The effect of forest to pasture conversion on soil biological diversity and function

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Recent declines in returns from primary forest products in New Zealand and projected increases in world food prices have led to the land-use conversion from plantation forest to pastoral farming in many lowland areas. After decades of forest cover the soils are in many cases less than adequate for pastoral farming, as they are acidic, with toxic levels of exchangeable aluminum, and contain low levels of available nitrogen (N), very high carbon (C):N ratio, and are devoid of earthworms and structural integrity. Overcoming the major site limitations of low soil pH and available N was a major priority and a field experiment was established in April 2005 to determine the impact of various rates of lime and N in relation to pasture establishment and production. Concerns about the short and long-term effects of these inputs on biological soil quality gave rise to the present study. The effects of land-use change and establishment inputs were assessed by comparison of selected treatment plots with two adjacent reference sites (long-term pasture and a 60–year *Pinus radiata* forest) on the same soil type. The effects of lime and N on soil biological quality were investigated under field and controlled environment conditions by determination of: microbial community structure (phospholipid fatty acids - PLFA), microbial biomass (total PLFA), and microbial activity (dehydrogenase activity). Soil physical (percentage water-stable aggregates) and chemical (pH, and total C and N) properties were also determined. Similarly, the effects of earthworm addition on soil biological properties were explored in a short-term glasshouse pot experiment. The role of earthworms as indicators of soil biological quality in the field was assumed by nematodes and these were assessed in field trial plots and the reference sites mentioned above. Land-use change and applications of lime and N contributed to changing the microbial community structure determined by principal component analysis of transformed PLFA data. However, the effect of lime was

more pronounced in the field, while N contributed most to changing microbial community structure in the glasshouse. Mean microbial activity in the field increased from 4 μg dwt/hr without lime to 16 and 21 μg dwt/hr where lime was applied at 5 and 10 tons/hectare (t/ha), respectively. Mean microbial activity in the field was markedly higher (7-fold) than in the glasshouse at similar rates of lime. Lime application also increased soil moisture retention in the field, mean gravimetric soil moisture increased from 0.33 in control plots to 0.38 and 0.39 in plots treated with 5 and 10 t/ha lime, respectively. Lime application was associated with greater soil aggregate stability. Soils from test plots treated with 5 and 10 tons/ha lime had 45-50% water-stable aggregates compared to 34% in treatments without lime. After 16 weeks in pots, earthworm treatments increased mean plant dry matter (DM)/pot by at least 19% above the control. The increase was attributed primarily to greater N mineralization in the presence of earthworms. For the duration of the trial the earthworm species tested (*Apporectodea caliginosa* and *Lumbricus rubellus*, individually or combined) did not affect any of the measured soil microbial properties. However, the survival rate of A. c*aliginosa* was 83% compared to 25% for *L. rubellus*. The control not receiving any lime or N and plots treated with 10t/ha lime and 200 kgN/ha had similar nematodes species composition, comprising 40% each of bacterial and fungal feeding nematodes. They differed markedly from the reference sites as the forest soil was dominated by plant associated species (38%) and the long-term pasture had 44% plant parasitic nematodes. Accordingly, the soil food web condition inferred from nematode faunal analysis characterized all test plots as basal, stressed and depleted, while the forest soil was categorized as highly structured and fungal dominated. The findings of this thesis demonstrated that land-use change from forest to pasture can have significant impacts on soil biological properties, earthworms can contribute to pasture productivity even in the short term, and nematode faunal analysis is a robust and reliable indicator of soil biological quality.

Key words: Forest; pasture; land-use change; microbial diversity, phospholipid fatty acid; earthworms; nematodes.
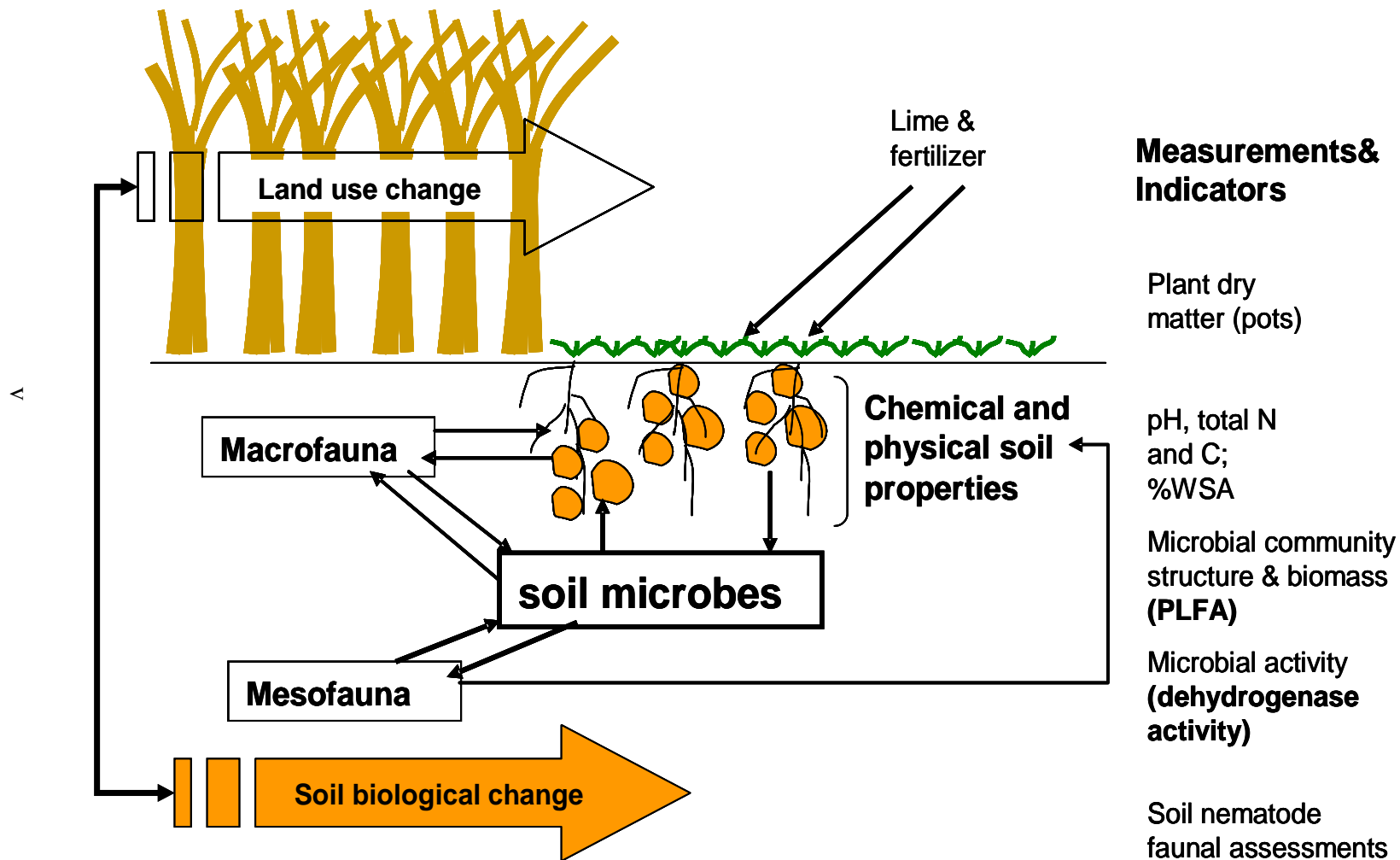
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Chapter 1
Introduction

The soil resource is fundamental to the existence of all life on earth. Soils provide, store and generate the biodiversity that is important for sustained and optimal functioning of the planet's ecosystems. The diversity of life forms occurring in soils are collectively responsible for key ecosystem processes such as decomposition of organic matter and cycling of nutrients, which support plant growth and food production. Since soil organisms are so crucial to these important processes, monitoring their activity in response to natural or human-induced impacts has become a key function of soil quality monitoring.

The soil quality concept advanced by Schjonning et al. (2004) likened soil quality to a vessel of desirable soil attributes. Good soil quality management is geared at maintaining or improving a range of desirable attributes while simultaneously removing undesirable attributes where possible. Failure to employ good soil quality management practices can to lead to loss of soil biodiversity and soil degradation. Such declines in soil quality are central to many of the problems facing agricultural production in many parts of the world. Land-use changes are inevitable owing to a rapidly expanding world population and increased competition among varied economic interests. However, successful transition between land-use options presents a major challenge for soil quality management.

Land-use changes are usually associated with critical impacts on soil quality, with repercussions that can be both positive and negative. Unfortunately these impacts are most often negative. During the early 1900s, sections of the Canterbury Plains were planted in pine forest plantations to meet the growing domestic requirements for wood and wood products and provide shelter from Norwest winds. The Selwyn Plantation Board Ltd (SPBL), owner of over 5,000 ha of forest on the Plains, has now determined that conversion to pasture and / or cropping would be most profitable. As a matter of policy it has pursued this option over the last five years. Lincoln University has for some time collaborated with the SPBL to develop appropriate

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management strategies for the conversion process, with an emphasis on optimal retention of organic matter in the soil.

The focus of the conversion strategy has been largely an 'above ground' effort, including seedbed preparation, application of lime and fertilizers, and crop rotations. However, in recognizing the key role of soil biodiversity (a 'below ground' concept) this study investigated the impacts of various management strategies on specific soil organisms representing the micro-flora (bacteria and fungi), meso-fauna (nematodes) and macro-fauna (earthworm). The principal objectives were to:

(1) Determine the effects of lime and N on soil microbial, physical and chemical properties;

(2) Determine the effects earthworms on plant productivity, as well as soil microbial, physical and chemical properties; and

(3) Evaluate the role of soil nematodes as indicators of soil biological quality.

This thesis focuses on soil biological quality and aims to contribute to a better understanding of soil biota and consequently facilitate successful conversion from plantation forest to sustainable pasture production.

Chapter 2
Literature Review

2.1 Land-use changes in New Zealand – forest to pasture

Generally, land-use changes between forest and pasture production in New Zealand have occurred along profitability gradients. For example, in the 1980s and early 1990s there was conversion away from pasture and into plantation forests. Depressed prices in the stock and dairy industries brought on by the removal of subsidies and attractive prices for forest products fuelled establishment of plantation forests across New Zealand (Tate et al., 2004). Present declines in the profitability of the forest industry [US$200/ m^3 (1992) vs. US$70/ m^3 (2006), a decrease of nearly 300%] have reversed this trend and shifted the majority of conversions to pasture (Condron, 2006). Cronshaw (2006) quoted SBPL Chief Executive, Kerry Ellen, as saying, "A better return can be made from finishing lambs and calves than growing trees on the plains". Profits appear to be the main driver of this conversion as the stated objective of the SPBL is "to operate a financially successful business in an environmentally and socially acceptable manner" (SPBL, 2006). The process of conversion involves:

(1) The initial felling of trees by excavator.

(2) Logs are then removed and graded and stumps are munched by stump grinders.

(3) This is followed by surface mulching, old windrows are also excavated ground down and mulched before being levelled by bulldozer and cultivated by giant discs.

2.2 Forest management and impacts on soil quality

Commercial forestry can be intrusive to the soil ecosystem in several respects, and the conversion process is particularly destructive to soil structural integrity (Figure 2). In studies considering the effects of land-use change from pasture to forest, forest cover was associated with declines in soil pH, microbial carbon (C) and nitrogen (N), soil C and N, soil C:N ratio, total nematodes, nematode functional groups, and nematode

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diversity (Beare et al., 2002). Yeates et al. (2000) found that earthworm numbers decreased with increasing time and tree stocking rate. Greater earthworm biomass is usually is indicative of improved soil quality (Condron, 2006). It can be argued that such changes in soil properties could be considered as a decline in overall soil quality.

Figure 2: Forest clearance on the Canterbury Plains.

In the context of the lands managed by the SPBL the soil quality challenge is immediately apparent from data presented in Table 1. Low pH, phyto-toxic levels of exchangeable aluminum (Al) and low levels of available N represent significant limitations to the establishment and maintenance of new pasture. Pasture requires neutral to basic pH, N and other nutrients as given in Table 1.

Table 1: Selected properties of topsoil (0-15cm) determined for Darfield site (after forest clearing and land preparation) with corresponding target levels for pasture.

Soil Properties	Forest	Pasture (target)
pH	4.6	6.0
Exch-Al (cmol+kg-1)	2.5	<0.1
Organic C (%)	5.9	-
Total N (%)	0.25	-
C:N	24	12-15
Total P (mg kg-1)	400	700
Olsen P (mg kg-1)	10	20
Total S (mg kg-1)	250	500
Sulphate-S (mg kg-1)	6	12

Reprinted with permission from (Condron, 2006)

2.3 Soil quality management

Soil is described as a dynamic, living, natural body that is vital to the function of terrestrial ecosystems and represents a unique balance between the living and the dead (Ingham, 2000). Carter et al. (1997) noted that the need to both characterize and assign quality to soil has been self evident from the beginning of agriculture. The Soil Science Society of America (SSSA) has defined soil quality as the capacity of a specific kind of soil to function within natural or managed ecosystem boundaries to sustain plant and animal productivity. According to Schjonning et al. (2004) the soil quality concept implies a value judgment (some measure of excellence). They argued that while most existing literature focused on assessments of soil quality, attention should be given to the management tools available to influence soil quality. They further recommended that efforts should be directed at identifying management thresholds as opposed to soil quality indicators and viewed this approach as an important means of implementing the soil quality concept.
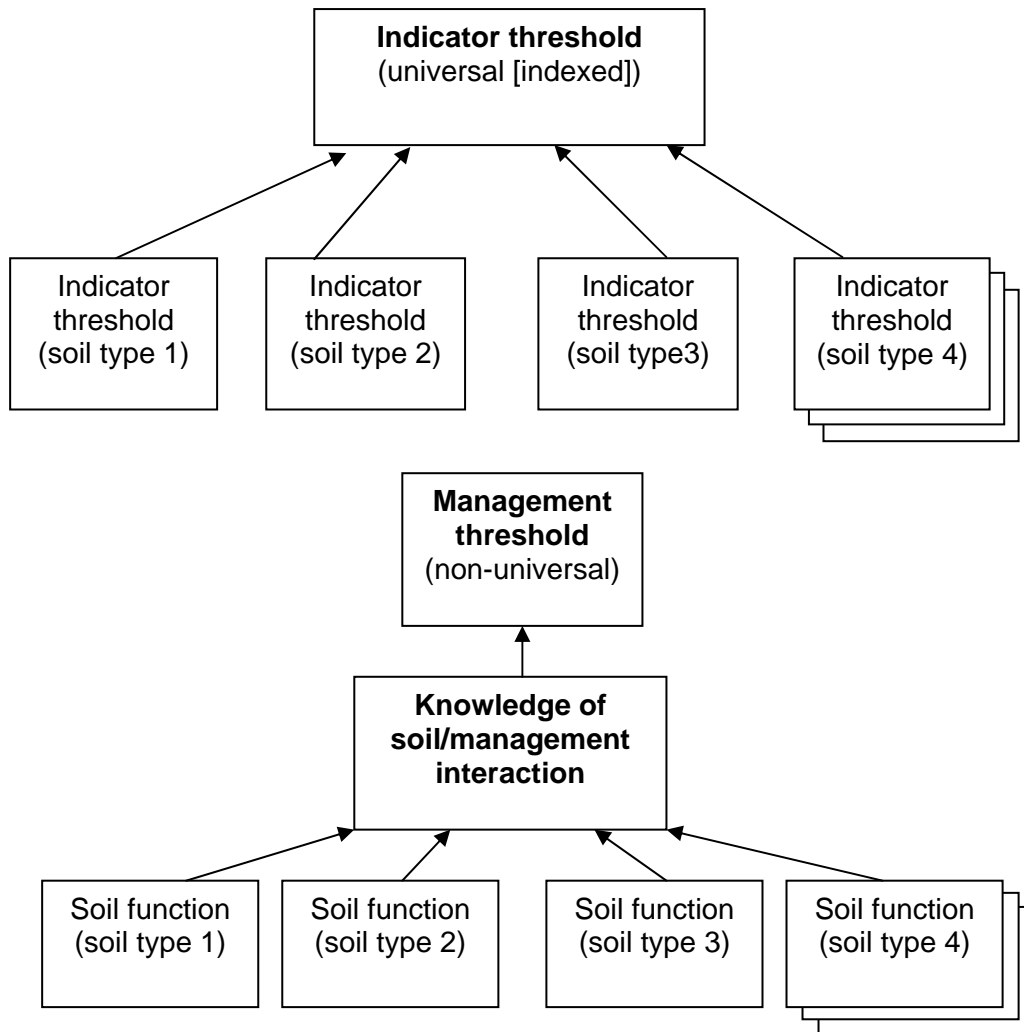
Figure 3: A schematic illustration reprinted from Schojonning et al. (2004).
It contrasts the predominant indicator threshold approach (top) with their proposed management threshold approach (bottom).The main aim of the threshold indicator approach is to identify thresholds, whereas focus of the management threshold approach is on identifying thresholds for specific management tools.

This document likens soil quality to a vessel of desirable attributes appropriate for a particular situation (Schjonning et al., 2004) In the context of lands converted to pasture by the SPBL the challenge is to reduce the variance between the desirable soil attributes for pasture production and the characteristics of remnant soil having undergone many years of forest cover (Table 1).

Doran & Parkin (1994) noted that an assessment of soil quality that includes soil biological, chemical and physical properties can provide valuable information for

evaluation of sustainability of land management practices. The value of monitoring soil quality indices for maintaining or improving soil quality in New Zealand was confirmed in the epic study by Haynes & Tregurtha (1999). Recognizing the dynamism of the soil complex and interactions between its components, this project is essentially a study of impacts of land management on the diversity and function of the soil microbial community.

2.4 The soil food web

The soil food web refers to the community of organisms living all or part of their lives in the soil (Schutter & Dick, 2002; Usher et al.2006). The soil food web theory attempts to simplify the myriad of energy and nutrient flows through the soil system and how these are affected by soil organisms (Figure 4).

Organisms in the food web can be broadly grouped as (energy) producers or consumers. Primary producers occurring at the first trophic level are plants and other autotrophs that are capable of synthesizing energy from the sun through photosynthesis. Consumers like, fungi and most bacteria appear at the second trophic level while anthropods occupy the higher trophic levels (3 and 4). Nematodes have special significance since they occur at the second, third and fourth trophic levels. This feature makes them excellent indicators of food-web conditions. Nearly all consumers are also secondary producers since they provide a ready food source for organisms at higher trophic levels.

The arrows in Figure 4 depict the energy flows of the soil web. Energy is the unit of exchange between trophic groups. Along with the energy provided directly from plant roots and shoots, decomposition of organic matter provides a significant source of energy soil systems. Decomposition occurs through two parallel pathways which are fungal or bacterial driven. A food-web can therefore be labeled as fungal or bacterial dominated based on its relative components of its energy drivers. Bacterial constituents (bacteria and bacterivores) are usually smaller than fungi and fungivores.

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Size determines sensitivity to environmental changes, which can in turn enhance food-web stability and maintenance of nutrient fluxes under stress conditions.
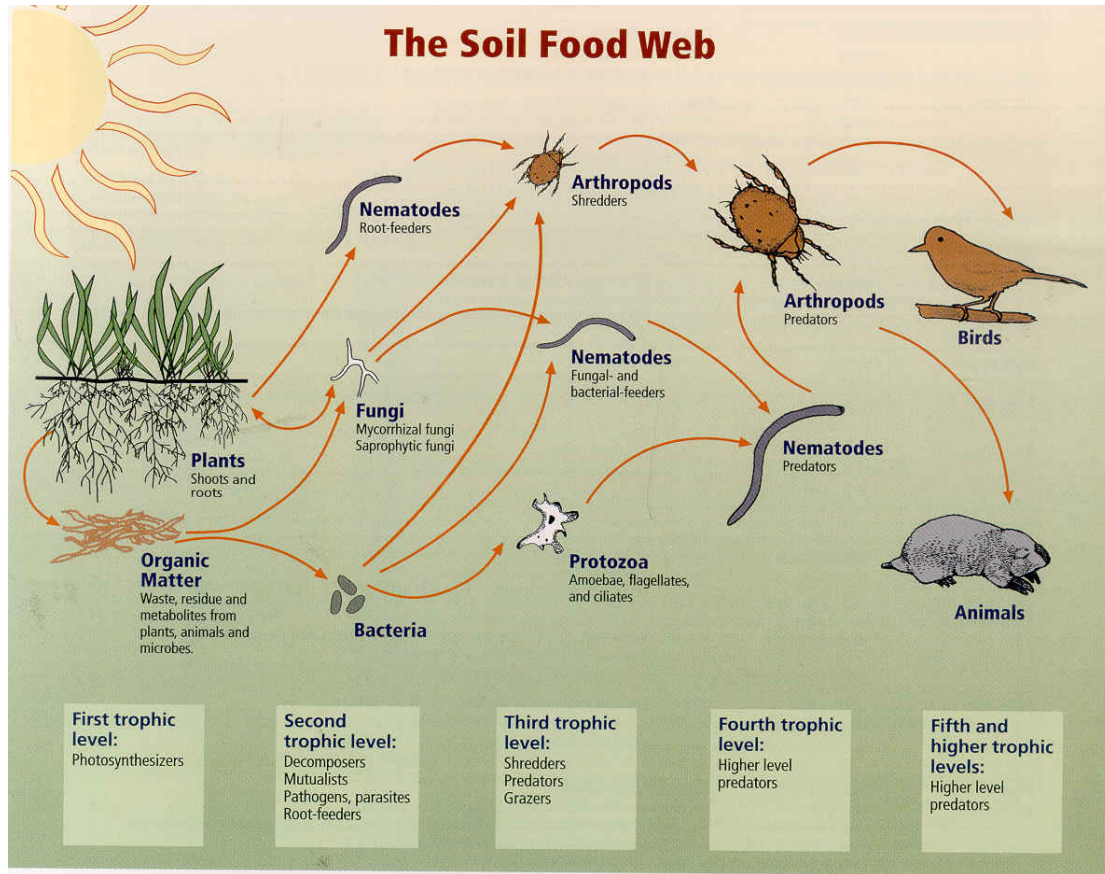
Figure 4: Diagram of the soil food web (Ingham, 2000).

The food-web structure is not fixed over time and space since its constituent organisms are distributed heterogeneously both temporally and spatially. This adds to the difficulty of food-web studies. However, it is accepted that the functioning of soil systems (and its productive capacity) hinges on many interactions among: plants (their roots and residues), physical structure of the soil, animals (and their residues), soil chemical composition and microorganisms. Microbial activity is usually high at the root-soil interface. Apart from feeding on root exudates some organisms like mychorrihza and rhizobium have symbiotic relationships with roots. This adds to the complexity of the soil system and the challenge of studies in this field.

An understanding of these interactions is important to manage the biological properties of soils for enhanced biological functioning, improved fertility and

sustainability. Three representatives of the soil food-web are the focus of this thesis, microorganisms mainly bacteria and fungi (micro-flora), nematodes representing the meso-fauna and earthworms the macro-fauna.

