



NSW DEPARTMENT OF
PRIMARY INDUSTRIES

Soil health: The foundation of sustainable agriculture
Workshop proceedings, Wollongbar, 20-21 June 2001 - Readers' Note

This document is part of a larger publication. The remaining parts and full version of the publication can be found at:

<http://www.dpi.nsw.gov.au/agriculture/resources/soils/structure/workshop>

Updated versions of this document can also be found at the above web address.

This document is subject to the disclaimers and copyright of the full version from which it is extracted. These disclaimers and copyright statements are available in the appropriate document at the above web address.

Soil health: a systems approach to soils

Peter Slavich

**Director, Wollongbar Agricultural Institute
NSW Agriculture, Wollongbar**

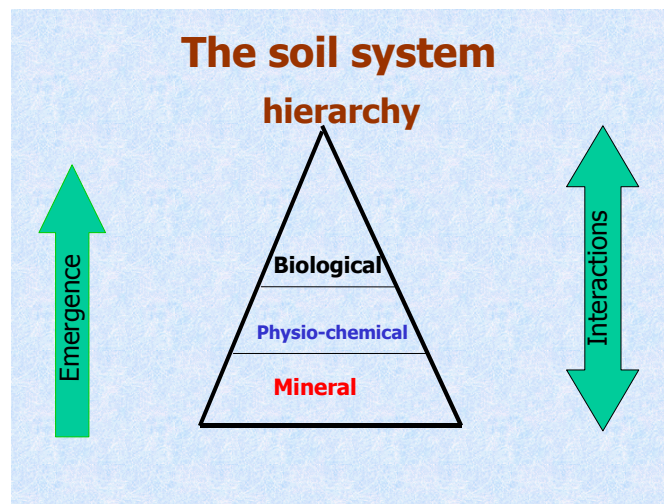
Healthy agricultural soils are able to balance a range of functions to meet the needs of both farmers and the community. Healthy soils function to sustain soil biota and plant life, store and cycle water and nutrients, decompose organic matter, inactivate toxic compounds, suppress pathogens and protect water quality. Soil health is a systems concept that implies that the soil functions as a balanced living system. It implies that the interactions among the soil's internal components are optimal and that the interactions of the soil with its external environment and the production system are sustainable. Soil degradation and poor water quality are symptoms of poor soil health. Soil biological activity, surface cover, organic matter content, pH and water availability are highly interactive and hence important soil health properties. This paper describes soil health and how it relates to soil properties and management practices in horticultural, cropping and grazing production systems.

Definition of soil health

Soil health has been defined by Doran and Zeiss (2000) as 'the capacity of soil to function as a vital living system, within ecosystem and landuse boundaries, to sustain plant and animal production, maintain or enhance water and air quality, and promote plant and animal health'. Healthy soil functions optimally through balanced interactions amongst its biological, physio-chemical and mineral components. The mineral component consists of sand, silt and clay particles; the physio-chemical component consists of soil aggregates, pore space, reactive surfaces, and organic and inorganic compounds; and the biological component consists of roots, insects, invertebrates and microorganisms. Healthy soils function to

- sustain biological productivity
- store and cycle water and nutrients
- decompose organic matter
- inactivate toxic compounds
- suppress pathogens
- protect water quality and enhance catchment health.

Hierarchy and emergence are properties of all systems including soils. These properties imply there are higher level components and functions of the system that depend on, and emerge from, lower level components and functions. They enable the whole to be more than the sum of the parts. The biological and organic component and functions of soils depend on, and emerge from, the physio-chemical and mineral components. Hence the abundance, diversity and functioning of these organisms is a key indicator of soil health.



The main function of soil organisms is to cycle and transform nutrients and energy by decomposing organic matter. This process occurs within a complex food chain and is highly dependent on environmental factors such as moisture content and temperature. Soils are part of the life cycles of many types of organisms including insects, earthworms, mites, springtails, bacteria, algae, blue-green algae, fungi, protozoa and nematodes. The microbial population of fertile grassland exceeds 3×10^{14} cells per square metre whilst the moist biomass may exceed 7 t/ha (Richards 1974). Earthworm biomass may exceed 900 kg/ha and produce more than 25 t/ha of worm casts (Russell 1973).

Soil degradation and soil health

Many agricultural practices increase the soil's vulnerability to degradation processes such as erosion, acidification, salinisation, soil structure decline and contamination. These degradation processes reduce the functional capacity of soils and, at a catchment level, can reduce the quality of water draining to streams and rivers. Hence soil and water quality degradation can be thought of as symptoms of poor soil health. The challenge for management of agricultural soils is to develop production systems that not only prevent soil degradation but also enhance soil health. The biological component of the soil system has a high dependence on the chemical and physical soil components and hence tends to be a sensitive indicator to disturbance or degradation processes.

There is a need for measurable indicators to evaluate the sustainability of resource use by particular management systems. Ecosystem functions can be characterised in terms of their *resistance* to change by an imposed disturbance and their *resilience*, or potential to recover following disturbance/degradation (Pimm 1984). These concepts are equally valid for assessing the sustainability of agricultural production systems (Herrick 2000). Useful indicators to evaluate the sustainability of different management practices may be the amount and rate of change in soil biological functions, and the amount and rate of recovery. The most sustainable practices will be those which cause little or no negative change in functional capacity and/or which enable rapid recovery.

Some soil properties and functions undergo changes when disturbed that are effectively irreversible within management time scales. Examples include the

impacts of extreme erosion or the oxidation of acid sulfate soil. The costs and benefits of practices which cause such changes need to be very carefully evaluated if we are to achieve a 'no regrets' approach to agricultural developments.

Properties of healthy soils

Soil health integrates all components of the soil system and is assessed by indicators that describe or quantify biological, chemical and physical properties. Soil characteristics that contribute to a healthy soil include

- protected soil surface and low erosion rates
- high soil organic matter
- high biological activity and biological diversity
- high available moisture storage capacity
- favourable soil pH
- deep root zone
- balanced stores of available nutrients
- resilient and stable soil structure
- adequate internal drainage
- favourable soil strength and aeration
- favourable soil temperature
- low levels of soil born pathogens
- low levels of toxic substances.

Measurement procedures and guideline optimum ranges for soil chemical, physical and biological properties are available (USDA 1999). However, agricultural productivity is determined by a large number of direct and indirect interactions between plant and animal characteristics, climatic conditions, soil properties, pest conditions and management practices. Satisfactory crop production may still occur if soil properties are outside guideline ranges because of plant tolerance, compensatory climatic conditions, or compensatory management practices. Hence it is usually not possible to predict animal or crop production from soil properties alone. This limits the value of generalised soil quality guidelines.

There is a need to develop tools that can assist interpretation of soil data in relation to crop/pasture type, stage of development, climatic & irrigation data, incidence of soil borne diseases, plant nutrient status, and rotation practices. Many types of information need to be combined to enable prediction of a desired outcome, eg response to an added amendment, or runoff water quality. A data analysis tool that could help achieve this is a neural network. Neural networks are a computer-based classification/prediction tools being used increasingly for agriculture and resource management (Shearer et al 1999). They are built using data sets with known properties and are an excellent device for capturing complex interactions and combining diverse information sources. There is potential to build neural networks which are trained to predict soil function, agricultural productivity or environmental indicators from a comprehensive knowledge base. These tools could be used help farmers identify the most critical limiting soil

factors in relation to their soil condition, plant type, climate and management practices.

The cost of fully characterising all soil properties that contribute to soil health is potentially very high. Given that soil-plant processes are complex, a reasonable strategy is to characterise the most interactive soil properties and/or biological properties that have a high level of dependence on physical and chemical properties. For example, soil water directly affects soil biological activity and indirectly affects a range of physical properties (mechanical resistance, soil oxygen, bulk density, thermal capacity, leaching rate) and chemical properties (salinity, ion speciation, breakdown pathways). Letey (1985) proposed the concept of the non-limiting water range (NLWR) to characterise the interactions between soil water content and soil physical properties. This concept identifies the range of soil water contents that limit plant growth. In wet soil, water content limits growth mainly by inadequate soil aeration, whereas in dry soils the main limiting factor is excessively high mechanical resistance to root growth. There is potential to expand the NLWR concept to include interactions between soil water and soil chemical and biological limitations to productivity. Other highly interactive soil properties are soil organic matter content and soil pH.

Integrated soil health research and extension strategy

It is important to distinguish soil health issues generic to all agricultural industries and production systems from those that are more specific to particular industries, regions, climates or farming systems. Sound management of soil erosion and organic matter is fundamental to the sustainability of all agricultural production systems. High levels of soil organic matter enable soils to supply water and nutrients to plants for longer, reduce the risk of soil loss via erosion, and provide the primary food source for soil biota. Management practices that enhance plant productivity generally sustain or enhance soil organic matter, provided cultivation is minimised. Effective methods of characterising soil biological functions such as organic matter decomposition rates, soil mixing by organisms, and nutrient release are also needed for all farming systems.

The potential to apply practices to enhance soil health varies with the type of production system (eg grazing, cropping, horticulture), local climate, landform and soil type, and socio-economic factors such as economic returns, attitude, education level. Practices that improve soil health within grazing systems include establishing deep-rooted perennial pasture species, applying adequate nutrients, liming regularly to manage acidification, adjusting stocking rates to prevent bare soil exposure, and introducing legumes and dung beetles. Management practices used in broadacre cropping systems to improve soil health include minimum cultivation, direct sowing, crop rotations, stubble retention, traffic lanes, soil conservation earth works, water and nutrient management plans. In horticultural production systems there is a growing interest in the use of ground covers, mulches, composts, biological fertilisers and biological inoculants to enhance and manipulate soil ecological processes.

The use of recycled organic materials is a key element of a sustainable agricultural systems and strongly promoted in 'alternative' agricultural production

systems (eg organic agriculture, pesticide-free products, naturally grown products). This interest is driven by a combination of factors, including community concern about soil degradation and food safety, loss of confidence in 'conventional' farming practices, ready availability of recyclable organic materials and a desire to supply growing markets for organic produce.

These practices can have significant effects on the physical, chemical and biological properties of the soil. Ground covers and mulches reduce soil loss by protecting the surface from rainfall impact and decreasing the velocity of overland water flow. Increasing soil organic matter content favours soil structural stability and can increase the numbers and diversity of soil biota (bacteria, fungi, nematodes, protozoa, arthropods, microinvertebrates).

The interaction of soil biological processes and fertilisers can have significant management implications. Microbial activity during organic matter decomposition can either release nutrients (mineralisation) for uptake by plants or decrease soil nutrient availability to plants through competition (immobilisation). Soil nitrogen immobilisation is common when organic materials with high carbon to nitrogen ratios are incorporated into soils. These processes may affect the most appropriate time to incorporate organic materials and timings to apply supplementary fertiliser. Microbial activity in the rhizosphere can also affect nutrient availability to the plant through a complex of interactions. For example many rhizosphere bacteria produce chelating agents such as ketogluconic acid which can make phosphates soluble (Richards, 1974). This process could be affected indirectly by the form of nitrogen available for uptake as this is known to affect rhizosphere pH which in turn may affect the microbial activity. The effects of microbial interactions on nutrient availability should have practical implications for improving the efficiency of use of fertilisers, but these are not well developed.

To maximise the soil health benefits through use of specific management practices (eg groundcovers, mulches, organic amendments, fertiliser strategies) there is a need to systematically evaluate and demonstrate their use across a range of production systems and climates. This can only be achieved through an integrated research and extension strategy that brings together the necessary disciplines, programs, organisations, and partnerships. This strategy needs to identify both generic and farming system specific issues and to establish linked project teams to address these issues. It is the intention of this workshop to develop and gain support for a strategically integrated approach to soil health management.

References

- Doran JW, Zeiss MR 2000. Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology* **15**:3-11.
- Herrick J 2000. Soil quality: an indicator of sustainable land management. *Applied Soil Ecology* **15**:75-83.
- Lety J 1985. Relationship between soil physical properties and crop production. *Advances in Soil Science* **1**:277-301.
- Pimm SL 1984. The complexity and stability of ecosystems. *Nature* **307** 321-326.
- Richards BN 1974. *Introduction to the soil ecosystem*. Longmans NY.
- Russell EW 1973. *Soil conditions and plant growth*. Longmans London.

Shearer SA, Thomasson JA, Mueller TG, Fulton JP, Higgins SF, Sampson S
1999. Yield prediction using a neural network classifier obtained using
soil landscape features and soil fertility data. ASAE-CSAE-SCGR Annual
International Meeting, Toronto, Ontario, Canada. ASAE Paper No.
993041.

USDA 1999. *Soil quality test kit*. Soil Quality Institute, Lincoln NE.

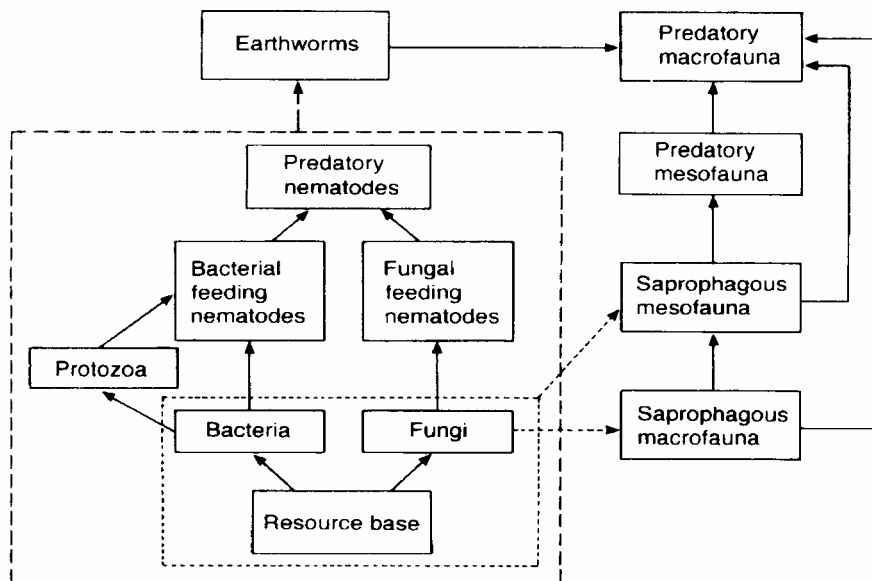
Soil biology and its importance in soil health

Marcelle Stirling
Biological Crop Protection Pty Ltd
Moggill Qld

Soil is taken for granted by many horticulturalists, who often think of it as an inert support for plants. In reality, it is a dynamic, living resource whose condition is vital for food production and for the function of the ecosystem as a whole. The physical and chemical components of soil are important, but the organisms that live in the soil ensure that it remains fertile and productive in the long term.

