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Impact of agricultural inputs on soil organisms—a review

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Abstract. External agricultural inputs such as mineral fertilisers, organic amendments, microbial inoculants, and pesticides are applied with the ultimate goal of maximising productivity and economic returns, while side effects on soil organisms are often neglected. We have summarised the current understanding of how agricultural inputs affect the amounts, activity, and diversity of soil organisms. Mineral fertilisers have limited direct effects, but their application can enhance soil biological activity via increases in system productivity, crop residue return, and soil organic matter. Another important indirect effect especially of N fertilisation is soil acidification, with considerable negative effects on soil organisms. Organic amendments such as manure, compost, biosolids, and humic substances provide a direct source of C for soil organisms as well as an indirect C source via increased plant growth and plant residue returns. Non-target effects of microbial inoculants appear to be small and transient. Among the pesticides, few significant effects of herbicides on soil organisms have been documented, whereas negative effects of insecticides and fungicides are more common. Copper fungicides are among the most toxic and most persistent fungicides, and their application warrants strict regulation. Quality control of organic waste products such as municipal composts and biosolids is likewise mandatory to avoid accumulation of elements that are toxic to soil organisms.

Additional keywords: fertiliser, compost, manure, biosolids, pesticide, soil biology.

Introduction

Agricultural inputs

External inputs to agricultural production systems include mineral fertilisers such as urea, ammonium nitrate, sulfates, and phosphates; organic fertilisers such as animal manures, composts, and biosolids; various other organic products such as humic acids and microbial inoculants, and pesticides including herbicides, insecticides, nematicides, fungicides, veterinary health products, and soil fumigants. All these products are applied with the ultimate goal of maximising productivity and economic returns.

Mineral fertilisers are a major physical input into Australian agricultural production and account for over 12% of the value of material and services inputs used (Fertilizer Industry Federation of Australia Inc., www.fifa.asn.au). In 1999, Australian farmers used around 5.25 million t of fertiliser products with a value of approximately AU$2 billion. Common types of mineral fertilisers and their abbreviations as used in this review are shown in Table 1. Manures from intensive animal industries are a major source of organic amendments for agricultural land. In Australia, beef and dairy cattle alone produce approximately 4 million t of manure every year. Human waste is another important source. Sydney, Australia's largest urban area, produces 185 000 t of biosolids each year (Sydney Water Annual Report 2004). Nearly all of this is used for land amendment, either as dewatered solids, lime-stabilised solids, or in composts with green wastes. Pesticides are a diverse group of inorganic and organic chemicals. More than 380 active constituent pesticides are currently registered in Australia (Record of Approved Active Constituents at: www.apvma.gov.au). Pesticide inputs constitute a major cost for Australian agriculture. For herbicide inputs alone it was estimated at AU$571 million to annual winter crops in the 1998–99 growing season (Jones *et al*. 2005).

Soil organisms: groups, activities, methods

Soil organisms consist of the microflora (bacteria and fungi) and the soil fauna (protozoa and invertebrate groups such as nematodes, mites, and earthworms). They influence the availability of nutrients for crop production via a range of activities such as the decomposition of crop residues, immobilisation of nutrients, mineralisation, biological nitrogen fixation, and bioturbation. The soil fauna is crucial for the initial comminution and mixing of residues into the soil, whilst the microflora has a greater suite of enzymes

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Table 1. Some common inorganic fertilisers and their abbreviations as used in this review

Name	Abbreviation
Ammonium nitrate	AN
Ammonium sulfate	AS
Calcium nitrate	CaN
Diammonium phosphate	DAP
Elemental sulfur	S^0
Phosphate rock	PR
Sodium dihydrogen phosphate	NaHP
Superphosphate	SP
Triple superphosphate	TSP
Urea	U

for chemical breakdown of organic material (Paul and Clark 1996). Bacteria and fungi are often considered as a labile pool of nutrients (C, N, P, S) called the soil microbial biomass that has a pivotal role in nutrient immobilisation and mineralisation. The release of nutrients from the microbial biomass is partly regulated through grazing by the soil fauna.

The effect of agricultural inputs on soil organisms can be measured either as changes in the amount of single organisms, organism groups or methodologically defined pools such as the microbial biomass, or as changes in biological activity, e.g. soil respiration and enzyme activities. The most commonly used methods are listed and explained in Table 2. Variable effects of a given amendment on different organisms may change the composition of the microbial (or faunal) community without changing total amounts or activities. However, most studies have focussed on the soil microbial biomass as the central pool in nutrient cycling.

Concept of this review

In this paper we summarise the current understanding of the effects of inorganic and organic agricultural inputs on soil organisms. The underlying concept is that these inputs

Table 2. Common methods to assess the amount, activity, and diversity of soil organisms

Name	Method description	Reference
	Enumeration/amount	
Microbial C	C in microbial biomass by fumigation–extraction or microwave methods	Vance *et al*. (1987), Islam and Weil (1998)
Microbial N	N in microbial biomass by fumigation–extraction methods	Vance *et al*. (1987), Amato and Ladd (1988)
Microbial P	P in microbial biomass by fumigation–extraction methods	Brookes *et al*. (1982), Kouno *et al*. (1995)
Adenosine triphosphate	Extractable ATP indicates size of microbial biomass	Contin *et al*. (2001)
Total bacterial DNA	PicoGreen dsDNA	Angersbach and Earp (2004)
CFU	Colony forming units; plate counting techniques, e.g. Gram −ve bacteria, actinomycetes, fungi	e.g. Sarathchandra *et al*. (1993)
AMF	Arbuscular mycorrhizal fungi; usually root colonisation observed	e.g. Ryan *et al*. (2000)
Soil fauna	Nematodes, collembola (springtails), enchytraeids, earthworms (sometimes in combination with avoidance tests)	e.g. Martikainen *et al*. (1998), Van Zwieten *et al*. (2003)
	Activity	
Soil respiration	CO_2-release from incubated soil	Alef (1995)
Metabolic quotient	Ratio of soil respiration to microbial C; higher values can indicate physiological stress	Anderson and Domsch (1990)
Soil enzyme activities	Dehydrogenase, acid and alkaline phosphatase, amidase, urease, arylsulfatase, etc.	Tabatabai (1994)
FDA hydrolysis	Fluorescein diacetate (FDA) hydrolysis as a measure of total microbial activity	Adam and Duncan (2001)
Acetylcholin-esterase activity in earthworms	Activity of the enzyme that hydrolyses acetylcholine in the nervous system; reduced activity indicates toxicity	e.g. Panda and Sahu (2004)
	Diversity	
PLFA and FAME	Phospholipid fatty acid and fatty acid methyl esters and analysis: indicate changes in microbial community composition	Drenovsky *et al*. (2004)
DGGE	Microbial diversity assessed by DNA extraction, amplification with polymerase chain reaction (PCR) and differentiation by denaturing gradient gel electrophoresis (DGGE)	e.g. Marschner *et al*. (2004)
Biolog	Soil microbial substrate utilisation potential	Konopka *et al*. (1998)

can affect soil organisms through direct or indirect effects (Table 3). Direct effects via changes in nutrient availability or toxicity will become already apparent in the first season after the application or in the longer term if repeated additions are required to reach a threshold above which effects are seen. Indirect effects will usually take more than one season to establish, especially when changes in soil organic matter levels are involved. In the case of long-term data, it can be difficult to separate direct and indirect effects.

Existing data are presented for the different amendments separately but discussed together. The evidence from Australia is rather limited, and therefore the review includes literature from overseas, in an attempt to establish the main principles and to draw some conclusions applicable to agro-ecosystems in Australia.

Mineral fertilisers

Most mineral fertiliser in Australia and elsewhere is applied to systems with regular and significant nutrient exports in harvested products, i.e. to grasslands and land under arable cropping. Experimental approaches to assess the effect of mineral fertilisers range from laboratory incubations, pot experiments, and 1-season studies in the field to long-term field experiments and sampling of paired sites under different management, thus covering time frames from days to more than 100 years. In an attempt to separate direct from indirect effects, in the following sections we have compiled studies according to their experimental approach and time frame.

Laboratory incubations

Laboratory incubations allow the study of short-term effects under controlled conditions, i.e. in the absence of plants, climatic variation, and external inputs or losses. We found, however, very limited and often contradictory results from laboratory studies. For example, the addition of 200 mg N/kg soil as ammonium sulfate to 2 pasture soils of varying P status from New Zealand resulted in a decrease in microbial P, no change in the turnover of added C, and an increase in N mineralisation during 168 days of incubation (Saggar *et al*. 2000). An earlier study from New Zealand had, however, found an increase in soil respiration and microbial P, but no effect on microbial N and a decrease in various enzyme activities upon addition of 500 mg P/kg soil as calcium diphosphate (Haynes and Swift 1988). The addition of N, P, K, and S at 100, 20, 100, and 20 mg/kg soil, respectively, to a range of soils from southern Australia, followed by incubation for 20 days, resulted in minor changes (increase or decrease) of soil respiration and microbial C, N, and P that remained within 20% difference from the non-amended controls (Bünemann, unpublished). Remarkably, changes in microbial C, N, and P were not interrelated.

Contradicting evidence such as an increase in microbial P while microbial C and N are unaffected might be interpreted as shifts in the composition of the microbial community. This possibility has been investigated in recent studies using biochemical markers and molecular techniques. The addition of N did not change the community composition as indicated by the phospholipid fatty acid (PLFA) profile in a study where total soil respiration was unaffected, but peroxidase activity and the preferential use of older, more stable soil organic matter increased after N addition (Waldrop and Firestone 2004). In 2 studies from Germany, ammonium addition did not change the composition of the microbial community during 28 days of incubation (Avrahami *et al*. 2003*a*), but led to community shifts after 16 weeks of incubation (Avrahami *et al*. 2003*b*). Using molecular techniques in a range of pot experiments, Marschner *et al*. (2004) showed that soil pH and N and P fertilisation can affect the microbial community composition, but that substrate availability, e.g. in the form of root exudates in the rhizosphere, appears to be the main factor determining the community composition in the rhizosphere. It is thus important to consider the potential feedback from improved plant nutrition when examining fertiliser effects on soil organisms.

