Agricultural Food Chemistry* 40, 289–293.

Narain Rai, J.P. (1992) Effect of long-term 2,4-D application on soil microbial populations. *Biology and Fertility of Soils* 13, 187–191.

Nemec, S. and Tucker, D. (1983) Effects of herbicides on endomycorrhizal fungi in Florida citrus (*Citrus* sp.). *Weed Science* 31, 427–431.

O'Bannon, J.H. and Nemec, S. (1978) Influence of soil pesticides on vesicular arbuscular mycorrizae in citrus. *Nematropica* 8, 56–61.

OECD (2001) OECD Dossier Guidance: Section 6 Ecotoxicological Studies and Risk Assessment. Available at: http://www.oecd.org/findDocument/0,2350,en_2649_34383_1_119820_1_1_1,00.htm

Omar, S.A. and Abdel-Sater, M.A. (2001) Microbial populations and enzyme activities in soil treated with pesticides. *Water, Air and Soil Pollution* 127, 49–63.

Perucci, P. and Scarponi, L. (1996) Side effects of rimsulfuron on the microbial biomass of a clay-loam soil. *Journal of Environmental Quality* 25, 610–613.

Perucci, P., Vischetti, C. and Battistoni, F. (1999) Rimsulfuron in a silty clay loam soil: effects upon microbiological and biochemical properties under varying microcosm conditions. *Soil Biology and Biochemistry* 31, 195–204.

Perucci, P., Dumontet, S., Bufo, S.A., Mazzatura, A. and Casucci, C. (2000) Effects of organic amendment and herbicide treatment on soil microbial biomass. *Biology and Fertility of Soils* 32, 17–23.

Racke, K.D. and Coats, J.R. (eds) (1990) *Enhanced Biodegradation of Pesticides in the Environment*. American Chemical Society Symposium Series 426, American Chemical Society, Washington, DC, 326 pp.

Reddy, K.N. and Locke, M.A. (1994) Prediction of soil sorption K_{oc} of herbicides using semi-empirical molecular properties. *Weed Science* 42, 453–461.

Reddy, K.N. and Locke, M.A. (1996) Imazaquin spray retention, foliar washoff, and runoff losses under simulated rainfall. *Pesticide Science* 48, 179–187.

Reddy, K.N. and Locke, M.A. (1998) Sulfentrazone sorption, desorption, and mineralization in soils from two tillage systems. *Weed Science* 46, 494–500.

Reddy, K.N. and Zablotowicz, R.M. (2003) Glyphosate-resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules. *Weed Science* 51, 494–502.

Reddy, K.N., Zablotowicz, R.M. and Locke, M.A. (1995a) Chlorimuron adsorption, desorption, and degradation in soils from conventional tillage and no-tillage systems. *Journal of Environmental Quality* 24, 760–767.

Reddy, K.N., Locke, M.A., Wagner, S.C., Zablotowicz, R.M., Gaston, L.A. and Smeda, R.J. (1995b) Chlorimuron ethyl sorption and desorption kinetics in soils and herbicide-desiccated cover crop residues. *Journal of Agricultural Food Chemistry* 43, 2752–2757.

Reddy, K.N., Locke, M.A. and Gaston, L.A. (1997a) Tillage and cover crop effects on cyanazine adsorption and desorption kinetics. *Soil Science* 162, 501–509.

Reddy, K.N., Locke, M.A. and Zablotowicz, R.M. (1997b) Soil type and tillage effects on sorption of cyanazine and degradation products. *Weed Science* 45, 727–732.

Reddy, K.N., Hoagland, R.E. and Zablotowicz, R.M. (2000) Effect of glyphosate on growth, chlorophyll content and nodulation in glyphosate-resistant soybeans (*Glycine max*) varieties. *Journal of New Seeds* 2, 37–52.

Rhoton, F.E. (2000) Influence of time on soil response to no-till practices. *Soil Science Society of America Journal* 64, 700–709.

Schäffer, A. (1993) Pesticide effects on enzyme activities in the soil ecosystem. In: Bollag, J.-M. and Stotzky, G. (eds) *Soil Biochemistry*. Marcel Dekker, New York, pp. 273–340.

Seifert, S., Shaw, D.R., Zablotowicz, R.M., Wesley, R.A. and Kingery, W.L. (2001) Effect of tillage on microbial characteristics and herbicide degradation. *Weed Science* 49, 685–693.