The biological component of soil (Figure 1) is concentrated mainly in the topsoil, occupying only a tiny fraction (<0.5%) of the total soil volume and making up less than 10% of total soil organic matter. A small part of this living component of soil consists of plant roots, with microorganisms and soil animals forming the bulk of the biomass. The soil biota are therefore much more important than is usually recognised, and this paper provides an overview of their function in soil and the role they play in maintaining soil health.

Figure 1. Simplified functional food web for detritus-based systems in soil.
Organisms in the dotted and dashed boxes may be consumed by organisms that utilise substrates from more than one trophic level.
From Wardle (1995).



Soil microorganisms

Bacteria, fungi, protozoa and algae (mostly blue green algae or cyanobacteria) form the major part of the soil biomass. There is an enormous number of species

in each of these groups, but our knowledge of each of them is limited because many species have not been described taxonomically and most have not been cultured.

Bacteria

Typically, there are between 10^6 and 10^9 bacteria per gram of soil. Bacteria play an important role in soil because their diverse metabolic capabilities enable them to exploit many sources of energy and carbon in soil. They are the principal agents for the global cycling of inorganic compounds such as nitrogen, sulfur and phosphorus.

Fungi and actinomycetes

About 70% of soil microbial biomass is contributed by fungi, whose numbers vary typically from 10^4 to 10^6 per gram of soil. Fungi may be free-living or have a mutually beneficial or parasitic relationship with plant roots. They exploit a diversity of substrates because of their filamentous nature and are decomposers of large molecules such as cellulose and lignins produced by plants. Other microorganisms such as bacteria then utilise the resulting smaller molecules. Mycorrhizal fungi are involved in a mutually beneficial association with plant roots. They derive nutrients from roots and in turn aid the plant in the uptake of relatively immobile nutrients such as phosphorus and zinc. By acting as agents of nutrient transport, they form a vital link between plants and soil and therefore play an important role in soil fertility.

Actinomycetes are filamentous bacteria that are found in soil at populations of 10^5 to 10^8 per gram of soil. They are involved in the decomposition of organic compounds including cellulose, chitin and recalcitrant substances such as humic acids.

Nitrogen fixing bacteria and actinomycetes

Biological fixation of nitrogen occurs predominantly but not exclusively in symbiotic associations between plant roots and bacteria. The symbiotic bacterial genera most commonly involved are *Rhizobium* and *Bradyrhizobium*, which specifically infect leguminous plant roots. Actinomycetes in the genus *Frankia* are also symbionts but have a much wider host range that includes several non-legume plant groups. However, our knowledge of these organisms is still limited (compared to *Rhizobium* and *Bradyrhizobium*) because they are difficult to culture. Among the free-living bacteria, *Azotobacter*, *Azospirillum* and *Bacillus* may also contribute relatively small amounts of nitrogen to soils.

Algae

Estimates show that populations of algae in soil vary between 10 and 10^6 per gram of soil. Valuable nitrogen inputs to soil are made by the blue green algae due to their capacity to fix nitrogen. Because of their photosynthetic capacity, they contribute to the organic carbon input of soil, and also produce extracellular polymers that may help to conserve soil structure.

Soil microfauna

The main components of the soil microfauna are the protozoa and nematodes, the largest members of the soil microbiota. They feed on bacteria and fungi (the primary decomposers) and are involved in nutrient recycling in soil.

Protozoa

Protozoa are water-dependent, unicellular organisms possessing a nucleus, and are classified as ciliates, flagellates and naked amoebae. They feed on soil bacteria and fungi and their populations range from 10 to 10⁶ per gram of soil. Although protozoa constitute only a small proportion (~5%) of soil microbial biomass, they are important ecologically because of their rapid turnover rates and the large grazing pressure they exert on the microflora. This grazing pressure maintains microbial populations that are physiologically young and in a high state of metabolic activity. Thus microbial turnover is stimulated, resulting in increased rates of nutrient mineralisation, especially nitrogen and phosphorus.

Nematodes

Nematodes are ubiquitous in soil and are among the most abundant soil microfauna. Some species are parasites of plants and animals but the majority are beneficial, playing an important role in nutrient cycling processes. This latter group of nematodes, commonly known as 'free-living nematodes', feed on bacteria or fungi and/or prey on various soil microfauna. They therefore obtain their nourishment from organisms that are associated with decaying organic matter.

Thus free-living nematodes are part of the natural food web of organisms, the protozoans, earthworms, mites, insects, fungi and bacteria that reduce the organic remains of animals and plants to their primary constituents.

Soil mesofauna

The soil mesofauna comprise mites, collembola (springtails), enchytraeids (small worms from 1 mm to 5 cm in length), tardigrades (water bears) and small insects. They are a diverse group with a wide range of feeding habits, but collectively they play a role in regulating microbial populations and disseminating microbial propagules. They also accelerate decomposition of plant residues by fragmenting large pieces of organic matter and reworking the faeces of larger fauna.

Microarthropods (mainly collembola and mites) are usually the most obvious of the mesofauna. Population densities vary from 10 to 10⁷ per square metre of soil. Populations are generally highest in the top 5 cm of soil and decline with increasing depth. Apart from fragmentation of residues, enchytraeids also affect soil porosity through their burrowing activities and influence soil aggregation via production of faecal pellets.

Soil macrofauna

These are the most conspicuous of the soil animals and, because of their size, have the greatest potential for direct effects on soil functional properties. Members of this group include ants, termites, millipedes, adult and larval insects, earthworms, snails and slugs. Through their feeding habits and their movement through soil, they help to comminute and redistribute organic residues in the soil profile.

This activity results in an increase in the surface area of organic substrates available for microbial activity. Certain groups, especially ants, termites, and earthworms, can greatly modify soil structure through the formation of macropores and aggregates. These effects may influence water infiltration and solute leaching through soil and hence the soil's capacity to function as an environmental buffer.

How soil biota contribute to soil health

Maintenance of soil structure

The binding substances that hold soil particles together have both mineral and organic origins. Some of the organic 'binding agents' are contributed by soil biota. Fungal hyphae, along with fibrous roots from plants, bind soil particles and small aggregates together into larger units. Polysaccharides produced by microorganisms (eg many commonly occurring soil bacteria such as *Bacillus*, *Pseudomonas*, *Agrobacterium*, *Azotobacter* and *Rhizobium*) act as the gums that bind and stabilise aggregates. Plant residues are also broken down by soil biota to create soil aggregates. Mycorrhizal fungi (VAM) contribute significantly to soil aggregate formation and soil stability at both micro and macro levels by enmeshing mineral and organic debris in a network of external hyphae.

Nutrient cycling

Most of the nutrients contained in soil organic matter are in complex organic forms that have to be mineralised to an inorganic form before they can be used by plants. Soil microorganisms play a dominant role in the decomposition of organic materials such as cellulose, hemicellulose, polysaccharides, hydrocarbons, lignins, proteins and amino acids, and are also responsible for nearly all nitrogen and carbon transformations in soil. They are also important in transforming nutrients such as phosphorus, sulfur, iron, potassium, calcium, magnesium, manganese, aluminium and zinc into forms that can be used by plants. Soil microorganisms therefore have a beneficial impact on plant health by releasing nutrients that would otherwise be 'locked away' in dead plant and animal tissue.

The decomposition of organic matter is brought about by a succession of microbial communities. For instance, the sugar fungi (*Mucor* spp) are the primary colonisers and the cellulose and lignin degraders then follow them. Different nutrients are cycled at different rates by soil biota. Thus amino acids and various oligosaccharides turn over rapidly and form a fast/active nutrient pool whereas humified soil organic matter forms a slow/passive pool that may take several decades to be degraded.

The fast/active pool, which cycles 8-10 times per year, has the greatest impact on plant growth.

Nutrient cycling involves a complex array of interactions between soil organisms that are often referred to as the detritus food web. In agricultural soils, the way these food webs operate depend on the type of farming system. When plant residues are incorporated into soil by cultivation, bacteria start the decomposition process. This immediately results in an increase in protozoa and bacterial feeding nematodes and nutrient cycling proceeds rapidly. On the other hand, when plant

residues remain on the surface of the soil (eg minimum tillage), a fungal flora develops and populations of fungal feeding organisms increase in the surface layers. In this case mineralisation of nutrients proceeds much more slowly.

Suppression of plant pathogens

Soil-borne disease problems are common in soils that have been intensively cropped for decades. Such soils are said to be ‘conducive’ to disease. They have lost much of their microbial diversity and biological buffering capacity, so many competitors of fungal pathogens and root-feeding nematodes have disappeared. In contrast, a ‘disease suppressive soil’ has a full complement of beneficial organisms, and the pathogens that cause disease are unable to increase to levels that will cause damage.

The organisms involved in disease suppression act in many different ways. Fungi and bacteria are able to displace each other from specific ecological niches in soil by competing for nutrients. A group of bacteria known as the fluorescent pseudomonads (*Pseudomonas* spp), for example, inhibit the growth of pathogens by limiting their access to soluble ferric iron. Other bacteria (eg *Bacillus* spp, and some actinomycetes) produce antibiotics that are detrimental to pathogens. Fungi such as *Trichoderma* and *Gliocladium* are able to parasitise fungal pathogens and are therefore useful biological control agents. Other fungi can parasitise or prey on nematodes. The ‘nematode trapping fungi’, for example, are able to capture nematodes by producing unique trapping structures in which nematodes become ensnared. Some small arthropods (eg mites and collembola) consume fungal spores or feed on parasitic nematodes.

Conclusions

The interactions that occur between soil organisms and their relationships with the soil environment are so complex that they will never be fully understood. Nevertheless, our current knowledge shows unequivocally that the soil biota play an important role in nutrient cycling and in improving soil structure. Some components are detrimental to plants but, from a horticultural perspective, there are other components that provide an active form of defence against these pests and pathogens. Thus the soil biota play a vital role in sustaining a healthy, productive soil capable of supporting a level of plant growth that is appropriate for a particular soil and climate.

References

- Atlas RM, Bartha R 1987. *Microbial Ecology: Fundamentals and Applications*. Cummings, California.
- Doran JW, Coleman DC, Bezdicek DF, Stewart BA (eds) 1994. *Defining Soil Quality for a Sustainable Environment*. Soil Science Society of America Inc. Wisconsin.
- Freckman DW 1988. Bacterivorous nematodes and organic-matter decomposition. *Agriculture, Ecosystems and Environment* **24**:195-217.
- Greenland DJ, Szabolcs I (eds) 1994. *Soil Resilience and Sustainable Land Use*. CAB International, Wallingford.
- Gregorich EG, Carter MR (eds) 1997. *Soil Quality for Crop Production and Ecosystem Health*. Elsevier, Amsterdam.

- Lynch JM 1983. *Soil Biotechnology: Microbial Factors in Crop Productivity*. Blackwell Scientific Publishers, Oxford.
- Pankhurst CE, Doube BM, Gupta VVSR (eds) 1997. *Biological Indicators of Soil Health*. CAB International, Wallingford.
- Pankhurst CE, Doube BM, Gupta VVSR, Grace PR (eds) 1994. *Soil Biota: Management in Sustainable Farming Systems*. CSIRO, Australia.
- Pfleger FL, Linderman RG (eds) 1996. *Mycorrhizae and Plant Health*. APS Press, St Paul Minnesota.
- Wardle DA 1995. Impacts of disturbances on detritus food webs in agroecosystems of contrasting tillage and weed management practices. *Advances in Ecological Research* **26**:105-185.

Soil fauna and the sustainability of arable soils: the earthworm viewpoint

**Tim Kingston
Soil Mates Unlimited
Launceston, Tasmania**

The topic of the role of soil fauna in agricultural soils encompasses an extensive range of information for a large number of groups of organisms from mites to earthworms. While biological studies of soil fauna are in themselves of great interest and relevance, discussion in more recent years has emphasised the notion that soil organisms provide valuable information to land managers on two rather elusive concepts: soil health and sustainable production. Lobry de Bruyn (1997) provides an excellent overview of soil fauna, while the potential role of each of the major groups of soil organisms is given thorough review by Pankhurst et al (1994) and Pankhurst et al (1997). Useful entry points into the literature pertaining to specific groups of soil fauna are provided by Lee (1985) and Temple-Smith and Pinkard (1996) for earthworms, and by Lobry de Bruyn and Conacher (1990) for termites and ants. The current paper does not attempt to cover the breadth of territory reviewed in these works. Rather, the purpose is to demonstrate that soil fauna, particularly earthworms, have an impact on soil physical, chemical and microbiological properties; that agricultural practices have impacts on soil fauna; and that soil faunal abundances have implications for the long term biological sustainability of agricultural systems.

I. Interaction between soil fauna and agricultural management

Complexity of soil biota

Biological activity in soils is complex. The taxonomic diversity is immense and each species has its own complex requirements and influences according to its taxonomy, size, biology and lifestyle. In order for each to survive and reproduce, environmental conditions and dietary requirements within the soil must remain supportive throughout each season and through each successive intervention by land managers. Individuals of each species or group of species have the potential to interact in a number of possible ways with every other species at the same site. Such interactions may be direct, for example predation and parasitism, or indirect, including the construction of burrows by one and the use of them by another, or the provision by one of smaller fragments of organic matter for consumption by another. In restricting the focus to the soil fauna we need to treat the remainder of the soil biota, including the bacteria, protozoa, fungi and plant roots, together with the physical and chemical properties of the soil, as the total environment in which soil fauna operate. The number and species composition of the soil fauna present at a site will be affected by these environmental factors, by microclimatic conditions, and by the general climate as determined by latitude and altitude. Each of these levels of complexity holds implications for land managers each time an operational change is effected.

Soil fauna and the functioning of terrestrial ecosystems

All plant matter eventually dies and decomposes, releasing its constituent nutrients for re-use. An entire 'trophic level' of biological organisms of diverse taxonomy and form has evolved to take advantage of what is, for them, a resource. Associations of such organisms, centred on the soil, have developed into highly complex communities with great interdependence between members. Changes in the soil physical and chemical environment brought about by soil fauna, including 'improved soil structure' and the release of nitrogen-rich excreta, contribute to increased plant growth, which in turn improves the reliability of the supply of the very energy source that 'runs' the soil faunal community. Such a system depends only on sunlight, rainfall and gases, mainly carbon dioxide and oxygen, as external inputs. As long as these resources remain available and in balance the system is 'sustainable'. Such systems are the norm in undisturbed vegetation, so we can deduce that the presence of such decomposer communities is a desirable component of sustainable agronomic systems.

Soil fauna and plant growth

Studies of soil fauna, notably earthworms, in arable soils over the past 40 years have shown that the presence of fauna leads to increased growth rates by a variety of plant types, including pasture grasses, field crops and orchard trees (reviewed by Lee 1985). Many other studies have looked more closely at soil physical, chemical or biological changes that result from the presence of soil fauna, but these studies have been mostly short-term and narrowly focussed, making it difficult to extrapolate implications for either productivity or sustainability of production in the longer term.

