Effects of long- and short-term crop management on soil biological properties and nitrogen dynamics

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A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Doctor of Philosophy

at

Lincoln University

by

Christine Stark

Lincoln University

April 2005

And I tell you, if you have the desire for knowledge and the power to give it physical expression, go out and explore. If you are a brave man you will do nothing: if you are fearful you may do much, for none but cowards have need to prove their bravery. Some will tell you that you are mad, and nearly all will say, “What is the use?” For we are a nation of shopkeepers, and no shopkeeper will look at research which does not promise him a financial return within a year. And so you will sledge nearly alone, but those with whom you sledge will not be shopkeepers: that is worth a good deal. If you march your Winter Journeys you will have your reward, so long as all you want is a penguin’s egg.

Apsley Cherry-Garrard in “The Worst Journey in the World”

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To date, there has been little research into the role of microbial community structure in the functioning of the soil ecosystem and on the links between microbial biomass size, microbial activity and key soil processes that drive nutrient availability. The maintenance of structural and functional diversity of the soil microbial community is essential to ensure the sustainability of agricultural production systems. Soils of the same type with similar fertility that had been under long-term organic and conventional crop management in Canterbury, New Zealand, were selected to investigate relationships between microbial community composition, function and potential environmental impacts. The effects of different fertilisation strategies on soil biology and nitrogen (N) dynamics were investigated under field (farm site comparison), semi-controlled (lysimeter study) and controlled (incubation experiments) conditions by determining soil microbial biomass carbon (C) and N, enzyme activities (dehydrogenase, arginine deaminase, fluorescein diacetate hydrolysis), microbial community structure (denaturing gradient gel electrophoresis following PCR amplification of 16S and 18S rDNA fragments using selected primer sets) and N dynamics (mineralisation and leaching).

The farm site comparison revealed distinct differences between the soils in microbial community structure, microbial biomass C (conventional>organic) and arginine deaminase activity (organic>conventional). In the lysimeter study, the soils were subjected to the same crop rotation (barley (*Hordeum vulgare* L.), maize (*Zea mais* L.), rape (*Brassica napus* L. ssp. *oleifera* (Moench)) plus a lupin green manure (*Lupinus angustifolius* L.) and two fertiliser regimes (following common organic and conventional practice). Soil biological properties, microbial community structure and mineral N leaching losses were determined over 2½ years. Differences in mineral leaching losses were not significant between treatments (total organic management: 24.2 kg N ha^{-1}; conventional management: 28.6 kg N ha^{-1}). Crop rotation and plant type had a larger influence on the microbial biomass, activity and community structure than fertilisation. Initial differences between soils decreased over time for most biological soil properties, while they persisted for the enzyme activities (e.g. dehydrogenase activity: 4.0 and 2.9 µg g^{-1} h^{-1} for organic and conventional management history, respectively). A lack of consistent positive links between enzyme activities and microbial biomass size indicated that similarly sized and structured microbial communities can express varying rates of activity.

In two successive incubation experiments, the soils were amended with different rates of a lupin green manure (4 or 8t dry matter ha^{-1}), and different forms of N at 100 kg ha^{-1} (urea and lupin) and incubated for 3 months. Samples were taken periodically, and in addition to soil biological properties and community structure, gross N mineralisation was determined. The form of N had a strong effect on microbial soil properties. Organic amendment resulted in a 2 to 5-fold increase in microbial biomass and enzyme activities, while microbial community structure was influenced by the addition or lack of C or N substrate. Correlation analyses suggested treatment-related differences in nutrient availability, microbial structural diversity (species richness or evenness) and physiological properties of the microbial community.

The findings of this thesis showed that using green manures and crop rotations improved soil biology in both production systems, that no relationships existed between microbial structure, enzyme activities and N mineralisation, and that enzyme activities and microbial community structure are more closely associated with inherent soil and environmental factors, which makes them less useful as early indicators of changes in soil quality.

Key words: microbial community structure; soil biology; soil processes; function; past and current management; organic and conventional farming practices; lupin (*Lupinus angustifolius* L.) green manure; urea; intact monolith lysimeters; N dynamics.