2.5 Soil micro-organisms

There is much interest in relating soil micro-organisms to their physical environments so that habitat influences on communities and functional processes can be better understood. In the past two decades a major focus of research has been to: (1) characterize the roles of major groups within the soil biota in ecologically important processes such as the carbon and N cycles; (2) determine the extent to which reducing diversity of soil organisms may reduce their ability to perform essential ecosystem services (including the ability to cope with human inputs such as N); and (3) determine the extent to which indicators of soil biodiversity can be used as measures of soil ecosystem resilience (recovery after impacts) to land use management (UNEP, 1992).

The United Nations Convention on Biological Diversity defined biological diversity as "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems" (Bardgett et al., 2005; Torsvik et al., 1990a). Soil microbial diversity can be considered a subset of biological diversity. It includes the difference or variability among the soil's microbial community, within species, and between species and their myriad of interactions with and within the soil ecosystem. In terms of species number, the bulk of biological diversity in soils is made up of hundreds or thousands of species of bacteria and fungi (FAO, 1998).

A special report by (Heywood & Watson, 1995) highlighted the crucial role of soil microbial diversity in providing the foundation for sustenance of all terrestrial biodiversity. However, the report also lamented that this fact is seldom acknowledged in discussions of agricultural genetic resources. Balser & Firestone (2005) suggested that the potential for rapid microbial growth and the high degree of diversity and

genetic exchange in microbial systems contributed to the common assumption that microbial activity does not constrain the processes involved in ecosystem nutrient transfer and transformation. To emphasize the apparent apathy of the scientific community with regard to microbial diversity Miller (1992) noted that no one has ever documented the extinction of a bacterium.

Development of modern and advanced research tools and techniques have contributed to an increased understanding of life within soils (O'Donnell et al., 2005; Usher et al., 2006). However, Bardgett et al. (2005) concluded that the major limitation to ecologists trying to understand and develop theories on patterns and determinants of soil biodiversity was "the dearth of available information on the diversity of soil biota (especially at the species level and across different spatial and temporal scales)". Usher et al. (2006) considered that one of the principal reasons why so little was known about soil ecosystems is that they are very difficult to study. It is well known that the relationships between the soil system, its component organisms, the complex processes they mediate and corresponding environmental interactions are seldom, if ever, straightforward.

2.5.1 Soil ecosystem functions and processes controlled or mediated by soil micro-organisms

Soil micro-organisms play a key role in organic matter decomposition, nutrient cycling and other chemical transformations in soil. The vast diversity of microbial species and their ability to break a wide range of chemical bonds means they are responsible for important soil functions (Murphy et al., 2003). Some of the soil functions controlled or influenced by micro-organisms include:

- Decomposition of soil organic matter and plant/animal residues with subsequent mineralization of nutrients (N, sulphur and phosphorus).

- Transformation of nutrients between chemical forms. Such as nitrification $\left(NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-\right)$.

- Degradation of synthetic compounds such as pesticides and herbicides.

- Production of antibiotics, which can aid the suppression of soil borne diseases.

- Production of cementing agents, which may aid aggregation and lead to water repellence.

- Plant nutrient acquisition through symbiotic associations (mycorrhiza and rhizobia).

2.5.2 Relevance of soil microbial diversity

The global biodiversity assessment noted the relatively high diversity of soil biota and reported that, "a single gram of temperate forest soil could contain 10^9 individual cells comprising 4000-5000 bacterial types of which only 10% have been isolated and are known to science" (Palojarvi, 2006). Leake et al. (2005) reported that diversity-function relationships are starting to be elucidated for some key soil microbes. They showed that diversity of mycorrhizal fungi was of central importance to agro-ecosystem functioning. This was demonstrated by consideration of the multifunctional nature of mycorrhizal associations (assisting plants in nutrient acquisitions, mediating

carbon transfer between plants and protecting their roots from pathogens) and on the basis of emerging evidence of a combination of high specificity and dependency in many mycorrhizal associations. Conversely, Murphy et al. (2003) reported that even where identifiable components of the soil microbial community have been linked to specific transformation processes such as mycorrhizal fungi, there is still limited knowledge of the importance of diversity within such groups on the chemical transformations they mediate. They further argued that species composition of microbial communities may be of greater direct relevance to the rate of specific ecosystem processes than their diversity *per se*.

A primary objective of recent research on soil microbial diversity has been to determine the existence of functional relationships to critical processes occurring in soils. Several researchers have reported significant advances but admit that much more work is needed to arrive at a fuller understanding of the relationship between microbial diversity and function in soils (Palojarvi, 2006). While the search continues, it seems logical that soil management strategies, which conserve or increase soil microbial biomass, enable niche environments to develop within the soils microbial matrix and provide a range of organic compounds on a regular basis will also tend to maintain a diverse microbial population.

The controlling effect of microbes on various soil processes is well documented (Sparling, 1988). However, there are very few studies that quantitatively link microbial community characteristics to soil processes and rates (Balser & Firestone, 2005). This is due in part to the inherent difficulty of microbial community studies. The communities are innately complex and their structure and function are difficult to quantify let alone connect (Balser & Firestone, 2005; Balser et al., 2002). Microbial ecological studies relying on gene-based techniques (like polymerase chain reaction (PCR) and denaturing gradient gel gene electrophoresis-DGGE) can provide highly detailed taxonomic data sets of communities. However the goal here is not necessarily a characterization of community components but rather a parameterization of the community in a way that it can be related to function. Microbial biomass is more commonly used as the parameter of choice for soil quality assessments, but this measure provides very little information about the microbial community. Nothing is

revealed about the effects of individual community components. Although lipid biomarker (PLFA) analysis and substrate utilization have been increasingly used to represent aspects of microbial community structure and function, few studies have assessed the relationship of these parameters with ecosystem function and processes (Balser & Firestone, 2005).

The precise relevance of microbial characteristics (biomass, diversity) to the soil processes outlined earlier remains elusive and sometimes controversial in the absence of a full understanding of below ground microbial communities (Balser & Firestone, 2005; Balser, Kirchner, & Firestone, 2002; Heywood & Watson, 1995; Usher et al., 2006).

2.5.3 Phospholipids fatty acid analysis for microbial community characterization

The analysis of ester-linked phospholipid fatty acids (PLFAs) is an acknowledged and widely used biochemical approach to microbial community characterization (Palojarvi, 2006). Zelles & Bai (1993) recommended this technique as one of the most sensitive and reliable chemical measures of microbial biomass. Phospholipid fatty acids are useful bio-markers since they are found in the membranes of all living cells. They are an intricate part of the bi-lipid layer of cell membranes and possess great structural diversity coupled with high biological specificity. Unique fatty acids are indicative of specific groups of organisms.

Under conditions expected in naturally occurring communities, PLFAs represent a relatively constant proportion of cell mass. They are also quickly degraded upon cell death and are not found in storage products. These features make them ideal as a proxy for the living, and probably active, microbial biomass (Heywood & Watson, 1995).

Extraction of phospholipids from soil samples is followed by analysis using gas chromatography (GC) and mass spectrometry (MS). These techniques yield precise resolution, sensitive detection, and accurate quantification of a broad array of PLFAs (Heywood & Watson, 1995). Hill et al. (2000) attributed the existence of an extensive library of signature molecules (used for identifying microbial groups) to the use of fatty acid analysis for bacterial taxonomy, where specific fatty acid methyl esters (FAMEs) have been used as an accepted taxonomic discriminator for species identification.

The results of PLFA analysis are essentially a fingerprint of the soil microbial community at the time of sampling. There is the added advantage that sum total of PLFAs extracted from a soil sample could be a reliable estimate of soil microbial biomass. Where new or unidentified groups are found, further characterization, and identification may be possible with other techniques. These features have led to its

widespread use in investigating the effects of management and fertility on soil microbial communities, example bacterial:fungal ratios in soils (Frostegard & Baath, 1996).

2.5.3.1 Specific applications of PLFA analysis

It is generally accepted that many aspects of agricultural production including monocultures, soil compaction, tillage, use of pesticides and commercial fertilizers have long-term detrimental effects to microbial life and diversity in soils (Torsvik et al., 1990a). Earlier methods for assessing microbial impacts depended on culturing and counting of microbes. Culture dependent techniques considered only a small fraction of the microbial community as in many cases less than 1% could be studied (Frostegard & Baath1996). This inadequacy was exposed with the emergence of novel approaches such as molecular biology (Bossio & Scow, 1998; Fierer et al., 2003) and PLFA analysis (Leininger et al., 2006).

Phospholipid fatty acid analysis is now used by many research scientists in varied programmes aimed evaluating how different anthropogenic interventions impact on soil microbes. Waldrop et al. (2000) used PLFA to determine the possible linkage of microbial community composition to function in a tropical soil. They reported that changes occurred in the microbial community profile with changing land use and management. Conversion from forest to pineapple plantation increased the relative amount of fungi and actinomycetes and decreased the relative amount of Gram-positive bacterial biomarkers. Correlations of PLFA with specific enzyme activity provided useful insights into the linkage between community composition and function.

Frostegard et al. (1993) used PLFA to investigate the effects of heavy metals on two soil types (arable and forest). A major objective of previous studies was to determine what level of contamination would produce detectable changes in the soil microbiota. Since these attempts relied solely on measures of biomass and microbial activity they were unable to detect possible effects on individual groups of the soil biota, but PLFA analysis allowed for examination of the entire microbial community structure. Thus it

was possible to determine which groups were affected and how. For example, their results indicated that the bacterial PLFAs 15:0 and 17:0[1] increased in all metal contaminated samples in the arable soil, while they were unaffected in the forest soil. Proving the high sensitivity of PLFA analysis, the effects on PLFA patterns were found at levels of contamination similar to or lower than those at which ATP content, soil respiration, or total biomass had occurred.

In a study of a long-term (280,000 year) forested chronosequence (caused by upland shift of marine terraces in the Waitutu region of Fiordland National Park, New Zealand) Williamson et al. (2005) used PLFA to investigate the response of soil microbial communities to ecosystem in decline, a phase which is said to accompany the creation of new land surfaces. Their results suggested a decline in microbial activity and soil fauna and an increase in relative importance of the fungal-based (vs. bacterial based) energy channel during long-term ecosystem development on terraces of marine origin. This suggested that at the study sites there was a retrogressive shift in organic matter quality over a long-term chronosequence. Studies of natural ecosystems are of tremendous value especially to efforts geared at conserving biodiversity. However, an understanding of the microbial impacts of altered or managed agricultural systems is especially important to the sustenance of life on earth as known today, especially with regards to the maintenance of food security to feed a growing world population.

Murray et al. (2006) used PLFA analysis to determine the impact of added lime and N on the soil biota in an upland grassland system in the UK. They found no changes in fungal biomass, but bacterial biomass was reduced with increased N and pH. Although the context of this study is very different from the scenario on the Canterbury Plains, their findings are of tremendous importance for researchers concerned with the holistic improvement of soil quality especially on lands (with poor quality soils) recently converted to pasture. In another study looking at the impacts of added soil amendments of the microbial community Frostegard et al. (1993) showed that increased pH (effected by additions of lime and wood ash) in a forest soil

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[1] PLFA nomenclature is explained in the Appendices.

generated significant shifts in the PLFA bacterial community profile. As soil pH increased there was a shift to more Gram-negative and fewer Gram-positive bacteria. There was also evidence of an increase in the population of actinomycetes in the limed soils. Ingels et al. (2005) suggested that decreases in Gram-negative bacteria with concurrent increases in actinomycetes and Gram-positive bacteria may indicate a decrease in labile carbon availability. In other studies, Gram-negative bacteria were considered indicative of increased substrate availability (Moore, 2003; Ramsey et al., 2006).

The value of PLFA analysis was further highlighted when a comparison of methods for soil microbial community analysis revealed that PLFA maximizes power (i.e., the probability of detecting significant differences) when compared to community level physiological profiling (CLPP) and PCR-based molecular methods (Ramsey et al., 2006).

2.5.3.2 Further advantages of PLFA analysis.

- Detects the microbial community in an environmental sample without the problems associated with cultures and direct counting methods.

- Can be used to detect rapid changes in wide range of environments: soil, sediments, water and humus.

- Relatively easy and quick to perform so a large number of samples can be processed simultaneously.

- Relatively inexpensive if a gas chromatograph is available.

2.5.3.3 Limitations of PLFA analysis

As with most useful methods and techniques PLFA do have some shortcomings. Some disadvantages of this method are:

- PLFA profiles do not reveal species level information. This is primarily due to the overlapping of PLFA patterns within groups. Results generated will be representative of groups within the microbial community rather than for individual species within groups. For example, a group of fungi occurring in a soil sample will be reported on rather than a specific fungus. This can be a major constraint when the goal is to link a highly diverse microbial community to specific functions and processes. Individual species are likely to have different roles and species apparently redundant may become active on the trigger of certain stimuli. The inability to track changes at the species level could be a major limitation to truly understanding the varied roles of the constituents of the microbial community.

- Archaea cannot be determined with this method since they have ether-linked fatty acids while PLFA analysis considers only ester-linked fatty acids. Archaea were found to dominate among ammonia-oxidizing prokaryotes in soils (Yeates & Bongers, 1999).

- Determination of signature PLFAs for specific microbes requires their isolation in pure culture. It follows therefore that only microbes already catalogued or those which can be cultured using available techniques will be considered in PLFA analysis.

- As the analysis relies on a library of signature fatty acids (FAs) for specific microbes unusual FAs or those found in low concentrations may not be easily detected and could be ignored, however these could be representative of a functionally very important group.

- PLFA patterns for individual populations can vary in response to environmental stimuli. It is possible that an abnormal event at or just prior to the time of sampling could bias the results. Precautions are necessary to preserve the integrity of samples and subsequent results.

- In contrast to chemical and physical characteristics, microbial parameters may be more easily affected by handling and storage of collected samples. In the case of PLFA it has been established that the integrity of membrane lipids are significantly affected by environmental factors. Storage of samples at

temperatures below 25^0C has been proven to be effective, for best results it is recommended that samples should be extracted very soon after collection.

(Moore, 2003; Ramsey et al., 2006)

2.6 Soil meso-fauna – nematodes

Soil nematodes are worm-like animals usually measuring 0.3-2 mm as adults, but can reach lengths of 12 mm. Yeates & Pattison (2006) noted that 20,000 species were known, although Poinar (1983) estimated that 42,000 species may exist. Nematodes are among the most diverse and abundant organisms. One m^2 of soil may contain 100,000 to 10 million individuals with up to 200 species. Nematodes are aquatic creatures and depend on films of water for movement within the soil profile and on other soil organisms and plant roots for food. Therefore the physical and biological conditions of soils are critical to their survival and success (Lewandowski & Zumwinkle, 1999; Neher, 2001; Yeates, 1998). They are the most well-known member of the soil meso-fauna due primarily to the plant parasitic species of nematodes which attack many cultivated plants.

Historically nematode research has focused on the few species injurious to plants [e.g. the clover cyst nematode, *Heterodera trifoli* (Mercer, 1994)]. The importance of many other species central to important soil processes were only highlighted during the last two decades (Edwards, 2004; Lavelle & Spain, 2001). Yeates & Pattison (2006) suggested that there may be a net benefit of the nematode impact if the entire nematode community is considered. They argued that while nematode damage can effect significant reductions in plant yields, nutrients and energy generated from their excretion and death are leaked back to the rhizosphere. Nematode grazing or feeding on soil micro flora and fauna increases nutrient cycling and maintains microbial populations at higher growth rates. Nematodes can also feed on disease causing microbes and have the potential to change the relative abundance of microbial taxa. Such qualities make these animals major players in the soil world.

Nematodes occur at several trophic levels in the soil food-web and are strategic in key soil processes (Figure 4). Advances in the identification of nematode taxa and

characterization into feeding groups and guilds[1] have boosted the potential of these microscopic worms as important indicators of soil food-web conditions and biological soil quality. Advances in molecular biology allowed for the mapping of the genome of the nematode, *Caenorhabditis elegans.* This achievement adds to a greater understanding of the biology of nematodes and their functions in soil processes. Nematodes were described as promising biological indicators (Fraser, 1999), and Yeates (1998) noted further that they may be especially important in grasslands due to: (1) the rapid turnover of roots and shoots; (2) they are ubiquitous; (3) easily separated into functional or trophic groups; (4) respond quickly to changes in food supply; (5) but are stable (relative to microbes) in response to weather changes. In promoting the concept of nematode faunal analysis for soil food-web diagnostics Ferris et al. (2001) supported the suitability of nematodes as indicators and highlighted that they could be enumerated by standardized extraction techniques. In addition, they are readily identified from morphological and anatomical characters and their feeding habits are clearly related to oral structure from which their trophic roles are inferred (e.g. bacterivores, fungivores, predators and, plant parasitic nematodes).

Analyses of nematode communities investigating population size and diversity in different soil systems have generated conclusive evidence of the association of specific nematode groups and community structures with particular soil conditions and habitats. Food source and quality, soil type, soil moisture and temperature are some important criteria which can impact abundance and diversity. Graphical representation of the nematode faunal data was organized by Ferris (2007) to estimate soil food-web conditions. Nematodes are characterized along a colonizers/persisters (c-p) scale based on response to stress conditions and food sources. A weighting of 1-5 is issued based on the c-p group. Example c-p 1 would denote persisters who would be prevalent in resource poor conditions. They are usually smaller with relative large numbers offspring and have a high tolerance for stress conditions. Colonisers are usually larger and have much less offspring. Their populations can increase exponentially when conditions are favourable e.g. additions of fertilizers or high

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[1] An assemblage of species with similar biological attributes and response to environmental conditions.

quality organic matter with low C:N ratios. Such characterizations of soil nematode populations in relation to soil conditions make them ideal indicators of soil quality

2.7 Soil macrofauna - earthworms

Earthworms are soft-bodied segmented animals ranging in length from a few millimetres to over 1 m (Coleman et al., 2004). They are an important component of the soil ecosystem contributing significantly to the physical, chemical and biological properties of soils. Although not numerically dominant they account for a larger proportion of animal biomass in most soils because of their large size relative to other members of the soil food web (Haynes et al., 1995). They enhance the overall productivity of soils through their feeding, excrement (casting), and burrowing activities and several researchers have reported on the positive influence they exert on many important soil characteristics (Simms & Gerard, 1985). According to Stockdill (1982) the introduction of earthworms to pastures in New Zealand produced several desirable effects. These include improved mixing and vertical distribution of organic material, plant nutrients and lime, increased water holding capacity (17% greater in soils with worms) and increased infiltration rates associated with reduced run-off and the risk of soil erosion, improved root development, and significant increases in pasture production (up to 113%).

New Zealand pastures are dominated by the *Lumbricus* species introduced from Europe. They replaced the native *Megascolecidae* species when virgin forests and grasslands were converted to intensive pastoral and arable production systems by the early settlers. There are no early reports on intentional seeding of these imported earthworm species. It is believed that they made their journey as cocoons hidden in roots of plants and shrubs brought by the settlers. The ships used soil used as ballast and these were discarded at the ports once no longer needed. While nearly 200 different earthworm species (of the endemic *Megoscolecidae* family) have been found in New Zealand, this diversity is now limited to undisturbed areas such as forests, old gardens and in the hills and mountains (Simms & Gerard, 1985). In contrast Haynes et al. (1995) found only 4-5 species of *Lumbricus* earthworms during an extensive

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survey of pastoral and arable lands in Canterbury (*Apporectodea caliginosa, Lumbricus rubellus, Apporectodea trapezoids, Octalasion cyaneum and Apporectodea rosea*)   Their results are supported by the findings of Fraser et al. (1996).

Yeates (1981) reported further that *Apporectodea caliginosa* was the dominant species (76-93%) in all cropping histories studied (varying periods of pasture and arable management). The study identified an inversely proportional relationship of *A. caliginosa* to *Lumbricus rubellus* numbers with increasing time under pasture management.

Although both species occur in the top soil, they possess some degree of specialization. Lee (1985) noted that earthworm communities are usually stratified vertically and individual species display morphological, physiological, reproductive and behavioural differences according to the position occupied in the strata. *Lumbricus rubellus* is characterized as a surface dweller where it feeds on fragments of decomposing litter and animal dung, ingesting little or no soil. In pastures they are located under dung pats. Their favoured habitat is usually moist and high in organic content (Brown, 1995). *Aporrectodea caliginosa* is located lower in the profile at 20-30 cm depth (Beare et al., 2002) feeding on soil and available organic material. Postma-Blaauw et al. (2006) showed that when combined Rubellus and Caliginosa significantly increased soil bacterial. Earthworms on a whole are numerically dominant in gardens and most cultivated land. Small individuals are common in the top 70 mm where they live in temporary horizontal burrows and occasionally make small casts on the soil surface (Beare et al., 2003; de Jonge et al., 2007; Frostegard, 1993a). Earthworm activities can impact on other soil dwelling organisms such as nematodes (Yeates, 1981).

There is evidence that earthworms can effect reductions in soil nematode populations (Kear et al., 1967) and their impact on soil bacteria and fungi was reported by Byzov et al. (2007) and Fraser et al. (2003). A significant impact of earthworms on soil organisms may be secondary, as increased earthworm activity is associated with enhanced soil aeration and availability of nutrients to plants, thereby increasing plant growth and returns as litter and root exudates. An abundance of food resources

stimulates microbial activity and can possibly alter microbial community composition (Alef, 1995).

Chapter 3
Impacts of Lime and Nitrogen Inputs on Soil Microbial, Chemical and Physical Properties

3.1 Introduction

There is growing acceptance of the pivotal role of the soil microbial community in most soil ecosystem functions and processes. In reference to the importance of soil microbes to nutrient cycling, (Jenkinson, 1977) described them as the "eye of the needle through which all organic matter must pass". Although microbes can have significant impacts on soil system services such as nutrient cycling, the relationships between microbial, chemical and physical soil properties are for the most part mutual. Consequently, prevailing soil characteristics of pH, nutrient availability and structure can have crucial implications for soil microbial dynamics. Low pH and reduced availability of N were highlighted as the major limitations to the forest-pasture conversion at the Darfield experimental site. Remedial applications of agricultural lime and N were necessary for successful pasture establishment. The conversion process involved intrusive soil disturbance and mulching of woody forest material including needles, bark and, roots. The fate of the microbial community and its ability to provide essential services was unclear, and literature searches provided no specific answers. There is a paucity of studies investigating the effects of lime and N on soil biological properties and the available published data are mostly reflective of agroecosystems not directly comparable to our trial site (Clegg, 2006; Moore, 2003). Nonetheless, some results suggest that both lime and N can change the structure and functioning of the soil microbial community (Ingels et al., 2005; Moore, 2003) and in so doing influence key soil processes. Research findings are on the whole variable, since the maze of interconnectivity that is the soil ecosystem allows for spatial and temporal differences that preclude the same microbial response from virtually identical soil conditions.

We used a field trial and a controlled glasshouse pot experiment to determine the possible effects of applied lime and N on selected soil quality indices. The microbial component was particularly emphasized since the limitations to forest-pasture

conversion as mentioned earlier (Table 1) were already explored in the traditional 'above ground' dimension. Hence, we sought to investigate how these above ground solutions impacted the belowground properties and processes. Growing evidence that microbes are central to soil processes that are linked to sustained soil productivity also formed a basis for underscoring biological soil quality. It was expected that inputs of lime (increased pH) and N fertilizers would significantly change soil microbial community structure and function.