Soil formation

Bioturbation or soil mixing by soil fauna over long periods of time is one of the major processes by which soil profiles develop (Chittleborough 1992). Soil fauna may continue to have a defining influence on soil texture profile even in well developed soils either through the sorting of particles by both ants and termites (Lobry de Bruyn, Conacher 1990) or by mixing, as by earthworms (Laffan, Kingston 1997). The power of soil fauna to construct soils over geological time highlights the vital importance of minimising our degrading activities, especially as we have not yet devised economically viable means to artificially create productive soils in bulk.

Soil structure

Soil fauna has a major influence on soil structure by selective movement of particles of a certain size, mass movement during burrowing by larger species, and mineralisation and incorporation of organic matter. The influence of soil fauna on the structure of soils may be local, such as within an ant nest or termite mound, or extensive, as by earthworms. Many soils under rainforest and wet sclerophyll forest in Tasmania, for example, have been found to consist entirely of earthworm casts and burrows (Laffan, Kingston 1997). The earthworm species *Pontoscolex corethrurus*, when studied in pots at Kingaroy in Queensland, brought about decreases in bulk density and penetration resistance over a three

month period (Zund et al 1995). Subsequent image analysis revealed an abundance of burrows and fine pores associated with the lowest bulk densities. Many soils under dairy pasture and macadamias within the NSW northern rivers region have subsoils comprised entirely of open and in-filled earthworm burrows (Kingston, in prep). Experimental work elsewhere has demonstrated that ingress by both air and water into soils with earthworm burrows is more rapid than into soils without such channels. Where the burrows are both deep and interconnected they aid soil drainage and affect nutrient leaching and rainfall run off. At a finer scale earthworms increase the aggregate stability or 'crumb' structure of some soils. This is believed to be a consequence of colloids that coat soil particles during their passage through the earthworm gut, and fungal hyphae which preferentially expand into and around particles and crumbs, binding them together.

Soil pathogens

There is some evidence that earthworms can influence the pathology of agents causing root diseases, and the efficacy of beneficial soil organisms. The presence of the earthworm *Aporrectodea trapezoides* has been shown to reduce the severity of the potentially destructive fungal disease *Gaeumannomyces graminis* or 'Take-all' in wheat (Stephens, Davoren 1996), and the fungal pathogen *Rhizoctonia solani* on wheat (Stephens et al 1994) and subterranean clover and perennial ryegrass (Stephens, Davoren 1997). The latter study showed greater growth of both roots and shoots in the presence of earthworms. Earthworms also appear to allow greater dispersal of soil microorganisms used as agents in the control of pathogens, as shown for *Pseudomonas corrugata* when used against Take-all in wheat (Ryder et al 1993). More such studies are required for a greater range of crops and diseases and under a wider range of management regimes.

Impacts of agriculture on soil fauna

There are now several published studies of the impact of specific farm management alternatives on soil fauna, especially earthworms. Some practices have been shown to be deleterious, either because they directly damage individual animals, disturb their habitat or because they disrupt essential physiological processes. Other practices may be beneficial because they render the physical or chemical environment more conducive to soil fauna survival or because they supplement the organic food supply. Even if impacts are not detected immediately their effects may be delayed or only become apparent after some years, perhaps in a year of extreme climate. Alternatively the impact may be masked by the complexity of the system or the variability in the results of research. In reality, all changes in management practices are likely to affect faunal groups differentially and result in a shift in the balance of component groups. Some years of consistent management may be required before a new balance is achieved. If the result is undesirable it could take even longer to reverse. In theory at least, greatest notice should be given to studies that continue observations over a long time period and employ maximum taxonomic separation of the soil fauna. Such studies are rare, one good model being that of Longstaff et al (1999) undertaken at Cowra and Harden in New South Wales over a three-year period. This project recommended further work in two specific areas, 'DNA probes' for soil biological assessment and the production of user-friendly, computer based keys for identifying soil

fauna. Regrettably, the CSIRO soil biology group responsible for the study was instead disbanded soon after (B Longstaff, pers.comm).

Effect of lime

In several countries the addition of lime or dolomite to acidic soils to raise soil pH has increased populations of earthworms. Most recently, the addition of lime to highly acidic pasture soils in a high rainfall zone in South Australia increased earthworm numbers from 85 to 250 /m² over four years (White et al 2000).

Cultivation and crop residue management

There is now a large body of research showing that cultivation reduces earthworm numbers and that the greater the energy used, the greater the population decline. The use of rotary hoes has been found to be especially deleterious whereas mouldboard ploughs have much less impact. Results of a study from tropical Queensland, in which earthworms were sampled at four localities between 1987 and 1992, support this pattern (Robertson et al 1994). At each site, wheat or grain sorghum was grown over a range of tillage intensities, including zero till, reduced till and conventional cultivation. The abundance and biomass of one introduced and one native species were greatest under zero tillage cropping with retention of stubble. The presence of the introduced species increased the rate of water infiltration by a factor of three in zero tillage compared with conventionally cultivated soils. Longstaff et al (1999) examined 20 groups of soil fauna under conventional cultivation, direct drilling and stubble incorporation at two NSW sites. In all cases stubble incorporation and direct drilling supported more soil fauna than conventional cultivation. Mite and springtail populations shifted in favour of fungus-feeding species. Near Casino, in northern NSW, laser levelling of a paddock resulted in moribund earthworms with empty intestines. The scraping of the soil surface had either closed the earthworm burrows and cut off the air supply and access to food, or had removed the topsoil and food supply altogether (Kingston, personal observation, March 2001).

Irrigation

Irrigation has been found to increase earthworm numbers in areas of summer drought, for example in New South Wales (Noble, Mills 1974) and in Victoria (Tisdall 1985). The water appears to extend the earthworm feeding period into summer and reduces mortality from desiccation. In Tasmania however, irrigation applied to a krasnozem soil, immediately followed by grazing by dairy cattle, altered earthworm species composition dramatically. The mobile, surface-feeding species *Lumbricus rubellus* increased in abundance while the topsoil species *Aporrectodea caliginosa* was seriously depleted. Earthworm mortality occurred as a direct result of physical damage to the soil and increased exposure of earthworms during the summer to a seasonally active parasitic fly (Kingston 1989). In a follow-up study the interactions between irrigation, grazing, soil structure and earthworms were explored in both experimental plots and by farm survey (Lobry de Bruyn, Kingston (1997). Summer irrigation at the trial site led to a decline in soil structure: but areas protected from trampling were found to have higher infiltration rates and lower bulk densities than trampled areas. The same shift in earthworm species as found in the preliminary study was duplicated on these study plots.

Biocides and metals

The use of herbicides in Australian agriculture as a 'chemical plough' has increased greatly as an alternative to cultivation in the control of unwanted plant growth in both field crops and orchards. Fungicides are used with great regularity in some subtropical fruit orchards and insecticides are called upon when necessary. Consideration of the impact of these biocides on soil fauna in the field is thus of great relevance, but published field studies are few. Dalby et al (1995) tested a single application of the broad spectrum herbicide glyphosate, the broadleaf herbicide 2,4-DB and the insecticide dimethoate for impact on earthworms in a pasture soil. None of these biocides reduced earthworm numbers by more than 10%. A study of post-emergent herbicides (Mele, Carter 1999) found that applications in two consecutive years resulted in significant increases in earthworms in the soil below. These results, supported by many other informal observations, suggest that an indirect effect of herbicides, the provision of freshly dead plant matter, is more significant to earthworms than any harm from the chemical itself. However a cautious approach should be taken in generalising this to all earthworm species, let alone to all groups of soil fauna or to the longer term.

In a study of four insecticides, Choo et al (1998) found significant mortality in the earthworm *Aporrectodea trapezoides* when exposed to endosulfan and fenamiphos on filter paper. When applied to soil in pots the only effect was a loss of weight in earthworms over a five week period with fenamiphos. Two other chemicals, methiocarb and ridomil, caused neither mortality nor loss of weight. In an overseas study of a semi-arid tropical soil (Reddy et al 1995) the biomass of three species of earthworms was reduced drastically by applications of carbofuran and herbicides. The extent of the impact varied significantly according to soil management.

A fungicide study carried out in orchards in Italy has particular relevance to orchards elsewhere. The study focussed on the effects of copper sulfate on earthworm communities in a variety of settings including vineyards and apple, peach and kiwifruit orchards (Paoletti et al 1998). Both abundance and biomass of the earthworm *Aporrectodea caliginosa* were severely reduced by copper spray and by soil tillage. Another study of fungicide use in orchards (Heijne, Anbergen 1998) provides evidence of the potential complexity and disguising of impacts. The rapid removal of fallen leaves from the soil surface in an orchard by earthworms was found to have a significant role in the suppression of fungal diseases. While some short-term disease control could also be achieved by the use of either copper or benzimidazole, these chemicals also reduced earthworms, thus creating a greater dependency on chemicals for disease control. This is an excellent example of the kind of impact that soil biologists intuitively fear, but that is difficult to demonstrate.

Another important aspect of the interaction between soil fauna and chemical contaminants of soil is the demonstrated ability of earthworms to bioaccumulate organochlorines and heavy metals. Given the place of earthworms at the base of food chains involving all the major groups of vertebrates: birds, mammals, frogs, lizards and fish, the entry of the contaminants into food chains is of concern.

Vorobeichik (1998) looked at the effects on earthworms of the contamination of soils by copper, lead and cadmium. When levels of these metals were up to 2.5 times higher than in control soils, earthworm populations were reduced; at up to 4.5 times higher, earthworms were absent. In the Tasmanian Midlands, earthworms were collected from trial plots to which superphosphate had been applied at rates of up to 250 kg per annum over nine years and their tissue analysed for cadmium, a known impurity of superphosphate fertiliser. Levels in earthworms from the 250 kg/ha plots contained cadmium at 8.7 ppm compared with 3.4 ppm on the control plots (Kingston, unpublished data).

There is cause for concern about the use of copper sprays in subtropical orchards, particularly avocados, in northern NSW. Observations made during joint soils research by Tuckombil Landcare and NSW Agriculture suggest that fallen leaves under avocado trees are slow to decompose, that there is poor mixing of organic matter into the mineral soil and that earthworm populations are low. Soil analyses have revealed greatly elevated levels of both copper (834 ppm) and cadmium (10 ppm) from within an avocado orchard (Lukas Van Zwieten, pers. comm). The link, if any, between these observations is the subject of ongoing investigations.

II. The earthworm resource in Australia's arable soils

Earthworms are widespread, diverse and frequently abundant in Australian soils under both natural vegetation and agricultural production, as well as in gardens and compost. The major geographic limitation to their distribution appears to be low rainfall, few specimens having been collected in areas with an annual rainfall of less than 400 mm (Abbott 1994).

Surveys of earthworms of both arable and naturally vegetated areas have been undertaken in West Australia (Abbott, Parker 1980; Abbott 1985; McKenzie, Dyne 1991), South Australia (Baker 1992; Baker et al 1992), Victoria (Baker et al 1992; Mele 1991), and Tasmania (Kingston, Temple-Smith 1989, Garnsey 1994, Kingston 2000). One innovative community-based survey, the 'Earthworms Downunder' project (Baker et al 1997) was Australia-wide in its coverage. The project asked members of the CSIRO Double Helix Club to collect earthworms from gardens and farms near their homes and submit specimens to the CSIRO for identification. Other taxonomic and ecological studies of Australian earthworms have contributed many additional records to species' distribution maps.

Earthworms found in Australia belong to either native or introduced species. The introduced ones have been inadvertently imported from overseas during the past 200 years. Earthworm surveys in areas of native vegetation generally find native species, while those carried out in areas cleared for agriculture, at least in southern Australia, have found predominantly introduced species. In more tropical parts of Australia fewer surveys have been conducted but it appears that inter-mixing of native and introduced species is more common, at least under agricultural soils (Baker et al 1997; Kingston, unpublished data collected in

Australian native earthworms

The native earthworms of Australia are poorly known because, in general, surveys have been conducted in a patchy and low intensity manner relative to the small natural ranges occupied by the great majority of species. Perhaps 500 species have

now been described but many more are known to exist in museum collections, their number being enlarged by each new survey undertaken. Kingston and Dyne (1994) suggested that eventually more than 1000 species will be described for the continent. Since then a number of publications have brought the total number of described species to around 700. These results now enable a revised minimum estimate of 1500 Australian species.

Very few large geographical areas under native vegetation have been comprehensively surveyed for earthworms, and the collections fully documented, as has been done by the author and his colleagues at the Queen Victoria Museum, Launceston, for the whole of Tasmania (Kingston 2000). In this survey 400 sites were sampled between 1990 and 1994, almost 8000 specimens were collected, preserved and sorted to about 220 native species. No more than 30 of these were previously known. Other more localised or lower intensity surveys outside areas of human activity have been reported for Mt Kosciusko in New South Wales (Wood 1974), for Tasmania (Laffan, Kingston 1997) and Western Australia (Abbott 1985 and McKenzie, Dyne 1991). Some additional studies of earthworms in agriculture, conducted at a limited number of sites only, have reported the presence of native species in significant numbers: for example at one of three sites assessed in the Mount Lofty Ranges in South Australia (Baker et al 1993), at a single site in western Australia (Abbott et al 1985) and at 19 of 50 sites within a radius of 100 km from Alstonville in the NSW northern rivers region (Kingston, in preparation). The site with both the heaviest mean earthworm weight (1.4 grams) and the greatest biomass of earthworms (171 grams/m²) (weight of earthworms preserved in alcohol) was a dairy pasture near Kyogle. The population comprised a single native species. Not only was it one of the largest species encountered but it was also found to be living more deeply in the soil (Plate 1) than introduced species in the district. Species having this deep-burrowing characteristic are considered especially desirable under agriculture (Baker 1996). The reasons for the appalling state of knowledge of Australian native earthworms include such factors as the difficulty of collecting, the rapid deterioration of unpreserved specimens, the specialised knowledge required in their identification and the chronic shortage of specialists performing the overdue taxonomic work required.

'Introduced' earthworms in Australia

The most up-to-date list of introduced earthworms in Australia (Blakemore 2000) includes 63 species in eight families but many of the species listed have been rarely recorded and may be considered to be of academic interest only. In surveys of soils under agriculture in WA (Abbott, Parker 1980, Abbott 1985), South Australia (Baker 1992, Baker et al 1992), Victoria (Baker et al 1992, Mele 1991) and Tasmania (Kingston, Temple-Smith 1989, Garnsey 1994), the great majority of which have been carried out in the southern half of Australia, the most commonly encountered earthworms belong to a very small group of introduced species (Table 1).

Table 1. Frequently encountered introduced earthworm species.