Acknowledgements

After more than 3 years of gestation and 6 months of labour this thesis has finally seen the light of day; an event that would have never been possible without the help of a wide range of people who gave physical, moral and/ or financial support along the way. Many people in- and outside Lincoln University have been directly associated with this project and the order of their mentioning is in no way indicative of their contribution:

Most importantly, I like to thank my supervisors Dr Leo Condron, Professor Hong Jie Di, Professor Alison Stewart (all Lincoln University) and Dr Maureen O'Callaghan (AgResearch, Lincoln) for taking on this project and me as a student, for sharing their expertise and giving me their support and advice. I am especially grateful to Leo for taking care of the logistical side of this "sledge journey" and for weighing out the occasional soil sample. Thanks for your enthusiasm, great supervision and for sending me home when everything got too much.

For financial support, I would like to express my gratitude to the New Zealand Fertiliser Manufacturers' Research Association and to Professor Keith Cameron for his advice and assistance on this matter, to Lincoln University for granting me a Lincoln University Graduate Scholarship and, of course, to my parents.

Despite the lack of attendance at last year's Miss Lincoln competition (as five wise men once said: "You can't always get what you want"), many thanks to all staff and students in the Soil Science Group for providing a fantastic, exciting work environment and for being such wonderful people that almost never nick stuff from other people's labs. Special thanks are naturally due to Analytical Services – in particular Jason Breitmeier, Roger Cresswell, Joy Jiao, Kate Scott and David "Happy New Year!" Wales – for technical assistance and analysis of millions of leachate samples and soil extracts. Thanks for all those interesting and entertaining chats in the hallway. I learned a lot about O-rings. Apologies to all those in offices near-by for the occasional disturbance by mad laughter! I am grateful to Amanda for making research life easy by being so good at what she does.

I also have to thank the "guys downstairs", Neil Smith, Steve Moore and Trevor Hendry, for practical help and good advice whenever it was needed. I am particularly thankful to Trevor for customising and allowing me to use his macros to analyse the lysimeter data. Thanks to Roger McLenaghen for his help in choosing sites appropriate for lysimeter collection and for profiling the soils for me. The help I got from Jim, Carol, Rhys, Abie and Tim was also much appreciated.

Many thanks to the former Plant Pathology Group, in particular Kirstin McLean for introducing me to the art of soil dilution plating and Hayley Ridgway for teaching me a lot about DNA extractions, PCR and gel electrophoresis.

I am thankful to Bruce McKenzie for his advice and to Dave Jack because of whom I never had to learn how to use the rotary hoe.

The help and advice I received from Dr Chris Frampton and Dr Andrew McLachlan regarding statistical matters was very much appreciated.

I am most grateful to Nick Waipara, from Landcare Research, for being so enthusiastic about and letting me in on some of the secrets of fungal taxonomy. I appreciate your help with identifying the fungal isolates.

Special thanks to Emily Gerard, from AgResearch, for practical help and advice in using the DGGE system. Thanks to her and her colleagues for such a fun work environment.

Of course, the contribution of friends and family should never be ignored:

Big thanks to "the usual suspects" for fun & games and great parties. The last two years have been successful despite – or maybe because of – you! Thanks to everybody who stopped asking "How is it going?" and the dreaded "When…?" question a long time ago.

I am extremely grateful to Sean, Merf and Alice for putting up with a 4th flatmate without complaints, especially when times were tough and stressful. I particularly have to mention Peter and Tuck for looking after me, cheering me up when everything seemed pointless and feeding me on the, probably not so, odd occasion (same goes for Sean, Alice, Merf, Cat and Pavla). Thank you again, Kelly, for a great trip to Mount Richardson! And thanks to Miss Tuck and Pavla for proof reading this precious work of art, and to Peter for prompt delivery.

I am immensely grateful to Merf for his practical help and support, and especially for enduring me and my mean disposition. Things would have been much harder without you. Thanks for keeping me sane!

Thanks a lot to my family, Gisela, Peter, Silja and Jost-Michael, for accepting my decision to come here, for answering phone calls and txt messages at strange hours and for great medical advice and telephone diagnoses of all types of stress related diseases.

Finally, I want to express my gratitude to Professor David McNeil, who was not involved in this project, but without his encouragement and help I would never have come to Lincoln University and not considered a career in science.

Last but not least, thanks to Basil and Dr Kenbarth for sticking around and being such good fun, to Mick, Keith, Charlie and Ronnie, the source of never ending joy and entertainment, and to New Zealand in general for letting me into this country.

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Section 1 – General introduction

This section outlines the aims, objectives and structure of the thesis and details the context of the study with respect to sustainability, the associated impact of farming practices and the role of microorganisms in maintaining soil fertility.

