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The importance of the antagonistic potential in the management of populations of plant-parasitic nematodes in banana (*Musa* AAA) as influenced by agronomic factors

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Dedication:

This work is dedicated to the support given to me by family and friends. Especially to my wife Susan, daughters Katie and Emily for their patience while I completed this work. Also, to the friends I have made along the way, who have helped to make the world a little smaller.

The importance of the antagonistic potential in the management of populations of plant-parasitic nematodes in banana (*Musa* AAA) as influenced by agronomic factors

Plant-parasitic nematodes are a major obstacle to sustainable banana production around the world. The use of organic amendments was investigated as one method to stimulate organisms that are antagonistic to plant-parasitic nematodes. Nine different amendments; mill mud, mill ash (by-products from processing sugarcane), biosolids, municipal waste (MW) compost, banana residue, grass hay, legume hay, molasses and calcium silicate (CaSi) were applied in a glasshouse experiment. Significant suppression of *Radopholus similis* occurred in soils amended with legume hay, grass hay, banana residue and mill mud relative to untreated soil, which increased the nematode community structure index, indicating greater potential for predation.

A field experiment was established to determine the changes in soil properties following applications of compost, grass hay, mill mud and mill ash. At the termination of the experiment there was significant increase in bunch size in the mill ash treatment relative to the untreated plants. Furthermore, in the soil treated with additional organic matter there was an increase in labile C, the number of omnivorous nematodes and lower proportion of plant-parasitic nematodes relative to the untreated soil. The suppression of plant-parasitic nematodes resulting from the addition of organic matter appeared to be the result of a combination of two factors; nematoxic compounds produced in the early degradation of the organic matter, followed by an increase in nematode antagonists favoured by an increase in soil fungal activity.

A study was implemented on 10 banana plantations in north Queensland to determine differences in soil management, soil physical, chemical and nematode community properties. A principal component analysis could explain 61% of the variation between farms and identified the proportion of plant-parasitic nematodes, labile C, nitrate-N, and the number of fungal feeding nematodes as the most important soil factors. When used in combination the ratio of labile C and nitrate-N in the soil and the diversity of nematodes were able to explain 88.7% of the variation in the proportion of plant-parasitic nematodes in the soil. A similar survey of 21 banana plantations in Costa Rica using 34 soil variables was able to explain 71% of variation between plantations from five principle components. A bioassay of the soil collected, which was inoculated with *R. similis*, resulted in different populations of the nematode recovered from the different soils. The differences could be explained by soil pH, structure index and Zn using a multiple linear regression model, which explained 79.2% of the variation. Furthermore, the correlation of soil pH with nematode diversity suggested that pH was the factor limiting the biological suppression of *R. similis* in the Costa Rican banana plantations.

The development of soils capable of suppressing plant-parasitic nematodes requires and understanding of soil constraints in the farming system. In Australia, soil C appeared to constrain antagonists, whereas, in Costa Rica low soil pH constrained the diversity of the soil nematode community. Management options to increase soil C in Australia and to increase soil pH in Costa Rica are necessary to develop a more favourable soil environment for the suppression of plant-parasitic nematodes by antagonistic soil organisms.

Die Bedeutung des antagonistischen Potentials für die Kontrolle von Pflanzen-parasitären Nematoden in Bananen (Musa AAA) und dessen Beeinflussung durch pflanzenbauliche Maßnahmen.

Der Pflanzen-parasitäre Nematode (PPN) *Radopholus similis* gehört weltweit zu den bedeutendsten Schädlingen nachhaltiger Bananenproduktion. Traditionell, wurden diese Schädlinge mit Nematiziden bekämpft. Deren Gebrauch ist jedoch mit einer Gefährdung landwirtschaftlichen Personals und einer Reduzierung der Biodiversität im Boden und dessen antagonistischem Potential verbunden. Deshalb ist ein besseres Verständnis der Faktoren, die das antagonistische Potential eines Bodens beeinflussen, unabdingbar. In der vorliegenden Arbeit wurden verschiedene organische Bodenzusätze auf die Stimulierung des antagonistischen Potentials gegen *R. similis* in Musa AAA untersucht.

Gewächshausexperimente zeigten, dass Leguminosen Heu, Zuckermühlen-abfälle und Bananenrückstände als Bodenzusätze die Nematodendiversität erhöhte, die Anzahl an *R. similis* jedoch reduzierten. In Feldexperimenten konnte gezeigt werden, dass die Zugabe von Kompost, Heu, Nebenprodukten aus Zuckermühlen und Mühlenschlamm zu einer Erhöhung des instabilen Kohlenstoffs und omnivorer Nematoden, jedoch zu einer Verringerung von PPN führte.

Eine Hauptkomponentenanalyse mit Zehn unterschiedlich bewirtschafteten Bananenplantagen in Nord-Queensland, Australien, identifizierte instabilen Kohlenstoff, Nitrat und die Anzahl fungivorer Nematoden als Hauptbodenfaktoren in Bezug auf ihr antagonistisches Potential gegenüber PPN und konnte 61% der Variation zwischen den Plantagen erklären. In Kombination erklärte das Verhältnis von instabilem Kohlenstoff zu Nitrat im Boden und die Nematodendiversität 88.7% der Variation an PPN zwischen den Plantagen.

Eine ähnliche Studie in Costa Rica bei der 34 Bodenvariablen erhoben wurden konnte 71% der Variation zwischen den verschiedenen Böden in Bezug auf ihr antagonistisches Potential gegenüber PPN erklären. In bioassay´s mit diesen Böden zeigte sich, dass deren antagonistisches Potential vom pH-Wert, dem „Struktur-Index“ und Zink-Gehalt beeinflusst wurde und das sich mit Hilfe einer multiplen linearen Regression 79.2% der Variationen des antagonistischen Potentials zwischen den Böden erklären ließ. Darüberhinaus korrelierte der Boden pH mit der Nematodendiversität, was den Schluss zuließ, dass der Boden pH der limitierende Faktor biologisch suppressiver Böden in Costa Rica´s Bananenproduktion ist.

Zusammenfassend konnte gezeigt werden, dass das antagonistische Potential gegenüber PPN von verschiedenen Faktoren beeinflusst wird. Während in Australien instabiles Kohlenstoff der limitierende Faktor war, ist in Costa Rica der Boden pH entscheidend für Nematodendiversität und einhergehende Suppresion von PPN. Eine Steigerung des instabilen Kohlenstoffs in Australiens Böden und eine Erhöhung des Boden pH in Costa Rica scheinen deshalb notwendig um suppresive Böden mit hohem antagonisten Potential gegen PPN wie *R. similis* zu schaffen.

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1 Literature review

1.1 General introduction

1.1.1 The banana plant

The banana is a large herbaceous plant consisting of a branched underground rhizome and an erect pseudostem composed of tightly packed leaf bases (Jones 2000). Bananas are classified within the family Musaceae, genus *Musa*, in which the section Eumusa, containing the majority of cultivated bananas, is the biggest and geographically most widespread (Stover and Simmonds 1987). The area of greatest diversity, and so presumed to be the centre of origin of *Musa* species, is South East Asia (Price 1995b). Edible bananas originate from *Musa acuminata* and *M. balbisaina* and these species contain the most widely cultivated varieties (Stover and Simmonds 1987).

Growth of the banana plant arises from the apical meristem, located in the centre of the pseudostem at ground level, which gives rise to leaf primordial that grows upwards, differentiating into a leaf base, petiole and lamina (Jones 2000). At a certain stage in plant development the apical growing point stops producing leaves and develops an inflorescence. Following formation of the flower, the peduncle begins to develop and carries the flower upwards to emerge from the top of the psuedostem. The inflorescence consists of females nodes with flowers, covered by bracts and terminating with a male bud (Karamura and Karamura 1995). The bracts dry and fall, exposing the fruit which encircles the peduncle in clusters with two rows, which form the bunch. The size and weight of the bunch depend on cultivar, plant vigour and health (Jones 2000). After harvest of the bunch, the pseudostem dies back.

The subterranean growth of bananas is typical of many monocots with sympodial, horizontal extension of the rhizome, and the shoot turning up to form a new aerial stem (Stover and Simmonds 1987). The stems growing out of the rhizome are known as a suckers or daughter plants and grow to regenerate the plant (Jones 2000). Several suckers can arise from each rhizome and if allowed to grow unmanaged, will form a clump. However, in cultivated bananas, a single sucker or daughter plant is usually selected for the following crop.

The root axis arises from the rhizome to form lateral or primary roots, which may branch into secondary and tertiary roots (Price 1995a). The primary roots are typically 5-8 mm in diameter, extending 1-2 m horizontally and 0.6 m vertically from the rhizome (Jones 2000).

The yield of bananas is determined genetically by the division of biomass between fruit and other plant parts, but is reliant on the length of the vegetative phase, length of time for bunch development and the number and size of fruit (Stover and Simmonds 1987). It is believed that cultivation of bananas first occurred in South East Asia and Melanesia, but spread to Africa around 2000 years ago (Jones 2000; Price 1995b). Further spread of banana cultivars to the Americas occurred with Spanish and Portuguese colonisation (Price 1995b). The commercial cultivation of bananas as a monocrop occurred just over 100 years ago with the improvements in shipping transport leading to its development as an important export fruit (Jones 2000).

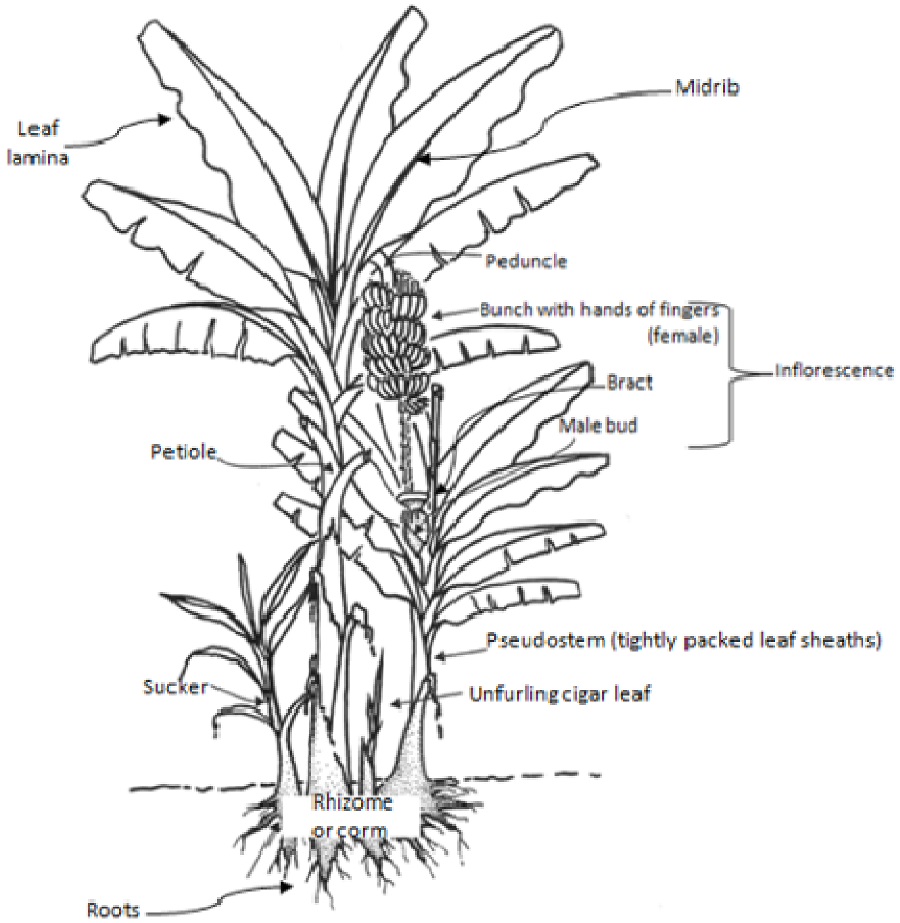

Figure 1-1: Diagrammatic representation of a fruiting banana plant with suckers (adapted from Kernot *et al.* (1998))

1.1.2 Banana production

Bananas are one of the world’s most important food crops (Price 1995b). Bananas are cultivated in over 100 countries and are the staple food and source of income for many developing nations in tropical areas as well as being an important international trade commodity (Frison and Sharrock 1999). Over 89 million tonnes of banana are produced every year, with production centred in tropical regions (http://faostat.fao.org/site/339/default.aspx). Bananas are used as a life sustaining crop in Africa, Asia and Latin America, where many of the bananas grown are local cultivar selections. The countries with the largest area of banana production are India, China, The Philippines, Brazil and Ecuador (Table 1-1). However, many of the major banana producing nations, such as India and China, do not export bananas.

Table 1-1: Banana production statistics of the 10 highest producing nations and Australia in 2007

Country	Production (tonnes)	Production area (ha)	Yield (tonnes ha^{-1})
India	23 204 800	646 900	35.9
China	8 038 385	317 776	25.3
Philippines	7 484 073	436 762	17.1
Brazil	7 098 350	515 346	13.8
Ecuador	6 002 302	197 410	30.4
Indonesia	5 454 226	337 831	16.1
Tanzania	3 500 000	480 000	7.3
Costa Rica	2 350 000	43 817	53.6
Thailand	2 000 000	153 000	13.1
Mexico	1 964 545	75 651	26.0
Australia	213 193	13 733	15.5

Source: http://faostat.fao.org/site/339/default.aspx

In 2007, 17.7 Mt of bananas were exported globally, which is 19.8% of the bananas produced with an export value of US$7249 million (http://faostat.fao.org/site/406/default.aspx). Ecuador, Costa Rica and the Philippines exported the greatest quantities of bananas, in 2007, 5.2, 2.3 and 1.8 million tonnes respectively (Table 1-2). The banana trade from Ecuador is worth more than US$1.2 billion (Table 1-2). Central and northern South America have many of the most important banana exporting nations, including Ecuador, Costa Rica, Guatemala, Honduras and Panama, which are able to export bananas under US$300 / tonne. Costa Rica has one of the highest yielding production areas, producing over 50 tonnes of fruit per hectare for export (Table 1-1).

Table 1-2: Banana export statistics of the 10 highest producing nations and Australia in 2007

Country	Quantity (tonnes)	Value (1000 $)	Unit value ($/tonne)
Ecuador	5 174 565	1 282 036	248
Costa Rica	2 272 332	675 406	297
Philippines	1 793 930	856 447	477
Colombia	1 639 833	531 765	324
Guatemala	1 408 804	300 484	213
Honduras	566 539	152 891	270
Panama	437 022	111 484	255
Côte d'Ivoire	290 757	126 465	435
Cameroon	224 546	185 927	828
Dominican Republic	208 653	71 277	342
Australia	3	12 000	4 000

Source: http://faostat.fao.org/site/342/default.aspx

In 2007, Australia was the 37th largest producing nation of bananas in terms of production, but the 41st largest producer in terms of area and the 50th nation in terms of yield (Table 1-1). The Australian banana industry produced 274,140 tonnes, valued at AU$295 million, from 13,733 ha, being primarily for domestic consumption (http://www.abgc.org.au/?industry/banana-industry). Only a small quantity of banana, 3 tonnes, was exported in 2007 (Table 1-2). Most of the Australian banana production occurs in Queensland (93%) due to favourable climate. However, there are smaller production zones in northern New South Wales (5%), Northern Territory (<1%) and Western Australia (2%) (http://www.abgc.org.au/?industry/banana-industry).

1.2 Diseases of bananas

Banana plants are susceptible to a range of pathogens that cause widespread loss of production (Gowen 1995; Jones 2000; Stover and Simmonds 1987). However, not all banana pathogens have global distribution. The major disease causing pathogens in commercial banana production are (Stover and Simmonds 1987);

- Black sigatoka (*Mycospharella fijiensis* Morelet),
- Panama disease (*Fusarium oxysporum* f.sp. *cubense*),
- Post harvest rots (various fungi),
- Moko (*Ralstonia solanacearum* Yabuuchi),
- Bacterial corm rot (*Erwinia chrysanthemi*),

- Plant-parasitic nematodes (*Radopholus similis, Pratylenchus coffeae, P. goodeyi, Helicotylechus multicinctus* and *Meloidogyne* spp).
- Banana Bunchy Top Virus.

Many of the diseases in commercial export banana production are managed by a combination of cultural and chemical methods. For example, sigatoka leaf spots (*Mycospharella* spp.) are managed by a combination of fungicide sprays and removal of infected leaf material (Jones 2000). However, diseases such as *Fusarium* wilt (*Fusarium oxysporum* f. sp. *cubense*) are more difficult to manage in commercial banana production and may lead to the abandonment of large scale commercial production in the region (Jones 2000; Stover and Simmonds 1987). For subsistence farmers growing bananas, the management options for diseases of bananas are more restricted.

Australia is free of many of the most devastating diseases of bananas due to strict quarantine restrictions and regulations governing the industry. In the Queensland production area of Australia, yellow sigatoka (*Mycospharella musicola* Leach) is the major limitation to banana production, although incursions of black sigatoka have occurred (Peterson *et al.* 2005). *Fusarium* wilt is a constraint to banana production in sub-tropical banana production areas of northern NSW and southern Queensland (Race 1 and 4) and the Northern Territory where the tropical race 4 form of the disease is present (Hennessy *et al.* 2005; Pegg *et al.* 1996). Occurrence of the bunchy top virus is also present in isolated areas in northern NSW and south-east Queensland (Hennessy *et al.* 2005). Plant-parasitic nematodes reduce production in north Queensland (*R. similis*) and the sub-tropical production areas (*P. goodeyi*) (Stanton *et al.* 2001).

1.3 Nematodes as parasites of banana

It is estimated that plant-parasitic nematodes cause yield loss of 19.7% annually to banana production around the world (Sasser and Freckman 1987). However, in particular situations, such as have occurred in Costa Rica, losses due to nematodes were as high as 80% (Araya and Moens 2005). The major nematode parasites for bananas globally are *R. similis*, *P. coffeae*, *P. goodeyi*, *H. multicinctus* and *Meloidogyne* spp. (Gowen *et al.* 2005; Jones 2000). The most devastating plant-parasitic nematode of commercial banana production in tropical countries is *R. similis*

(Gowen *et al.* 2005). However, plant-parasitic nematodes of bananas usually occur in mixed populations (Araya 2005).

1.3.1 *Radopholus similis*

R. similis is a vermiform, migratory endoparastic nematode (Figure 1-2) that causes root necrosis due to feeding on cells within the cortical tissue of banana roots (Gowen *et al.* 2005). *R. similis* is able to complete its life cycle, from egg to egg in about 20 – 25 days at 24-32 ^{o}C (Gowen *et al.* 2005). *R. similis* undergoes four moult stages, the first being within the egg, before becoming an adult (Hunt *et al.* 2005). Juveniles and females are able to penetrate the root and feed. The male has a degenerated stylet, but can still be isolated from banana root tissue (Hunt *et al.* 2005). The females reproduce either amphitically, parthenogenically or as hermaphrodites depositing an egg in the root tissue every 3-5 days (Kaplan and Opperman 2000).

Figure 1-2: *Radopholus similis* female

The feeding of *R. similis* on the cytoplasm of cortical cells of banana roots causes the cells to become necrotic (Figure 1-3). The necrotic areas within the roots may be associated with fungi, such as weakly pathogenic *Fusarium* spp. (Blake 1966). *R. similis* rarely enters the vascular tissue of the banana root (Blake 1969; Gowen *et al.* 2005); however, there have been reports of *R. similis* present in the steele with very high populations of the nematode (Araya and De Waele 2004). The damage of the cortical tissue within the roots of the banana plant develops into purplish – red lesions (Figure 1-3). The banana plant itself may appear stunted, have smaller bunches and take longer to cycle, due to a reduced capacity to absorb water and nutrients through the root system. Severe infestations of *R. similis* lead to plant toppling, due to poor anchorage or snapping of the root system (Figure 1-4).

Figure 1-3: Necrotic lesion development in the root cortex of bananas caused by *R. similis*.

Figure 1-4: Toppling of bananas due to a weakened root system caused by the feeding of *R. similis*.

R. similis can migrate from the roots into the rhizome, where they cause similar lesion development (Gowen *et al.* 2005). Infected rhizome material has been the primary means of distribution of *R. similis* around the world (Gowen *et al.* 2005). *R. similis* was first reported on bananas from Fiji by Cobb in 1893 (Blake 1969). It is currently recorded in most banana producing areas and has a wide host range of over 350 plant species (Holdeman 1986). Some variation is reported in the pathogenicity of *R. similis*, with the possible development of regional biotypes of the nematode (Elbadri *et al.* 2002).

1.3.2 Other plant-parasitic nematodes

Other plant-parasitic nematodes reported to cause loss of production of bananas include lesion nematodes, *Pratylenchus coffeae* and *P. goodeyi*. *P. coffeae* is widespread through many banana producing nations and is the most damaging nematode in Vietnam (Van den Berg *et al.* 2005). *P. goodeyi* causes losses in production in the African highlands, the Canary Islands and has been found in sub-tropical eastern banana production areas of Australia (Gowen *et al.* 2005; Pattison *et al.* 2002; Price and Bridge 1995). *H. multicinctus* is an important root pathogen in sub-optimal banana growing areas (Araya and Moens 2005; Blake 1966; Gowen *et al.* 2005) or in the absence of *R. similis* (McSorley and Parrado 1983). Root-knot nematodes are widespread and also reported cause to economic losses in banana plantations (Gowen *et al.* 2005). However, root-knot nematodes were not found to be economically important in Australia (Stanton and Cobon 2000).

1.4 Management strategies for plant parasitic nematodes

Management of plant-parasitic nematodes in commercial banana production since the 1960s has relied on the use of chemical control (Gowen *et al.* 2005; Stirling and Pattison 2008). However, recently there has been a move toward developing integrated strategies to reduce losses caused by plant-parasitic nematodes. The strategies that have been used in the Australian banana industry have been reviewed by Stirling and Pattison (2008). Similar options for managing nematodes on bananas have been reviewed previously (Gowen *et al.* 2005).

1.4.1 Clean planting material

The importance of clean planting material has been recognised since the 1960s with the development of paring and hot water treatment of vegetative planting material and then nematicide treatment of planting material (Blake 1961; Blake 1969; Broadley 1979a; Colbran 1964b). The development of *in vitro* techniques improved the reliability of planting material. Nevertheless, vegetative planting material derived from the corms or suckers of existing banana plants remains an important part of establishing banana plantations, particularly where *in vitro* technology is not available, replanting within existing plantations and where *in vitro* plants are too expensive.

1.4.2 Nematode monitoring

Nematode monitoring is an important part of any integrated nematode management program. Monitoring systems have been developed using counts of nematodes extracted from the roots, soil or the level of damage caused by nematodes in the root system (Araya and Moens 2005; Broadley 1979c; Speijer and De Waele 1997; Stanton *et al.* 2001). An economic threshold for damage levels caused by plant parasitic nematodes allowed growers to assess the need for chemical or other control applications (Moens *et al.* 2001; Pattison *et al.* 2002).

1.4.3 Chemical control

The use of chemical nematicides has been used to prevent yield loss. The fumigant nematicide DBCP (dibromochloropropane) was used during the 1960s in an effort to eradicate nematodes from banana plantations (Broadley 1979b; Gowen *et al.* 2005). The development of non-volatile nematicides in the 1970s and their application to nematode infested banana fields became common commercial practice and remains a common practice in many commercial banana plantations (Broadley 1979b; Burnett *et al.* 1974; Gowen *et al.* 2005). The application of 2-3 cycles of non-fumigant nematicides was reported to increase banana yields by 21-44% in Australia (Broadley 1979b), 48% in Côte d'Ivoire (Queneherve *et al.* 1991), up to 50% in Cameroon (Fogain *et al.* 1996) and 41% in Costa Rica (Araya and Cheves 1997).

However, the efficacy of the non-volatile chemical nematicides was observed to diminish with repeated use of chemicals as early as 1984, due to the development of enhanced biodegradation, the rapid microbial decomposition of the active ingredients in nematicides (Anderson 1989). Enhanced biodegradation of nematicides used in banana production was similarly noted in Australia and Costa Rica (Cabrera *et al.* 2010; Moens *et al.* 2004; Pattison *et al.* 2000). Simultaneously, concerns about the environmental impact of nematicide use on banana plantations and surrounding ecosystems were being expressed, due to the appearance of nematicides in aquatic environments (Castillo *et al.* 2000). With no new chemical nematicides available, strategies for improving the efficacy of the nematicides were developed. This included increasing the number of applications per year (Araya and Cheves 1997), application through irrigation systems (Schipke and Ramsey 1994) and the application of systemic liquid nematicides into the pseudostem of bananas (Araya 1999; Robalino *et al.* 1983). However, recent reports suggested that enhanced degradation due to bacterial decomposition of the active ingredient remains a continual constraint to use of non-fumigant nematicides (Cabrera *et al.* 2010).

There has been an attempt to develop “biological nematicides” that are safe to the environment and workers. One product ABG-9008 (DiTera®) was found to suppress the activity of *R. similis* on bananas in a greenhouse study (Marin *et al.* 2000). Biochemical extracts from soil microorganism have also been tested, such as Abamectin (Jansson and Rabatin 1997; Jansson and Rabatin 1998). Nevertheless, there are very few new options for chemical control of nematodes in bananas in the future, so other options for nematode management require investigation.

1.4.4 Crop destruction, fallows and crop rotation

Fallow periods between cropping cycles have been used to reduce the number of plant-parasitic nematodes carried over to the following crop. One of the most important aspects is the prompt destruction of the previous crop, which allowed a reduction in the number of nematodes surviving in the soil (Chabrier and Queneherve 2003). Blake (1969) suggested that *R. similis* was able to survive in decomposing root and corm material for up to 6 months. Furthermore, the growth of volunteer bananas could be seen as one source of carry over of *R. similis* (Chabrier and Queneherve 2003). Therefore, the complete decomposition of root and corm material is necessary prior to replanting bananas. Weeds common to banana plantations have also been found to host *R. similis* allowing the nematode to reinfect the following banana crop (Quénéhervé *et al.* 2006).

The use of a fallow period crop that is resistant to *R. similis* has been successfully used in Australia (Colbran 1964a; Hall *et al.* 1993; Stirling and Pattison 2008). In Australia, Brassica species were grown in the cooler months (May – August) in short fallows and Rhodes grass (*Chloris gayana*) in long fallow periods up to two years which reduced numbers of *R. similis* carried over to the following banana crop (Stirling and Pattison 2008).

1.5 Nematode suppression

Soil suppressiveness is the phenomenon which occurs when soil factors constrain pathogens like plant-parasitic nematodes, despite the presence of a susceptible host (Pyrowolakis *et al.* 2002). Soils suppressive to plant-parasitic nematodes have been reported in many crops for different species of nematodes (Kerry 1990; Pyrowolakis *et al.* 2002; Sikora 1992; Weibelzahl-Fulton *et al.* 1996; Westphal 2005), but there are few reports of soils suppressive to nematodes in commercial banana plantations, except in Guatemala (zum Felde 2008; zum Felde *et al.* 2005).

Suppression of pathogens is typically divided into two types; specific suppression where one or several organisms are responsible for the suppressive effects, and general suppression which is the sum of the overall activities of the soil biomass (Stone *et al.* 2004). Furthermore, suppression of plant-parasitic nematodes may be natural, where the agents have increased without intention or induced, where the agents have been manipulated by either addition of the agent or manipulation of the soil environment (Kerry 1987).

1.5.1 Specific suppression

Specific suppression occurs where one or several organisms are involved in suppressing plant-parasitic nematodes and are typical of classical approaches to biological control (Sikora 1992). Organisms that may produce specific suppression of plant-parasitic nematodes include fungi, bacteria, nematodes and microfauna (Stirling 1991). The process of developing biological controls for specific suppression of migratory endoparasites, such as *R. similis*, had not been as effective as sedentary endoparasites such as *Meloidogyne* spp. However, recent developments using endophytic organisms demonstrated that specific suppression may be possible (Sikora *et al.* 2008) and that suppression of *R. similis* in the roots of banana plants with endophytic fungi, may have lead to plant driven suppressiveness that was independent of the soil (zum Felde 2008).

1.5.1.1 Fungal egg pathogens

The egg pathogen *Paecilomyces lilacinus* has been the most widely studied fungal egg parasite for suppression of plant-parasitic nematodes in bananas, beginning with work in the Philippines (Davide 1988). Glasshouse and laboratory studies have confirmed that the fungus was able to reduce numbers of *R. similis* on the roots of bananas due to the chitinolytic activity of the fungus, which degraded the nematode egg shell (Khan *et al.* 2006a; Khan *et al.* 2006b; Mendoza and Sikora 2009; Mendoza *et al.* 2007). There are few reports of *P. lilacinus* being able to reduce numbers of *R. similis* on the roots of bananas in the field. In the field, Fernandez *et al.* (2005), reported a reduction in the population of *R. similis* by 82% and associated with yield improvements of bananas up to 25%.

1.5.1.2 Nematode predatory and trapping fungi

The vermiform nematodes in the soil are attacked and consumed by many different species of fungi (Stirling 1991). The nematode-trapping fungi *Arthrobotrys* spp., which produces adhesive networks or constricting rings for capturing and killing nematodes, has been one of the most widely studied organisms as a potential biological control of plant-parasitic nematodes (Jaffee 2004a; Jaffee *et al.* 1998; Kumar and Singh 2006; Sikora 1992). However, there have been studies investigating other predatory or trapping fungi for the control of plant-parasitic nematodes (Sikora 1992; Stirling 1991). However, the efficacy of predatory and trapping fungi is dependent on many external factors; such as density of the fungi in the soil, density of nematodes, soil nutrition and organic matter (Jaffee 2003; Jaffee 2004b; Jaffee and Strong 2005; Sikora 1992). This has meant that these organisms are usually not considered as acceptable alternatives to nematicide application (Meyer and Roberts 2002).

1.5.1.3 Fungal root endophytes

Endophytic root fungi may have the potential to suppress the damage to crops caused by plant-parasitic nematodes (Sikora *et al.* 2008). An endophyte has been described as an organism that can colonise internal living tissue of their host (Sikora *et al.* 2008). The pathogen *F. oxysporum* f. sp. *cubense* may be described as a deleterious endophytic root fungus of bananas. However, other endophytic fungi of bananas, such as non-pathogenic *Fusarium oxysporum* and *Trichoderma* sp., have been shown to suppress the activity and damage of plant-parasitic nematodes (Pocasangre 2000; Sikora *et al.* 2008; Sikora and Schuster 1999; Vu *et al.* 2006; zum Felde 2008). Furthermore, zum Felde (2008) found that when *in vitro* propagated banana plants were inoculated with isolates of *Fusarium* spp. and *Trichoderma* spp. they were able to transfer suppression to the plants when challenged with *R. similis*. Pocasangre (2000) reported a 76% reduction in the number of *R. similis* in the roots of banana plants inoculated with non-pathogenic *F. oxysporum*. Further research has demonstrated that it is also possible for banana plants to transfer endophytic fungi from the mother to the daughter plant and maintain suppression of plant-parasitic nematodes (zum Felde 2008).

Colonisation of banana roots by arbuscular mycorrhizae (AMF) have been shown to suppress endoparsitic nematodes of bananas (Elsen *et al.* 2003; Jaizme-Vega and Pinochet 1997; Umesh *et al.* 1988). Elsen *et al.* (2005) also found a varietal interaction which dictated the nematode species suppressed. AMF are however, obligate symbionts and difficult to produce commercially (Sikora *et al.* 2008).

1.5.1.4 Bacterial nematode antagonists

Bacteria are numerically the most abundant organisms in field soil and therefore have an important impact on populations of plant-parasitic nematodes (Siddiqui and Mahmood 1999). Soil bacteria can be divided into those having a direct or indirect effect on plant-parasitic nematodes.

Bacteria with direct activity include *Pasteuria penetrans,* an obligate nematode parasite where as *Bacillus* spp., are known to produce toxins fatal to nematodes. There have been few studies involving the use of *P. penetrans* to suppress plant-parasitic nematodes on bananas except to reduce populations of *Meloidogyne* spp. (Devrajan *et al.* 2003; Jonathan *et al.* 2000a). *Bacillus* spp. has been shown to produce secondary metabolites that are toxic to plant-parasitic nematodes (Mendoza *et al.* 2008). The application of *Bacillus thurigiensis* var. *kurstaki* was reported as giving an average reduction in *M. incognita* numbers of 87% in banana crops in Cuba (Fernandez *et al.* 2005). However, Esnard *et al.* (1998) suggested that *B. thuringiensis* decreased rapidly in the soil, requiring frequent reapplication to give suppression of *R. similis* and *H. multicinctus.*

Indirect activity of bacteria commonly occurs through the activity of rhizobacteria inhabiting the roots of plants. Rhizobacteria in bananas have been shown to enhance the growth of plants as well as increase pathogen suppression. Mia *et al.* (2005) reported that rhizobacteria treated plants were able to obtain a yield improvement with only 33% of the fertiliser needs. There are also reports of rhizobacteria used in bananas to reduce levels of fungal pathogens *Cylindrocladium* sp. (Sutra *et al.* 2000), *F. oxysporum* f. sp. *cubense* (Sun *et al.* 2008), banana bunchy top virus (Kavino *et al.* 2008) and plant-parasitic nematodes (Chaves *et al.* 2009; Jonathan *et al.* 2000a; Rodríguez-Romero *et al.* 2008). However, the specific activity of rhizobacteria on plant-parasitic nematodes often is difficult to ascertain because of indirect interactions over the host plant.

1.5.1.5 Predators of nematodes

Predatory nematodes and other soil invertebrates have been largely ignored in suppression of plant-parasitic nematodes (Bilgrami and Brey 2005). However, a better understanding of their role in the soil food web is being developed (Bilgrami and Brey 2005; Bilgrami *et al.* 2008; Khan and Kim 2005; Khan and Kim 2007). Khan and Kim (2005) suggested that predatory nematodes belonging to the Diplogastrida may be suitable for biological control, because of their ability to withstand environmental changes. Furthermore, Bilgrami *et al.*(2008) reported in a field study, a significant reduction in the number of plant-parasitic nematodes *Tylenchus* sp., *Ditylenchus* sp., *Tylenchorhynchus* sp. and *Aphelenchoides* sp. following the release of a predatory nematode, *Monochoides gaugleri*. Currently there are no reports on the importance and role of predatory nematodes in the suppression of plant-parasitic nematodes in banana production.

Other soil invertebrates found to prey on plant parasitic nematodes include mites, insects, turbellarians and tartigrades (Bilgrami and Brey 2005). Tartigrades were found to have an important impact on the soil food web in the absence of predatory nematodes (Sánchez-Moreno *et al.* 2008). However, Piskiewicz *et al.*(2008) found that predacious microarthropods did not have a significant effect on the reduction of plant-parasitic nematodes in dune sands.

1.5.1.6 Combination of specific organisms

Due to the variety of organisms able to give specific suppression of plant-parasitic nematodes, combinations of organisms have been suggested to give better suppression of plant-parasitic nematodes (Meyer and Roberts 2002). Various combinations of fungi and bacteria have been used to reduce numbers of plant-parasitic nematodes, in situations where the organisms were selected because they had different modes of action on the nematodes that could lead to greater levels of suppression (Chaves *et al.* 2009; Chen *et al.* 2000; Mendoza and Sikora 2009; Siddiqui and Akhtar 2008). By targeting organisms with different modes of action Mendoza and Sikora (2009) were able to enhance the suppression of *R. similis* in bananas.

1.5.2 General suppression

It has been suggested that instead of focusing on the role of individual organisms in suppressing nematode pests, research should concentrate on defining the level of activity and complexity within the food web that is needed to achieve suppression (Stirling *et al.* 2005). Unlike specific suppression, which can be attributed to one or several organisms, general suppression is the sum of the activities of the overall microbial biomass (Stone *et al.* 2004). It has been suggested that general suppression may be mediated through the development of stable ecosystems over many years, or through long term mono-cropping (Stone *et al.* 2004). Furthermore, general suppression may occur naturally or be mediated through the alteration of the physical, chemical and biological environment in the soil. Therefore, the farming system becomes an important regulator of general suppression of plant-parasitic nematodes (Sánchez-Moreno and Ferris 2007; Stirling 2008).

1.6 Suppressive cropping systems

Agricultural intensification has produced important changes in soil biological communities, reducing biodiversity and altering trophic relationships (Sánchez-Moreno and Ferris 2007). The intensification of agriculture has been attributed to an increase in the incidence of plant disease and the decline in the natural enemies of nematodes (Davies *et al.* 1991; van Bruggen and Semenov 2000). Therefore, by developing integrated agricultural production systems it may be possible to enhance general suppression to plant-parasitic nematodes (Stirling 2008; Stirling and Pattison 2008; Tixier *et al.* 2006).

Bananas can be grown in a range of climatic environments and cropping systems (Frison and Sharrock 1999). Cropping systems include mixed cropping of small gardens to supplement food and income (Sikora and Schuster 1999) and large scale monocultures for domestic and export markets (Robinson 1995). The focus in this section deals with monocultures of bananas for commercial production and the suppression of the main plant parasitic nematode of bananas, *R. similis*. While it is recognised that there are cultivars of banana with varying amounts of resistance to plant-parasitic nematodes (De Waele and Elsen 2002; Gowen *et al.* 2005), only Cavendish type bananas (*Musa* AAA) will be considered as they are the cultivars used in export banana production.

Bananas are a perennial crop where suckers will continue regenerating from the corm. However, the life of the plantation is dependent on the cropping systems in which they are grown and range from very long-term (20-30 years), medium term (4-6 years) to annual cropping (Robinson 1995). Plantation length will affect the management tools available to develop suppressive systems. However, soil organic matter management and maintaining diversity of soil organisms are seen as paramount to developing systems that suppress plant-parasitic nematodes (Sikora 1992; Stone *et al.* 2004).

1.6.1 Crop rotation

Crop rotation is the practice of growing a sequence of different plants (Bullock 1992). Bananas grown in large scale commercial production are rarely rotated (Gowen *et al.* 2005). However, a range of crops and pasture species have been shown to have resistances to *R. similis* and numbers of *R. similis* in bananas can be reduced in the following banana crop when rotation is used (Chabrier and Quénéhervé 2008; Colbran 1964a; Stirling and Pattison 2008; Tarte *et al.* 1981). Rotation of crops with bananas may have multiple benefits for increasing the general suppression of plant-parasitic nematodes, through increased carbon inputs, improved soil structure and diversity of organisms (Bullock 1992; Wang *et al.* 2008).