Table 3. Potential effects of inorganic and organic agricultural inputs on soil organisms

	Time frame[A]
Direct effects	
• Increased amount and/or activity after removal of nutrient limitations	Short- to long-term
• Decreased activity due to high nutrient availability	
• Decreased amount and/or activity due to toxicity	
Indirect effects	
• Change in pH	Long-term
• Change in soil physical properties (aggregation, porosity)	
• Change in productivity, residue inputs, and soil organic matter levels	

[A]Short-term, 1 season; long-term, more than 1 season.

Pot experiments and field studies

Pot experiments (Table 4) have mainly been used to investigate the effect of mineral P and N fertiliser on root colonisation by arbuscular mycorrhizal fungi (AMF). Whereas the addition of mineral N did not affect AMF, increasing additions of inorganic P decreased the rate of root length colonisation in 2 cases (Ryan and Ash 1999; Rubio *et al*. 2003). A decrease in AMF root colonisation was also observed in pastures after 15–17 years of mineral P and N fertilisation (Ryan *et al*. 2000).

Many field experiments have shown a lack of response of the microbial biomass and earthworms to mineral fertilisers (Table 4), even in cases where pasture production increased (e.g. Perrott *et al*. 1992; Sarathchandra *et al*. 1993). Where a decrease in microbial C was observed, it was usually accompanied by a decrease in soil pH after application of N or S fertilisers (e.g. Gupta *et al*. 1988; Ladd *et al*. 1994;

Table 4. Effects of inorganic fertilisers on soil organisms as observed in pot experiments and field studies

Reference and location	Soil type and characteristics[A]	Vegetation/ test plant	Time frame	Fertiliser (kg/ha)	Effect on soil organisms (amount and activity; % of control)			Other changes
					Negative	No change	Positive	
Ryan and Ash (1999), Australia	Red-brown earth, pH 6, OC 26 g/kg	Pot exp with white clover and ryegrass	5 weeks	180 N[B] (AN)		% Clover and rye grass root length colonized by AMF		
				200 P[B] (NaHP)	% Clover root length colonised by AMF (20–30%)	% Ryegrass root length colonised by AMF		
Rubio *et al.* (2003), Chile	Volcanic soil, pH 5.5, 18% SOM	Pot exp with wheat	6 months	17–86 P (TSP, PR)	% Root colonisation (60–90%)			
Sarathchandra *et al.* (1993), NZ	Not given	Pasture	2 weeks	0–120 P (SP, PR)		Microbial P, earthworms	Fungi (480%), Gram −ve bacteria (140%)	
			3 years			Microbial P, earthworms		Pasture production ↑
Lovell and Hatch (1997), UK	36% clay	Pasture	10 weeks	40 N (AN)		Microbial C and N	Nitrification, ammonification	
Lupwayi *et al.* (2001), Canada	Gray Luvisols and Black Chernozems, pH 5.7 and 7.3, OC 28 and 47 g/kg	Wheat–canola	1–2 seasons	20 S (S^0 or AS)	Diversity (Biolog)	Microbial C		
Perrott *et al.* (1992), NZ	Yellow-brown earth, silt loam, pH 5.8, OC 76 g/kg	Pasture	2 years	61 P (SP)		Microbial P and S		Herbage ↑
Sarathchandra *et al.* (2001), NZ	Umbric Dystrochrept, sandy loam, pH 5.7, OC 63 g/kg	Pasture	4 years	400 N (U)	Microbial C (80%), diversity (Biolog), *Meloidogyne* (10%), plant-associated (68%) and fungal-feeding (82%) nematodes	Total nematodes	*Paratylenchus* (1677%)	OC ↓, pH ↓ by 0.4 units
	Typic Hapludand, silt loam, pH 4.9, OC 77 g/kg	>10 years	100 P (SP)		Microbial C, microbial diversity (Biolog), total nematodes			
Gupta *et al.* (1988), Canada	Grey Luvisols, pH 5.4 and 5.7, OC 11 and 34 g/kg	Canola–fallow rotation	2 years	50–100 S (S^0)	Fungal CFUs (38%), protozoa (4–25%), microbial C (49–98%), respiration (67–86%)		Microbial S (136–168%), acid phosphatase (106–130%)	pH ↓ by 0.15 units
Gupta and Germida (1988); Gupta *et al.* (1988), Canada	Grey Luvisol, pH 5.7, OC 28 g/kg	Pasture	5 years	44 S (S^0)	Microbial C (60%), respiration (54%), hyphal length (24%), fungal CFUs (23%), protozoa (29–71%)	Bacterial and actinomycetes CFUs	Microbial S (178%), acid phosphatase (141%)	pH ↓ by 1.0 units, OC ↓

Table 4 Contd

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Reference and location	Soil type and characteristics[A]	Vegetation/ test plant	Time frame	Fertiliser (kg/ha)	Effect on soil organisms (amount and activity; % of control)			Other changes
					Negative	No change	Positive	
Ladd *et al.* (1994), Australia	Red-brown earth, 14% clay, pH 6.8, OC 10 g/kg	Wheat rotations	9–13 years	0–80 N (AN)	Microbial C (68–86%)	C and N mineralisation		pH ↓ by 0.4–1.0 units
Parfitt *et al.* (2005), NZ	Typic Dystrudepts, pH 5.6–6.0, OC 32–41 t/ha in 0–7.5 cm	Pasture	7–23 years	12–37 P, 12–26 S		Earthworms	Microbial P (168–300%), microbial N (106–163%), total nematodes (123–223%)	
Ryan *et al.* (2000), Australia	Mostly red-brown earths, mean pH 6.2, mean OC 2.6 g/kg	Pasture	15–17 years	27 P (SP, DAP), 17 N (U)	% Clover and grass root length colonised by AMF (67–79%)			
Moore *et al.* (2000), USA	Hapludoll, 22% clay and 31% sand, mean pH 6.9, mean OC 21 g/kg	Maize–soybean–oat–meadow rotations	19 years	180 N (U)		Microbial C		pH ↓ by 0.1–0.9 units; microbial C related to OC
	Haplaquoll, 33% clay and 24% sand, mean pH 6.2, mean OC 34 g/kg		44 years	180 N (U)		Microbial C		
Graham *et al.* (2002); Graham and Haynes 2005, S. Africa	Chromic Vertisol, 58% clay, pH 5.8, OC 42 g/kg	Sugarcane	59–60 years	140 N, 20 P, 140 K	Dehydrogenase, arylsulfatase, alkaline phosphatase	Protease, respiration	microbial C (119–136%), FDA hydrolysis rate, acid phosphatase	OC ↑, pH ↓ by 0.7 units

[A]Soil classification, texture, pH, and OC content as far as given.
[B]mg/kg soil.

Sarathchandra *et al*. 2001). Other methods such as microbial enumeration by plate counts (Sarathchandra *et al*. 1993), enzyme activities (Graham and Haynes 2005), and nematode counts (Parfitt *et al*. 2005), which are possibly more sensitive than measurements of microbial biomass, show variable changes due to mineral fertilisation (Table 4). For example, although the total number of nematodes was not affected by N fertilisation and a concomitant decrease in pH, some nematode species increased, whereas others were decreased (Sarathchandra *et al*. 2001).

The absence of changes in microbial C in response to N fertilisation and a related decrease in pH in the 2 long-term field experiments studied by Moore *et al*. (2000) are interesting, because in the same study microbial C was found to be correlated to levels of organic C (OC) as induced by different crop rotations. Several long-term field experiments in which mineral and organic fertiliser inputs have been compared (Table 5) have likewise shown good correlations between the microbial biomass and soil organic C (Witter *et al*. 1993; Houot and Chaussod 1995; Leita *et al*. 1999). Although soil organic C levels are often increased by mineral fertilisation compared with the non-fertilised control, even greater increases in soil organic C are usually achieved in treatments receiving organic amendments. This is also reflected in the fact that whereas mineral fertilisers show variable effects on soil organisms, organic amendments have only been reported to have insignificant or positive long-term effects (Table 5). The only exception was a decrease in microbial C after sewage sludge application, which also decreased soil pH (Witter *et al*. 1993). These observations point towards the role of C inputs, either with the organic amendment, or indirectly via increased plant growth and resulting plant residue input.

Graham *et al*. (2002) investigated the amounts of microbial C and N under sugarcane after 59 years of differential crop residue management and NPK fertilisation and showed that the microbial biomass was directly influenced by residue management and indirectly by NPK fertilisation through increased residue inputs. A follow-up study in the same trial revealed the interaction of soil acidification with negative effects and organic matter accumulation with positive effects on soil organisms and enzyme activities (Graham and Haynes 2005). The long-term field experiment studied by Houot and Chaussod (1995) exemplifies that agro-ecosystems can be relatively slow to respond to changes in management and thus illustrates the value of long-term field experiments. The excellent correlation between microbial C and soil organic C found after >100 years of constant management practices remained disturbed 2 years after a change in crop rotation and crop residue management. The time required to reach a new equilibrium is a factor that may confound the results from many short-term studies.

Another potential indirect effect of fertiliser inputs was investigated in a long-term fertilisation experiment without plants (Pernes-Debuyser and Tessier 2004). The comparison of various N, P, and K fertilisers, liming, and manure treatments revealed that ammonium fertilisers decreased pH and CEC, causing a degradation of hydraulic properties, whereas basic amendments increased pH and CEC. Aggregate stability was lowest in acid plots, intermediate in basic plots, and highest in plots treated with manure. A short-term study suggested that ammonium nitrate enhanced soil porosity by 18%, compared with 46% increase in a manure treatment. Since soil respiration almost doubled in the mineral fertiliser treatment compared with the unfertilised control, the authors discussed a potential priming effect of N addition on the decomposition of soil organic matter. Although such a priming effect is often observed (Kuzyakov *et al*. 2000), it seems to be rather short-lived, which might explain why we did not find much evidence for it (Table 4).

A decreased amount or activity of soil organisms after mineral fertilisation could be due to the toxicity of metal contaminants contained in mineral fertilisers. In general, N and K fertilisers contain very low levels of contaminants, whereas P fertilisers often contain significant amounts of cadmium, mercury, and lead (McLaughlin *et al*. 2000). Metal contaminants are, however, most prevalent in waste products from urban and industrial areas and will be dealt with more in-depth in the section on organic fertilisers. Long-term chronic toxicity due to gradually accumulating metals appears to be far more common than immediate, acute toxicity (Giller *et al*. 1998). Quality control of fertiliser products is therefore required. This applies in particular to any new products. For example, the application of rare earth elements such as lanthanum, which is increasing in China, was shown to decrease soil respiration and dehydrogenase activity at high application rates (Chu *et al*. 2003). Such observations warrant more detailed investigation into processes of accumulation, bioavailability, and threshold levels of elements contained in fertilisers that can be toxic to soil organisms.