FAMILY/SPECIES	DISTRIBUTION	WEIGHT	NOTES
LUMBRICIDAE			
<i>Aporrectodea caliginosa</i>	WA, SA, VIC, TAS, NSW, QLD	1.2 gm	One of the six most common species in Australia-wide survey ² . Widespread southern Australia, rare in tropical areas, most frequent species in urban and orchard habitats ² . Dominant species in northern Tasmanian pastures ¹ .
<i>Aporrectodea longa</i>	WA, SA, VIC, TAS, NSW, QLD	5 gm	Northern European origin. Only in Tasmania ² . Locally distributed species in northern Tasmanian pastures, usually with <i>A. caliginosa</i> ¹ . Described as 'deep burrowing' but in Tasmania is found near the surface when active ¹ .
<i>Aporrectodea rosea</i>	WA, SA, VIC, TAS, NSW, QLD	0.4 gm	One of the six most common species in Australia-wide survey ² . Equal dominant (with <i>A. trapezoides</i>) in croplands ² . Occasional species in northern Tasmanian pasture, often with <i>A. caliginosa</i> ¹ .
<i>Aporrectodea trapezoides</i>	WA, SA, VIC, TAS, NSW, QLD	1.5 gm	Mediterranean origin in Europe. One of the six most common species in Australia-wide survey, especially in Mediterranean climate ² . Most common species in pastures and (with <i>A. rosea</i>) in cropland ² . Most common species in permanent pasture near Adelaide ⁵ . most common species in pasture – cereal rotations in South Australia and western Victoria ⁴ . Most common species in Tasmanian Midlands, replaces <i>A. caliginosa</i> in lower rainfall areas becoming dominant below 600 mm ³ .
<i>Dendrodrilus rubidus</i>	WA, SA, TAS, NSW, QLD		
<i>Eisenia fetida</i> Tiger Worm	WA, SA, VIC, TAS, NSW, QLD, NT	1 gm	
<i>Lumbricus rubellus</i> 'Red Worm' 'Dung Worm'	WA, SA, VIC, TAS, NSW	2 gm	Northern European origin. One of the six most common species in Australia-wide survey ² .
<i>Octolasion cyaneum</i>	WA, SA, VIC, TAS, NSW, QLD	2.5 gm	
GLOSSOSCOLECIDAE			
<i>Pontoscolex corethrurus</i>	WA, NSW, QLD, NT	1 gm	Only recorded coastally from northern New South Wales, Queensland and NT ² .
ACANTHODRILIDAE			
<i>Microscolex dubius</i>	WA, SA, VIC, TAS, NSW, QLD		Mediterranean origin in Europe. One of the six most common species in Australia-wide survey, especially in Mediterranean climate ² . Most common species in Perth ² .
<i>Microscolex phosphoreus</i>	WA, SA, VIC, TAS, NSW, QLD		
MEGASCOLECIDAE			
<i>Perionyx excavatus</i>	SA, VIC, TAS, NSW, QLD		
<i>Amyntas corticis</i>	WA, SA, VIC, TAS, NSW, QLD		
<i>Amyntas rodericensis</i>	WA, VIC, NSW, QLD		One of the six most common species in Australia-wide survey, most abundant coastally in New South Wales and Queensland ² . Most common species in Sydney

1. Kingston, Temple-Smith 1989. 2. Baker et al 1997. 3. Garnsey 1994. 4. Baker et al 1996. 5. Baker et al 1992

**Plate 1 Native earthworm and soil block from 30 cm depth below pasture, Casino.
Note numerous open and in-filled burrows**



Introduced species fall into two subgroups according to their place of origin and climatic zone of their distribution within Australia. Their origin is, to a large degree, reflected in their taxonomy with members of the first subgroup, belonging to the family Lumbricidae, having their origin in eastern and Mediterranean Europe. Within Australia they are concentrated in regions of temperate and mediterranean climate; in the east of the continent they are rarely found north of the New South Wales – Queensland border. The second subgroup is a more heterogeneous mixture derived from several families and contains species from widely separated locations including South America and Asia. The species predominate in Australia's tropical north, extending as far south as northern New South Wales. Many members of the group belong to the same family as the majority of Australian native species: the Megascolecidae. This family is endemic to an area from eastern Russia, through Asia, Malaysia, Indonesia, New Guinea, Australia and New Zealand, and contains perhaps 2000 species. The overlay of introduced over native members of the same family, within Australia, has caused unresolved difficulties in determining the true origin of some species.

From the distributions of these two groups of introduced earthworm species it is apparent that there is a broad transition zone within which the species dominant under arable soils shift from predominantly European to predominantly tropical ones. The zone stretches from south of Sydney to the Queensland border. Distribution maps of species (Baker et al 1997) show that some species within each group traverse this entire zone, rather than there being a sequence of species replacing each other across the zone. Some species are apparently able to tolerate a wide range of maximum and minimum temperatures and rainfall. Within this extensive area it is presumed that microclimatic conditions, notably soil temperature and moisture, as mediated by altitude, aspect and distance from the ocean, combined with local soil conditions, determine the presence or absence of

a species. Considerably more earthworm survey is required, particularly in the north and west of the continent, in order to reveal the key factors that determine the distributions of the two subgroups.

Interactions between native and introduced species

Within southern Australia at least there appears to be an incompatibility between native earthworms and the changes made to soils in the process of preparing them for agriculture. Whether any particular change, such as soil disturbance *per se*, removal of native plants or alteration of pH and/or fertility is a key factor is unknown. The equivalent picture for tropical and especially subtropical Australia is only now emerging and is still based on very inadequate surveys. Published results for the region come only from the 'Worms Downunder' project (Baker et al 1997) but because of a lack of consistency and rigour in the collecting, and taxonomic difficulties in separating some of the introduced, as well as most of the native species, little information on interrelationships between the two groups can be deduced. However, an as yet unpublished survey in northern NSW in recent months by the author does have the potential to do so. In a preliminary survey of earthworms at 50 sites under a variety of orchard crops, sugar cane and pasture, within about 100 km of Alstonville on the NSW north coast, Kingston (in prep.) found that lumbricids, 'tropical' exotics and native species were found at seven, 41, and 19 sites. Lumbricids and tropical exotic species were not mutually exclusive, tropical species being present at six of the seven sites occupied by lumbricids. Of the 19 sites at which native species were found, exotics were also found at 14. This level of intermixing of native species with both groups of exotic species at the same sites has not previously been reported.

Conclusions

The current state of knowledge of the major groups of soil fauna is extremely poor, whether it be taxonomy, biology, ecology, distributions, or their inter-relationship with other groups or with agricultural management. This statement applies generally for Australia but is especially true of the northern half of NSW. There are some useful data sets for earthworms but they come mostly from uncoordinated studies in temperate and mediterranean regions and largely relate to species introduced from Europe. Next to nothing is known about more tropical exotics and rather less about native species. Considering the demonstrated benefits available from soil fauna, the ease with which populations can be depleted by unsympathetic management practices, and their potential use as indicators of soil conditions, this lack of knowledge is surprising. The onfarm consequence of this data deficit is that land managers are unable to realistically take account of the welfare of their underground 'stock' in the way they are used to doing for their more traditional above-ground plants and animals.

As farmers increasingly move towards ecologically-based management they will be rewarded with soil conditions compatible with increased abundance and diversity of soil fauna, including species exterminated during times of harsher practices. If remnant soil fauna populations have survived locally along fence lines, creeks or small patches of remnant bush then they are likely to recolonise without intervention. If no such reserves exist, then experimental reintroductions from further afield or of proven overseas species may be required. There are

obvious advantages in using native soil faunal species that have evolved under local conditions.

Taking into account the recent confirmation of widespread, diverse and locally abundant populations of native and introduced earthworms persisting under agricultural soils in subtropical NSW, and the fact that some of these species clearly possess the desirable qualities of large size and deep-burrowing habits, the potential benefits to be derived from earthworms in the region is as high as for any other region of Australia. With such potential from the native species it no longer makes sense to focus our efforts on seeking additional overseas species for active introduction, as proposed by Baker (1996), a mindset perhaps overly influenced by the success of the dung beetle story.

While much more needs to be known about almost all aspects, review of the current literature has strongly endorsed the soil biologist's perspective that greater combined abundance and diversity of soil fauna (and one lacking in bioaccumulated chemicals), both supports and reflects 'biologically sustainable' agricultural systems and soils that a majority of observers would describe as 'healthy'. It needs to be appreciated that these latter terms are by no means synonymous with 'most productive'. Indeed, taking care of soil fauna and attaining biological sustainability are likely to come at a cost to production. On marginally productive properties this cost may well be the 'final straw' that breaks the back of economic sustainability. Socio-economic considerations must then be invoked in the formation of a new paradigm for the use and management of the land, one that may involve continuing the operation with an organic matter 'subsidy' or alternatively, a move to a lower intensity usage encouraged and supported by financial incentives.

Recommendations

There is a dire need for a significantly increased effort, in both research and education, on all aspects of soil fauna throughout Australia, but the need is greatest in tropical and subtropical regions. Examples of the kind of research required under a variety of climate and crops include:

- surveys of earthworms and other soil fauna in soils under both agricultural management and bushland remnants
- studies of the impact on soil fauna, over a minimum of three years, of frequently employed management practices including irrigation (including with dairy effluent), drainage, cultivation, land levelling, mulches, herbicides, insecticides and fungicides
- studies of the life cycle of representatives of the major groups of soil fauna, particularly of native earthworms
- screening of native soil fauna to test their capacity for reintroduction into soils under agricultural management
- studies of the introduction of both native and exotic earthworms, alone and in combination, into study plots and the monitoring of their populations over a period of 5-10 years
- revision of economic models of analysis of agricultural operations to account for the declining 'ecological capital' of depleted soil organic matter and soil biota.

References

- Abbott I 1985. Distribution of introduced earthworms in the northern jarrah forest of Western Australia. *Australian Journal of Soil Research* **23(2)**:263-270.
- Abbott I 1994. Distribution of the native earthworm fauna of Australia: a continent-wide perspective. *Soil Biology and Biochemistry* **32**:117-126.
- Abbott I, Parker CA 1980. The occurrence of earthworms in the wheat belt of Western Australia in relation to land use and rainfall. *Australian Journal of Soil Research* **18**:343-52.
- Abbott I, Ross JS, Parker CA 1985. Ecology of the large indigenous earthworm *Megascolex imparicystis* in relation to agriculture near Lancelin, Western Australia. *Journal of the Royal Society of Western Australia* **68**:13-15.
- Baker GH 1992. Optimising earthworm activity in soils. In *Proceedings of the 33rd Annual Grassland Society of Victoria Conference*. James G (ed). pp. 59-66. Henry Cotton Press, Warragul.
- Baker GH 1996. Introduction of earthworms to agricultural soils in southern Australia. In *The role of earthworms in agriculture and land management: Report of a national workshop, Launceston, June 1993*. Temple-Smith M, Pinkard T (eds). pp 90-98. Department of Primary Industry and Fisheries, Tasmania.
- Baker GH 1996. The ecology and management of earthworms in agricultural soils, with particular reference to southern Australia. In *Earthworm Ecology*. Edwards C (ed). Soil and Water Conservation Service, Ankeny.
- Baker GH, Buckerfield JC, Grey-Gardner R, Merry R, Doube B 1992. The abundance and diversity of earthworms in pasture soils in the Fleurieu Peninsula, South Australia. *Soil Biology and Biochemistry* **24(12)**:1389-1395.
- Baker GH, Barrett VJ, Grey-Gardner R, Buckerfield JC 1993. Abundance and life history of native and introduced earthworms (Annelida: Megascolecidae and Lumbricidae) in pasture soils in the Mount Lofty Ranges, South Australia. *Transactions of the Royal Society of South Australia* **117(1)**: 47-53.
- Baker GH, Thumlert TA, Meisel LS, Carter PJ, Kilpin GP 1997. Earthworms downunder: a survey of the earthworm fauna of urban and agricultural soils in Australia. *Soil Biology and Biochemistry* **29(3-4)**:589-597.
- Blakemore RJ 2000. Diversity of exotic earthworms in Australia - a status report. In *The other 99%: the conservation and biodiversity of invertebrates*. Ponder W, Lunney D (eds). pp. 182-187. Transactions of the Royal Society of New South Wales.
- Chittleborough DJ 1992. Formation and pedology of duplex soils. *Australian Journal of Experimental Agriculture* **32**: 815-25.
- Choo LP, Baker GH 1998. Influence of four commonly used pesticides on the survival, growth, and reproduction of the earthworm, *Aporrectodea trapezoides* (Lumbricidae). *Australian Journal of Agricultural Research* **49**:1297-1303.
- Dalby PR, Baker GH, Smith SE 1995. Glyphosate, 2,4-DB and dimethoate: effects on earthworm survival and growth. *Soil Biology and Biochemistry* **27**:1661-2.

- Garnsey RB 1994 Seasonal activity and aestivation of lumbricid earthworms in the Midlands of Tasmania. *Australian Journal of Soil Research* **32(6)**:1355-1366.
- Heijne B, Anbergen RHN 1998. Scab control after the harvest can be effective. *Fruitteelt* **88**:9-11.
- Kingston TJ 1989. *Aporrectodea caliginosa* and *Lumbricus rubellus* populations under irrigated and dryland pastures in northern Tasmania. *Proceedings 5th Australasian conference of grassland invertebrate ecology*. Stahle PP (ed). pp 199-205.
- Kingston TJ, Temple-Smith MG 1989. Earthworm populations under Tasmanian pastureland. *Proceedings 5th Australasian conference of grassland invertebrate ecology*. Stahle PP (ed). pp 192-198.
- Kingston TJ, Dyne G 1996. Potential for agronomic exploitation of Australian native earthworms. In *The role of earthworms in agriculture and land management: Report of a national workshop, Launceston, June 1993*. Temple-Smith M, Pinkard T (eds). pp 29-37. Department of Primary Industry and Fisheries, Tasmania.
- Kingston TJ 2000. *Distribution and conservation status of Tasmanian native earthworms*. Report to the Heritage Commission, Queen Victoria Museum Vol. 1 (text), 40 pp. Vol. 2 (figures and distribution maps) 420 pp.
- Laffan MD, Kingston TJ 1997. Earthworms in some Tasmanian forest soils in relation to bioturbation and soil texture profile. *Australian Journal of Soil Research* **35**:1231-43.
- Lee KE 1985. *Earthworms - Their Ecology and Relationships with Soils and Land Use*. Academic Press, Sydney.
- Lobry de Bruyn LA 1997. The status of soil macrofauna as indicators of soil health to monitor the sustainability of Australian agricultural soils. *Ecological Economics* **23**:167-78.
- Lobry de Bruyn LA, Conacher AJ 1990. The role of termites and ants in soil modification: a review. *Australian Journal of Soil Research* **28**:55-93.
- Lobry de Bruyn LA, Kingston TJ 1997. Effects of summer irrigation and trampling in dairy pastures on soil physical properties and earthworm number and species composition. *Australian Journal of Agricultural Research* **48**:1059-79.
- Longstaff BC, Greenslade PJM, Colloff M, Reid I, Hart P, Packer I 1999. *Managing soils in agriculture: the impact of soil tillage practices on soil fauna*. Rural Industries Research and Development Corporation Publication No 99/18, 65 pp.
- McKenzie NL, Dyne GR 1991. Earthworms of rainforest soils in the Kimberley, Western Australia. In *Kimberley Rainforests*, McKenzie NL, Johnston RB, Kendrick PG (eds). Surrey Beatty & Sons, Chipping Norton.
- Mele PW 1991. What species, and how many, on local farms? In *Earthworms. Improving soils for agriculture*. Haines PJ (ed). pp. 20-26. Australian Institute of Agricultural Science, Occasional Publication No.62.
- Mele PM, Carter MR 1999. Impact of crop management factors in conservation tillage farming on earthworm density, age structure and species abundance in southeastern Australia. *Soil and Tillage Research* **50(1)**:1-10.