1.6.2 Intercropping

Intercropping is the practice of growing two or more crops simultaneously on the same land (Bullock 1992). Banana intercropping is more common in small land holder situations (McIntyre *et al.* 2001; Ssennyonga *et al.* 1999; Wortmann and Sengooba 1993). The importance of intercrops in banana cultivation for nematode management was mixed. It was found to have no benefit for nematode suppression and banana growth in Africa (McIntyre *et al.* 2001), but was able to suppress plant-parasitic nematodes in a plantain production system in Puerto Rico. The use of intercrops has also been able to host beneficial nematode antagonists, which can aid nematode suppression (Van der Veken *et al.* 2008; Vargas-Ayala *et al.* 2000). The effect of intercrops on nematode suppression was dependent on the intercrop species and the environment in which they were grown.

1.6.3 Crop residues

The residue from banana production can be twice that removed in the banana bunch (Lekasi *et al.* 1999). The residue from bananas is an important source of nutrients that can be recycled in the soil and mineralised for crop growth (Dorel *et al.* 2008; Lekasi *et al.* 1999), but can also contribute organic matter to the soil (Cordeiro *et al.* 2004). Investigations into the composting of banana residue have been conducted to determine the effects on the nutrient supply to bananas (Ultra *et al.* 2005). However, the effects of crop residue application on bananas gave mixed results. The retention of crop residues as a mulch around the base of the banana plant produced a positive effect on the growth of banana, increasing production (Gaidashova *et al.* 2009). However, plant-parasitic nematode were suppressed in some studies (Talwana *et al.* 2003), but found to be higher in other studies (Gaidashova *et al.* 2009). However, the long-term impacts of retention of banana residue close to the plant on the suppression of plant-parasitic nematodes are still to be determined (Stirling and Pattison 2008)

1.6.4 Organic amendments

Akhtar and Malik (2000) suggested that organic amendments can stimulate the naturally occurring nematode antagonists in the soil and provide suppression of plant-parasitic nematodes. However, additions of organic matter may have side effects. Also, large quantities of organic material are often required, making it difficult to interpret the modes of suppression on plant-parasitic nematodes (Kerry 1987; Kerry 1990). There are very few studies on the application of organic amendments in the control plant-parasitic nematodes in bananas. However, there are reports that applications of neem as an organic amendment were able to reduce plant-parasitic nematodes in bananas (Jonathan *et al.* 2000b; Zarina 2007). The application of organic amendments relies on the supply of large quantities of material which need to be in close proximity to the location where they will be used (Kerry 1990).

1.6.5 Tillage

Tillage is used to prepare land prior to planting bananas. There has been increasing evidence suggesting that minimum tillage systems are able suppress plant-parasitic nematodes in a number of cropping systems although results have been mixed (Conway 1996; Kimpinski and Sturz 2003; Stirling 2008; Stirling and Eden 2008). In subsistence farms tillage may be done using hand hoes with minimal disturbance of the soil (Dowiya *et al.* 2009). Tillage in export banana production is dependent on the topography and may occur only rarely in long-term plantations (Robinson 1995). However, tillage in mechanised banana production has been attributed to a decline in the physical quality of the soil (Cattan *et al.* 2006; Rasiah *et al.* 2009). Since tillage may only occur once every 4-5 years in mechanised banana production systems, there are no studies that have investigated the long term impact of the tillage systems on suppression of plant-parasitic nematodes, particularly *R. similis*. However, Dowiya *et al.*(2009) attributed increased root damage and reduced production to the use of hand hoes to remove weeds from banana plots.

1.6.6 Nutrient management

Nutrient management is an important component in the management of productive banana plantations (Lahav 1995). Nutrient depletion is a major constraint to production of bananas in many countries, while on the other hand high inputs of nutrients in intensive banana production may lead to environmental degradation (Delvaux 1999). The form of nitrogen utilised as, either NH_4^+ or NO_3^-, was found to impact on the growth of banana and the number of *R. similis* in the root systems of hydroponic bananas. However, the possible role played by other soil organisms was not considered when higher numbers of nematodes was measured in the banana roots of the NO_3^-N treatments. Smithson *et al.*(2001) reported no effect of fertiliser on the populations of plant-parasitic nematodes in the roots of bananas in Uganda. The impact of nutrient management on the suppression of plant-parasitic nematodes of bananas is difficult to determine directly and may be specific to banana production regions due to the complex interactions that occur in the soil.

1.6.7 Analysis of cropping systems

Innovative cropping systems for bananas need to be developed to meet the challenges of production, environmental protection and suppression of pests and diseases (Blazy *et al.* 2009; Delvaux 1999). This requires the assessment of multiple criteria (Delvaux 1999; Gaidashova *et al.* 2009; Tixier *et al.* 2008a). One method is the modelling of production systems with emphasis on suppressing plant-parasitic nematodes (Tixier *et al.* 2008a; Tixier *et al.* 2006; Tixier *et al.* 2008b; Zander and Kachele 1999). Another method is to develop an understanding of the soil constraints in the production system through an understanding of soil health and quality (de Lima *et al.* 2008; Shukla *et al.* 2006). A thorough knowledge is needed to determine how management decisions impacts on soil physical, chemical and biological soil properties and how this in turn may impact on the suppression of important plant-parasitic nematodes such as *R. similis*.

2 Research objectives

It is the aim of this thesis to describe practical management strategies that can be implemented by banana growers to suppress *R. similis* and to determine the changes these management practices have on soil properties. The hypothesis for this research is that organisms that are antagonistic to plant-parasitic nematodes exist in agricultural soils, but their activity is suppressed by the agronomic practices utilised on banana plantations. Furthermore, it is believed that plant-parasitic nematode problems in banana plantations are a symptom of the farming system suppressing the activity of nematode antagonists through less than optimal soil conditions. Therefore, the investigations in this thesis were targeted at identifying soil conditions that favour antagonistic organisms, which provide general suppression of *R. similis* and not at identifying the specific organisms responsible for suppression.

The objectives of the following investigations were:

1. To examine various soil amendments readily available to banana growers in the wet tropics region of Australia to determine which amendments are able to suppress *R. similis* on bananas and what changes in soil properties are associated with nematode suppression in a glasshouse experiment.

2. To determine changes in selected soil chemical properties and the nematode community following amendment of soil with organic matter and the effect this has on the suppression of *R. similis* on bananas in a glasshouse experiment.

3. To ascertain changes in soil physical, chemical and biological properties in the field following the addition of amendments to the soil and to determine changes in populations of plant-parasitic nematodes and production of bananas.

4. To survey banana farm productions systems using farm management and soil health indicators to define farm typologies and indicators relating to the suppression of *R. similis* on bananas.

5. To use the knowledge of soil indicators and plant-parasitic nematode suppression gained in Australia to investigate the effects of different soil management practices and soil physical, chemical and biological properties on the suppression of *R. similis* in Costa Rica.

3 Effects of soil amendments on suppression of the burrowing nematode, *R. similis,* and on the growth of bananas

3.1 Introduction

The Australian banana industry is composed of subtropical and tropical components. However, the wet tropics region of north Queensland is the most important production area with 88% of the country’s banana production (Collins, *et al*. 2004). The wet tropics is located in a region receiving an average of 3 800 mm of rainfall annually, often in heavy downpours. The high rainfall and close proximity to world heritage listed rainforest and coral reefs make the area an environmentally sensitive zone for agriculture. Therefore, environmentally responsible farming practices are required to ensure grower profitability and sustainable management of pests, diseases and nutrients and in particular *Radopholus similis*.

To prevent losses in banana production in Australia caused by *R. similis*, banana farmers apply organophosphate or carbamate nematicides, which cost AU$10-15 million annually (Pattison 1994). Also, the application of nematicides is potentially hazardous to the local environment as they can be readily transported in soil water and attached to colloidal soil particles (Cáceres *et al.* 2002). Furthermore, concerns for worker safety have meant that alternative methods for managing plant parasitic nematodes on bananas are required.

The application of organic amendments to soil is an environmentally favourable waste management strategy that can potentially improve soil quality (Flavel and Murphy 2006). Considerable research has shown the benefits of using composts and other organic amendments to improve soil physical (water holding capacity, porosity and bulk density), chemical (pH, electrical conductivity and nutrient content) and biological properties such as soil microbial populations and plant growth (Flavel and Murphy 2006; Kennedy *et al*. 2004; Moss *et al*. 2002). Amendments have also been investigated for the suppression of soil borne diseases in a range of cropping systems (Stone *et al.* 2004). Furthermore, there have been investigations to determine the usefulness of soil amendments to reduce plant-parasitic nematode populations in agricultural crops (Akhtar and Malik 2000; Stirling *et al.* 2003; Widmer *et al.* 2002). However, the results of plant-parasitic nematode suppression are variable and sometimes crop or site specific. The application of amendments may need to be applied continuously at high rates to reduce plant-parasitic nematode populations (Vawdrey and Stirling 1997), which would impact on physical, chemical and biological soil properties.

The chemical composition of the amendments influences the rate of microbial decomposition of organic matter, which can regulate the stability and release of nutrients (Moss *et al.* 2002). Easily degraded forms of C are preferentially utilised by bacterial populations (Flavel and Murphy 2006; Valenzuela-Solano and Crohn 2006). The rate of nutrient release may impact on the amendment’s potential to stimulate organisms that are antagonistic to plant-parasitic nematodes. Furthermore, due to their inherent qualities, some soils have a greater potential to suppress plant-parasitic nematodes than others (Westphal 2005; Widmer *et al*. 2002).

An integrated nematode management system has been developed for the Australian banana industry to reduce losses caused by *R. similis* (Stirling and Pattison 2008). However, further work is required to improve the resilience of the banana production system to damage by plant-parasitic nematodes, particularly *R. similis*.

3.1.1 Aim

The objectives of the following investigations were to examine various soil amendments readily available to banana growers in the wet tropics region of Australia to determine which amendments were able to suppress *R. similis* on bananas and what changes in soil properties were associated with nematode suppression in a glasshouse experiment.

3.2 Materials and methods

3.2.1 Soils

Three soils, representative of major soil types for banana production in the wet tropics region of north Queensland, were chosen for a pot experiment; Mundoo, Innisfail and Coom soils series (Table 3-1). The Mundoo soil is a red uniform, clay loam structured Ferrosol soil, with good drainage, derived from basalt (Cannon *et al.* 1992). The Innisfail soil was a structured brown clay loam and the Coom soil was a poorly structured, mottled uniform clay loam to clay soil with a silty texture and poor drainage. The later two soils were dermosols (Cannon *et al*. 1992). All soils used in the experiment were collected from the top 15 cm of commercial banana farms, prior to addition of amendments.

Table 3-1: Location, classification, pH, sand, silt and clay content of typical banana producing soils used in the pot experiment to determine the effect of different soil amendments.

Soil series	Classification (Isbell 1996)	Latitude (S)	Longitude (E)	pH	Sand %	Silt %	Clay %
Coom	Dermosol	17°40′58″	146° 3′38″	4.7	5	44	50
Innisfail	Dermosol	17°58′ 9″	146°49′50″	5.3	35	25	40
Mundoo	Ferrosol	17°36′14″	146° 0′41″	4.9	49	10	41

3.2.2 Amendments and nematode inoculation

Nine different amendments; mill mud, mill ash, molasses (all by-products from sugarcane processing), biosolids, municipal waste compost (MW compost), banana residue (*Musa* AAA, Cavendish subgroup), grass hay (*Chloris gayana*), legume hay (*Medicago sativa*), and calcium silicate (CaSi) were mixed with the three different soils and compared to untreated soil. The amendments were analysed for their chemical composition, listed in Table 2-2, using the methods described by Rayment and Higgins (1992). The biosolids, compost, mill mud, banana residue, grass hay and legume hay were mixed at a rate equivalent to 40 t ha^{-1} (Table 3-2). Two rates of mill ash were incorporated with the soil, 40 and 120 t ha^{-1}. Furthermore, CaSi was applied at 5 t ha^{-1} and molasses at 300 L ha^{-1}. The rate of amendment applied per pot was calculated using the surface area of the 200 mm diameter pots with a depth of 150 mm. The amendments were incorporated and thoroughly mixed with the soil prior to placement in the pots. Approximately 3 kg of the soil and amendment mix was placed in each pot. Pots were tapped several times to achieve a uniform bulk density.

The pots were then placed in the glasshouse for two weeks to allow the amendments to settle before planting 12-week old *in vitro* bananas (*Musa* AAA, Cavendish subgroup, cv. Williams) into each pot. Half of the soil was removed from the pot to allow sufficient room to plant the *in vitro* plantlets and backfilled with the remaining soil. The banana plants were allowed to grow in the amended soil for a further 3 days and then inoculated with 860 motile *R. similis*, taken from carrot cultures (Moody *et al.* 1973).

3.2.3 Design and management

All treatments comprising of soil type and amendment combinations were replicated four times in a randomised block design. Pots were maintained in the glasshouse at 20-30 °C and received 5 mm of water daily through an automated sprinkler system. Fertiliser was applied (5 g Osmocote Plus Mini™ 16:8:11 N:P:K plus trace elements) at planting. Additional soluble fertiliser (Thrive™ 27:5.5:9 N:P:K) was applied four and eight weeks after bananas were planted in pots. The plants were harvested 12-15 weeks after planting and plant growth and nematode parameters determined.

3.2.4 Plant growth

The plant height and number of leaves of banana plants were determined when plants were inoculated with nematodes. The height and the number of leaves were determined at four weekly intervals until the termination of the experiment after 12 weeks (84 days). The relative change in plant height and leaf number for each treatment was calculated for each four week period, by subtracting plant height or leaf number at the beginning of the four week period from the height or leaf number at the end of the four week period. The change in height or leaf number was divided by the number of weeks between assessments, to obtain an average weekly change in plant height and leaf emergence.

At the termination of the experiment, as well as plant height and leaf number, the area of the last fully emerged leaf, plant dry weight and fresh root weight were determined. The area of the last fully emerged leaf was estimated by determining the length and width of the leaf at its widest point and multiplying by 0.83 (Turner 1972). Plant dry weight was determined by placing shoots of banana plants in an oven at 75 °C for 5 days. Banana fresh root weight was determined by washing the soil from the roots and allowing the roots to air dry for 30 minutes before weighing. After determining the fresh root weight nematodes were extracted from the banana roots.

3.2.5 Nematode extraction

R. similis was extracted from the roots of banana plants by cutting the roots into 1-2 cm pieces and placing them on a coarse screen in a misting cabinet for seven days (Hooper 1986). *R. similis*, washed from the roots, were collected at the base of the container with excess misting water. Nematodes were captured by passing the water through a 25 µm sieve. The nematodes were backwashed from the sieve and collected in a 30 mL vial for counting.

Soil nematodes were extracted by placing 200 g of soil collected from pots on a single layer of tissue, contained within a mesh basket (Whitehead and Hemming

1965). The basket was placed in 200 mL of water within a tray and maintained at 25 ^{o}C. After 48 hours, nematodes contained within the water of the tray were collected on a 25 µm sieve. The nematodes were backwashed from the sieve and collected in a 30 mL vial. The total number of nematodes extracted from 200 g of soil was determined. Using a compound microscope, nematodes were identified to genera for plant-parasitic nematodes or families for non-parasitic nematodes and assigned to trophic groups according to Yeates *et al*. (1993) and indices calculated.

3.2.6 Nematode indices

Indices of the nematode community composition were calculated from the number of nematode taxa extracted from the soil of each pot. Nematode diversity was determined using the Shannon-Weiner index, $H' = -\Sigma\ p_i \log_e p_i$ and dominance calculated using Simpson's index of dominance $\lambda = \Sigma\ (p_i)^2$, where p_i is the proportion of individuals in the i^{th} taxon (Yeates and Bongers 1999).

Additionally, the weighted faunal analysis concept was applied, without plant-parasites, to determine the basal, structure and enrichment conditions of the soil food web (Ferris *et al*. 2001). The enrichment index (EI) assesses the resources available to the soil food web and response by primary decomposers to those resources. The structure index (SI) is a measure of the number of trophic layers in the soil food web and the potential for regulation by predators. These indicators were calculated as EI = 100 [e/(e+b)] and SI = 100[s/(s+b)], where e, s and b are the abundance of nematodes in guilds representing enrichment (e) [B1 and F2, where B = bacterivores, F = fungivores, and numbers represent the coloniser–persister (c-p) value 1-5 (Bongers 1990)], structure (s) (B3-B5, F3-F5, O3-O5, P2-P5, where O = omnivores, P = predators) and basal (b) (B2 and F2) nematode communities (Ferris *et al*. 2001). The channel index (CI), is an indication of the decomposition channel of nutrients calculated as CI = 100[0.8F2/(3.2B1 + 0.8 F2] (Ferris *et al*. 2001). A low value suggests a primarily bacterial decomposer community whereas a high value indicates a fungal-dominated decomposer nematode community (Hohberg 2003).

3.2.7 Statistics

Significant differences were determined using a two-way ANOVA for treatment and soil effects. If statistical differences were found, the means were separated using the LSD method using Genstat 8 statistics package (Lawes Agricultural Trust). Total nematode numbers and number of nematodes in each trophic group per 100 g of soil were transformed using ln(x+1), prior to analyses, to comply with assumptions of normally distributed data. The major nutrient (N, P, K, Ca, Mg and C) content of amendments was subjected to principal component analysis (PCA) and a biplot was constructed using the correlation matrix method. All statistical analysis was performed using Genstat 8 (Lawes Agricultural Trust).

3.3 Results

3.3.1 Amendments composition

The banana residue, grass hay and legume hay all had carbon contents greater than 40%, suggesting that at a rate of 40 t ha^{-1}, greater than 16 t ha^{-1} of carbon was mixed in with the soil (Table 3-2). Conversely, biosolid applied at 40 t ha^{-1} contained 7% carbon, so that only approximately 3 t ha^{-1} of carbon was mixed with the soil (Table 3-2). Biosolids also had the lowest C:N ratio (8.7) of the amendments, where as mill ash had the highest C:N ratio (64.7) (Table 3-2). Legume hay had the highest nitrogen content (3.2%) of the amendments, which meant that at a rate of application of 40 t ha^{-1}, 1 280 kg N ha^{-1} was potentially available if all nitrogen in the legume hay was mineralised. The grass hay, banana residue and compost also contained relatively high amounts of N and could potentially supply more than 700 kg N ha^{-1} if all N was mineralised. The compost, sourced from a municipal supplier, contained 32% Ca, five times more than the next highest amendment, mill mud, which contained 6.3% Ca (Table 3-2). The compost was also found to have high amounts of lead, 780 mg kg^{-1}, which is above an acceptable level and 50 times higher than any other amendment. The mill mud, mill ash and banana residue all contained high levels of metals, such as Fe and Al, relative to other amendments (Table 3-2).

The PCA of the macronutrient composition of the amendments (C, N, P, K, Mg and Ca) was able to explain over 63% (PC1 = 35.3 and PC2 = 28.0) of the variation of nutrient composition of the amendments (Figure 2-1). The PCA grouped legume hay, grass hay and banana trash together. The N, C and Mg vectors of the biplot appeared to be positively associated with the grouping of legume hay, grass hay and banana residue (Figure 3-1). Conversely, mill ash was on the opposite side of the biplot from the origin relative to legume hay, grass hay and banana trash (Figure 3-1). The N and C content of the amendments gave the two highest latent vector loadings (-0.52 and -0.51 respectively), which suggested that these two nutrients were able to explain most of the variation in nutrient content of the amendments.

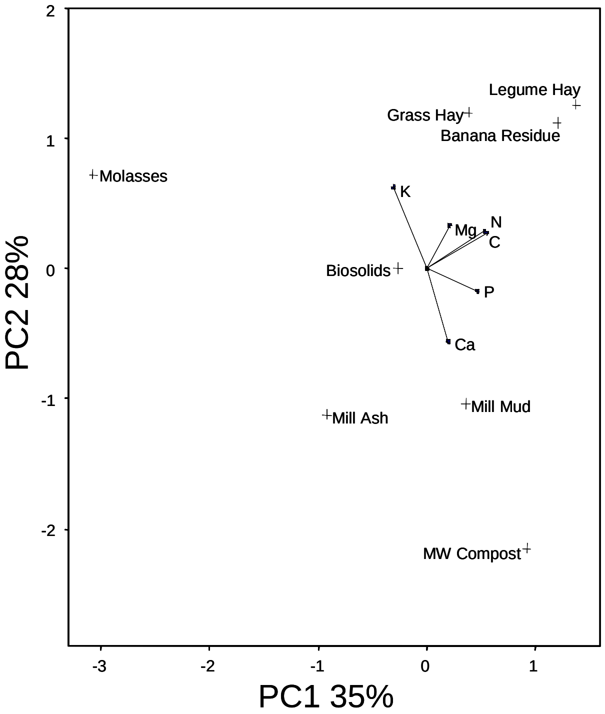

Figure 3-1: Principal component biplot for the distinction between amendments used in a pot experiment for suppression of *R. similis* in bananas based on macronutrient content of the amendments.

3.3.2 *R. similis* suppression

There was no significant interaction of the soil type and the amendment's ability to suppress *R. similis* in the roots of the banana plants. Therefore, the results of *R. similis* recovery from the three different soils used in the experiment were pooled (Figure 3-2). The amendments legume hay, grass hay, banana residue and mill-mud significantly reduced ($P<0.05$) the number of *R. similis* in the roots of banana plants relative to the untreated control (Figure 3-2). The legume hay was significantly better than all other treatments, with less than 6% of the number of *R. similis* relative to the untreated plants. The grass hay, banana residue and mill mud treatments had a 5-fold reduction in the number of *R. similis* recovered from the roots of bananas compared to the untreated plants (Figure 3-2). The CaSi, molasses, MW compost, mill ash and biosolid treatments all had statistically similar numbers of *R. similis* in the roots of bananas relative to the untreated control (Figure 2-2).

Table 3-2: Amendments applied to bananas, the rate at which they were applied and their chemical composition including heavy metal composition of biosolid, compost mill mud and mill ash.

Amendment	**Rate** **t ha^{-1}**	**C** **%**	**N** **%**	**C:N** **ratio**	**P** **%**	**K** **%**	**Ca** **%**	**Mg** **%**	**S** **%**	**Na** **%**	**Si** **%**	**Cu** **mg kg^{-1}**	**Zn** **mg kg^{-1}**	**Mn** **mg kg^{-1}**	**Fe** **mg kg^{-1}**	**Al** **mg kg^{-1}**	**B** **mg kg^{-1}**
Banana residue	40	46.8	1.8	25.7	0.35	2.0	1.5	0.72	0.10	0.4	2.3	59.3	110	1300	24000	55000	< 0.3
Biosolid	40	7.1	0.8	8.7	0.39	1.5	1.0	0.77	0.64	2.24	1.4	14.9	95	180	330	90	54
Grass hay	40	44.7	2.3	19.6	0.29	3.2	0.5	0.16	0.54	0.76	1.3	9	22	190	170	140	6
Legume hay	40	45.8	3.2	14.5	0.25	1.7	1.7	0.52	0.35	0.30	0.6	6.1	15	61	880	700	45
Mill ash	40 & 120	19.4	0.3	64.7	0.27	0.3	0.7	0.16	0.05	0.01	25.3	32.4	76	930	13000	38000	< 0.3
Mill mud	40	19.8	1.4	13.7	0.39	0.5	6.3	0.23	0.40	0.52	15.7	247.8	460	270	13000	11000	< 0.3
Molasses	300	-	0.5	-	0.06	3.8	0.7	0.25	0.43	0.04	-	2	8.8	70	350	110	3
MW Compost	40	29.0	1.8	15.7	0.28	0.1	32.0	0.19	0.13	0.05	15.9	56.8	120	83	670	2200	11

Amendment	**Cd** **mg kg^{-1}**	**Pb** **mg kg^{-1}**	**Al** **mg kg^{-1}**	**Co** **mg kg^{-1}**	**Cr** **mg kg^{-1}**	**Cu** **mg kg^{-1}**	**Ni** **mg kg^{-1}**	**Zn** **mg kg^{-1}**
Biosolid	0.48	9.2	2104	1.1	7.6	62.2	5.4	139
Mill ash	0.20	15.7	48110	10.8	55.6	74	27.4	140
Mill mud	0.17	14.9	63060	14	74.4	76.1	48.2	143
MW Compost	2.12	780	11150	17	117	293	84.9	785

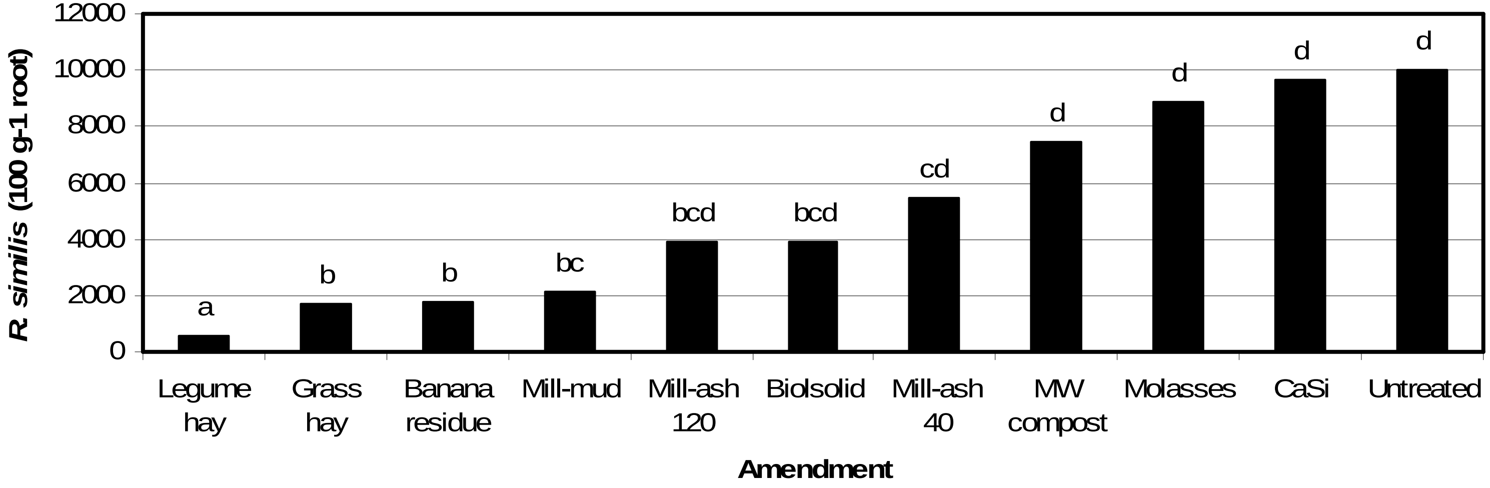

Figure 3-2: Recovery of *R. similis* from the roots of banana plants grown in soil with different amendments for 12 weeks. (Bars, with the same letter above are not significantly different from one another at *P*=0.05).

3.3.3 Plant growth

All treatments, except the mill mud, mill ash applied at 120 t ha^{-1} and CaSi, slowed the growth of banana plants in the first 4-week period (Table 3-3). In the second 4-week period, between 28 and 56 days after commencing the experiment grass hay, legume hay and banana residue all significantly increased the growth of the banana plants relative to the untreated plants, but there was no difference in the emergence of leaves over the same period (Table 3-3). In the final 4-week period of the experiment, between 56 and 84 days since commencement, grass hay, legume hay and banana residue as well as the biosolid treatments had significantly greater plant height increases and leaf emergence rates relative to the untreated soil (Table 3-3). The application of molasses was also able to increase the leaf emergence rate of bananas in the final 4-week period of the experiment (Table 3-3).

At the termination of the experiment, banana plants grown in mill mud amended soil had grown significantly greater shoot and root weights relative to the untreated plants, having 1.4 times greater dry shoot weight and fresh root weight than the untreated plants (Table 3-3). Banana plants grown with additional banana residue had significantly greater shoot dry weight than the untreated soil (Table 3-3). The incorporation of banana residue also produced plants with the greatest area of the last fully emerged leaf, but this was not significantly greater than the untreated plants (Table 3-3). Furthermore, the application of mill ash at 120 t ha^{-1} produced significantly greater fresh root weight than the untreated soil (Table 3-3).

The application of biosolid to the soil significantly reduced the growth of plants relative to the untreated soil (Table 3-3). The plants grown in the biosolid amended soil were approximately two-thirds of the shoot dry weight and root fresh weight of the untreated plants and exhibited an 18% decrease in the size of the last fully emerged leaf (Table 3-3). In the first four weeks after planting the bananas in the biosolid treated soil caused a four-fold reduction in plant height and significantly reduced leaf emergence relative to the untreated soil (Table 3-3). The height and leaf emergence of the plants grown in the biosolid treated soil were not significantly different from the untreated plants in the mid 4-week period (28 to 56 days) and were significantly better in the final 4-week period of the experiment (56 to 84 days) (Table 3-3).

The soil used in the experiment had a significant effect on the growth of banana plants at the beginning and end of the experiment (Table 3-4). Banana plants grown in the Innisfail soil series had a significantly greater increase in plant height in the first 28 days relative to the Mundoo and Coom soil series (Table 3-4). In the final 28 days of the experiment, plants grown in the Coom soil series had a significantly reduced leaf emergence rate relative to the Mundoo soil series. Similarly at the conclusion of the experiment the plants grown in the Coom soil series had at significantly reduced last fully emerged leaf area, and reduced shoot and root weights, relative to the other soils used in the experiment (Table 3-4).

Table 3-3: Growth of banana plants in different amendments applied in a pot experiment

Amendment	Rate (t ha^{-1})	Increase in plant height (mm week^{-1})			Leaf emergence (leaves week^{-1})			Last fully emerged leaf (cm^2)	Shoot dry weight(g)	Root fresh weight (g)
		28 days	56 days	84 days	28 days	56 days	84 days			
Untreated	0	36 f	22 a	7 a	1.1 bcd	0.7 n.s.	0.4 ab	43.6 ab	17.8 bcd	53.8 bc
Banana residue	40	27 cd	37 c	14 bc	1.1 abc	0.8 n.s.	0.6 de	47.7 a	24.5 e	66.9 cde
Biosolid	40	8 a	24 ab	13 bc	1.0 a	0.7 n.s.	0.6 de	35.8 c	11.6 a	36.0 a
CaSi	5	31 def	25 ab	6 a	1.1 bcd	0.7 n.s.	0.4 abc	40.6 bc	16.8 bc	56.4 bcd
Grass hay	40	24 bc	39 c	16 cd	1.1 bc	0.8 n.s.	0.6 e	46.8 a	21.2 cde	61.4 bcde
Legume hay	40	21 b	29 bc	19 d	1.0 ab	0.8 n.s.	0.6 de	40.6 bc	15.7 ab	50.5 abc
Mill ash	120	33 ef	26 ab	9 ab	1.2 de	0.7 n.s.	0.5 bcd	42.8 ab	22.0 de	71.4 de
Mill ash	40	29 cde	26 ab	8 a	1.1 cde	0.7 n.s.	0.5 cd	40.6 bc	21.1 cde	52.9 abc
Mill mud	40	36 f	28 ab	8 a	1.2 e	0.8 n.s.	0.4 abc	42.5 ab	25.9 e	75.9 e
Molasses	300	24 bc	28 ab	7 a	1.1 abc	0.8 n.s.	0.5 cd	38.6 bc	16.6 bc	46.1 ab
MW Compost	40	27 cde	25 ab	6 a	1.1 cde	0.8 n.s.	0.4 a	41.5 b	18.1 bcd	54.8 bcd

Means in columns followed by the same subscript are not significantly different from one another at *P*=0.05.

Table 3-4: Growth of banana plants in a pot experiment using soils common in banana production in north Queensland

Soil	Increase in plant height (mm week^{-1})			Leaf emergence (leaves week^{-1})			Last fully emerged leaf (cm^2)	Shoot dry weight(g)	Root fresh weight (g)
	28 days	56 days	84 days	28 days	56 days	84 days			
Coom	26 a	26 n.s.	9 n.s.	1.1 n.s.	0.8 n.s.	0.4 a	39.5 b	17.2 a	43.5 a
Innisfail	29 b	28 n.s.	9 n.s.	1.1 n.s.	0.8 n.s.	0.5 ab	43.0 a	20.3 b	54.9 b
Mundoo	26 a	30 n.s.	11 n.s.	1.1 n.s.	0.8 n.s.	0.5 b	42.8 a	19.6 ab	69.5 c

Means in columns followed by the same subscript are not significantly different from one another at *P*=0.05.

3.3.4 Amendment effects on soil nematode community structure

There was a significant interaction between the application of amendments and the three different soils used in the experiment in terms of number of soil nematodes recovered from different nematode trophic groups (Figure 3-3).

Plant parasitic nematodes identified from soil extracts included *R. similis*, *Helicotylenchus dihystera*, *Rotylenchulus reniformis* and *Meloidogyne* spp. The application of banana residue was able to reduce the number of plant-parasitic nematodes in all three soils relative to the untreated soil (Figure 3-3A). The legume hay, grass hay and biosolid amendments reduced the number of plant-parasitic nematodes relative to the untreated Innisfail, Mundoo or Coom soil (Figure 3-3A). However, some amendments were more effective at reducing the population of plant-parasitic nematodes in particular soil types. For example, the application of mill mud reduced the number of plant-parasitic nematodes in the Coom and Innisfail soils, but did not significantly reduce plant-parasitic nematode numbers in the Mundoo soil (Figure 3-3A). Conversely, CaSi and molasses had no impact on plant-parasitic nematode numbers in any of the soils relative to the untreated soil (Figure 3-3A).

Bacterivores extracted from soil samples were identified and belonged primarily to the nematode families Cephelobidae, Rhabditidae, Pangrolaimidae, Wilsonematidae and Prismatolaimidae. The application of grass hay, compost and mill mud to the Mundoo soil increased the number of bacterivores, relative to the untreated, but the amendments had little effect on bacterivore numbers in the Innisfail and Coom soils (Figure 2-3B). There were relatively high numbers of bacterial feeding nematodes in the untreated Coom soil and the addition of amendments did not significantly change the number of bacterivores recovered from the soil (Figure 3-3B). The biosolid treatment applied to Coom soil had the highest recovery of bacterivores and significantly greater numbers of bacterivores than recovered from the mill mud treated Coom soil (Figure 3-3B). Similarly, the legume hay applied to the Innisfail soil series had a significantly greater number of bacterivores recovered from soil relative to the mill mud treated Innisfail soil (Figure 3-3B).

Fungivores recovered from soil extracts were identified as belonging to the families Aphenchidae or Tylechidae. The application of mill mud significantly reduced the number of fungivorous nematodes in the Innisfail soil relative to the untreated soil (Figure 3-3C). The application of grass hay, MW compost, banana residue and mill ash at 120 t ha^{-1}, to the Mundoo soil were all able to significantly increase the number of fungivores in the soil relative to the application of CaSi, but not the untreated soil (Figure 3-3C). The mill mud treatment had the lowest number of fungivorous nematodes in the Coom and Innisfail soils. There were significantly fewer fungivores in the mill mud treated Coom soil relative to the legume hay treatment (Figure 3-3C). Similarly, there were fewer fungivores in the mill mud treated Innisfail soils relative to mill ash 40 t ha^{-1}, MW compost, grass hay, legume hay and untreated soil (Figure 3-3C).

All omnivores identified belonged to the family Dorylaimidae. Very low numbers of omnivorous nematodes were extracted from the untreated Mundoo soil and only the application of grass hay was able to significantly increase the number of omnivores (Figure 3-3D). Furthermore, the application of grass hay to the Coom soil was able to significantly increase the number omnivorous nematodes relative to the untreated soil (Figure 3-3D). Conversely, the application of biosolid and CaSi significantly reduced the number of omnivores in the Coom soil relative to the untreated soil (Figure 3-3D). Four amendments, mill ash 40 t ha^{-1}, MW compost, grass hay and banana residue applied to the Innisfail soil, all significantly increased the number of omnivorous nematodes recovered relative to the untreated Innisfail soil (Figure 3-3D).

Predatory nematodes extracted from soil samples belonged to the families Mononchidae or Tripylidae. There were significantly more predatory nematodes in the untreated Coom and Innisfail soils relative to the untreated Mundoo soil (Figure 3-3E). No amendment applied to the any of the three soils was able to significantly increase the number of predatory nematodes relative to the corresponding untreated soil (Figure 3-3E). However, the number of predatory nematodes was significantly reduced in Coom and Innisfail soils following the application of biosolid, grass hay and legume hay relative to the untreated soil (Figure 3-3E). Furthermore, the application of mill ash 120 t ha^{-1} and CaSi to the Coom soil significantly reduced the number of predatory nematodes relative to the untreated Coom soil (Figure 3-3E).

Unlike the number of nematodes in each trophic group, there was no significant interaction between amendments and soil type in relation to nematode community indices and therefore, the data for the different indices have been pooled for amendment effects (Table 3-5). The application of biosolids significantly decreased the diversity index and increased the dominance index of soil nematode community relative to the untreated soil (Table 3-5). Conversely, the application of banana residue was the only treatment to significantly increase the diversity index of nematodes in the soil compared to the untreated soil (Table 3-5). Furthermore, mill mud was the only treatment to significantly reduce the dominance index of nematode taxa in the soil compared to the untreated soil (Table 3-5).

Mill mud, banana residue, MW compost and grass hay treatments all significantly increased the structure index relative to the untreated soil (Table 3-5). Conversely, the application of biosolids significantly reduced the structure index and increased the enrichment index compared to the untreated soil (Table 3-5). Furthermore, banana residue, MW compost and legume hay significantly increased the enrichment index relative to the untreated soil (Table 3-5). The application of biosolid and legume hay exhibited a predominantly bacterial decomposition pathway of nutrients having a significantly lower channel index relative to the untreated soil (Table 3-5). Seven treatments, banana residue, biosolid, MW compost, grass hay, legume hay, mill ash 40 t ha^{-1} and mill mud all significantly reduced the proportion of plant-parasitic nematodes recovered from the soil relative to untreated soil (Table 3-5). The mill ash 120 t ha^{-1}, molasses and CaSi treated soils all had statistically similar proportion of plant-parasitic nematodes in the soil community compared to the untreated soil (Table 3-5).