3.2 Materials and Methods

3.2.1 Field trial

3.2.1.1 Experimental site

The trial was located on a 30 ha dryland research block at Darfield, Canterbury ($43^o49'$S, $172^o13'$ E) (Figure 6). The site had been through three rotations of radiata pine for timber production between 1890 and 2003. *Pinus radiata* was felled and timber cleared during 2004. After the removal of larger timber waste to burn piles stumps, roots and other woody debris were mulched from September 2004 to March 2005. The site was then cultivated in preparation for planting crops and pastures in April 2005.

The soil was a Lismore stony silt loam. It is shallow, with stones in the top soil, free-draining and susceptible to drought during the summer months (Ingels et al., 2005). The soil was littered with wood debris both along the profile and on the surface. The quantity of wood debris was difficult to quantify and varied widely across the site but would have exceeded 50 tons/ha. Initial soil analyses from samples taken after mulching and land preparations highlighted several limitations to pasture establishment including low pH (4.6), high levels of exchangeable aluminium, low available N and high C:N (Table 1). The area is an un-irrigated dryland with an average annual rainfall of 780 mm (Figure 5).
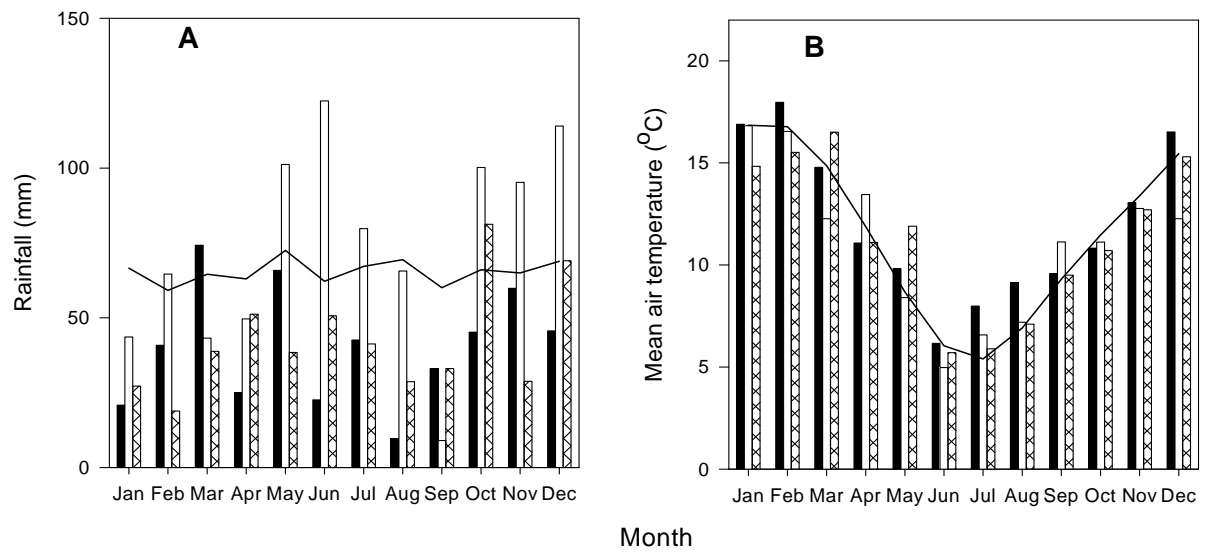
Figure 5: Mean monthly (A) rainfall (mm) and (B) mean air temperature (^{o}C) in 2005 (■), 2006 (□) and 2007 (▨) at Darfield, Canterbury. Long term (1919-2005) means (—) were recorded on site.

Figure 6: Darfield trial site indicating the lime x nitrogen trial plot (before forest removal) and two reference sites forest and long term pasture.

3.2.1.2 Experimental design

A trial was established on the site in March 2005 to determine the impact of various ratios of lime and N in relation to pasture establishment and production. The trial was a split plot factorial design with lime as the main factor and N the sub-factor. The treatments were standard agricultural lime at four rates (0, 2.5, 5 and 10 t/ha) and five rates of N (0, 50, 100, 200, 400 kg N/ha) as calcium ammonium nitrate (26%N), giving a total of 60 plots (4 lime × 5 N × 3 replicates) with dimensions 6m x 5m (Figure 3). Nitrogen applications continued on an annual basis with three split applications and the trial was periodically grazed by sheep.

Six treatments including lime at (0, 5 and 10 t/ha) × M at (0 and 200 kg/ha) were selected to assess the impact of lime and N on biological, chemical and physical soil properties. Each treatment was replicated three times (3 lime × 2 N × 3 replicates) for a total of 18 plots. Selected treatments included the recommended rates of lime and N (based on soil analysis). Previous assessments revealed that significantly higher pasture yield (kg DM/ha) in treated plots compared to the control. We sought to investigate whether this was linked to changes in specific soil properties.

Figure 7: Lime x nitrogen field trial at Darfield showing replicate treatment plots 6m x 5m.
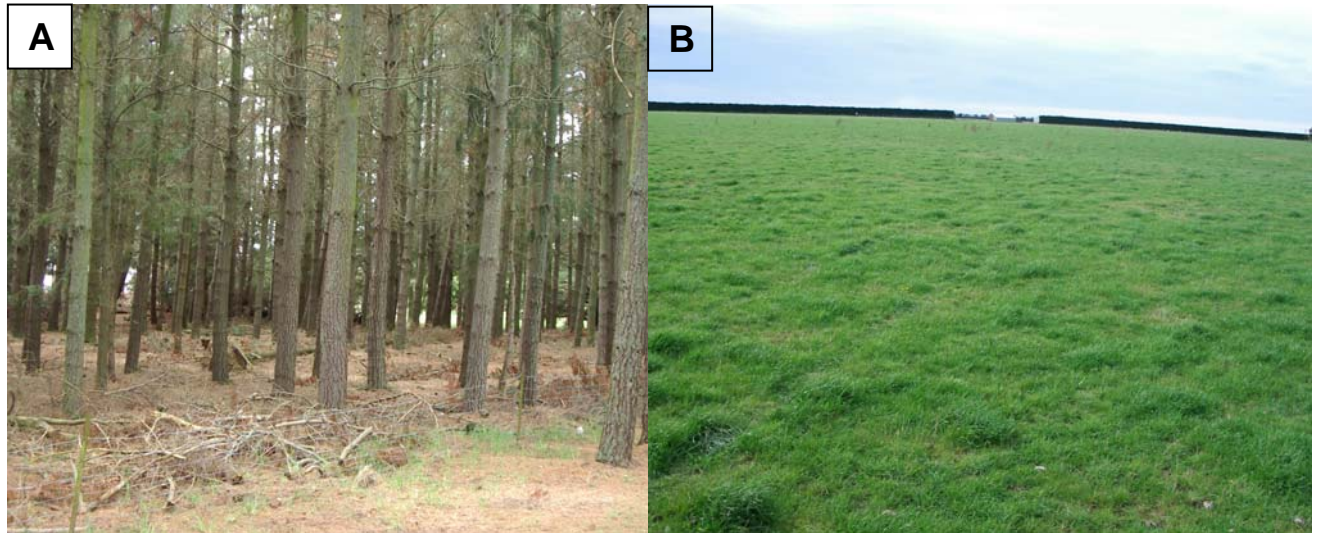
Figure 8: Reference sites (A) Forest and (B) Long-term pasture
Forest is 60 year old *Pinus radiata* block 25 years into the second rotation. The
pasture site had been in pasture crop rotation for 100 years and was in the 11th
year of a pasture rotation cycle.

Soil sampling

Soil samples were collected on September 10, 2007 from five 25mm diameter cores to a depth of 75mm taken from each plot of the selected treatments. On the same day 10 cores of the same dimensions were randomly sampled from each reference site. At the forest site the top layer of litter was cleared before sampling. The samples were kept in sealed plastic bags and stored in a portable cooler, followed by immediate storage at 4°C. Sub samples were taken for moisture determination and dehydrogenase activity within 24 hrs.

3.2.1.3 Measurement of soil microbial, chemical and physical variables

Microbial

Phospholipids fatty acid (PLFA) analysis and dehydogenase activity (DHH) were used to measure soil microbial community structure and activity, respectively.

PLFAs were measured for each experimental unit described by Bligh and Dyer (1959) and as modified by White et al. (1979) and used by (Bardgett et al., 1996). Lipids

were extracted from 1.5 g of fresh soil using a mix of chloroform, methanol and citrate buffer (1:2:0:8 by volume). The supernatant from this was split into two phases by adding chloroform and citrate buffer. The lower chloroform phase containing the lipids was recovered and evaporated under a stream N_2 gas. These lipids were re-suspended in chloroform, and then separated into neutral lipds, glycolipids and phospholipids (eluted individually, with chloroform, acetone and methanol) by fractionation on silicic acid columns (Isolute; 500 mg silicic acid in 6-ml reservoirs). The phospholipids were retained and evaporated under a stream of N_2 gas, and then mild alkaline methanolysis was performed to create methyl esters. These samples were also evaporated under N_2 gas and stored at -20°C until analysis by gas chromatography (GC).

After GC analysis, peaks were identified by calculating retention times relative to two added internal standards (C13 and C19) and comparing these with peaks from a bacterial methyl ester standard (Supelco Bacterial Acid Methyl Esters CP Mix 47080-U). The abundance of individual of individual fatty acids was calculated as relative ηmoles per gram of dried soil, and characterized by standard nomenclature (Tunlid et al. 1989). PLFAs used to represent bacteria were; cyclic fatty acids (cy-17:0, cy-19:0), branched fatty acids (i-15:0, a-15:0, i-16:0, i-17:0) and 15:0. A relative measure of the fungal: bacterial ratio was calculated by dividing fungal PLFA (18:2ω9,12) by bacterial PLFA. All identified peaks were summed to form a measure of total PLFA.

Dehydrogenase activity was determined for each experimental unit as described by Alef (1995) based on Thalmann (1968). In summary, it involved measurement of the rate of reduction of triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF). Field-moist soil (5 g) was mixed with 5 ml TTC solution in 82 ml glass tubes which were then sealed with glass stoppers and incubated at 30 ^{o}C for 24 hrs. After 24 hrs 40 ml acetone was added to each tube and the contents thoroughly mixed. The tubes were again incubated for 2 hrs in the dark at room temperature and shaken at intervals. The mixture was then filtered and samples and blanks analyzed by dip probe on a Varian Cary 50 UV-Vis spectrophotometer at 546 nm. Standards were made using TPF solution (50 mg TPF in 80 ml AR grade acetone), 8.3 ml tris buffer and acetone. Standard concentrations of 0, 5, 10, 20 and 30 μg TPF ml^{-1} were prepared.

The standard curve was corrected for the control value and used to calculate dehydrogenase activity for each sample:

$$TPF(\mu g) \Big/ dwt(g) = \left(\frac{TPF(\mu g^{-1})}{dwt \times 5} \times 45 \right)$$

Where:

dwt = dry weight of 1g moist soil

5 = Amount of moist soil used (g)

45 = Volume of solution added to the soil sample in the assay

Chemical and physical analyses

Each soil sample was analysed for pH (water) (Blakemore et al., 1987) and total C and N, (LECO CNS-2000 element analyser) Leco Australia Pty Ltd NSW Australia).

Aggregate stability describes the capacity of soil aggregates to withstand the degrading impact of water. Aggregate stability was determined for each soil sample, as percentage water-stable aggregates, using a modified method based on Beare et al. (2002) and Niewczas & Witkowska-Walczak (2005). In short, soil samples were air dried and sieved to a range of 2.0 mm to 4.mm. We determined the moisture content of the aggregates by drying a sub sample (approx. 10g) at 105°C for 24hrs. We added 25 g of the air dried aggregates to 2 mm mesh sieves (diameter 100 mm and depth 45 mm) and were allowed to be slowly re-wetted before being repeatedly submerged in water using a wet sieve apparatus. Samples were kept on the machine for 4 minutes and completed 25 strokes (vertical up-down movements in and out of a water bath) per minute. The soil remaining on the sieve was carefully collected and dried at 105 ^{o}C and weighed to determine the percentage aggregate stability determined using the following equation.

$$\text{Aggregate stability \%} = \frac{\text{Soil weight (oven dry equivalent) retained on sieve}}{\text{Total soil weight (oven dry equivalent) added to sieve}} \times 100$$

3.2.2 Glasshouse experiment

In consideration of the inherent variability of the field conditions, a pot trial to investigate the effects of lime and N was included. In June 2007, soil from the top 0-25 cm depth was retrieved from an untreated section (with no added lime or fertilizers) of the Darfield site. The soil was passed through a 6.3mm sieve and homogenized to remove any resident earthworms, grass grubs and other macrofauna.

A 3 x 2 factorial design was employed for this trial. Three lime treatments:

1. (**L0**) untreated pH 5.1;

2. (**L1**) pH 5.8[1];

3. (**L2**) pH 6.5[2] equivalent to $CaCO_3$ at 10 t/ha.

Two N treatments:

1. (**N0**) untreated, no added N;

2. (**N1**) (N at 200 kg /ha applied as urea).

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Soil pH in the lime treatments were adjusted by thorough mixing of measured quantities of analytical grade calcium hydroxide. The soil was then thoroughly wetted and packed into 4-Litre rectangular pots of dimensions (top area 17×17 cm and height 16 cm). The pots were maintained at a constant moisture content of 80% field capacity by watering every other day to a constant weight. After incubation in the glasshouse for 11 days annual ryegrass seeds *Lolium perene* were sown at a rate of 20 kg/ha or 18 plants per pot. Potassium phosphate and potassium sulphate equivalent to 300 kg/ha of postassium superphosphate were applied as basal fertilizers 10 days after planting, in an attempt to simulate field operations. Nitrogen was applied as two split applications at 10 days and 2 weeks after planting. All pots were sprayed for aphids with natural pyrethrum on August 28 and September 26 2007 and were again sprayed with Neemazal (neem extract) on October 23 2007.

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[1] pH adjusted with analytical grade calcium hydroxide equivalent to $CaCO_3$ at 5 t/ha.
[2] pH adjusted with analytical grade calcium hydroxide equivalent to $CaCO_3$ at 10 t/ha.

Soil sampling

The glasshouse trial was destructively sampled on November 23, 2007, 22 weeks after planting. After removal of roots the soil was homogenized and samples bagged for determination of microbial assays, chemical analysis and aggregate stability. All samples were stored at 4^oC and sub-samples were taken for moisture determination and dehydrogenase activity analysis within 24 hrs.

3.2.2.1 Measurement of soil microbial, chemical and physical variables

See section 3.2.1.3 above.

3.2.2.2 Measurement of plant variables

Plant variables were only assessed for the pot trial. Shoots were clipped to 2 cm from the base six times during the trial. The harvested shoots were collected and dried for 48 hrs at 65^oC and weighed. The cummulative dry weight from each clipping over the duration of the trial provided the shoot biomass. At the end of the trial each pot was emptied, all roots were collected and bagged separately and stored at 4^oC. Within 4 days the roots were washed, dried for 48 hrs at 65^oC and weighed to determine root biomass. Root to shoot biomass was also calculated. Dried leaf and soil samples were ground and analyzed for total C and N by LECO.

3.2.3 Data analysis

The effects of treatments on soil microbial, chemical and physical properties were determined using ANOVA with block and treatment as factors for the field study (split plot) and only treatments as factors for the glasshouse pot experiment (completely randomized). The least significant difference test at $P<0.05$ was used to determine differences between treatments where ANOVA indicated a significant overall effect. Principal component analysis (PCA) was performed on PLFA data to determine the effect on treatments on soil microbial community structure. The proportion that each PLFA made up of total PLFA was used for this analysis to avoid confounding results with differences in biomass. Proportions of PLFA groups as a percentage total PLFA was also used to assess community structure impacts by conducting ANOVA of the same. Canonical variate analysis was used to compare the microbial community structure at the test plots with reference sites. Pearson's correlation coefficient was used to correlate each PLFA with PC 1 and PC 2 to determine which PLFAs contributed most to the variation along each axis.

3.3 Results

3.3.1 Field trial

3.3.1.1 Effect of lime and nitrogen on soil microbial biomass, community structure and activity

Management practice in the conversion process had significant impacts on some of the soil properties considered in our trial. No significant differences were observed in total soil microbial biomass (estimated by the sum of PLFAs ηmoles rel. C19) or bacterial and fungal biomass in response to lime and N (Table 2). Significant treatment effects were observed for only two fatty acids: C16:1ω9 increased with lime ($P<0.05$) and iC15:0 was reduced ($P<0.05$) by N (Table 2). Interactions of lime and N did not have a significant effect on any of the microbial biomass measurements (data not shown). However, the percentage contribution of branched fatty acids (BFA) and

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cyclic PLFA to total PLFA (biomass) were significantly lowered by lime (P<0.01) (Table 2). There was an observed trend of increasing fungal composition (PLFA) of total biomass (PLFA) compared to bacterial (PLFA), in response to lime (Table 2).

Changes in microbial community structure in response to lime and N were shown by PCA of transformed PLFAs data (proportions relative to total PLFA). ANOVA of the first and second principal components (PCs) showed that the microbial community structure was significantly impacted by both lime (P<0.001) and N (P<0.01) in PC1 (Table 2). There were no significant effects of lime and fertilizer interactions (data not shown). The ordination plot in Figure 9 illustrates differences in PLFA composition under different ratios of lime and N, where PC1 and PC2 accounted for 40.4% and 16.1% of the variation, respectively. The treatments without lime (L1) are clustered to the right and the lime treatments L3 and L4 (5 and 10 ton/ha, respectively) are to the left along PC1 while N treatments 200 kg/ha (N4) shift upward along PC2.

Principal component loadings revealed that separation of treatments effects were largely attributable to the branched PLFAs (BFA), iC16:0 along PC1 and aC15:0 along PC2. These are branched fatty acids which are indicative of gram positive bacteria. Correlation analysis revealed significant negative correlations between PLFA community structure (PC1) and dehydrogenase activity (P<0.01) and soil moisture (P<0.01) but there was no significant relationship with pH and aggregate stability (Table 3).

In comparison to the reference sites, the microbial community structure appeared similar across the control and lime × N treated plots but appeared distinctly different from the reference sites (Figure 10).
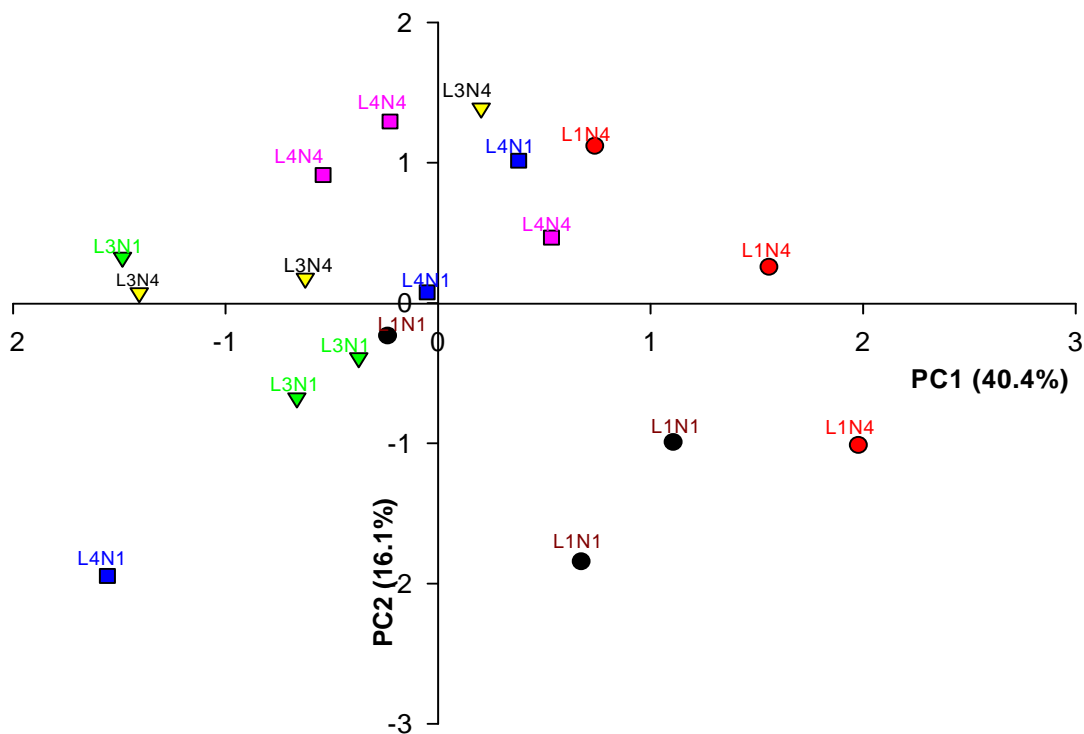
Figure 9: Effect of lime and nitrogen applications on the principle component (PC) scores of phospholipid fatty acids (PLFA) from selected plots of the Darfield trial. L1 (0t/ha lime); L3 (5t/ha lime); L4 (10t/ha lime); N1 (0kgN/ha), N4 (200kgN/ha).

Table 2: Mean values for microbial properties and soil moisture determined for topsoil (0-7.5 cm) taken from lime x N pasture treatments plots and compared to 2 reference sites. Means within rows followed by the same letter are not significantly different to each other at P < 0.05.

	Units	Lime				Nitrogen			Reference sites	
		L1	**L3**	**L4**	**F-Stat**	**N1**	**N4**	**F-Stat**		
		(0t/ha)	(5t/ha)	(10t/ha)		(0kg/ha)	(200kg/ha)		*Forest*	*Pasture[5]*
Microbial biomass[6]	rel. C19 ηmoles /g d.w.	70.20	77.50	75.70	0.79	77.50	71.40	1.50	*62.60*	*27.80*
Total soil bacteria	rel. C19 ηmoles /g d.w.	42.90	47.40	45.00	0.79	46.60	43.60	1.02	*36.60*	*17.70*
Total soil fungi	rel. C19 ηmoles /g d.w.	5.47	7.21	7.55	2.32	7.38	6.11	2.23	*7.78*	*2.09*
% Soil bacteria	% of total PLFA	61.18	61.27	59.63	2.23	60.31	61.08	1.18	*36.60*	*17.70*
% Soil fungi	% of total PLFA	7.67a	9.27ab	9.76b	3.49†	9.32	8.48	1.54	*7.78*	*2.09*
Fungi: bacteria ratio		0.13	0.15	0.16	3.05	0.16	0.14	1.58	*0.21*	*0.12*
% Cyclic PLFA	% of total PLFA	6.01a	4.95b	5.34b	11.20**	5.26c	5.61c	3.59†	*2.70*	*1.38*
% Branched PLFA	% of total PLFA	29.14a	26.55b	26.08b	15.88**	27.32	27.2	NS	*16.61*	*6.41*
iC15[7]	rel. C19 ηmoles /g d.w.	9.46	9.47	8.92	0.28	10.12c	8.44d	5.93*	*6.74*	*2.38*
C16ω9[8]	rel. C19 ηmoles /g d.w.	7.02a	9.52b	9.22b	5.90*	8.89	8.28	0.89	*6.60*	*4.00*
PC1 (40.4%)		0.97	-0.73	-0.24	45.33***	-0.25c	0.25d	10.76**		
PC2 (16.1%)		-0.45	0.15	0.3	0.97	-0.52d	0.52d	4.94†		
Gravimetric moisture		0.33a	0.38b	0.39b	5.53*	0.37	0.37	0.03	*0.32*	*0.22*

† P<0.1, * P<0.05, ** P<0.01, *** P<0.001

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[5] Long-term pasture.
[6] Total soil microbial biomass as measured by the sum of PLFA.
[7] iC15 was the only PLFA that showed a significant response to nitrogen in the field trial.
[8] C16ω9 was the only PLFA that showed a significant response to lime applications in the field trial.