Organic fertilisers

Since most organic fertilisers are waste products, their application rate is often determined by availability rather than demand. Most amendments are applied primarily to benefit plant growth. In contrast to mineral fertilisers, however, effects on the soil's physical, chemical, and biological properties are sometimes intended as well (Table 6). In the following sections, we try to establish some links between the properties of various organic inputs and their effects on soil organisms.

Compostable organics

Compostable and composted materials vary widely in characteristics such as dry matter content, pH, salinity,

Table 5. Comparative effects of inorganic and organic fertilisers on soil organisms as concluded from field experiments

FYM, farmyard manure

Reference and location	Soil type and characteristics[A]	Vegetation	Time frame (years)	Fertiliser (kg/ha.year)	Effect on soil organisms (amount and activity; % of control)			Other changes
					Negative	No change	Positive	
Peacock *et al.* (2001), USA	Typic Fragiudalf, silt loam, pH 6.0, OC 15 g/kg	Maize–pasture rotation	5	18 N (AN)	Gram −ve bacteria (85%)	Total PLFAs	Gram +ve bacteria (120%)	pH ↓ by 0.6 units
				FYM (252 N)		Gram +ve bacteria	Total PLFAs (170%), Gram −ve bacteria (115%)	OC ↑
Leita *et al.* (1999), Italy	Calcic Cambisol, 29% clay, 47% sand, pH 7.8, OC 7.9 g/kg	Cover crop	12	100 N (AN), 75 P (SP), 150 K (PS)		Microbial C		Microbial C correlated to OC
				Compost (500–1500 N)			Microbial C (200–350%)	
				FYM (500 N)			Microbial C (243%)	
Witter *et al.* (1993), Sweden	35% clay, 21% sand, pH 6.2, OC 10 g/kg	Arable crops	34–36	80 N (CaN)			Microbial C (142%)	pH ↓ with AS and sewage sludge; microbial C and respiration related to OC
				80 N (AS)	Microbial C (13%)			
				FYM (4 t/ha.year)			Microbial C (209%)	
				Sewage sludge 4 t/ha.year)	Microbial C (69%)			
Dick *et al.* (1988), USA	Haploxeroll, coarse-silty, pH 6.5	Wheat–fallow rotation	55	90 N	Amidase, urease	Acid and alk. phosphatase, arylsulfatase, glucosidase		pH ↓ by 0.6 units
				Straw + manure			Acid and alk. phosphatase, arylsulfatase, glucosidase, amidase, urease	pH ↑ by 0.6 units, OC ↑
Parham *et al.* (2002, 2003), USA	Paleustoll, 23% clay, 38% sand, pH 5, OC 6.7 g/kg	Wheat	69	67 N, 15 P, 28 K	Fast-growing bacteria	Bacterial and fungal CFU, alk. phosphatase, phosphodiesterase, pyrophosphatase	Microbial C, acid phosphatase, slow-growing bacteria	pH ↓ by ~0.5 units
			>100	FYM (269 N/ha.4 years		Fungal CFU, acid phosphatase, fast-growing bacteria	Microbial C, alk. phosphatase, phosphodiesterase, pyrophosphatase, bacterial CFU, slow-growing bacteria	pH ↑ by ~0.6 units
Colvan *et al.* (2001); O'Donnell *et al.* (2001), UK	Clay loam, pH 5.2, OC 34 g/kg	Pasture	100	35 N (AS)	Microbial P, C		Phosphatase	pH ↓ by 2 units
				60 P		Phosphatase	Microbial P	
				67 K			Phosphatase	
				35 N, 60 P, 67 K			Microbial P, phosphatase	
				FYM (20 t/ha.year)			Microbial P, phosphatase	pH ↑ by 0.6 units
Houot and Chaussod (1995), France	Agrudalf, 22–30% clay, 40–44% sand, pH 8.0–8.3	Wheat–sugar beet	112	87 N, 40 P, 75 K			Microbial C	Microbial C related to OC
				FYM (1 t/ha.year)			Microbial C	

[A]Soil classification, texture, pH, and OC content as far as given.

Table 6. Intended benefits of organic amendments

Reason for organic amendment	Examples
(*a*) Supply bulk nutrients for plant production	Animal manures, sewage sludge, and other composted organics supply N, P, K for plant uptake
(*b*) Increase availability of existing soil nutrients	Bacteria solubilise P and S from soil minerals (Grayston and Germida 1991; Gyaneshwar *et al*. 2002). Mycorrhizae extend root exploration and uptake of immobile nutrients (Dodd and Thomson 1994)
(*c*) Increase the availability of applied fertilisers	Humic acid products may increase fertiliser P availability (Delgado *et al*. 2002)
(*d*) Fix N from air	Symbiotic and free-living N_2-fixing bacteria (Brockwell 2004; Kennedy *et al*. 2004)
(*e*) Improve soil chemical fertility	Manure, sewage sludge, and compost can increase soil organic matter and cation exchange capacity. Humic substances can enhance micronutrient availability (Chen *et al*. 2004)
(*f*) Improve soil physical condition	Mulches prevent erosion and improve water infiltration and water storage (Buerkert *et al*. 2000). Manures and mycorrhizae enhance aggregate stability and pore structure (Tisdall and Oades 1982)
(*g*) Improve soil biology	Manures and composts can add significant quantities of readily decomposable C substrate for microbes, and add microbes as well (Semple *et al*. 2001). 'Helper' bacteria can stimulate mycorrhizal and rhizobial symbioses (Garbaye 1994)
(*h*) Plant growth promoters	Rhizobacteria and possibly humic substances can supply plant growth-promoting hormones (Bowen and Rovira 1999)
(*i*) Direct suppression of plant disease	Composted manure and brewed compost leachates may suppress plant diseases (Scheuerell and Mahaffee 2002). Mycorrhizal fungi can control nematodes and root diseases (Siddiqui and Mahmood 1995; Whipps 2004)
(*j*) Indirect suppression of plant disease	Rhizobacteria can be added to seed or soil to enhance plant resistance to disease. Organic substrates may stimulate plant-beneficial microbial populations
(*k*) Decontaminate polluted soils	Microbially catalysed reactions in soil can breakdown organic pollutants or precipitate metals making them unavailable for plant uptake or water transport (Romantschuk *et al*. 2000)
(*l*) Degrade crop residues and other compostable materials	Microbial inoculants may enhance breakdown of crop residues and waxes that cause water repellency (Damodaran *et al*. 2004; Roper 2004)

carbon content, plant nutrient concentrations, non-nutrient elements, and microbial types, numbers, and activity. Although studies of amendments vary widely in nature of materials, application rates, and experimental conditions (Albiach *et al*. 2000), amendment with raw and composted organics generally results in increased microbial proliferation in the soil (Table 7). The duration of observed increases in soil organisms depends on the amount and proportions of readily decomposable carbon substrates added and the availability of nutrients, particularly nitrogen (Hartz *et al*. 2000; Adediran *et al*. 2003). However, microbial characteristics of amended soils often return to their baseline within a few years (Speir *et al*. 2003; Garcia Gil *et al*. 2004). Sustained changes in microbial biomass, diversity, and function are more likely where organic amendments are ongoing, as is the case in organic and biodynamic farms (Mäder *et al*. 2002; Zaller and Kopke 2004). Ryan (1999) argues, however, that an increase in microbial populations may not be seen when system productivity is limited by nutrient input or water supply.

Manures and sewage sludge generally have higher salinity than municipal garden wastes, and salts can build up in soil with repeated heavy applications (Hao and Chang 2003; Usman *et al*. 2004). Sewage sludges (biosolids) often contain heavy metals such as copper, zinc, or cadmium, especially where industries contribute to the waste stream. Heavy metals can affect microbial processes more than they affect soil animals or plants growing on the same soils. For example, nitrogen-fixing rhizobia were far more sensitive to metal toxicity than their host plant clover. This resulted in N deficiency of clover due to ineffective rhizobia in sludge-amended soils (Giller *et al*. 1998). Sewage sludge and livestock manure may also contain active residues of therapeutic agents used to treat or cure diseases in humans and animals (Jjemba 2002). Green wastes from farms and gardens are typically lower in nutrient concentrations than manures or sewage sludges, but may contain residues of synthetic compounds such as herbicides, insecticides, fungicides, and plant growth regulators. Composting degrades some but not all such compounds, depending on the nature of the pesticide and the specific composting conditions (Buyuksonmez *et al*. 2000). Negative effects of heavy metals (Giller *et al*. 1998) can persist for many years following cessation of application (Abaye *et al*. 2005), since metals persist in soil practically indefinitely (McLaughlin *et al*. 2000). Such observations warrant strict regulations of organic fertiliser quality and applied quantity, especially of waste products such as sewage sludge and biosolids, in order to minimise contamination of agricultural land with toxic metals.