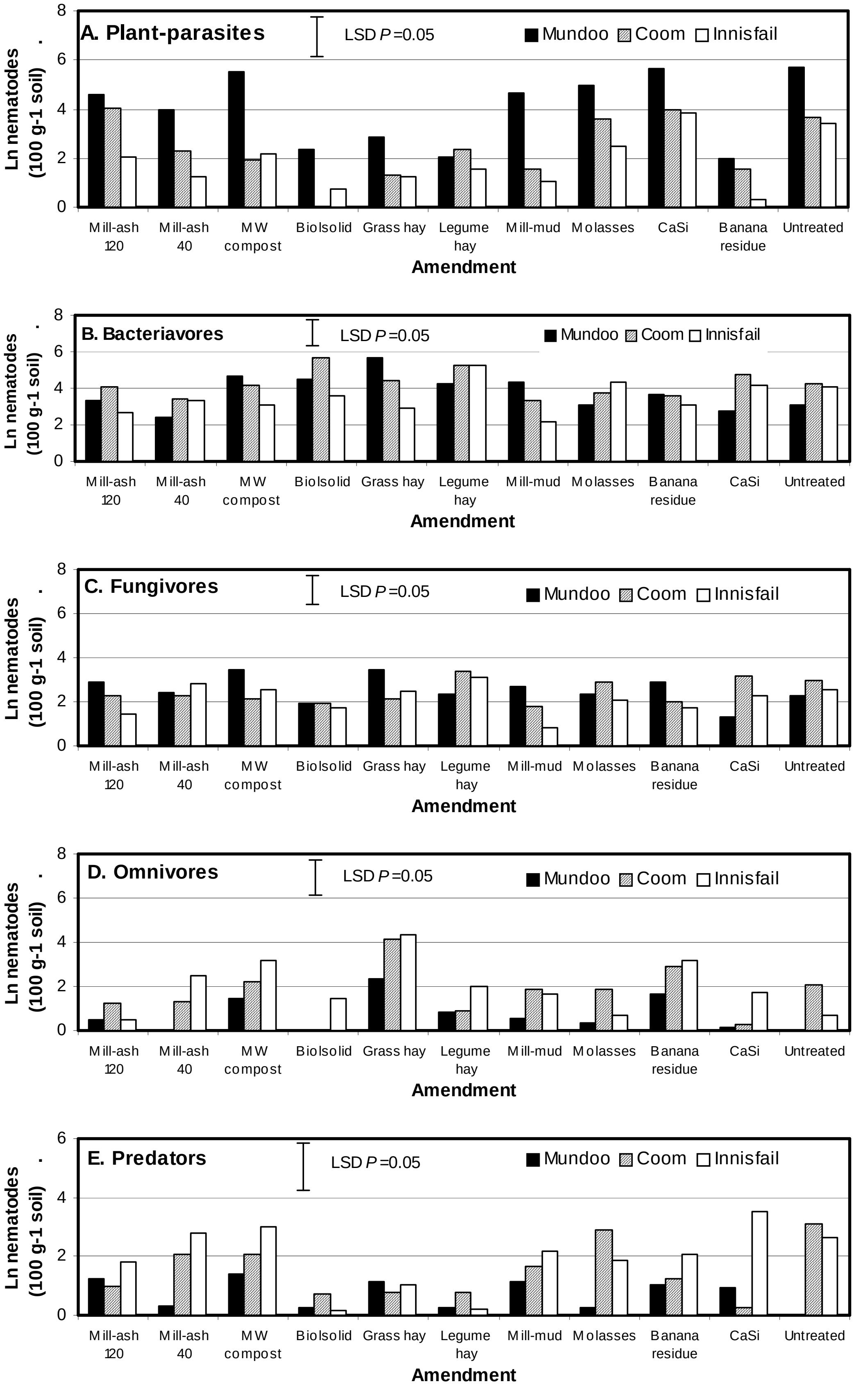

Figure 3-3: Effects of amendments applied to three different soil types on the trophic groups of soil nematodes (Bars represents LSD values at *P*=0.05)

Table 3-5: Effects of soil amendments on soil nematode indices

Amendment	Rate (t ha^{-1})	Diversity (H')	Dominance (λ)	Structure index	Enrichment index	Channel index	Plant parasitic nematodes (%)
Untreated	0	1.48 bc	0.49 bcd	47 b	67 def	20 abc	48 a
Banana residue	40	1.70 a	0.43 cde	78 a	81 b	10 cde	9 e
Biosolid	40	0.93 e	0.62 a	33 c	96 a	1 e	6 e
CaSi	5	1.24 d	0.55 ab	42 bc	71 cdef	17 bc	50 a
Grass hay	40	1.32 cd	0.52 bc	80 a	75 bcde	22 ab	3 e
Legume hay	40	1.32 cd	0.52 bc	55 b	93 a	5 de	6 e
Mill ash	120	1.40 cd	0.52 bc	46 bc	65 f	31 a	47 a
Mill ash	40	1.67 ab	0.49 bcd	51 b	67 ef	29 a	33 bc
Mill mud	40	1.69 ab	0.37 e	72 a	67 ef	16 bc	24 d
Molasses	300 L	1.46 c	0.50 bc	52 b	77 bcd	14 bcd	42 ab
MW Compost	40	1.67 ab	0.41 de	71 a	79 bc	11 bcde	28 cd

Means in columns followed by the same subscript are not significantly different from one another at *P*=0.05.

3.4 Discussion

The application of amendments to soil was found to affect different soil properties depending on the type of amendment applied. Amendments that had high organic carbon contents gave the best results for suppressing the population of *R. similis* in the roots of banana plants. In particular, legume hay, grass hay, banana residue and mill mud treatments were able to significantly reduce the number of *R. similis* in the roots of banana plants relative to the untreated plants. These treatments also had the lowest proportion of plant-parasitic nematodes in the soil, relative to the untreated soil. The suppressive mechanism, which reduced the number of plant-parasitic nematodes in the soil, was unknown, so it was categorised as general suppression. The application of amendments that were high in organic carbon was able to change many of the biological properties in the soil. However, the application of grass hay and banana residue was able to increase the number of omnivorous nematodes, so predation by other nematodes may have had a role in the suppression of *R. similis*.

Stirling *et al* (2005), similarly found suppression of plant-parasitic nematodes in sugar cane soil amended with high amounts of organic carbon. This research was not able to define the suppressive mechanism causing the reduction of plant-parasitic nematodes but postulated that there was an overall shift in the soil food web causing a cascade of changes in biological properties in the soil that could lead to nematode suppression. A change in biological properties in amended soils was also evident in the current investigation, with significantly different numbers of nematodes belonging to different trophic groups, found in soils with different amendments. Similarly, the nematode community indices were also significantly different with the different amendment treatments, with amendments high in C increasing the structure index. Applications of amendments high in carbon appear to be able to induce suppression of plant-parasitic nematodes in the soil and roots of banana plants through changes to the biological composition of the soil food web.

The PCA of the nutrient content of the amendments grouped legume hay, grass hay and the banana residue treatments together (Figure 3-1). The macronutrient content of the amendments was able to explain 63% of the variation in nutrient composition of the amendment (Figure 3-1). The N and C content of the amendments had the highest latent vector loadings from the PCA analysis, which suggested that these two nutrients had the greatest effect on discriminating between amendments. The grass hay, legume hay and banana residue tended to have higher C and N contents, determined by the position and direction of PCA vectors (Figure 3-1). Conversely, the application of mill ash did not suppress *R. similis* (Figure 3-2) and had opposing macronutrient properties relative to the grass, legume hay and the banana residue. From this experiment it appeared that grass hay, legume hay and banana residue, with high carbon content, was able to increase the number of beneficial organisms in the soil.

The biosolid had the lowest C:N ratio, but was able to significantly reduce the number of plant-parasitic nematodes in the soil. However, the addition of biosolid was phytotoxic to banana plants, causing a reduction in plant growth in the first 8-weeks of the experiment. The application of biosolids also caused significant changes in nematode community composition with an increase in the number of bacterivores, a decline in the number of predators and omnivores and reduction in the diversity and structure indices of the nematode community. This suggested that the reduction in plant-parasitic nematode populations in the soil caused by biosolids may have been due to chemical effects from the decomposition of the biosolid that were toxic to most soil organisms as well as the banana plant and that suppression of plant-parasitic nematode was not due to increased nematode antagonistic organisms. Increased bacterial activity was suggested by Chavarria-Carvajal *et al* (2001) and Lazarovits *et al* (2003) to favour plant-parasitic nematode suppression. However, the increase in bacterial feeding nematodes, mediated through the application of biosolids, was not the best method to develop suppression of *R. similis* in bananas, as the increased bacterial activity was associated with poor plant growth. Therefore, some knowledge of how amendments affect soil biology and how their degradation impacts on the soil biology is important in order to develop strategies to suppress plant-parasitic nematodes in agricultural crops.

Early growth of the banana plants was slowed by most amendments, except mill mud, mill ash and CaSi, relative to plants grown in untreated soil. This is possibly because of the nutrient release rate from the amendments, with nutrient draw-down reducing nutrient availability for early plant growth for amendments with high C:N ratios. Even though the amendments were allowed to stand for two weeks prior to planting bananas, this was not sufficient time to prevent nutrients being immobilised during the initial decomposition of the amendments. However, after 28 days, any effects of nutrient draw-down were overcome and amendments with high carbon contents, banana residue, grass hay and legume hay, produced significantly taller plants than the untreated control. The size of the last fully emerged leaf indicated how the plants were growing at the termination of the experiment. Only the biosolid treatment had a significantly smaller leaf area than the untreated soils. This suggested that any draw-down effects by amendments on early plant growth had been overcome at the conclusion of the experiment, 12-15 weeks after planting. In fact, plant growth had been stimulated by the use of mill mud producing significantly greater shoot and root dry weight. Therefore, the application of amendments could act as a slow release source of nutrients to sustain banana growth. This is particularly important when bananas are grown in the wet tropics, as nutrient leaching due to heavy rainfall can be a serious problem (Kleise *et al*, 1997; Moody and Aitken, 1997).

The soil type used in the experiment was an important variable in determining the growth of the plant and structure of the nematode community following the addition of amendments. Better plant growth was observed in the Innisfail soil series in the first four weeks of the experiment. This could have been due to a higher initial nutrient content relative to the other two soils. At the termination of the experiment poor plant growth was measured in the Coom soil relative to the Innisfail and Mudoo soil types. The Coom soil is described as being poorly drained with poor structure (Cannon *et al* 1992). The poor structure of the Coom soil appears to have been the factor contributing to the reduction in plant growth at the termination of the experiment. There were no significant interactions between amendments and soil type affecting plant growth, which suggested that the rate and types of amendments, or the time period for the experiment were insufficient to improve the structure of the Coom soil for the growth of bananas.

The different soils also had a different inherent nematode community structure. The application of amendments to the soil changed the nematode community structure, but structure of the nematode community responded differently in the different soils. The application of molasses to the Coom soil had little affect on the nematode community composition relative to the untreated soil. In the untreated Mundoo soil, CaSi, molasses and mill ash (40 t ha^{-1}) all had similar nematode community structures. However, grass hay and banana residue had a different nematode community structure. These results highlighted that the application of amendments to different soils may not produce the same result in terms of nematode suppression because of inherent soil biological properties that existed before the amendments were applied. Therefore, there needs to be a better understanding of the soil conditions that favour the increase plant-parasitic nematode antagonistic organisms and how they are stimulated by the addition of amendments.

Further work is required to determine efficacy of amendments in field conditions and to determine how amendments high in C, such as grass hay, can be applied more efficiently and reliably. Furthermore, investigations are required that allow the use of amendments to be incorporated with current agronomic practices in the production of bananas and integrated with other nematode management practices. For example, the use of grassed fallows that are resistant to *R. similis* followed by the incorporation of the organic material may give greater, durable suppression of plant-parasitic nematodes in commercial banana production systems.

3.5 Summary

Radopholus similis is a major obstacle to sustainable banana production in Australia. Traditionally, the nematode has been managed through the use of nematicides, which have the potential to move into the local environment and pose a health risk to farm workers. The use of organic amendments is one method that may reduce the need for nematicides by improving the soil physical, chemical and biological properties and stimulating organisms that are antagonistic to plant-parasitic nematodes. Nine different amendments, mill mud, mill ash (by-products from processing sugarcane), biosolids, compost, banana residue, grass hay, legume hay, molasses and calcium silicate (CaSi) were applied to the three major soil types used to grow bananas in the wet tropics region in a glasshouse experiment. A sample of the different amendments was used to determine their nutrient content and potential to contribute heavy metals to the environment. Banana plants were inoculated with *R. similis* and grown in the soil-amendment mix for 12-weeks before being harvested and assessed for plant growth, plant-parasitic nematodes and soil nematode community characteristics. Significant suppression of plant-parasitic nematodes occurred in soils amended with legume hay, grass hay, banana residue and mill mud relative to untreated soil. These amendments were found to have the highest N and C content. The application of banana residue and mill mud significantly increased shoot dry weight at the termination of the experiment relative to untreated soil. Furthermore, the applications of banana residue, grass hay, mill mud and MW

compost were able to increase the nematode community structure index, indicating greater potential for suppression of plant-parasitic nematodes through antagonistic activity. The application of amendments that are high in C and N appear to be able to induce suppression of plant-parasitic nematodes in bananas, by developing a more favourable environment for antagonistic organisms.

4 Organic matter induced changes in soil chemical properties and nematode community composition associated with the suppression of *Radopholus similis* on banana.

4.1 Introduction

One method of manipulating soil conditions is the use of amendments. There are many reports of amendments being added to soil and causing suppression of plant-parasitic nematodes (Akhtar and Mahmood 1996; Akhtar and Malik 2000; Hallmann *et al.* 1999; Sikora 1992; Stirling *et al.* 2005). The use of amendments has been able to change many soil properties (Moss *et al.* 2002), but it is unclear which of these changes are related to the suppression of nematodes. The previous chapter (Chapter 3) identified amendments that were high in organic carbon as having the ability to suppress *R. similis* within the roots of banana plants regardless of soil type.

Organic matter has a complex composition of different compounds that do not behave the same way in soil (Weil and Magdoff 2004). The application of organic matter has been an unreliable method of managing plant-parasitic nematodes giving differences in efficacy of nematode control (Chapter 1). Because of the complex nature of organic materials, the diversity of different sources and the variation in its composition, there are inconsistent results regarding their effectiveness for suppression of plant-parasitic nematodes (Akhtar and Malik 2000). Organic matter can be composed of compounds that are easily degraded such as sugars and starches and compounds that are resistant to degradation such as tannins, lignins and other compounds rich in polyphenolics (Coleman *et al.* 2004). Cellulose and hemi cellulose compounds are considered to be intermediate in their degradability (Coleman *et al.* 2004). Organic matter is made up of a range of organic compounds and so it tends to be decomposed by a range of different organisms (Coleman *et al.* 2004; Dilly 2004). Organic matter, freshly added to the soil, has vastly different properties compared to organic matter that has already undergone some decomposition (Kennedy *et al.* 2004).

It has been suggested that the quality of organic amendments is largely determined by its carbon to nitrogen ratio (Chen and Ferris 2000; Ferris and Matute 2003; Ferris *et al.* 1998; Wang *et al.* 2006; Wang *et al.* 2007). Organic amendments with a C:N ratio less than 20:1 are easily mineralised (Ferris and Matute 2003; Wang *et al.* 2006). When C:N ratios exceed 22:1-32:1 N immobilisation of soil nutrients occurs (Ferris *et al.* 1998). Two scenarios are possible when additional N is added with amendments. Firstly, the addition of nitrogen will increase microbial activity, therefore increasing the suppressive potential of the amendment. Secondly, the high availability of N may favour opportunistic *r* selective organisms, reducing the suppressive potential of the amendments. In general, predatory and parasitic organisms which favour balanced nematode communities, survive best in stable soil conditions and hence tend to be more K selected than organisms produced by transient high N levels (Sánchez-Moreno and Ferris 2007; Stirling and Pattison 2008). The application of nitrogen is often used to optimise agriculture production. Therefore, the effects of nitrogen application may be positive in terms of plant nutrition, but detrimental to the ability of the soil to suppress disease organisms.

Following amendment of the soil with organic matter it is unclear which of the changes in soil properties are associated with the nematode suppression. However, it is known that addition of organic matter causes changes in nematode community compositions and that soil nematodes are key organisms involved in the mineralisation of nutrients from organic material (Yeates and Pattison 2006). It has been suggested that changes in soil properties, like bacterial composition or nutritional status of the soil following the addition of amendments, may change the suppressive potential of agricultural soils (Chavarria-Carvajal *et al.* 2001; Hallmann *et al.* 1999; Lazarovits *et al.* 2003). Furthermore, secondary compounds produced from the decomposition of organic matter have also been shown to have nematicidal effects on plant-parasitic nematodes (Browning *et al.* 2004; Browning *et al.* 2006). Stirling *et al.* (2005) suggested that large quantities of amendments were needed, in the range of 10 t C ha^{-1}, to successfully alter soil properties in favour of nematode antagonistic organisms. However, this investigation did not determine the characteristics of organic amendments or determine which soil properties were influenced by the additions of amendments to increase the suppressive potential of agricultural soils to plant-parasitic nematodes.

4.1.1 Aim

The objectives of the following greenhouse trials were to determine changes in selected soil chemical properties and the nematode community following amendment of soil with organic matter and the effect this had on the suppression of *R. similis* on bananas.

4.2 Materials and methods

4.2.1 Experiment 1: Additional C and N in the form of organic matter and urea fertiliser

4.2.1.1 Establishment

A pot experiment was established in January 2004 where *in vitro* banana plants (*Musa* spp. AAA subgroup Cavendish cv. Williams) obtained from Slocombe's QBan nursery, Gordonvale north Queensland were grown in a 50% field soil (Mundoo series, 49% sand, 10 % silt, 41% clay, pH 4.9) and coarse sand mix. Additional organic matter, equivalent to 10 t ha^{-1} of carbon (69.8 g of grass hay in 200 mm diameter pot, 45% C, 2.9% N, 1.3% P, 2.3% K) was added or the soil was left untreated. The grass hay was mixed in with the soil 2 weeks prior to planting bananas to allow microbial degradation to occur and settling of pot contents. Plants were inoculated with 509 motile *R. similis* per pot at the time of planting bananas. A basal rate of 2 g per pot of Osmocote® Mini Plus was also applied at planting. This was equivalent to 102 N, 53 P and 70 K kg ha^{-1}. A further application of nitrogen, equivalent to 400 kg N ha^{-1}yr^{-1} was applied to half of the pots in a weekly application of urea as a solution (0.0525 g urea plant^{-1} week^{-1}). All treatments were replicated six times in a randomised block. Plants were watered with a fixed sprinkling system twice a day for 10 minutes. A tray was placed at the base of the pots to capture any leachate. Plants were maintained in the glasshouse at an ambient air temperature 22-31 ^{o}C, in a randomised block for 12-weeks after inoculating with nematodes.

4.2.1.2 Trial assessment

During the progress of the experiment, 4 weekly measurements were made on plant height and leaf emergence rate. At the end of the experiment plants were destructively harvested. Plant height, leaf emergence, the size of the last fully emerged leaf (width at widest point x length x 0.83 (Turner 1972), shoot dry weight (dried at 75 ^{o}C for 5 days) and root fresh weight were determined. Soil samples were taken from each pot at harvest for soil chemical properties and nematode community analysis. A soil sample was used to determine soil pH, electrical conductivity (EC) and labile carbon. The soil pH and EC were determined from a 1:5 soil to water mix using distilled water, shaken for 2 minutes and tested using a pH and EC meter (TPS Pty Ltd). The labile C was determined as the amount of carbon readily oxidised by $KMnO_4$, using the method described by Weil *et al.* (2003). A 5 g sample of air dried soil was added to a tube containing 2.0 mL of a 0.2 M $KMnO_4$ solution, in 1 M $CaCl_2$ (pH 7.2) and made up to 20 mL with distilled water. This was shaken for 2 minutes and then allowed to stand for 10 minutes. A 0.5 mL aliquot, taken from the upper 1 cm of the suspension, was transferred to 45 mL of distilled water and made up to 50 mL and mixed thoroughly. The absorbance of the solution was then determined on a spectrophotometer (Hach) at 550 nm and compared to a standard curve to determine the amount of Labile C in the soil. Since carbon was used to measure and determine organic matter content, further references in the succeeding text and tables will be to carbon which was the property measured.

The root systems of the banana plants were washed free of soil and the fresh weight determined before nematodes were extracted using a misting technique for seven days (Hooper 1986). *R. similis* were extracted from the root systems and viewed using a compound microscope and recorded as males and females. Juvenile *R. similis* that were not able to be able to be morphologically separated into sex were assumed to be female.

Nematodes were extracted from the soil by placing 200 g of field moist soil on a single layer of tissue, contained within a mesh basket. The basket was placed in 200 mL of water within a tray. After 48 hours nematodes contained within the water of the tray were collected on a 25 µm sieve (Whitehead and Hemming 1965). The nematodes were backwashed from the sieve and collected in a 30 mL vial. The total number of nematodes extracted from 200 g of soil was determined. Nematodes were identified to genera using a compound microscope and assigned to trophic groups (Yeates 2001; Yeates *et al.* 1993). Plant-parasitic nematodes were identified to species. The Shannon-Weiner diversity index, dominance index and ratio of bacterial to fungal feeding nematodes were calculated from identification data (Yeates and Bongers 1999).

4.2.2 Experiment 2: Different forms of carbon

4.2.2.1 Trial establishment

A pot experiment was established in July 2004 where in vitro banana plants (*Musa* spp. AAA subgroup Cavendish cv Williams) were grown in a Ferrosol soil from Redlands Research Station in south-east Queensland (36% sand, 21% silt, 43% clay, pH 5.3, 3% organic C). Carbon was added to the soil in three different forms; sucrose, cellulose or lignin or an equal mixture of all 3 (90 g of amendment in 1.5 L of soil in 200 mm pots) or the soil was left untreated. The lignin was added as very finely chopped balsa wood. The sucrose and cellulose used were laboratory grade (Aldrich Chemical Company Inc., USA). The treatments were allowed to stabilise for 2 weeks in the glasshouse after the amendments were added and then planted with 13-week old *in vitro* banana plants (*Musa* spp. AAA subgroup Cavendish cv. Williams) obtained from Slocombe's QBan nursery, Gordonvale north Queensland. Banana plants were inoculated 17 days after planting with 500 motile *R. similis* per pot. All treatments were replicated six times in a randomised block design. A basal rate of 2 g per pot of Osmocote® mini plus was also applied at planting. This was equivalent to 102 N, 53 P and 70 K kg ha^{-1}. Plants were hand watered in the glasshouse, with a tray at the base of each pot to capture any leachate. Plants were maintained in the glasshouse at an ambient air temperature 17-28 ^{o}C, for 12-weeks after inoculating with nematodes.

4.2.2.2 Assessment

During the progress of the experiment, 4-weekly measurements were made on plant height and leaf emergence rate. At the end of the experiment plants were destructively harvested. Plant height, leaf emergence, the size of the last fully emerged leaf (width at widest point x length x 0.83 (Turner 1972), shoot dry weight (75 ^{o}C for 5 days) and root fresh weight were determined.

The root systems of the banana plants were washed free of soil and the fresh weight determined before nematodes were extracted using a misting technique for seven days (Hooper 1986). *R. similis* were extracted from the root systems and viewed using a compound microscope and recorded as males and females. Again juvenile *R. similis* that were not able to be able to be morphologically separated into sex were assumed to be female.

Soil samples were taken from each pot for soil chemical properties (pH, EC and Labile C). Soil pH and EC were determined in a 1:5 soil to water mixture using 30 g of soil, which was shaken for 2 minutes with 150 mL of distilled water. The pH and EC were measured using portable meters (TPS Pty Ltd). Soil NO_3-N was determined from the filtered extract of the soil water mixture using test strips (Aquacheck™, Hach company, Loveland, USA).

The soil nematode community composition was determined as described previously. Nematodes were extracted from the soil by placing 200 g of field moist soil on a single layer of tissue, contained within a mesh basket. The basket was placed in 200 mL of water within a tray. After 48 hours nematodes contained within the water of the tray were collected on a 25 µm sieve (Whitehead and Hemming 1965). The nematodes were backwashed from the sieve and collected in a 30 mL vial. The total number of nematodes extracted from 200 g of soil was determined. Nematodes were identified to genera using a compound microscope and assigned to trophic groups (Yeates 2001; Yeates *et al.* 1993). Plant-parasitic nematodes were identified to species. The Shannon-Weiner diversity index, dominance index and ratio of bacterial to fungal feeding nematodes were calculated from identification data (Yeates and Bongers 1999).

Additionally, the weighted faunal analysis concept was applied, as previously described in chapter 2, without plant feeders, to determine the basal, structure, (SI) enrichment (EI) and channel indices (CI) of the nematode community (Ferris *et al.* 2001).

4.2.3 Statistics

Plant, soil and nematode properties were analysed using a three-way ANOVA for experiment 1 with carbon, nitrogen and *R. similis* as the main factors and tested for interactions. In experiment 2, a one-way AOV was used with carbon type as the main factor. If significant differences were found, means were separated using the LSD test using Genstat 8 statistics package (Lawes Agricultural Trust). Nematode numbers from roots and soil were transformed using ln(x+1), prior to analyses to comply with assumption of normally distributed data. Back transformed means were then presented. Simple linear correlation of soil properties and nematode measurements were determined using the statistical package Genstat 8 (Lawes Agricultural Trust).

4.3 Results

4.3.1 Experiment 1: Additional C and N in the form of organic matter and urea fertiliser

4.3.1.1 Soil chemistry

The addition of organic matter to the soil significantly altered soil chemical and biological properties relative to the untreated soil. The additional organic matter reduced pH by 0.4 units, increased EC 10-fold and doubled the amount of labile C measured in the soil (Table 4-1). The addition of extra nitrogen as weekly urea applications had no significant effect on the soil chemical properties (Table 4-1). Although nitrate-N was higher in the treatments with extra nitrogen treatment, the difference was not significant.

Table 4-1 Soil chemical properties from soil with and without additional C and N in the form of organic matter and urea fertiliser

Treatment		**pH**	**EC (dS cm^{-1})**	**Labile C (mg kg^{-1})**	**Nitrate N (mg kg^{-1})**
Carbon	+	5.8 b	0.022 a	594 a	27.5 ns
	-	6.2 a	0.002 b	281 b	17.9 ns
Nitrogen	+	6.0 ns	0.012 ns	433 ns	27.3 ns
	-	6.0 ns	0.012 ns	441 ns	18.1 ns
R. similis	+	6.0 ns	0.015 ns	451 ns	29.4 ns
	-	6.0 ns	0.009 ns	423 ns	16.0 ns
LSD		0.09	0.005	46.8	5.6

Means in columns with the same subscript are not significantly different from one another at P=0.05.
ns indicates means are not significantly different from one another P>0.05.

4.3.1.2 Nematode community

The addition of carbon to the pots significantly reduced the number of female, male and total *R. similis* recovered from the roots of banana plants (Figure 4-1). The addition of both carbon with nitrogen significantly reduced *R. similis* numbers in the roots relative to the addition of carbon without additional N (Figure 4-1). However, the addition of the carbon alone was able to reduce numbers of *R. similis* by almost 10-fold compared to treatments not receiving additional carbon (Figure 4-1). Additional nitrogen had little impact on the *R. similis* population in the roots without the presence of the extra carbon (Figure 4-1). There were no significant differences between male and female *R. similis* in their response to additional organic matter.

There was no significant difference in the total number of nematodes recovered from the soil, with or with out additional organic matter, probably due to the disturbance of the soil by mixing with sand (Table 4-2). Furthermore, there was not a well developed nematode community structure. However, changes were observed in trophic groups and the diversity of nematodes where the addition of the organic matter significantly altered the soil nematode community (Table 4-2). The additional organic matter decreased the number of plant-parasitic nematodes, predominately *Rotylenchulus reniformis*, and increased the number of bacterial and fungal feeding nematodes relative to soil that did not receive additional organic matter (Table 4-2). Furthermore, there was an increase in the diversity, decrease in the dominance and increase in the ratio of bacterial to fungal feeding nematodes in the organic matter treated soil relative to the untreated soil (Table 4-3).

Figure 4-1: *Radopholus similis* females, males and total, recovered from the roots of banana plants grown for 12 weeks in soil with or without additional carbon added as organic matter (+/-C) and nitrogen added as urea (+/-N). (Columns with the same letter above, in the same gender nematode category, were not significantly different from one another at *P*=0.05).

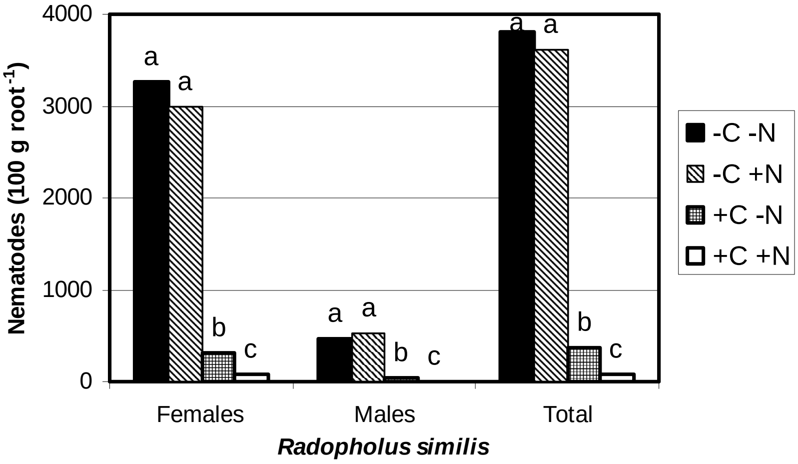

Table 4-2: Soil nematode community composition recovered from soil with or without additional carbon added as organic matter, nitrogen added as urea and *R. similis*.

Treatment	Nematodes 100 g soil^{-1}											
	Carbon				Nitrogen				*R. similis*			
	+		-		+		-		+		-	
Total nematodes	212	ns	204	ns	268	ns	226	ns	263	ns	231	ns
Total plant parasites	32	b	163	a	97	ns	76	ns	69	b	105	a
R. similis	5	ns	5	ns	5	ns	5	ns	10	a	0	b
R. reniformis	25	b	153	a	90	ns	67	ns	56	b	104	a
Total bacterivores	223	a	24	b	102	ns	99	ns	138	a	69	b
Cephalobidae	115	a	17	b	70	ns	47	ns	76	ns	43	ns
Panagrolaimidae	22	ns	1	ns	6	ns	15	ns	18	ns	3	ns
Rhabditidae	81	a	2	b	31	ns	41	ns	50	a	23	b
Total fungivores	40	a	14	b	30	ns	22	ns	25	ns	28	ns
Aphelenchidae	40	a	14	b	30	ns	22	ns	25	ns	28	ns

Means in rows with the same subscript are not significantly different from one another at *P*=0.05. ns indicates that means are not significantly different from one another *P*>0.05. Means are back-transformed from an ln(x+1) transformation used prior to ANOVA.

Table 4-3: Nematode diversity, dominance and ratio of bacterivores to fungivores recovered from soil with or without additional carbon added as organic matter, nitrogen added as urea and *R. similis*.

Treatment		**Diversity**		**Dominance**		**B/(B+F) ratio**	
Carbon	+	1.13	a	0.38	a	0.82	a
	-	0.73	b	0.06	b	0.64	b
Nitrogen	+	0.93	ns	0.23	ns	0.76	ns
	-	0.93	ns	0.21	ns	0.71	ns
R. similis	+	0.96	ns	0.27	a	0.77	ns
	-	0.90	ns	0.17	b	0.70	ns
LSD		0.21		0.09		0.15	

Means in columns with the same subscript are not significantly different from one another at *P*=0.05. ns indicates that means are not significantly different from one another *P*>0.05.

4.3.1.3 Plant growth

The improved plant growth of bananas following the addition of organic matter was evident at the 28-day assessment, by the increased plant height and number of leaves that had emerged (Table 4-4). The additional nitrogen, added weekly, significantly increased the plant growth parameters at the termination of the experiment (Table 4-4 and 4-5). The weekly applications of nitrogen had little impact on plant growth in the first 55 days of the experiment (Table 4-4).

The additional organic matter significantly increased the weight of shoots, roots and the leaf area of the last fully emerged leaf of the banana plants compared to the untreated plants (Table 4-5). Similarly, the addition of nitrogen increased the shoot and root weight and the leaf area (Table 4-5). However, there was no interaction between additional nitrogen and organic matter on plant growth parameters of banana plants. The addition of *R. similis* significantly reduced root weight and the leaf area of the banana plants relative to the uninoculated plants, but did not reduce shoot dry weight (Table 4-5).

Table 4-4: Vegetative growth parameters of bananas grown in soil with and without additional carbon, nitrogen and *R. similis*.

Treatments		**Plant height (cm)**			**Leaf emergence rate**		
		28 days	**55 days**	**90 days**	**28 days**	**55 days**	**90 days**
Carbon	+	27.9 b	33.8 b	50.5 b	5.2 b	2.6 b	2.0 ns
	-	17.5 a	23.4 a	32.9 a	4.4 a	2.3 a	2.2 ns
Nitrogen	+	23.3 ns	29.5 ns	43.4 b	4.9 ns	2.6 b	2.1 ns
	-	22.0 ns	27.6 ns	40.0 a	4.8 ns	2.4 a	2.1 ns
R. similis	+	22.0 ns	27.9 ns	40.4 a	4.8 ns	2.4 a	2.1 ns
	-	23.4 ns	29.2 ns	42.9 b	4.8 ns	2.6 b	2.0 ns
LSD		2.50	2.50	2.26	0.24	0.21	0.28

Means in columns with the same subscript are not significantly different from one another at *P*=0.05. ns indicates that means are not significantly different from one another *P*>0.05.

Table 4-5: Plant growth parameters of bananas grown for 12 weeks in soil with and without additional carbon, nitrogen and *R. similis*.

Treatment		**Dry shoot weight (g)**	**Fresh root weight (g)**	**Leaf area (cm^2)**
Carbon	+	55.2 b	184.8 b	1031 b
	-	17.8 a	83.0 a	435 a
Nitrogen	+	39.9 b	142.4 b	782 b
	-	33.0 a	125.4 a	684 a
R. similis	+	35.1 ns	123.2 a	707 a
	-	37.8 ns	144.6 b	759 b
LSD		3.83	10.25	53.65

Means in columns with the same subscript are not significantly different from one another at *P*=0.05. ns indicates that means are not significantly different from one another *P*>0.05.

4.3.1.4 Correlations

There were significant correlations between the soil chemical properties that were measured in the experiment, labile C, pH and EC (Table 4-6). Soil pH decreased with increasing labile C, whereas the soil EC increased with increasing labile C (Table 4-6). Increasing the labile C content in the soil was positively correlated with increased plant growth parameters (Table 4-6). However, the plant growth parameters were negatively correlated to increasing soil pH (Table 4-6).

The number of *R. similis* in the roots of banana plants and the number of plant-parasitic nematodes in the soil were negatively correlated with labile C measured in the soil (Table 4-6 and Figure 4-2). Conversely, the number of bacterivores and the dominance index increased with increasing soil labile C (Table 4-6).

Table 4-6: Linear regression of soil properties, plant growth responses and nematode community composition where banana plants were grown in soil with or without additional carbon added as organic matter, nitrogen added as urea and *R. similis*.

Response variates	Constant	Explanatory variable		R^2	Probability
Soil properties					
EC	-0.01343	0.00006	x Labile C	50.7	<0.001
pH	6.41	-0.0009	x Labile C	48.7	<0.001
Plant growth properties					
Height	21.59	0.0457	x Labile C	66.1	<0.001
Leaf area	71.2	1.509	x Labile C	68.5	<0.001
Root fresh wt.	17.8	0.267	x Labile C	71.4	<0.001
Shoot dry wt.	-6.49	0.0983	x Labile C	70.9	<0.001
Height	208	-27.71	x pH	41.3	<0.001
Leaf area	6240	-916	x pH	42.9	<0.001
Root fresh wt.	1134	-166.3	x pH	45.5	<0.001
Shoot dry wt.	378	-56.8	x pH	39.5	<0.001
Nematode properties					
Bacterivores (soil)	-0.342	0.0024	x Labile C	32.3	<0.001
Dominance (soil)	-0.165	0.0009	x Labile C	48.3	<0.001
Plant-parasitic nematodes (soil)	1.48	-0.0019	x Labile C	46.4	<0.001
R. similis (roots)	8872	-14.245	x Labile C	50.9	<0.001

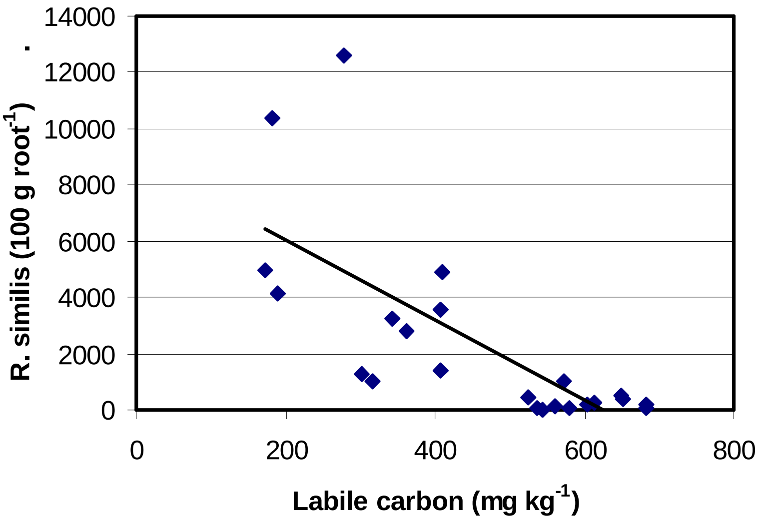

Figure 4-2: Linear regression of labile C and the number of *R. similis* recovered from the roots of bananas, with and without additional carbon added as organic matter and nitrogen added as urea (R^2=0.51 *P*<0.001).

4.3.2 Experiment 2: Different forms of carbon

4.3.2.1 Plant growth

The sucrose treatment caused plant death within the first 28 days of replanting bananas in amended soil. Therefore, no plant measurements were possible from this treatment and it was not included in the analysis of plant growth. A reduction in plant growth was evident within the first 28 days in the soils with additional carbon, particularly in the lignin and the mixed carbon treatments, causing a significant reduction in plant height relative to the untreated soil (Table 4-7). The plants remained significantly shorter in the lignin and mixed carbon source treatments than the untreated plants throughout the experiment (Table 4-7). The cellulose treatment reduced plant height, but the banana plants were not significantly shorter than the untreated soil until the final plant assessment at the termination of the experiment (Table 4-7).

The leaf emergence rates were reduced in the untreated soil relative to the soils amended with cellulose throughout the experiment (Table 4-7). The lignin and mixed carbon treatments initially had a leaf emergence rate superior to the untreated soil at the 28 day assessment (Table 4-7). However, at the termination of the experiment the leaf emergence rate of the lignin and mixed carbon treatments were similar to the untreated plants, with the lignin treatment having a leaf emergence rate significantly lower than the cellulose treatment (Table 4-7).

The application of the different forms of carbon caused a reduction in plant growth compared to the untreated soil (Table 4-8). Furthermore, the dry shoot weight in the lignin and mixed carbon treatments was significantly less than the cellulose treated soil (Table 4-8).

Table 4-7: Vegetative growth parameters of bananas grown in soil with and without additional carbon, nitrogen and *R. similis*.