Table 3: Correlation coefficients of PLFA groups with the first two principle components and measured soil indices from the lime x nitrogen field trial.

PLFA groups	Principle components		Measured soil indices			
	PC1	PC2	pH	DHH	Agg Stab	Grav
Total PLFA (Biomass)	-0.74**	-0.21	-0.43	0.40	-0.28	0.34
Bacterial PLFA	-0.72**	-0.15	-0.10	0.37	-0.28	0.34
Fungal PLFA	-0.76**	-0.26	0.19	0.61**	-0.21	0.49
Branched PLFA	-0.50*	-0.23	-0.24	0.17	-0.36	0.14
Cyclic PLFA	-0.30	-0.23	0.01	0.08	-0.09	0.10
PC1	-	-	0.02	-0.69**	0.33	-0.62**
PC2	-	-	0.36	0.21	0.42	0.17

All data transformed to proportions of total PLFAs. (PC) principle component, (DHH) - dehydrogenase enzyme activity (Agg Stab) - aggregate stability %, (Grav) – gravimetric soil moisture. Pearson correlation (2-tailed) significance * at 0.05 level, ** at 0.01 level.
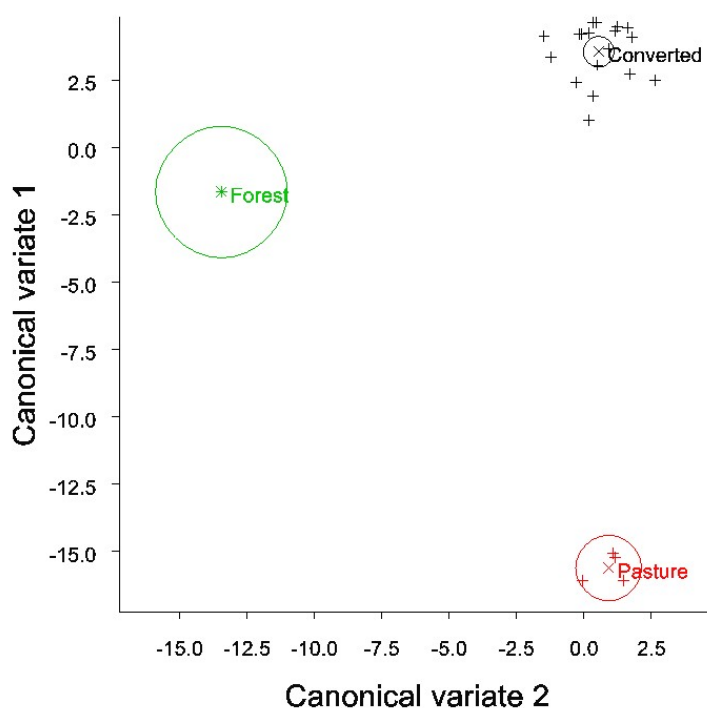
Figure 10: Canonical variate analysis of PLFAs comparing the microbial community structure of the converted trial site to the reference sites (forest and long-term pasture).

There was a strong positive correlation between soil pH and microbial activity as measured by DHH activity (r = 0.9061). Lime applications produced significant increases in microbial activity (P<0.001). Nitrogen appeared to have worked in the opposite direction but this effect was not significant. The highest rate of microbial activity was observed in L4/N1 (10 tons/ha lime and 200 kg/ha) at 23.27 μg dwt/hr and the lowest for L1/N4 (no added lime and 200 kg/ha N) at 3.7 μg dwt/hr (Figure 11). Microbial activity observed for the (L × N) treatment plots is compared with that obtained for the forest and long-term pasture reference sites (Figure 11). We detected comparable levels of microbial activity in the un-limed treatments and the forest site. The lime treated plots had similar levels of microbial activity to the long-term pasture site.
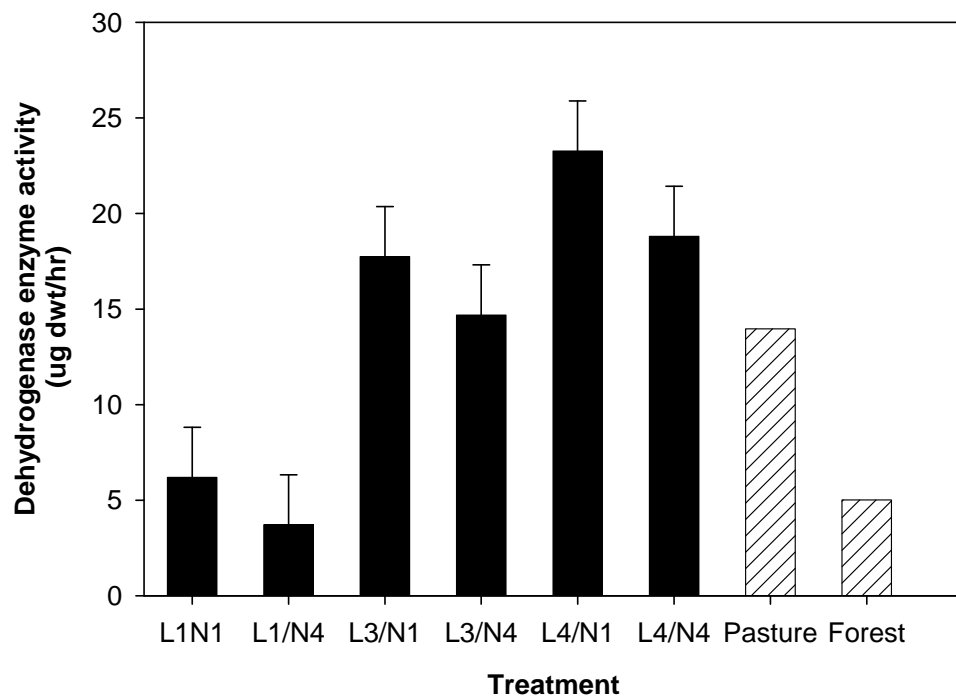
Figure 11: Mean microbial activity determined as dehydrogenase enzyme activity in lime × N treatment plots (■) and reference sites (▨). Error bars show the least significant difference between means at 5%.

Effects of lime and nitrogen on soil chemical and physical properties

Soil samples from the replicate plots of each treatment were bulked to obtain a single treatment sample for determination of soil pH, total C, total N and C:N (Table 4). Soil pH ranged from 4.9 in L1/N4 to 6.24 in L4/N1. Total C ranged from 6.34 to 7.36 in L1/N1 and L3N4, respectively. Total N% was consistent across the trial and reference sites. The C:N ratio was highest in L4/N1 (23.14) and lowest in L3/N4 18.90.

Soil pH under long-term pasture was 6.06 and this was within the upper range of the trial treatments, while the forest at 5.06 was similar to the treatments not receiving any lime applications (L1). Total C was highest on the conversion trial site (average 6.85) which was greater than the forest site 4.91 and long-term pasture 3.58. Total N was similar at all sites (Table 4).

Table 4: Mean values for soil chemical and physical properties determined for topsoil (0–7.5 cm) taken from lime × N pasture treatment plots and two reference sites.

Treatment	Rates (Lime×N)	C%	N%	C:N	pH	% WSA[1]
L1 N1	0 t/ha L × 0 kg N/ha	6.34	0.31	20.6	5.06	34.92
L1 N4	0 t/ha L × 200 kg N/ha	6.64	0.33	20.05	4.79	36.86
L3 N1	5 t/ha L × 0 kg N/ha	7.14	0.33	21.54	5.64	45.76
L3 N4	5 t/ha L × 200 kg N/ha	6.59	0.35	18.9	5.6	50.6
L4 N1	10 t/ha L × 0 kg N/ha	7.02	0.3	23.16	6.24	43.61
L4 N4	10 t/ha L x 200 kg N/ha	7.36	0.34	21.86	6.2	46.49
Long-term pasture		3.6	0.3	11.1	6.1	49.8
Forest		4.91	0.3	16.49	5.02	77.99

Soil aggregate stability was not significantly affected by the treatments. However greater aggregate stability was observed in treatments with lime. The lowest aggregate stability 34.9% was returned from the L1/N1 treatment and the highest 50.6% from L3/N4.Aggregates from the forest soil were very stable at 78% compared to aggregates from the long-term pasture at 49.8% (Table 4).

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[1] Percentage water-stable aggregates.

3.3.2 Glasshouse experiment

3.3.2.1 Effect of lime and nitrogen on soil microbial biomass, community structure and microbial activity

Lime and N treatments in the pot trial had significant impacts on the soil microbial community structure as measured by PLFA. Nitrogen had the greatest impact on PLFAs causing reductions in the fungal biomass represented by C18:2ω9,12 (P=0.017), fungal to bacterial ratio (P=0.006), and in several bacterial PLFAs (Table 5). Lime was responsible for a significant reduction in the Cy C19:0 (P=0.003). The combined effect of lime and N interactions were not significant. Treatment impacts on the microbial community structure were reflected in the PC analysis (Figure 12). The effects of lime and N on the microbial community structure in pot treatments were confirmed by ANOVA of the first two principle component factors. For PC1 only N had a significant impact (P=0.006) while both lime and N had significant (P<0.001) effects in PC2.

Table 5: Mean values for microbial properties determined for soil samples taken from lime × N treatments (glasshouse pot experiment).

	Units	Lime			F-Stat	Nitrogen		F-Stat
		L0(0 t/ha)	L1(5 t/ha)	L2(10 t/ha)		N0 (0 kgN/ha)	N1 (200 kgN/ha)	
Total Biomass	rel. C19 ηmoles /g d.w.	28.15	27.22	28.58	0.14	29.82	26.15	2.99
Total soil bacteria	rel. C19 ηmoles /g d.w.	17.54	16.89	17.62	0.16	18.12	16.57	1.81
Total soil fungi	rel. C19 ηmoles /g d.w.	1.71	1.65	1.99	0.65	2.12c	1.44d	6.91*
Fungal: Bacterial ratio		0.10	0.09	0.11	1.23	0.12c	0.09d	9.78**
% Soil bacteria	% of total PLFA	62.36	62.53	61.96	0.31	61.07c	63.5d	15.97***
% Soil fungi	% of total PLFA	6.02	5.85	6.81	1.29	7.00c	5.45d	8.84**
% Branched PLFA	% of total PLFA	34.58	34.52	33.34	0.44	32.89c	35.41d	8.46**
% Cyclic PLFA	% of total PLFA	6.37	6.23	6.43	0.34	5.62c	7.06d	47.40***
PC1 (49.2%)		0.3	0.11	-0.41	1.47	-0.54c	0.54d	9.66**
PC2 (18.1%)		0.84a	0.06b	-0.9c	23.40***	0.51d	-0.51e	23.84***

Means within the row followed by the same letter are not significantly different to each other at P<0.05.

† P<0.1, * P<0.05, **P<0.01, ***P<0.001

Figure 12 shows the difference in PLFA composition of the treatments where PC1 and PC2 accounted for 49.2% and 18.1% of the variation, respectively. Treatments without nitrogen (N0) are bundled towards the left and N treatments (N1) to the right along PC1. Along the axis of PC2 the nitrogen treatment (N1) and added Lime (L1and L2) are bundled downwards, and to the right.
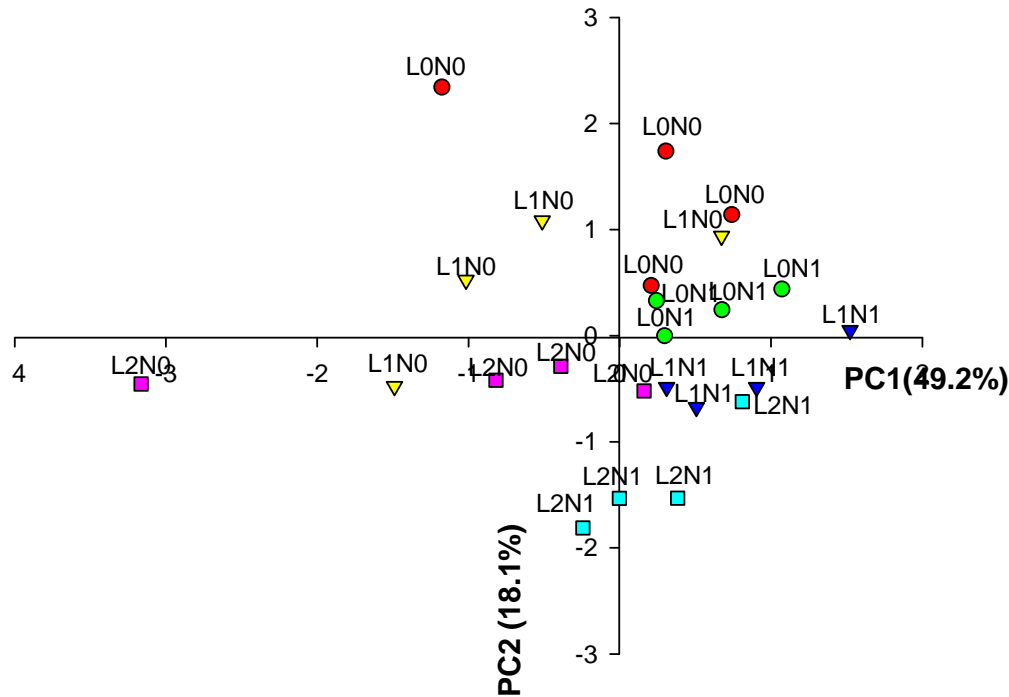
Figure 12: Effect of lime and nitrogen applications on the principal component (PC) scores of phospholipid fatty acids (PLFA) in the glasshouse pot experiment L0 (0t/ha lime); L1 (5t/ha lime); L2 (10t/ha lime); N0 (0kgN/ha); N1 (200kgN/ha).

Principal component loadings indicate that the outliers contributing most to the separation of treatments are the BFAs, iC16:0 and iC15:0 along the PC1 axis and C16:1ω9 along PC2. Correlation analysis revealed that PC1 was significantly negatively correlated with microbial activity (DHH) ($P<0.05$) and soil moisture ($P<0.05$) and positively correlated to aggregate stability ($P<0.01$). Principal component 2 was significantly negatively correlated to pH ($P<0.01$) and microbial activity ($P<0.01$) (Table 6).
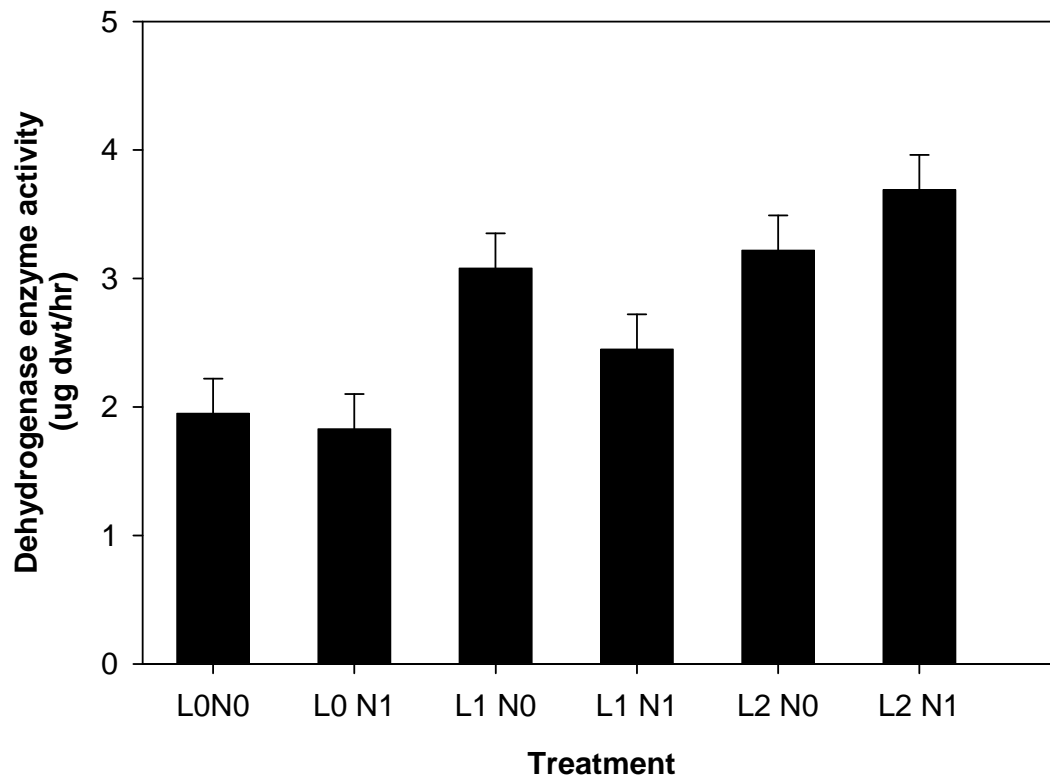
Figure 13: Mean soil microbial activity per treatment in glasshouse pot experiment measured as dehydrogenase enzyme activity. Error bars show the least significant difference between means at 5%.

Figure 13 shows that microbial activity measured by DHH activity was relatively low in the pot trial, however the inclusion of lime accounted for a significant difference between lime-treated and un-limes treatments P<0.001. Microbial activity was significantly positively correlated to soil pH (r = 0.8183).

Table 6: Correlation coefficients of PLFAs with the first two principal components and soil indices determined from the lime × nitrogen glasshouse experiment

	Principal components		Measured soil indices			
	PC1	PC2	pH	DHH	Agg Stab	Grav
Total PLFA (Biomass)	-0.82**	-0.04	0.07	0.27	0.43*	0.35
Bacterial PLFA	-0.75**	-0.08	0.04	0.26	-0.39	0.34
Fungal PLFA	-0.76**	-0.26	0.30	0.35	-0.55**	0.39
Branched PLFA	0.52**	-0.06	-0.09	0.17	-0.32	0.31
Cyclic PLFA	-0.26	-.52**	0.07	0.20	0.17	-0.03
PC1			-0.35	-0.44*	0.58**	-0.42*
PC2			-0.70**	-0.59**	-0.27	0.10

(PC) principal component, (DHH) – microbial activity as dehydrogenase enzyme activity (Agg Stab) – wet aggregate stability %, (Grav) – gravimetric soil moisture. Pearson correlation (2-tailed) significance * at 0.05 level, ** at 0.01 level.

3.3.2.2 Effects of lime and nitrogen on soil chemical and physical properties and plant variables

Results of soil chemical properties measured at the end of the trial are given in (Table 7). Soil pH ranged from low in untreated (L0) to medium 5 t/ha (L1) and high in 10 t/ha (L3). Soil C:N ratio was reduced by added N (P=0.003) and increased by liming (P=0.021) However soil total N and C% were not significantly affected by the treatments. Wet aggregate stability measured from pot samples was significantly higher in pots treated with N (<0.001). Mean aggregate stability ranged from 13% without N to 41.1% with N added. Lime did not affect percentage aggregate stability. Nitrogen and or lime had significant effects on all the plant parameters measured (Table 7). However N effects were more pronounced and widespread, affecting total plant biomass (root and shoot dry matter). Lime impacted significantly on shoot biomass. A significant lime ×N interaction was only observed for shoot:root ratio.

Table 7: Mean values for soil chemical, physical and microbial properties and plant response determined for soil samples taken from the glasshouse pot experiment.

	Units	L0 (0t/ha)	L1 (5t/ha)	L2 (10t/ha)	F-Statistic	N0 (0kg/ha)	N1 (200kg/ha)	F-Statistic	F-statistic (L×N[j])
Soil C	%	3.73	3.85	3.81	1.14	3.85	3.74	2.49	3.31
Soil N	%	0.20	0.20	0.19	1.97	0.20	0.20	0.51	1.05
Soil C:N		19.01a	19.37ab	19.80b	4.85*	19.75c	19.03d	11.82**	2.72
Root biomass	g	4.66	5.02	4.90	0.26	3.12c	6.60d	71.99***	2.65
Shoot biomass	g	14.83a	16.05b	16.31b	12.74***	8.88	22.58	2884.61***	0.09
Shoot: Root		3.18	3.20	3.33	0.32	2.85c	3.42d	6.96*	4.20*
pH		5.08a	5.41b	5.9	854.34***	5.48	5.45	4.74*	1.61
Microbial activity	µg dwt/g soil/h	1.89a	2.76b	3.45c	37.16***	2.75	2.66	0.37	0.02
Wet aggregate stability	%	29.40	28.80	22.80	0.72	12.90c	41.10d	33.21***	0.64

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

[j] Significance of interactions between lime and nitrogen.

3.4 Discussion

A major finding of this study was that additions of lime and N altered soil microbial community structure in pasture test plots converted from forestry after 2 years. This agrees with the work of several researchers investigating microbial impacts of lime and N in different agro-ecological systems, (Frostegard et al., 1993a) limed forest soil, managed grasslands (Clegg, 2006; Murray et al., 2006) and a coniferous forest soil (Demoling et al., 2008). Although the recently converted pasture site provided a unique soil environment, the observed microbial effects to applied lime and N were not exclusive. We now consider the microbial, chemical and physical response to lime and N in relation to previous observations in different environments.

Lime and N were applied at equivalent rates in both experiments, so it was unexpected that lime appeared to be the major driver of shifts in the microbial community structure in the field while N was the most important driver in pots. In addition, analysis of raw PLFA data indicated that lime × N significantly changed the proportions of two PLFAs in the field trial while six PLFAs were significantly altered in pots. The discrepancies between the pots and field trials may be reflective of the different time scales involved (2 year field trial compared to a 22 week pot experiment), and the handling of soil material before potting (example sieving), or they may also be related to the inherent difficulty of simulating field conditions in pots. This could have implications for interpretation of results and comparison with other related studies.

Branched PLFAs (BFAs) contributed significantly to the separation of lime and N effects (on microbial community composition) in both experiments. Since BFAs are indicative of Gram–positive bacteria we inferred that these bacteria were most affected by the treatments (Clegg, 2006; Moore, 2003). Gram–positive bacteria have been associated with reduced substrate availability (Bossio & Scow, 1998, Ingels et al., 2005). Thus the impact of lime and N on substrate availability (carbon flows) within the soil ecosystem is likely to be of major significance in this study.

In the field, lime applications reduced the relative proportions of BFAs and cyclic PLFAs (CFAs) to total soil microbial biomass (total PLFA). Cyclic PLFAs are indicative of Gram-negative bacteria (Clegg, 2006; Moore, 2003). Ingels et al. (2005) noted that decreases in Gram-negative bacteria with simultaneous increases in actinomycetes and Gram-positive bacteria could be indicative of lower substrate availability. Actinomycetes were not included in this part of the study, but there were observed reductions in the relative proportions of BFAs and CFAs (indicators of Gram-positive and Gram-negative bacteria, respectively). The contribution of lime to substrate availability in this trial was not confirmed. However, added lime produced a concomitant increase in fungal biomass and fungal PLFA %, which suggests that fungi may be replacing BFAs and CFAs. Fungi usually tend to dominate in low pH environments (Murray et al., 2006; Tate, 1987), but apparently also favour higher soil pH (Baath & Anderson, 2003). It is known that fungi are specialized in the decomposition of recalcitrant carbon material. They also form important symbiotic relationships with plant roots that are mutually beneficial (Brady & Weil, 2008). Consequently, the effect of lime to increase fungal growth is desirable in a soil such as the trial site, which is littered with wood debris and where pasture establishment is required.