Humic substances

Humus in soil has traditionally been separated into humin, humic acid, and fulvic acid based on extraction with an alkaline solution and subsequent precipitation after addition of an acid (Swift 1996). The fractions typically rank in their resistance to microbial decomposition in the order humic

Table 7. Effects of animal manures, biosolids, and composts on soil organisms

Reference and location	Soil type and characteristics	Compared treatments	Effects
Trochoulias *et al*. (1986), Australia	Red basaltic soil	Poultry manure, gypsum + dolomite, others	Manured treatment had highest microbial C
Poll *et al*. (2003), Germany	Luvic Phaeozem (FAO), 8% clay, 72% sand, pH 5.6, OC 10 g/kg	Long-term annual application of farmyard manure, control	Manure addition enhanced microbial biomass and xylanase and invertase activity
Dinesh *et al*. (2000), India	5 soils; pH 5.7–6.4, OC 6–9 g/kg	3 years poultry manure, FYM, sesbania and gliricidia residues, control	Organic manures increased microbial biomass, activity, diversity, and C turnover
Wu *et al*. (2004), China	3 soils: Calcaric Cambisols, Haplic Greyxems, and Calcic Kastanozems (FAO); 25–30% clay, pH 8.3–8.4, OC 7–17 g/kg	Manure, mineral fertiliser, combined manure and fertiliser	Manure ± N and P fertiliser treatments restored OC and microbial C to the level of the native sod
Min *et al*. (2003), USA	Mesiq Achic Hapludult, fine loamy, pH 6.6, OC 15.8 g/kg	5 years of dairy manure slurry, mineral fertiliser, control	Dairy manure slurries increased OC and microbial biomass and decreased metabolic quotient compared with mineral fertiliser treatments
Thomsen *et al*. (2003), Denmark	3 soils: 11–34% clay, 11% sand, pH 6.4–7.4, OC 13.7–15.4 g/kg	Lab. incubation of soil amended with sheep manure at various soil matric potentials and clay contents	Manure increased soil respiration in all combinations of soils and matric potentials. Microbial biomass increased most with the addition of manure to the sandiest soil
Yang *et al*. (2003), USA	Thermic Typic Xerothent; coarse loam; pH 8.1, OC 2 g/kg	Surface mulches of grass clippings, lucerne stems, composted manure, eucalyptus, oleander or pine chip waste, chipped construction waste	Only grass clippings stimulated dehydrogenase activity in the soil measured after 1 year. Eucalyptus yardwaste and grass clippings caused shifts in bacterial populations and increased bacterial diversity but only at the soil surface
Villar *et al*. (2004), Spain	3 Typic Haplumbrepts; sandy loam to sandy clay loam; pH 4.6–6.3; OC 7.1–19.9 g/kg	Single application of poultry manure, NPK fertiliser to soil after wildfire	Poultry manure application increased microbial biomass C, particularly at high dose. Little or no changes as a consequence of inorganic fertilisation
Baker *et al*. (2002), Australia	3 soils: Aeric Kandiaqualf, Typic Natraqualf, Oxic Ustropept	Biosolids (30–120 t/ha)	Increase in earthworm abundance
Munn *et al*. (2001), Australia	6 soils: 6–54% clay, pH 4.2–6.4, OC 7–32 g/kg	Single application of biosolids from 5 treatment plants applied to one soil. Biosolids from 1 plant applied to 6 soils	Symbiotic effectiveness of rhizobium dependent on soil type and level and source of biosolids, not on basis of heavy metal concentrations
Abaye *et al*. (2005), England	Typic Udipsamment; sandy loam; 8% clay; pH 6.5–7.1; OC 5.2–14.8 g/kg	Long-term FYM, metal-contaminated sewage sludge, NPK mineral fertiliser	Microbial biomass-C and total bacterial numbers greater in the FYM-treated soil than in NPK and sludge-amended soils. Relatively small heavy-metal concentrations decreased microbial C and bacterial numbers, increased metabolic quotient, and changed microbial community 40 years after metal inputs ceased
Chaudhuri *et al*. (2003), India	Acid lateritic soil; pH 5.2; OC 5.4 g/kg	Several combinations of sludge and coal ash, control, NPK fertiliser	Microbial C and soil enzyme activities increased with all amendments; highest at equal proportions of coal ash and sludge. Mobile fractions of Cd and Ni correlated with microbial C
Usman *et al*. (2004), Germany	Calcareous soil; 3.5% clay, 87% sand; pH 8.16; OC 3.1 g/kg	Short-term incubation, sewage sludge, composted turf and plant residues	Compared with compost, sewage sludge caused greater increases in soil respiration, microbial C, and metabolic quotient, especially with increasing application rate

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Table 7. *Continued*

Reference and location	Soil type and characteristics	Compared treatments	Effects
Alvarez *et al*. (1999), Argentina	Thermic Vertic Argiudol; clay 42%, sand 14%; pH 5.8; OC 17 g/kg	2 years of sewage sludge applied to de-surfaced soils	Microbial biomass not affected by sludge, but metabolic activity and organic matter mineralisation enhanced. Increased soil respiration from sludge-amended soil represented 21% of C applied that year and 15% of C applied the year before
Barbarick *et al*. (2004), USA	2 soils: Aridic Argiboroll, Aridic Argiustoll; pH 7.3, 5.9; OC 1.5 g/kg	Single application of biosolids	6 years after application, amended plots had increased microbial respiration, nitrogen mineralisation, root colonisation by AM, microbial biomass. No change in metabolic quotient
Garcia Gil *et al*. (2004), Spain	Calcareous sandy loam; sand 41%, clay 29%; pH 8.1; OC 10.1 g/kg	Single application of sewage sludge	Microbial biomass, basal respiration, metabolic quotient, and enzymatic activities increased in soil 9 months after sludge application, but increases had disappeared after 36 months, presumably due to the loss of energy sources
Speir *et al*. (2003), New Zealand	Typic Udipsamment; coarse sand; pH 6.1; OC 39 g/kg	Compost of biosolids, wood waste and green waste	Soil basal respiration, microbial C, and anaerobically mineralisable N were significantly increased in the amended plots. No effects on rhizobial numbers or microbial biosensors (Rhizotox C and lux-marked *Escherichia coli*)
Canali *et al*. (2004), Italy	Sandy loam; pH 7.8; OC 17.3 g/kg	Composts of distillery waste and livestock manure, poultry manure, mineral fertiliser control	Parameters related to potentially mineralisable C showed significant differences among the treatments. No differences were observed in biodiversity indexes
Wells *et al*. (2000), Australia	Luvic Ferrasol (yellow earth); 77% sand, 15% clay; pH 5.4; OC 11.9 g/kg	Composts of woody material with either manure (poultry and horse) or sewage sludge, several mineral fertiliser treatments	Both composted treatments higher in microbial C than mineral fertiliser treatments, but trial was of systems so there were also other differing factors
Franco *et al*. (2004), Italy	10 soils: 4 Inceptisols, 3 Mollisols, 3 Entisols; 10–60% clay; pH 5.2–8.3; OC 13.6–58.5 g/kg	Glucose, maize stalks, or maize stalk compost added to soils contaminated with crude oil	The addition of organic substrates (glucose, maize stalks, and maize stalk compost) to contaminated soils had no synergistic effect on the decomposition of crude oil but produced a marked increase in microbial biomass, although the increase was smaller than in uncontaminated soils. Compost decreased the stress conditions caused by oil contamination as measured by a reduction in metabolic quotient
Lalande *et al*. (2003), Canada	Orthic Humo-Ferric Podzol; loamy sand; pH 5.4; OC 26–35 g/kg	Single application of co-composted papermill sludge and hog manure applied alone or in combination with mineral fertilisers	Activities of β-glucosidase, β-galactosidase, acid phosphatase, urease, and fluorescein diacetate hydrolysis, microbial C and soil respiration all increased compared with the control. Addition of fertiliser to compost resulted in a greater increase in enzyme activities than compost alone but had little effect on microbial biomass. Enzyme activities and microbial biomass decreased in the second season

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Table 7. *Continued*

Reference and location	Soil type and characteristics	Compared treatments	Effects
Zaller and Kopke (2004), Germany	Fluvisol; pH 5.35	9-year study of traditionally composted FYM, 2 types of biodynamically composted manure	FYM increased microbial biomass, dehydrogenase activity, decomposition (cotton strips), but not saccharase activity, microbial basal respiration, or metabolic quotient. Biodynamic manure preparation decreased soil microbial basal respiration and metabolic quotient compared to non-biodynamic manure. After 100 days, decomposition was faster in plots which received biodynamic FYM than in plots which received no or non-biodynamic FYM
Miyittah and Inubushi (2003), Japan	Typic Hapludand; pH 4.87; OC 64.9 g/kg	Composts of soymilk residues, cow manure, poultry manure, and sewage sludge	Soil respiration increased rapidly initially, but patterns differed among the composts. Composted soymilk treatment gave higher CO_2-evolution and lower metabolic quotient than the other composts
Tiquia *et al*. (2002), USA	Silt loam; 29% sand, 29% clay; pH 5.5; OC 29 g/kg	Soil mulched with composted yard waste, ground wood pallets, bare soil control, with or without chemical fertiliser	Microbial respiration rate was highest in soils mulched with composted yard wastes. Mulching with compost strongly influenced the structure of the microbial rhizosphere community

acid > fulvic acid > humin (Qualls 2004). Concentrated sources of organic material such as peat, composts, and brown coal (oxidised coal, lignite, leonardite) also contain humic substances and are often marketed on the basis of their humic and fulvic acid contents as determined by similar procedures. Contents of humic acids vary, however, widely (Riffaldi *et al*. 1983). Some of the chemically extracted humic and fulvic acid separates are themselves sold as soil amendments. In discussion of organic amendments, a clear distinction must be made between products containing humic substances and those products that are humic (or fulvic) acids extracted from the primary sources listed above.

Humic substances can stimulate microbial activity directly through provision of carbon substrate, supplementation of nutrients, and enhanced nutrient uptake across cell walls (Valdrighi *et al*. 1996). Several studies showed that increasing amounts of compost or brown coal-derived humic acid stimulated aerobic bacterial growth, but had only slight effects on actinomycetes and no effect on filamentous fungi (Vallini *et al*. 1993; Valdrighi *et al*. 1995, 1996). Differences in microbial response were related to the molecular weight of the humic acids, with the lower weight fractions, typical of composts, causing greater microbial stimulation than the higher molecular weight fractions extracted from brown coal (Garcia *et al*. 1991; Valdrighi *et al*. 1995). Application of humic substances may induce changes in metabolism, allowing organisms to proliferate on substrates which they could not previously use (Visser 1985). Both heterotrophic and autotrophic bacteria can be stimulated by humic acid addition, mostly through the enhanced surfactant-like absorption of mineral nutrients, although heterotrophs also benefit from the direct uptake of organic compounds (Valdrighi *et al*. 1996). Vallini *et al*. (1997) showed that nitrifiers (chemotrophs) cannot use humic acids as an alternative carbon and energy source. Microbial activity may even be inhibited if humic acid is the sole carbon source (Filip and Tesarova 2004).