Treatments	**Plant height (cm)**			**Leaf emergence rate**		
	28 days	**56 days**	**91 days**	**28 days**	**56 days**	**91 days**
Untreated	10.6 a	10.6 a	12.1 a	0.2 b	0.2 b	0.2 b
Cellulose	9.6 ab	9.8 ab	7.4 b	0.3 ab	0.6 a	0.6 a
Lignin	7.3 c	7.3 c	5.3 c	0.4 a	0.2 b	0.2 b
Mixed	8.7 bc	8.7 bc	6.7 b	0.5 a	0.6 a	0.4 ab
LSD	1.5	1.5	1.3	0.2	0.2	0.3

Means in columns with the same subscript are not significantly different from one another at *P*=0.05.

Table 4-8: Plant growth parameters of bananas grown for 12 weeks in soil with and without additional carbon, nitrogen and R. similis.

Treatment	**Dry shoot weight (g)**	**Fresh root weight (g)**	**Leaf area (cm^2)**
Untreated	5.24 c	40.3 b	97.6 b
Cellulose	2.76 b	31.2 a	37.2 a
Lignin	2.10 a	29.8 a	33.2 a
Mixed	2.20 a	31.5 a	35.3 a
LSD	0.52	7.73	9.34

Means in columns with the same subscript are not significantly different from one another at *P*=0.05.

4.3.2.2 Soil chemistry

The soil chemical properties were measured at the time of planting bananas, four weeks after incorporation of carbon amendments into the soil and again at the termination of the experiment, 12 weeks after planting bananas. There was a difference in the pH, EC and labile C measured in the different treatments at the 4 and 12 week assessment times (Table 4-9). Four weeks after the incorporation of the carbon sources, sucrose significantly decreased the soil pH relative to all other treatments (Table 4-9). The sucrose treatment also had the highest EC and labile C measurements relative to the other treatments in the experiment (Table 4-9). The cellulose had the lowest labile C measured in the soil two weeks after incorporating the carbon sources (Table 4-9). At the final assessment, 12 weeks after the commencement of the experiment, the mixed carbon sources had the lowest soil pH and the lignin treatment had the highest labile C, significantly greater than the untreated soil (Table 4-9). Conversely, at the termination of the experiment the cellulose treatment had the highest soil pH and the lowest labile C content (Table 4-9).

Table 4-9: Soil pH, EC and labile C in soil treated with sucrose, cellulose and lignin or a mixture of carbon sources relative to untreated soil 4 and 12 weeks after commencement of the experiment.

Treatment	pH		EC (µS cm^{-1})		Labile C (mg kg^{-1})	
4-weeks						
Untreated	6.4	a	0.12	b	617	abc
Sucrose	4.9	b	0.52	a	669	a
Cellulose	6.4	a	0.11	b	552	c
Lignin	6.4	a	0.11	b	643	ab
Mix	6.6	a	0.10	b	584	bc
LSD	0.4		0.14		65.6	
12-weeks						
Untreated	6.8	ab	0.25		566	cd
Sucrose	6.7	ab	0.26		591	bc
Cellulose	6.8	a	0.26		549	d
Lignin	6.7	bc	0.24		635	a
Mix	6.6	c	0.28		628	ab
LSD	0.12		n.s.		40	

Means in columns with the same subscript are not significantly different from one another at *P*=0.05.

4.3.2.3 Nematode community

Due to the death of banana plants treated with sucrose in the first 28 days of the experiment, no results were obtained on the effect of sucrose on the suppression of *R. similis* in the roots of banana plants. However, the number of *R. similis* recovered from the roots of bananas grown in the cellulose and mixture of carbon treatments was significantly lower than the untreated or the lignin amended soil (Figure 4-3). The application of lignin gave some reduction in nematode numbers, but this was not significantly different from the untreated soil (Figure 4-3).

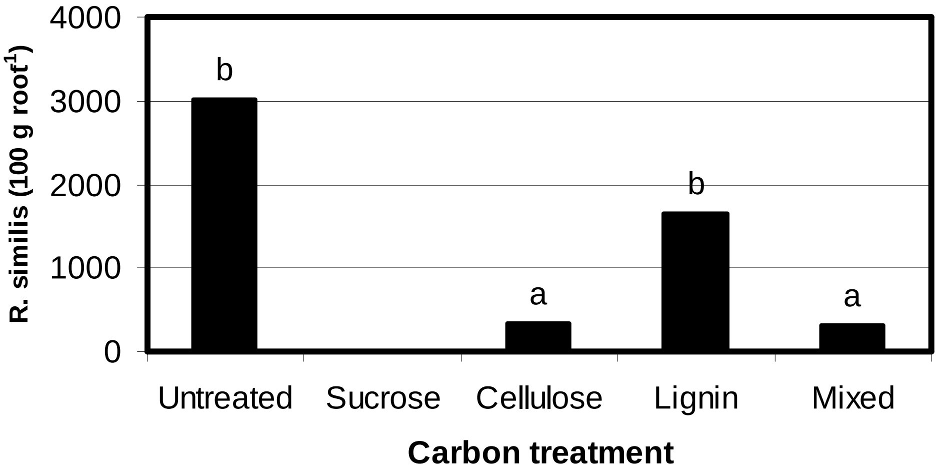

Figure 4-3: *R. similis* recovered from the roots of banana plants grown for 13 weeks in soil with different forms of carbon added or mixed relative to untreated soil. (Columns with the same letter are not significantly different from one another at *P*=0.05).

The addition of the different carbon forms significantly altered the nematode community composition in the unsterilized field soil (Table 4-10). There were no plant-parasitic nematodes recovered from the soil samples from the different carbon treated soils. Therefore, this group of nematodes was not included in the analysis of nematode trophic groups. There was a significantly greater total nematode population in the cellulose treated soil relative to the untreated, lignin and mixed carbon treatments (Table 4-10). Furthermore, the addition of cellulose significantly increased the number of fungivores and omnivores in the soil compared to the other forms of carbon and the untreated soil (Table 4-10). The addition of sucrose significantly increased the number of bacterivores in the soil, doubling the numbers in the untreated soil, whereas the lignin and mixed carbon sources significantly reduced the number of bacterivores, relative to the untreated soil (Table 4-10). The highest number of predators was found in the mixed carbon sources treatment, with no predatory nematodes recovered from the sucrose treatment (Table 4-10).

The change in the different numbers of fungivores and bacterivores resulted in a change in the B/(B+F) ratio and the channel index (Table 4-10). The diversity of nematodes was greatest in the untreated soil and lowest in sucrose amended soils (Table 4-10). Conversely, the dominance of nematode genera was lowest in the untreated soil and greatest in the sucrose amended soil (Table 4-10). The structure index was the greatest where a mixture of carbon types had been added to the soil and no nematode community structure was determined in the sucrose treatment (Table 4-10).

Table 4-10: Soil nematode trophic groups and nematode community indices from soil amended with different forms of carbon compared to untreated soil, 12-weeks after planting bananas.

Nematodes	**Untreated**	**Sucrose**	**Cellulose**	**Lignin**	**Mix**
Total nematodes	221 (2.95) b	436 (4.78) ab	574 (8.45) a	77 (1.63) c	75 (1.10) c
Bacterivores	138 (1.96) b	411 (3.47) a	178 (2.31) ab	13 (0.37) c	10 (0.21) c
Fungivores	15 (0.29) b	0 (0.01) c	168 (2.84) a	14 (0.84) b	1 (0.02) c
Omnivores	8 (0.19) bc	2 (0.12) cd	80 (1.75) a	13 (0.29) b	1 (0.04) d
Predators	17 (0.32) ab	0 (0.00) c	8 (0.39) b	5 (0.18) b	46 (0.83) a
Indices					
Diversity	1.60 a	0.50 d	1.37 ab	1.08 bc	0.76 cd
Dominance	0.28 c	0.72 a	0.33 bc	0.44 b	0.60 a
B/F ratio	0.85 b	1.00 a	0.52 c	0.49 c	0.90 ab
Enrichment	84 n.s.	97 n.s.	74 n.s.	75 n.s.	77 n.s.
Structure	60 b	0 c	62 b	76 b	97 a
Channel	7 c	0 c	30 ab	37 a	14 bc

Means in rows with the same subscript are not significantly different from one another at *P*=0.05. n.s. denotes no statistical difference between treatments. Numbers in parenthesis are the back transformed means on which statistical analysis was conducted.

4.4 Discussion

The addition of organic matter with high carbon content was able to suppress the population of *R. similis* in the roots of bananas. This finding confirmed previous results in Chapter 3, where general suppression of plant-parasitic nematodes could be induced in soil by the addition of amendments that had a high carbon to nitrogen ratio. The addition of carbon to the soil, in the form of grass hay, was able to significantly reduce the number of *R. similis* in the roots of banana plants. Furthermore, it was found that carbon amendments that were high in cellulose were most effective at inducing suppression of *R. similis* over a 12 week period. The decomposition of the organic amendments tended to increase the labile C component in the soil. In the first experiment, where grass hay was added to the soil, there was a significant decrease in *R. similis* numbers recovered from the roots of banana plants and an increase in the amount of labile C measured in the soil. However, this relationship did not occur in the second experiment where different forms of carbon were added to the soil, even though it was expected that the different forms of carbon would provide a range of soil labile C levels. This suggested that the amount of labile C in the soil may be an important indicator of soil conditions that lead to suppression of plant-parasitic nematodes, but that other factors also are involved in the suppression of the nematodes.

The mechanism by which the organic matter caused the suppression of nematodes is unknown as the additional organic matter caused a variety of significant changes in the soil chemical and biological soil parameters measured in this experiment. The addition of grass hay decreased soil pH, increased EC, increased labile C and led to higher soil N levels detected in the soil. However, different forms of carbon induced different reactions within the soil. Rapidly degradable forms of carbon such as sucrose, produced a rapid decrease in soil pH and increase in EC and labile C. This appeared to stimulate fast growing organisms, with a greater number of bacterivores in the sucrose treatment, but no change in the nematode community structure. However, the slower degrading forms of carbon, like lignin, did not change soil chemical and biological properties. In soil treated with lignin, the pH and EC were not significantly different to the untreated soil throughout the experiment. Similarly, there was no increase in different trophic groups of nematodes or change in the structure index of the nematode community when lignin was added to the soil relative to untreated soil. This confers with the findings of Wang *et al.* (2007) who found that cowpea with high lignin content had little impact on soil microbial biomass N and C. This suggested that lignin was relatively inert in this experiment, sucrose was labile and rapidly decomposed and the cellulose was intermediate causing shifts towards a more fungal dominated nematode community. The cellulose treatment may have achieved a C:N ratio closer to 20:1, which was suggested to be optimal for mineralisation of organic matter (Ferris and Matute 2003; Wang *et al.* 2006).

Stirling *et al.* (2005) found greatest suppression of plant-parasitic nematodes in sugar cane soil where fungal activity was greatest. The most efficacious treatments for reduction of the number of *R. similis* in both experiments, additional grass hay in experiment 1 and cellulose in experiment 2, increased the number of fungivorous nematodes in the soil relative to the untreated soil, conferring with the findings of Stirling *et al.* (2005). Furthermore, Stirling *et al.* (2005) suggested that addition of organic matter had a cascading effect on all trophic levels in the soil food web, through diverse interactions between the components ultimately affecting plant-parasitic nematodes. The results from these experiments agreed with complex effects on soil organisms, but suggested that effects can be pushed along different pathways depending on the source of carbon added to the soil and its degradability.

The additional nitrogen added with the organic matter had little impact on soil chemical properties and the nematode community indices. This suggested that the amount of nitrogen applied as weekly applications was insufficient to cause a shift in the biological activity in the soil. Wang *et al.* (2006) similarly found that addition of ammonium nitrate had little impact on nematode community composition in the first year of a field experiment. The quantity of nitrogen applied in the glasshouse experiment was sufficient to stimulate additional plant growth and it is possible that the plants were able to utilise the added nitrogen before it had a significant effect on the nematode community indices. The small amounts of nitrogen added as frequent applications did not decrease the suppressive potential of plant-parasitic nematodes, but conversely increased the suppression of *R. similis* when applied in conjunction with organic matter additions. The extra nitrogen may have stimulated biological activity and decomposition of the organic matter, which may have increased the number of organisms present in the soil that were antagonistic to plant-parasitic nematodes. The additional nitrogen applied in the grass hay may have produced similar soil conditions as those that were developed when cellulose was applied to soil in the second experiment. That is, the activity of soil organisms was not as rapid as occurred with the addition of sucrose, but was more rapid than when only lignin had been added to the soil. This would suggest that to develop conditions that favour general suppression of plant-parasitic nematodes, amendments applied to the soil need to have a specific carbon to nitrogen ratio range as suggested by (Wang *et al.* 2006). Furthermore, the suppression of plant-parasitic nematodes following addition of amendments that were difficult to decompose would benefit from additional nitrogen application to develop a favourable C:N ratio close to 20:1.

It is possible that nematode suppression, as well as being biologically mediated, may have been the result of nemostatic chemicals produced by the decomposition of the additional organic matter. The production of volatile fatty acids, such as butyric acid from the fermentation of organic matter by anaerobic soil bacteria, was found to be a powerful nematicidal product (Browning *et al.* 2004). The decomposition of organic matter, leading to labile C fractions in the soil, was highly correlated with soil pH in both experiments. Where additional organic matter had been applied there was also a reduction in soil pH, particularly when it stimulated bacterial activity in the soil, as occurred in the first experiment and in the second experiment following the addition of sucrose. This suggested that organic acids may have contributed to the reduction in plant-parasitic nematodes and the production of organic acids would explain the decrease in the soil pH, particularly in the sucrose treated soil which resulted in the death of the banana plants. Browning *et al.*(2004) also found that some nematodes, such as bacterivores, were tolerant of high levels of butyric acid relative to plant-parasitic nematodes. The volatile fatty acids are rapidly decomposed under aerobic conditions, which could explain the increase in soil pH between the two soil assessments at week 4 and week 12, in the sucrose treatment. The volatility of the chemicals that result from the decomposition of organic matter makes it difficult to correlate their levels with reduction of nematodes within the roots of the banana plants. However, the reduction of *R. similis* in the roots of the banana plants from the addition of grass hay is most likely a result of combined biological and chemical effects occurring in the soil. The chemical changes are most likely to occur shortly after the addition of organic amendments and to be relatively short lived, whereas the biological changes are longer term mechanisms, which may take more time to reduce nematode populations but may be more persistent over time.

The mixture of organic matter sources exhibited influences on soil parameters that reflected properties the organic matter sources added to the soil. In the mixed treatment, there was a significant suppression of *R. similis* in the roots of the banana plants, which could be related to the cellulose component, but there was also an increase in the amount of labile C and a decrease in soil pH, which occurred when the soil was amended with lignin and sucrose. The mixture of carbon sources was also the treatment with the highest number of predatory nematodes in the soil, which resulted in a significantly higher structure index. As most organic amendments added to agricultural soil, including grass hays, would be a combination of different types of carbon, it would be expected that they would have similar effects on soil properties as the mixed carbon treatment. The mixture of carbon sources would be expected to require a more diverse group of organisms to decompose the organic matter. Complexity of nematode community structure, which is developed by increasing predatory and omnivorous nematodes, was found to be an important indicator in the suppression of plant-parasitic nematodes (Sánchez-Moreno and Ferris 2007).

Although the diversity of soil organisms was not reflected in the nematode diversity index or structure indices, the contribution of omnivores and predators may have been a significant factor relating to the suppression of *R. similis* in the cellulose and mixed carbon treatments.

The addition of organic matter may have led to the immobilisation of nutrients and restricted plant growth in the second experiment. When different forms of carbon were added to the soil, there was a significant reduction in the plant height at the first assessment, 4 weeks after the commencement of the experiment, in all treatments except the cellulose treated soil. However, by the termination of the experiment all treatments had significantly reduced the growth of the banana plants, including plant height, dry shoot weight, leaf area and fresh root weight. Over the 12-week course of the experiment there was no increase in plant height except in the untreated soil, which suggested that soils may have not had sufficient nutrients to sustain decomposition of the amendments and provide for plant growth. Conversely, there was increased growth of banana plants in the first experiment where the grass hay had been added to the soil. This may have resulted from mineralisation of the organic matter applied, which had the potential to add another 2.02 g N, 0.91 g P and 1.60 g K per plant (equivalent to 644 kg N, 288 kg P and 511 kg K ha^{-1}).

The additional organic matter increased the tolerance of banana plants to the damage caused by *R. similis* early in the experiment and helped maintain the dry shoot weight. *R. similis* was pathogenic to banana plants causing a reduction in plant height, root weight and leaf area at the termination of each experiment. However, where organic matter was applied as grass hay in the first experiment or cellulose in the second experiment, there was a delay of at 28 – 56 days before *R. similis* reduced the plant height relative to the untreated soil. This suggested that the application of organic matter to banana soil was able to buffer the plants, albeit temporarily, from the effects of *R. similis*. Furthermore, where *R. similis* was added to the soil in the first experiment, there was a significant increase in the number of bacterivores detected in the soil. This may have been an experimental anomaly or may be a result of increased leakage of nutrients from the roots system caused by the feeding of the nematode and resulting in stimulation of bacterial activity and bacterivorous nematodes.

The results from the two experiments helped to explain the observations from the first experiment, that *R. similis* was suppressed in soils that received amendments with high carbon contents. The suppression of *R. similis* by the addition of organic matter induced greater fungal activity within the soil but caused a simultaneous increase in general microbial activity. Amendments that were rich in cellulose-like compounds, but which had a range in their degradability, appeared to be able to give the greatest and most prolonged suppression of plant-parasitic nematodes. The suppression of nematodes from these types of amendments is most likely mediated through the production of nematoxic compounds that result from decomposition and an increased number of nematode antagonistic organisms. Further work is required to determine if the suppression of plant-parasitic nematodes induced by the addition of organic matter is transferrable to field conditions.

4.5 Summary

Radopholus similis is a major obstacle to sustainable banana production in Australia. Previous work found that amendments that had high carbon contents were able to suppress the number of *R. similis* recovered from the roots of banana plants. Two pot experiments were established to validate and elucidate the suppression that could be induced by the addition of amendments with high carbon content by measuring the changes in soil chemical and biological properties. The first experiment investigated the addition of grass hay with and without additional nitrogen. The second experiment investigated different forms of carbon; sucrose, cellulose, lignin and a mixture of the three, added to the soil. The addition of grass hay significantly altered the chemical and biological properties in the soil. Where additional organic matter was applied there was a decrease in the number of *R. similis* recovered from the roots of banana plants and an increase in the growth of banana plants, confirming the results from previous experiments. There was also a decrease in the soil pH, and an increase in EC and labile C. Furthermore, there was an increase in the number of bacterivores, fungivores and the diversity of nematodes. In the second experiment there was a decrease in the number of *R. similis* when cellulose and a mixture of carbon sources, which included cellulose, were added to the soil. The addition of lignin was not able to significantly suppress the number of *R. similis* relative to the untreated soil, whereas the sucrose treatment was phytotoxic, resulting in complete mortality of the banana plants. The cellulose treatment increased the number of fungivores and omnivores in the soil, but resulted in a lower labile C level in the soil relative to the untreated soil. Rapidly degradable forms of carbon, such as sucrose, increased bacterivores, whereas inert forms of carbon such as lignin had little effect on soil properties, suggesting that biological activity could be manipulated based on the type of organic matter applied to the soil. The suppression of plant-parasitic nematodes following the addition of moderately degradable organic matter, like cellulose and grass hay, appeared to be a combination of nematoxic compounds produced in the early degradation of the organic matter, followed by an increase in nematode antagonists favoured by an increase in soil fungal activity.

5 Suppression of plant-parasitic nematodes on bananas by manipulation of biotic and abiotic soil properties using organic amendments.

5.1 Introduction

The application to soil of organic amendments is viewed as an environmentally favourable waste management strategy that can potentially improve soil quality and benefit agricultural production (Flavel and Murphy 2006; Moss *et al.* 2002). Amendments include a wide range of products such as organic; animal manures, solid wastes and composts and inorganic; fly ash and rock minerals (Janvier *et al.* 2007; Moss *et al.* 2002).

Work in the previous chapters (Chapter 3 and 4) found that amendments with a high carbon content had a suppressive effect on *Radopholus similis* on bananas grown in glasshouse experiments. In particular, the results in Chapter 3 demonstrated that amendments such as grass hay, legume hay, banana trash and mill mud were able to significantly reduce *R. similis* in the roots of bananas. The effects corresponded to changes in the soil nematode community structure. Furthermore, in Chapter 3, amendments with high cellulose content had the greatest suppression of *R. similis*. However, in commercial banana production, farmers must consider whole farm management in the strategies they choose. Therefore, the addition of any amendments must sustain banana production by overcoming inherent soil constraints, such as compaction and low nutrient holding capacity, as well as suppressing pests and diseases.

The addition of amendments to the soil has been used in a number of agriculture crops to manipulate soil properties and overcome soil constraints (Moss *et al.* 2002). Furthermore, amendments have successfully suppressed plant-parasitic nematodes (Akhtar and Malik 2000; Oka and Yermiyahu 2002; Stirling 2008; Stirling *et al.* 2005). However, to understand how amendments may develop conditions that are suppressive to plant-parasitic nematodes and overcome inherent soil constraints to banana production, it is necessary to determine changes that occur in the physical, chemical and biological soil environment. Applications of amendments have been related to changes in physical soil conditions such as the increase in aggregate stability (Albiach *et al.* 2001; Moss *et al.* 2002), increased soil water retention (Moss *et al.* 2002) and increased porosity (Bulluck III *et al.* 2002). Similarly, the application of amendments have been shown to alter chemical soil properties, increasing the nutrient holding capacity (Moss *et al.* 2002; Seiter and Horwath 2004), altering soil pH (Seiter and Horwath 2004), increasing soil N concentrations (Rodriguez-Kabana 1986; Valenzuela-Solano and Crohn 2006) and increasing organic C (Seiter and Horwath 2004).

The application of amendments may be used to manipulate specific soil properties, which could be related to changes in the soil biology (Kennedy *et al.* 2004). For example the application of fly ash was found to enhance crop growth, develop a neutral soil pH and benefit fungi and gram-negative bacteria (Schutter and Fuhrmann 2001). Similarly, the application of coal ash increased soil pH and improved water infiltration but suppressed phosphatase activity in eroded soil (Cox *et al.* 2001). However, in the same study Cox *et al.* (2001) found that the addition of compost gave the greatest improvement in soil quality, increasing soil microbial respiration. The addition of organic substrates in general tended to increase microbial activity. However, the chemical composition of the amendments, particularly the C:N ratio, has been a significant factor in determining the biological changes occurring in the soil (Carpenter-Boggs *et al.* 2000; Chavarria-Carvajal *et al.* 2001; Degens 1998; Valenzuela-Solano and Crohn 2006).

To ensure that the cost of amendments is not prohibitive for use in the agricultural industries, they are usually sourced as wastes from local industries. In north Queensland it is possible to source amendment as waste products from the sugar and poultry industries. By-products from the processing of sugar cane are readily applied as amendments to sugarcane fields (Barnes 1974) and have been shown to increase crop production and reduce numbers of plant-parasitic nematodes (Meunchang *et al.* 2006; Vawdrey and Stirling 1997). Mill mud (also called filter press or filter cake), a by-product from washing the sugarcane before crushing, has been shown to have a favourable chemical composition for promoting plant growth and stimulating microbial activity in the soil (Calcino 1995; Meunchang *et al.* 2006; Rasul *et al.* 2008). Mill ash, resulting from the combustion of dried, crushed sugarcane from the furnaces of the sugar mill, has also been used as an amendment (Barnes 1974; Calcino 1995). Various types of fly ashes, mostly from coal fired power stations, have been investigated for suppression of plant-parasitic nematodes (Ahmad and Alam 1997; Khan *et al.* 1997; Wang *et al.* 2006). Manures, which have been composted in a thermophylic process with a carbon source, are the most common source of compost (Aryantha *et al.* 2000; Canali *et al.* 2004; Jonathan *et al.* 2000). However, the properties of the compost can vary depending on time of processing and feed stocks (Noble and Coventry 2005) and accordingly, have had mixed effects on the suppression of plant-parasitic nematodes (McIntyre *et al.* 2000; McSorley and Gallaher 1995). Crop residue returned to the soil has also been shown to be able to increase microbial activity in the soil and suppress plant-parasitic nematodes (Akhtar and Alam 1992; McSorley and Gallaher 1995; Stirling 2008; Stirling *et al.* 2005). More specifically to bananas, the use of the leaf and pseudostem material, either as a mulch or composted material, improved plant and soil relationships increasing crop production (Formowitz *et al.* 2007; Lekasi *et al.* 1999; McIntyre *et al.* 2000; Ultra *et al.* 2005).

5.2 Objectives

This experiment was established to ascertain changes in soil physical, chemical and biological properties following the addition of amendments to the soil and to determine changes in populations of plant-parasitic nematodes and production of bananas. The experiment was not designed to determine optimum rates of amendments for use in banana production.

5.3 Materials and methods

5.3.1 Site and soils

The experimental site was located on a commercial banana plantation approximately 20 km south of Innisfail, Australia (146^{o} 03′ 33″ E; 17^{o} 42′ 55″S) on a dermosol soil described as a Coom series soil (28% sand, 42% silt and 30% clay) (Cannon *et al.* 1992). The soil was described as being seasonally wet, with moderate drainage and low permeability and requiring drainage and special management for crop production (Simpson *et al.* 2004). The average annual rainfall for the site was 3 384 mm. The site had never grown bananas and had previously been cropped to sugar cane.

5.3.2 Treatments

Four locally available amendments were chosen for the experiment; mill ash, mill mud, composted chicken manure (manufactured on farm) and an organic matter treatment consisting initially of grass hay applications followed by use of crop residues once the crop had become established (Table 5-1). The amendment treatments were compared to a control where the soil surface was kept bare of crop residue. The chemical composition of the four amendments is given in Table 5-1. Soil amendment treatments; compost, mill ash and mill mud were applied on August 28, 2003, prior to planting bananas. The amendments were incorporated into the soil using two passes with a tyned scarified. Bananas (*Musa* AAA, Cavendish sub-group cv. Williams) were planted from vegetative corm pieces on September 7, 2003. Grass hay was applied to the soil surface of the organic matter treatment on September 9, 2003, prior to the emergence of bananas. A repeat application of grass hay was made on January 5, 2004. All amendment treatments were reapplied on November 4, 2004 to the soil surface around the bananas (Table 5-2). The rates and dates of the different soil amendment applications are given in Table 5-2.

Following the first harvest of the banana crop, all plant residue from the untreated plots was removed and placed on the soil surface of organic matter treated plots. This procedure continued throughout the experiment, leaving the untreated plots void of all banana residue on the soil surface. Surface organic matter samples were taken in October 2005 and May 2006, by collecting all surface organic matter in a 0.1 m^2 quadrant. The organic material was then dried in an oven at 75 ^{o}C for 5 days, before the weight was determined.

Table 5-1: Chemical composition of macronutrients and micronutrients of soil amendments.

Amendment	**Total C**	**Total N**	**C/N**	**P**	**K**	**Ca**	**Mg**	**S**
	(%)	**(%)**		**(%)**	**(%)**	**(%)**	**(%)**	**(%)**
Compost	30.5	2.9	10.5	1.28	2.30	4.30	0.67	0.60
Grass hay	45.0	2.2	20.5	0.17	2.50	0.51	0.49	0.49
Mill ash	8.8	0.1	88.0	0.17	0.87	0.86	0.04	0.04
Mill mud	24.7	1.8	13.7	0.70	0.31	1.60	0.12	0.12

Amendment	**Na**	**Cu**	**Zn**	**Mn**	**Fe**	**Al**	**B**	**Si**
	(%)	**(mg kg^{-1})**	**(mg kg^{-1})**	**(mg kg^{-1})**	**(mg kg^{-1})**	**(g kg^{-1})**	**(mg kg^{-1})**	**(%)**
Compost	0.44	168.8	280	410	2800	6.8	16.0	7.4
Grass hay	0.36	5.2	14	200	340	0.5	5.1	1.3
Mill ash	0.05	28.8	55	560	17000	16	21.0	34.5
Mill mud	0.02	38.6	110	1100	19000	18	2.8	14.8

Table 5-2: Treatment application dates, rates of application and amount of C added in the amendments.

Treatment	September 9, 2003		January 5, 2004		November 4, 2004	
Mill ash	220 t ha^{-1}	19 t C ha^{-1}			220 t ha^{-1}	19 t C ha^{-1}
Compost	175 t ha^{-1}	53 t C ha^{-1}			175 t ha^{-1}	53 t C ha^{-1}
Mill mud	120 t ha^{-1}	30 t C ha^{-1}			120 t ha^{-1}	30 t C ha^{-1}
Grass hay	35 t ha^{-1}	16 t C ha^{-1}	50 t ha^{-1}	23 t C ha^{-1}	50 t ha^{-1}	23 t C ha^{-1}

5.3.3 Experimental design and management

The experiment was designed in a randomised block with six replicates, with 22 banana plants in each plot. Each plot consisted of double row of bananas spaced 2 m apart and 2.2 m between plants within rows. The distance between the centres of double rows was 6.5 m, giving a plant density of approximately 1 650 plant ha^{-1}.

The banana plants were maintained under commercial conditions using standard district management practices. Herbicide, (Basta® 200 g L^{-1} glufosinate-ammonium, Bayer CropScience), was used 6-weekly to reduce weed growth around banana plants. Banana plants were fertilised using granular fertiliser through the irrigation system following normal commercial practices to achieve an annual application of 350 kg N ha^{-1}, 36 kg P ha^{-1} and 750 kg K ha^{-1}. Additional irrigation was applied when needed in drier months (August to January). Irrigation was applied through under tree mini-sprinklers in the centre of banana rows. All banana leaf and pseudostem residue remaining after the harvest of banana bunches and during leaf hygiene operations were placed in the row area next to banana plants and kept off the interrow area. Fungicides were applied according to standard commercial practice to reduce the foliar disease yellow sigatoka (*Mycospharella musicola*).

5.3.4 Plant growth

Vegetative plant growth measurements commenced on November 10, 2003. Plant leaf emergence rates were measured on a monthly basis for the plant crop only. Bunch emergence commenced on March 17, 2004 and bunch harvest commenced on July 14, 2004 for the plant crop. The dates the flower emergence and bunch harvested were recorded for the first and two successive ratoon crops. The commercial practice on the farm was to trim the lowest three hands from each bunch at the time of bag placement over the bunch. The finger number per bunch was determined using a modified method described by Turner *et al.* (1988). The number of hands on each bunch was determined by counting the total number of hands present on trimmed bunches or on untrimmed bunches deducting 3 from the total number of hands present. The number of fingers on the third hand from the top was determined and the number of fingers on the last hand of trimmed bunches and fourth from the bottom of untrimmed bunches. The estimation of finger number was then calculated from the average of the third hand from the top and the lower hand, multiplied by the number of hands on each bunch (Turner *et al.* 1988). Recording of bunching times and finger number was conducted on the first two ratoon crops.

5.3.5 Soil measurements

Soil observations commenced on October 20, 2003, 7 weeks after amendments were incorporated into the soil. Soil observations were repeated on March 29, 2004, September 20, 2004, October 10, 2005, and May 8, 2006; 6, 11, 24 and 31 months respectively after bananas were planted. A soil health survey method was developed using basic soil quality indicators (Sarrantonio *et al.* 1996) (http://soils.usda.gov/sqi/soil_quality/assessment/kit2.html). On-site water infiltration was measured with aluminium infiltration rings (150 mm diameter x 125 mm) inserted 75 mm into the soil. The ring was placed 100 mm from the following sucker of the plant, so that it was in line with the following sucker and the mother plant. Soil respiration was measured from the head space of the covered infiltration rings for 30 minutes using CO_2 gas sampling tubes (0.01-2.6%, 126SA, Kitigawa, Japan) prior to determining water infiltration rates. Following measurement of infiltration rates the ring was then inserted to be level with the soil surface and excavated. All roots within the ring were washed free of soil and banana roots were divided into classes, < 1mm, 1-5mm and > 5mm diameter (Araya and Blanco 2001) and expressed as the weight of root in one litre of soil. Soil temperature at 100 mm depth was also recorded.

A second aluminium ring (75 mm diameter x 75 mm) was inserted into the soil until level with the soil surface. The ring was excavated from the soil and the soil within the ring was used in the laboratory determination of soil bulk density, gravimetric water content and aggregate stability (Sarrantonio *et al.* 1996). An additional four soil cores were collected from each plot using a 50 mm diameter tube to a depth of 100 mm, and then used to determine pH, EC, NO_3-N, labile C and the number of soil nematodes. Soil pH and EC were determined in a 1:5 soil-water mixture using 30 g of soil and 150 mL of deionised water. Soil NO_3-N was determined from the filtered extract of the soil water mixture using test strips

(Reflectoquant, Merck company). The amount of NO_3-N kg ha^{-1} was calculated by equation 5-1:

Equation 5-1:

$$NO_3\text{-N kg ha}^{-1} = \frac{\underline{(5 \text{ x ppm } NO_3\text{-N}) \text{ x (depth of soil in cm) x (bulk density)}}}{10}$$

The value determined from the test strip was multiplied by 5 as this was the dilution factor of the soil solution. The depth of soil sampled was normally 10 cm and the bulk density was determined from separately from oven dried soil.

The labile C was determined as the amount of carbon readily oxidised by $KMnO_4$ using the method described by Weil *et al.* (2003). A 5 g sample of air dried soil was added to a tube containing 2.0 ml of a 0.2 M $KMnO_4$ solution, in 1 M $CaCl_2$ (pH 7.2) and made up to 20 mL with distilled water. This was shaken for 2 minutes and then allowed to stand for 10 minutes. A 0.5 mL aliquot, taken from the upper 1 cm of the suspension, was transferred to 45 mL of distilled water and made up to 50 mL and mixed thoroughly. The absorbance of the solution was then determined on a spectrophotometer (Hach) at 550 nm and compared to a standard curve to determine the amount of Labile C in the soil. A 200 g sub-sample was kept for nematode community analyses.

5.3.6 Soil nematodes

Soil nematode community analyses were determined by placing 200 g of field moist soil on a single layer of tissue, contained within a mesh basket (Whitehead and Hemming 1965). The basket was placed in 200 mL of water within a tray and maintained at 25 ^{o}C. After 24 hours nematodes contained within the water of the tray were collected on a 25 µm sieve. The nematodes were backwashed from the sieve and collected in a 30 mL vial. The total number of nematodes extracted from 200 g of soil was determined. Using a compound microscope, nematodes were identified to genera for plant-parasitic nematodes or families for non-parasitic nematodes and assigned to trophic groups according to Yeates *et al.* (1993).

5.3.7 Nematode indices

Indices of the nematode community composition were calculated from nematodes extracted from the soil. Nematode diversity was determined using the Shannon-Weiner index $H' = -\Sigma\, p_i \text{loge}\, p_i$. and dominance calculated using Simpson's index of dominance $\lambda = \Sigma\, (p_i)^2$, where p_i is the proportion of individuals in the i^{th}

taxon (Yeates and Bongers 1999). The bacterial - fungal ratio was determined using B/(B+F) ratio, where B and F are, respectively, the relative number of bacterivorous and fungivorous nematodes in the total nematode population, which is constrained to values between 1 (totally bacterivore dominated) and 0 (totally fungivore dominated) (Yeates 2003). Additionally, the proportion of plant-parasitic nematodes relative to the total soil nematode population was determined P-p/Tot nematodes; where P-p is the total number of plant-parasitic nematodes and Tot nematodes is the total number of nematodes recovered from the soil sample.

The weighted faunal analysis concept was applied, without plant feeders, to determine the basal, structure and enrichment conditions of the soil food web (Ferris *et al.* 2001). The enrichment index (EI) assesses the resources available to the soil food web and response by primary decomposers to those resources. The structure index (SI) is a measure of the number of trophic layers in the soil food web and the potential for regulation by predators. These indicators were calculated as EI = 100 x [e/(e+b)] and SI = 100 x [s/(s+b)], where e, s and b are the abundance of nematodes in guilds representing enrichment (e) (B1 and F2, where B = bacterivores, F = fungivores, and numbers represent the coloniser – persister (c-p) value 1-5 (Bongers 1990), structure (s) (B3-B5, F3-F5, Om3-Om5, P2-P5, where Om = omnivores, P = predators) and basal (b) (B2 and F2) nematode communities (Ferris *et al.* 2001). The channel index (CI), is an indication of the decomposition channel of nutrients calculated as CI = 100 x [0.8F2/(3.2B1 + 0.8 F2] (Ferris *et al.* 2001). A low value indicates a primarily bacterial decomposer community and high value a fungal dominated decomposer community (Hohberg 2003).

5.3.8 Statistics

Measurement from soil sites were analysed using a repeated analysis to determine differences between sampling times and treatments and their interaction. If significant differences were found using the repeated analysis for treatments, a one-way ANOVA for treatment differences was used. If significant F values ($P<0.05$) were found, the means were separated using the LSD method using Genstat 8 statistics package (Lawes Agricultural Trust). Total nematode numbers and number of nematodes in each trophic group per 100 g of soil were transformed using ln(x+1), prior to analyses, to comply with assumptions of normally distributed data.

5.4 Results

5.4.1 Crop growth and yields

There were no significant differences in the vegetative growth of the banana plants in the first crop following planting in September 2003, up until flower emergence at March 17, 2004. The addition of the amendments prior to planting had not significantly affected the vegetative growth of the banana plant (Table 5-3).

Table 5-3: Vegetative growth, leaf emergence and plant height, of bananas in the plant crop until first bunch emergence

Treatment	**8 Dec 2003**		**5 Jan 2004**		**16 Feb 2004**		**17 Mar 2004**	
	Leaf emergence	**Height (m)**	**Leaf emergence**	**Height (m)**	**Leaf emergence**	**Height (m)**	**Leaf emergence**	**Height (m)**
Mill ash	1.27	0.45	*	1.05	1.29	1.52	0.93	1.90
Compost	1.31	0.51	*	1.08	1.29	1.50	0.98	1.85
Organic matter	1.32	0.47	*	1.22	1.30	1.64	0.86	1.93
Mill mud	1.36	0.51	*	1.26	1.36	1.74	0.93	2.05
Untreated	1.33	0.42	*	1.00	1.31	1.51	0.93	1.88
LSD	n.s.	n.s.	.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. denotes no significant difference (*P*>0.05) between means in columns. * denotes missing data.

Measurement of the time taken from planting until bunch emergence in the first crop revealed that mill mud treated plants flowered on average two-weeks earlier than other treatments (Figure 5-1 A). However, in the second ratoon crop, the mill mud and compost treated plants took the longest time to flower relative to other treatments (Figure 5-1 A).

The compost and the mill mud treated plots had significantly more fingers per bunch in the first crop after planting relative to the untreated plots (Figure 5-1 B). There were on average 10% more fruit per bunch in the mill mud and compost treated plants compared to the untreated plants in the first crop. However, in the first ratoon crop there were no significant differences in the number of fingers per bunch (Figure 5-1 B). Furthermore, in the second ratoon crop mill ash treated plants had an average 20 fingers more per bunch than the untreated and 38 fingers more than the compost and mill mud treatments (Figure 5-1 B). In the second ratoon the compost and mill mud treated plants had significantly fewer fingers relative to the untreated and the mill ash treated plants (Figure 5-1 B). The organic matter treated plants were intermediate.