On the other hand N induced an opposite effect on the soil microbial community in the glasshouse pot experiment, with reductions in fungal biomass, fungal:bacterial ratio and simultaneous increases in percentage contribution of BFAs and CFAs to total microbial biomass. The N effect on the microbial community in the glasshouse pot experiment agrees with Demoling et al. (2008) who found reduced fungal PLFA in N fertilized forest soil. Clegg (2006) referred to the uncertainty of the mechanisms by which N affects the microbial community, but we suspect that the N effects observed in this trial may be related to soil pH. Nitrogen addition is known to lower soil pH (Brady & Weil, 2008), and Clegg (2006) noted the possible contribution of N-induced acidity to spatial differences in microbial community structure. The acidifying effect was probably magnified in the condensed pot environment, where N contributed, albeit minimally, to reducing soil pH. The type of N fertilizer used may also have been a contributing factor since urea (46%N) was used in pots while CAN was applied to the field plots. An inverse relationship between soil pH and BFAs (% of total PLFA) was established from the lime response observed from the field trial.

Evidence of the acidifying effect of N treatments and a microbial community structure with greater (relative proportions) of BFAs in the pot experiment suggest that the soil pH – BFA relationship is consistent across the two experiments. It follows that BFAs, and therefore Gram-positive bacteria, are likely to be more abundant in conditions of low soil pH and could be influenced by both lime and N.

In a study of upland grasslands Murray et al. (2006) found that bacterial biomass (PLFA) was reduced by lime and N while the fungal biomass (PLFA) was not affected. Working with coniferous soils, Demoling et al. (2008) reported reductions in both bacterial biomass and fungal biomass (PLFA C18:2ω9, 12) after N-fertilizer applications. We did not observe any significant changes to the bacterial biomass (PLFA) in any of the trials, however, in the pot experiment N reduced fungal biomass (PLFA 18:2ω9, 12). This effect on fungal biomass was not observed in field samples.

Under field conditions, the relative proportions of BFAs and CFAs were reduced in lime treatments with a simultaneous increase in fungal proportions of total PLFA. Frostegard et al. (1993a) reported similar reductions of BFAs and CFAs in response to added lime, but they did not observe higher fungal PLFA in lime treatments as we have done in this experiment.

The N induced decline in fungal biomass (PLFA) observed in pots was largely responsible for the marked reduction of the fungal:bacterial ratio. According to Bardgett et al. (1996) reductions of the fungal:bacterial ratios in a soil may be indicative of increasing bacterial dominance and declining food-web stability. A trend of reduced fungal:bacterial ratio in response to N fertilizers was also evident in the field, but this difference was not significant. It is possible that microbial interactions with dung and urine deposits of grazing sheep could have contributed to reducing this effect under field conditions or it was temporary and occurred prior to our assessments. Nonetheless, secondary effects of N and lime applications on pasture growth and plant species diversity can significantly impact organic C quantity and quality returned from root exudates (Lee et al., 2006). Ultimately, the subsequent lime

and N induced changes are likely to be reflected in the activity and composition of the soil microbial community.

In both treatments the soil microbial community structure as captured in PC1 was significantly negatively correlated to microbial activity (DHH) and gravimetric soil moisture. Since lime and N were the major drivers of change in the field and pots, respectively, the desirable parameters of microbial activity and soil moisture were enhanced by lime (field trial) and declined in the presence of N (pot trial). In a dry land system such as the Darfield trial site soil moisture could be major driver of soil processes and the impacts of the treatments could likely be moisture related. Murray et al. (2006) showed that microbial effects of applied N were linked to soil moisture loss (in an upland grassland) and suggested that such moisture deficits could have an impact on microbial community structure. In the field trial we unexpectedly observed significantly higher soil moisture in lime treated plots. This could have implications for soil microbial activity (measured as dehydrogenase enzyme activity, see below).

Enzyme activity in soils is commonly used in soil microbial studies to estimate the activity of the soil microbial community. Dehydrogenase activity is indicative of soil microbial respiration. Our results indicate that lime, and particularly its effect of increasing soil pH, increased soil microbial activity in the field and pot experiments. However, at similar pH levels, activity in the field was approximately 7 times greater than in the pots. Soil pH and microbial activity were strongly positively correlated in both experiments indicating the importance of soil pH irrespective of different growing conditions. Greater microbial activity in response to liming is likely linked to greater pasture dry matter (DM) yield recorded from pots treated with lime. Condron et al. (2007) compared pasture DM responses from the different treatments at the Darfield site and reported increased pasture DM in response to lime applications. Similar to our findings for the pot trial, there were no differences between DM for higher and lower rates of lime. It was interesting to note that the yield (DM) response to lime was observed in the field during spring and summer, months commonly associated with increased microbial activity (Bardgett et al., 1999). Edmeades & Perrot (2004) concluded that improvements to pasture (DM yield) after liming was a common response on acid soils such as our trial site. However our data suggests that the impact of increased DM yield may not necessarily be a lime response in itself but

the effect of increased soil pH on the soil microbial community. Greater microbial activity usually coincides with increased nutrient cycling, and a subsequent boost in the nutrient supply to growing plants. Another possibility influencing pasture DM production in the field trial could be soil moisture (as mentioned earlier). Although this argument is supported by the findings of Murray et al. (2006), soil moisture and microbial activity were significantly positively correlated only in the field trial and not in glasshouse experiment. This suggests that factors other than soil moisture may be responsible for increased microbial activity in response to lime applications.

In both trials microbial activity measured by DHH activity was lowered (though not significant) in response to applied N. This agrees with Demoling et al. (2008) who found similar declines in microbial activity (basal respiration) in response to fertilizers in a coniferous forest soil. There was disagreement with Bardgett et al. (1999) and Murray et al. (2006) determined that fertilizers applied to grasslands did not affect soil microbial activity.

Lime and N impacted on some of the soil chemical indices measured in both experiments. Lime and N contributed to reducing the C:N ratio in the pots but no significant differences were observed in the field. Nonetheless, the highest C: N ratio (23), in the field trial was observed in a treatment not receiving N and the lowest (18.1) was observed in the L3N1 which had 200 kg N/ha, this may an obvious indication of reduced C:N. However, further gains were probably limited by the large quantity of wood debris incorporated in the field soil. Soil used in the pots was sieved and would thus have less woody debris, and as noted earlier, the N effects were probably exaggerated in the pots, but were likely temporary in the field.

Nitrogen significantly increased aggregate stability in the pots, and lime had no effect on aggregate stability in pots or in the field. In the field, however, there was a trend of increasing aggregate stability with higher rates of lime. The highest percentages of water-stable aggregates (43.6%-50.6%) were obtained from plots treated with the highest rates of lime and N and were comparable to the aggregate stability measurements for the long-term pasture reference site (49.8%). The results suggest

that both lime and N may be beneficial in promoting water-stable soil aggregates and, therefore, have a positive effect on soil physical properties. However, Grieve et al. (2005) found that applications of lime to an acid grassland soil had no impact on aggregate stability, this may have been because the soil aggregate stability was already high (50%) at the start of their experiment. In a comprehensive review capturing the effects of lime and fertilizers on soil physical properties, Haynes & Naidu (1998) concluded that conflicting results from several lime and fertilizer investigations could be explained by a simplification of the of interactions that are likely to occur on a temporal scale (Figure 14).
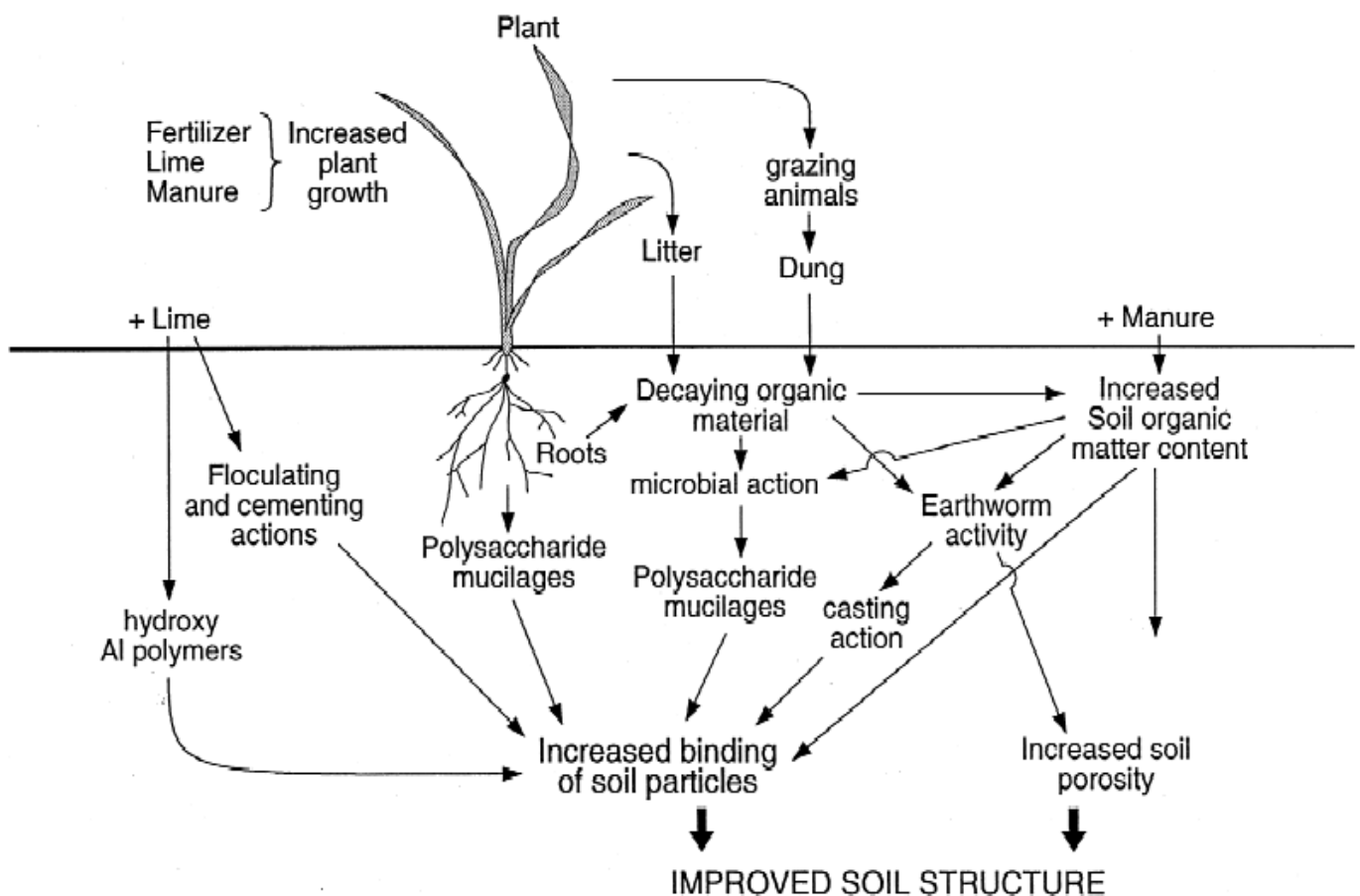
Figure 14: A conceptual model for the effects of fertilizer and lime on soil physical properties reprinted with permission from Haynes & Naidu (1998).

They argued that, in the short-term increased pH due to liming can result in dispersion of soil clay particles and reduce aggregate stability. Cations are attracted to the surfaces of negatively charged soil particles and can form an electrostatic double layer around the soil colloid, which has a slight negative charge. This causes repellence or dispersion of soil particles. Since these reactions occur in a flux state, the exchange of cations between clay particles and the soil solution continues and settles to a point where the electrostatic double layer is compressed and particles begin to flocculate, resulting in formation and stabilization of soil aggregates. There is also the direct positive cementing effect of liming agents like $CaCO_3$ as well as the increased precipitation of Al^- and H^+ ions on the clay particles by OH^- ion from the liming agent. This can lead to precipitation of Al-polymer compounds, which also act as cementing agents for the formation of stable soil aggregates. Over the long-term lime has the capacity to improve plant yield and returns of carbon to the rhizopshere thus increasing microbial activity and breakdown of organic matter while promoting polysaccharide release which also aids the formation of stable aggregates (Figure 14).

Increased plant biomass in response to applied lime and N was demonstrated in the pot trial. Although total soil C was not affected by the treatments, potentially there can be significant returns of soil C in managed pastoral systems (Neher, 2001). Distinct differences in soil C were probably not observed in the pot trial because the harvested aboveground biomass was not returned to the pots, and also because of the relatively short duration of the trial (22 weeks).

3.5 Summary and conclusions

The contribution of lime and N fertilizer to a degraded soil in a dryland pasture system (in conversion from forestry) is near irreplaceable if pasture establishment and biomass (DM) production are major priorities. However, the major aim of this investigation was to assess below ground impacts of these two inputs. Assessments of the soil microbial community, and physical and chemical soil attributes indicate that the inputs of lime and N have critical impacts on below ground dynamics. Lime and N changed the microbial community composition. Lime increased microbial activity

while N had a tendency to reduce microbial activity. Nitrogen and lime contributed directly or indirectly to increasing the percentage of water-stable soil aggregates, and also reduced soil C: N ratio. The pivotal role of soil microbes in ecosystem processes that underscores plant growth and sustainability justifies efforts to determine the possible impacts soil management practices. The scenario of changing land-use (forest to pasture) provided a unique context for investigating above and below ground relationships, but we have shown that most of the relationships observed were also common in other agro-ecosystems as described in other published studies. It can be expected that soil quality in the converted pasture will improve over time as the returns of plants and grazing stock increases both the quantity and quality of soil organic matter and the soil food web shifts to a stable equilibrium.

Chapter 4
Impact of Earthworms on the Soil Microbial Community

4.1 Introduction

The low pH (4.9) of the remnant forest soil at Darfield would be limiting to earthworms, since they thrive at pH range 5.5 -8.5 (Lee, 1985). It was expected that the resulting rise in pH (4.9 to 6.0) from the applied lime would be helpful in encouraging the return of nature's tillers, but 2 years after converting from forest to pasture the Darfield trial site remained devoid of a detectable earthworm population. The tremendous value of earthworms to drive changes in the soil physico-chemical and biological status was reviewed by Brown (1995) and their value to pasture production and quality in New Zealand was demonstrated through the early contributions of Waters (1951)and Stockdill (1982) and more recently by Fraser et al. (2003) and Haynes et al. (2003). Yeates et al. (1997) noted that deleterious effects of prolonged plantation forestry on soils such as reduced pH, nematode diversity and earthworm populations were reversible. However, some specific questions must be answered in the case of Darfield and the Canterbury Plains by extension:

1. How long will this reversal take?

2. What mechanisms are involved? and

3. Can these mechanisms be controlled?

The mulching of wood debris coupled with intense cultivation is unconventional in forest to pasture conversions and presented a unique scenario worthy of investigation. Owing to the short duration allowed for this study (less than 1 year) we used a pot experiment to test the hypothesis that the presence of *lumbricid* earthworms increased soil microbial diversity and improved plant productivity.

4.2 Materials and Methods

4.2.1 Experimental design

For this experiment, eight replicates each of four treatments were used to determine effects of earthworms on plant and soil variables:

Treatment 1 (**Lumbricus**) earthworms from the epigeic group (*Lumbricus rubellus*),

Treatment 2 (**Caliginosa**) earthworms from the endogeic group (*Aporrectodea calignosa*)

Treatment 3 (**Lumbricus + Caliginosa**) earthworms from both epigeic and endogeic groups and,

Treatment 4 (**Control**) no earthworms added

Soil was collected and treated similarly as per the lime x N pot trial described in Chapter 3 with modifications. A layer of fine sand was glued to the inside walls of the pots to prevent preferential burrowing of worms along the walls of the pots and N was applied in the form of urea at a rate of 150 kg N/ha divided into two applications, the first at 10 days and the second application two weeks after planting.

4.2.1.1 Earthworm collection and preparation

Earthworms were collected from the Lincoln University Dairy Farm on 28 and 30 June, 2007. Approximately 300 each of *A. caliginosa* and *L. rubellus* were collected and stored in 20 L plastic buckets (150 worms per bucket). For acclimatization to the trial conditions 5 L of soil from the same batch used in the trial pots were added to the buckets together with a dressing of dried ryegrass (*Lolium perenne*) as a food source. The buckets were stored in a glasshouse at 15°C until ready for further processing.

A 20 L plastic container was modified into an earthworm gut voidance chamber (Figure 15). Ten litres de-ionized water was added to the chamber which held 9 worm cages. The cages were made from 400 ml polyethylene terephthalate (PET) jars. Retangular incisions measuring 5 x 2.5 cm were made on all four sides of the jars and

a stainless steel (1mm) wire mesh was glued over the cut area with araldite glue (Figure 15). A small electric aquarium pump was used to keep the water aerated. Sixteen worms were placed in each cage which was then laid lengthwise at the bottom of the chamber. After 24-hours in water the worm had released most of their intestinal contents and tissue moisture content was standardized (Dalby et al., 1996). The chamber design was based on systems used by Crop and Food Research (P. Fraser, personal communication, 16 March 2007) and recommended by Dalby et al. (1996).
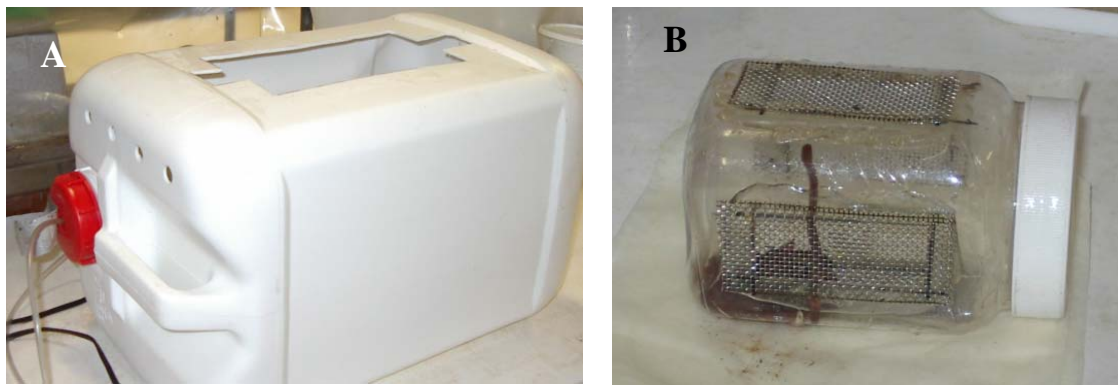
Figure 15: Gadgets used for gut voidance of earthworms, (A) The gut voidance chamber made from a 20 L plastic container. (B). Worm cage.

Three 24-hour cycles were run to complete gut voidance for a total of 27 treatments (3 earthworm treatments with 9 repetitions). After 24 hours the cages were removed, and the worms placed on tissue paper for a few seconds to remove excess water. They were then weighed and quickly transferred to plastic cups containing 10 -15mls distilled water. The cups were covered with perforated plastic lids for transport to the glasshouse and immediate seeding into pots (Figure 16). Worms were seeded on 19, 20 and 21 July, 2007. The seeding rates and average weight of worms per pot in each treatment is given in Table 8 below.
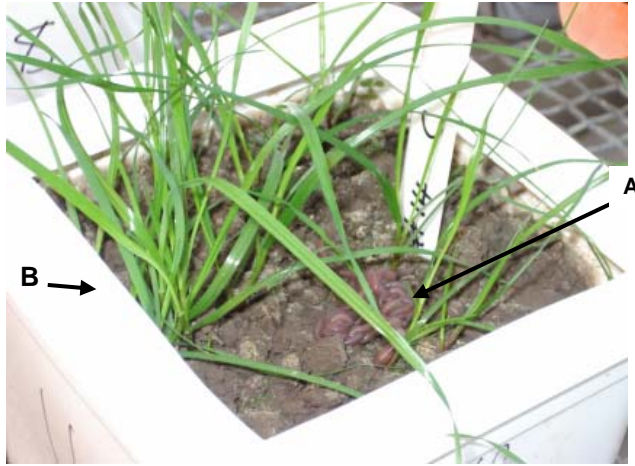
Figure 16: Earthworm inoculation in treatment pots.
(A) Earthworms on soil surface in pots. (B) Cut-out lid on pots with
worm treatments to prevent worm escape.

Table 8: Summarized data of earthworm inoculation and recovery for treatments (R), (C) and (RC) determined 4 and 16 weeks after inoculation.

	(R)		(C)		(RC)	
Sampling dates (weeks)	4 wks	16 wks	4 wks	16 wks	4 wks	16 wks
Inoculation rate (no. per pot) [1]	16.00	16.00	16.00	16.00	16.00	16.00
Average fresh wt (g) at inoculation [2]	0.46	0.46	0.33	0.33	0.43	0.43
Recovered [3] **worms per pot (%)** [4]	14.00	25.10	98.40	82.70	64.00	67.10
Average wt of worms (g) [5]	0.33	0.23	0.27	0.26	0.30	0.34
Percentage fresh wt reduction (%)	28.26	50.00	18.18	21.21	30.23	20.93
Total fresh wt worms per treatment (g)	2.97	3.74	16.86	13.77	11.22	14.73

R = *Rubellus*, **C** = C*aliginosa*, **RC** = *Rubellus + Caliginosa*, **wt** = weight, **wks** = weeks after inoculation

[1] The worm inoculation rate was 16 worms per pot, equivalent to 550 worms per m^2.
[2] Average live fresh weight (g) of worms at inoculation, after gut voidance.
[3] Worms collected from each treatment at the two sampling dates.
[4] Average number of worms recovered per pot expressed as a percentage of the worms inoculated at the start of the experiment. High recovery percentage indicates low mortality and vice versa.
[5] Average live fresh weight of worms in each treatment at the two sampling dates.

4.2.2 Sampling and analyses

Four replicates from each treatment were randomly selected and destructively sampled 4 weeks after worm seeding. The remaining four replicates were sampled at the end of the experiment 16 weeks after seeding. Pots were emptied and earthworms were hand-sorted. Worms from each treatment were stored separately in ventilated containers and immediately transferred to the lab for gut voidance and weighing as described earlier. Plant and soil samples were taken from each pot to determine the effects of earthworm treatments on soil and plant variables as described in the section 3.2 above.

4.2.3 Data analysis

The effects of earthworm treatments on soil microbial, chemical and physical properties were determined using ANOVA with treatments as factors (completely randomized design). The least significant difference test at $P<0.05$ was used to determine differences between treatments where ANOVA indicated a significant overall effect.