The principal indirect effects of humic substances on soil organisms are through increased plant productivity by mechanisms as listed in Table 6, but excessive applications can negatively affect plant growth (Fagbenro and Agboola 1993; Vallini *et al*. 1993; Valdrighi *et al*. 1995; Atiyeh *et al*. 2002), possibly through reduced availability of chelated nutrients (Chen *et al*. 2004). Field studies vary widely in the applied amounts of humic substances and in outcomes. Kim *et al*. (1997*a*) found no effect of commercial humate applied at 8.2 t/ha on microbial activity or microbial functional groups (total fungi, actinomycetes, total Gram-negative bacteria, fluorescent pseudomonads, and *P. cupsici*) in a sandy soil used to grow bell peppers. Similarly, after 5 years of annual applications of 100 L/ha liquid humic acid to a horticultural soil, Albiach *et al*. (2000) found no effect on microbial biomass or enzyme activity. They ascribed the lack of effect to the low rates recommended by the manufacturer because of high product costs. Municipal solid waste compost and sewage sludge were more affordable

and led to significant increases in microbial biomass in the same study. Only fungi were stimulated by humate added to soil being restored post-mining (Gosz *et al*. 1978), whereas Whiteley and Pettit (1994) found that lignite-derived humic acid inhibited decomposition of wheat straw. Chen *et al*. (2004) calculated from laboratory studies that 67.5 kg/ha of humic substances were needed for effective application to a sandy soil, but thought beneficial effects to plants may only occur in semi-arid or arid areas when applied in combination with irrigation and mineral nutrients.

Microbial inoculants

Inoculation with natural or genetically engineered microbial formulations can be broadly categorised according to whether they are intended to (*a*) exist on their own in the bulk soil, (*b*) populate the rhizosphere, (*c*) form symbiotic associations with plants, or (*d*) promote microbial activity on leaf or straw surfaces. To achieve the desired effect in the field, the inoculant organism must not only survive but establish itself and dominate in the soil or rhizosphere. Survival depends firstly on the quality of the inoculant itself, i.e. purity, strain trueness, viable numbers, the degree of infectivity, and level of contaminants (Abbott and Robson 1982; Kennedy *et al*. 2004). Secondly, the establishment and proliferation of inoculant in the soil environment are determined by many edaphic and climatic factors, the presence of host organisms (for symbionts and endophytes) and, most importantly, by competitive interactions with other microorganisms and soil fauna (Stotzky 1997; Slattery *et al*. 2001; McInnes and Haq 2003). Effects of inoculation on indigenous soil organisms can therefore either result from direct addition effects and interactions with indigenous soil organisms, or from indirect effects via increases in plant growth by one or several of the mechanisms listed in Table 6.

Positive effects of inoculants on the soil microbial biomass may be short-lived (Kim *et al*. 1997*b*), and increases in biomass or activity can even be due to the indigenous population feeding on the newly added microorganism (Bashan 1999). The most successful and widely studied inoculants are the diazotroph bacteria (Rhizobium, Bradyrhizobium, Sinorhizobium, Frankia) used for symbiotic fixation of N_2 from air. Provided soil conditions are favourable for rhizobia survival (Slattery *et al*. 2001), inoculation can increase microbial C and N in the rhizosphere compared with uninoculated soils (Beigh *et al*. 1998; Moharram *et al*. 1999). Population changes can be limited to the season of inoculation if the newly added organism is not as well adapted to the soil conditions as the indigenous population (McInnes and Haq 2003).

Inoculant application research is increasingly focussing on co-inoculation with several strains or mixed cultures enabling combined niche exploitation, cross-feeding, complementary effects, and enhancement of one organism's colonisation ability when co-inoculated with a rhizosphere-competent strain (Goddard *et al*. 2001). An example is the use of phosphorus-solubilising bacteria to increase available phosphorus along with mycorrhizae that enhance phosphorus uptake into the plant (Kim *et al*. 1997*b*). Saini *et al*. (2004) achieved maximum yields of sorghum and chickpea at half the recommended rates of inorganic fertiliser when a combination of mycorrhizae, N_2-fixing bacteria, and phosphorus-solubilising bacteria was added. Increases in microbial biomass C, N, and P in soils of inoculated treatments were strongly correlated with N and P uptake of the plants. Garbaye (1994) suggested that specific 'helper' bacteria may improve the receptivity of the root to the fungus to enhance mycorrhizal colonisation and symbiotic development with plant roots (e.g. Founoune *et al*. 2002). Similarly, legume root nodulation can be enhanced by co-inoculation with *Azospirillum*, which increases root production and susceptibility for rhizobium infection and may also increase secretion of flavonoids from roots that activate nodulation genes in *Rhizobium* (Burdman *et al*. 1996). Conn and Franco (2004) found a significant reduction in indigenous actinobacterial endophytes upon inoculation of soil with a commercial multi-organism product, compared with no change in diversity after inoculation with a single species. Trial with 'effective microorganisms' (EM), a proprietary combination of photosynthetic bacteria, lactic acid bacteria, and yeasts used as a soil and compost inoculant, showed enhanced soil microbial biomass, plant growth, and produce quality (Daly and Stewart 1999; Cao *et al*. 2000). The interactions of microbial inoculants with indigenous soil organisms are likely to be complex, and a better mechanistic understanding is necessary to predict short- and long-term effects.

Pesticides

The results from our literature survey on the effects of selected pesticides on soil organisms are shown in Table 8 (herbicides), Table 9 (insecticides and nematicides), Table 10 (fungicides), and Table 11 (veterinary health products, fumigants, and biological/non-chemical products). Although more than 380 active constituent pesticides are currently registered in Australia, this current review has found data on the effects of only 55 of these on soil organisms. There is clearly a paucity of data in both the Australian and international literature on the effects of a large number of pesticides on soil organisms. Additional data may be available in the chemical reviews of the Australian Pesticide and Veterinary Medicines Authority (www.apvma.gov.au), but much of the information is contained within confidential company reports. Some of the chemicals such as DDT and chloropicrin are no longer registered for use in Australia; however, data have been included in this review as their use continues in many countries.

Table 8. Impact of herbicides on non-target soil organisms

Reference and location	Soil type and characteristics	Active chemical	Effects
Sannino and Gianfreda (2001), Italy	22 soils from Campania region, sandy to clayey soils, OC 1.2–34.2 g/kg	Atrazine	Significant activation of soil urease activity (up to 100-fold increase), and suppression of invertase enzyme
Seghers *et al*. (2003), Belgium	Soils from a field site in Melle, Belgium. No further descriptions	Atrazine, metolachlor	Altered community structure of several groups of bacteria and actinomycetes
Panda and Sahu (2004), India	Soil from upland non-irrigated paddy field. Sandy-loam with pH 6.8, OM 2.7%	Butachlor	Very toxic, suppressing growth, sexual maturation and cocoon production of the earthworm *Drawida willsi* following single dose at recommended rate
Araujo *et al*. (2003), Brazil	Two Brazilian soils: sandy clay, pH 5.9, OM 2.3%, and clay soil, pH 5.2, OM 2%	Glyphosate	Bacteria reduced. Fungi and actinomycetes increased. Microbial activity increase by 9–19%. Increased glyphosate degradation with repeated application
Busse *et al*. (2001), USA (forestry)	Three soils: 18–34% clay, pH 5.4–5.9, OM 2.0–6.7%	Glyphosate	Short term changes to community structure. Increased microbial activity and no long-term changes to community structure
Sannino and Gianfreda (2001), Italy	Described above	Glyphosate, paraquat	Activation of urease and invertase soil enzymes, but glyphosate suppressed phosphatase activity (up to 98%)
Dalby *et al*. (1995), Australia	Yellow, duplex loam (Palexeralf)	Glyphosate and 2,4-DB	No effect of single dose to soil on growth or survival of the earthworms *Aporrectodea trapezoides, A. caliginosa, A. longa* or *A. rosea*
Reid *et al*. (2005), UK	Three soils: Icknield (silty clay loam, OM 4.8%), Shellingford (sandy loam, OM 4.3%) and Evesham (clay, OM 6.0%)	Isoproturon	Catabolic activity induced in soils not previously treated with this herbicide
Mosleh *et al*. (2003), France	43.9% clay, 28.7% sand, pH 8.16, OC 7.7%	Isoproturon	Affected earthworms at very high soil concentrations (not agricultural rates) with LC50 for *Eisenia fetida* >1000 mg/kg
Das *et al*. (2003), India	Clayey Typic Fluvaquent, pH 7.1, OC 5.8%	Oxyfluorfen, oxadiazon	Both herbicides stimulated microbial populations, and increased availability of phosphorus in rhizosphere soil of rice
Strandberg and Scott-Fordsmand (2004), Denmark	Review paper covering several soil types	Pendimethalin	Soil nematodes and other invertebrates reduced, plant-rhizobium symbiosis reduced at herbicide rates as low as 0.5–1.0 kg/ha
Amorim *et al*. (2005), OECD standard soil and several European test soils	OECD standard soil: pH 6, OM 8%. 17 other test soils: pH 3.2–6.9, OM 1.7–15.9%	Phenmedipham	Enchytraeid worms avoided these chemicals in standard avoidance test procedures. In some soil types, avoidance behaviour exhibited at low concentrations (1 mg/kg)
Heupel (2002), Germany: standard soil	Laboratory standard soil described as a loamy sand	Phenmedipham	Dose-dependent avoidance of the collembolan *Isotoma anglicana*, *Heteromurus nitidus*, *Lepidocyrtus violaceus*, *Folsomia candida*, and *Onychiurus armatus*
Kinney *et al*. (2005), USA	Remmit fine sandy loam (Ustollic camborthids)	Prosulfuron	Significant reduction in production of N_2O and NO following N-based fertiliser application: significant reduction in nitrification