Sustainability of agricultural production systems is essential to ensure high productivity and a clean environment over a long period of time, and microbial diversity in soils is considered to be important to sustain the functioning of the soil ecosystem (i.e. maintain soil fertility and productivity). Several questions remain regarding the links between microbial diversity and ecosystem processes. In this study, microbial community structure and soil processes were studied in the context of organic and conventional farming systems. As research results suggest that microbial populations and activities significantly differ in soils farmed under contrasting management regimes, this provided an opportunity to study different microbial communities and their response to varying management practices.

1 Background

1.1 Sustainability and soil quality

"Sustainability" and "soil quality" are terms discussed when describing the condition of soil as an important resource in agricultural production systems. Definitions have changed over time due to new research and changed perceptions of agriculture (Carter *et al.* 1997; Lal 1998). The large number of publications on these subjects reflects the significance of, and the increasing interest in, these issues. Both sustainability and soil quality are closely related to soil degradation and the loss of productivity. They should, therefore, be key concerns to agronomists, politicians, scientists, and society as a whole (e.g. Parr *et al.* 1992; Hatfield and Stewart 1994; Cameron *et al.* 1996; Doran and Jones 1996; Elliott *et al.* 1996; Gregorich and Carter 1997; Lal 1998, 1999; Kirchmann and Thorvaldsson 2000; Schloter *et al.* 2003; Schjønning *et al.* 2004b).

1.1.1 Sustainability

Maintenance of high productivity, soil conservation and environmental protection are central issues of sustainability. Intensive agricultural production systems often encounter problems related to soil degradation, erosion and the associated decrease in productivity (Reeve 1990; Kirchmann and Thorvaldsson 2000). As stated by Reeve (1990), soil degradation "represents a loss of potential to provide food and employment. It represents the theft from future

generations of a food-producing resource." To secure high levels of production and ensure productivity for future generations, agricultural systems have to be sustainable. Problems of defining the term "sustainability" adequately have been expressed in various publications (e.g. Hendrix *et al.* 1990; Reeve 1990; Swift 1994; Christen 1996; Schjønning *et al.* 2004a). Lal (1994) described two central requirements that any sustainable system has to fulfil:

- the increase in per capita productivity (and linked with that, the maintenance of soil productivity); and
- the decrease in risk of soil and environmental degradation.

Kirchmann and Thorvaldsson (2000) summarised existing literature on sustainable agriculture and identified four general aims, which cover socio-economic, environmental, ethical and political aspects:

- sufficient food and fibre production;
- environmental stewardship;
- economic viability;
- social justice.

A sustainable agricultural system should, thus, prevent soil degradation, minimise the loss of non-renewable resources, be environmentally sound and socially acceptable, while, at the same time, providing the economical basis for living by producing sufficient food for the world population (Douglass 1985).

Sustainability is often mentioned as a characteristic of alternative production systems, such as reduced or low-input systems, organic/ biological, bio-dynamic, ecological agriculture and agroforestry systems (Reeve 1990; Cook and Lee 1995). Although modern agricultural systems (Chapter 1.2, this section) do not seem to be sustainable in many regards, it has to be stressed that sustainability is not an exclusive characteristic of alternative farming systems and that these systems are not necessarily sustainable (Reganold 1995). However, the adjustment of existing conventional farming practices by placing more emphasis on protection of the environment is inevitable for the development of more sustainable agricultural systems (Reeve 1990). Sustainability of agricultural production is also closely linked with soil quality as soil is a limited and non-renewable resource (Lal 1994). It is essential to protect cropping soils by applying cultivation and management techniques that enhance fertility and decrease soil loss through erosion. Agricultural systems cannot be sustainable unless the soil – the basis of production – is maintained in good condition, i.e. of high quality and fertility.

1.1.2 Soil quality

The simplest definition of soil quality is the capacity of a soil to fulfil a certain function (Mausbach and Seybold 1998). Blum and Santelises (1994) and Blum (1998) described the concept of soil quality by grouping soil into six categories according to its functions. Three of these functions are of an ecological nature: (1) production of biomass, (2) capacity to filter, buffer and transform matter and (3) genetic reserve and biological habitat for plants, animals and microorganisms. The other three are related to non-agricultural human activity: (4) physical medium for technical and industrial structures, (5) source of raw materials, e.g. water, clay, gravel, sand, and (6) cultural heritage. This indicates that soil quality cannot and should not be assessed out of context as it always depends on the soil's specific use (e.g. agricultural, urban, industrial, recreational, athletic, environmental, and waste disposal) and conditions (e.g. crop type, soil type, climate, management and cultivation regime).