- Noble JC, Mills PA 1974. Soil moisture status and its effect on earthworm populations under irrigated pastures in southern Australia. *Proc. XII International Grassland Congress*. Moscow, June 11-20, 1974.
- Pankhurst CE, Doube BM, Gupta VVSR (eds) 1997. *Biological Indicators of Soil Health*. CAB International, Wallingford.
- Pankhurst CE, Doube BM, Gupta VVSR, Grace PR (eds) 1994. *Soil biota: Management in Sustainable Farming Systems*. CSIRO, Australia.
- Paoletti MG, Sommaggio D, Favretto MR, Petruzzelli G, Pezzarossa B, Barbaferi M 1998. Earthworms as useful bioindicators of agroecosystem sustainability in orchards and vineyards with different inputs. *Applied Soil Ecology* **10**:137-150.
- Reddy MV, Kumar VP, Reddy VR, Balashouri P, Yule DF, Cogle AL, Jangawad LS 1995. Earthworm biomass response to soil management in semi arid tropical Alfisol agroecosystems. *Biology and Fertility of Soils* **19**(4):317-21.
- Robertson LN, Radford BJ, Bridge B, McGarry D, Blakemore RJ, Sabag M 1994. Tropical earthworms under cropping in Queensland. In *Soil biota: Management in Sustainable Farming Systems* Pankhurst CE (ed). pp. 33-34. CSIRO, East Melbourne.
- Ryder MH, Terrace TE, Bird AF, Stephens PM, Doube BM 1993. Physical and biological determinants of the success of soil bacteria as biocontrol agents. *Australian Microbiologist* **14**(4):27.
- Stephens PM, Davoren CW, Ryder MH, Doube BM, Correll RL 1994. Physical and biological determinants of the success of soil bacteria as biocontrol agents. *Soil Biology and Biochemistry* **26**(11):1495-1500.
- Stephens PM, Davoren CW 1996. Effect of the lumbricid earthworm *Aporrectodea trapezoides* on wheat grain yield in the field, in the presence or absence of *Rhizoctonia solani* and *Gaeumannomyces graminis*. *Soil Biology and Biochemistry* **28**:561-67.
- Stephens PM, Davoren CW 1997. Disease severity of *Rhizoctonia solani* on subterranean clover and ryegrass. *Soil Biology and Biochemistry* **29**:511-516.
- Temple-Smith M, Pinkard T 1996. *The role of earthworms in agriculture and land management*. Report of a national workshop, Launceston, June 1993. Department of Primary Industry and Fisheries, Tasmania.
- Tisdall JM 1985. Earthworm activity in irrigated red-brown earths used for annual crops in Victoria. *Australian Journal of Soil Research* **23**:291-9.
- Vorobeichik EL 1998. Populations of earthworms (Lumbricidae) in forests of the middle Urals in conditions of pollution by discharge from copper works. *Russian Journal of Ecology* **29**:85-91.
- Wood TG 1974. The distribution of earthworms (Megascolecidae) in relation to soils, vegetation and altitude on the slopes of Mt Kosciusko, Australia. *Journal of Animal Ecology* **43**:87-106.
- White RE, Helyar KR, Ridley AM, Chen D, Heng LK, Evans J, Fisher R, Hirth JR, Mele PM, Morrison GR, Cresswell HP, Paydar Z, Dunin FX, Dove H, Simpson JR (2000). Soil factors affecting the sustainability and productivity of perennial and annual pastures in the high rainfall zone of south-eastern Australia. *Australian Journal of Experimental Agriculture* **40**:267-83.

Zund PR, Pillai-McGarry U, McGarry D, Bray SG 1995. Repair of a compacted Oxisol by the earthworm *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta). *Biology and Fertility of Soils* **19**:317-21.

Role of organic amendments in disease control

Percy Wong

**Principal research scientist
NSW Agriculture, Richmond**

In agriculture and horticulture, organic amendments such as composts and green manures have long been valued as good sources of organic matter and slow-release fertilisers. Organic matter is important for improving soil structure and ensuring a microbiological balance that is vital to the health of the soil and the plants it sustains. However, the quality of the incorporated organic amendments determines the eventual microbial composition and diversity developing in the soil, which then impact on the interactions between beneficial microorganisms and plant pathogens. Some crop residues have also been found to be superior to others. For example, brassica crops have been used in many countries for crop rotation and as green manures. The seed meal, following the extraction of oil, has a high protein content and has served as an important agricultural fertiliser in China and the Indian sub-continent. The fortuitous disease-suppressive effects of the seed meal and crop residue amendments were probably not fully realised in the past but contributed to the production of healthy crops and sustainable systems of agriculture, which have lasted for thousands of years in those countries. In the last few decades, increasing scientific research has been carried out to explain the disease-suppressive effects of composts and brassica amendments. This paper will explore some of this research.

Mechanisms of pathogen suppression

Certain types of composts have been shown to suppress a range of plant pathogens and pathogenic nematodes. The diverse antagonistic microflora present in these composts are thought to mediate this suppression. The beneficial microorganisms achieve this through the production of antibiotics that suppress the pathogens either in the resting stage or when the pathogens are at the infection site. Other microorganisms parasitise the resting bodies or actively growing stages of the pathogens. There is also evidence that some microorganisms present in the roots of plants can induce localised or systemic resistance in plants and thereby ward off infection either in below-ground parts or in the foliage. For example, the suppression of the take-all pathogen of wheat by closely related non-pathogenic fungi is due to induced localised resistance. There is no evidence of antibiosis, parasitism or the production of phytoalexins (Deverall et al 1978) and the presence of the non-pathogenic fungi in the wheat root cortex induces greater lignification (strengthening) of the vascular tissues directly below the colonised cortex (Speakman, Lewis 1978). As this is a localised response, a large proportion of the root system would have to be colonised in order to provide adequate protection. Therefore, competition for root cortical tissues is critical. This was confirmed when a dose response in disease control was obtained by the

application of increasing rates of the non-pathogenic fungi in field experiments (Wong et al 1996).

There are increasing examples of compost microorganisms inducing systemic resistance, that is, their activities in the root systems can cause aerial plant parts to become resistant to such diseases as anthracnose and grey mould (Wei et al 1991, Zhang et al 1996, De Meyer et al 1998). This phenomenon may account for numerous observations that plants are more resistant to diseases when grown in compost or green manure amended soils. Scientists are hopeful that it may be possible to isolate microorganisms that are responsible for inducing systemic resistance and apply them to the soil under the right environmental conditions to obtain effective disease control.

Biofumigation

Biofumigation is a term used to describe the suppression of soil-borne pests and pathogens by *Brassica* crops (Angus et al 1994). There is considerable interest in biofumigation as an alternative to synthetic soil fumigants in horticulture and for the control of intractable soil-borne pathogens in broadacre agriculture. It may also become a cheap replacement for the common chemical fumigant, methyl bromide, which will soon be banned. The main mechanism of suppression of plant pathogens and pests by brassica amendments is through the production of a range of toxic gases called isothiocyanates, released when the brassica residues decompose in soil. These compounds are selectively biocidal, being more effective against some pathogens and pests than others. The superior growth and yield of wheat following brassica crops such as canola (*B. napus*) and Indian mustard (*B. juncea*) has been attributed to the suppression of soil-borne fungal pathogens by the gases released from the crop residues (Kirkegaard et al 1996).

Apart from the direct biocidal effects of the toxic gases on fungal pathogens, the brassica amendments appear to enhance the populations of antagonistic microflora such as bacteria, actinomycetes and fungi (Ramirez-Villapudua, Munnecke 1987). Sharma and Trivedi (1987) found that a parasite of nematodes, *Paecilomyces lilacinus*, was unaffected by mustard meal and proliferated in a medium of rice husks and oilseed meals. My preliminary studies also show a succession of fungi following the incorporation of mustard meal into soil, beginning with growth of tolerant fungi such as *Rhizopus* and *Mucor* species, followed several days to a week later by a flush of *Trichoderma* species and actinomycetes (Wong, unpublished). The overall increase in biological activity and, in particular, the increase in antagonistic microflora would serve the useful role of ‘mopping up’ the pathogenic fungi which may have escaped the toxic effects of the gases. The duration of this suppression is not known but is expected to last several weeks, if not months. Therefore, the application of these amendments on a regular basis could ensure that disease and pest pressures do not become high enough for chemical pesticides to be required. Research is in progress to investigate the types of brassica residues to use, the brassica species that release the most toxic isothiocyanates for targeted pathogens, and the methods and rates of application for optimal efficacy without phytotoxic effects on crops.

Disease-suppressive composts

The nature of the disease-suppressiveness of composts is not fully understood but it appears that composts that have been matured for a long time (more than six months) tend to be highly disease-suppressive (Hoitink, Boehm 1999). These composts contain large and diverse populations of mesophilic microflora that usually grow at temperatures of 20-35°C and include large proportions of microbial antagonists of plant pathogens. However, mature composts are not all equally disease-suppressive (Chen et al 1988, Inbar et al 1991). In attempts to produce highly suppressive composts consistently, various microbial antagonists such as *Trichoderma*, *Flavobacterium* and *Enterobacter* species, have been added to the composts at the last stages of composting so that these mesophilic antagonists would predominate (Hoitink, Fahy 1986). It has now been confirmed that these added antagonists significantly increase the suppressiveness of well-matured and stabilised composts (Hoitink, Boehm 1999). It may be possible in the future to tailor-make composts for various glasshouse or field situations.

Some factors affecting pathogen suppression

Temperature

Most of the serious pathogens of economically important crops are mesophiles, active at temperatures of 20-35°C. However, the temperature range for growth and infection of these pathogens can extend to lower temperatures, eg *Botrytis cinerea* (16°-21°C), *Pythium* species (7°-30°C), *Phytophthora infestans* (5°-30°C), *Rhizoctonia solani* (10°-32°C) and *Sclerotinia sclerotiorum* (4°-26°C). Therefore, for microbial antagonists present in organic amendments to be effective in the field, they have to be active throughout the whole temperature range or at those ranges that favour infection. In attempts to select specific biocontrol agents for disease control, potential agents screened at 25°C in the laboratory failed when tested at soil temperatures of 10-15°C in field experiments because they were not cold tolerant (Wong et al 1996). The greater diversity of microbial antagonists in organic amendments would improve the chances of successful disease control.

Moisture

Pathogens such as *Pythium* and *Phytophthora* species flourish in wet soil conditions, so the microbial antagonists required to combat these pathogens would need to grow in the same conditions. Bacteria and protozoa are most active in very moist to wet soils and would, therefore, be more effective against these pathogens than fungi and actinomycetes, which generally require drier soil conditions. It is unlikely then that one biocontrol agent will be suitable for the control of a number of plant pathogens, especially when the environmental conditions for their pathogenic activities are dissimilar. As such, the great diversity of antagonistic microflora in mature composts could account for the control of a broader spectrum of pathogens.

pH

Some *Trichoderma* species have been commercialised as biocontrol agents for several crop diseases (Fravel 1999). However, the various species of this fungus tend to be active only under acid soil conditions of pH 5-6. Their efficacy is

reduced or non-existent at higher pH of 7-8. As such, other groups of fungi or bacteria will have to be selected for alkaline soils. Again, the microbial diversity in mature composts would overcome this deficiency in a single biocontrol agent.

Substrate quality

The maturity of composts and the freshness of incorporated green manures can affect the behaviour of plant pathogens. Hoitink and Grebus (1994) found that immature composts provide a food source for plant pathogens such as *Pythium* species and *R. solani*, and exacerbate disease severity. This would also be the case for freshly incorporated green manures. However, excessively stabilised compost did not support the activities of the antagonistic microflora and also resulted in more disease. Therefore, the age and quality of organic amendments are of the utmost importance. In addition, Erhart and Burian (1997) show that the degree of suppression of *Pythium* damping-off disease is related to the organic matter content of the compost.

Management of organic amendments

Traditionally, large annual incorporations of more than 50 tonnes/ha of green manures and composts have been considered necessary for disease control and fertility. However, in the turf industry, Nelson and Craft (1992) have shown that monthly applications of relatively small amounts of suppressive composts, around 500kg/ha, have suppressed turf diseases such as dollar spot (*Sclerotinia homoeocarpa*), brown patch (*Rhizoctonia solani*) and Pythium root rot (*Pythium* species). Moreover, the application of regular top-dressings and root-zone amendments of suppressive composts were as effective as chemical fungicides in suppressing Pythium root rot in established turfgrasses (Nelson et al 1994). As such, regular or strategic applications of highly suppressive composts may provide a way to control some notably intractable soil-borne diseases. This is encouraging for broadacre agriculture and may also explain the success of the organic farming practice of the regular use of side-dressings of composts as fertiliser and for disease control.

Conclusions

The control of plant diseases by organic amendments is founded on the encouragement of large, diverse populations of antagonistic microorganisms that reduce the activities of pathogens in crops and fallow, when the resting stages of the pathogens may be attacked. The amendments have to be suitably aged or mature before cropping as the quality of the substrates may alter the population dynamics of the pathogen-antagonist relationship and, therefore, the expression of disease. A good knowledge of the ecology of the pathogens and microbial antagonists may also assist in the provision of the most suitable environmental conditions for pathogen suppression.