Table 9. Impact of insecticides and nematicides on non-target soil organisms

Reference and location	Soil type and characteristics	Active chemical	Effects
Hart and Brookes (1996), UK	Silty clay loam, pH 6.4, OC 13.6 g/kg	Aldicarb, chlorfenvinphos	Aldicarb caused a long-term increase of 7–16% in microbial C. No other effects found on respiration or N mineralisation
Van Zwieten *et al*. (2003), Australia (contaminated site)	Sand, sandy clay loam and clay loam soils. No further details	Arsenic	Arsenic co-contamination was shown to inhibit the breakdown of DDT, and a concomitant reduction in microbial activity was found
Ghosh *et al*. (2004), India	Range of clay loam to clay soils, pH 6.9–7.5, OC 8.7–10.7%	Arsenic	Arsenic between 11–36 mg/kg in soil reduced microbial biomass, respiration, fluorescein diacetate hydrolysis and dehydrogenase activity, and induced microbial stress measured by increased metabolic quotient
Amorim *et al*. (2005), OECD standard soil and several European test soils	Described previously	Benomyl	Enchytraeid worms avoids benomyl in standard avoidance test procedures
Ribera *et al*. (2001), France: OECD standard soil	OECD artificial soil was prepared	Carbaryl	Significant reductions in acetylcholinesterase and other biotransformation enzymes in earthworms
Sannino and Gianfreda (2001), Italy	Described previously	Carbaryl	Activation of urease and invertase soil enzymes, but suppression of phosphatase enzyme
Kanungo *et al*. (1998)	Typic Haplaquept (deltaic alluvium), pH 6.7, OM 17%	Carbofuran	Appears to have an inhibitory effect on nitrogenase activity in *Azospirillum* sp. at higher application rates
Panda and Sahu (2004), India	Described previously	Carbofuran, malathion	Significant reduction in acetylcholinesterase activity in earthworms (*D. willsi*) for up to 45 days (carbofuran) and 75 days (malathion)
Pandey and Singh (2004), India	Sandy loam, pH 6.75, OC 0.49%	Chlorpyrifos, quinalphos	Reduced bacterial numbers, but significantly increased fungal numbers with chlorpyrifos and slightly reduced fungal numbers (short-term) with quinalphos
Menon *et al*. (2005), India	Loamy sand, pH 8.2, and sandy loam, pH 7.7, both soils from semi-arid regions	Chlorpyrifos, quinalphos	Reduced oxidative capability of the soils as measured by reduced dehydrogenase activity and inhibited iron reduction
Endlweber *et al*. (2005), Germany	No data on soil types provided	Chlorpyrifos, dimethoate	Chlorpyrifos reduced collembolan density to a greater extent than dimethoate. Both changed the dominance structure of the collembolan community, but had no effects on species composition

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Table 9. *Continued*

Reference and location	Soil type and characteristics	Active chemical	Effects
Edvantoro *et al*. (2003), Australia (contaminated site)	11 soils, sandy to clayey, pH 4.9–6.0, OC 0.14–5.2%	DDT, arsenic contamination	Bacterial and fungal numbers, and biomass carbon were reduced in contaminated soils compared to controls
Megharaj *et al*. (2000), Australia (contaminated site)	Sandy soil, pH 7.1, OC 1.77–3.6%	DDT	Reduced bacterial and soil algal populations, but may have increased fungal counts
Singh and Singh (2005), India	Silty sand, pH 6.98–7.22, OM 0.63–0.93%	Diazinon	Significant increase in dehydrogenase activity and decrease in alkaline phosphomonoesterase for up to 30 days following treatment
Martikainen *et al*. (1998), Finland	Soil from a pesticide free grain field in central Finland, no further description	Dimethoate	Short term reduction in microarthropod numbers (Collembola), but recovery in numbers after time. Community structure remained differentiated. Slight reduction in soil microbial biomass (measured by ATP)
Dalby *et al*. (1995), Australia	Described previously	Dimethoate	Single dose on soil had no measured effect on the growth or survival of the earthworms *Aporrectodea trapezoides*, *A. caliginosa*, *A. longa*, or *A. rosea*
Singh and Singh (2005), India	Described previously	Imidacloprid	Significant increases in dehydrogenase and phosphomonoesterase activities when used as seed dressing, effect lasting up to 60 days
Capowiez and Berard (2006), France	Artificial soil with pH 8.3	Imidacloprid	Significantly reduced burrowing activity in two earthworm species at sub-lethal concentrations (0.5–1 mg/kg), but no avoidance of the insecticide
Singh and Singh (2005), India	Described previously	Lindane	Significant decreases in both dehydrogenase and phosphomonoesterase enzyme activities for up to 90 days
Loureiro *et al*. (2005), Portugal	Silty sand, pH 5.03, OM 1.28%. Also soils from metal contaminated mine site, pH 4.14–4.47, OM 2.88–5.07%	Lindane, dimethoate	Collembola avoid soil with lindane (10–20 mg/kg) and dimethoate (5–20 mg/kg), while earthworms avoided dimethoate at 2.5 mg/kg
Panda and Sahu (1999), India	Sandy loam, pH 6.8, OM 2.7%	Malathion	Short-term impacts of standard application rates of malathion on earthworm reproduction lasting for 105 days

Table 10. Impact of fungicides on non-target soil organisms

Reference and location	Soil type and characteristics	Active chemical	Effects
Chen *et al*. (2001), USA	Silt-loam Luvisol, pH 6.3, OM 4%	Benomyl	Suppression of respiration, stimulation of dehydrogenase, effects were less noticeable with organic matter addition
Smith *et al*. (2000), USA	Smectitic silt loam soil	Benomyl	Significant long-term effects on mycorrhizal colonization (80% reduction), reduction in fungal to bacterial ratios and nematode numbers
Loureiro *et al*. (2005), Portugal	Previously described	Benomyl	Earthworms avoid benomyl at 1mg/kg soil
Martikainen *et al*. (1998), Finland	Previously described	Benomyl	Total numbers of enchytraeids and nematodes, soil respiration and mineral N were not affected, but the collembolan community structure was affected
Hart and Brookes (1996), UK	Silty clay loam, pH 6.9, OC 1.36%	Benomyl, triadimefon	Microbial biomass, SIR and mineralization of soil organic N to ammonium and then nitrate mostly unaffected by the pesticide treatments
Chen *et al*. (2001), USA	Previously described	Captan	Suppression of respiration and dehydrogenase; increases in ammonium N
Hu *et al*. (1995), USA	A well-drained sandy clay loam, OC 0.8%	Captan	Fungal length and density, and microbial C and N significantly reduced
Van Zwieten *et al*. (2004), Australia (field and OECD soil)	Ferrosols with kaolinitic clay minerals. pH 6.3–6.8, OC 4.9–7.1%	Copper	Earthworm populations avoid soils with concentrations as low as 34 mg/kg. Lack of breakdown of organic carbon suggest potential long-term implications
Merrington *et al*. (2002), Australia	Ferrosols with kaolinitic clay minerals. pH 4.5–6.0, OC 4.88–8.82%	Copper	Increased metabolic quotient indicating microbial stress at 280–340 mg Cu/kg. Significantly reduced microbial biomass and ratio of microbial biomass to OC
Loureiro *et al*. (2005), Portugal	Previously described	Copper, carbendazim	Earthworm avoidance at concentrations >100 mg/kg. Test collembolan species far less sensitive to copper. Carbendazim avoidance by earthworms at 10 mg/kg
Gaw *et al*. (2003, 2006), New Zealand	Mainly silty soils, pH 5.3–6.3, OC 1.5–9.4%	Copper	Reduced performance of soil functions resulting in reduction of DDT degradation
Belotti (1998), Germany	Loamy to clayey soils, pH 5.5–7.2, OM 3.1–7.4%	Copper	Bioavailable copper concentration of 0.677 mg Cu/kg soil established as the critical concentration for soil impairment (irrespective of OC content)
Eijsackers *et al*. (2005), South Africa	Clayey soils, pH 5.7–6.8, OM 0.6–1.3%	Copper	Increasing copper resulted in reduced burrowing of earthworms and decreased growth of earthworms, resulting in increased soil bulk densities in a vineyard
Chen *et al*. (2001), USA	Previously described	Chlorothalonil	Suppression of respiration, stimulation of dehydrogenase
Kinney *et al*. (2005), USA	Previously described	Mancozeb, chlorothalonil	Significant reduction in production of N_2O and NO following N-based fertiliser application: Significant reduction in nitrification
Fravel *et al*. (2005), USA	*In vitro* studies not using soil	Chlorothalonil, azoxystrobin	Both fungicides toxic to the biocontrol agent *Fusarium oxysporum* strain CS-20 which has been used to reduce incidence of Fusarium wilt
Monkiedje *et al*. (2002), Germany	Silty clay soil, pH 7.2, OC 1.69%	Metalaxyl, mefenoxam	Reduced enzyme activity, in particular dehydrogenase, for up to 90 days. Increased bacterial numbers with increasing doses, but toxic to N fixers at 1 mg/kg (mefenoxam) and 2 mg/kg (metalaxyl)

Table 11. Impact of veterinary health products, fumigants, and other biological and non-chemical plant protection measures on non-target soil organisms

Reference and location	Soil type and characteristics	Active chemical	Effects
		Veterinary health products	
Svendsen *et al*. (2005), Denmark	OECD standard soil substrate	Ivermectin, fenbendazole	It was concluded that earthworm populations will not be affected in the field following normal use of these products
Jensen *et al*. (2003), Denmark	Sandy-loamy soil, pH 6.2, OC 1.6%	Tiamulin, olanquindox, metronidazole, ivermectin	Threshold values for toxicity (10% reduced reproduction or EC10 values) of antibacterials tiamulin, olanquindox and metronidazole were in the range of 61–111 mg/kg soil for springtails and 83–722 mg/kg soil for enchytraeids. Ivermectin more toxic with EC10 values of 0.26 mg/kg soil for springtails and 14 mg/kg soil for enchytraeids
Radl *et al*. (2005), Germany	Marine sediments, 78% <0.01 mm, pH 7.5, redox 21 mV	Trenbolone (TBOH) from cattle production	N-acetyl-glucosaminidase activity was almost 50% lower in sediments receiving trace quantities of TBOH. Biolog substrate utilisation was reduced, and this appeared to be permanent
Vaclavik *et al*. (2004), Denmark	Sandy loam, pH 6.1, OC 1.6%	Tylosin, oxytetracycline, sulfachloropyridazine	Tylosin and sulfachloropyridazine significantly impact on gram positive bacteria, while oxytetracycline inhibits general microbial respiration at levels as low as 1mg/kg soil
Westergaard *et al*. (2001), Denmark	Sandy soil, pH 6.8, OC 1.2%	Tylosin	Long term changes to microbial community structure, and short term reduction in total microbial numbers
		Fumigants	
Massicotte *et al*. (1998), USA	Gravelly sandy loam, no further descriptions	Chloropicrin	Chloropicrin did not adversely affect the formation of ectomycorrhizae on young Douglas fir seedlings by naturally occurring fungi for up to 5 years following treatment
Ingham and Thies (1996), USA	Gravelly sandy loam, no further descriptions	Chloropicrin	Limited effects on fungal biomass and amoebae were found, but no major impact on the soil food web
Spokas *et al*. (2005), USA	Sandy soil, pH 5.0–6.0, OC 1.11–1.39%	Methyl isothiocyanate, chloropicrin	Altered soil biology leading to 10–100-fold increases in N_2O production lasting >48 days. No effects on methane production. Methyl isothiocyanate suppressed soil respiration in laboratory trials
Karpouzas *et al*. (2005), Greece	Silty sand, pH 7.2–7.5, OM 3.74–6.6%	Metham sodium, methyl bromide	Inhibited degradation of an organophosphate nematicide applied to soil 9 months following fumigation, suggesting long term impacts
Dungan *et al*. (2003), USA	Sandy loam, pH 7.2, OM 0.92%	Propargyl bromide, 1,3 dichloropropene	Significant decline in dehydrogenase activity; however, this recovered after 8 weeks in manure amended soil. Bacterial community diversity decreased with increasing fumigant concentration
Klose and Ajwa (2004), USA	Two sandy loam soils, pH 7.75–7.82, OC 0.6–0.7%	Propargyl bromide, InLine, Midas, Chloropicrin	Organic matter turnover and nutrient cycling, and thus, the long-term productivity largely unaffected in soils repeatedly fumigated with these products, except for a reduction in several key enzymes activities