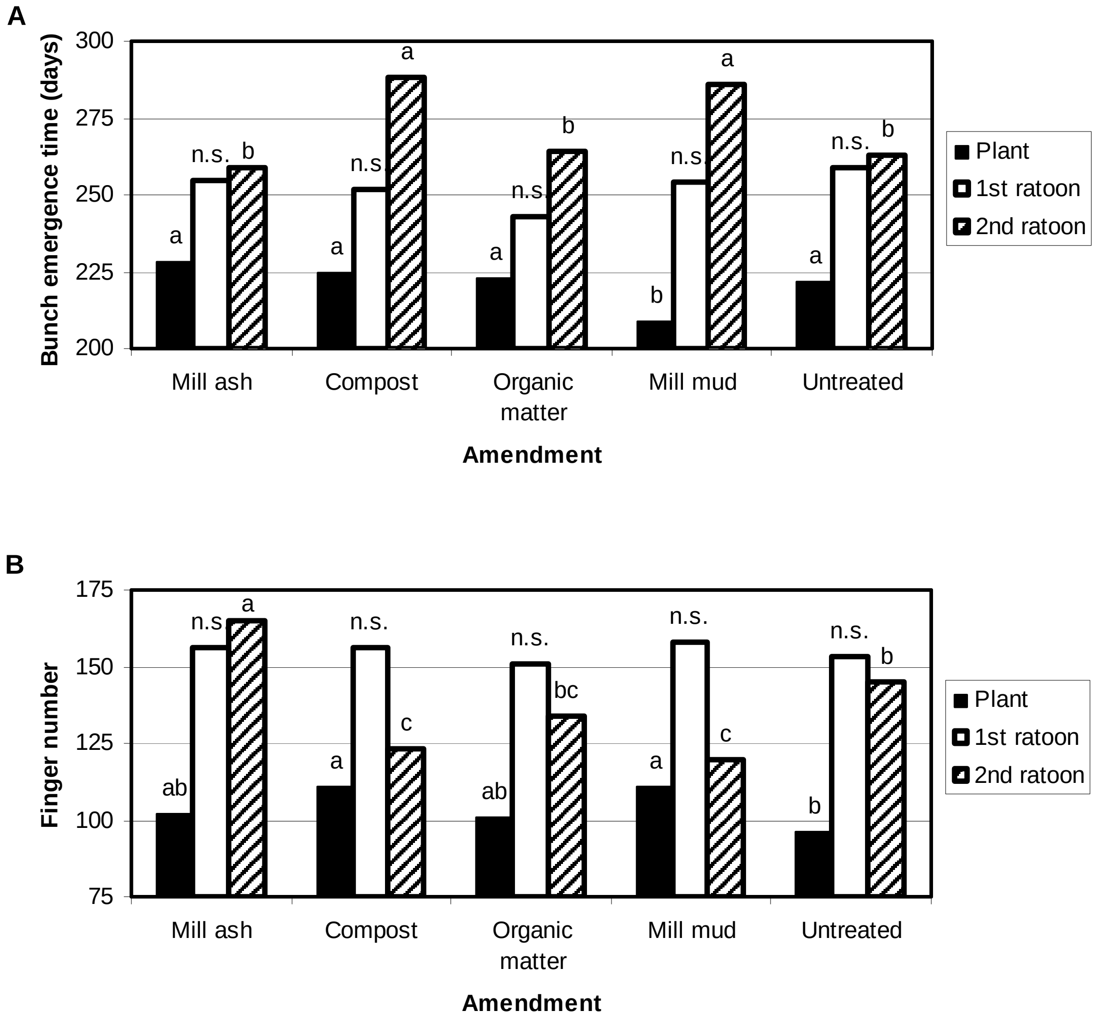

Figure 5-1: Time for flower emergence (A) and finger number per bunch (B) of bananas in the first and two successive ratoon crops in a field experiment investigating the use of soil amendments on soil properties and the growth of bananas.

On March 20, 2006 the experimental site was destroyed by tropical cyclone *Larry*, with wind gusts of 300 km hr^{-1}. An assessment was made of the number of plants that were uprooted, rather than just having the stem bent over. Relative to the untreated soil (9.4% plants up-rooted), the mill ash treated soil had a significantly lower proportion of plants uprooted (2.2%) (Figure 5-2). The organic matter treated soil had the highest proportion of plants up rooted (11.2%) but this was not significantly more than the untreated soil (Figure 5-2).

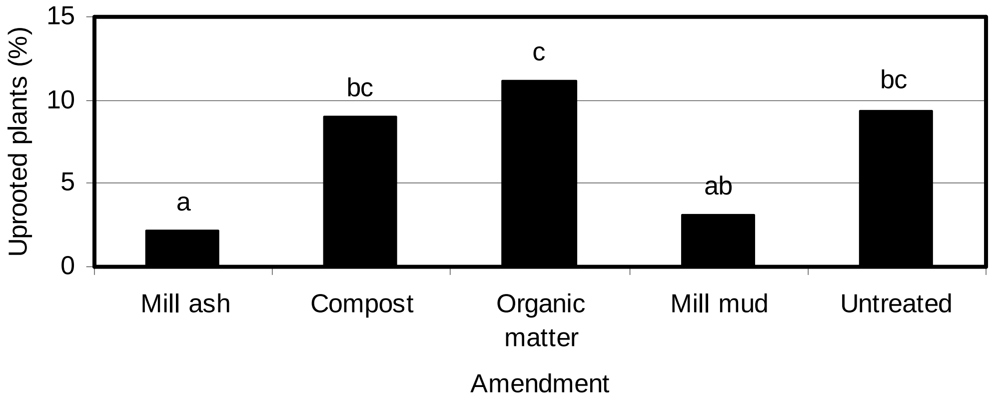

Figure 5-2: Percentage of banana plants uprooted following cyclone Larry in March 2006 in soil with different amendments compared to untreated bare soil.

Assessments were made of root growth at each sampling period by washing soil from the 150 mm rings used for respiration and infiltration measurements. Small differences were detected in the weight of roots less than 1 mm. However, this was not consistent over time and there were no trends that were related to the application of amendments (Table 5-4).

5.4.2 Soil measurements

There was twice as much organic matter on the soil surface in the organic matter treated plots relative to other treatments and ten times more than the untreated plots at the two assessment periods in October 2005 and May 2006 (Figure 5-3). This was the result of the continual removal of crop residue from the untreated plots to organic matter plots following the harvest of the first crop in August 2004.

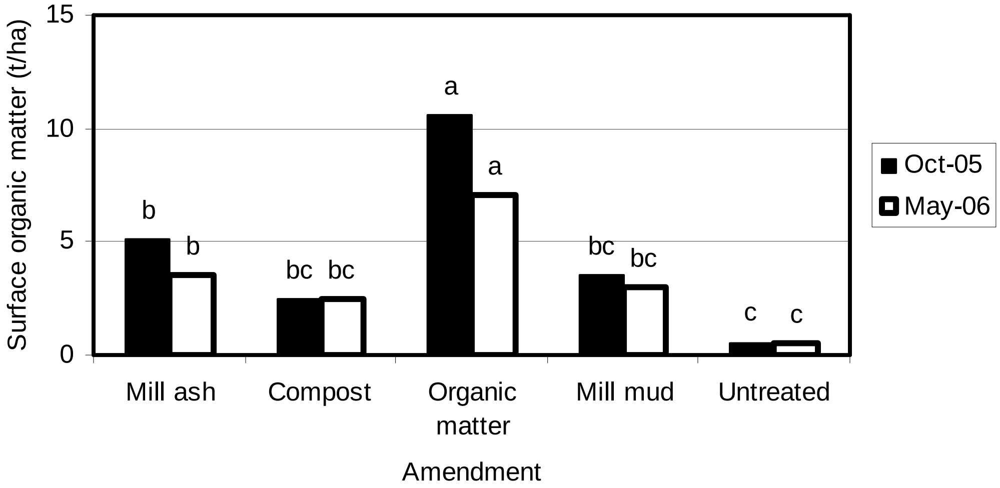

Figure 5-3: Surface organic matter in bananas treated with different soil amendments in October 2005 and May 2006.

The repeated analysis of variance found that there was a significant variation over time for all soil properties measured, except the channel index and the assessment of banana roots (Table 5-3). There were significant treatment effects determined in the repeated analysis of variance for the soil properties aggregate stability, EC, labile C, the ratio of labile C to NO_3-N, nematode enrichment and channel indices and for growth of root less than 1 mm in diameter (Table 5-3). There were also significant interactions between the different amendment treatments and the sampling time for the soil properties aggregate stability, bulk density, labile C, soil respiration and number of bacterivores (Table 5-3).

Table 5-4: Repeated measures analysis of soil properties determined in an experiment to determine the differences in soil properties due to the application of amendments to banana plants.

Soil Parameter	Treatment	Time	Time x treat
Physical soil properties			
Aggregate stability (%)	*	**	*
Bulk density (g cm^3)	-	***	***
Infiltration (cm min^{-1})	-	***	-
Temperature (^{o}C)	-	***	-
Volumetric water (cm cm^{-1})	-	***	-
Water filled pore space (%)	-	***	-
Chemical soil properties			
pH	-	***	-
EC (µS cm^{-1})	*	***	-
NO3-N (kg ha^{-1})	-	*	-
Labile C (mg kg^{-1})	***	***	***
Labile C/ NO_3-N	*	***	-
Biological soil properties			
Respiration (kg CO2 ha^{-1} d^{-1})	-	***	*
Total nematodes	-	**	-
Plant parasites	-	***	-
Bacterivores	-	***	*
Fungivores	-	**	-
Omnivores	-	-	-
Predators	-	***	-
H' diversity	-	***	-
λ dominance	-	**	-
Plant parasites (%)	-	***	-
B/F ratio	-	***	-
Enrichment index	*	*	-
Structure index	-	***	-
Channel index	*	-	-
Banana roots			
All roots (g 1 soil^{-1})	-	-	-
>5mm roots (g 1 soil^{-1})	-	-	-
1-5mm roots (g 1 soil^{-1})	-	-	-
<1mm roots (g 1 soil^{-1})	*	**	-

* $P<0.05$, ** $P<0.01$, ***$P<0.001$,

5.4.3 Physical soil properties

Aggregate stability was significantly different between treatments and over time, with a significant interaction between treatment and time (Table 5-2). There was a continual increase in the aggregate stability in the organic matter treatment, but it was not significantly greater than other treatments until May 2006 (39%), 31 months after the experiment commenced (Table 5-5). The application of mill mud produced more stable aggregates (26%) relative to the bare, untreated soil (14%) at the completion of the experiment, but was not significantly greater than the mill ash (21%) or compost (16%) treatments and was significantly less than the organic matter treated soil (Table 5-5). The aggregate stability in the mill ash and the compost treatments was never significantly greater than the untreated, bare soil throughout the experiment (Table 5-5).

The soil bulk density in the untreated bare soil increased over the duration of the experiment. At the beginning of the experiment, in October 2003, soil bulk density was 1.11 g cm^{-3}. At the termination of the experiment, in May 2006, the bulk density had increased to 1.38 g cm^{-3} and was significantly higher than all other treatments, except the compost and mill mud treated soil (1.23 and 1.24 g cm^{-3} respectively) (Table 5-5). Bulk density in the mill ash treated plots in October 2005 and May 2006 (1.17 and 1.18 g cm^{-3} respectively) was significantly lower than the untreated control. However, in May 2006, at the termination of the experiment, the lowest bulk density was recorded in the organic matter treated plots (1.09 g cm^{-3}), 0.3 g cm^{-3} lower than the untreated bare plots (Table 5-5).

Water infiltration was significantly greater in October 2005 in the mill ash treated plots relative to all other treatments and times (Figure 5-4). This corresponded with the significantly lower bulk density in the mill ash treated plots in October 2005. During October 2005 the soil exhibited lower volumetric soil water content (data not shown) relative to other assessment times, which may have allowed a faster infiltration of water into the soil.

Table 5-5: Changes in soil physical properties, aggregate stability and bulk density over the experimental period from October 2003 to May 2006 in soils treated with soil amendments compared to an untreated bare control.

Indicator	Treatment	Sampling date				
		Oct 03	Mar 04	Sep 04	Oct 05	May 06
Aggregate stability (%)	Mill ash	17 n.s.	12 n.s.	13 n.s.	16 n.s.	21 bc
	Compost	15 n.s.	15 n.s.	21 n.s.	14 n.s.	16 bc
	Organic matter	14 n.s.	16 n.s.	27 n.s.	24 n.s.	39 a
	Mill mud	19 n.s.	15 n.s.	23 n.s.	15 n.s.	26 b
	Untreated	19 n.s.	15 n.s.	17 n.s.	16 n.s.	14 c
LSD (*P*=0.05)						12
Bulk density (g cm^{-3})	Mill ash	1.16 n.s.	1.21 n.s.	1.17 n.s.	1.17 a	1.18 b
	Compost	1.15 n.s.	1.26 n.s.	1.24 n.s.	1.34 b	1.23 ab
	Organic matter	1.22 n.s.	1.25 n.s.	1.18 n.s.	1.25 ab	1.09 b
	Mill mud	1.19 n.s.	1.22 n.s.	1.26 n.s.	1.21 a	1.24 ab
	Untreated	1.11 n.s.	1.24 n.s.	1.21 n.s.	1.32 b	1.38 a
LSD (*P*=0.05)					0.12	0.15

Means in columns for each indicator followed by the same letter are not significantly different (*P*=0.05). n.s. denotes no significant difference (*P*>0.05) between means.

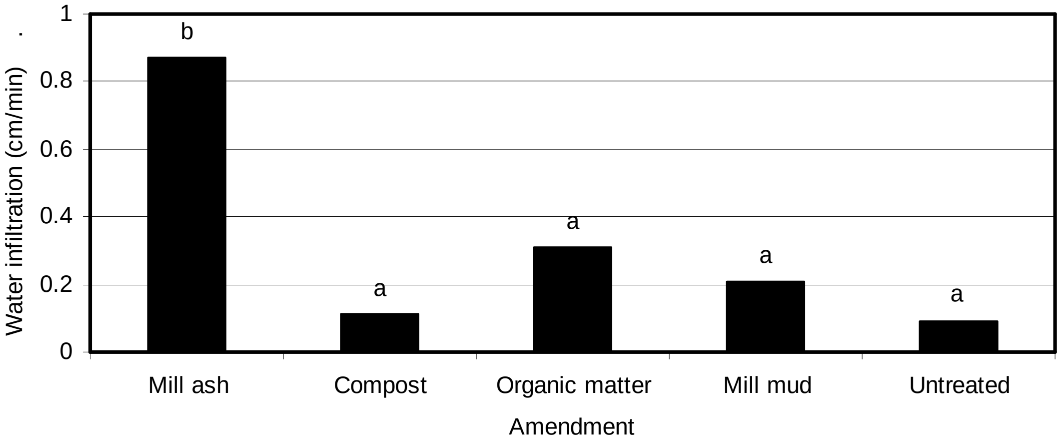

Figure 5-4: Differences in infiltration rate of water in October 2005 in soils treated with soil amendments compared to an untreated bare control.

5.4.4 Chemical soil properties

There was a significant change in the EC between sampling dates (Table 5-6). In particular, in September 2004, there was a large increase in the EC in all treatments relative to other sampling periods (Table 5-6). However, this corresponded with high within treatment variability so that there were no significant differences between treatments (Table 5-6). The EC measurements throughout the experiment were significantly correlated with high soil NO_3-N measurements ($R^2 = 0.66$ $P<0.05$). At the termination of the experiment the organic matter treated plots had a significantly greater EC (0.05 µS cm^{-1}) than the untreated and the mill mud treated plots (0.03 and

0.04 µS cm^{-1} respectively). However, the final soil assessments recorded the lowest EC measurements for the whole experiment (Table 5-6).

Soil NO_3-N measurements mirrored EC measurements (Table 5-6). There was a significant difference between treatments at the commencement of the experiment. In particular, the compost treatment had double the amount NO_3-N measured in the soil (93.4 kg ha^{-1}) relative to all other treatments. The lowest NO_3-N measurements were recorded at the end of the experiment, but unlike EC there were no significant differences between treatments (Table 5-6).

Labile C measurements were significantly lower in the untreated, bare banana plots relative to the organic matter, mill mud and compost treatments throughout the experiment, except following planting (Table 5-6). Similarly, the organic matter, mill mud and compost all tended to have greater labile C measurements, relative to the mill ash treatment up until the termination of the experiment. The amount of labile carbon measured under mill ash increased as the trial progressed, initially at 390 mg kg^{-1} in March 2004, increasing to 668 mg kg^{-1} in May 2006 (Table 5-6). At the termination of the experiment there was no significant difference in the amount of labile C between amendment treatments. However, all amendment treatments had labile C measurement significantly greater then the untreated plots (Table 5-6).

The ratio of labile C to NO_3-N was significantly different between treatments at the beginning and at the end of the experiment (Table 5-6). At the beginning of the experiment the ratio of labile C to NO_3-N was significantly greater in the mill ash and mill mud treatments relative to the compost (Table 5-6). However, at the termination of the experiment the organic matter treated soil had a significantly greater ratio of labile C to NO_3-N relative to the untreated soil. The ratio of labile C to NO_3-N had increased in the final assessment (Table 5-6).

Table 5-6: Changes in chemical soil properties; EC, NO3-N, labile C and labile C/NO_3-N ratio over the experimental period from October 2003 to May 2006 in soils treated with soil amendments compared to an untreated bare control.

Indicator	Treatment	Sampling date				
		Oct 03	Mar 04	Sep 04	Oct 05	May 06
EC	Mill ash	0.08 n.s.	0.04 n.s.	0.19 n.s.	0.07 n.s.	0.04 ab
(µS cm)	Compost	0.16 n.s.	0.05 n.s.	0.48 n.s.	0.08 n.s.	0.04 ab
	Organic matter	0.09 n.s.	0.04 n.s.	0.39 n.s.	0.08 n.s.	0.05 a
	Mill mud	0.07 n.s.	0.04 n.s.	0.26 n.s.	0.09 n.s.	0.04 bc
	Untreated	0.09 n.s.	0.05 n.s.	0.46 n.s.	0.19 n.s.	0.03 c
	LSD (*P*=0.05)					0.006
Nitrate N	Mill ash	31.2 b	54.7 n.s.	40 n.s.	41 n.s.	23 n.s.
(kg ha^{-1})	Compost	93.7 a	53.7 n.s.	149 n.s.	47 n.s.	23 n.s.
	Organic matter	44.6 b	41 n.s.	103 n.s.	48 n.s.	21 n.s.
	Mill mud	35.2 b	60.5 n.s.	70 n.s.	43 n.s.	22 n.s.
	Untreated	38.7 b	37.2 n.s.	172 n.s.	42 n.s.	22 n.s.
	LSD (*P*=0.05)	34.5				
Labile C	Mill ash	390 bc	429 ab	541 b	548 b	668 a
(mg kg^{-1})	Compost	436 abc	464 ab	679 a	680 a	676 a
	Organic matter	472 a	500 a	672 a	673 a	714 a
	Mill mud	443 ab	421 bc	676 a	635 a	680 a
	Untreated	362 c	352 c	483 b	399 c	443 b
	LSD (*P*=0.05)	76	74	75	63	47
Labile C /	Mill ash	24.3 a	12.7 n.s.	15.8 n.s.	13.9 n.s.	31.4 a
NO3-N	Compost	8.6 b	11.5 n.s.	7.1 n.s.	14.9 n.s.	30.2 a
	Organic matter	15.6 ab	19.7 n.s.	12.2 n.s.	15.2 n.s.	39.3 a
	Mill mud	20.4 a	11.8 n.s.	14.8 n.s.	22.1 n.s.	32.5 a
	Untreated	14.2 ab	13.7 n.s.	9.4 n.s.	10.2 n.s.	20.1 b
	LSD (*P*=0.05)	11.2				9.1

Means in columns for each indicator followed by the same letter are not significantly different from one another (*P*=0.05). n.s. denotes no significant difference (*P*>0.05) between means.

5.4.5 Biological soil properties

Soil respiration changed significantly over the time of the experiment. The highest soil respiration occurred at the commencement of the experiment when amendments had been applied and then declined at subsequent sampling times. There was a significant difference in the soil respiration between treatments at the initial sampling in October 2003 and 24 months later in October 2005 (Table 5-7). At the first assessment following the application of treatments there was significantly greater production of CO_2 from the compost treated soil relative to the untreated soil, mill ash and organic matter treatments. The organic matter had the lowest respiration measurement, significantly less than the mill mud and compost treatments (Table 5-7). However, in October 2005, the mill ash treated soil had the greatest soil respiration, relative to the compost, organic matter and untreated soil (Table 5-7).

The number of plant-parasitic nematodes was low through out the experiment and *Radopholus similis* was not detected in any samples. The only plant parasitic nematodes present were *Meloidogyne* spp. and *Helicotylenchus dihystera*, both regarded as minor plant parasites on bananas in north Queensland. There was no significant difference in plant-parasitic nematode numbers until the final sampling period in May, 2006. The lowest numbers of plant-parasitic nematodes were found in the organic matter plots relative to all other treatments (Table 5-7).

The highest number of bacterivores occurred at the commencement of the experiment following the application of amendments (Table 5-7). The high number of bacterivores corresponded with the high respiration. The number of bacterivores recovered from the soil samples at March 2004 was significantly lower in the organic matter treated soil relative to all other amendment treatments and the untreated, bare soil (Table 5-7).

There was no significant difference in the number of fungivores throughout the experiment (Table 5-7). Similarly there was no significant difference in the number of omnivores and predators until the final assessment conducted in May 2006 (Table 5-7). In May 2006 the number of predatory nematodes was significantly higher in the mill ash treated soil relative to all other treatments including the untreated soil (Table 5-7). Furthermore, in May 2006 the number of omnivorous nematodes was significantly higher in the mill ash, mill mud and organic matter treatments relative to the untreated soil (Table 5-7). The compost treated soil had an intermediate number of omnivores relative to the untreated soil and the other amendment treatments (Table 5-7).

Table 5-7:Changes in soil respiration and nematode trophic groups, plant-parasites, bacterivores, fungivores, predators and omnivores from October 2003 to May 2006 in soils treated with soil amendments compared to an untreated, bare control.

Indicator	Treatment	Sampling date				
		Oct 03	**Mar 04**	**Sep 04**	**Oct 05**	**May 06**
Soil respiration (kg CO_2 ha^{-1} d^{-1})	Mill ash	8.9 ab	5.2 n.s.	4.3 n.s.	6.8 a	4.9 n.s.
	Compost	16.2 c	6.3 n.s.	4.0 n.s.	4.5 b	3.0 n.s.
	Organic matter	7.1 a	4.9 n.s.	5.1 n.s.	4.2 b	3.9 n.s.
	Mill mud	12.5 bc	4.7 n.s.	4.4 n.s.	5.5 ab	3.5 n.s.
	Untreated	10.9 ab	4.9 n.s.	3.7 n.s.	3.8 b	2.3 n.s.
	LSD (*P*=0.05)	4.8			1.9	
Plant-parasites[A] (100 g $soil^{-1}$)	Mill ash	54 (4.01)n.s.	8 (2.24)n.s.	14 (2.73)n.s.	112 (4.73)n.s.	28 (3.38)a
	Compost	12 (2.58)n.s.	10 (2.41)n.s.	31 (3.45)n.s.	86 (4.47)n.s.	30 (3.44)a
	Organic matter	65 (4.19)n.s.	35 (3.58)n.s.	14 (2.68)n.s.	49 (3.91)n.s.	12 (2.59)b
	Mill mud	31 (3.48)n.s.	12 (2.59)n.s.	25 (3.25)n.s.	27 (3.32)n.s.	26 (3.29)a
	Untreated	39 (3.69)n.s.	14 (2.73)n.s.	39 (3.69)n.s.	190 (5.25)n.s.	43 (3.78)a
	LSD (*P*=0.05)					(0.52)
Bacterivores (100 g $soil^{-1}$)	Mill ash	124 (4.83)n.s.	32 (3.50)a	76 (4.35)n.s.	40 (3.72)n.s.	31 (3.48)n.s.
	Compost	749 (6.62)n.s.	40 (3.72)a	80 (4.40)n.s.	25 (3.25)n.s.	38 (3.66)n.s.
	Organic matter	132 (4.89)n.s.	6 (2.00)b	50 (3.99)n.s.	48 (3.89)n.s.	33 (3.52)n.s.
	Mill mud	180 (5.20)n.s.	58 (4.07)a	54 (4.01)n.s.	20 (3.03)n.s.	35 (3.58)n.s.
	Untreated	203 (5.32)n.s.	47 (3.87)a	64 (4.17)n.s.	32 (3.49)n.s.	31 (3.48)n.s.
	LSD (*P*=0.05)		(1.05)			
Fungivores (100 g $soil^{-1}$)	Mill ash	62 (4.14)n.s.	66 (4.20)n.s.	107 (4.68)n.s.	90 (4.51)n.s.	70 (4.26)n.s.
	Compost	147 (5.00)n.s.	88 (4.49)n.s.	27 (3.34)n.s.	59 (4.10)n.s.	40 (3.72)n.s.
	Organic matter	146 (4.99)n.s.	81 (4.41)n.s.	67 (4.22)n.s.	105 (4.66)n.s.	39 (3.68)n.s.
	Mill mud	122 (4.81)n.s.	94 (4.55)n.s.	46 (3.86)n.s.	43 (3.79)n.s.	54 (4.00)n.s.
	Untreated	144 (4.98)n.s.	95 (4.56)n.s.	67 (4.22)n.s.	66 (4.20)n.s.	30 (3.44)n.s.
	LSD (*P*=0.05)					
Predators (100 g $soil^{-1}$)	Mill ash	1 (0.70)n.s.	6 (1.94)n.s.	7 (2.11)n.s.	15 (2.75)n.s.	37 (3.64)a
	Compost	0 (0.00)n.s.	1 (0.84)n.s.	20 (3.04)n.s.	5 (1.77)n.s.	15 (2.75)b
	Organic matter	0 (0.00)n.s.	5 (1.85)n.s.	18 (2.96)n.s.	11 (2.48)n.s.	15 (2.79)b
	Mill mud	0 (0.00)n.s.	3 (1.37)n.s.	16 (2.85)n.s.	3 (1.38)n.s.	8 (2.23)b
	Untreated	1 (0.79)n.s.	14 (2.68)n.s.	12 (2.54)n.s.	3 (1.41)n.s.	14 (2.27)b
	LSD (*P*=0.05)					(0.77)
Omnivores (100 g $soil^{-1}$)	Mill ash	5 (1.74)n.s.	3 (1.49)n.s.	15 (2.77)n.s.	15 (2.80)n.s.	25 (3.25)a
	Compost	4 (1.54)n.s.	10 (2.41)n.s.	20 (3.06)n.s.	3 (1.44)n.s.	8 (2.17)ab
	Organic matter	11 (2.47)n.s.	7 (2.07)n.s.	21 (3.11)n.s.	26 (3.29)n.s.	25 (3.24)a
	Mill mud	7 (2.10)n.s.	2 (1.22)n.s.	6 (1.98)n.s.	9 (2.27)n.s.	13 (2.61)a
	Untreated	9 (2.27)n.s.	8 (2.16)n.s.	28 (3.36)n.s.	10 (2.42)n.s.	3 (1.46)b
	LSD (*P*=0.05)					(1.13)

Means in columns for each indicator followed by the same letter are not significantly different (*P*=0.05). n.s. denotes no significant difference (*P*>0.05) between means.

[A] Means presented are back transformed with values in parentheses being the transformed means ln(x+1)

There were no significant differences in the diversity or the structure index of nematodes between the treatments at the five samplings times throughout the experiment (Table 5-8). However, there was a significant difference in the enrichment index, at the commencement of the experiment (Table 5-8). The enrichment index was significantly higher in the compost amended soil relative to the untreated, mill ash and organic matter treatments (Table 5-8). The mill ash, mill mud and untreated soils had an intermediate channel index between the compost and organic matter treatments at the commencement of the experiment. At the following assessment, March 2004, the organic matter treatment had a significantly higher value for the channel index relative to the other treatments (Table 5-8).

Similarly, there was a significant variation in the ratio of bacterial to fungal feeding nematodes over the course of the experiment (Table 5-8). There was a significantly lower ratio of bacterivores in the organic matter treated soil relative to all other treatments soon after the amendments were applied. Conversely, the compost treated soil had the highest ratio of bacterivores. There was a significantly higher ratio of bacterivores relative to fungivores in the compost treatment compared to the organic matter treatment for the first 12 months of the experiment, from October 2003 until September 2004 (Table 5-8). This coincides with differences measured using the channel index. Initially, the compost treatment had a greater proportion of bacterivores and the organic matter treatment had a greater proportion of fungivores. Other treatments were intermediate. However, by October 2005, there were no significant differences in the ratio of bacterivores to fungivores between treatments through to the termination of the experiment (Table 5-8).

There was an initially a significantly lower ratio of plant-parasitic nematodes to total nematode numbers in the soil in the compost treatment (Table 5-8). However, at the termination of the experiment in May 2006, there was a significantly higher ratio of plant-parasitic nematodes in the untreated plots (0.33) relative to the mill ash (0.18) and organic matter (0.09) amended soil, with the compost (0.26) and mill mud (0.20) treatments having intermediate proportions of plant-parasitic nematodes relative to other soil nematodes (Table 4-8). In the October 2005 sampling, the untreated soil (0.60) had double the proportion of plant-parasitic nematodes relative to the organic matter (0.24) and mill mud (0.30) treatments (Table 5-8). However, the variability within treatments meant that there were no significant differences.

Table 5-8: Changes in nematode community indices; diversity, structure index, enrichment index, channel index, B/(B+F) ratio, plant-parasitic to total nematode ratio from October 2003 to May 2006 in soils treated with soil amendments compared to an untreated, bare control.

Indicator	Treatment	Sampling date				
		Oct 03	Mar 04	Sep 04	Oct 05	May 06
Diversity	Mill ash	1.63 n.s.	1.50 n.s.	1.70 n.s.	1.59 n.s.	1.94 n.s.
	Compost	1.33 n.s.	1.59 n.s.	1.66 n.s.	1.55 n.s.	1.91 n.s.
	Organic matter	1.70 n.s.	1.61 n.s.	1.61 n.s.	1.77 n.s.	1.97 n.s.
	Mill mud	1.49 n.s.	1.43 n.s.	1.94 n.s.	1.70 n.s.	1.91 n.s.
	Untreated	1.76 n.s.	1.73 n.s.	1.86 n.s.	1.23 n.s.	1.93 n.s.
	LSD (*P*=0.05)					
Structure index	Mill ash	15 n.s.	61 n.s.	52 n.s.	77 n.s.	87 n.s.
	Compost	17 n.s.	57 n.s.	80 n.s.	62 n.s.	76 n.s.
	Organic matter	24 n.s.	75 n.s.	84 n.s.	70 n.s.	84 n.s.
	Mill mud	28 n.s.	30 n.s.	62 n.s.	75 n.s.	64 n.s.
	Untreated	26 n.s.	68 n.s.	81 n.s.	73 n.s.	69 n.s.
	LSD (*P*=0.05)					
Enrichment index	Mill ash	63 a	74 n.s.	55 n.s.	61 n.s.	66 n.s.
	Compost	90 b	71 n.s.	61 n.s.	58 n.s.	76 n.s.
	Organic matter	57 a	50 n.s.	53 n.s.	47 n.s.	72 n.s.
	Mill mud	74 ab	72 n.s.	54 n.s.	62 n.s.	66 n.s.
	Untreated	66 a	67 n.s.	64 n.s.	63 n.s.	71 n.s.
	LSD (*P*=0.05)	17				
Channel index	Mill ash	37 ab	33 a	56 n.s.	45 n.s.	43 n.s.
	Compost	8 a	44 a	44 n.s.	54 n.s.	31 n.s.
	Organic matter	55 b	84 b	49 n.s.	71 n.s.	34 n.s.
	Mill mud	34 ab	39 a	52 n.s.	33 n.s.	39 n.s.
	Untreated	34 ab	45 a	31 n.s.	51 n.s.	44 n.s.
	LSD (*P*=0.05)	30	34			
B/(B+F) ratio	Mill ash	0.65 ab	0.70 b	0.45 b	0.34 n.s.	0.31 n.s.
	Compost	0.82 a	0.57 b	0.71 a	0.30 n.s.	0.49 n.s.
	Organic matter	0.15 c	0.29 a	0.47 b	0.33 n.s.	0.46 n.s.
	Mill mud	0.61 ab	0.45 ab	0.54 ab	0.32 n.s.	0.40 n.s.
	Untreated	0.62 ab	0.61 b	0.48 b	0.32 n.s.	0.51 n.s.
	LSD (*P*=0.05)	0.19	0.25	0.17		
Plant-parasites / total nematodes	Mill ash	0.12 ab	0.11 n.s.	0.10 n.s.	0.43 n.s.	0.18 bc
	Compost	0.05 b	0.11 n.s.	0.19 n.s.	0.47 n.s.	0.26 ab
	Organic matter	0.18 ab	0.20 n.s.	0.09 n.s.	0.24 n.s.	0.09 c
	Mill mud	0.11 ab	0.12 n.s.	0.14 n.s.	0.30 n.s.	0.20 abc
	Untreated	0.11 ab	0.15 n.s.	0.18 n.s.	0.60 n.s.	0.33 a
	LSD (*P*=0.05)	0.08				0.14

Means in columns for each indicator followed by the same letter are not significantly different (*P*=0.05). n.s. denotes no significant difference (*P*>0.05) between means.

5.5 Discussion

The effects of amendments on the production of bananas changed over the course of the experiment. Initially, the compost and the mill mud treatments produced plants with a greater number of fingers per bunch. This is thought to be due to the initial influences of the amendments on soil properties. The application of compost and mill mud amendments resulted in greater bunch size in the first crop following planting, possibly through greater nutrient availability following the decomposition of the compost and mill mud. There was a significantly higher recording of soil NO_3-N in the compost and mill mud treatments relative to the untreated soil. This suggested that soil chemical properties, like NO_3-N tended to have the greatest impact on banana production following planting. Furthermore, the application of mill mud was also able to reduce the time until flower emergence, reducing the vegetative phase of the first crop.

The application of amendments with high nitrogen content, such as compost and mill mud, stimulated bacterial activity in the soil resulting in a greater respiration and greater numbers of bacterivores. However, many of the changes induced by the applications of the pre-plant amendments were not sustained past 12 months. Some of the changes in soil properties which occurred after the application of amendments may have been masked by commercial crop management inputs such as fertilisers and therefore were not detected after 12 months. The commercial crop management inputs may have also contributed to the variability in soil properties at the 12 month sampling period, in September 2004. The variability within treatments meant that it was difficult to determine the impact of amendments on the first ratoon bunch size and therefore, no statistical difference was measured in banana bunches or banana bunch emergence time.

The application of mill ash produced significantly larger bunches in the second ratoon banana crop and had a shorter time to flowering relative to the compost and mill mud treated plants. The application of mill ash resulted in a lower soil bulk density relative to the untreated soil in the last two assessments of soil properties, in October 2005 and May 2006. Interestingly, soil bulk density measurements were increasing in the untreated bare soil, with increasing time, whereas they tended to remain constant in the mill ash treated soil. It is possible that high soil bulk density is a limiting factor to the long term production of bananas, which goes unrecognised because of the slow nature of this change. In this case, it took two years for soil bulk density to be significantly different between the soil treatments and affect banana production. The application of mill ash was the only treatment, which improved the infiltration rate of water into the soil, similar to the findings of (Cox *et al.* 2001). This only occurred in October 2003 during a dry period. The application of ash to the soil can have differing affects on soil properties depending on the source of the ash. Application of fly ash, a by-product from coal fired power stations tended to increase bulk density relative to other treatments (Cox *et al.* 2001), whereas the ash from sugar mills resulted in a similar bulk density to cultivated soil. The reapplication of the mill ash to the soil surface after one year appeared to be more effective than the pre-plant application for maintaining bulk density and sustaining crop production. The mill ash treated soil also reduced the number of uprooted banana plants following destructive winds caused by cyclone Larry. This could be due to the plants having a larger support structure, although root measurements and assessments of the following sucker did not support this hypothesis.

The application of organic amendments is reported to increase soil biological diversity and disease suppression (Janvier *et al.* 2007). The application of organic matter, firstly as grass hay followed by additional banana residue, was able to significantly increase the amount of surface organic matter, labile C and increase aggregate stability. The organic matter applications were related to a lower number of plant-parasitic nematodes relative to the bare soil, which suggested better general suppression of plant diseases. This was partially supported by the significant increase in the number of omnivorous nematodes in the organic matter treated soil relative to the bare, untreated soil. However, it would appear that factors other than the presence of predatory nematodes were contributing to the suppression of plant-parasitic nematodes. The levels of plant-parasitic nematodes recovered from soil samples were not regarded as being able to limit banana yield, both in the number of individuals quantified and the species identified (*H. dihystera* and *Meloidogyne* spp.). However, these species can be used as a surrogate measurement for other plant-parasitic nematodes that cause losses in production of bananas such as *R. similis*. The suppression of plant parasitic nematodes appears to be a general suppression that is not related to any specific suppressive mechanism such as the number of predatory nematodes. The mechanisms causing nematode suppression needs to be clarified with further investigations involving the addition of organic amendments.

Soil bulk density was lower in the organic matter treated plots at the termination of the experiment in May 2006. Soil bulk density was an excellent indicator for many of the physical soil properties that were limiting banana growth. Therefore, if the experiment were to continue for more crop cycles, up to four to six, which is normal commercial practice for the north Queensland banana industry, additional organic matter may contribute to improved plant productivity through increased aggregate stability and decreased bulk density.

High levels of organic matter in the soil may have also contributed to high EC in the organic matter treated plots at the termination of the experiment. EC measurements are typically used to determine problems with salinity, but in high rainfall areas such as in the north Queensland banana production zone, EC may indicate nutrient availability. Organic matter is able to hold onto nutrients (Seiter and Horwath 2004). Therefore, it is possible, following heavy rains after cyclone Larry, that the additional organic matter was able to hold onto nutrients, preventing leaching and resulting in a significantly higher EC relative to the untreated bare soil. Changes in soil EC may also serve as an indicator that there have been changes in soil conditions, which may affect the soil nematode community structure (Neher *et al.* 2005; Pattison *et al.* 2008).