References

- Angus JF, Gardner PA, Kirkegaard JA, Desmarchelier JA 1994. Biofumigation: isothiocyanates released from *Brassica* roots inhibit growth of the take-all fungus. *Plant and Soil* **162**:107-112.
- Chen W, Hoitink HAJ, Madden LV 1988. Microbial activity and biomass in container media for predicting suppressiveness to damping-off caused by *Pythium ultimum*. *Phytopathology* **78**:1447-1450.
- De Meyer G, Bigirimana J, Elad Y, Hofte M 1998. Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*. *European Journal of Plant Pathology* **104**:279-286.
- Deverall BJ, Wong PTW, McLeod S 1978. Failure to implicate antifungal substances in cross-protection of wheat against take-all. *Transactions of the British Mycological Society* **72**:233-236.
- Erhart E, Burian K 1997. Evaluating quality and suppressiveness of Austrian biowaste composts. *Compost Science and Utilisation* **5**:15-24.
- Fravel DR 1999. Hurdles and bottlenecks on the road to biocontrol of plant pathogens. *Australasian Plant Pathology* **28**:53-56.
- Hoitink HAJ, Boehm MJ 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. *Annual Review of Plant Pathology* **37**:427-446.
- Hoitink HAJ, Fahy PC 1986. Basis for the control of soilborne plant pathogens with composts. *Annual Review of Phytopathology* **24**:93-114.
- Hoitink HAJ, Grebus ME 1984. Status of biological control of plant diseases with composts. *Compost Science and Utilisation* **2**:6-12.
- Inbar Y, Boehm MJ, Hoitink HAJ 1991. Hydrolysis of fluorescein diacetate in sphagnum peat container media for predicting suppressiveness to damping-off caused by *Pythium ultimum*. *Soil Biology and Biochemistry* **23**:479-483.
- Kirkegaard JA, Wong PTW, Desmarchelier JM 1996. *In vitro* suppression of fungal root pathogens of cereals by Brassica tissues. *Plant Pathology* **45**:593-603.
- Nelson EB, Craft CM 1992. Suppression of Pythium root rot with top-dressings amended with composts and organic fertilisers. *Biological and Cultural Tests for the Control of Plant Diseases* **7**:104.
- Nelson EB, Burpee LL, Lawton MB 1994. Biological control of turfgrass diseases. In *Integrated Pest Management for Turf and Ornamentals*. Leslie AR (ed). pp 409-427. Lewis Publishers, Ann Arbor USA.
- Ramirez-Villapudua J, Munnecke DE 1987. Control of cabbage yellows (*Fusarium oxysporum* f.sp. *conglutinans*) by solar heating of fields soils amended with dry cabbage residues. *Plant Disease* **71**:217-221.
- Sharma A, Trivedi PC 1987. Screening of substrates suitable for the growth of *Paecilomyces lilacinus*. *International Nematology Network Newsletter* **4**:24-26.
- Speakman JB, Lewis BG 1978). Limitation of *Gaeumannomyces graminis* by wheat root responses to *Phialophora radicola*. *New Phytologist* **80**:373-380.
- Wong PTW, Mead JA, Holley MP 1996. Enhanced field control of wheat take-all using cold tolerant isolates of *Gaeumannomyces graminis* var. *graminis* and *Phialophora* sp. (lobed hyphopodia). *Plant Pathology* **45**:285-293.

Zhang W, Dick WA, Hoitink HAJ 1996. Compost-induced systemic acquired resistance in cucumber to *Pythium* root rot and anthracnose. *Phytopathology* **86**:1066-1070.

Molecular techniques for measuring soil microbial diversity

Tony Vancov
Research officer
NSW Agriculture Wollongbar

More than one million bacterial species exist on this planet, yet fewer than 4500 have been described (Heywood 1995). Despite the importance of microbial activity in the health of soil, the diversity of microbial species and the circumstances that govern their activities have not been explored in depth. These have been treated as a 'black box', an approach that ignores possible controls of processes by species or community interactions.

The inability, however, to identify greater than 90% of community members through cultivation makes this job intimidating (Hugenholtz et al 1998). The technical difficulties associated with defining species in soil have led to a number of culture-independent approaches that measure biodiversity of microbial communities. These procedures are either process-orientated, such as carbon utilisation profiles appraised via BIOLOG plates (Heuer, Smalla 1997), or census-based approaches such as comparative analysis of ester-linked fatty acids (Cavigelli et al 1995), or single or multiple genetic markers.

This paper focuses on the genetic marker approach. The intention is not to appraise the various techniques but to provide an overview of current molecular approaches in exposing microbial diversity.

Measuring biodiversity

Techniques that do not rely on culturing, such as molecular biology, have become a powerful means to describe microbial diversity and potentially unearth its role in ecosystem maintenance. There are three distinct phases in the application of molecular methods in soil microbiology. The first phase is characterised by the development of methods to isolate and amplify nucleic acid molecules from soil in the presence of inhibitors. The second phase reflects microbial evolutionary relationships, where microbes are grouped according to similarities in genes fundamental to all life forms (eg rDNA genes) or unique to certain physiologies (eg nitrogen fixation). The third and arguably the most central phase is combining the molecular information with soil process measurements to provide an understanding of what constitutes a healthy soil (O'Donnell, Gorres 1999).

Isolation of microbial nucleic acid

There are a number of reports in the literature describing methods for isolating microbial community DNA from several different environments. The reader is referred to Trevors 1992, and Coutinho et al 1999 for detailed reviews. Generally, DNA isolation methodologies derive from two approaches, *in situ* lysis and DNA recovery, whereby the microorganisms are lysed in the soil matrices and the DNA

is subsequently isolated and purified (More et al 1994, Zhou et al 1996, Cullen, Hirsch 1998). The alternative approach is microbial fractionation which relies on removing microorganisms from soil before cell lysis and DNA retrieval (Hopkins et al 1991). This method is seldom used today largely due to inefficient recoveries.

Most molecular microbiologists favour the direct isolation procedure because yields are higher, which consequently increases the sensitivity of the measurement. *In situ* nucleic acid extraction procedures also offer quicker turn around times and lower cost per unit extraction, not to mention the convenience associated with using commercially available kits. As with most methods, problems do arise, particularly when dealing with soils high in organic matter. Soluble humic acids and other organic compounds tend to be co-extracted with the DNA, thereby inhibiting subsequent molecular analyses. For the additional cost of a cafe latte, soil DNA can be promptly decontaminated using a range of commercially available purification kits.

16S rRNA genes

One of the most powerful ways to explore microbial diversity in nature is to analyse DNA sequences that encode the bacterial 16S ribosomal RNA (rRNA) molecule. This molecule is found in all life forms and is essential for translating genes into functional proteins. Since certain structural features of the 16S rRNA molecule must be preserved for its function, it follows that 16S rRNA gene sequences are highly conserved. Indeed, within the bacterial kingdom, this is what one finds. Structural constraints ensure against mutational changes that may compromise the functional integrity of the 16S rRNA molecule. However, throughout the course of hundreds of millions of years of evolution, non-deleterious mutations have slowly accumulated in segments of the 16S rRNA gene known as divergent regions (Woese 1987). Sequencing these divergent regions in the 16S rRNA genes and then comparing the sequences to a database of known 16S rRNA sequences can lead to the rapid identification of bacterial isolates.

This pattern of gene conservation has been exploited in determining microbial diversity through amplification of these regions via polymerase chain reaction (PCR), cloning the products and subsequently sequencing the library clones. The power of this technique rests in its ability to reveal uncultivable bacteria and measure the diversity of the soil community. A number of researchers have isolated from total DNA soil libraries rDNA sequences that have not been found in cultured bacteria (Stackebrandt et al 1993, Rheims et al 1996, Chandler et al 1997, Felske et al 1997, Kuske et al 1997).

As a result of the widespread adoption of the 16S rDNA approach, the size of ribosomal DNA sequence databases has increased. Besides resolving phylogenetic issues, expansions in the field of comparative genomics (bioinformatics) have facilitated the isolation of novel organisms for the biotechnology industry, and advanced the development and application of DNA hybridisation and rapid genetic fingerprinting techniques to dissect microbial soil communities and study

successional changes. Comparative genomics imparts an indispensable insight towards the designing of PCR amplification primers and hybridisation probes. Prior to the dawning of the PCR age, these sequence collections were primarily used to devise rRNA-targeting oligonucleotide probes. These probes could target signature sites of the rRNA molecule characteristic for defining phylogenetic entities such as species, genera, families, orders and even domains. Indeed, within the context of microbial ecology, one of the earliest exploits of such gene probes was the identification of ruminant bacteria using phylogenetic targeting rRNA probes (Stahl et al 1988). Identification of individual microbial cells has greatly profited by advancements made in fluorescent chemistry, particularly the ability to fluorescently tag probe molecules. This gave rise to a new visual technique, namely fluorescence *in situ* hybridisation (FISH) analysis. The technique consists of fixing the sample material onto a microscope slide and hybridising with a fluorescently labelled probe. The probe traverses through the cell wall and membrane, and binds to the complementary region within the 16S ribosomal RNA molecule (DeLong et al 1989). With the aid of an epifluorescent microscope, fluorescent emissions corresponding to microbial cells are visualised and counted. Given that ribosome numbers are proportionally related to protein synthesis (actively growing cells contain between 1000-10,000 ribosomes), the strength of the fluorescent signal may also be correlated with recent microbial activity (Wallner et al 1993).

DNA fingerprinting techniques

Several fingerprinting techniques have been developed with a view to providing comparative microbial community profiles of different environments and or to follow the behaviour of one population over time. These have been reviewed recently (Coutinho et al 1999, Marsh 1999, Muyzer 1999, Theron, Cloete 2000). The general strategy for genetic fingerprinting of microbial communities consists of isolating the nucleic acid, amplifying the target gene, and finally separating/analysing the PCR products on the basis of their composition (DGGE/TGGE) or size (T-RFLP).

Denatured gradient gel electrophoresis (DGGE) and its close cousin temperature gradient gel electrophoresis (TGGE) are capable of detecting sequence polymorphism by separating the PCR amplified DNA fragments based on their melting behaviour (Myers et al 1987). When subjecting double stranded DNA (dsDNA) to an increasing denaturing gradient, the double strands do not melt in a uniform fashion but in discrete units called melting domains. The PCR amplified products possess two domains: an artificially created high melting region, introduced via the PCR amplification primers (a GC clamp at the 5' termini) and the lower melting domain which comprises the region of interest (up to 450bps). When the melting temperature of the lowest melting region is reached, the double strand of this domain becomes partially melted, creating branched molecules, which effectively stops further migration through the gel. The position in the gel at which melting takes place, therefore, depends on base pair composition and sequence of the lower melting domain. Provided they melt at different denaturing conditions, the respective positions of equally sized dsDNA molecules on the gel should differ. For a more detailed account the reader is referred to Muyzer and Smalla (1998).

A method currently gaining wide acceptance for separating PCR amplified sequences is terminal restriction fragment length polymorphism (T-RFLP) analysis. As the name implies, T-RFLP analysis measures the size polymorphism of terminal restriction fragments from PCR amplified products (Liu et al 1997, Marsh 1999). In essence, the soil DNA is amplified from a high background of sample DNA and subsequently digested with judiciously selected restriction endonucleases. The choice of enzymes to be used in differentiating sequence variants of the target gene depends on the level of discrimination required. The resulting digests produce appropriately sized terminal fragments, which are resolved by automated sequencing or capillary electrophoresis systems that provide digital output. The use of fluorescently tagged primers limits the analysis to only the terminal fragments of the digestion. Because size markers bearing different fluorophore from the samples can be included in every lane, the sizing is extremely accurate (± 1 base). One sequence variant therefore corresponds to a single T-RFLP fragment. Comparison with terminal fragment sizes derived from RDP (Ribosome Database Project [Maidak et al 2000]) sequence entries allow for phylogenetic inferences to be made about the sequence variants within the T-RFLP profiles of mixed community PCR products.

Besides assessing amplification product diversity within a community, genetic fingerprinting techniques have been used to study specific microbial activities by targeting functional genes, and comparatively measuring microbial distribution across communities. A summary outlining the types of measurements, the entities under study (specific microbial activities/groups and changes brought about by management practices), and the molecular techniques employed, is presented in Table 1.

Conclusion

Molecular techniques in their current format have undoubtedly contributed to our awareness of the extent of microbial diversity in soils and to the largely untapped microbial resource. The long-term goals of using such approaches is to increase our understanding of important microbial processes, characterising the cues to which they respond and the mechanisms by which they are regulated, for the express purpose of diagnosing the health of soils and intervening when required. Realising these goals will require additional technology, extensive data collection, sophisticated computational tools, and efforts to discern cause and effect.

Table 1. List of molecular techniques used to measure microbial/gene diversity.

Measurements	Microbial community or genes under study or management practice	Techniques	References
Microbial diversity		16S rDNA sequencing	Borneman et al 1996 Borneman, Triplett 1997 Dunbar et al 1999.
		DGGE/TGGE	Muyzer et al 1993 Felske et al 1998 Smalla K et al 1998 Heuer et al 1999 Smit et al 1999
		T-RFLP	Liu et al 1997 Dunbar et al 2000 Osborn et al 2000 Derakshani et al 2001
Functional microbial diversity	Actinomycetes	DGGE/TGGE	Heuer et al 1997
	Ammonia-oxidising bacteria	FISH DGGE/TGGE T-RFLP	Juretschko et al 1998 Bruns et al 1999 Kowalchuk et al 1999 Horz et al 2000
	Denitrifiers	T-RFLP	Braker et al 2001
	Coryneform bacteria	DGGE/TGGE	Felske et al 1999.
	Cellulolytic bacteria	16S rDNA sequencing	Ulrich, Wirth 1999
	Methanotrophs	DGGE/TGGE	Jensen et al 1998 Henckel et al 2000
	Plant rhizosphere microbes	DGGE/TGGE	Duineveld et al 1999 Smit et al 1999 Miethling et al 2000 Yang et al 2001
Soil disease suppressiveness / conductiveness	16S rDNA sequencing DGGE	Shiomi et al 1999 Yang et al 2001	
Changes in community diversity	Crop rotations	ERIC-PCR	Achouak et al 2000
	Composting	DGGE	Kowalchuk et al 1999
	Flooding	T-RFLP	Lueders & Friedrich 2000
	Heavy metal contamination, pollution and soil bioremediation	TGGE/DGGE	Torsvik et al 1998 Brim et al 1999 MacNaughton et al 1999 Rasmussen, Sorensen 2001
	Nitrogen fertiliser and tilling	DGGE, Group specific 16S rDNA PCR and sequencing	Ceccherini MT et al 1998 Bruns et al 1999 McCaig et al 1999 Phillips et al 2000
	Spatial and temporal and oxygen effects	16S rDNA sequencing, FISH and T-RFLP	Chin et al 1999a Chin et al 1999b Fey, Conrad 2000 Ludemann et al 2000 Lukowi et al 2000
	Pesticides	TGGE	Engelen et al 1998 el Fantroussi et al 1999
	Waste water	T-RFLP	Kerkhof et al 2000
Functional gene diversity	amoA and amoB	DNA hybridisation PCR T-RFLP	Bruns et al 1998 Hastings et al 1998 Horz et al 2000
	dsz	DGGE	Duarte et al 2001
	mcrA/mrtA	T-RFLP	Lueders et al 2001
	nifH	DGGE RFLP	Rosado et al 1998 Widmer et al 1999
	[NiFe] hydrogenase	DGGE	Wawer et al 1997