Smith, M.D., Hartnett, D.C. and Rice, C.W. (2000) Effects of long-term fungicide applications on microbial properties in tallgrass prairie soil. *Soil Biology and Biochemistry* 32, 935–946.

Staddon, W.J., Locke, M.A. and Zablotowicz, R.M. (2001) Microbiological characteristics of a vegetative bufferstrip soil and degradation and sorption of metolachlor. *Soil Science Society of America Journal* 65, 1136–1142.

Stearman, G.K., Lewis, R.J., Tortorelli, L.J. and Tyler, D.D. (1989) Herbicide reactivity of soil organic matter fractions in no-tilled and tilled cotton. *Soil Science Society of America Journal* 53, 1690–1694.

Suter, G.W. (1993) *Ecological Risk Assessment*. CRC Press, Boca Raton, Florida, pp. 156–159.

Taylor, A.W. and Spencer, W.F. (1990) Volatilization and vapor transport processes. In: Cheng, H.H. (ed.) *Pesticides in the Soil Environment*. Soil Science Society of America Book Series 2, Madison, Wisconsin, pp. 213–270.

Thirup, L., Johnsen, K., Torsvik, V., Spliid, N.H. and Jacobsen, C.S. (2001) Effects of fenpropimorph on bacteria and fungi during the decomposition of barley roots. *Soil Biology and Biochemistry* 33, 1517–1524.

Tiedje, J.M. and Zhou, Z. (1996) Analysis of non-culturable bacteria. In: Hall, G.S. (ed.) *Methods for the Examination of Organismal Diversity in Soils and Sediments*. CAB International, Wallingford, UK, pp. 53–66.

USEPA (1996) Ecological Effects Test Guidelines. Available at: http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/index.html

Wagner, S.C. and Zablotowicz, R.M. (1997a) Effect of organic amendments on the bioremediation of cyanazine and fluometuron in soil. *Journal of Environmental Science and Health Part B – Pesticides, Food Contaminants and Agricultural Wastes* 32, 37–54.

Wagner, S.C. and Zablotowicz, R.M. (1997b) Utilization of plant material for remediation of herbicide-contaminated soils. In: Kruger, E.L., Anderson, T.A. and Coats, J.R. (eds) *Phytoremediation of Soil and Water Contaminants*. American Chemical Society Symposium Series 664, American Chemical Society, Washington, DC, pp. 65–76.

Wagner, S.C., Zablotowicz, R.M., Locke, M.A., Smeda, R.J. and Bryson, C.T. (1995) Influence of herbicide-desiccated cover crops on biological soil quality in the Mississippi Delta. In: Kingery, W.L. and Buehring, N. (eds) *Conservation Farming: a Focus on Water Quality*. Mississippi Agricultural Forestry Experiment Station Special Bulletin 88–7, pp. 86–89.

Wagner, S.C., Zablotowicz, R.M., Gaston, L.A., Locke, M.A. and Kinsella, J. (1996) Biodegradation of bentazon in soil, effects of tillage and bentazon history. *Journal of Agricultural and Food Chemistry* 44, 1593–1598.

Wardle, D.A., Yeates, G.W., Nicholson, K.S., Bonner, K.I. and Watson, R.N. (1999) Response of soil microbial biomass dynamics, activity, and plant litter decomposition to agricultural intensification over a seven-year period. *Soil Biology and Biochemistry* 31, 1707–1720.

Zablotowicz, R.M., Locke, M.A. and Smeda, R.J. (1998a) Degradation of the herbicides 2,4-D and fluometuron in herbicide-desiccated cover crop residues. *Chemosphere* 37, 87–101.

Zablotowicz, R.M., Hoagland, R.E. and Locke, M.A. (1998b) Biostimulation: enhancement of cometabolic pathways in pesticide-contaminated soils. In: Kearney, P.C. and Roberts, T.R. (eds) *Pesticide Remediation in Soils and Water*. John Wiley & Sons, Chichester, UK, pp. 217–250.

Zablotowicz, R.M., Locke, M.A., Gaston, L.A. and Bryson, C.T. (2000) Interactions of tillage and soil depth on fluometuron degradation in a Dundee silt loam soil. *Soil Tillage Research* 57, 61–68.