Soil quality underpins economic viability (i.e. productivity), environmental sustainability and, consequently, food quality and human and animal health (Figure 1). Preserving and improving soil quality should be one of the central tasks of good management practices (Doran 1996; Schjønning *et al.* 2004a).

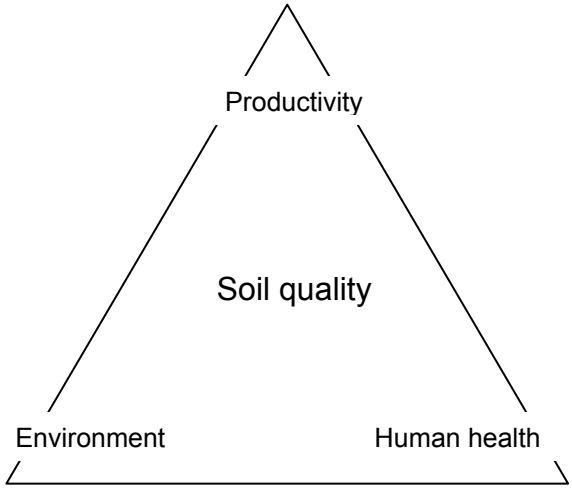

Figure 1: Soil quality and the three related concerns (after Schjønning *et al.* 2004a).

There has been increasing interest in the use of microbial soil properties as soil quality indicators (Hatfield and Stewart 1994; Doran and Jones 1996; Elliott *et al.* 1996; Rayns *et al.* 2002; Schjønning *et al.* 2002; Johnson *et al.* 2003; Schloter *et al.* 2003; Bending *et al.* 2004; Brussaard *et al.* 2004). Gregorich (1996) recognised that, in the context of agricultural production, soil quality is determined by an interaction of biological, physical and chemical soil properties. The evaluation of soil quality has traditionally focused on physical and chemical properties since agreement on suitable biological indicators is lacking (Cameron *et al.* 1996; Bending *et al.* 2004) and standardised methods exist for the assessment of these parameters (Kennedy and Smith 1995; Filip 1998). However, it has been widely recognised

that the biological component is very important for the concept of soil fertility, soil quality and health because microorganisms affect and/ or control most soil processes and properties (see Chapter 1.3, this section).

1.2 Land management systems

1.2.1 Problems with intensive agriculture

Sustainability and soil quality are key concerns of modern agricultural production systems. To ensure human survival, it is necessary to maximise crop production, while minimising negative effects on human health and the environment. This requires the development of sustainable farming practices that are productive, environmentally friendly and economically viable on a long-term basis. However, modern farming systems, if not managed properly, can have adverse environmental and social consequences (Ponting 1992; Schjønning *et al.* 2004b).

Modern agricultural systems, particularly in the industrialised world, follow intensive, high-input management regimes to increase crop production. They rely on the extensive use of synthetic fertilisers, high pesticide applications and energy inputs and on the use of non-renewable resources (fossil fuels). In addition, modern agriculture raises questions regarding ethical (e.g. animal welfare) and social issues (e.g. destruction of traditional social structures and "de-humanisation" of the workplace) (Oehlaf 1978; Domsch 1986; Smyk *et al.* 1986; Ponting 1992; Schachtschabel *et al.* 1992; Drinkwater *et al.* 1995; IFOAM 1997; Hansen *et al.* 2001).

Cultivation and management techniques that are commonly associated with conventional farming practices include the use of mineral fertilisers and pesticides, monocultures, narrow or absence of crop rotations, frequent tillage and irrigation (Oehlaf 1978; Ponting 1992; Mäder *et al.* 1999; Wells *et al.* 2000). Although these management regimes result in high productivity, they can create new problems for the environment and society (IFOAM 1997):

- soil degradation, i.e. irreversible damage to the soil structure and loss of fertility;
- soil erosion, including damage to the natural environment and landscapes;
- loss of biodiversity;
- accumulation of toxic compounds in the soil, eutrophication of surface waters and contamination of groundwater due to runoff and leaching (e.g. nitrate);
- contribution to ozone depletion;
- residues on food due to pesticide applications

  (Herman and Maier 2000; Kirchmann and Thorvaldsson 2000; Tinker 2000b; Wells *et al.* 2000; Hansen *et al.* 2001; Hole *et al.* 2005).