The results of increased organic inputs into the banana production system required at least 2 years of consistent application to create significant changes in soil properties. The application of additional organic matter resulted in a change in a number of key soil indicators; soil bulk density, aggregate stability and EC. This highlights the long term investment needed for soil improvement through the addition of organic matter and the time needed for biological processes to change soil properties, particularly physical properties such as aggregate stability and soil bulk density.

The use of amendments with low C:N ratios such as composts and mill mud tended to have only a short term influence (6 and 12 months) on soil properties in the banana production area of the wet tropics. These amendments tended to increase bacterial decomposition of organic matter and did not result in long term changes in soil properties. However, the addition of amendments with a high C:N ratio, such as grass hay and banana residue, tended to slow down biological activity resulting in less bacterial decomposition of the organic matter in the soil and help to restore degraded soil properties over a 2-year period. Mill ash appeared relatively chemically inert, due to the very high C:N ratio, but provided improved physical soil properties, such as bulk density and water infiltration, allowing improved soil water and root exchanges. The practice of keeping the soil bare around banana plants resulted in a slow degradation of the soil. This was evident from the significant decline in the soil indicators by the end of the experiment, such as increased bulk density, reduced aggregate stability, poor nutrient holding capacity and increased levels of plant-parasitic nematodes. Therefore, banana growers need to find an alternative practice to keeping bare soil within their plantations. The use of grass hay was meant to simulate the use of grassed fallows prior to planting bananas. Grassed fallows act as mulch for plant banana crops, and when followed by retention of crop residue in the banana row area, constitute a low cost method of increasing organic C levels in the soil.

Changes in farm management, such as the application of amendments, lead to changes in soil properties that were detected using on-farm soil health indicators. Short term increases in NO_3-N, EC, soil respiration and the levels of bacterial feeding nematodes indicated improved short-term growing conditions for plants. However, bulk density, aggregate stability, labile C and proportion of plant-parasitic nematodes in the soil nematode community were better indicators of sustainability of banana production.

5.6 Summary

The application of soil amendments have previously been shown to be able change soil properties and suppress plant-parasitic nematodes. However, the effects of the amendments on commercial banana production and soil factors were unclear. A field experiment was established to determine the changes in soil properties following application of four commonly available soil amendments; compost, grass hay and by-products from sugar mills, mill mud and mill ash. The amendments were applied at the time of planting bananas and re-applied again 12 months later. After 12 months all crop residue from the untreated plots were removed from the soil surface and placed in the rows of plots receiving the grass hay to increase organic matter inputs. Measurements of soil properties were made after 1, 6, 11, 24 and 31 months after planting bananas. Assessments of plant growth and production were made for the plant, first ratoon and second ratoon crops. The application of compost initially increased soil respiration and was linked to greater bacterivore nematode activity in the soil. The application of grass hay and banana residue increased the proportion of fungivore nematodes relative to the compost treatment. The application of compost and mill mud resulted in a significantly greater number of fingers in the first crop relative to the untreated soil. In the first ratoon crop, there were no significant differences between treatments in terms of production and changes in soil properties. However, at the termination of the experiment, at the end of the second ratoon crop, there was significant increase in bunch size in the mill ash treatment and decline in bunch size in the compost and mill mud treatments relative to the untreated plants. Furthermore, there was a significant increase in aggregate stability and decrease in soil bulk density in the organic matter treated soil relative to the untreated soil. This corresponded with increased labile C and number of omnivorous nematodes and lower number of plant-parasitic nematodes in the organic matter treatment relative to the untreated soil. Therefore, practices that are able to increase soil carbon and improve soil structure may give greater suppression of plant-parasitic nematodes and increase sustainability of banana production.

6 Impact of banana farming system on the soil food web and suppression of plant-parasitic nematodes.

6.1 Introduction

Banana production in the Australian environment faces multiple constraints associated with productivity, labour efficiency, environmental protection and pest and disease management. It has been suggested that soils used for crop production in tropical regions are particularly sensitive to degradation due to the climatic influences (Primavesi 2006). Within the north eastern Australian banana production area there is a need to reduce the impacts of farming on the Great Barrier Reef (Anon 2003). Therefore, crop management systems have evolved to adapt to changing constraints, which has led to a divergence of management practices and farm inputs (Blazy *et al.* 2009).

Agricultural practices have been shown to have a large impact on the soil environment (Doran 2002; Westphal 2005). In order to maximise crop production synthetic fertilisers have been used to increase the nutrient availability, tillage is used to provide a fine seed bed and pesticides are used to manage pest and disease problems. The use of chemical nematicides, mostly organophosphates and carbamates, has traditionally been the main method of managing plant-parasitic nematodes in banana crops (Stirling and Pattison 2008). However, the reduction in the use of chemical nematicides is necessary because of their toxicity to human health and concerns about their impact on the environment. Furthermore, chemical nematicides are becoming less effective at controlling plant–parasitic nematodes in bananas due to enhanced biodegradation of the chemicals (Moens *et al*. 2004; Pattison *et al*. 2000).

Uncertainty about new farm management practices reduces adoption and innovation. Blazy *et al.*(2009) suggested that participatory on-farm research was one of the main methods of prototyping innovative crop management systems to reduce some of the uncertainty. However, Janvier *et al* (2007) also suggested that the links between improved plant health and measurable soil properties were not sufficiently clear. The way in which specific agricultural practices affect soil properties, particularly in respect to sustainability requires closer scrutiny (Upoff *et al*. 2006). A set of soil health indicators developed for the Australian banana industry could assist in the assessment of banana farming practices on soil health (Pattison *et al.* 2008).

However, the relationship between each type of farm practice, the effect on soil properties and the effect on plant-parasitic nematodes is not well understood.

A methodology framework using multivariate statistics and factor analysis can aid in the selection of soil health indicators and determine impacts of farming systems on soil health (de Lima *et al.* 2008; Shukla *et al.* 2006). The authors were able to reduce the number of indicators needed to a minimum set which could discriminate between different types of agricultural production systems. Furthermore, Idowu *et al.* (2009) were able to discriminate between tillage management systems using principle component analysis with a set of physical, chemical and biological indicators. The concept was also applied to develop indicators for the suppression of soil borne diseases and nematodes (Borrero *et al.* 2004; Garbeva *et al.* 2004; Janvier *et al.* 2007)

The findings from previous chapters suggested that farming practices, which were able to increase carbon inputs, particularly labile C, caused greater suppression of plant-parasitic nematodes, particularly burrowing nematode (*Radopholus similis*). Alternative methods for managing plant-parasitic nematodes must be developed and integrated into farming systems to sustain productivity and profitability of banana growing. An integrated nematode management programme based on cultural and biological controls needs to be developed so that the banana industry can adapt to the pressures of the current economic, environmental and political climate.

6.2 Objectives

The aim of this survey was to use farm management and soil health indicators to define farm typologies and to relate these indicators to the suppression of *R. similis* on bananas.

6.3 Materials and methods

6.3.1 Farm typology

The data used to develop farm typology was derived from interviews with banana plantation managers. Ten banana plantations were selected in north Queensland, Australia with a range of soil management systems. Banana plantation managers were asked questions relating to land preparation, use of amendments, fertiliser practices in the banana crop, pesticide application, crop age, interplant vegetation and crop residue management (Table 6-1). The survey was qualitative and used a scoring system that was developed for the managers’ response to each question allowing statistical characterisation of the plantation typology (Table 6-1).

Table 6-1: Criteria used to determine banana plantation typology relating to soil management practices.

Cultivation	**Scoring categories**
Do you have a fallow period between banana crops?	1. < 6 months 2. 6 month – 1 year 3. > 1 year
Do you grow something in the fallow period?	1. Yes 2. No
Approximately how many tillage operations did you use in preparing the soil to plant bananas (e.g. disking, rotary hoeing, deep ripping, V ploughing)?	1. < 3 2. 3 - 6 3. > 6
Amendments	
Did you add an amendment to the soil prior to planting bananas (e.g. mill ash, mill mud, compost etc)?	1. Yes 2. No
If yes how much did you apply?	1. < 40 t/ha 2. > 40 t/ha
Fertiliser	
How much nitrogen was applied to the bananas?	1. < 200 kg N/ha 2. 200-400 kg N/ha 3. > 400 kg N/ha
How long since the last fertiliser application?	1. < 1 week 2. 1 – 4 weeks 3. > 4 weeks
Pesticides	
Do you apply insecticide / nematicides to your soil?	1. Yes 2. No
If yes, when was the last application made?	1. < 1 week 2. 1 – 4 weeks 3. > 4 weeks
Crop	
What age is the banana crop?	1. Plant 2. 1st ratoon 3. 2nd ratoon 4. 3rd ratoon 5. >3rd ratoon
How do you manage the inter-row between bananas?	1. Grassed 2. Bare
Where do you place the crop residue (trash)?	1. Inter-row 2. Row 3. Where it falls

6.3.2 Soil sampling

Ten commercial banana plantations were surveyed with soil samples collected from three fields on each plantation (Table 6-2). The environment and location for each plantation were recorded (Table 6-2). In each field, soil samples were collected from 20 different locations by selecting plants that had recently flowered. The soil was collected using a 50 mm diameter soil core to a depth of 150 mm at a distance of 100 mm in front of the following sucker.

6.3.3 Soil health indicators

Eight indicators were selected as key soil health indicators for the Australian banana industry (Pattison *et al.* 2008). These included a physical soil indicator (bulk density), chemical soil indicators (labile C, NO_3-N, pH, electrical conductivity (EC)) (Table 6-3) and biological indicators (diversity of the nematode community, number of plant-parasitic nematodes and proportion of plant-parasitic nematodes) (Table 6-4). Bulk density measurements were not recorded for Farms 8, 9 and 10 (Table 6-3).

Soil properties were measured using on-farm assessments described in section 5.3 (Sarrantonio *et al*. 1996, http://soils.usda.gov/sqi/soil_quality/assessment/kit2.html). The soil nematode community was assessed from a 200 g sub-sample using the methods described in section 5.3.6 and assigned to functional groups and nematode community indices calculated as described in section 5.3.7 (Ferris *et al* 2001;Yeates 2003). The proportion of plant-parasitic nematodes in the soil was a useful indicator that may be related to the suppressive potential of the soil (Abawi and Widmer 2000; Stirling *et al*. 2005).

6.3.4 Statistics

A hierarchical cluster analysis was used to determine farming system typology based on group averages and weighted by cluster size using the group average method. The similarity between farm management practices was used to construct a dendrogram and plantations with a similarity greater than 0.7 were regarded as having the same typology. The mean and standard error of soil physical, chemical and biological parameters was determined for each plantation. A correlation matrix was used to determine linear relationships between soil parameters included in the survey. The mean values were used in a multivariate principle component analysis to determine key factors related to the suppression of plant-parasitic nematodes using the methods described by de Lima *et al.* (2008) and Shukla *et al.* (2006). The factors were subject to varimax rotations and parameters with a weighted value greater than 0.3 were regarded as important principal components.

A cluster analysis was used to determine the similarity of plantations based on the important principal components. A stepwise forward regression was used to determine the soil health indicators that were significantly related to the proportion of plant-parasitic nematodes in the soil. All statistical analyses were conducted using Genstat 11 (Laws Agricultural Trust).

6.4 Results

6.4.1 Farm typology

Farms included in the survey used some similar practices such as: growing ground cover in the fallow period, no plantations applied soil amendments prior to planting bananas, all plantations had applied fertiliser between 1 and 4 weeks prior to the survey and plantations that had applied nematicide had applied it greater than 4 weeks prior to the survey. The location, rainfall and soil types of plantations were similar and were not used in the analysis of farm typology (Table 6-2). However, Farms 9 and 10 were taken from the southern region of the north Queensland banana growing region, which is marginally drier (Table 6-2). Banana plantations included in the survey were predominately situated on Dermosols except for two plantations Farms 2 and 5, which were on Ferrosols (Table 6-2). Only seven criteria were used to determine the soil management typology for the 10 banana plantations included in the survey (Table 6-2).

Seven banana plantations used a fallow period greater than one year between subsequent banana crops. Farms 1 and 3 used a short fallow system less than 6 months and Farm 10 used an intermediate fallowing period of 6 months to 1 year (Table 6-2). Farms 5, 6 and 7 used less than 3 tillage operations to establish new banana planting, whereas Farms 2, 4, 8, 9 and 10 used between 3 and 6 tillage operations. Two farms, 1 and 3, used greater than 6 tillage operations to establish new banana plantings (Table 6-2). Only Farm 1 used greater than 400 kg of N ha^{-1} yr^{-1}, whereas Farms 4, 5, 6, 7 and 8 used less than 200 kg of N ha^{-1} yr^{-1} (Table 6-2). Four plantations had used nematicide to manage plant-parasitic nematodes, Farms 1, 3, 4 and 6, with the remaining 6 plantations not using nematicide (Table 6-2). Farm 7 was a recently established banana plantation producing the first crop since replanting. Most banana plantations were 2nd ratoon crops (Farms 2, 4 and 6) or a mix of different ratoons. Farm 5 was a long term planting, older than the 3rd ratoon crop (Table 6-2). All plantations, except Farms 6 and 10, maintained vegetated ground cover in the interrow spaces between banana plants (Table 6-2). Two plantations, Farms 5 and 10 removed the crop residue from around banana plants, placing it in the interrow space. Farms 1, 2 and 4 actively retained their crop residue around the base of the banana plants, whereas Farms 3, 6, 7, 8 and 9 left the crop residue where it fell following de-leafing and harvesting operations.

From the responses of the seven soil management criteria and using a similarity threshold of 0.7, the cluster analysis of the responses grouped the typologies of plantations into four different groups (Figure 6-1). Plantations typology 1 was made up of Farms 1 and 3, plantation typology 2 was made up of 6 banana plantations Farms 2, 4, 6, 8, 9 and 10 (Figure 6-2). Typologies three and four were made up of single plantations, typology 3 was Farm 5 and typology 4 was Farm 7 (Figure 6-2).

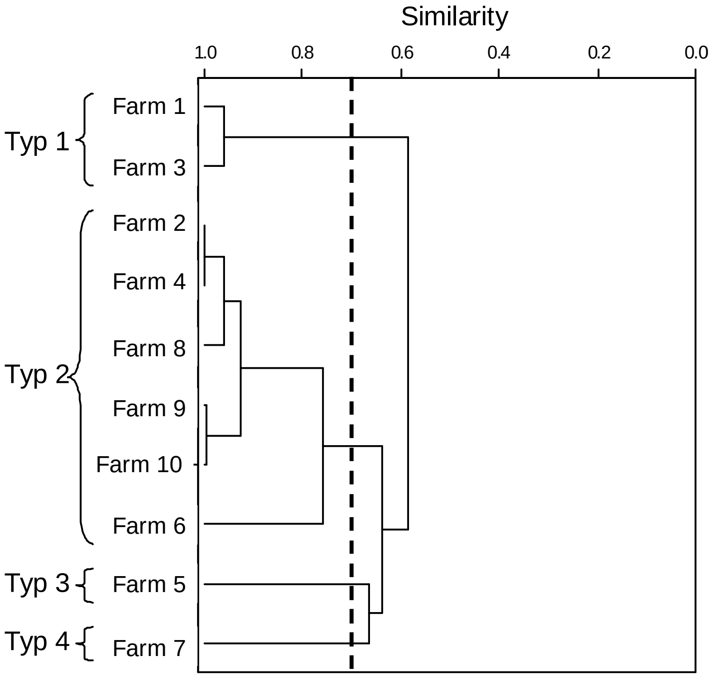

Figure 6-1: Cluster analysis dendrogram of 10 banana plantations grouped based on soil management typology (dashed line represents a similarity of 0.7 used to form groups)

Table 6-2: Characteristics of 10 banana plantations included in a survey to determine the impact of soil management on the soil food web and plant-parasitic nematodes.

Farm	Latitude (S)	Longitude (E)	Rainfall (mm)	Soil Order	Soil Description	Fallow length	Tillage operations	Nitrogen applications	Nematicide application	Crop age	Interrow vegetation	Crop residue
Farm 1	17° 34' 37"	146° 01' 17"	3 442	Dermosol	Friable clay or clay loam	1	3	3	1	2.6	1	2
Farm 2	17° 36' 42"	146° 00' 03"	3 390	Ferrosol	Friable clay or clay loam	3	2	2	2	3	1	2
Farm 3	17° 36' 17"	146° 00' 33"	3 390	Dermosol	Friable clay or clay loam	1	3	2	1	2.5	1	3
Farm 4	17° 27' 42"	145° 51' 50"	4 048	Dermosol	Friable clay or clay loam	3	2	1	2	3	1	2
Farm 5	17° 37' 33"	145° 56' 49"	3 482	Ferrosol	Friable clay or clay loam	3	1	1	1	4	1	1
Farm 6	17° 56' 18"	145° 47' 04"	3 245	Dermosol	Friable clay or clay loam	3	1	1	2	3	2	3
Farm 7	17° 56' 18"	145° 47' 04"	3 245	Dermosol	Friable clay or clay loam	3	1	1	1	0	1	3
Farm 8	17° 36' 01"	146° 01' 05"	3 390	Dermosol	Friable clay or clay loam	3	2	1	2	2.8	1	3
Farm 9	18° 14' 35"	145° 57' 12"	2 157	Dermosol	Grey loam, poorly drained	3	2	2	2	2.6	1	3
Farm 10	18° 13' 51"	145° 52' 54"	2 144	Dermosol	Grey loam, poorly drained	2	2	2	2	2	2	1

Fallow length: 1 = < 6 months; 2 = 6-1 year; 3 = >1year. Tillage operations: 1 = < 3; 2 = 3-6; 3 = >6. Nitrogen applications: 1 = 200 kg N ha^{-1} yr^{-1}; 2 = 200 = 400 kg N ha^{-1} yr^{-1}; 3 = >400 kg N ha^{-1} yr^{-1}. Pesticide applications: 1 = yes; 2 = no. Crop age; 0 = plant crop; 1 = 1st ratoon; 2 = 2nd ratoon; 3 = 3rd ratoon; 4 = >3rd ratoon. Interrow vegetation: 1 = vegetated; 2 = bare. Crop residue: 1 = inter-row; 2 = row; 3 = where it falls

Table 6-3: Soil physio-chemical characteristics and standard errors of 10 banana plantations included in a survey to determine the impact of soil management on the soil food web and plant-parasitic nematodes.

Plantation	Labile C (mg kg^{-1})	Nitrate-N (kg ha^{-1})	Labile C / NO3-N	pH	EC (dS m^{-1})	Bulk density (g cm^{-3})
Farm 1	348 ± 14	94 ± 9	3.9 ± 0.5	6.5 ± 0.3	0.078 ± 0.009	1.19 ± 0.02
Farm 2	444 ± 10	50 ± 8	9.5 ± 1.3	7.1 ± 0.1	0.055 ± 0.006	0.95 ± 0.04
Farm 3	319 ± 14	89 ± 15	4.0 ± 0.8	5.3 ± 0.3	0.090 ± 0.021	1.15 ± 0.02
Farm 4	357 ± 21	29 ± 5	13.4 ± 2.3	6.8 ± 0.1	0.103 ± 0.009	1.27 ± 0.02
Farm 5	608 ± 16	24 ± 5	28.7 ± 8.6	5.7 ± 0.1	0.027 ± 0.003	0.89 ± 0.01
Farm 6	483 ± 53	64 ± 19	10.3 ± 3.0	7.0 ± 0.1	0.035 ± 0.010	1.13 ± 0.03
Farm 7	474 ± 49	30 ± 11	19.5 ± 9.1	7.1 ± 0.2	0.010 ± 0.000	1.03 ± 0.05
Farm 8	509 ± 40	47 ± 5	11.1 ± 1.0	6.5 ± 0.2	0.044 ± 0.007	n.a.
Farm 9	289 ± 35	120 ± 15	2.5 ± 0.4	7.3 ± 0.2	0.058 ± 0.008	n.a.
Farm 10	430 ± 10	125 ± 6	3.4 ± 0.1	7.3 ± 0.0	0.043 ± 0.007	n.a.

n.a. = not available

Table 6-4: Nematode community characteristics and standard errors of 10 banana plantations included in a survey to determine the impact of soil management on the soil food web and plant-parasitic nematodes.

Plantation	Total nematodes	Bacterial feeding	Fungal feeding	Predators	Omnivores	Plant parasites		Diversity	Structure index	Enrichment index	Channel index
	100 g soil^{-1}	100 g soil^{-1}	100 g soil^{-1}	100 g soil^{-1}	100 g soil^{-1}	100 g soil^{-1}	Proportion				
Farm 1	1385 ±444	80 ±12	22 ±6	21 ±9	19 ±13	1243 ±436	0.86 ±0.04	0.94 ±0.20	68 ±7	70 ±11	22 ±8
Farm 2	1294 ±587	234 ±86	20 ±11	0 ±0	16 ±6	1024 ±573	0.67 ±0.09	1.37 ±0.30	63 ±21	87 ±8	6 ±5
Farm 3	651 ±208	49 ±16	35 ±18	6 ±2	9 ±2	551 ±218	0.74 ±0.11	1.21 ±0.40	52 ±13	51 ±9	59 ±17
Farm 4	587 ±119	80 ±16	58 ±18	48 ±11	126 ±41	275 ±72	0.45 ±0.04	2.06 ±0.09	88 ±3	68 ±6	32 ±6
Farm 5	640 ±163	168 ±12	137 ±49	57 ±7	35 ±1	243 ±99	0.34 ±0.07	2.30 ±0.07	66 ±5	67 ±2	33 ±7
Farm 6	752 ±126	98 ±8	55 ±28	58 ±24	74 ±16	467 ±92	0.62 ±0.05	1.68 ±0.12	88 ±2	79 ±6	21 ±10
Farm 7	282 ±172	25 ±19	79 ±40	23 ±8	6 ±0	150 ±121	0.43 ±0.17	1.52 ±0.04	57 ±22	59 ±2	64 ±8
Farm 8	1308 ±335	195 ±66	71 ±20	68 ±20	77 ±20	896 ±279	0.63 ±0.09	1.45 ±0.24	80 ±6	66 ±8	18 ±4
Farm 9	500 ±84	89 ±13	10 ±2	38 ±15	9 ±3	354 ±67	0.69 ±0.03	1.43 ±0.13	77 ±6	64 ±13	27 ±18
Farm 10	353 ±102	59 ±12	13 ±3	10 ±4	20 ±8	251 ±90	0.68 ±0.05	1.59 ±0.15	80 ±1	77 ±3	7 ±3

(Nematode diversity was determined using the Shannon-Weiner index $H' = -\Sigma\, p_i \log_e p_i$. where p_i is the proportion of individuals in the i^{th} taxon. The proportion of plant-parasitic nematodes was determined from the number of plant-parasitic nematodes relative to the total number of nematodes recovered from the soil sample. The enrichment index assesses the resources available to the soil food web and response by primary decomposers to those resources. The structure index is a measure of the number of trophic layers in the soil food web and the potential for regulation by predators. The channel index is an indication of the decomposition channel of nutrients; a low value indicates a primarily bacterial decomposer community and high value a fungal dominated decomposer community.)

Table 6-5: Correlation coefficients matrix of physical, chemical and biological soil parameters of 10 banana plantations from a survey to determine the impact of soil management on the soil food web and plant-parasitic nematodes.

Soil parameters	Bulk density (g cm^{-3})	pH	EC (µS m^{-1})	Nitrate-N (kg ha^{-1})	Labile C (mg kg^{-1})	Labile C/ NO$_3$-N	Nematodes (100 g^{-1})	Bacterial feeding (100 g^{-1})	Fungal feeding (100 g^{-1})	Plant parasites (100 g^{-1})	Predatory (100 g^{-1})	Omnivores (100 g^{-1})	Parasites / Nematodes	Diversity	Enrichment index	Structure index	Channel index
Bulk density (g cm^{-3})	1																
pH	0.08	1															
Electrical conductivity (µS m^{-1})	0.71	-0.28	1														
Nitrate -N (kg ha^{-1})	0.42	-0.31	0.45	1													
Labile C (mg kg^{-1})	-0.79*	0.07	-0.83	-0.64	1												
Labile C/ NO$_3$-N	-0.62	-0.02	-0.64	-0.86*	0.86*	1											
Nematodes (100 g^{-1})	0.00	0.11	0.32	0.56	-0.26	-0.54	1										
Bacterial feeding (100 g^{-1})	-0.63	0.11	-0.11	-0.23	0.44	0.20	0.55	1									
Fungal feeding (100 g^{-1})	-0.50	-0.26	-0.56	-0.70	0.80*	0.95**	-0.61	0.03	1								
Plant parasites (100 g^{-1})	0.10	0.08	0.36	0.70	-0.43	-0.69	0.96**	0.35	-0.74	1							
Predatory (100 g^{-1})	0.08	0.06	-0.24	-0.44	0.52	0.55	-0.39	-0.06	0.66	-0.55	1						
Omnivores (100 g^{-1})	0.54	0.26	0.38	-0.34	-0.08	0.08	-0.20	-0.04	0.11	-0.34	0.68	1					
Parasites / Nematodes	0.39	-0.09	0.48	0.95**	-0.68	-0.92**	0.73	-0.05	-0.86*	0.86*	-0.58	-0.33	1				
Diversity	-0.27	-0.03	-0.25	-0.83*	0.65	0.82*	-0.54	0.23	0.82*	-0.74*	0.76*	0.58	-0.90**	1			
Enrichment index	-0.24	0.64	-0.15	-0.14	0.29	-0.03	0.59	0.76*	-0.23	0.42	0.11	0.24	0.09	0.08	1		
Structure index	0.47	0.43	0.20	-0.19	0.04	0.01	0.03	0.06	0.03	-0.13	0.73	0.91**	-0.17	0.44	0.48	1	
Channel index	0.11	-0.38	-0.13	-0.08	-0.12	0.17	-0.78*	-0.77	0.30	-0.61	-0.10	-0.27	-0.28	-0.01	-0.92**	-0.50	1

* Significant r value *P*<0.05 ** significant r value *P*<0.01

6.4.2 Soil measurements

Labile carbon measurements from the 10 farms varied from 608 mg kg^{-1} at Farm 5 to 289 mg kg^{-1} at Farm 9, with the majority of plantations having a labile C measurement between 300 and 500 mg kg^{-1} (Table 6-3). The nitrate-N readings ranged from 125 kg ha^{-1} at Farm 5 to 24 kg ha^{-1} at Farm 10, with half of the plantations having 50 kg ha^{-1} of nitrate-N or less and the remainder between 51 and 125 kg ha^{-1} (Table 6-3). The variations in labile C and nitrate-N between the plantations resulted in a range in the labile C to nitrate-N ratio of the two parameters from 2.5 at Farm 9 to 28.7 at Farm 5 (Table 6-3). Only two plantations had a soil pH less than 6.0, Farms 3 (5.3) and 5 (5.7) (Table 6-3). The remainder of the farms had a soil pH close to neutral, between 6.5 and 7.5 (Table 6-3). The EC on all plantations was low with the highest recordings on Farm 4 (Table 6-3). Similarly, the soil bulk densities were regarded as low for clay loam soils with only one plantation having a soil bulk density greater than 1.2, Farm 4 at 1.27 g cm^{-3} (Table 6-3).

The highest total nematode population was on Farm 1 with 1 385 nematodes in 100g of soil (Table 6-4). Farm 1 also had the highest number and proportion of plant-parasitic nematodes (Table 6-4). The lowest number of plant-parasitic nematodes was on Farm 7, however, Farm 5 had the lowest proportion of plant-parasitic nematodes (Table 6-4). In general, plant-parasitic nematodes made up around two-thirds of the total nematode population on banana farms included in this survey (Table 6-4). The highest number of bacterial feeding nematodes were from Farm 2 (234 nematodes 100 g of $soil^{-1}$) and the highest number of fungal feeding nematodes were on Farm 5 (137 nematodes 100 g $soil^{-1}$) (Table 6-4). The greatest numbers of predatory nematodes were on Farm 8 (68 nematodes 100 g $soil^{-1}$) and the greatest number of omnivorous nematodes on Farm 4 (126 nematodes 100 g $soil^{-1}$); the higher number of omnivores resulted in a greater structure index at Farm 4.

The correlation analysis of the 16 soil parameters representing physical, chemical and nematode community structure in the soil resulted in 18 significant correlation coefficients ($P<0.05$) out of a possible 136 (Table 6-5). However, 11 of these were indices related to the values used in their calculations (Table 6-5). Significant correlations ($P>0.05$) of non related indices and values included labile C with bulk density (r = -0.79), nitrate-N with plant-parasite / total nematode ratio (r = 0.95), nitrate-N with diversity ratio (r = -0.83) and labile C with fungal feeding nematodes (r = 0.80). The ratio of labile C and nitrate-N was significantly correlated to three other variables; the number of fungal feeding nematodes (r =

0.95), ratio of plant-parasitic to total nematode population (*r*= -0.92) and nematode diversity (*r* = 0.82) (Table 6-5).

The 16 soil parameters considered in the principal component analysis were able to separate the 10 plantations; however, there was no obvious grouping of the plantations (Figure 6-2). The soil parameters could be grouped into two components, which accounted for a total of 61 % of the variation in the data set (Table 6-6). The first principal component accounted for 38 % of the variation and parameters with a high positive loading for the ratio of labile C to nitrate-N, nematode diversity, number of fungivores and the amount of labile C and a high negative loading for the proportion of plant-parasitic nematodes and amount of nitrate-N (Table 6-6). Because the first principal component contained the parameters related to the proportion of plant-parasitic nematodes at each plantation it was identified as the primary component with parameters related to plant-parasitic nematode suppression (Table 6-6) and used to determine similarities in soil properties between plantations in a cluster analysis.

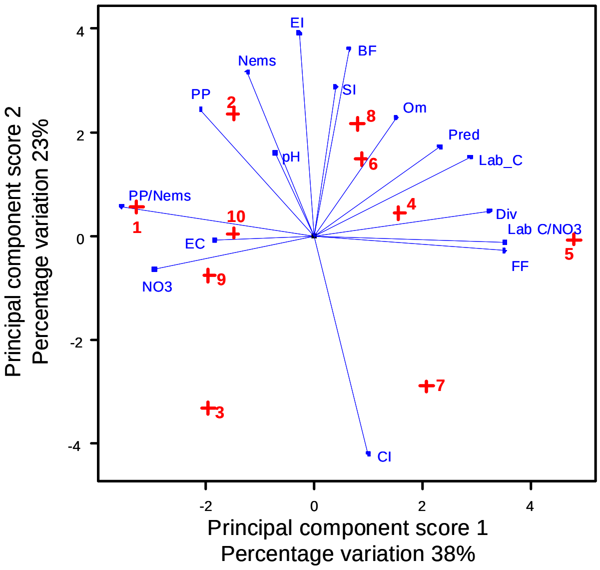

Figure 6-2: Scores on the two significant principal component functions based on 16 soil parameters for physical, chemical and nematode community properties to determine nematode suppression at banana plantations in north Queensland.

(numbers coincide with plantation numbers; BF = bacterial feeding nematodes 100 g soil^{-1}, CI = Channel index, Div = nematode diversity, EC = electrical conductivity, EI = enrichment index, FF = fungal feeding nematodes 100 g soil^{-1}, Lab C = Labile carbon, Lab C/NO_3 = labile C / nitrate-N, PP/Nems = plant-parasitic nematodes / total nematodes, Nems = Total nematodes 100 g soil^{-1}, NO_3 = nitrate-N, OM = omnivorous nematodes 100 g soil^{-1}, pH = pH, PP = plant-parasitic nematodes 100 g soil^{-1}, Pred = predatory nematodes 100 g soil^{-1}, SI = Structure index)

Table 6-6: Rotated component loadings of physical, chemical and nematode parameters on the first two principal components to determine nematode suppression at banana plantations in north Queensland.

Rotated factors	**PC1**	**PC2**
Parasites / Nematodes	-0.38	-0.12
Labile C/ Nitrate-N	0.38	0.07
Diversity	0.36	0.00
Fungal feeding (100 g^{-1})	0.37	0.08
Labile C (mg kg^{-1})	0.34	-0.12
Nitrate-N (kg ha^{-1})	-0.33	0.03
Predatory (100 g^{-1})	0.28	-0.15
Omnivores (100 g^{-1})	0.20	-0.22
Electrical conductivity (µS m^{-1})	-0.20	-0.02
Plant parasites (100 g^{-1})	-0.19	-0.29
Bacterial feeding (100 g^{-1})	0.12	-0.38
Structure index	0.08	-0.31
Nematodes (100 g^{-1})	-0.08	-0.36
pH	-0.05	-0.18
Channel index	0.05	0.47
Enrichment index	0.03	-0.43
Variation (%)	38	23

A cluster analysis was used to determine plantation typology based on five soil parameters; proportion of plant-parasitic nematodes, nematode diversity, fungal feeding nematodes in 100g of soil, labile C and nitrate-N (Figure 6-3). The ratio of labile C to nitrate-N was not included in the cluster analysis as it was derived from two parameters that had already been included in the analysis. From the cluster analysis the plantations could be grouped into three types. The first type had four plantations, Farms 1, 3, 9 and 10 (Figure 6-3). These farms were characterised by tending to have a high nitrate-N and greater proportion of plant-parasitic nematodes in the soil (Figure 6-2). The second group of plantations was made up of Farms 2, 6, 8, 4 and 7 with intermediate soil properties (Figure 6-3). The third group was made up of a single plantation, Farm 5. Farm 5 was characterised by having high labile C and high nematode diversity (Figure 6-2).

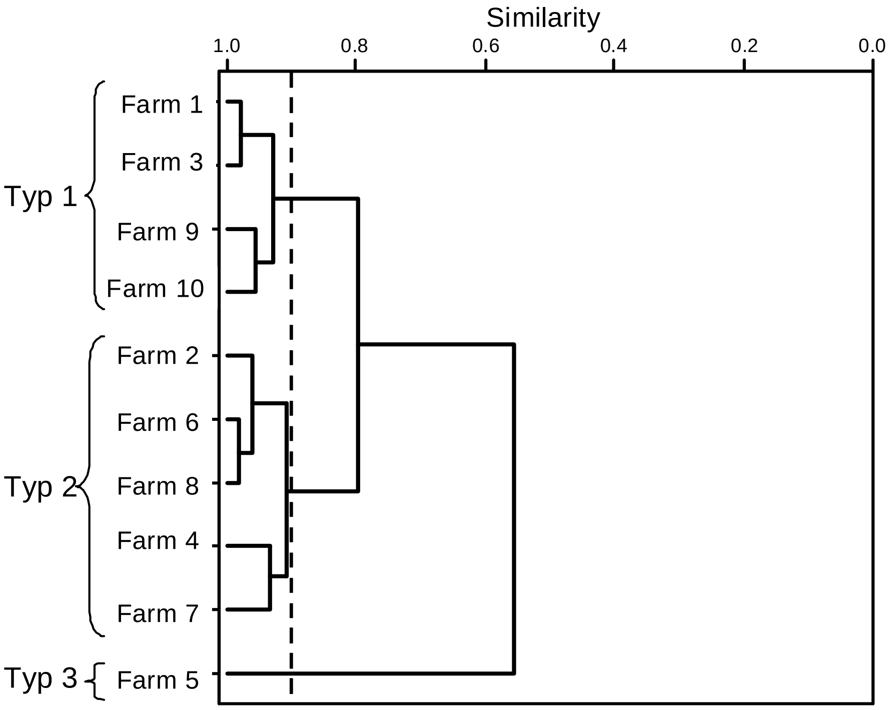

Figure 6-3: Cluster analysis dendrogram of 10 banana plantations based on proportion of plant-parasitic nematodes, nematode diversity, fungal feeding nematodes in 100g of soil, labile C and nitrate-N typology (dashed line represents a similarity of 0.9 used to form groups).

A forward stepwise regression of soil parameters was used to explain the difference in the proportion of plant-parasitic nematodes in the soil. Two parameters; the ratio labile C to nitrate-N ($P = 0.014$) and nematode diversity ($P = 0.028$) gave a significant multiple regression result, with adjusted R^2 coefficient of 88.7 ($P = 0.029$). The proportion of plant-parasitic nematodes in the soil could be predicted using equation 6-1.

Equation 6-1

Proportion of plant-parasitic nematodes = 1.0151+(-0.01049 * (labile C / nitrate-N)) - (0.187*nematode diversity)

Adjusted R^2 =88.7 (P=0.029)

6.5 Discussion

Farm management was found to be an important factor in the development of favourable soil conditions to suppress plant-parasitic nematodes of bananas in north Queensland. Analysis of farm typology based on fallow length, number of tillage operations, pesticide applications, crop age, interrow vegetation and placement of crop residue, allowed plantations to be grouped into four types, with Farm 5 having a distinctly separate farm management system relative to other plantations. Similarly, plantations grouped on the soil parameters; proportion of plant-parasitic nematodes, nematode diversity, fungal feeding nematodes in 100g of soil, labile C and nitrate-N also found that Farm 5 had distinctly different soil properties relative to the other banana plantations included in the survey. Farm 5 was found to have the lowest proportion of plant-parasitic nematodes in the soil, which was a function of the amount of labile C, nitrate-N and the diversity of nematodes in the soil community. Farm 5 also had the highest labile C measurement, lowest nitrate-N and highest nematode diversity of the plantations included in the survey (Tables 6-3 and 6-4). The management of Farm 5 was characterised by using fallows greater than one year, using less than three tillage operations to prepare ground, application of less than 200 kg N ha^{-1} yr^{-1}, no nematicide applied, older plantations greater than 3rd ratoon, vegetated interrows and removal of crop residue from around the base of the banana plants. The retention of crop residue around the base of banana plants is another source of carbon inputs, which if practiced on Farm 5 may have further increased labile C measurements and suppressed of plant-parasitic nematodes. The practices used on Farm 5, which increased soil C and reduced N appeared to have contributed to the favourable soil conditions leading to the lower proportion of plant-parasitic nematodes in the soil community.

Previous research in Chapters 3, 4 and 5 have all indicated that the amount of labile C in the soil is an important factor in the suppression of plant-parasitic nematodes on bananas. The results from this on-farm survey coincide with the work in the previous chapters, where increasing labile C in the soil lead to greater general suppression of plant-parasitic nematodes, but additionally in this study nitrate management and the diversity of the soil nematode community were indicators that could be used to identify production systems, which suppress plant-parasitic nematodes on bananas. Therefore, farm management practices that are able to increase labile C such as long fallows, use of interrow vegetation and reduced tillage and careful nitrogen management can all contribute to the suppression of plant-parasitic nematodes in bananas.