Continued next page

Table 11. *Continued*

Reference and location	Soil type and characteristics	Active chemical	Effects
		Biological and non-chemical products	
Cohen *et al*. (2005), USA	Clay loam soil, pH 7.4	*Brassica napus* seed meal	Reduced *Rhizoctonia* root infection and *Pratylenchus spp* nematodes. Suggested mechanism was altered bacterial community supporting nitrous oxide production and induction of plant systemic resistance
Cortet *et al*. (2006), Denmark and France	Three soils: silty sand, pH 6.2, OM 6.4%; silty clay, pH 8.1, OM 4.8%; clayey silt, pH 8.2, OM 1.5%	Bt toxin	No effect on litter bag decomposition or N mineralisation
Accinelli *et al*. (2004), Italy	Two soils: a loam, pH 7.9, OC 0.92%; sandy loam, pH 8.1, OC 0.7%	Bt toxin	In laboratory studies, the presence of the Bt toxin inhibited the breakdown of glufosinate-ammonium and glyphosate
Gelsomino and Cacco (2006), Italy	A clay loam soil, pH 7.2, OC 1.49%	Solarisation with transparent polyethylene film	Decreased bacterial diversity as measured by DGGE fingerprinting over time, although short term increases in diversity were noted
Patricio *et al*. (2006), Brazil	A fertile peat, pH 5.9, OM around 20%	Solarisation	Reductions in microbial C, and fungal and bacterial numbers, but no effect on fluorescent pseudomonas

Herbicides

The herbicides (Table 8) generally had no major effects on soil organisms, with the exception of butachlor, which was shown to be very toxic to earthworms at agricultural rates (Panda and Sahu 2004). The authors showed, however, that butachlor had little effect on acetylcholinesterase activity. Butachlor is not registered for use in Australia. Phendimedipham induced avoidance behaviour in earthworms (Amorim *et al*. 2005) and collembola (Heupel 2002). These effects are expected to be relatively short lived, as phendimedipham is broken down moderately rapidly (25-day half-life) in soil (Tomlin 1997). Other effects of herbicides on soil organisms were mainly isolated changes in enzyme activities. Glyphosate, for example, was shown to suppress the phosphatase activity by up to 98% (Sannino and Gianfreda 2001) in a laboratory study; however, urease activity was stimulated by glyphosate as well as atrazine.

Insecticides

Insecticides (Table 9) were generally shown to have a greater direct effect on soil organisms than herbicides. Organophosphate insecticides (chlorpyrifos, quinalphos, dimethoate, diazinon, and malathion) had a range of effects including changes in bacterial and fungal numbers in soil (Pandey and Singh 2004), varied effects on soil enzymes (Menon *et al*. 2005; Singh and Singh 2005), as well as reductions in collembolan density (Endlweber *et al*. 2005) and earthworm reproduction (Panda and Sahu 1999). Carbamate insecticides (carbaryl, carbofuran, and methiocarb) had a range of effects on soil organsism, including a significant reduction of acetylcholinesterase activity in earthworms (Ribera *et al*. 2001; Pandey and Singh 2004), mixed effects on soil enzymes (Sannino and Gianfreda 2001), and inhibition of nitrogenase in *Azospirillum* species (Kanungo *et al*. 1998). Persistent compounds including arsenic, DDT, and lindane caused long-term effects, including reduced microbial activity (Van Zwieten *et al*. 2003), reduced microbial biomass, and significant decreases in soil enzyme activities (Ghosh *et al*. 2004; Singh and Singh 2005).

Fungicides

Fungicides (Table 10) generally had even greater effects on soil organisms than herbicides or insecticides. As these chemicals are applied to control fungal diseases, they will also affect beneficial soil fungi and other soil organisms. Very significant negative effects were found for copper-based fungicides, which caused long-term reductions of earthworm populations in soil (Van Zwieten *et al*. 2004; Eijsackers *et al*. 2005; Loureiro *et al*. 2005). Merrington *et al*. (2002) further demonstrated significant reductions in microbial biomass, while respiration rates were increased, and showed conclusively that copper residues resulted in stressed microbes. Other observed effects included the reduced degradation of the insecticide DDT (Gaw *et al*. 2003). These negative effects are likely to persist for many years, as copper accumulates in surface soils and is not prone to dissipative mechanisms such as biodegradation. Negative effects were also found for benomyl, which caused long-term reductions in mycorrhizal associations (Smith *et al*. 2000). Two fungicides, chlorothalonil and

azoxystrobin, have recently been shown to affect on a biocontrol agent used for the control of Fusarium wilt (Fravel *et al*. 2005), illustrating potential incompatibilities of chemical and biological pesticides.

Veterinary health products, soil fumigants, and non-chemical products

Veterinary health products (Table 11) include a range of nematicides, hormones, and antimicrobials. Data on the potential effect of these compounds on soil organisms are quite limited. The antimicrobials tylosin, oxytetracycline, and sulfachloropyridazine reduced Gram-positive bacterial populations and inhibited microbial respiration (Vaclavik *et al*. 2004), which is in accordance with changes in the microbial community structure after tylosin addition (Westergaard *et al*. 2001). The broad-spectrum anti-parasite Ivermectin was shown to be toxic to collembola at concentrations as low as 0.26 mg/kg soil; however, it was far less toxic to enchytraeid worms (Jensen *et al*. 2003) and earthworms (Svendsen *et al*. 2005).

Soil fumigants are designed to eliminate harmful soil organisms and any competition for soil resources between soil organisms and the crop. In spite of this, soil fumigants have not always been found to have significant effects on soil organisms (Table 11). Confirmed long-term effects on various soil functions (Karpouzas *et al*. 2005) are, however, a serious concern. The long-term effects of fumigants were shown to be reduced by the addition of composted steer manure, with normal biological activity being observed 8–12 weeks following high application rates of the fumigant (Dungan *et al*. 2003). In the absence of the organic amendment, little recuperation (resilience) of soil function was detected even after 12 weeks.

Microorganisms have been used to control plant diseases for over 100 years (Winding *et al*. 2004). However, risks of biological control agents are often forgotten. Although the selected microbes may occur naturally in the environment, there are concerns that altering the proportion of soil microbes will affect non-target species including mycorrhizal and saprophytic fungi, soil bacteria, plants, insects, aquatic and terrestrial animals, and humans (Brimner and Boland 2003). In a recent review of non-target effects of bacterial control agents suppressing root pathogenic fungi, Winding *et al.* (2004) concluded that significant non-target effects occurred that were, however, generally short lived. Residues from genetically modified maize expressing a protein from *Bacillus thuringiensis* (Bt) that is toxic to corn borers were found to decompose similarly to residues from conventional maize (Cortet *et al*. 2006), although the Bt toxin did inhibit some decomposition processes under laboratory conditions (Accinelli *et al*. 2004). Other methods for pest control include technologies such as solarisation (Table 11). This method uses plastic sheeting to heat-sterilise the surface soil. Several authors found reductions in microbial biomass and bacterial diversity (Gelsomino and Cacco 2006; Patricio *et al*. 2006).

Pesticide formulation

In addition to the active ingredient, the formulation of a pesticide may also influence soil organisms. This is, however, an aspect that is rarely investigated. Little is known about the environmental fate of adjuvants after application on agricultural land. Adjuvants constitute a broad range of substances, of which solvents and surfactants are the major types. Non-ionic surfactants such as alcohol ethoxylates (AEOs) and alkylamine ethoxylates (ANEOs) are typical examples of pesticide adjuvants (Krogh *et al*. 2003). Tsui and Chu (2003) demonstrated that the surfactant in the Roundup formulation polyoxyethylene amine (POEA) was significantly more toxic to Microtox bacterium than glyphosate acid or the IPA salt of glyphosate. Even Roundup was found to be less toxic. The toxicity of glyphosate acid was concluded to be a result of its inherent acidity. In another study, dos Santos *et al*. (2005) demonstrated that the presence of ethylamine in a glyphosate formulation had major effects on *Bradyrhizobium*, whereas the active ingredient (glyphosate) had little if any effect. In formulation, effects included reduced nodulation in a soybean crop.

General discussion

Main findings and knowledge gaps

In agreement with the main focus of this journal, we attempted to base our review primarily on results from Australia and New Zealand. However, we found that the existing database on the effect of agricultural inputs on soil organisms in this region was far too limited to draw sound conclusions. Even when considering the global literature, we identified several knowledge gaps.

There was little evidence for significant direct effects of mineral fertilisers on soil organisms, whereas the main indirect effects were shown to be an increase in biological activity with increasing plant productivity, crop residue inputs, and soil organic matter levels, and a depression with decreasing soil pH as a result primarily of N fertilisation. This is in accordance with a review by Wardle (1992) who suggested that soil organic matter is the main factor governing levels of microbial biomass in soil, followed by soil pH. Long-term field experiments comparing mineral and organic fertilisers illustrated the role of indirect and direct carbon inputs into the soil in supporting biological activity. There is, however, a lack of such experiments in Australia and New Zealand.