4.3 Results

4.3.1 Earthworm growth and survival

All earthworms recovered from trial plots were mature clitellate worms. *Caliginosa* fared best and survived significantly ($P<0.001$) better than the other treatments, with 98% and 83% survival rate at 4 and 16 weeks after inoculation, respectively. The survival rate for *Rubellus* was relatively low as only 14% and 25% of seeded worms were recovered at 4 and 16 weeks, respectively. The combined treatment of *Rubellus* and *Caliginosa* had a survival rate of 64% and 67% due largely to the high survival of *caliginosa* (>90%) at the weeks 4 and 16. Mortality rates were consistent at the two sampling points (Table 1). The average wet weight of worms was reduced at each destructive sampling compared to the inoculation weights. *Rubellus* had the highest

average weight loss from 0.46g to 0.3gand 0.2g representing declines of 28% and 50% in weeks 4 and 16, respectively. Average wet weight reduced in *Caliginosa* from 0.3g to 0.27g and 0.26g 4 and 16 weeks after inoculation representing weight reductions of 18 and 21%, respectively (Table 8).

4.3.2 Earthworm impacts on soil microbial properties

After 16 weeks there were increases in all microbial parameters measured compared to the assessments made 4 weeks after worm seeding (Table 8). Nonetheless, there was no evidence that earthworms contributed to any change in the microbial community structure. The microbial biomass measured as total PLFAs, fungal and bacterial biomass (PLFA) and PLFA marker for actinomycetes were also unaffected by earthworms (Table 9). Though not statistically significant there was a tendency for *Caliginosa* treatment to yield the highest biomass for nearly all microbial assessments measured, and there was a similar tendency for the control to yield the lowest biomass measurements. One exception to this trend was the low fungal: bacterial ratio when trial pots were treated with RC (Table 9). Microbial activity measured by dehydrogenase activity was also highest in *Caliginosa* but not significantly better than the control or the other earthworm treatments (Table 9).

Table 9: Soil microbial properties measured as PLFA and Dehydrogenase enzyme activity as impacted by earthworm treatments 16 weeks after inoculation.

Treatments	C	R	RC	Control	P=0.05
Microbial biomass ηmoles rel C19[1]	25.46	23.33	22.63	20.38	P=0.80
Fungal PLFAs[2]	4.08	3.61	3.36	3.15	P=0.60
Bacterial PLFAs[3]	15.07	14.04	13.91	12.31	P=0.87
Fungal:Bactrial raito[4]	0.11	0.11	0.09	0.10	P=0.10
Actinomycetes[5]	1.89	1.82	1.73	1.61	P=0.90
Microbial activity (DHH)	5.30	5.10	4.55	5.08	P=0.20

C = C*aliginosa*, R = *Rubellus*, RC = *Rubellus + Caliginosa*, NE = Control (No earthworms), P=0.05 = 95% significance level.

4.3.3 Plant and soil analyses

There were significant treatment differences in plant biomass and total percent carbon in plant shoots. Dry matter production measured as accumulated dry matter over 16 weeks was significantly higher (P<0.05) in all earthworm treatments compared to the control (Figure 18). *Caliginosa* had the highest overall DM production, followed by RC and R but there was no significant difference between the worm treatments. Total C in shoots measured at 16 weeks was higher in the earthworm treatments (P<0.05) and followed a similar trend to the accumulated dry matter results (Figure 19). These results demonstrate the significant contribution of earthworms to increasing plant biomass and overall plant productivity.

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[1] Estimate of total microbial biomass determined by summation of bacterial and fungal PLFAs

[2] An estimate of fungal biomass, mean value of the fungal PLFA marker (C18:2ω9,12)

[3] An estimate of bacterial biomass determined by the summation of bacterial PLFAs extracted from

[4] An estimate of the mean fungal : bacterial biomass ratio determined from PLFAs. It gives an indication of decomposition pathways most dominant in soil samples, and therefore also a measure of stability

[5] An estimate of actinomycete biomass as represented by the PLFA, 10 Me16:0.
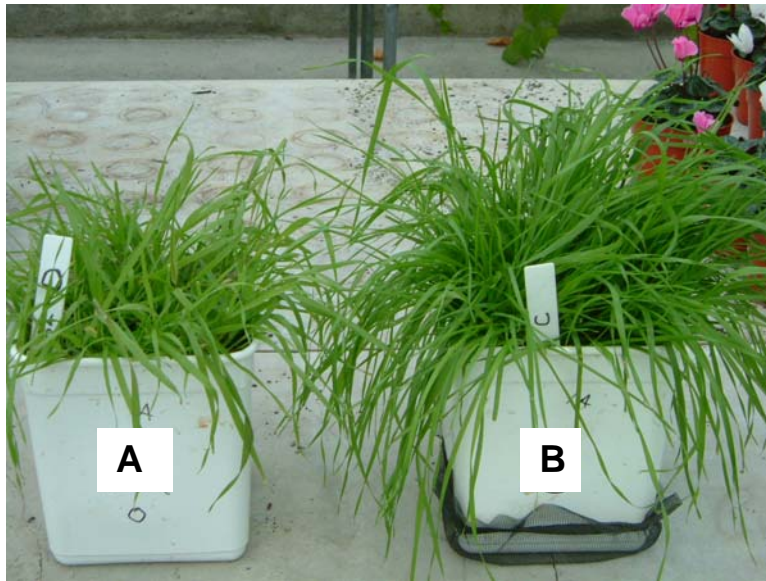
Figure 17: Earthworm effect on plant growth.
(A) Control pot without earthworms. (B) Pot inoculated with
A. caliginosa.
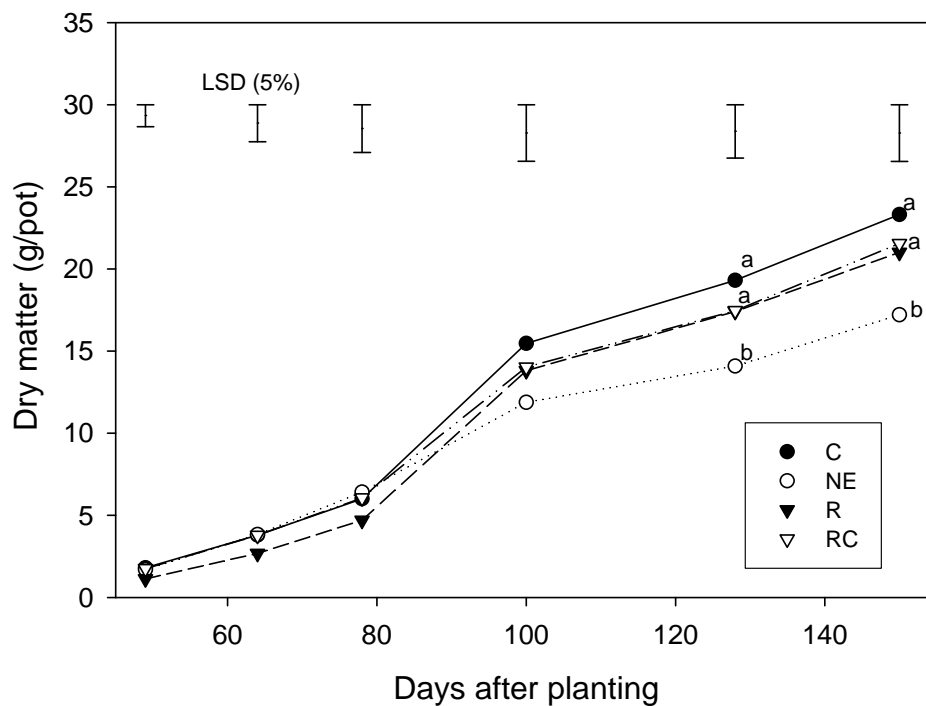
Figure 18: Mean accumulated shoot DM per earthworm treatment
[*Caliginosa* (C) *Rubellus* (R) and *Rubellus + Caliginosa*] compared to the control
(NE). Error bars indicate least significant difference (LSD) between means at
5% from 4 replicates. Means per harvest day with the same letter indicate that
they are not significantly different at (P<0.05).
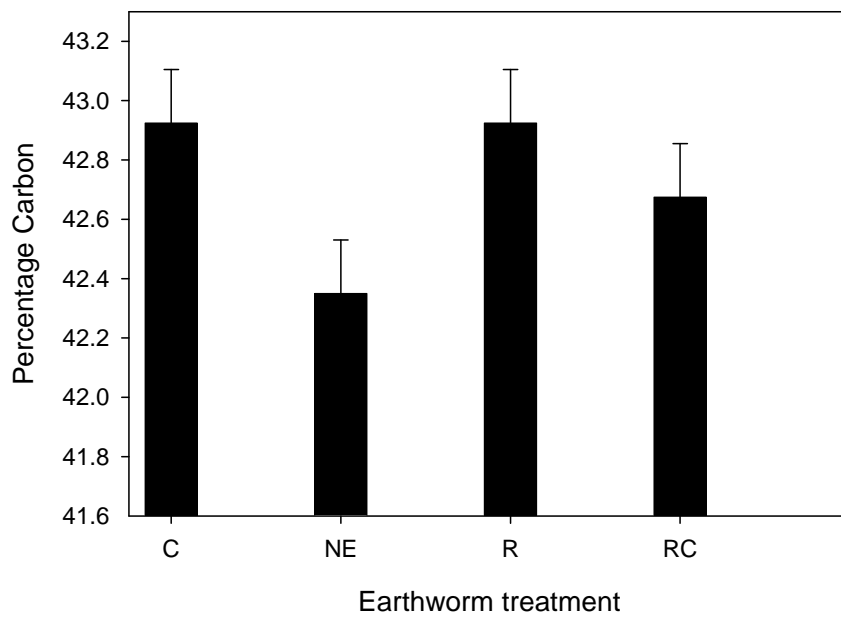
Figure 19: Mean shoot biomass carbon per earthworm treatment. Error bars indicate least significant difference (LSD) between means at 5% from 4 replicates.

4.3.4 Aggregate stability

Earthworm treatments apparently had no significant effect on soil aggregate stability as measured by the percentage water-stable aggregates (%WSA) (Table 10). The highest %WSA (44.7%) was observed in the control (NE) and the lowest 34% in the *Rubellus + Caliginosa* treatment.

Table 10 Mean values of plant and soil parameters determined from earthworm treatments 4 and 16 weeks after inoculation.

	Units	C 4 wks	C 16 wks	NE 4 wks	NE 16 wks	R 4 wks	R 16 wks	RC 4 wks	RC 16 wks	Significance 4 wks	Significance 16 wks	LSD 16 wks
Microbial Activity	μg/dwt/hr	3.96	5.30	3.90	5.08	3.20	5.10	3.79	4.55	NS	NS	0.76
Total C soil	%	3.51	3.48	3.56	3.52	3.70	3.54	3.57	3.83	NS	NS	0.28
Total N soil	%	0.21	0.17	0.21	0.18	0.22	0.18	0.21	0.18	NS	NS	0.01
C:N soil	ratio	16.71	20.24^x	16.95	19.76^x	16.82	19.83^x	17.00	21.33^y	NS	**	0.89
Total C in herbage	%	-	42.93^x	-	42.35^y	-	42.93^x	-	42.68^y	NS	*	0.36
Total N in herbage	%	-	3.54	-	3.46	-	3.32	-	3.80	NS	NS	1.51
Soil pH	pH	-	5.94	-	5.95	-	6.00	-	5.98	NS	NS	0.05
Soil moisture[21]	ratio	-	0.28	-	0.27	-	0.28	-	0.29	NS	NS	0.04
% WSA[22]	%	-	41.40	-	44.70	-	34.10	-	37.80	NS	NS	17.42

R = *Rubellus*, **C** = C*aliginosa*, **RC** = *Rubellus* + *Caliginosa*, **wks** = weeks after inoculation, ** significant at (P<0.05), * Significant at (P<0.01), **NS** not significant at (P=0.05), **LSD** = Least significant difference between means at (P=0.05).

[x y] The same letter indicate that the means within a row are not statistically different at 4 and 16 weeks.

[21] Gravimetric soil moisture determined from fresh soil samples
[22] Percentage water-stable aggregates (2-4mm)

4.4 Discussion

4.4.1 Impact of earthworms on soil microbial properties

The soil microbial parameters considered in this trial and measured 16 weeks after inoculation confirmed that the three earthworm treatments tested did not have any significant effects on the biomass, activity and community structure of the soil microbial community. Phospholipid fatty acid analysis did not support the hypothesis that earthworms cause changes to the structure and constituents of the microbial community (Brown, 1995). The results obtained were probably impacted by the high mortality or escape of *Rubellus*, and being absent they could not have an effect.

The findings of this study are in contrast to that of Saetre (1998) who found that *A. caliginosa* inoculated in microcosms along a birch-spruce soil gradient significantly affected microbial community structure (PLFA) and reduced bacterial biomass. A similar decline in bacterial biomass in the presence of lumbricid worms such as *A caliginosa* was reported by Scheu (1987) and Fraser et al. (2003). However Postma-Blaauw et al. (2006) reported that combinations of *Rubellus* and *Caliginosa* significantly increased soil bacterial counts although individually they had no effect. Scheu et al. (2002) observed an opposite effect of interactions between epigeic and endogeic worms where individually they caused significant reductions in microbial biomass but did not have any significant effect when combined. In this study, the two earthworm species investigated (individually or combined) did not have a significant effect on soil bacterial biomass. The bacterial biomass was estimated from PLFAs extracted from soil samples. These findings may have been due to the short duration of the trial (16 weeks) as Sheu et al. (2002) and Sheehan et al. (2008) observed significant earthworm effects on soil microbial properties after 48 and 30 weeks, respectively. However, the notion of a requisite duration of at least 30 weeks is dispelled by Saetre (1998) who observed effects in an experiment lasting only 14 weeks (along a birch + soil gradient). It should be noted that Saetre had to maintain at least 25% birch in his birch-soil mixture in order to maintain worm activity over the duration of his experiment. This highlights the point made by several researchers that the effect of earthworms on soil microbial activity, structure and eventually function is largely dependent on the food source. In the presence of earthworms additions of high quality organic matter enhances microbial activity and nutrient cycling, and contribute to greater

plant productivity. Perhaps it was unrealistic to expect a greater impact of earthworms in a pot trial with limiting food resources. It was explained earlier in section 2.7 that *Caliginosa* and *Rubellus* occupy different strata in the soil profile. In addition, *Caliginosa* is geophagus, preferring to feed on the mineral component of the soil while *Rubellus*, occupying the soil surface, favours high quality organic matter. This may explain the ability of *Caliginosa* to survive and yield better results in this trial, though the effects were not statistically significant.

Another explanation for our results could be the absence of stratification in our sampling process. In reviewing the work of Fraser et al. (2003) and Postma-Blaauw et al. (2006) we observed that they conducted microbial analyses only on the upper 13 cm of mesocosms. The depth of our pots was 16 cm and since we homogenized the soil before taking samples for analysis the probability of diluting concentrations of microbes and other materials accumulated by the worms may have increased, and could be a contributor to some of our results. This would not be an important consideration for determination of earthworm effects on plant parameters.

4.4.2 Earthworm effects on plant productivity

The most striking result from this trial was the impact of earthworms on plant productivity, measured as cumulative plant DM determined from 5 harvests. All earthworm treatments performed significantly better (P=0.015) than the control. *Caliginosa* returned the highest DM yield 16 weeks after inoculation but this performance was not significantly better than *Rubellus* or *Rubellus + Caliginosa*. Increased pasture productivity (DM yield) in the presence earthworms was demonstrated through pot experiments (Yeates & Pattison, 2006) and in the field (Yeates, 1998). Improvements to soil fertility and other desirable soil attributes, such as physical structure, have been linked to earthworms (Jenkins, 1964). Brady and Weil (2008) noted three major pathways may be involved in earthworm contribution to improved soil fertility:

1. Bacterial biomass and overall microbial activity are usually higher in earthworm cast compared to mineral soil. Bacteria increase the rate of nutrient cycling thus making nutrients more available for plant uptake.

2. Earthworm bodies can contain very high concentrations of key nutrients like N, P and S even when feeding on nutrient poor materials. Upon death, these stored nutrients become readily available for uptake by plant roots.

3. By mixing nutrients and organic matter worms can reduce nutrient losses especially via erosion and volatization.

It is likely that a combination of 1 and 2 above contributed to the positive effect on plant dry matter observed in all earthworm treatments in this pot trial.

Since only mature clitellate worms were recovered from pots we assumed that the worms did not reproduce during the trial. This indicates that conditions within the pots may have been less than ideal for both test species. The soil used was very poor (low pH, low available N), with high levels of recalcitrant carbon (as wood fragments) creating high C:N ratio (>19). We recognised that this would not be a reliable food source for the worms and incorporated 1 gram dried ground ryegrass to the top 1 cm of all pots. This was applied for 2 consecutive weeks after inoculation but was promptly stopped after the appearance of fungal hyphal growth on the soil surface of most pots. Consequently, the food resource in pots was limited and of poor quality particularly for *L. rubellus,* which prefers environments with high quality organic material near the surface (Ferris et al., 2001). Dead ryegrass shoots were not used as a food source in this experiment, as all shoots were periodically harvested and not returned. It is therefore not surprising that after 16 weeks we observed the highest mortality rates (75%) and weight reductions (50%) in the *Rubellus* treatment. It may be correct to deduce that nutrients from worms that died early in the experiment played a significant role in later productivity gains in the treatments containing *Rubellus*. Conversely the *Caliginosa* mortality rate was under 20%, indicating that its contribution to soil fertility was not totally reliant on the nutrients released by earthworm death. Brady & Weil's point 1 above may have played a more prominent role in this study, and is supported by the slightly higher bacterial biomass and activity observed for *Caliginosa*.

In a pot experiment McColl et al. (1982) found that the presence of *Caliginosa* not only increased ryegrass dry matter production but also resulted in higher plant uptake of nutrients, thus enhancing the nutritional value for livestock. In our trial total C and N were measured from the accumulated ryegrass herbage, and the total C was found to be

significantly higher in *Caliginosa* and *Rubellus* treatments compared to the control. There was no significant difference in N uptake (measured as total N%), which is consistent with McColl et al. (1982), who compared pots with earthworms to those without. They instead found much higher herbage concentrations of sulphur and potassium in the earthworm treatments. Although our trial lasted 16 weeks compared to McColl's 12 months it is possible that similar assimilations of nutrients occurred.

4.4.3 Earthworm effects on soil physical properties

At the end of the trial, measurements of % wet stable aggregates showed no significant difference between treatments (Table 10). This is in agreement with Fraser et al. (2003), who observed from a pot experiment that earthworms did not affect aggregate stability. Marashi & Scullion (2003), however, found that earthworms significantly increased aggregate stability after five years in a pasture field trial using severely physically degraded soil recovered from mining operations. The extreme difference in trial duration and conditions between or trial and that of Marashi & Scullion (2003) precludes direct comparison of results. We explain our negative results as a consequence of food shortage in pots and a short experimental period.

The soil aggregate stability obtained in all treatments and the control were comparable to the highest levels obtained in the lime × N experiment reported earlier (section 3.3). In that experiment, N applications significantly increased root biomass and were mainly responsible for increases in percentage water-stable aggregates. As similar rates of N were used in the two experiments, we can assume that N rather than earthworms was the major driver of aggregate stability in the earthworm trial. This could explain why the control, without earthworms, had the highest percentage water-stable aggregates.

4.5 Summary and conclusions

Of the two earthworm species tested *A. caliginosa* adapted better to the conditions than *L. rubellus* and had a better survival rate. The impact of earthworm was most pronounced on aboveground plant yield. All earthworm treatments yielded greater shoot biomass (g/pot) than the control. *Caliginosa* treated pots had the highest yield. Presence of the two species tested (individually or combined) did not change the soil microbial community structure (PLFA) and had no effect on soil microbial activity (DHH). However, *Caliginosa* showed a consistent trend of generating the greatest total microbial biomass (PLFA), and biomass of individual groups (fungi, bacteria, and actinomycetes) and also the highest microbial activity.

It was concluded that the positive impacts of earthworms on pasture productivity were likely to occur in the short-term (within months), but the effect on the microbial community structure and activity may require more time. Worm seeding could contribute significantly to pasture production in the forest-pasture conversions. Of the two species tested here, *A. caliginosa* should be the species of choice.

Chapter 5
Soil Nematodes as indicators of Soil Quality

5.1 Introduction

Current and projected declines in the price of primary forest products in New Zealand has lead to conversion from exotic *Pinus radiata* forest to pastoral farming in some areas. Several decades of forest cover coupled with intrusive harvest operations, and mulching of residue wood material in the process of conversion to pasture, have produced a remnant soil that is acidic, contains toxic levels of exchangeable Al and low available N, very high C:N ratio, and is devoid of earthworms and structural integrity (Table 1). In the absence of earthworms we sought to use nematodes as a surrogate for assessing soil biological quality in response to lime and N inputs.

Nematodes are key constituents of soil biota and a prime representative of meso-fauna. Nematode grazing was highlighted by Ingham et al. (1985) and Ferris et al. (2001) as critical in controlling microbial mediated release of plant nutrients. Nematode abundance and species diversity are usually strongly linked to soil quality conditions, thus making them ideal soil quality indicators (Fontaine et al., 2003; Hunt et al., 2004). In this experiment we assessed nematode abundance and community structure from two selected treatments from the field trial established in 2005 to determine possible effects of soil management strategies employed during the forest-pasture conversion (Section 3.2). These were compared with two reference sites: long-term pasture and long-term *Pinus radiata* forest. The aim of this experiment was to test the reliability of nematode faunal analysis as a diagnostic tool for soil quality determination (in a forest-pasture conversion) by comparing and contrasting the inferred quality conditions generated from nematode assessments with other measured indices including chemical, physical and biological parameters reported earlier in Section 3.3.

5.2 Materials and Methods

5.2.1 Soil sampling, nematode extraction and counting

The treatments selected field trial were: (**1**) Control (no lime, no N fertilizers) and (**2**) Lime+N (10t lime/ha + 200 kg N/ha). Each treatment was applied to three replicate plots for a total six experimental plots. These treatments were chosen because they yielded the lowest and highest dry matter (DM) production hereby implying the greatest possibility for observing differences in nematode community structure (Condron et al., 2007). Two reference sites nearby (within 700 m), a long-term pasture and a 60-year forest block 25 years into its second rotation were selected and compared to the selected treatment plots (Figure 6, section 3.2.1.1). The reference sites were sampled and assessed for nematode abundance, community structure and composition. The data generated was then used to conduct a nematode faunal analysis of each treatment and reference site to determine soil quality status under different soil management practice incorporated in the forest-pasture conversion and land use patterns in the general area.

Soil samples were collected on 10 September 2007, using a (25 mm diameter × 100 mm depth corer). Five cores were taken from each replicate plot of the selected treatments. One composite sample of 20 cores (of the same dimensions as for the treatment plots) was collected from each reference site at the time of sampling the treatment plots. On the forest block surface litter was cleared before sampling so that samples contained mostly soil material. Samples were taken using a zigzag pattern to ensure maximum site coverage. The reference sites were in the same area as the test plots described in Section 3.2.1.1, (Figure 6). All samples were kept in sealed plastic bags and stored in a portable cooler, followed by immediate storage at 4°C until extraction of nematodes.