References

- Achouak W, Thiery JM, Roubaud P, Heulin, T 2000. Impact of crop management on intraspecific diversity of *Pseudomonas corrugata* in bulk soil. *FEMS Microbiol Ecol.* **31(1)**:11-19.
- Borneman J, Skroch PW, O'Sullivan KM, Palus JA, Rumjanek NG, Jansen JL, Nienhuis J, Triplett EW 1996. Molecular microbial diversity of an agricultural soil in Wisconsin. *Appl Environ Microbiol.* **62(6)**:1935-43.
- Borneman J, Triplett EW 1997. Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Appl Environ Microbiol.* **63(7)**:2647-53.
- Braker G, Ayala-Del-Rio HL, Devol AH, Fesefeldt A, Tiedje JM, Bruce KD, Hughes, MR 2001. Community structure of denitrifiers, bacteria, and archaea along redox gradients in Pacific Northwest marine sediments by terminal restriction fragment length polymorphism analysis of amplified nitrite reductase (*nirS*) and 16S rRNA genes: terminal restriction fragment length polymorphism monitoring of genes amplified directly from bacterial communities in soils and sediments. *Appl Environ Microbiol: Mol Biotechnol.* **16(4.3)**:1893-901.
- Brim H, Heuer H, Krogerrecklenfort E, Mergeay M, Smalla K 1999. Characterisation of the bacterial community of a zinc-polluted soil. *Can J Microbiol.* **45(4)**:326-38.
- Bruns MA, Fries MR, Tiedje JM, Paul EA 1998. Functional gene hybridisation patterns of terrestrial ammonia-oxidising bacteria. *Microb Ecol.* **36(3)**:293-302.
- Bruns MA, Stephen JR, Kowalchuk GA, Prosser JI, Paul EA 1999. Comparative diversity of ammonia oxidiser 16S rRNA gene sequences in native, tilled, and successional soils. *Appl Environ Microbiol.* **65(7)**:2994-3000.
- Ceccherini MT, Castaldini M, Piovaneli C, Hastings RC, McCarthy AJ 1998. Effects of swine manure fertilisation on autotrophic ammonia oxidising bacteria in soil. *Applied Soil Ecology* **7(2)**:149-157.
- Chin K, Lukow T, Stubner S, Conrad R 1999(a). Structure and function of the methanogenic archaeal community in stable cellulose-degrading enrichment cultures at two different temperatures (15 and 30 degrees C). *FEMS Microbiol Ecol.* **30(4)**:313-326.
- Chin KJ, Lukow T, Conrad R 1999 (b). Effect of temperature on structure and function of the methanogenic archaeal community in an anoxic rice field soil. *Appl Environ Microbiol.* **65(6)**:2341-2349.
- Coutinho HL, De Oliveira VM, Manfio GP, Rosado AS 1999. Evaluating the microbial diversity of soil samples: methodological innovations. *An Acad Bras Cienc.* **71(3 Pt 2)**:491-503.
- Derakshani M, Lukow T, Liesack W 2000. Novel bacterial lineages at the (sub)division level as detected by signature nucleotide-targeted recovery of 16S rRNA genes from bulk soil and rice roots of flooded rice microcosms. *Appl Environ Microbiol.* **67(2)**:623-31.
- Duarte GF, Rosado AS, Seldin L, de Araujo W, van Elsas JD 2001. Analysis of bacterial community structure in sulfurous-oil-containing soils and detection of species carrying dibenzothiophene desulfurization (*dsz*) genes. *Appl Environ Microbiol.* **67(3)**:1052-62.

- Duineveld BM, Rosado AS, van Elsas JD, van Veen JA 1998. Analysis of the dynamics of bacterial communities in the rhizosphere of the chrysanthemum via denaturing gradient gel electrophoresis and substrate utilization patterns. *Appl Environ Microbiol.* **64(12)**:4950-7.
- Dunbar J, Takala S, Barns SM, Davis JA, Kuske CR 1999. Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. *Appl Environ Microbiol.* **65(4)**:1662-9.
- Dunbar J, Ticknor LO, Kuske CR 2000. Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. *Appl Environ Microbiol.* **66(7)**:2943-50.
- el Fantroussi S, Verschuere L, Verstraete W, Top EM 1999. Effect of phenylurea herbicides on soil microbial communities estimated by analysis of 16S rRNA gene fingerprints and community-level physiological profiles. *Appl Environ Microbiol.* **65(3)**:982-8.
- Engelen B, Meinken K, von Wintzingerode F, Heuer H, Malkomes HP, Backhaus H 1998. Monitoring impact of a pesticide treatment on bacterial soil communities by metabolic and genetic fingerprinting in addition to conventional testing procedures. *Appl Environ Microbiol.* **64(8)**:2814-21.
- Faegri A, Torsvik VL, Goksoyr J 1977. Bacterial and fungal activities in soil: separation of bacteria and fungi by a rapid fractionated centrifugation technique. *Soil Biology and Biochemistry* **9(2)**:105-112.
- Felske A, Akkermans AD, De Vos WM 1998. Quantification of 16S rRNAs in complex bacterial communities by multiple competitive reverse transcription-PCR in temperature gradient gel electrophoresis fingerprints. *Appl Environ Microbiol.* **64(11)**:4581-7.
- Felske A., Vancanneyt M, Kersters K., Akkermans AD 1999. Application of temperature-gradient gel electrophoresis in taxonomy of coryneform bacteria. *Int J Syst Bacteriol.* **49(1)**:113-21.
- Fey A, Conrad R 2000. Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil. *Appl Environ Microbiol.* **66(11)**:4790-7.
- Hastings RC, Saunders JR, Hall GH, Pickup RW, McCarthy AJ 1998. Application of molecular biological techniques to a seasonal study of ammonia oxidation in a eutrophic freshwater lake. *Appl Environ Microbiol.* **64(10)**:3674-82.
- Henckel T, Jackel U, Schnell S, Conrad R 2000. Molecular analyses of novel methanotrophic communities in forest soil that oxidise atmospheric methane. *Appl Environ Microbiol.* **66(5)**:1801-8.
- Heuer H, Hartung K, Wieland G, Kramer I, Smalla, K 1999. Polynucleotide probes that target a hypervariable region of 16S rRNA genes to identify bacterial isolates corresponding to bands of community fingerprints. *Appl Environ Microbiol.* **65(3)**:1045-9.
- Horz HP, Rotthauwe JH, Lukow T, Liesack W 2000. Identification of major subgroups of ammonia-oxidising bacteria in environmental samples by T-RFLP analysis of amoA PCR products. *J Microbiol Methods.* **39(3)**:197-204.
- Jensen S, Ovreas L, Daae FL, Torsvik V 1998. Diversity in methane enrichments from agricultural soil revealed by DGGE separation of PCR amplified 16S rDNA fragments. *FEMS Microbiology Ecology.* **26(1)**:17-26.

- Juretschko S, Timmermann G, Schmid M, Schleifer KH, Pommerening-Roser A, Koops HP, Wagner M 1998. Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: *Nitrosococcus mobilis* and *Nitrospira*- like bacteria as dominant populations. *Appl Environ Microbiol.* **64(8)**:3042-51.
- Kerkhof L, Santoro M, Garland J 2000. Response of soybean rhizosphere communities to human hygiene water addition as determined by community level physiological profiling (CLPP) and terminal restriction fragment length polymorphism (TRFLP) analysis. *FEMS Microbiol Lett.* **184(1)**:95-101.
- Kowalchuk GA, Naoumenko ZS, Derikx PJ, Felske A, Stephen JR., Arkhipchenko IA 1999. Molecular analysis of ammonia-oxidising bacteria of the beta subdivision of the class Proteobacteria in compost and composted materials. *Appl Environ Microbiol.* **65(2)**:396-403.
- Liu WT, Marsh TL, Cheng H, Forney LJ 1997. Characterisation of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environ Microbiol.* **63(11)**:4516-22.
- Ludemann H, Arth I, Liesack W 2000. Spatial changes in the bacterial community structure along a vertical oxygen gradient in flooded paddy soil cores. *Appl Environ Microbiol.* **66(2)**:754-62.
- Lueders T, Chin KJ, Conrad R, Friedrich M 2001. Molecular analyses of methyl-coenzyme M reductase alpha-subunit (*mcrA*) genes in rice field soil and enrichment cultures reveal the methanogenic phenotype of a novel archaeal lineage. *Environ Microbiol.* **3(3)**:194-204.
- Lueders T Friedrich M 2000. Archaeal population dynamics during sequential reduction processes in rice field soil. *Appl Environ Microbiol.* **66(7)**:2732-42.
- Lukowl T, Dunfield PF, Liesack W 2000. Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants. *FEMS Microbiol Ecol.* **32(3)**:241-247.
- MacNaughton SJ, Stephen JR, Venosa AD, Davis GA, Chang YJ, White DC 1999. Microbial population changes during bioremediation of an experimental oil spill. *Appl Environ Microbiol.* **65(8)**:3566-74.
- Marchesi JR, Sato T, Weightman, AJ, Martin TA, Fry JC, Hiom SJ, Dymock D, Wade WG 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl Environ Microbiol.* **64(2)**:795-9.
- McCaig AE, Glover LA, Prosser JI 1999. Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. *Appl Environ Microbiol.* **65(4)**:1721-30.
- Miethling R, Wieland G, Backhaus H, Tebbe CC 2000. Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L33. *Microb Ecol.* **40(1)**:43-56.
- More MI, Herrick JB, Silva MC, Ghiorse WC, Madsen EL 1994. Quantitative cell lysis of indigenous microorganisms and rapid extraction of microbial DNA from sediment. *Appl Environ Microbiol.* **60(5)**:1572-80.

- Muyzer G, de Waal EC, Uitterlinden AG 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol.* **59(3):**695-700.
- Muyzer G, Smalla K 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Van Leeuwenhoek* **73(1):**127-41.
- Muyzer G 1999. DGGE/TGGE a method for identifying genes from natural ecosystems. *Curr Opin Microbiol.* **2(3):**317-22.
- Myers RM, Maniatis T, Lerman LS 1987. Detection and localisation of single base changes by denaturing gradient gel electrophoresis. *Methods Enzymol.* 155:501-27.
- O'Donnell AG, Gorres HE 1999. 16S rDNA methods in soil microbiology. *Curr Opin Biotechnol.* **10(3):**225-9.
- Osborn AM, Moore ER, Timmis KN 2000. An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. *Environ Microbiol.* **2(1):**39-50.
- Phillips CJ, Harris D, Dollhopf SL, Gross KL, Prosser JI, Paul EA 2000. Effects of agronomic treatments on structure and function of ammonia-oxidising communities. *Appl Environ Microbiol.* **66(12):**5410-8.
- Rasmussen LD, Sorensen SJ 2001. Effects of mercury contamination on the culturable heterotrophic, functional and genetic diversity of the bacterial community in soil. *FEMS Microbiol Ecol.* **36(1):**1-9.
- Rosado AS, Duarte GF, Seldin L, Van Elsas JD 1998. Genetic diversity of nifH gene sequences in *paenibacillus azotofixans* strains and soil samples analysed by denaturing gradient gel electrophoresis of PCR-amplified gene fragments. *Appl Environ Microbiol.* **64(8):**2770-9.
- Shiomi Y, Nishiyama M, Onizuka T, Marumoto T 1999. Comparison of bacterial community structures in the rhizoplane of tomato plants grown in soils suppressive and conducive towards bacterial wilt. *Appl Environ Microbiol.* **65(9):**3996-4001.
- Smalla K, Wachtendorf U, Heuer H, Liu WenTso, Forney L, Liu WT 1998. Analysis of BIOLOG GN substrate utilisation patterns by microbial communities. *Applied and Environmental Microbiology.* **64(4):**1220-1225.
- Smit E, Leeftang P, Glandorf B, van Elsas JD, Wernars K 1999. Analysis of fungal diversity in the wheat rhizosphere by sequencing of cloned PCR-amplified genes encoding 18S rRNA and temperature gradient gel electrophoresis. *Appl Environ Microbiol.* **65(6):**2614-21.
- Theron J, Cloete TE 2000. Molecular techniques for determining microbial diversity and community structure in natural environments. *Crit Rev Microbiol.* **26(1):**37-57.
- Torsvik V, Daae FL, Sandaa RA, Ovreas L 1998. Novel techniques for analysing microbial diversity in natural and perturbed environments. *J Biotechnol.* **64(1):**53-62.
- Ulrich A, Wirth S 1999. Phylogenetic diversity and population densities of culturable cellulolytic soil bacteria across an agricultural encatchment. *Microb Ecol.* **37(4):**238-247.

- Wawer C, Jetten MS, Muyzer G 1997. Genetic diversity and expression of the [NiFe] hydrogenase large-subunit gene of *Desulfovibrio* spp. in environmental samples. *Appl Environ Microbiol.* **63(11)**:4360-9.
- Widmer F, Shaffer BT, Porteous LA, Seidler RJ 1999. Analysis of nifH gene pool complexity in soil and litter at a Douglas fir forest site in the Oregon cascade mountain range. *Appl Environ Microbiol.* **65(2)**:374-80.
- Woese CR 1987. Bacterial evolution. *Microbiol. Rev.* **51**:221-271.
- Yang C, Crowley DE, Menge JA 2001. 16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and *Phytophthora* infected avocado roots. *FEMS Microbiol Ecol.* **35(2)**:129-136.
- Zhou J, Bruns MA, Tiedje JM 1996. DNA recovery from soils of diverse composition. *Appl Environ Microbiol.* **62(2)**:316-22.

Biologically active soils help suppress nematode pests

Graham Stirling
Biological Crop Protection Pty Ltd
Moggill Qld

Most of the horticultural crops grown in subtropical and tropical regions of north-eastern Australia are attacked by plant-parasitic nematodes (Table 1). In many of these industries, nematodes are serious pests, and nematicides and soil fumigants are used routinely to achieve control.

Table 1. Major nematode pests of horticultural crops in tropical and sub-tropical regions of north-eastern Australia.