Conversely, Farm 1 had the highest number and proportion of plant-parasitic nematodes, lowest nematode diversity, low labile C and high nitrate-N (Table 6-3 and 6-4). Farms 1 and 5 had divergent soil properties and soil management typologies, with only around a 60% similarity in both of these aspects of banana production (Figure 6-1 and 65-3). Farms 1 and 3 were grouped similarly (<10% difference) in the cluster analysis of both the key soil indicators and the soil management (Figure 6-1 and 6-3). These two farms could be regarded as traditional intensive, high input farms, and were representative of conventional banana farming operations in Australia. Farms 9 and 10 also had very similar (<5% difference) soil parameters and soil management practices of bananas and were a part of a closely related group. These farms used fewer inputs and were experimenting with practices to improve the health of their soil. However, management practices did not always consistently match differences in soil health indicators (Figure 6-3), which suggested other factors may have also impacted on the key soil health indicators and that some caution is needed when using single point measurements to determine the health of banana soils if it cannot be related to some other factor such as nematode suppression.

The proportion of plant-parasitic nematodes was related to soil management that could be identified by soil health parameters using a forward stepwise regression. Using this method, the proportion of plant-parasitic nematodes was determined to be a function of the amount of nitrogen applied to the crop and carbon inputs. Increasing the amount of nitrogen and reducing soil carbon in the plantation increased the proportion of plant-parasitic nematodes. The number of fungal feeding nematodes was also correlated to increased labile C, nematode diversity and a reduction in the proportion of plant-parasitic nematodes in the soil. Therefore, increasing the labile C content in the soil may be related to soil conditions that favour fungal growth leading to an increase in fungal antagonists and predators of nematodes. The survival and increase in fungal feeding nematodes could be seen as an indicator of suppression of plant-parasitic nematodes. Therefore, the general suppression of plant-parasitic nematodes observed in this survey may be related to a greater number of fungal nematode antagonists in the soil, which were promoted under the conditions of high labile C and low nitrate-N.

Low levels of nitrate-N in the soil were found to be an important contributor in developing banana cropping systems that suppress plant-parasitic nematodes. Banana growers in Australia have implemented a number of practice changes, which have led to a reduction in the amount of nitrogen fertilisers applied to banana crops. At the same time they have managed to maintain the same level of production. The average amount of N applied in

1995 was 519 kg ha^{-1} (Daniells 1995) with an average of 2 300, 13 kg cartons ha^{-1} yr^{-1} (http://www.abgc.org.au/?industry/banana-industry). The average amount of nitrogen currently being used by banana growers in 2004, in tropical banana production is 305 kg ha^{-1} (Mark Warmington, *pers. comm.*) with an average production of 2 520, 13 kg cartons ha^{-1} yr^{-1} (http://www.abgc.org.au/?industry/banana-industry). These figures do not account for seasonality differences, but are indicative of the previous overuse of nitrogen-based fertilisers by the banana industry and suggest that reductions in N application can be made without sacrificing production.

Only two plantations, Farms 6 and 10 did not have vegetative ground cover between rows of banana plants. The inclusion of managed vegetated ground covers reduces soil and nutrient movement and has been shown to reduce compaction due to machinery traffic in banana plantations (Rasiah *et al.* 2009). Furthermore, increasing the diversity of ground cover has been linked to increasing diversity of soil organisms (Van der Putten *et al.* 2009; Wardle *et al.* 2004). However, when ground covers are not managed and are allowed to compete with banana plants they reduce production (Firth *et al.* 2003; Johns 1994). While there are many factors contributing to the diversity of nematodes in the soil, by maintaining a non-competitive vegetative ground cover, greater microbial diversity can be achieved. Increases in the diversity of soil organisms have been shown to contribute to the suppression soil borne diseases (Alabouvette *et al.* 2004; Garbeva *et al.* 2004; van Bruggen and Semenov 2000). Therefore, the management of a diverse vegetated ground cover, together with management of carbon and nitrogen inputs are farm management options that can significantly contribute to the suppression of plant-parasitic nematodes of bananas.

The inherent properties of different soil types contribute significantly to their suitability for crop production and have been shown to develop distinctly different soil microbial profiles (Adesina *et al.* 2007). Two plantations, Farms 5 and 2 were situated on Ferrosols of volcanic origin. These are described as friable clay soils that are well drained (Cannon *et al.* 1992). The remaining plantations were situated on dermosol soils of alluvial origin. However, Farms 9 and 10 were on poorly drained soils. The inherent soil properties associated with the soil types may have contributed to differences in the suppression of plant-parasitic nematodes. Farm 5, which had distinctly different soil and management properties resulted in the greatest reduction in the proportion of plant-parasitic nematodes in the soil. While soil management appeared to be a contributing factor, differences due to soil type cannot be disregarded because of the limited number of sites included in this study.

Therefore, further work is needed to clarify the effect of inherent soil properties due to soil type and to farm management systems on plant-parasitic nematode suppression.

The approach of using multivariate statistics to determine differences in banana production systems and soil properties allowed the determination of factors that were contributing to differences in the proportion of plant-parasitic nematodes to total nematodes. The survey questionnaire of farm managers allowed separation of plantations depending on differences in soil management. While this information is categorical and semi-qualitative it was useful in determining differences due to farm management. Similarly, the measurement of soil physical, chemical and nematode community parameters using a pre-determined set of indicators allowed the separation of plantations based on soil properties. Furthermore, parameters that were indicative of suppression of plant-parasitic nematodes could be determined. By matching soil management system with soil properties, such as the case with Farm 5, a greater understanding of how farm management decisions can impact on development and suppression of soil borne pest and diseases such as plant-parasitic nematodes can be developed.

The management practices which suppressed plant-parasitic nematodes included the increasing carbon inputs, managing nitrogen inputs and practices that could increase diversity of soil organisms. Soil indicators that can be used to monitor achievements following implementation of these practices include labile C, nitrate-N and the diversity of soil nematodes in the soil community. These results are consistent with studies in previous chapters on suppression of *R. similis*, and therefore give banana growers guidance in selection of inputs for developing practical management practices.

6.6 Summary

Agricultural practices have been shown to have a significant impact on the soil environment. Therefore, by characterising banana soil management typologies and soil properties it may be possible to determine soil management practices and indicators that can lead to the suppression of plant-parasitic nematodes in banana plantations. Previous work had suggested that increasing the labile C fraction in the soil was correlated with suppression of *R. similis*. A study was implemented on 10 banana plantations in the north Queensland banana production area to determine differences in soil management, physical, chemical and nematode community properties using a multivariate statistical approach to determine factors that demonstrated differences between plantations and suppression of plant-parasitic nematodes. A cluster analysis was used to separate plantations based on soil management based on fallow length, number of tillage operations, nitrogen application, nematicide applications, crop age, inter-row vegetation and placement of crop residue. Four management typologies were formed from the resulting dendrogram which separated a plantation, Farm 5, from other plantations as having a unique soil management approach. Measurement of soil physical, chemical and nematode community parameters were used to determine the parameters that were associated with the suppression of plant-parasitic nematodes. A principal component analysis was able to explain 61% of the total variation and identified the soil factors with the highest loadings: the proportion of plant-parasitic nematodes, labile C, nitrate-N, and the number of fungal feeding nematodes in the soil community. Again a cluster analysis of these factors was able to separate Farm 5 as having unique soil properties. A forward stepwise regression of the soil properties associated with the proportion of plant-parasitic nematodes in the soil community found that the ratio of labile C and nitrate-N in the soil and the diversity nematodes was able to explain an adjusted 88.7% of the variation in the proportion of plant-parasitic nematodes in the soil. The identification of Farm 5 as having the lowest proportion of plant-parasitic nematodes, unique soil properties and management typology suggested that management could be used to develop soils suppressive to plant-parasitic nematodes. Farm practices that increase carbon inputs, manage nitrogen fertiliser applications and promote soil biodiversity, can increase soil labile C, reduce nitrate-N and increase nematode diversity leading to a suppression of plant-parasitic nematodes on banana plantations in Australia.

7 The effect of banana farm management on soil health and plant-parasitic nematodes in Costa Rica.

7.1 Introduction

Bananas are typically grown in areas of high rainfall, high soil temperatures and on acid soils (Simmonds 1959). It has been suggested that intensively managed monocrop agriculture, practiced close to the equator can be unsustainable, due primarily to soil degradation and erosion (Primavesi 2006), which places most of the banana production areas around the world at risk. Costa Rica is the third largest banana exporting nation in the world, located between 8° and 10° north of the equator and producing 2.3 million tonnes of bananas in 2007, worth US$675 million from 43 817 ha (http://faostat.fao.org/site/339/default.aspx). However, in terms of banana production Costa Rica is only the 8th largest banana producing nation behind India (21.7 million tonnes), China (8.0 million tonnes), Philippines (7.4 million tonnes), Brazil (7.1 million tonnes), Ecuador (6.0 million tonnes), Indonesia (5.4 million tonnes) and United Republic of Tanzania (3.5 million tonnes) (http://faostat.fao.org/site/567/default.aspx#ancor). There is an increasing need to demonstrate that banana production practices in Costa Rica, which are typical for export monoculture production systems, can be sustainable and can overcome inherent constraints of soil and climate with minimal impact on the environment.

Plant-parasitic nematodes, particularly the burrowing nematode (*Radopholus similis*), remains a serious constraint to sustainable banana production in Costa Rica (Araya 2005). The burrowing nematode is endemic to the banana production areas of Costa Rica being found on greater than 95% of plantations (Araya *et al.* 2002; Araya and Moens 2005) and is responsible for declining banana production (Araya and Moens 2005; Cabrera *et al.* 2006; Moens *et al.* 2001). The nematode has traditionally been controlled through the use of nematicides applied, on average, twice per year (Araya and Lakhi 2004; Moens *et al.* 2004). However, there has been a decline in the efficacy of the nematicides in Costa Rica due to enhanced biodegradation (Cabrera *et al.* 2010; Moens *et al.* 2004). This has meant that alternative methods must be developed to manage nematode populations and minimise yield losses in banana plantations, methods that do not rely on the use of nematicides (Pocasangre 2000; Sikora and Pocasangre 2004). The use of endophytic fungi has been shown to be able to reduce the populations of burrowing nematodes in banana plantations and has potential for use in commercial plantations (Pocasangre 2000; Sikora *et al.* 2008; zum Felde *et al.* 2005). However, research in previous chapters has demonstrated that soil properties are also important in the suppression of *R. similis* and careful management of bananas can increase general suppression of plant-parasitic nematodes.

There have been many investigations to determine the physical and chemical soil properties that constrain banana production in Costa Rica (Turner and Rosales 2005). In particular, poor drainage and compaction leading to restriction of roots has been identified as a major physical constraint to sustainable banana production in Costa Rica (Gauggel *et al.* 2005). Furthermore, low pH and soil acidity have been shown to reduce plant growth with the problem becoming more apparent in older plantations (Serrano 2005). However, there is lack of information on how the biological components of the soils in Costa Rica change under commercial banana production and how management of soil biology may contribute to a healthy soil.

It has been suggested that a healthy soils are better able to buffer plants from biotic and abiotic stresses, thus sustaining crop production levels (van Bruggen and Termorshuizen 2003). This requires the development of methods to assess soil health and to determine the stresses imposed on the plants, such as soil borne diseases (Janvier *et al.* 2007). Soil health assessment requires holistic interpretation of the physical, chemical and biological soil properties, which are related to suppression of soil borne pathogens such as plant-parasitic nematodes (Janvier *et al.* 2007). An approach, such as this, measures a general suppressive mechanism, which is not usually transferable between soils (Weller *et al.* 2002). Research in Australia, presented in previous chapters, had identified that labile soil C and nitrate-N management, together with increased nematode diversity were important in developing soils with general suppression to plant-parasitic nematodes under bananas. However, under a different banana production system, with different soil conditions, such as in Costa Rica, it was unclear if the same mechanisms and soil factors that produce nematode suppression in Australia would be relevant.

The methodological framework using multivariate statistics and factor analysis for selection of soil health indicators suggested by de Lima *et al.* (2008) and used in Chapter 5 was considered appropriate to determine soil properties related to suppression of *R. similis* in Costa Rican banana production. Furthermore, the soil indicators suggested by Pattison *et al.* (2008) and found to be useful in determining soil properties related to plant-parasitic nematode suppression was used in the context to provide a practical and rapid method of assessing banana plantations.

7.2 Objectives

The aim of the study was to use the knowledge of soil indicators and plant-parasitic nematode suppression gained in Australia to investigate the effects of different soil management practices and soil physical, chemical and biological properties on the suppression of *R. similis* in Costa Rica.

7.3 Materials and methods

7.3.1 Survey sites

Twenty one banana plantations in Costa Rica, involved in commercial production for the international export market, were surveyed between February and June 2007. All plantations surveyed were growing Cavendish bananas (*Musa* AAA Cavendish sub-group) of the varieties Williams, Gran Naine or Valery. The location, soil type, soil moisture and soil temperature at the time of the survey are given in Table 7-1.

All banana farms surveyed had extensive drainage systems in place and had plant density between 1500 and 1700 plants ha^{-1}. All farms were using systemic and contact fungicides to control the leaf disease black Sigatoka (*Mycospharella fijiensis*). Bunch bagging and trimming were carried out as standard practice one and two weeks after flowering, on all farms. On each bunch, three true hands plus the false hand and the bell were eliminated. Fruit deflowering was conducted before bagging, when fruits had begun to curl upwards.

Table 7-1: Location, soil type, volumetric moisture content and soil temperature of Costa Rican banana farms surveyed for soil health characteristics.

Farm	**Latitude (N)**	**Longitude (W)**	**Soil Type**	**Volumetric soil moisture (%)**	**Soil temperature (°C)**
Ag1	10° 21′ 19″	83° 41′ 46″	Dystrandept	47	26.1
Ag2	09° 40′ 09″	82 ° 47′ 48″	Tropaquept	44	29.0
Ba1	10° 25′ 48″	83 ° 42′ 00″	Dystropept	56	24.6
Ba2	10° 07′ 56″	83 ° 28′ 51″	Eutropept	52	23.5
Ca1	08° 28′ 12″	82 ° 56′ 24″	Eutropept	46	-
Ca2	10°:19′ 17″	83 ° 36′ 32″	Dystropept	47	-
Ca3	10° 07′ 25″	83 ° 26′ 19″	Eutropept	57	23.0
Ca4	10° 38′ 12″	83° 38′ 12″	Dystropept	46	26.7
Ca5	08° 27′ 55″	82 ° 56′ 15″	Eutropept	50	23.5
Co1	09° 46′ 37″	82 ° 54′ 01″	Tropaquept	45	-
Es1	10° 04′ 59″	83 ° 22′ 01″	Tropaquept	44	27.8
Es2	10° 09′ 42″	83 ° 27′ 49″	Eutropept	50	24.8
La1	10° 20′ 59″	83 ° 44′ 01″	Dystrandept	47	25.9
La2	10° 09′ 37″	83 ° 28′ 51″	Eutropept	45	26.4
Mi1	10° 02′ 01″	83 ° 15′ 36″	Tropaquept	38	28.3
Pa1	10° 02′ 43″	83 ° 13′ 19″	Tropaquept	49	26.7
Pe1	10° 25′ 23″	83 ° 54′ 24″	Dystrandept	50	26.0
Re1	10° 28′ 32″	84 ° 00′ 31″	Dystropept	52	24.1
Sa1	10°:29′ 17″	83 ° 55′ 41″	Dystrandept	59	24.5
Sa2	10° 06′ 45″	83 ° 22′ 54″	Eutropept	55	24.6
Ve1	09° 43′ 25″	83 ° 03′ 25″	Tropohumult	40	25.4

7.3.2 Soil health indicators

7.3.2.1 Sampling soils and plants

On each plantation an area was selected for the study that was described by the plantation owner or manager as being average for the plantation. Sampling sites in each area were determined by selecting five banana plants at the same physiological stage of development, that is, plants that had recently flowered and that had the lower hands removed. The fruit on selected plants had not yet begun to curl upwards and the lower hands usually had female flower ends still intact. At each sampling site measurements of infiltration, bulk density and root density were taken 10 cm from the base of the selected plant in front of the daughter plant, in the zone where fertiliser and pesticide were applied and most root activity was thought to occur. A composite soil sample for laboratory analysis of chemical and biological soil properties was taken by collecting three 15 mm soil cores, from the selected plant and four additional neighbouring plants, to a depth of 10 cm in a zone 30 cm from the base of the plant and in front of the daughter plant. The soil was divided into sub-samples from each sampling site for different chemical and biological soil tests. A 200 g sub-sample was kept for nematode community analyses and 1.25 kg was used for a pot experiment. A 100 g composite sample from each plantation over the five sampling sites was used for analysis for chemical properties by a commercial laboratory.

7.3.2.2 Plant measurements

The finger number per bunch was determined using a modified method described by Turner *et al.* (1988). The commercial practice on the farm was to trim the lower three hands from each bunch. Therefore, the number of hands on each bunch was determined by counting the total number of hands present on trimmed bunches. The number of fingers on the third hand from the top and the number of fingers on the last hand of trimmed bunches was determined. The estimation of finger number was then calculated from the average of the third hand from the top and the lower hand, multiplied by the number of hands on each bunch (Turner *et al.* 1988).

Three additional plant measurements were made; the height of the following sucker from the ground to the intersection of the second and third youngest leaves; the height of the bunched plant was determined by measuring from the highest point of the curve of the underneath of the peduncle to the ground at the base of the plant and the circumference of the plant which was measured at a point which was determined to be one-third of the height of the plant.

7.3.2.3 Soil measurements

A soil health survey method was developed using basic soil quality indicators as described previously in section 4.3 (Pattison *et al.* 2008; Sarrantonio *et al.* 1996). The soil measurements made included physical soil measurements; bulk density (section 5.3.5) and water infiltration times (section 5.3.5), chemical soil measurements included; pH, electrical conductivity (EC), nitrate-N and labile C (Weil *et al.* 2003) as described in section 5.3.5. Furthermore, a composite soil sample from each plantation was analysed for extractable nutrients using Mehlich 3 methods by the laboratories of the Corporación Bananera Nacional (Corbanan, Centro de Investigación, La Rita, Pococí, Limon, Costa Rica) and included pH, extractable acidity, Al, Ca, Mg, K, P, Fe, Zn, Mn and organic matter (OM). Biological measurements included analysis of the soil nematode community and calculation of community indices (Ferris *et al.* 2001; Yeates and Bongers 1999) as described in sections 4.3.6 and 4.3.7.

7.3.2.4 Root nematodes

Banana roots collected from the field were washed free of soil. Excess water was dried from the surface of the roots using paper towel. The functional root weight was then calculated by removing any dead roots (Moens *et al.* 2001). The disease index was determined by splitting the roots open and assessing the amount of necrotic tissue in the root cortex (Stanton *et al.* 2001). Root ratings were assigned: 0, no lesions;1, 1-25% of root cortex occupied by lesions; 3, 26-50%; 5, 51-75%; and 7, 76-100%. The ratings were then used to calculate the disease index using equation 7-1:

Equation 7-1:

$$\text{Disease index} = \frac{\sum \text{ratings}*100}{\text{total number of roots}*7}$$

Nematode populations in the root were then determined by cutting the roots into 10-20 mm length and macerating in a blender for 10 secs at low speed and 10 secs at high speed (Araya *et al.* 1999). Nematodes were recovered from the macerated root tissue by passing the blended roots through a series of sieves 300, 150 and 25 µm. The nematodes were washed from the 25 µm sieve into a container and suspended in 50 mL of water. A 2 mL aliquot was used to determine the total number and genera of nematodes present.

7.3.2.5 Glasshouse bioassay

Soil collected from the 21 banana plantations was used in a glasshouse experiment to determine the suppressive potential of the soil to prevent *R. similis* penetrating into the roots of banana plants. 250 g of soil was placed into plastic cups (75 mm d x 100 mm) with drainage holes in the base. A 7-week old *in vitro* banana plant (*Musa* AAA Cavendish subgroup, Gran Naine) was planted in the soil of each cup. From each farm there were 5 replicate pots, except ES1 due to insufficient soil. Soil had been stored at 4 ^{o}C from the time the soil had been collected until the commencement of the glasshouse experiment.

Fertiliser was applied seven days after planting the bananas using 3 g of a slow release fertiliser (Hydrocomplex, Barco Vingingo, 12%N, 11% P_2O_5, 18% K_2O, 3% Mg, 8% S, 1% minor elements (B, Zn, Cu, Mn), 47% inert ingredients). The following day, 8 days after planting, all plants were inoculated with 250 motile *R. similis*, obtained from carrot disc cultures (Moody *et al.* 1973).

Plant height was measured 4-weeks after planting and the experiment was terminated 8 weeks after planting and 7-weeks after banana plants were inoculated with nematodes. At the termination of the experiment the fresh weight of roots and shoots of banana plants were determined and nematodes were extracted from the roots using the maceration and sieving technique described earlier.

7.3.3 Statistics

The mean and standard error was determined for all measurements of soil properties from plantations. A correlation matrix was used to determine linear relationships between soil and plant parameters included in the survey. The mean values were used in a multivariate principle component analysis to determine key factors related to the suppression of plant-parasitic nematodes using the methods described by de Lima *et al.* (2008) and Shukla *et al.* (2006). The factors were subject to varimax rotations and parameters with a weighted value greater than +/- 0.3 were regarded as important variates for the principal component. The variables with a weighted value greater than +/- 0.3 were used in a hierarchical cluster analysis for each of the principal components weighted by cluster size using the group average method. The similarity between soil properties was used to construct a dendrogram and plantations with a similarity greater than 0.9 were regarded as having similar soil properties, unless otherwise specified. A stepwise forward regression was used to determine the soil health indicators that were significantly related to the number of *R. similis* recovered from the roots of bananas grown in the bioassay experiment and used to construct a multiple linear regression model. All statistical analyses were conducted using Genstat 11 (Laws Agricultural Trust).

7.4 Results

7.4.1 Soil health indicators

Banana plants measured in the survey were at a similar physiological stage of development, however, there was a range of banana agronomic characteristics between plantations (Table 7-2). The plantation with the highest number of hands and fingers per bunch was Mi1, where trimmed bunches had on average 9.2 hands and 179 fingers per bunch. The tallest following sucker occurred at Ve1 (214 cm) and the tallest mother plant occurred at Sa2 (392 cm) (Table 7-2). The plantation with the shortest following sucker, mother plant and circumference was Sa1 (Table 7-2). Root density was greatest at Ba1 and least at Mi1, however, there was a large error associated with measurement of the root density (Table 7-2).

Physical soil measurements included water infiltration rates and bulk density. The fastest infiltration rate was recorded at Pa1, 21.2 cm min^{-1} and the slowest at Ba2, 0.4 cm min^{-1} (Table 7-2). There was a small range in soil bulk density from 0.81 to 1.17 g cm^{3} at Ag1 and Ve1 respectively (Table 7-2). The in-field chemical measurements produced a range of EC measurements from 71 to 909 µS cm^{-1}at Pa1 and Ca5 respectively (Table 7-2). There was a 19 fold difference in the amount of nitrate-N between Es2 and Ca5, which represented 10 kg NO_3-N ha^{-1} and 192 kg NO_3-N ha^{-1} respectively (Table 7-2). Soil pH ranged from 4.3 at Ag2 to 6.8 at two plantations Ca4 and Mi1 (Table 7-2). There was a two-fold difference in the amount of labile carbon measured, with Ve1 and Mi1, 305 and 685 mg kg^{-1} respectively (Table 7-2).

Differences were observed in the number of plant-parasitic nematodes and damage observed on the roots of bananas from the 21 plantations. The lowest disease index was recorded at Mi1 where no root damage due to nematodes was observed and the highest was at Pa1, where over half of the root cortex was occupied by nematode lesions (Table 7-3). *R. similis* was not detected at two plantations Ca3 and Pe1 (Table 7-3). However, high numbers of *R. similis* were extracted from the roots of Re1 (Table 7-3). Re1 also had the greatest total plant-parasitic nematode number extracted from the roots (Table 7-3). In the soil, plant-parasitic nematodes generally made up around 50% of the soil nematode population. However, the greatest proportion of plant-parasitic nematodes was found at Ca1, which had 86% of the soil nematode population as plant-parasites (Table 7-3). Conversely, Ca4 had the lowest proportion of plant-parasitic nematodes, 20% of the soil nematodes population. Furthermore, Ca4 had the greatest proportion of fungivores, 44% of the nematode population, but the proportion of fungivores was generally below 15% of the nematode population on most plantations (Table 7-3). The proportion of predators and omnivores was typically under 25% of the total nematode population. However, Mi1 had 26% of the soil nematode populations as predators and omnivores (Table 7-3). The proportion of bacterivores was generally between 25 and 50 % of the soil nematode population, but ranged from 7% at Ca1 to 49% at Ca2 (Table 7-3).

The diversity of soil nematodes, determined by the Shannon-Weiner index, ranged from 1.12 to 2.32 at Ve1 and Ca 3 respectively (Table 7-3). Similarly, there was a range in the enrichment index from 28 to 75 at La 2 and Sa2 respectively (Table 7-3). There were three plantations with a structure index of 80, Ca4, Mi1, and Re1. The lowest structure index was observed at La2, which was 19 (Table 7-3). The channel index was generally below 50 for most banana plantations included in the study. However, two plantations, Ca1 and Sa1 had channel indices of 58 and 69 respectively. (Table 7-3).

Soil nutrient tests were only performed on composite soil samples from each plantation. There was a range in extractable nutrients Ca, Mg, K, P Fe, Cu, Zn, Mn and Al recorded between plantations (Table 7-4). Similarly, as observed with in-field soil chemical measurements, there was a range in soil pH values from 4.3 at Ag2 and Ca4 to 6.3 at Ve1 (Table 7-4). Furthermore, there was a range in exchangeable acidity from 3.1 cmol (+) L^{-1} at Ag2 to 0.1 cmol (+) L^{-1} at four plantations Ba2, Ca3, Re1 and Ve1 (Table 6-4). Soil organic matter, similarly, ranged between plantations from 1.9% at Ve1 to 8.2% at Ca2 (Table 6-4).

Table 7-2: Differences in soil properties assigned to soil functions, productivity, plant health and disease suppression on 21 banana farms in Costa Rica.

Farm	**Plant measurements**					**Physical measurements**			**Chemical measurements**			
	Hands	**Fingers**	**Sucker height (cm)**	**Height mother (cm)**	**Circumference (cm)**	**Root density (g L^{-1})**	**Water infiltration (cm min^{-1})**	**Bulk density (g cm^{-3})**	**Electrical conductivity (µS cm^{-1})**	**pH**	**Nitrate nitrogen (kg ha^{-1})**	**Labile C (mg kg^{-1})**
Ag1	7.8 ± 0.2	137 ± 5	145 ± 11	293 ± 9	72 ± 2	6.6 ± 1.0	6.8 ± 3.0	0.81 ± 0.06	259 ± 40	5.4 ± 0.1	38 ± 9	668 ± 25
Ag2	7.6 ± 0.4	131 ± 10	183 ± 10	299 ± 7	70 ± 2	3.9 ± 3.1	5.6 ± 3.4	1.01 ± 0.06	320 ± 22	4.3 ± 0.2	62 ± 17	515 ± 67
Ba1	6.0 ± 0.3	105 ± 8	144 ± 11	302 ± 17	66 ± 2	9.4 ± 4.2	4.5 ± 1.1	0.92 ± 0.05	414 ± 20	5.3 ± 0.1	103 ± 13	667 ± 15
Ba2	7.4 ± 0.4	129 ± 10	172 ± 11	316 ± 5	75 ± 1	5.2 ± 1.5	0.4 ± 0.1	1.23 ± 0.03	140 ± 38	6.2 ± 0.1	36 ± 15	428 ± 40
Ca1	8.4 ± 0.4	144 ± 12	183 ± 10	322 ± 6	73 ± 2	2.6 ± 1.5	1.5 ± 1.1	1.04 ± 0.03	273 ± 45	4.8 ± 0.2	101 ± 19	652 ± 13
Ca2	7.0 ± 0.4	116 ± 12	159 ± 18	307 ± 16	73 ± 4	7.6 ± 1.7	4.2 ± 1.5	0.86 ± 0.06	291 ± 47	5.8 ± 0.1	52 ± 9	597 ± 20
Ca3	8.8 ± 0.4	163 ± 14	140 ± 6	310 ± 4	73 ± 3	2.0 ± 0.9	8.7 ± 3.3	1.15 ± 0.06	228 ± 42	5.8 ± 0.2	54 ± 14	559 ± 23
Ca4	7.6 ± 0.3	148 ± 10	126 ± 13	341 ± 11	80 ± 2	1.3 ± 0.4	1.8 ± 0.7	1.14 ± 0.02	147 ± 14	6.8 ± 0.2	27 ± 4	467 ± 12
Ca5	7.4 ± 0.2	118 ± 5	142 ± 14	288 ± 6	69 ± 1	2.3 ± 0.4	11.8 ± 2.7	1.07 ± 0.04	909 ± 136	4.5 ± 0.1	192 ± 8	603 ± 22
Co1	7.8 ± 0.2	137 ± 6	184 ± 9	294 ± 8	69 ± 1	2.5 ± 1.2	6.4 ± 1.2	1.00 ± 0.02	352 ± 29	5.5 ± 0.3	72 ± 15	585 ± 36
Es1	7.2 ± 0.7	125 ± 22	123 ± 13	282 ± 14	64 ± 3	6.0 ± 3.6	3.7 ± 2.8	0.87 ± 0.06	255 ± 22	5.2 ± 0.1	72 ± 13	572 ± 22
Es2	8.2 ± 0.2	160 ± 8	174 ± 16	334 ± 32	77 ± 2	4.5 ± 1.3	5.3 ± 1.2	1.13 ± 0.03	125 ± 26	5.0 ± 0.1	10 ± 1	570 ± 23
La1	6.4 ± 0.2	104 ± 8	179 ± 10	360 ± 6	69 ± 1	3.6 ± 1.4	12.4 ± 5.1	0.99 ± 0.05	78 ± 8	6.2 ± 0.1	12 ± 1	588 ± 19
La2	7.4 ± 0.6	142 ± 16	163 ± 10	320 ± 12	76 ± 3	4.4 ± 2.2	14.4 ± 7.3	1.14 ± 0.04	241 ± 27	4.6 ± 0.1	14 ± 3	444 ± 39
Mi1	9.2 ± 0.2	179 ± 7	150 ± 14	341 ± 5	77 ± 2	1.0 ± 0.3	1.9 ± 1.3	1.04 ± 0.03	185 ± 34	6.8 ± 0.2	35 ± 8	685 ± 12
Pa1	7.4 ± 0.2	135 ± 7	147 ± 14	311 ± 8	75 ± 1	3.7 ± 1.3	21.2 ± 4.9	1.18 ± 0.02	71 ± 4	5.6 ± 0.2	11 ± 1	527 ± 24
Pe1	7.4 ± 0.2	144 ± 8	168 ± 20	334 ± 3	80 ± 2	3.9 ± 1.5	5.8 ± 2.7	0.96 ± 0.04	321 ± 44	5.5 ± 0.2	129 ± 16	694 ± 9
Re1	7.4 ± 0.2	134 ± 5	206 ± 15	329 ± 20	77 ± 2	3.2 ± 1.3	7.5 ± 2.7	1.05 ± 0.03	165 ± 26	5.6 ± 0.1	46 ± 7	681 ± 14
Sa1	6.4 ± 0.5	110 ± 12	118 ± 4	281 ± 25	65 ± 2	4.9 ± 2.0	1.5 ± 0.6	0.85 ± 0.03	57 ± 12	4.9 ± 0.1	15 ± 5	649 ± 12
Sa2	7.0 ± 0.2	128 ± 5	194 ± 11	392 ± 9	71 ± 2	2.2 ± 0.9	6.8 ± 3.0	0.96 ± 0.06	317 ± 38	5.9 ± 0.2	108 ± 26	516 ± 31
Ve1	6.2 ± 0.4	107 ± 10	214 ± 10	351 ± 7	66 ± 2	8.6 ± 2.9	5.6 ± 3.4	1.17 ± 0.06	161 ± 25	6.6 ± 0.5	25 ± 10	305 ± 59

Table 7-3: Differences in soil properties assigned to soil stability and nutrient supply and recycling on 21 banana farms in Costa Rica.

Farm	**Root nematode measurements**			**Soil nematode trophic groups**				**Soil nematode indices**			
	Disease index	***R. similis* (100 g root)**	**Total nematodes (100 g root)**	**Plant-parasites (%)**	**Predators & omnivores (%)**	**Bacterivores (%)**	**Fungivores (%)**	**Nematode diversity**	**Enrichment index**	**Structure index**	**Channel index**
Ag1	30 ± 8	26 239 ± 4864	28 091 ± 4978	32 ± 6	18 ± 3	37 ±4	13 ± 3	2.20 ± 0.05	46 ± 7	68 ± 5	41 ± 15
Ag2	34 ± 16	2 880 ± 2398	18 044 ± 8169	64 ± 13	3 ± 1	27 ±13	6 ± 1.	1.12 ± 0.09	54 ± 8	28 ± 12	30 ± 7
Ba1	16 ± 5	19 074 ± 6516	20 450 ± 5890	42 ± 5	7 ± 1	41 ±4	10 ± 3	1.82 ± 0.05	67 ± 4	46 ± 5	14 ± 4
Ba2	12 ± 4	35 533 ± 15440	38 659 ± 16198	63 ± 3	6 ± 2	25 ±5	7 ± 2	1.96 ± 0.09	71 ± 6	57 ± 11	8 ± 2
Ca1	3 ± 2	3 403 ± 1885	32 042 ± 15217	86 ± 3	5 ± 2	7 ±2	3 ± 1	1.23 ± 0.11	36 ± 6	68 ± 10	58 ± 17
Ca2	23 ± 9	4 749 ± 1397	8 033 ± 1984	34 ± 2	10 ± 2	49 ±4	7 ± 1	2.03 ± 0.06	64 ± 8	55 ± 10	11 ± 3
Ca3	8 ± 4	0 ± 0	4 739 ± 1975	40 ± 1	22 ± 3	27 ±3	12 ± 2	2.32 ± 0.04	46 ± 3	81 ± 4	11 ± 7
Ca4	3 ± 3	2 710 ± 2613	5 307 ± 2923	20 ± 5	11 ± 3	25 ±7	44 ± 11	1.39 ± 0.15	57 ± 15	80 ± 5	8 ± 4
Ca5	28 ± 12	11 742 ± 8549	27 094 ± 7918	80 ± 2	4 ± 1	14 ±3	2 ± 1	1.49 ± 0.16	54 ± 6	49 ± 13	15 ± 5
Co1	33 ± 13	7 787 ± 2749	20 678 ± 10954	67 ± 8	7 ± 3	19 ±3	7 ± 4	1.69 ± 0.07	42 ± 6	49 ± 15	43 ± 15
Es1	2 ± 1	9 442 ± 5344	16 187 ± 4620	67 ± 4	9 ± 2	17 ±2	6 ± 1	1.86 ± 0.05	41 ± 3	67 ± 2	37 ± 9
Es2	12 ± 5	1 993 ± 792	4 213 ± 1589	47 ± 6	6 ± 2	40 ±4	7 ± 1	2.10 ± 0.05	50 ± 6	41 ± 7	15 ± 2
La1	28 ± 16	25 725 ± 11723	26 851 ± 12291	44 ± 4	16 ± 2	33 ±2	7 ± 2	1.17 ± 0.30	72 ± 5	79 ± 3	13 ± 5
La2	7 ± 7	4 218 ± 3994	4 667 ± 4126	49 ± 14	2 ± 1	28 ±9	2 ± 1	2.13 ± 0.07	28 ± 9	19 ± 10	34 ± 18
Mi1	0 ± 0	4 661 ± 4141	7 034 ± 3615	34 ± 8	26 ± 3	34 ±5	7 ± 1	2.23 ± 0.14	37 ± 12	80 ± 3	49 ± 21
Pa1	56 ± 13	75 610 ± 37388	79 033 ± 36004	41 ± 3	21 ± 5	28 ±2	10 ± 1	1.78 ± 0.12	54 ± 5	74 ± 8	28 ± 6
Pe1	3 ± 3	0 ± 0	7 544 ± 3226	50 ± 7	8 ± 1	26 ±4	15 ± 6	2.00 ± 0.10	59 ± 3	57 ± 6	21 ± 4
Re1	10 ± 8	69 612 ± 67964	69 193 ± 67956	27 ± 8	18 ± 4	40 ±6	15 ± 3	1.85 ± 0.05	79 ± 5	80 ± 3	7 ± 3
Sa1	19 ± 7	2 413 ± 991	10 307 ± 4148	59 ± 5	11 ± 2	18 ±3	12 ± 3	1.83 ± 0.17	39 ± 4	65 ± 7	69 ± 9
Sa2	18 ± 13	10 101 ± 7222	11 172 ± 797	45 ± 6	9 ± 2	44 ±8	5 ± 2	2.20 ± 0.05	75 ± 6	61 ± 7	7 ± 15
Ve1	22 ± 12	9 881 ± 4738	12 692 ± 5199	48 ± 8	10 ± 4	36 ±4	6 ± 1	1.12 ± 0.09	45 ± 8	45 ± 13	16 ± 7

Table 7-4: Analytical chemical soil analysis from 21 banana farms in Costa Rica

Farm	pH	O.M	Ca	Mg	K	CEC	P	Fe	Cu	Zn	Mn	Al	Extract acidity
		(%)	cmol (+) L^{-1}				mg L^{-1}					cmol (+) L^{-1}	
Ag1	5.5	7.6	7.9	2.7	1.3	11.9	47	112	4	15	22	0.2	0.2
Ag2	4.3	3.4	18.0	5.6	5.5	29.2	118	380	7	50	163	2.3	3.1
Ba1	4.5	7.1	7.2	2.6	2.5	12.2	98	143	3	13	48	1.1	1.3
Ba2	6.0	2.5	13.6	5.4	2.5	21.4	157	358	6	26	43	0.0	0.1
Ca1	4.7	3.2	25.0	7.7	1.8	34.5	67	427	45	8	32	0.7	1.0
Ca2	5.2	8.2	6.0	3.1	1.2	10.2	25	87	3	30	15	0.1	0.2
Ca3	5.1	2.8	22.8	8.1	0.8	31.7	54	444	9	18	59	0.3	0.5
Ca4	6.1	4.0	12.8	4.2	2.1	19.1	200	204	8	21	42	0.1	0.1
Ca5	4.3	3.1	17.8	6.4	3.3	27.5	119	470	35	10	32	1.2	2.0
Co1	5.0	4.2	21.1	7.5	2.0	30.6	655	341	10	29	118	0.6	0.8
Es1	4.5	3.9	22.8	7.1	2.0	31.9	45	369	8	10	159	0.8	1.6
Es2	4.9	3.5	9.8	6.0	2.1	17.8	388	460	6	9	60	0.7	0.8
La1	5.4	5.8	10.9	5.5	1.3	17.7	62	240	6	10	34	0.2	0.3
La2	4.6	2.5	5.6	5.6	2.6	13.9	491	466	5	5	40	1.9	2.1
Mi1	5.6	4.7	28.6	7.1	0.9	36.6	59	292	7	24	110	0.0	0.2
Pa1	5.4	2.9	22.0	8.1	0.9	31.0	47	297	7	11	142	0.2	0.5
Pe1	5.6	4.1	13.2	5.3	2.2	20.7	139	421	17	25	22	0.0	0.2
Re1	5.6	4.2	11.6	4.9	1.7	18.2	116	350	11	11	32	0.1	0.1
Sa1	5.0	5.8	9.7	4.1	1.1	14.9	64	353	8	5	35	1.1	1.4
Sa2	4.9	3.6	26.5	7.0	2.8	36.3	293	305	8	21	49	0.4	0.7
Ve1	6.3	1.9	33.3	4.6	2.0	39.9	108	269	6	33	84	0.0	0.1

Table 7-5: Correlation coefficients matrix of plant measurements, physical, chemical and biological soil parameters of 21 banana plantations in Costa Rica from a survey to determine the impact of soil management on the soil food web and plant-parasitic nematodes.