The soil samples were hand crumbled and 100 g of field moist soil was placed onto Whitehead & Hemming (1965) trays for 72 hours. The extract was collected in a 1000 ml beaker and left to settle for 4 hours. The water level was reduced to c. 100 ml, settled for a further 2 hours, reduced to 10ml and transferred to 30 ml scintillation vials. 10 ml hot 4% formalin solution was then added to the scintillation to kill and fix the nematodes. The

extract was cloudy with soil material which got in from the sides of the Whitehead & Hemming (1965) trays which were not properly covered with tissue paper. The samples were cleaned by a modified rapid centrifugation method used by Ferris et al. (2001).

The fixed samples in scintillation vials were left to stand for 2 hours and then reduced via suction to 8 ml and the contents and transferred to 15 ml centrifuge tubes. The scintillation vials were rinsed with distilled water and the rinsate also transferred to the 15 ml tubes. The tubes were then topped with distilled water and centrifuged at $1800 \times g$ for 90 seconds to separate nematode from debris. The volume in the tubes was reduced (by suction) to 5 ml and sucrose solution (specific gravity 1.1 g) was added to fill each tube. Tubes were centrifuged at $1800 \times g$ for 1 min and the supernatant quickly poured into 100 ml beaker containing 50 ml tap water. The pellet at the bottom of the tubes was gently rinsed to remove all of the supernatant. The beaker was left to stand for 2 hrs before aspiration and transfer to a clean 15 ml centrifuge tube. Tubes were spun at $1800 \times g$ for 90 seconds, and all but 1 ml of the supernatant was removed by suction. We used a 1000 µl auto pipette, with a plastic tip cut to widen the opening to 2–3 mm, to agitate the sample and transfer 100 µl (representing 10 %) of the sample to a 50 ×76 mm slide. The slide was then covered with a cover slip on a wax square and heated lightly to seal the contents. Using an Olympus microscope model CX41 we counted total nematodes in each sample and identified 100 individuals per slide to family, using an updated version of the electronic key of Bell (2002) and allocated them to a trophic group based on morphology of the head, stoma and pharynx according to Ferris et al. (2001).

5.2.2 Faunal analysis of the soil food web

In this section of the report we adopted the definitions proposed by Ferris et al. (2001) for description of soil food webs (Table 11).

Table 11: Definition of terms for description of soil food web reprinted with permission from Ferris et al. (2001).

Colonizer–persister (cp) scale: Assignment of taxa of soil and freshwater nematodes to a 1–5 linear scale according to their r and K characteristics.

cp-**1**: Short generation time, small eggs, high fecundity, mainly bacterivores, feed continuously in enriched media, form *dauerlarvae* as microbial blooms subside.

cp-**2**: Longer generation time and lower fecundity than the cp-1 group, very tolerant of adverse conditions and may become cryptobiotic. Feed more deliberately and continue feeding as resources decline. Mainly bacterivores and fungivores.

cp-**3**: Longer generation time, greater sensitivity to adverse conditions. Fungivores, bacterivores and carnivores.

cp-**4**: Longer generation time, lower fecundity, greater sensitivity to disturbance. Besides the other trophic roles, smaller omnivore species.

cp-**5**: Longest generation time, largest body sizes, lowest fecundity, greatest sensitivity to disturbance. Predominantly carnivores and omnivores.

Faunal profile: A graphical representation of the condition of a food web in relation to its structure and enrichment as indicated by weighted nematode faunal analysis.

Functional guild: Nematode taxa with the same feeding habits, and inferred function, in the food web.

Bax, Fux, Cax, Omx (where x = 1–5): Functional guilds of nematodes that are bacterivores, fungivores, carnivores or omnivores where the guilds have the character indicated by x on the cp scale.

Functional stability: is the stability of a biological function to perturbation.

Guild: An assemblage of species with similar biological attributes and response to environmental conditions.

Resilience: The ability of the food web to recover from perturbation.

Resistance: The ability of the food web to withstand the immediate effects of perturbation.

Stability: Lack of change in a food web function following perturbation; it is the integral of both resistance and resilience.

Nematode faunal analysis was conducted using the Microsoft Excel generated faunal assessment software developed by Ferris (2007) (Figure 20). The model is based on the integration of information on the nematode feeding groups (Yeates, 1993) and life history characteristics of nematode families expressed along a colonizer-persister (c-p) continuum (Bongers, 1990) into a matrix classification of nematode guilds (Table 11, Figure 21) (Ferris, 2007). Three basic qualitative food web conditions are used to describe the nematode indicator guilds associated with a particular soil as determined from nematode assessment and categorization (Ettema & Yeates, 2003).

1. **Basal** – a food web that has been diminished due to stress, including limitation of resources, adverse environmental conditions, or recent contamination. The nematode guilds that feature in this category are those that characterize stress conditions and represented in the cp-2 class of the c-p scale (Table 11).

2. **Structured** – food webs in which resources are more abundant or where recovery from stress is occurring. These webs are more structurally diverse and with more species and include guilds that represent cp classes 3–5 (Table 11).

3. **Enriched** – food webs develop when disturbance occurs and resources become more available due to organism mortality, turnover, or favourable shifts in the environment, (the so called 'priming effect') (Fontaine et al., 2003; Hunt et al., 2004). The guilds in this category are characterized by cp1 (Table 11).

From the nematode identification data we determined the average number of nematodes per family for the selected treatments. The nematode family groups for the treatments and reference sites were allocated to their respective faunal guilds. For example, nematodes identified as belonging to *Rhabditidae, Diplogasteridae* or *Panagrolaimidae* were combined to determine the number of individuals in the Enrichment indicator bacterial feeder guild, while *Cephalobidae* alone comprised the Basal bacterial indicator guild (Figure 20).

The total number of individuals per guild was entered in a separate faunal analysis worksheet for each treatment and reference site (Figure 20). The programme automatically

assigns weightings (Figure 21) to the functional guilds and ordinates them along a structure and enrichment trajectory as explained by (Coleman et al., 2004) (Figure 21; Table 11).

The enrichment and structure trajectories have a common start point in cp-2 (indicators of basal conditions) (Figure 21). The enrichment index (EI), is determined by the expected response of opportunistic non-herbivorous guilds (Ba_1 and Fu_2) to food resources and is plotted along the enrichment trajectory. The structure index (SI) determines the structure trajectory and is derived from an aggregate of disruption sensitivity, body size, and longevity of the functional guilds so expressed in the cp classification of taxa (Table 11).

In Figure 21 distances along the enrichment trajectory show the activity and abundance of primary detrital consumers. Along the structure trajectory distances were weighted based on food web complexities as indicated by the functional guilds found during sampling and identification of nematodes to families. Food webs are therefore characterized as structured (indicating stability) if they appear at the distal end along the structure trajectory or considered basal (indicating stressed or degraded environment) when they appear at the proximal end.

Ferris et al. (2001) also highlighted the usefulness of nematode faunal assessments for higher resolution diagnostics. The relative proportions of bacterivores to fungivores were proposed as indicators of the agents of organic matter decomposition in the soil ecosystem. On opposite ends of the decomposition spectrum in soils there can be fungal dominance where organic matter is recalcitrant, lignified material with high cellulose content, and bacterial dominance where more moist and N-enriched material is mostly available. Nematode faunal analysis of C and detrital flows in soils were used provide deeper insights into the nature of microbial dynamics occurring in soil samples.

Figure 20: An example of a faunal analysis data entry worksheet for analysis of nematode data to generate graphical representations of food web conditions. Reprinted with permission from Ferris (2007).
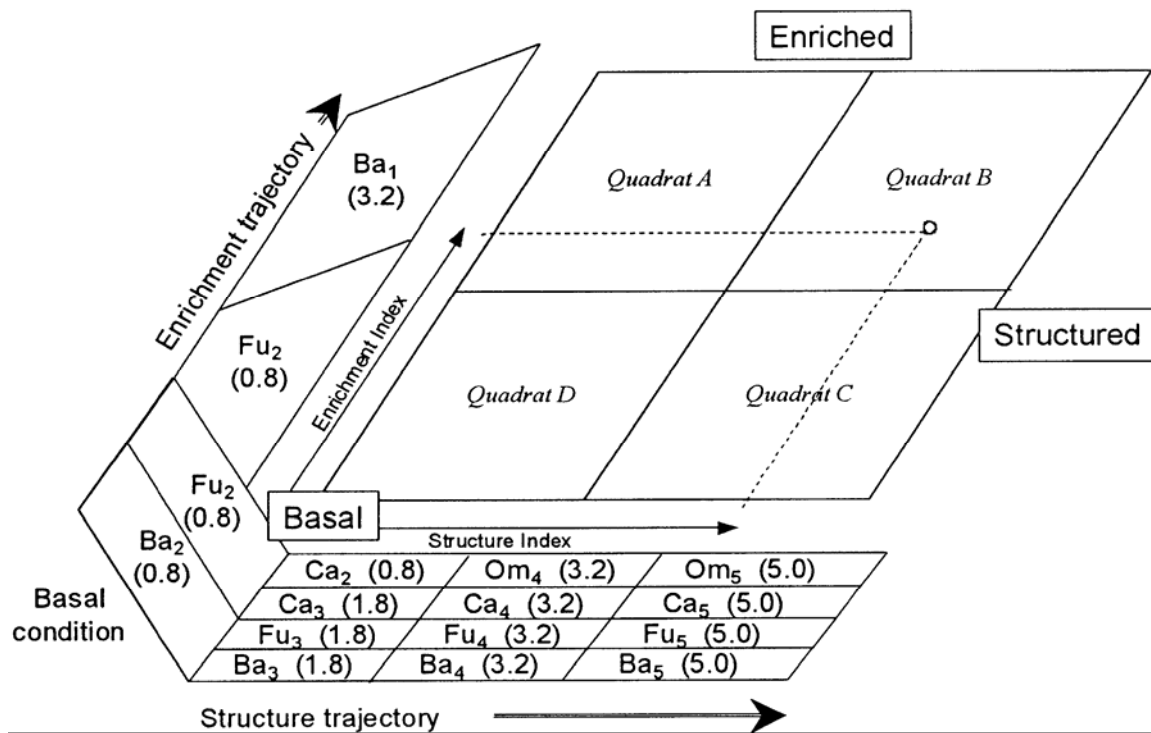
Figure 21[1] Functional guilds of soil nematodes characterized by feeding habit and life history characteristics expressed along a colonizer-persister (cp) scale (after Bongers and Bongers, 1998). Indicator guilds of soil food web condition (basal, structured, enriched) are designated and weightings of the guilds along the structure and enrichment trajectories are provided, for determination of the enrichment index (EI) and structure index (SI) of the food web.

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[1] Reprinted with permission from Ferris et al. (2001).

Table 12: Inferred condition of food web environment based on weighted nematode analysis Quadrant refers to faunal ordination in the faunal profile graphically displayed in (Figure 21). Reprinted with permission from Ferris et al. (2001)

General diagnosis	Quadrant A	Quadrant B	Quadrant C	Quadrant D
Disturbance	High	Low to moderate	Undisturbed	Stressed
Enrichment	N-enriched	N-enriched	Moderate	Depleted
Decomposition channels	Bacterial	Balanced	Fungal	Fungal
C:N ratio	Low	Low	Moderate to high	High
Food web condition	Disturbed	Maturing	Structured	Degraded

5.2.3 Data analysis

The effect of lime and N on nematode abundance and community composition was determined using ANOVA with block and treatment as factors. The least significant difference test at $P<0.05$ was used to determine differences between the lime+N treatment and the control. The test plots could not be statistically compared to the reference sites because only one composite sample was taken from each reference site.

5.3 Results

5.3.1 Effect of lime and nitrogen on nematode abundance and community composition

Nematode abundance was not significantly different between the control and lime+N treatment (Table 13). Abundance at the reference sites was within the range of the test plots (Table 13). A total of 18 nematode families was identified from the samples analysed. They were placed into 6 trophic groups (Table 13). Fungal and bacterial feeders were equally dominant in the two treatment plots. Each feeding group accounted for approximately 40% of all nematodes in the samples. The pasture and forest sites were

dominated by plant parasitic (44%) and plant associated nematodes (38%), respectively (Table 13). Eight nematode families were identified in lime+N treatment compared to 10 in the control, while 12 and 11 were identified in the forest and pasture sites, respectively.

Table 13: Comparison of percentage contribution of nematode feeding types, families and genera, between selected treatments (control and lime+N) and reference sites.

Feeding group/genera	Family	Forest-Pasture conversion		Significance	Reference sites	
		Control	lime+N	P<0.05	*Forest*	*LT Pasture*
Plant associated		**10**	**13**	NS	**38**	**22**
Tylenchid	*Tylenchidae*	10	13		38	22
Plant Parasites		**10**	**4**	NS	**1**	**44**
Paratylenchus	*Paratylenchidae*	9	3		1	6
Pratylenchus	*Pratylenchidae*	-	-		-	18
Helicotylenchus	*Hoplolaimidae*	-	1		-	6
Heterodera	*Heteroderidae*	-	-		-	13
Meloidogyne	*Meloidogynidae*	-	-		-	1
Unidentified	*Tylenchidae*	1	-		-	-
Fungal feeders		**40**	**44**	NS	**30**	**16**
Aphelenchoides	*Aphelenchoididae*	38	44		24	15
Tylencholaimus	*Leptonchidae*	-	-		3	-
Unidentified	*Anguinidae*	1	-		1	-
Ditylenchus	*Anguinidae*	1	-		2	1
Bacterial feeders		**39**	**39**	NS	**12**	**14**
Cephalobids	*Cephalobidae*	29	25		3	9
Plectus	*Plectidae*	5	5		2	3
Rhabditids	*Rhabditidae*	1	4		-	-
Prismatolaimus	*Prismatolaimidae*	-	-		1	-

Genus	Family					
Alaimus	*Alaimidae*	1			4	
Panagrolaimus	*Panagrolaimidae*	1	1			
Unidentified	*Cephalobidae*	2	4		2	2
Predators -Mononchs					**1**	**-**
Mononchidae[i]	*Mononchidae*	0	0		1	0
Omnivores		**1**	**0**	NS	**18**	**4**
Dorylamidae[i]	*Dorylamidae*	1	-		17	4
Steinernema	*Steinernematidae*	-	-		1	-
Total nematodes (Thousands/m²)		**2450**	**2420**	NS	**1840**	**1520**
Total individuals identified		**100**	**100**		**100**	**100**
Total genera (family)	**20(18)**	**13(10)**	**9(8)**		**14(12)**	**12(11)**

5.3.2 Faunal analysis

Analysis of faunal data showed similar food web conditions in the control and lime+N treatment, but distinct differences were apparent between the test plots and reference sites (Figure 22). Both treatments have food web conditions that feature in quadrant D and nearing A (Figure 22[1] & [2], Figure 21). These food webs are characteristic of basal soil systems that are stressed, depleted (low OM and nutrients), with high C:N ratios (Table 7, Section 0). Comparison of Figure 22[1] to Figure 22[2] shows that the food web condition of the control was more structured than lime+N. Ordination of food web constituents in (Figure 22[1]) depicts the control about 10 to 15% along the SI trajectory while lime+N is more basal and remains at zero in the SI trajectory (Figure 22[2]). Along the enrichment trajectory the EI is near 50% in the control and lime+N, nonetheless lime+N is slightly more enriched (Figure 22[1] & Figure 22[2]*)*. By comparison, the food web condition of forest site appeared in quadrant B and bordering quadrant C, such faunal profiles are indicative of highly structured systems that are fungal dominated, mature and undisturbed (Table 12).
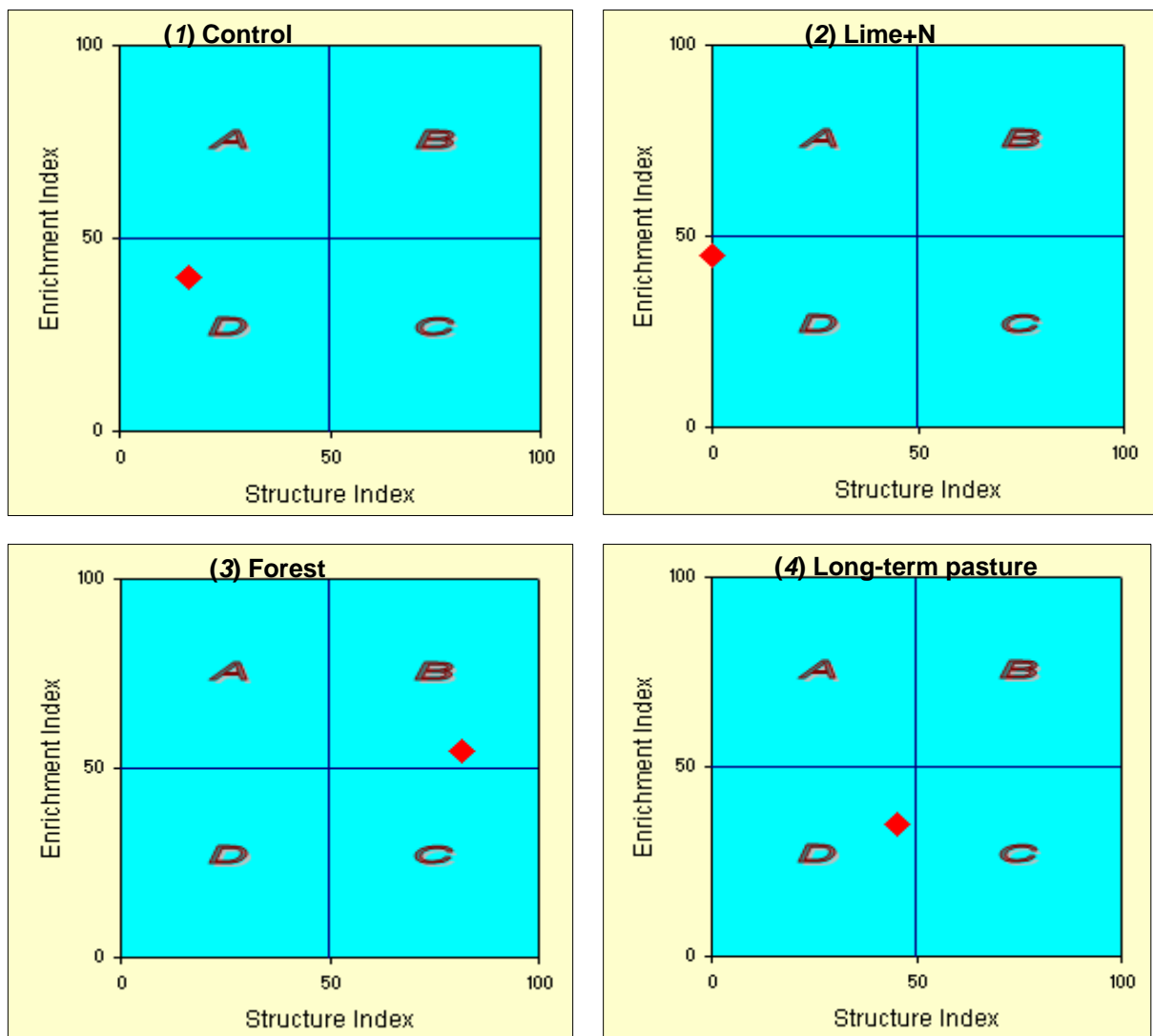
Figure 22: Nematode faunal profiles determined at sampling representing the structure and enrichment conditions of the soil food web for (*1*) Control determined by analysis of the average of nematode composition and diversity data collected from 3 replicate test plots, (2) treatment lime+N determined similarly to (1) and the reference sites (3) Forest and (4) long-term pasture. The inferred condition of the soil web as depicted by the quadrants A, B, C and D is given in Table 12.

5.3.3 Carbon and detrital channel flows

The control and lime+N appear to have similar C and detrital flows (Figure 23 & Figure 24). Detritus forms the major carbon flow in control and lime+N treatments (Figure 23). In the long-term pasture plant material (roots and shoots) was the major carbon resource (Figure 23). Detrital material also appeared to be the major resource in the reference forest system.

The decomposition pathways in the pastoral systems assessed from either recently converted to pasture or long-term pasture (reference site) appears to be dominated by bacteria, while the forest reference system is fungal dominated. This indicates that significant shifts in microbial dynamics may have occurred as a result of forest to pasture conversion.
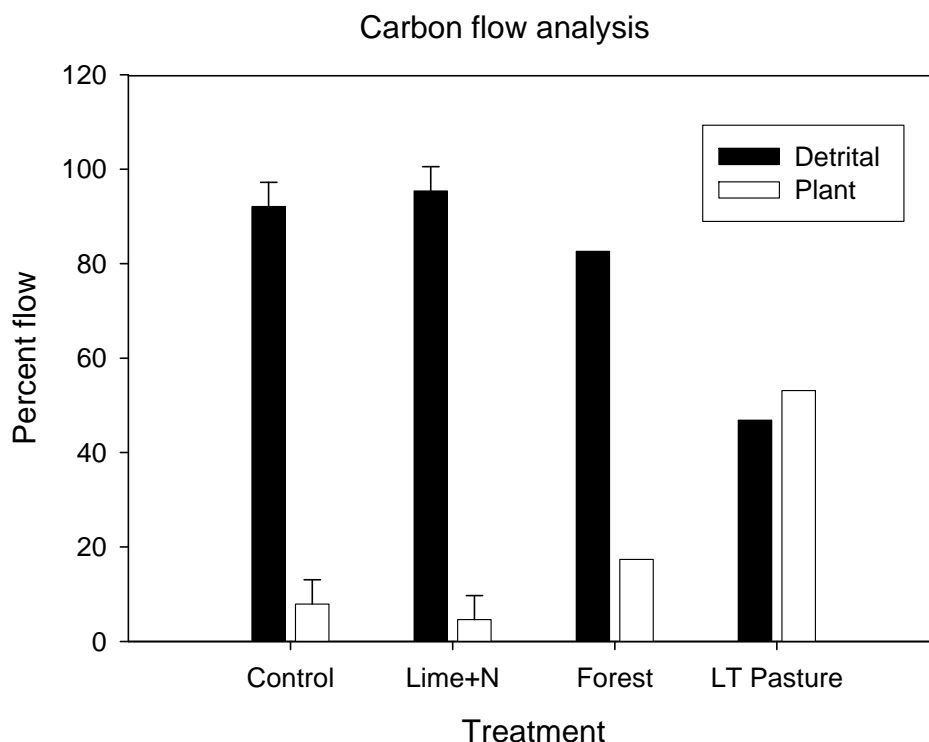
Figure 23: Mean percentage carbon flows for the control and lime+N treated plots and reference sites (forest and (LT) Long-term pasture). Carbon flow analysis considers the relative contributions of detrital and plant material to food web resources.