Although direct C addition with the various organic amendments plays a major role in stimulating soil organisms, the role of C quality is not yet well understood. Compostable organics are an extremely diverse commodity with many

potential benefits to soil organisms but also potential harmful effects, particularly with long-term application. Proper composting negates many potential harmful effects but not all. The toxic components that are not degraded or deactivated need to be identified and their specific effects better quantified. Australian standard AS 4454–2003 (Composts, soil conditioners and mulches) specifies threshold limits of heavy metals, pathogens, and organic compound contaminants based on demonstrated effects on plants and animals, not microorganisms, which may have a much lower threshold (Giller *et al*. 1998). As more and more of this material is used as a soil amendment rather than landfill, more research must be done on the long-term effects of the various contaminants on microorganisms.

The main problem with evaluating effects of specific products such as humic substances lies in the variety of materials of various origins, and in the fact that the properties are often defined by extraction methods that vary among laboratories and product manufacturers. Very few studies have investigated how humic substances affect soil organisms, and a closer examination of the effects of humic substances in laboratory cultures and soil cultures is required for an improved process understanding.

Microbial inoculants have mainly been studied under the aspect of inoculant survival and efficiency rather than with respect to effects on indigenous soil organisms. Apart from rhizobial and some mycorrhizal inoculants, much of the potential for microbial inoculants is yet to be realised. Possibly, the conventional scientific approach has been too reductionist, producing single strain organisms that often cannot compete in complex field situations (Marx *et al*. 2002). Since there is evidence that multi-organism products may be in a better position to compete with indigenous microorganisms, it is necessary to investigate the mechanisms in order to derive a causal understanding. Non-target effects of inoculants appear to be small and transient. However, Winding *et al*. (2004) point out that not enough is known about some marketed products aimed at disease control whose antimicrobial effects may extend beyond the growth season.

Among the pesticides, herbicides appeared to have the least significant effects on soil organisms, whereas some insecticides and especially some fungicides proved to be quite toxic. Few studies have investigated long-term effects of pesticide application, and even less discuss measured or observed changes to soil processes. One example is the lack of bioturbation noted recently in a copper-contaminated orchard (Van Zwieten *et al*. 2004). Copper has been shown to reduce the burrowing activity of earthworms, which in turn led to increased soil bulk density in a vineyard (Eijsackers *et al*. 2005). Likewise, Gaw *et al*. (2003) described the lack of pesticide breakdown in soils where copper was a co-contaminant. There is clear evidence that soil organisms and thus soil functions can be affected by pesticides, but comprehensive data showing which of these changes are long-term and reduce soil health are lacking.

Methodological issues

A broad range of tests has been used to evaluate effects of agricultural inputs on soil organisms, measuring the amount, activity, and diversity of soil organisms (Table 2). The lack of standardised methods often precludes a direct comparison between the various studies. Even if a similar method is used, slight variations in environmental conditions during the assay may change the outcome considerably, resulting, for example, in threshold levels of metal toxicity that can vary among studies by several orders of magnitude (Giller *et al*. 1999). Microbial endpoints have therefore sometimes been deemed to have limited use in risk assessment (Kapustka 1999). Ideally, endpoints should be highly sensitive to the respective contaminant while at the same time being robust, i.e. showing little variation among soils in the absence of the contaminant. However, when testing 8 ecotoxicological endpoints on 2 sets of soils, one metal-contaminated and one non-contaminated, Broos *et al*. (2005) observed a negative relationship between sensitivity and robustness of an endpoint. Therefore, a reasonable compromise might be to use endpoints of average sensitivity and good robustness. In their study, the lag-times of substrate-induced respiration, clover yield, and N fixation in clover were the most suitable endpoints for metal toxicity.

The most commonly measured variable, the microbial biomass, generally appears to be less sensitive to the various agricultural inputs than microbial activities such as soil respiration and enzyme activities. In the context of using microbial parameters to monitor soil pollution by heavy metals, Brookes (1995) suggested that the ratio of microbial activity and biomass, i.e. the metabolic quotient (Table 2), is more sensitive as an indicator of stress than either of the measurements alone.

Interpretation of enzyme activities in soil is complicated by the fact that enzymes may remain active when stabilised on organic matter or mineral surfaces. In addition, enzyme assays are usually based on the hydrolysis of artificial substrates such as *p*-nitrophenyl phosphate, but enzyme activity against natural substrates and under soil rather than assay conditions may be different. Enzyme activity against an artificial substrate must therefore be viewed as a potential activity and cannot be translated into actual reaction rates, and soil respiration may be a more direct measurement of microbial activity.

Methods to determine the microbial diversity have greatly advanced in recent years with the development of DNA-based techniques. However, even these methods still suffer from shortcomings such as the dependence of results on the extraction protocol (Martin-Laurent *et al*. 2001). Inoculation research has benefited from recent methodological advances, especially the development of molecular methods that allow following specific microorganism after addition into the soil–plant system (Marx *et al*. 2002; Conn and Franco 2004).

Another technique is to genetically 'tag' newly released organisms to monitor the effects of introducing genetically modified organisms into the rhizosphere (Hirsch 2005). At the cellular level, direct staining techniques and advanced microscopy can provide high-resolution data on the metabolic activity and growth of inoculants (Schwieger *et al*. 1997).

Although laboratory studies are important to investigate basic processes, only field studies can fully elucidate the complex interactions of plants, soil, and climatic variation. Extrapolation from short-term tests is often not possible, especially when the mechanisms behind observed changes are not fully understood. This is especially true when long-term chronic toxicity poses a different stress on soil organisms than the immediate shock effect in laboratory tests (Giller *et al*. 1999). Only long-term monitoring in the field can provide the information required to establish regulatory guidelines, and an improved understanding of the system is mandatory for a sound risk assessment.

Interpreting changes in measured variables: where is the limit?

Our review has shown that most agricultural management strategies and external inputs can cause changes in the measured variables, whether they represent the amount, activity, or diversity of soil organisms. The challenge lies in interpreting the findings: we need to establish the limits for changes that are acceptable in view of that fact that agricultural inputs are a necessity, and those that are unacceptable, e.g. because they decrease biodiversity, impede soil functions, and diminish system productivity. Ultimately, the question is: what do we want to protect?

Terrestrial endpoints are often based on sensitive, threatened, and endangered species, such as the charismatic megafauna (Kapustka 1999). Measurements on soil organisms are, however, complicated by great spatial and temporal variation as well as complexity, since 1 g of soil can host more than 10 000 species of bacteria and an unknown diversity of fungi. In aquatic toxicology, an underlying assumption has sometimes been that if thresholds for toxic substances are based on the most sensitive species, then all species will be protected. However, the relative sensitivity of 2 species to chemical A may differ from that to chemical B. This concept is additionally complicated by the fact that an identified most-sensitive species may not be present in another ecosystem, making the application in regulatory terms questionable (Cairns 1986).

Protection of soil organisms based on their roles in nutrient cycling may be more practical and relevant for agroecosystems, even though it carries the risk that functional redundancy may mask changes in a population. Loss of specific functions that can only be carried out by very few species such as the loss of symbiotic nitrogen fixation due to application of metal-contaminated sewage sludge (Giller *et al*. 1998) or decreased decomposition due to detrimental effects of copper on earthworms (Van Zwieten *et al*. 2004) is obviously the biggest concern. Complete loss of function is, however, an exception rather than the rule.

When judging whether a change in a measured variable is of concern or not, the concept of Domsch *et al*. (1983) provides a good framework: a decrease in biological activity by up to 30% is deemed negligible, whereas a decrease by up to 90% could still be considered acceptable if it is followed by recovery within 30–60 days. This concept acknowledges the natural variation in many of the biological variables measured. It also places more emphasis on resilience than on resistance, where resistance is defined as the ability of the soil to withstand the immediate effects of perturbation, and resilience as the ability of the soil to recover from perturbation (Griffiths *et al*. 2001). Therefore, even laboratory tests should be run for a minimum of 30 days (Somerville *et al*. 1987). However, Giller *et al*. (1998) stress that a fundamental difference remains between acute toxicity (disturbance) and long-term chronic toxicity (stress), i.e. studying an adapting *v.* an adapted community. Thus, only long-term monitoring and field experiments can provide the information required to develop a sound risk assessment.

An increase in the amount, activity, or diversity of soil organisms is generally viewed as positive. However, an increase in the microbial biomass often goes along with increased nutrient immobilisation, at least temporarily, and an increase in soil organic matter can increase populations of detrimental organisms such as parasitic nematodes and root diseases. As stated above, it is the resilience of the system that matters. In terms of biodiversity, a mild stress can actually increase species diversity by reducing competition effects, before diversity decreases at higher stress levels (Giller *et al*. 1998). This exemplifies the difficulties in interpreting changes, especially those in biodiversity.

Dahlin *et al*. (1997) observed that detrimental effects of metal contamination at one site were seen at metal concentrations below the background concentrations at the other site and asked in exasperation: 'Where is the limit?' One answer may be that there is no distinct threshold for metal toxicity, or for detrimental effects of other inputs, partly because the effects depend on site-specific characteristics such as climate and soil type. In testing procedures for the side effects of pesticides on soil microorganisms it has long been recognised that effects are more likely to be seen on light-textured soils that are low in organic matter than on heavier soils, and it is therefore recommended to use at least 2 contrasting soil types (Somerville *et al*. 1987). Likewise, changes in soil pH are more likely to have detrimental effects on soil organisms closer to the extreme points of the scale. For these reasons, it is mandatory to always choose a valid control, i.e. to allow for site-specific differences in the baseline, and to interpret changes in the context of the given site-specific characteristics.

An approach to assess the relative risk of pesticides to an agroecosystem (EcoRR) has been developed in Australia (Sanchez-Bayo *et al*. 2002). The methodology

uses site-specific data and accounts for chemical dose, partitioning (air, soil, vegetation, surface and ground water), degradation, bioconcentration, and toxicity. Another model (PIRI) has been developed in Australia to assess the risk of pesticides entering groundwater (Kookana *et al*. 1998) and thus affecting the environment and human health. Neither of these models assesses, however, the risk of pesticides to soil organisms or even more broadly, soil quality.

Concluding remarks

The underlying principle for the protection of soil organisms should be to limit or prevent exposure of organisms to unacceptable hazards (McLaughlin *et al*. 2000). Our review has shown that some drastic negative effects such as those of copper fungicides and, to a lesser degree, soil acidification on soil organisms, have to be considered urgently if soil health is to be maintained. For some classes of inputs such as humic acids and various pesticides, the existing database is simply too small to draw sound conclusions. The main lesson learnt from the fertiliser section, however, is that any practice that increases levels of soil organic matter will also increase soil biological activity.

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