Crop	Nematodes	Pest status
tomato, potato, sweet potato, capsicum, zucchini, rockmelon	root-knot nematode (<i>Meloidogyne</i> spp.)	A serious pest in most vegetable crops grown in sandy or well-structured clay loam soils. Of major importance in Bundaberg and other coastal regions of Queensland and NSW.
pineapple	root-knot nematode (<i>Meloidogyne javanica</i> .)	A major limiting factor on about 25% of pineapple farms.
banana	burrowing nematode (<i>Radopholus similis</i>) and lesion nematodes (predominantly <i>Pratylenchus goodeyi</i>)	Serious pests in both tropical and sub-tropical regions.
apple	lesion nematodes (<i>Pratylenchus penetrans</i> and <i>P. jordanensis</i>)	One of the factors causing apple replant problems in the Granite Belt, Queensland.
citrus	citrus nematode (<i>Tylenchulus semipenetrans</i>)	Occurs on many farms in the Burnett region of Queensland. The importance of the nematode is underestimated by industry.
grape	root-knot nematodes (<i>Meloidogyne</i> spp.)	Widespread on wine grapes in the Granite Belt. Commonly found on table grapes in Queensland and the Northern Territory.
strawberry	root-knot nematodes (<i>Meloidogyne</i> spp.) and lesion nematode (<i>Pratylenchus vulnus</i>)	An occasional problem because of the widespread use of soil fumigation.
ginger	root-knot nematodes (<i>Meloidogyne</i> spp.)	Important on many farms, particularly in late-harvested crops and market ginger.
turf	many species	Common on turf and an important component of the root disease complex.
ornamentals	root-knot nematode (<i>Meloidogyne</i> spp.)	A serious problem on some crops (e.g. riceflower).

Health and environmental effects of nematicides

Several soil fumigants have been removed from the market in the last 25 years (eg DD, DBCP and EDB) and many of the remaining chemicals used against nematodes are detrimental to human health and the environment. All nematicides and soil fumigants are relatively mobile in soil and therefore have the potential to contaminate groundwater (Thomason 1987). Atmospheric pollution is an ongoing problem with the fumigants because they are extremely volatile materials. Methyl bromide is being phased from use because of its ozone-depleting properties while its potential replacements (metham sodium, chloropicrin and 1,3 D) will readily drift off-site if fumigated soil is not sealed adequately. The non-volatile organophosphate and carbamate nematicides have the highest mammalian toxicity of all the chemicals currently used in horticulture. Another problem from a grower's perspective is that the usefulness of nematicides is threatened by enhanced microbial degradation. Microorganisms capable of degrading fenamiphos (Nemacur®) are already widely distributed in Australia (Stirling et al 1992), while enhanced degradation of metham sodium has recently been reported from Western Australia (Warton et al 2001). The above problems will almost certainly result in a continuing decline in the number of chemicals available for use against nematodes.

IPM as it applies to nematodes

As the number of registered nematicides and soil fumigants declines, growers will have little choice but to start using integrated pest management (IPM) to achieve nematode control. The appeal of IPM is that it provides acceptable procedures for managing pests in sustainable agricultural systems. In the case of nematodes, population densities are measured and control tactics are implemented only when infestation levels are above the economic threshold. When measures are required to reduce nematode populations, the economic and environmental impacts of possible control options are considered and the safest and most effective tactics are chosen. These tactics include crop rotation, fallowing, resistant varieties, various cultural and biological controls, and the strategic use of nematicides.

In north-eastern Australia, the IPM systems currently used against nematodes are in their infancy. The banana and pineapple industries have taken their first tentative steps towards adopting IPM by establishing monitoring programs that determine whether nematode population densities in particular fields have reached damaging levels. Control is still achieved mainly with nematicides, but monitoring is enabling growers to use them strategically rather than routinely. In the vegetable industry, an increasing number of growers collect samples from fields before planting and have them analysed for nematodes. Non-chemical management strategies are also being used more frequently. Some vegetable growers reduce nematode and soil-borne disease problems by rotating with sugarcane or nematode-resistant green manure crops such as forage sorghum. Others prepare beds for planting and then use appropriate fallowing, solarisation and irrigation techniques to reduce nematode populations to acceptable levels by the time the crop is planted.

Biological control of nematodes

Biological control is a vital component of IPM programs for some insect pests, but there are no examples anywhere in the world of the successful use of introduced natural enemies to control plant-parasitic nematodes. Many bacteria, fungi and other soil organisms parasitise or prey on nematodes, but many of these organisms cannot be grown in culture, are difficult to mass-produce or cannot be formulated in a commercially acceptable manner. However, the main limiting factor with laboratory-cultured natural enemies is that they often fail to establish or are relatively ineffective when they are introduced into soil (Stirling 1991). The reason for this is that the introduced organism must compete with the multitude of other organisms already established in the soil environment. Progress has been made with some fungi in recent years (Stirling et al 1998a, 1998b; Stirling, Smith 1998), but biocontrol systems based on one or a few mass-produced organisms have never been consistently successful. Some Australian companies have experimented with or marketed products based on egg-parasitic fungi (eg *Paecilomyces lilacinus* and *Verticillium chlamydosporium*) or nematode-trapping fungi (eg *Arthrobotrys* species), but these products have never been registered in Australia and there is no experimental evidence that they are effective. Thus the most practical way to use biological control is to conserve and enhance the activity of the natural enemies of nematodes that occur naturally in all horticultural soils.

Nematode-suppressive soils

Fungi that produce specialised trapping structures to capture nematodes are present in most Australian soils (McCulloch 1977), while fungal parasites of nematode eggs and the bacterial parasite *Pasteuria penetrans* are also commonly found (Stirling, White 1982, Stirling, West 1991). However, survey data indicate that these indigenous natural enemies of nematodes tend to have their greatest impact as biological control agents in perennial rather than annual cropping systems (Stirling, White 1982, Mertens, Stirling 1993). The reasons for this are not known, but one possibility is that perennial crops are subject to minimal disturbance (Stirling 1999). Specialised parasites of nematodes occupy the same ecological niche as their nematode hosts and cultivation tends to destroy the intimate relationship that develops between host and parasite. Thus circumstantial evidence suggests that the activity of indigenous natural enemies of nematodes will be enhanced if cultivation is minimised.

Another way of manipulating the environment to favour the natural enemies of nematodes is by adding crop residues, animal manures, composts and other organic materials to soil. Organic inputs induce a succession of microbiological changes in soil and as decomposition proceeds, populations of various parasites and predators of nematodes increase and biological control activity is enhanced. Mechanisms other than parasitism and predation are probably also involved (Stirling 1991). For example, some of the chemicals released from organic materials during the decomposition process (eg ammonia, nitrites and various organic acids) are toxic to nematodes. Plants also tend to become more resistant to attack by nematodes following the addition of organic matter to soil, possibly because microorganisms in the rhizosphere activate natural nematode-resistance mechanisms in the plant.

The detrimental effects of nitrogenous amendments on plant-parasitic nematodes have been known for many years (Rodriguez-Kabana 1986). Proteinaceous materials, poultry manure, residues from leguminous crops and other nitrogenous waste materials produce ammonia when they decompose in soil, and ammonia is nematicidal at concentrations in excess of 300 mg/kg soil. Since the amount of ammonia produced varies with the level of nitrogen in the amendment, the effectiveness of nitrogenous amendments against nematodes will increase as the nitrogen content increases. The usefulness of high-nitrogen containing materials such as poultry manure has been demonstrated in Australia (Stirling, Nikulin 1998), while Lazarovits et al (2001) have shown that soybean meal and meat and bone meal are useful in field trials in Canada. However, to be effective, such materials must be added to soil at relatively high application rates (at least 2% of soil mass or more than 40 tonnes of dry matter/ha). Since high concentrations of nitrogen in soil can cause phytotoxicity, planting may need to be delayed when nitrogenous amendments are applied. Plant-back periods can be reduced by adding a source of carbon, as this balances the carbon nitrogen ratio and allows soil microorganisms to more effectively convert the excess nitrogen into proteins and other less toxic compounds.

Although there has been less experimental work with high-carbon amendments, results of recent studies suggest that they can also be used to suppress nematodes. Pine bark was effective against root-knot nematodes in glasshouse experiments in the USA (Kokalis-Burelle, Rodriguez-Kabana 1994), while sawdust and molasses reduced galling caused by root-knot nematodes in a field trial on tomatoes at Bundaberg (Vawdrey, Stirling 1997). The mechanism of action of high-carbon materials is not known, but fungi are mainly responsible for their decomposition and it is thought that some of these fungi may also be antagonistic to plant-parasitic nematodes. Pine bark and some wood products are also rich in phenolic compounds that may be directly toxic to nematodes. The main problem in using such amendments is that the nitrogen status of soil must be managed carefully so that nitrogen drawdown does not become a problem.

Despite the fact that scientists continue to demonstrate the benefits of using organic matter for nematode control, the practice of adding organic amendments to soil is still not widely accepted in horticulture. One of the main reasons for this is that the effectiveness of amendments depends on their chemical composition and application rate, and there are currently no guidelines on how locally-available organic materials can be prepared and used for nematode control. It is clear from studies of fungal pathogens such as *Pythium* (see review by Hoitink, Boehm 1999) that the concentration and availability of nutrients in soil organic matter regulates the activity of disease-suppressive microbial communities. Sustained biological control of certain soil-borne fungal pathogens is achievable, but a minimum threshold level of microbial activity must be maintained. The situation with nematodes is likely to be similar. The critical question is what type and amount of organic matter must be added to soil to maintain a biological community capable of providing a consistent and useful level of nematode suppression.

Current research

Currently, I am trying to determine the level of biological activity that is required to reduce populations of root-knot nematodes to densities that are not economically important. Plots that have received different organic inputs have been established in two different soils in Bundaberg and changes in various biological parameters are being measured over time. Initial results from one trial (Table 2) clearly show that total numbers of free-living nematodes and microbial activity increase as increasing amounts of organic carbon are added to soil. Populations of root-knot nematodes were reduced and tomato plants had fewer galls in soils receiving carbon inputs of more than 23 t/ha. These inputs were achieved by adding sugarcane trash and growing a green manure crop of forage sorghum and lab lab.

Table 2. Biological status of organically amended soils at Bundaberg, and the effects of various organic inputs on root-knot nematodes.

C applied (t/ha)	No. of free-living nematodes/200 ml soil	Microbial activity ($\mu\text{g FDA/g/min}$)	No. of root-knot nematodes/200 ml soil	Root gall rating on tomato
0	1320 d	0.145 d	977 a	7.83 a
10	3160 c	0.197 d	417 b	7.00 ab
17	3890 bc	0.202 cd	331 bc	7.00 ab
23	4790 bc	0.262 bc	407 b	6.50 b
33	5750 ab	0.323 ab	282 c	5.33 bc
43	5250 a	0.370 a	245 c	5.00 c

Within each column, numbers followed by the same letter are not significantly different ($P=0.05$)

Conclusions

A trend towards monoculture, excessive use of the rotary hoe and other tillage implements, lack of replenishment of organic matter, widespread use of plastic ‘mulches’, and over-dependence on herbicides and chemical fertilisers have reduced the fertility of many soils used for horticulture. These soils are susceptible to erosion, poorly drained, inadequately aerated, and have limited biological activity and therefore provide an environment that is ideal for the development of chronic nematode and root disease problems. There is experimental evidence to show that increasing the amounts of labile organic carbon in soil will increase microbial activity, reduce nematode problems and have many other positive effects on soil health. However, the changes in soil biology that are required to improve degraded soils cannot be achieved quickly or with limited organic inputs. Organic amendments can help in the fight against nematodes but they may not provide the level of nematode control that is achievable with nematicides. Thus they should always be used as a component of an IPM program for nematodes rather than as a stand-alone control procedure.

References

- Hoitink HAJ, Boehm MJ 1999. Biocontrol within the context of soil microbial communities: a substrate dependent phenomenon. *Annual Review of Phytopathology* **37**: 314-322.
- Kokalis-Burelle N, Rodriguez-Kabana R 1994. Changes in populations of soil microorganisms, nematodes and enzyme activities associated with the application of powdered pine bark. *Plant and Soil* **162**: 169-175.
- Lazarovits G, Tenuta M, Conn KL 2001. Organic amendments as a disease control strategy for soil-borne diseases of high value agricultural crops. *Second Australasian Soilborne Diseases Symposium, Keynote Papers* 37-44.
- McCulloch JS 1977. A survey of nematophagous fungi in Queensland. *Queensland Journal of Agricultural and Animal Sciences* **34**: 25-33.
- Mertens MC, Stirling GR 1993. Parasitism of *Meloidogyne* spp. on grape and kiwifruit by the fungal egg parasites *Paecilomyces lilacinus* and *Verticillium chlamydosporium*. *Nematologica* **39**: 400-10.
- Rodriguez-Kabana R 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. *Journal of Nematology* **18**: 129-135.
- Stirling AM, Stirling GR, MacRae IC 1992. Microbial degradation of fenamiphos after repeated application to a tomato-growing soil. *Nematologica* **38**: 245-254.
- Stirling GR 1991. *Biological Control of Nematodes*. CAB International, Wallingford.
- Stirling GR 1999. Increasing the adoption of sustainable, integrated management strategies for soilborne diseases of high-value annual crops. *Australasian Plant Pathology* **28**: 72-79.
- Stirling GR, Nikulin AN 1998. Crop rotation, organic amendments and nematicides for control of root-knot nematodes (*Meloidogyne javanica*) on ginger. *Australasian Plant Pathology* **27**: 234-243.
- Stirling GR, Smith LJ 1998. Field tests of formulated products containing either *Verticillium chlamydosporium* or *Arthrobotrys dactyloides*. *Biological Control* **11**:231-239.
- Stirling GR, West LM 1991. Fungal parasites of root-knot nematode eggs from tropical and sub-tropical regions of Australia. *Australasian Plant Pathology* **20**:149-54.
- Stirling GR, White AM 1982. Distribution of a parasite of root-knot nematode in South Australian vineyards. *Plant Disease* **66**: 52-53.
- Stirling GR, Licastro KA, West LM, Smith LJ 1998a. Development of commercially acceptable formulations of the nematophagous fungus *Verticillium chlamydosporium*. *Biological Control* **11**:217-223.
- Stirling GR, Smith LJ, Licastro KA, Eden LM 1998b. Control of root-knot nematode with formulations of the nematode-trapping fungus *Arthrobotrys dactyloides*. *Biological Control* **11**:224-230.
- Thomason IJ 1987. Challenges facing nematology: environmental risks with nematicides and the need for new approaches. In *Vistas on Nematology*. Veech JA, Dickson DW (eds). pp. 469-476. Society of Nematologists, Hyattsville, Maryland.

- Vawdrey LL, Stirling GR 1997. Control of root-knot nematode (*Meloidogyne javanica*) on tomato with molasses and other organic amendments. *Australasian Plant Pathology* **26**:179-187.
- Warton B, Matthiessen JN, Roper MM 2001. Enhanced biodegradation of metham sodium soil fumigant-occurrence, influences and implications. *Proceedings, Second Australasian Soilborne Diseases Symposium*, Lorne, Vic., pp. 83-84.