Detrital channel flow
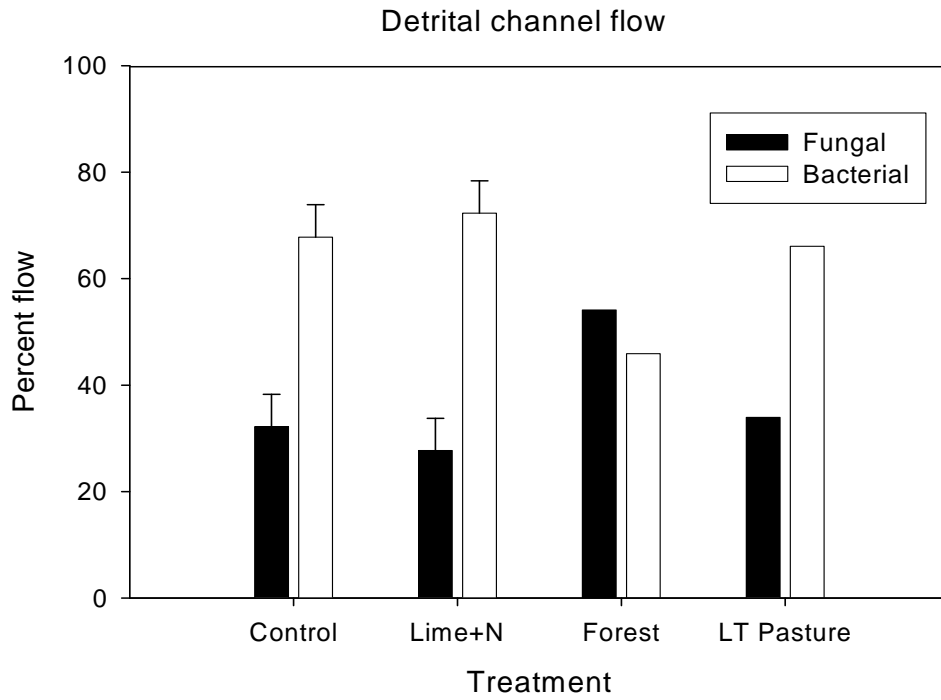
Figure 24: Mean percentage detrital flows for the control and lime+N treatment plots compared to the reference sites (forest and (LT) Long-term pasture). Detrital channel flows gives an indication of relative importance of bacteria and fungi to the decomposition of detritus material

5.4 Discussion

Assessments of nematode populations in the two (lime × N) treatments suggest that abundance and community structure of nematodes were not significantly affected by the inputs at the time of sampling. Nematode faunal analysis shows that the treatments did not have a significant effect on the soil quality as indicated by conditions of the soil food webs in the test plots. Two years into the conversion process the food web conditions in treated plots, L4/N4 receiving 10 tons lime and 200 Kg N/ha, is not different from the control (L1/N1) with no added lime or N. This may not be an unreasonable scenario as restoration of soil nematode communities have been shown to be delayed and inconsistent with plant species (aboveground) restoration (Kardol et al., 2005) The patchy dynamics of the soil nematode community (Nannipieri et al., 2002) coupled with high responsiveness to changing conditions such as temperature, food resources (enrichment) and soil pH and nutrients (Bardgett et al., 1998; Batten et al., 2008; Wardle et al., 1999) are key factors that determines real change in soil nematode populations.

Nematode abundance did not change significantly across the different land use types assessed. Nonetheless, our data suggest that nematode community composition (in similar soil type and climatic conditions) can be altered by land use changes in a relatively short time period (under 5 years). This is evident from the observed difference in nematode community composition when we compared the test plots to the forest reference site. These findings agree with a comparison of pasture and forest systems made by Ferris et al. (2001). Environmental conditions at the forest reference site would probably be similar to the trial site before conversion, but two years into the conversion a very different community composition now obtains. Since there is no significant difference across the lime+N and the untreated control plots we can assume that the primary reason for the difference in community structure between the trial plots and the forest site is land use. Hence at the time of sampling land use was more important than soil additions of lime and N in relation to the state of the soil food web. We can expect that with time the converted site will move towards the conditions observed in the long-term pasture site. However, the high levels of plant parasitic nematodes (44%) observed in the long-term pasture site could be limiting to pasture production (Mercer, 1994) and are not desirable.

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Results of C and detrital flow analysis show that decomposition in the treatment plots was dominated by bacteria but also with significant contribution from fungi. This scenario was expected since the soil had a high content of woody debris mulched in. The consequent high levels of recalcitrant carbon are preferably broken down by fungi however plant material (roots and shoots), root exudates and excrete from grazing sheep is increasingly becoming a food source encouraging a growing bacterial population. The long-term pasture site is dominated by bacteria with a much smaller contribution from fungi relative to the test plots, and plant material is also the major carbon source, which is typical of pasture systems (Tate, 1987) and it can be expected that the converted site will move towards a similar decomposition pathway dynamic over time.

We expected comparable faunal profiles for the control and treated plots, because their nematode abundance and composition were similar. Furthermore, the food web condition in all test plots irrespective of treatment were characterized as basal, being highly stressed, degraded, nutrient poor, with high C:N ratio. The inferred condition of the soil food web based on nematode faunal analysis agrees with our earlier reported findings on measurements of selected soil quality indices.

5.5 Conclusion

Unlike the PLFA analysis, the nematode faunal analysis was unable to detect significant impact of added lime and N. However, the inferred soil conditions ascribed to the treatment plots and reference sites were in agreement with actual measurements made. This indicates the usefulness of nematode community assessments as a reliable indicator of biological soil quality. These findings underscore the value of combining nematode faunal assessments with soil microbial community assessments, like PLFA, to develop a more comprehensive picture of biological soil quality. In a dry land system such as this, devoid of earthworms, nematode analysis offers another option for assessing soil quality and monitoring the impacts of management practices over time.

Chapter 6
Summary, Conclusions and Recommendations for Future Research

The quality of soil is inherently linked to its composition. The relative proportions and structure the soil biota, organic matter and minerals (living and non-living components) determine key capabilities and functions. Changes in soil quality can be reliably assessed by careful evaluation of various soil attributes. Shifts in indicator characteristics over time and space can provide guidance for sustainable management of soil resources, particularly in relation to agricultural production. Aboveground indicators such as plant growth used in tandem with soil nutrient analyses has historically been the dominant approach for soil quality evaluations. Advances in modern science and the development of molecular biological markers have created a paradigm shift in the approach to soil biological quality management (Ferris et al., 2001). There is now greater emphasis on soil biota, but this has in no way simplified the task of soil quality evaluation and management. Coleman et al., (2004) also noted that "the major lesson to be learned for soil ecologists is one of paying attention to details yet considering them in a holistic perspective." In so doing the inherent complexity of the soil system necessitates some simplification to understand functions and determine how these can change in response to natural and human induced impacts. In this study, representatives from three soil biota groups (micro, meso and macro-organisms) were selected and assessed. The context of changing land use from exotic forest plantations (*Pinus radiata*) to grazed pasture, in a dryland temperate environment provided a unique template for investigating the effects of two commonly used inputs (lime and N) on biological soil quality.

6.1 Summary

This study aimed to identify changes in soil microbial and nematode communities in response to lime and N applications and the presence of earthworms. Emphasis was placed on soil biological properties and the indicators used in this study are interconnected by virtue of the intricate relationships of the soil ecosystem as shown in Figure 25. We used PLFA analyses to determine soil microbial community structure and to estimate the biomass of microbial groups (bacteria, fungi). Microbial activity was determined by dehydrogenase enzyme activity. Plant interactions with the microbial community was

assessed through nematode faunal analyses including nematode abundance and identification, food web characterization, and detrital and carbon flow analysis. Soil microbes (their activity and community structure) are linked to plants by the ecosystem services they mediate, such as decomposition and mineralization. It is in most cases a two-way relationship as the plants provide much needed resources through root exudates and other plant material that stimulates microbial activity and function. These relationships are at the core of this study and were used to monitor the impacts land use change and management.
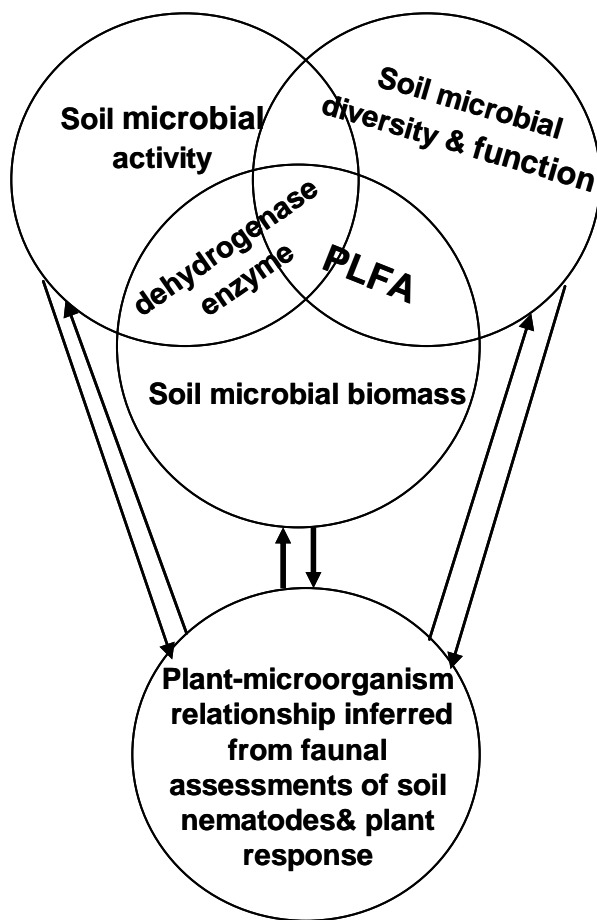
Figure 25: A schematic depiction of the relationship between the different soil microbiological parameters measured in our study. PLFA, phospholipid fatty acid. Adapted from Benedetti & Dilly (2006).

The basic hypotheses behind the objectives of this thesis are that:

(1) Conversion from plantation forest to pasture and applications of lime and N are associated with changes in microbial and nematode community structure and therefore ecosystem functions;

(2) The presence of earthworms (in pastoral lands converted from forestry) can contribute to increased soil microbial biomass, activity and function (plant productivity).

The impacts of land-use changes and inputs (lime and N) on soil biological, chemical and physical properties were assessed using two experiments (field and glasshouse) which were compared to two selected reference sites (long-term pasture and forest).

To accomplish the objectives of this research, the first step, as presented in Chapter 2, was to review the literature to find a theoretical framework for the study. This review included a description of the context for the study and considered the major factors driving land-use changes on the Canterbury Plains. The unique method of land clearing and soil preparation to retain optimal organic mater levels was highlighted. The soil quality constraints of the remnant forest soil was demonstrated by comparing soil chemical analysis for the trial site with the fertility requirements for pasture establishment and growth. A definition of soil quality was then given and the intention to focus on biological soil quality was made clear. The soil food web model for characterizing soil biota was explored. This was followed by a review of selected representatives from different trophic (feeding) groups of the soil food web: (1) micro-flora (bacteria and fungi), (2) meso-fauna (nematodes) and (3) macro-fauna (earthworms). Micro-organisms were particularly emphasized because of their crucial role in most soil ecosystem processes.

Chapter 3 reported the methodology, results, and discussion of investigations into the impacts of lime and N on soil microbial, chemical and physical properties. This study involved examining an existing field trial (two years after establishment) and conducting a 22-week glasshouse pot experiment, each with different combinations of lime and N. The effects of lime contributed most to changing the microbial community structure in the field. As determined from PCA analysis of transformed PLFA data ($P<0.001$). On the other

hand, N was the main driver changing the microbial community structure in the glasshouse pot experiment, also determined from PCA analysis of transformed PLFA data ($P<0.001$). Applied lime in the field was associated with greater microbial activity (DHH) and increased moisture retention (gravimetric). Applied lime also increased microbial activity (DHH) in the pots, however, microbial activity in the field increased 7-fold compared to the glasshouse experiment at the same rate of lime.

Apart from the expected increase in soil pH from lime application there was no observed difference between treated and control plots for the other measured indices (total C and N). The percentage water-stable aggregates were also similar across the treated (L × N) and control plots. However, higher rates of lime were associated with greater soil aggregate stability. Soil from test plots treated with 5 and 10 t/ha had 45-50% water-stable aggregates compared to 34% in treatments without lime. The impacts of land-use change on soil microbial dynamics was most evident from comparison of converted test plots with the two selected reference sites. After two years the microbial community structure in converted pasture test plots was distinctly different from the long-term forest and long-term pasture reference sites.

The findings from investigating the impact of earthworms on soil microbial, chemical and physical properties, and plant productivity were reported in Chapter 4. The tremendous contribution of earthworms to improving soil conditions and pasture production is well known (Edwards, 2004; Lee, 1985). It was expected that earthworm populations would be at least noticeable at the converted site within a few years after liming and pasture establishment. Annual checks did not show this to be the case and earthworm seeding was seriously considered and eventually trialled over a small area. The environment in the converted land, low soil moisture and high levels of recalcitrant forest debris presented an interesting model for investigating earthworm impacts. Due to time constraints a controlled glasshouse pot experiment was used. Of the two species tested *A. caliginosa* had the best survival rate (83%) and seemed better suited to the soil conditions than *L. rubellus* (25%). The most striking result from this trial was the impact of earthworm on plant DM yield. After 16 weeks all earthworm treatments produced higher ($P<0.05$) total plant DM than the control. Microbial community structure and activity were not significantly affected by the treatments but *Calignosa* had greater values for all measured microbial indices. This

supported the initial assumption that earthworms could contribute to improved soil quality in the conversion from forest to pasture.

Chapter 5 presented the nematode assessments conducted on replicate field plots treated with lime and N and the control (no lime or N). These were then compared to the two reference sites, forest and pasture. Mean nematode abundance was similar in all converted test plots (control and treated), and the two reference sites, and ranged from 1520 to 2450 thousand/m^2, in the long-term pasture and control test plots, respectively. The converted test plots had similar nematode composition with bacterial and fungal feeding nematodes each comprising 40% of all nematodes identified. Clear differences were observed between the converted plots and the two reference sites. The forest was dominated by plant associated species (38%) and long-term pasture had 44% plant parasitic nematodes. Consequently, the soil food web condition as inferred from nematode faunal assessments were similar for the control and treated plots but differed from the reference sites. The test plots were characterized as basal, stressed and depleted, with high C:N ratios, while the forest soil categorized as highly structured and fungal dominated. Soil nematode faunal assessments were shown to be a robust and reliable indicator of biological quality.

6.2 Conclusions

Herewith the major conclusions derived from this study:

- Microbial community structure was affected by both lime and N. Findings from the glasshouse pot experiment essentially supported our field observations. However, the effects of N were more dominant in the pot trial, while the effect of lime was greater in the field. Lime and N apparently had opposite effects on some biological measurements (PLFA) which were attributed to their impact on soil pH.

- Generally, lime (higher soil pH) was associated with greater microbial activity (DHH) and fungal biomass (PLFA), and lower branched fatty acids (indicator of Gram-positive bacteria). Nitrogen application reduced fungal biomass, and increased branched fatty acids (PLFA) (Gram-positive bacteria). Overall, conversion from forest to pasture resulted in more significant change to the soil microbial community structure compared to the effects of lime and N applications.

- Microbial activity (DHH) at the forest (reference) site was comparable to the activity observed in the control test plots, 5.02 and 6.19 μg TPF/g dwt soil/hr, respectively. Microbial activity in the lime treated plots ranged from 14.7 – 23 μg TPF/g dwt soil/hr and slightly higher than the measured activity in the long-term pasture (13.97 μg TPF/g dwt soil/hr). Soil pH correlated strongly ($r = 8.183$) with microbial activity throughout the experiment, and the association of lime with increased soil moisture retention are indications of the positive impacts of lime to the soil ecosystem.

- Earthworm presence is likely to increase pasture yields in the short-term (months). *Apporectodea caliginosa* adapted better to the soil conditions better than *L. rubellus* and had a better survival rate. Earthworm presence did not affect the microbial properties measured in this trial.

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- Nematode faunal assessment was shown to be a reliable indicator of biological soil quality in a forest to pasture conversion that was left devoid of earthworms after several decades of plantation forest rotations. The identification of nematode faunal groups for characterization of soil food web conditions produced results that were comparable with the microbial, chemical and physical assessments conducted in this trial.

During the course of this study some constraining factors became apparent. These are now highlighted and to some extent clarified for the benefit of persons who may wish to embark on a similar exercise.

1. The reliability of enzyme assays as a measure of microbial activity has been questioned (Nannipieri et al., 2002), nonetheless the appearance of consistency and repeatability (across field and glasshouse conditions) in this study could be indicative of its value.

2. Unfortunately the reference sites in this study could not be compared to the test plots using rigid statistical methods, as only one composite sample (each) from the forest and long-term pasture sites were processed and analysed. Even so, the results of nematode faunal assessments contained therein are in agreement with Ferris (2007) (Figure 26).
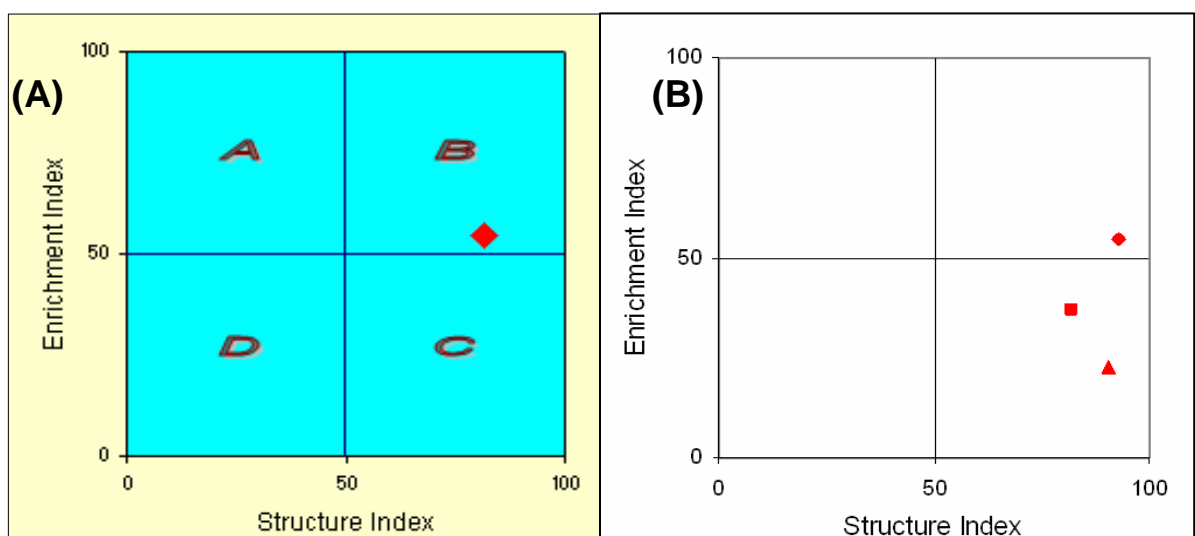
Figure 26: Similarity of nematode faunal assessment determined for (A) the forest reference site compared to (B) An assessment of New Zealand forest reported by Ferris (2007).

3. Limited knowledge and expertise in nematode identification meant that only a minimum number of samples could be processed. It would be interesting to know whether the other rates of lime and N used in the field trial made a difference to the wider nematode community structure.

6.3 Recommendations

Based on the research described in this thesis, there are several areas that warrant further investigation:

- More work is needed to analyse the effects of applied inputs (lime and N) on the soil ecosystem, in the context of this study. Focus was on the identifying change to microbial community but not necessarily on which organisms were changing. Molecular assessments of soil biota using PCR could be beneficial in answering questions such as, which microbes are impacted most by management and how? This could assist in the identification of innovative management options for improving soil biological quality. An option could be the use of spent mushroom compost, biochar or rotations with leguminous plants that could fix N and add high quality organic matter to the soil.

- Apart from the dry matter yield data obtained from the glasshouse experiments, very little emphasis was placed on functionality of the soil ecosystem. Assessments of ecological functions such as decomposition of cellulose paper (Orwin et al., 2006) could be helpful in assessing the impacts of soil amendments and earthworms on soil processes.

- There could be tremendous value in increasing the frequency of monitoring (from one to two or three times per year). It may also be worthwhile to do continued monitoring over a long-term period (in excess of 10 years). While only few studies have focused on conversions from pasture to forest there seems much less work on conversions from plantation forest to pasture, and the effects of lime and N fertilizer.

- From preliminary assessments lime and N applications appear to be impacting on plant species diversity in treated plots (data not shown). In addition, several studies have confirmed the presence of strong relationships between aboveground plant diversity and soil microbial dynamics (Bardgett et al., 1998; Wardle et al., 1999). This highlights the need to explore the significance of such relationships (above and belowground) in the context of forest to pasture conversions and applications of lime and N.

- The introduction of irrigation to parts of the converted lands on the Canterbury Plains may offer the opportunity to examine how conversion process is impacted by irrigation. Increased soil moisture is likely to facilitate and even increase the rate of most microbial mediated processes and possibly hasten the soil food web to a more stable state. Such conditions may also be favourable enough to increase earthworm populations, but confirmation that these will occur and at what rates can be deduced from proper investigations.

Current trends indicate that changing land use will continue in as many forms, directions and permutations that prevailing economic circumstances allow. Apart from advising of impending consequences for soil quality where poor land use choices are imminent, soil ecologists or biologists may need to have a more intimate understanding of soil ecology to either restore degraded systems, improve existing systems to function optimally or maintain those performing suitably. This study is a small step in the quest for that intimate understanding and appreciation of the soil ecological system.

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6.4 Appendices

Phospholipid fatty acid nomenclature

Table 14: Common fatty acid signatures, adapted from Moore (2003).

Microbial Group	Fatty Acid
Gram positive bacteria	15:0i, 17:0i, 15:0a, etc.
Gram negative bacteria	cy17:0, cy19:0,
Actinomycetes	10 Me18:0, 10 Me17:0, 10 Me16:0
Fungi	18:2ω6,9, 18:2 ω9,12, 18:1ω9c
Protozoan	20:4 ω6
Arbuscular mycorrhizal fungi	16:1 ω5
Methanotrophs	18:1ω8c

As shown in

Table 14 fatty acids are designated in terms of the total number of carbon atoms, number of double bonds, followed by the position of the double bond from the methyl end of the molecule (Figure 27). The letters '*c*' and 't' denote *cis* and *trans* configurations, respectively. The prefixes '*a*' and '*I*' indicate antieso and iso branching, 'Br' indicates unknown methyl branching position, 10Me indicates a methyl group on the 10th carbon atom from the carboxyl end of the molecule, and '*cy*' refers to cyclopropane fatty acids.
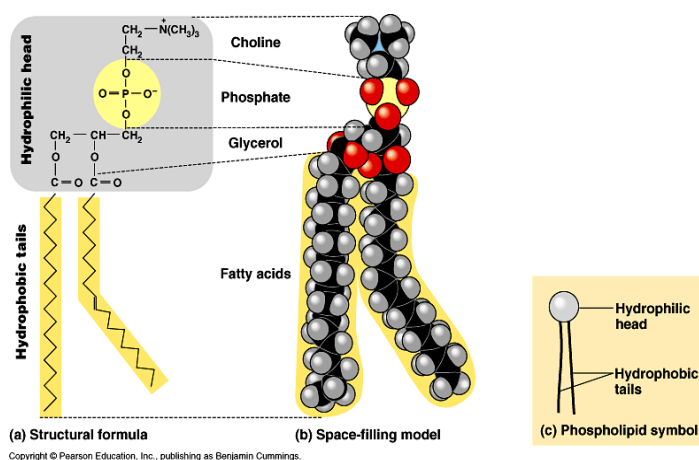

Figure 27: Graphic depiction of PLFA structure (Cummings, 2006)