	Plant measurements				Physical		Field chemical tests				Root nematodes (100g)			Soil nematode trophic groups (%)				Soil nematode indices				Laboratory chemical tests										
	Hands	Fingers	Sucker height	Mother height	Infiltration	Bulk density	pH	NO3_kg_ha	EC	Labile_C	Disease Index	*R. similis*	Total nematodes	Parasitic	Predatory	Bacterivores	Fungivores	Diversity	Enrichment	Structure	Channel	Al	Ca	Cu	Fe	K	Organic matter	Mg	Mn	P	Zn	pHlab
Hands	1.00																															
Fingers	0.93*	1.00																														
Sucker height	-0.08	-0.10	1.00																													
Mother height	-0.03	0.13	0.55	1.00																												
Infiltration	0.27	0.39	0.07	0.17	1.00																											
Bulk density	0.27	0.33	0.28	0.31	-0.25	1.00																										
$\mathrm{pH_{field}}$	0.02	0.13	0.11	0.55	-0.02	0.25	1.00																									
NO3_kg_ha	-0.01	-0.14	-0.01	-0.13	0.24	-0.22	-0.34	1.00																								
EC	0.00	-0.16	-0.08	-0.29	0.12	-0.14	-0.43	0.88*	1.00																							
Labile_C	0.23	0.17	-0.29	-0.26	0.37	-0.61*	-0.23	0.32	0.18	1.00																						
Disease Index	-0.30	-0.40	0.11	-0.20	-0.27	-0.03	-0.17	-0.09	0.07	-0.16	1.00																					
R. similis	-0.42	-0.56*	0.09	-0.16	-0.51	-0.19	-0.06	-0.10	0.02	-0.04	0.61*	1.00																				
Total nematodes	-0.28	-0.52	0.08	-0.34	-0.55*	-0.20	-0.22	0.08	0.12	-0.05	0.65*	0.81*	1.00																			
Plant parasites	0.01	-0.21	0.06	-0.39	-0.10	0.00	-0.58*	0.49	0.45	-0.03	0.04	0.16	0.48	1.00																		
Predators/Om	0.33	0.35	-0.17	0.15	0.12	0.00	0.56*	-0.38	-0.42	0.29	0.08	-0.07	-0.17	-0.61*	1.00																	
Bacterivores	-0.23	-0.08	0.30	0.44	0.09	-0.14	0.36	-0.29	-0.23	-0.02	0.11	0.12	-0.24	-0.74*	0.28	1.00																
Fungivores	0.02	0.18	-0.35	0.10	-0.14	0.06	0.42	-0.20	-0.26	0.00	-0.20	-0.30	-0.31	-0.57*	0.23	0.03	1.00															
Diversity	0.04	0.13	-0.11	0.24	0.02	-0.06	0.64*	-0.46	-0.56*	0.17	0.07	0.10	-0.11	-0.68*	0.81*	0.55	0.23	1.00														
Enrichment	-0.38	-0.35	0.30	0.39	-0.13	-0.04	0.29	0.19	0.03	0.09	0.15	0.25	0.13	-0.32	0.07	0.49	0.20	0.34	1.00													
Structure	0.23	0.21	-0.24	0.19	-0.03	-0.10	0.55*	-0.08	-0.31	0.42	-0.15	-0.07	-0.02	-0.36	0.75*	-0.05	0.45	0.64*	0.29	1.00												
Channel	0.22	0.12	-0.29	-0.45	0.07	-0.38	-0.32	-0.12	-0.14	0.34	0.01	0.06	0.24	0.41	0.05	-0.53	-0.21	-0.21	-0.70*	0.03	1.00											
Al	-0.11	-0.16	-0.10	-0.41	0.00	-0.10	-0.82*	0.17	0.35	-0.09	0.14	-0.05	0.06	0.47	-0.60*	-0.29	-0.34	-0.75*	-0.37	-0.73*	0.27	1.00										
Ca	0.28	0.21	0.28	0.28	0.04	0.29	0.31	0.17	0.03	-0.29	0.00	-0.15	0.09	0.26	0.22	-0.26	-0.21	-0.01	-0.20	0.21	0.16	-0.21	1.00									
Cu	0.28	0.08	0.08	-0.09	-0.08	0.09	-0.37	0.64*	0.52	0.27	-0.17	-0.09	0.21	0.66*	-0.29	-0.67*	-0.19	-0.54	-0.17	0.10	0.26	0.11	0.28	1.00								
Fe	0.38	0.35	0.11	-0.06	0.22	0.44	-0.48	0.23	0.18	-0.08	-0.25	-0.38	-0.20	0.57*	-0.31	-0.57	-0.33	-0.47	-0.33	-0.24	0.11	0.38	0.23	0.48	1.00							
K	-0.15	-0.18	0.26	-0.04	-0.02	0.09	-0.49	0.38	0.46	-0.33	0.10	-0.08	0.12	0.40	-0.70	-0.12	-0.16	-0.72*	0.12	-0.66*	-0.19	0.69*	0.00	0.12	0.28	1.00						
OM	-0.27	-0.29	-0.31	-0.22	0.04	-0.80*	0.04	-0.04	-0.01	0.59*	0.10	0.29	0.12	-0.36	0.20	0.43	0.14	0.34	0.23	0.19	0.13	-0.13	-0.56*	-0.30	-0.76*	-0.31	1.00					
Mg	0.56*	0.47	0.10	0.12	0.20	0.42	-0.07	0.14	0.03	-0.07	0.01	-0.17	0.02	0.40	0.15	-0.48	-0.32	-0.11	-0.27	0.17	0.17	0.02	0.66*	0.39	0.66*	-0.04	-0.66*	1.00				
Mn	0.17	0.14	-0.03	-0.22	0.01	0.11	-0.06	-0.15	-0.11	-0.26	0.30	0.01	0.24	0.20	0.10	-0.19	-0.17	-0.11	-0.28	-0.13	0.26	0.27	0.52	-0.21	0.15	0.27	-0.33	0.49	1.00			
P	0.03	0.23	0.20	0.38	0.16	0.38	-0.22	-0.13	-0.03	-0.37	-0.26	-0.17	-0.45	-0.08	-0.45	0.17	-0.05	-0.33	-0.12	-0.57*	-0.29	0.34	-0.25	-0.12	0.40	0.34	-0.39	0.02	-0.19	1.00		
Zn	0.04	0.02	0.38	0.10	0.12	0.06	0.23	0.05	0.05	-0.36	0.18	-0.20	0.04	-0.03	-0.12	0.21	0.03	-0.08	0.18	-0.23	-0.19	0.01	0.29	-0.25	-0.20	0.48	-0.07	-0.08	0.36	-0.19	1.00	
$\mathrm{pH_{lab}}$	-0.01	0.11	0.20	0.43	-0.10	0.34	0.84*	-0.45	-0.54	-0.28	-0.08	-0.05	-0.13	-0.53	0.46	0.24	0.50	0.57*	0.24	0.48	-0.25	-0.80*	0.15	-0.29	-0.34	-0.44	-0.08	-0.20	-0.19	-0.15	0.22	1.00

* significant r value $P<0.01$

A linear correlation matrix was used to determine the relationship between plant measurements and soil physical, field chemical, laboratory chemical measurements and nematode measurements made in both the soil and roots of bananas (Table 7-5). Soil pH measured in the field and laboratory were found to be significantly correlated ($P<0.01$) to each other as well as Al, nematode diversity, proportion of plant-parasitic, proportion of predatory and omnivorous nematodes and the nematode structure index (Table 7-5). The proportion of plant-parasitic nematodes in the soil was also found to be significantly correlated ($P<0.01$) with proportions of other nematode trophic groups, Cu and Fe measurements determined in the laboratory chemical measurements (Table 7-5). The diversity of soil nematodes was significantly ($P<0.01$) positively correlated with pH determined in the field and laboratory, proportion of predatory and omnivorous nematodes and structure index and was negatively correlated with EC, proportion of plant-parasitic nematodes, Al, and K (Table 7-5).

Thirty four soil and crop growth parameters were used in the principal component analysis. The first five principle components were able to explain 71% of the variation in the entire data set (Table 7-6). The first principal component explained 24% of the variation and had high positive rotated loadings with Al and exchangeable acidity and high negative loadings with structure index, proportion of predatory nematodes, pH and diversity (Table 7-6). This principal component was regarded as a "*soil acidity*" component, because of the large number of components correlated to soil pH, which included nematode community diversity and structure.

The second principal component explained 17% of the variation and was identified as an "*organic matter*" component, because it had high positive loadings with organic matter and labile C and high negative loading with soil bulk density (Table 7-6). The third principal component was indentified as "*recycled nutrients*" and explained 12% of the variation (Table 7-6). The recycled nutrients component had high positive loadings for enrichment index, mother plant height and daughter plant height and negative loading for channel index (Table 7-6). The fourth principal component explained 10% of the variance and had the highest negative loadings for total nematodes and number of *R. similis* in 100 g^{-1} of root and disease index and high positive loadings for plant circumference and finger number per bunch. This component was identified as a "*root nematode*" component because of the correlations between root nematode measurements and number of fingers. The fifth principal component was identified as "*soil nutrients*" because it had high negative loadings for Cu, nitrate-N and Ca and was able to explain 8% of the variation between plantations.

Table 7-6: Rotated component loadings of plant measurements, physical, chemical and nematode parameters on five principal components (PC) to determine nematode suppression at banana plantations in Costa Rica.

	PC1	PC2	PC3	PC4	PC5
Component identifier	Acidity	Organic matter	Recycled nutrients	Parasitic nematodes	Soil nutrients
Variation	24%	17%	12%	10%	8%
Cumulative variation %	24%	41%	53%	63%	71%
Structure index	-0.36	0.10	-0.05	0.04	-0.14
Predators (%)	-0.31	0.00	-0.16	0.02	0.02
$\mathrm{pH}_{\mathrm{field}}$	-0.30	-0.09	0.14	-0.01	0.02
Diversity	-0.29	0.03	-0.04	-0.03	0.14
$\mathrm{pH}_{\mathrm{lab}}$	-0.26	-0.14	0.11	-0.02	0.09
Ca	-0.18	-0.22	0.00	-0.09	-0.30
Mother height	-0.11	-0.12	0.30	0.11	-0.02
Fungivores (%)	-0.10	0.04	0.02	0.13	0.13
Enrichment index	-0.10	0.11	0.40	-0.05	-0.03
Mg	-0.09	-0.22	-0.13	0.04	-0.27
Hands	-0.09	-0.09	-0.20	0.25	-0.13
Labile C	-0.09	0.36	-0.11	0.16	-0.12
Finger number	-0.07	-0.12	-0.18	0.32	-0.02
Circumference	-0.06	-0.07	0.08	0.33	0.07
Total nematodes 100 g root	-0.05	0.00	-0.02	-0.40	-0.13
Organic matter	-0.05	0.37	-0.02	-0.04	0.13
R. similis 100 g roots	-0.03	0.03	0.02	-0.37	0.03
Bacterivores (%)	-0.02	0.06	0.22	0.01	0.25
Disease index	-0.01	-0.06	0.00	-0.34	0.02
Cu	-0.01	0.06	0.04	0.08	-0.38
Bulk density	-0.01	-0.38	0.06	0.05	0.01
Channel index	-0.01	0.05	-0.41	-0.07	-0.06
Zn	0.00	-0.10	0.20	-0.08	-0.05
Mn	0.00	-0.22	-0.20	-0.18	-0.05
Sucker height	0.00	-0.15	0.30	-0.05	-0.08
Infiltration	0.01	0.12	0.02	0.29	-0.08
Nitrate-N	0.05	0.22	0.24	0.09	-0.37
Plant-parasites (%)	0.13	-0.03	-0.06	-0.12	-0.29
EC	0.13	0.18	0.19	0.05	-0.28
Fe	0.14	-0.18	-0.09	0.17	-0.16
P	0.26	-0.15	0.09	0.21	0.21
K	0.28	-0.07	0.21	-0.02	-0.06
Exchangeable Acidity	0.32	-0.01	-0.12	-0.04	-0.02
Al	0.35	-0.01	-0.12	-0.02	0.06

The soil and plant measurements with the highest loadings from each of the five principal components were used in a cluster analysis to determine the groupings of the plantations (Figure 7-1). Using the first principal component, "*acidity*" and the variables Al, exchangeable acidity, pH, structure index, predators (%) and diversity, plantations could be arranged into three groups, with a similarity greater than 0.9 (Figure 7-1A). The first group consisted of 12 plantations, which tended to have slightly acid soils, but high nematode diversity and structure. The second group of farms consisted of seven plantations and had acid soils with moderate nematode diversity and structure. The third group consisted of two plantations with very acid soils and low nematode diversity and structure (Figure 7-1A).

A cluster analysis of the second principal component based on components related to "*organic matter*", OM, labile C and bulk density, gave 4 groups of farms with a similarity less than 0.9. The first group of four plantations tended to have high organic matter, but low labile C and bulk density. The second group of 10 plantations tended to have low bulk density but high labile C, while the third group tended to have high bulk density, but medium levels of organic matter and labile C. The fourth group consisted of a single plantation, Ve1 with high soil bulk density and low labile C (Figure 7-1B).

The third principal component, "*recycled nutrients*" was assembled into three groups with a similarity of 0.85 using the variates enrichment and channel indices and mother and daughter plant height. The first group consisted of 8 plantations, the second group 9 plantations and the third group had four plantations, which tended to have greater plant heights and a high enrichment index (Figure 7-1C).

A cluster analysis of the fourth principal component produced four groups based on "*parasitic nematodes*" using the variables disease index, *R. similis* and total nematodes 100 g of root, plant circumference and finger number per bunch. The first group consisted of 12 plantations, which tended to have high nematode counts, and low finger number and plant circumference. The second group of three plantations had medium levels of nematodes and plant characteristics. The third group consisted of a single plantation, Pa1, which had a high disease index but also a high number of fingers per bunch and plant circumference. The fourth group of plantations had low plant-parasitic nematode indices and a high number of fingers per bunch and plant circumference (Figure 7-1D).

The fifth principal component, "*soil nutrients*", resulted in three groups with a similarity of 0.88 after performing the cluster analysis using the variates Cu, nitrate-N and Ca. Two plantations Ca1 and Ca5 made up group three having high Cu and nitrate-N and had a similarity less than 0.65 relative to the other plantations (Figure 7-

1D).

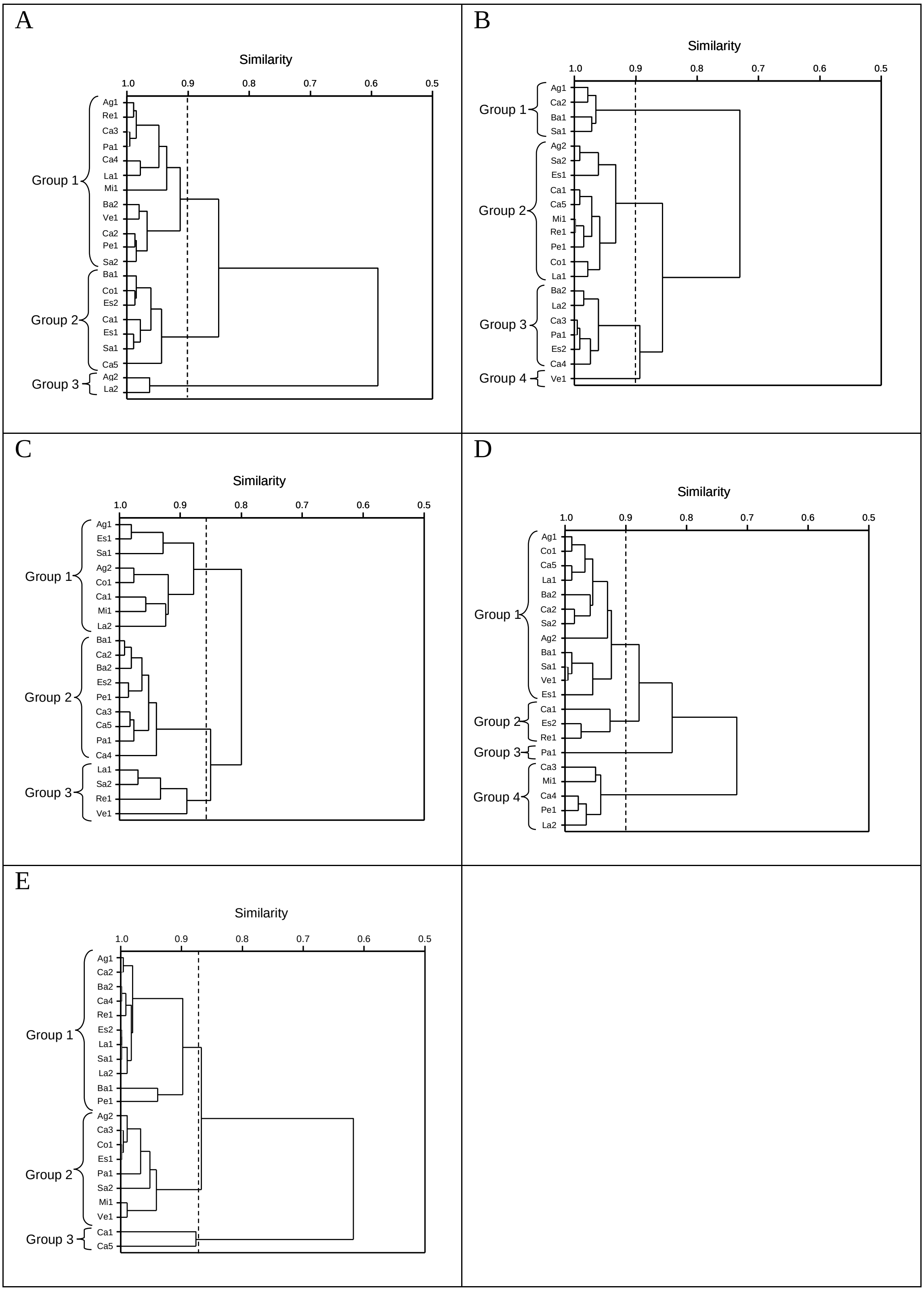

Figure 7-1: Cluster analysis dendrograms of 21 banana plantations in Costa Rica based on principal component rotated loadings for PC 1 “acidity” (A), PC 2 “organic matter” (B), PC3 “recycled nutrients” (C), PC4 “parasitic nematodes” (D) and PC5 “soil nutrients” (E). (dashed line represents a similarity of 0.9 used to form groups, except in C and E where the dashed line represents a similarity of 0.86 and 0.88 respectively)

7.4.2 Glasshouse bioassay

The growth of banana plantlets in the glasshouse experiment varied between the soils collected from the different plantations (Table 7-7). The initial growth determined by plant height after 4 weeks was greatest in plants grown in soil from Ag2 and least in soil from plantation Sa1 (Table 7-7). Fresh shoot weight at the termination of the experiment was greatest in plants grown in soil from Ca5, but least in plants grown in soil from Ca3 (Table 7-7). The plantation Ca3 also had lower root weight similar to Ca4 and Pa1 (Table 7-7). The greatest fresh root weights were recorded at Ag1 and Pa1 (Table 7-7).

The soils with the greatest number of *R. similis* per plant also tended to record the greatest number in 100 g of root (Table 7-7). Banana plants grown in soil from Sa1and Ca5 had the highest number of *R. similis* recovered per plant and from 100 g of root. Similarly, banana plants grown in soil from Ca4 had both the lowest number of *R. similis* recovered per plant and in 100 g of root (Table 7-7).

Table 7-7: Growth and recovery of plant-parasitic nematodes from banana plantlets grown in soil collected from 21 banana plantations in Costa Rica and inoculated with *R. similis*.

Farm	4-week plant height (cm)		Fresh shoot weight (g)		Fresh root weight (g)		*R. similis* recovered*					
							Total per plant			100 g root		
Ag1	5.3	bcd	16.9	bc	8.4	a	927	(6.83)	ab	11486	(9.35)	abc
Ag2	6.7	a	17.4	b	7.2	abc	876	(6.78)	abc	12809	(9.46)	ab
Ba1	5.1	bcde	16.5	bcd	6.5	abcde	869	(6.77)	abc	13642	(9.52)	ab
Ba2	4.4	cdef	11.9	bcde	4.3	efg	458	(6.13)	bcde	11567	(9.36)	abc
Ca1	5.7	abc	15.9	bcd	6.2	abcdef	877	(6.78)	abc	14690	(9.60)	ab
Ca2	5.3	bcd	10.5	de	4.8	cdefg	618	(6.43)	abcd	13779	(9.53)	ab
Ca3	3.7	efg	9.6	e	3.6	g	263	(5.58)	e	7207	(8.88)	bcd
Ca4	4.9	bcde	10.4	de	3.8	g	221	(5.40)	e	4646	(8.44)	d
Ca5	6.2	ab	25.5	a	7.6	ab	1338	(7.20)	a	19830	(9.90)	a
Co1	5.2	bcd	13.8	bcde	4.3	efg	417	(6.04)	bcde	9906	(9.20)	abcd
Es1	5.4	abcd	14.1	bcde	7.8	a	1106	(7.01)	a	15382	(9.64)	ab
La1	4.5	cdef	11.4	bcde	6.4	abcde	629	(6.45)	abcd	10362	(9.25)	abcd
La2	4.8	bcde	14.7	bcde	7.0	abcd	860	(6.76)	abc	12682	(9.45)	ab
Mi1	5.0	bcde	11.7	bcde	4.8	cdefg	243	(5.50)	e	5161	(8.55)	cd
Pa1	3.4	fg	10.4	de	3.7	g	725	(6.59)	abcd	20251	(9.92)	a
Pe1	4.4	cdef	12.5	bcde	3.9	fg	334	(5.81)	de	9729	(9.18)	abcd
Re1	4.5	cdef	11.3	bcde	4.5	efg	464	(6.14)	bcde	10915	(9.30)	abc
Sa1	2.9	g	10.9	cde	5.3	bcdefg	1118	(7.02)	a	21719	(9.99)	a
Sa2	5.1	bcd	14.0	bcde	6.3	abcdef	612	(6.42)	abcd	11069	(9.31)	abc
Ve1	4.3	def	13.8	bcde	4.7	defg	407	(6.01)	cde	11259	(9.33)	abc

Values in columns with the same subscript are not significantly different from one another at $P=0.05$.
* values presented are back-transformed from transformed means using (ln (x+1)), which were used in statistical analysis and are presented in parenthesis.

A forward stepwise regression was used to determine the relationship between soil variables measured from the plantations and the number of *R. similis* recovered from the banana root systems in the glasshouse bioassay. Three variables were found to have a significant relationship with the number of *R. similis* recovered from the roots of banana plants; pH, Zn and structure index (Table 7-8). As individual soil measurements, pH, Zn and structure index had a significant R^2 statistic ($P<0.05$), however, they were only able to explain 69.2, 17.2 and 22.5 % of the variation in the number of *R. similis* recovered from the roots respectively (Table 6-8). As a combined multiple linear regression model, pH, Zn and structure index were able to explain 79.2 % of the variation in the number of *R. similis* recovered from the roots of bananas (Table 7-8).

Table 7-8: Linear regression models of soil variables to explain the variation in the number of *R. similis* recovered from the roots of banana plants used in a glasshouse experiment using soil from 21 banana plantations in Costa Rica.

Model	$\mathbf{R^2}$	***P***
pH	69.2	<0.001
Zn	17.2	0.039
Structure index	22.5	0.020
pH.Zn.Structure index	79.2	<0.001

7.5 Discussion

Management of soil pH appeared to be the critical factor in the suppression of the plant-parasitic nematode, *R. similis* in bananas in Costa Rica. From the soils collected, banana plantations with acid soils had a greater number of *R. similis* recovered from the roots of the bioassay banana plants. The low soil pH appeared to restrict beneficial biological soil functions by decreasing diversity and nematode community structure, determined by measuring soil nematode diversity and the nematode structure index. The structure index is an indicator of the number of links in the soil food web and potential for regulation of nematode populations by predators (Ferris *et al.* 2001). Therefore, the low structure index in acid soils, such as plantations Ag2 and La2, suggested these soils had poor potential for regulation by predators due to a simplified soil food web. Highly acid soils with a reduced nematode structure, showing little predator potential, allowed *R. similis* to penetrate the roots of banana plants, resulting in a greater number of nematodes being recovered from the roots of banana plants in the banana bioassay.

A principal component and correlation analysis found that soil acidity measurements; low pH, high exchangeable acidity and available aluminium were significantly correlated with low nematode diversity, low proportion of predatory soil nematodes and low nematode community structure. The correlation of soil acidity measurements with nematode diversity and community structure in the field provided further evidence of the impact low soil pH had on the biology of the soil. However, in the final multiple linear regression model of recovery of *R. similis* from bioassay plants, pH, structure index and Zn was able to explain 79.2% of the variation in the *R. similis* population. The role of Zn in the suppression of nematodes is unclear, other than the change in availability of Zn with changes in soil pH and consequently Zn is possibly limiting for soil organisms in soils with very low pH. The results from the field survey and bioassay demonstrated that very low soil pH changes soil biological characteristics, reducing the ability of soil organisms to suppress plant-parasitic organisms such as *R. similis*.

Factors associated with soil pH in Costa Rica were the most important variables for separating the 21 banana plantations. Using a PCA, the first principal component was able to explain 24% of the variation between banana plantations. The soil variables with the greatest loadings in this principal component were associated with acidity, and included pH, Al, exchangeable acidity, structure index and percentage of predatory nematodes in the soil. This suggested that there was a lot of variation associated with soil acidity between the 21 banana plantations. This was further evident when a cluster analysis was able to separate the plantations into three separate groups based on the variables associated with “*acidity*”. Two plantations Ag2 and La2 had soil “*acidity*” characteristics that had a similarity of 0.6 relative to the other 19 plantations included in the survey (Figure 7-1A). Both of these farms had very acid soils, with a pH less than 5.0, high exchangeable acidity, high available Al, a structure index below 30 and a low proportion of predatory nematodes, less than 3%. Using the structure and enrichment indices and the classification proposed by Ferris *et al.* (2001), the plantation Ag2 would be considered as a “*stressed*” soil because both enrichment and structure indices were below 50. Therefore, both Ag2 and La2 plantations could be considered as having poor soil health, which was associated with soil acidity and poor soil nematode community structure.

The populations of plant-parasitic nematodes collected from the roots of bananas in the field did not reflect the same relationship with soil pH and diversity as

R. similis recovered from the roots of bananas grown in the glasshouse bioassay. This may be because other management factors used in commercial banana production, such as the use of nematicides, may compensate for the lack of "*biological*" suppression of plant-parasitic nematodes. In this study, it was difficult to ascertain if low numbers of plant-parasitic nematodes recovered from the root samples collected from the field were due to biological suppression or the application of nematicides. However, the *in vitro* banana plants grown in the glasshouse experiment were not subjected to nematicides. Therefore, the mechanism for suppression of *R. similis* observed in the glasshouse experiment must be due to an inherent soil condition and not from recent farm management decisions like nematicide application. When relying solely on field assessments of nematode populations it is difficult to separate impacts due to farm management from mechanism that may increase the soil's general suppression. This may explain why the numbers of plant-parasitic nematodes recovered from the field did not correlate with numbers of nematodes recovered in the glasshouse experiment and why soil pH and diversity did not correlate with populations of plant-parasitic nematodes recovered from field samples of banana roots.

The importance of soil pH in the suppression of *R. similis*, in Costa Rica, contrasted with the results found in Australia (Chapter 6). In Costa Rica, suppression of *R. similis* on bananas was related to soil pH, whereas in Australia suppression of plant-parasitic nematodes on bananas was related to labile C and nitrate-N content in the soil. However, in both countries either nematode community diversity or nematode community structure were co-variables related to suppression of plant-parasitic nematodes. This suggested that nematode community structure and diversity were important components and indicators of suppression of plant-parasitic nematodes. Therefore, to increase nematode community structure and diversity, and to develop nematode suppressive soils requires overcoming the most limiting soil factors. In Costa Rica the most limiting soil factor was low soil pH, whereas, in Australia the most limiting soil factors were low soil carbon and excess nitrogen. This highlighted the importance of quantifying and understanding the soil environment and how it impacts on soil biology, before recommending management practices on commercial banana plantations.

The investigation of the soil nematode community is a powerful tool in the understanding of soil biological interactions with soil physical and chemical properties and land management practices. In order to develop soils that are suppressive to plant-parasitic nematodes it is important to also understand the impacts that soil management may have on other nematode trophic groups, nematode community structure and diversity. The suppression of plant-parasitic nematodes on bananas in Costa Rica was linked to an increase in the number of predatory or omnivorous nematodes in the soil. While predatory and omnivorous nematodes had previously been regarded as poor biocontrol agents (Stirling 1991), they may contribute to nematode suppression *in situ,* as well as act as indicators of other non-nematode parasites and predators of plant-parasitic nematodes (Yeates and Wardle 1996). The role that predatory nematodes play in the regulation of plant-parasitic nematodes has gained increased attention and it was suggested that the migratory stages and possibly juveniles are more susceptible to predation (Khan and Kim 2007). However, it is difficult to correlate activities of predatory nematodes directly to the suppression of plant-parasitic nematodes due to the complexities in predator:prey relationships (Yeates and Wardle 1996). However, in this study increasing soil pH increased the proportion of predatory and omnivorous nematodes in the soil, which was related to greater nematode community structure and greater suppression of *R. similis*. Furthermore, omnivorous and predatory nematodes may be indicators of increased microbial antagonism of *R. similis*. The omnivorous and predatory nematodes mainly feed on nematode trophic groups that consume bacteria and fungi, the main source of microbial antagonists that are important in *R similis* density regulation. Therefore, increases in the omnivorous and predatory nematode number in the soil may also be an indirect indicator of increased suppression by microbial antagonists.

Although soil measurements associated with organic matter were not significantly correlated with nematode suppression, they were still important variables used to separate plantations in the principal component analysis. Variables associated with the "*organic matter*" principal component; organic matter, labile C and soil bulk density were able to explain 17% of the variation between plantations. Furthermore, a cluster analysis was able to separate the plantations into four groups, based on differences in "*organic matter*" components with a similarity less than 0.9. However, soil organic matter did not appear to limit soil nematode community structure and was not found to be related to suppression of *R. similis* as had been found in Australia (Chapter 6).

Soil nutrition, other than soil acidity, was not a major component separating soil plantations in Costa Rica. While Zn was found to be significant component in the final model related to the suppression plant-parasitic nematodes, overall soil nutrition was not largely different between plantations. A "*soil nutrient*" component was the fifth principal component, but was only able to explain 8% of the variation between plantations. The soil nutrients that were contributing to the differences between plantations were Cu, nitrate-N and Ca.

The recycling of soil nutrients and its contribution to vegetative plant growth was an important component for separating banana plantations in Costa Rica. Recycling of nutrients occurs through a biological process (Nannipieri and Paul 2009) and the recycled nutrients contribute to the growth of bananas. Nutrient recycling can be mediated either through bacterial or fungal decomposition of organic matter and the soil nematodes present reflect how the organic matter is decomposed (Ferris *et al.* 2001; Yeates and Pattison 2006). The range in the enrichment and channel indices between banana plantations in Costa Rica suggest there is variation between efficiency and availability of recycled nutrients. The enrichment index indicates the response of opportunistic bacterivorous and fungivorous nematodes to food resources and the activity of the detritus consumers (Ferris *et al.* 2001). Therefore, if there are higher levels of nutrient and resources available for recycling through the soil food web, there will be an increase in the enrichment index. The channel index indicates the predominate pathway through which nutrients are recycled. A low value, close to zero indicates bacterially dominated decomposition and a high value close to 100 indicates fungal dominance of nutrient decomposition. Therefore, plantations that have a high enrichment and low channel indices, such as La1, Re1 and Sa2, may indicate greater nutrient availability, which was recycled rapidly through bacterial activity, increasing the availability of nutrients and contributing to the vegetative growth of banana plants resulting greater vegetative growth of the mother and daughter plants.

The factors affecting where banana plantations have been established in Costa Rica were primarily based on climatic characteristics and physical soil properties, particularly drainage (Gauggel *et al.* 2005). Therefore, it was not surprising to find little differences in soil physical measurements of banana plantations in this study. Only one plantation Ve1, had soil with significantly different properties and it formed a separate group in the soil "*organic matter*" principal component. The soil from Ve1 was classified as a tropohumult, whereas soils in other plantations were either dystrandepts or eutropepts. Therefore, differences in soil properties between plantations, except Ve1, could be attributed to differences in farm management rather than differences in soil types.

Multivariate statistics allowed the identification of differences in banana production systems and soil properties and hence, allowed the determination of factors that were contributing to differences in the suppression of *R. similis*. The measurement of soil physical, chemical and nematode community parameters using a pre-determined set of indicators allowed the separation of plantations based on soil properties and determination of the soil properties that were limiting the suppression of plant-parasitic nematodes. A forward stepwise regression allowed the most important and interacting soil properties, pH, structure index and Zn, to be determined. However, further investigation is needed to determine conclusively the benefits from increased soil pH in the development of favourable soil biology and its effect on the suppression of *R. similis* in banana plantations in Costa Rica.

Farm management practices have an important impact on soil pH. Low soil pH has been attributed to poor root growth and reduced production through an imbalance in nutrient availability and increased toxicity of trace elements (Serrano 2005). Moody and Aitken (1997) suggested that acidification in Australian banana soils was associated with carbon and nitrogen cycles. The carbon cycle factors affecting soil acidity included the removal of bases in harvested fruit and reductions in levels of soil organic matter, whereas the addition of introduced organic material could increase alkalinity. The major nitrogen cycle factors contributing to acidification of banana soil was the leaching out of nitrate from the root zone when it has been originally applied as ammonium-based fertiliser (Moody and Aitken 1997). Farm management practices to reduce the rate of acidification or neutralise acidity, such as use of alkalising amendments, conservation of organic matter and reduced application ammonium based nitrogen, could foster beneficial soil biology, which could encourage the suppression of *R. similis* on banana plantations in Costa Rica.

7.6 Conclusion

Bananas grown in equatorial climates as monocultures are susceptible to soil and environmental constraints, which can reduce the sustainability of their production. In particular, biological constraints such as plant-parasitic nematodes tend to increase as a result of unsustainable farm practices. A survey of 21 banana plantations in Costa Rica measured soil chemical and physical properties as well as the nematode community composition. A principal component analysis using 34 soil variables was able to explain 71% of variation between plantations using the first five principle components. The variables identified in the principle components determined to be related to soil "*acidity*", "*organic matter*", "*recycled nutrients*", "*parasitic nematodes*" and "*soil nutrients*" and were able to explain 24, 17, 12, 10 and 8 % of the variation between plantations respectively. A cluster analysis of the plantations for each of the principal components using the variables with the greatest vector loadings allowed the grouping of plantations with similar soil properties. A banana bioassay of the soil from the 21 plantations, inoculated with *R. similis*, resulted in different populations of the nematode recovered from the root system. A forward stepwise regression of the number of nematodes recovered from the roots of bioassay plants with soil properties revealed that pH, structure index and Zn gave a significant multiple linear regression model, which was able to explain 79.2% of the variation in recovery of *R. similis* from the roots of bioassay plants. Furthermore, the correlation of soil pH with nematode diversity, the proportion of predatory and omnivorous nematodes and the structure of the nematode community suggested that pH was the factor limiting the biological suppression of *R. similis* in banana plantations. Therefore, management options to increase soil pH need to be investigated to determine if they are able to contribute to the suppression of *R. similis* in Costa Rican banana plantations.

8 General conclusions

Changes in soil properties that resulted from agronomic management of bananas were shown to influence the suppression of plant-parasitic nematodes. Suppression was constrained by low biological diversity and the lack of predators. Therefore, manipulation of soil properties through agronomic management may overcome these soil constraints. The studies outlined in this thesis found that:

1. The use of organic amendments that are high in C and N appear to be able to induce suppression of plant-parasitic nematodes in bananas, by developing a more favourable environment for antagonistic organisms in a glasshouse experiment.
2. The suppression of plant-parasitic nematodes following the addition of moderately degradable organic matter, like cellulose and grass hay, appeared to be due to a combination of nematoxic compounds produced in the early degradation of the organic matter, followed by an increase in nematode antagonists favoured by an increase in soil fungal activity.
3. Amendment of soil with additional organic matter significantly increased the labile C, increased the number of omnivorous nematodes and decreased the number of plant-parasitic nematodes relative to the untreated soil in a field experiment.
4. Suppression of plant-parasitic nematodes on banana plantations in Australia occurred where the soils had high labile C, low nitrate-N and high nematode diversity, which corresponded to farms having agronomic practices that increased carbon inputs, used lower nitrogen applications and promoted soil biodiversity.
5. Low soil pH limited suppression of *R. similis* in Costa Rican banana plantations by constraining nematode diversity, the proportion of predatory and omnivorous nematodes and the structure index of the nematode community.
6. Consistent increases in densities of fungivorous, omnivorous and predacious nematodes followed addition of organic amendments high in carbon which underscores the importance of fungal and bacterial

antagonists in nematode suppression in the soil either through direct or indirect mechanisms.

The development of suppression towards plant-parasitic nematodes in commercial banana plantations relies on identification of the factors constraining biological diversity and adopting management practices to overcome inherent soil constraints, such as low soil carbon or low pH. The mechanisms by which plant-parasitic nematodes were suppressed were not specifically identified. There remains a need to gain a better understanding of which antagonists are involved in the actual suppression of plant-parasitic nematodes of bananas once soil constraints are removed. Furthermore, there is a need to understand how to develop and validate farming systems that can suppress plant-parasitic nematodes of bananas using investigations under controlled conditions, over longer time periods (5-10 years). To develop soils that are suppressive to plant-parasitic nematodes requires a more comprehensive understanding of the soil ecological interactions, starting from a recognition of inherent soil constraints, how farm management decisions impact on soil properties, how changes in soil properties impact on soil organisms and how the antagonists impact on plant-parasitic nematodes, pathogens and the host plant.

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