Alternative farming systems that are being widely promoted as more sustainable include, for example, organic farming, conservation tillage, low-input, biodynamic systems and precision farming (e.g. Kristensen *et al.* 1995; Unwin *et al.* 1995; Shepherd *et al.* 2000a; Stolze *et al.* 2000; Tinker 2000a; Hansen *et al.* 2001; Stockdale *et al.* 2001; Robson *et al.* 2002; Shepherd *et al.* 2003; van Steensel *et al.* 2004; Hole *et al.* 2005).

1.2.2 Organic cropping systems

Current research situation

Organic production systems avoid or minimise the use of synthetic fertilisers, pesticides and antibiotics. They instead rely on biological pest control and specific soil management techniques, such as crop rotations including grass/ clover leys, winter break and catch crops and/ or green manures, crop residue management and manuring, to control plant diseases and pests, maintain soil fertility and improve physical soil properties (Shepherd *et al.* 2000a; Watson *et al.* 2002). Organic farming practices aim to increase sustainability by reducing pesticide residues on crops (cf. Figure 1: human health), improving biodiversity in the soil (productivity, environment), avoiding leaching losses and runoff (environment), and reducing soil erosion by enhancing soil structure (productivity, environment) (Lampkin 1994; Tinker 2000b). It has to be stressed, however, that alternative farming systems are not sustainable *per definitionem*. Incorporation of leys, legumes or animal manures, for example, can lead to a nitrate surplus and cause leaching or runoff and weed control by intensive land cultivation can result in soil compaction (Greenland 2000; Schjønning *et al.* 2004c).

There is an expansive range of reviews evaluating soil fertility, crop production and environmental issues (biodiversity, nitrate and pesticide leaching, gaseous emissions, etc.) on organic and conventional farms in New Zealand and overseas (e.g. Unwin *et al.* 1995; Saunders *et al.* 1997; Condron *et al.* 2000; Shepherd *et al.* 2000a; Stolze *et al.* 2000; Tinker 2000a; Hansen *et al.* 2001; Stockdale *et al.* 2001; Di and Cameron 2002b; Shepherd *et al.* 2003; van Steensel *et al.* 2004; Hole *et al.* 2005). Most authors have come to broadly similar conclusions, as follows.

- There is inconsistent evidence regarding the impact of organic farming on soil structure; Stockdale *et al.* (2001) reported positive effects on soil aggregate stability, while, for example, Alföldi *et al.* (1995) and Raupp (1995a) found no differences in soil structure.
- Organic farming practices are reported to have a positive effect on the soil organic matter, mainly because of high organic matter inputs to the soil (e.g. animal or green manures, crop residue), which is beneficial for soil microbial biomass and activity. However, the addition of organic material is a farming practice not restricted to, and not solely used by, organic production systems and has the same effect on conventional farms (Stolze *et al.* 2000; Stockdale *et al.* 2001; Shepherd *et al.* 2003).

- Organic management supports above- and below-ground biodiversity, however, some studies suggest that in particular intensive soil cultivation used in organic farming systems can have a negative influence on soil biodiversity and that beneficial effects depend on farm location, climate, and species investigated and are often a result of lower stocking rates and less intensive production (Mäder *et al.* 1996; Greenwood 2000; Shepherd *et al.* 2003; Hole *et al.* 2005).
- Lower nitrate losses have been measured under organic compared to conventional farming systems resulting from lower nitrogen (N) inputs and/ or lower stocking densities. Although nitrate losses can be increased after ploughing of ley phases and incorporation of green manure crops, it has been suggested that nitrate losses are similar from both systems when assessing the organic rotation as a whole instead of this one aspect (Dalgaard *et al.* 1998; Di and Cameron 2002b; Shepherd *et al.* 2003).
- Organic farming practices are beneficial for microbial biomass and activity and soil fertility (Condron *et al.* 2000; Shepherd *et al.* 2000a; Stolze *et al.* 2000). The effect of different management regimes on soil biological properties have been investigated by a number of researchers (Fliessbach 1995; Fliessbach and Mäder 1997; Gunapala and Scow 1998; Albiach *et al.* 1999; Stolze *et al.* 2000; Poudel *et al.* 2001; Mäder *et al.* 2002; Watson *et al.* 2002; Jordan *et al.* 2004; Parfitt *et al.* 2005) and will be discussed in more detail later in this section (Chapter 1.3.5).

Most of these findings are relative depending on their assessment on a per area unit (e.g. hectare) or per unit of yield (e.g. tons) basis. In some instances no differences or negative effects (i.e. higher leaching losses, lower diversity) of organic management systems were observed when assessing the parameters per unit of yield (Stolze *et al.* 2000; Shepherd *et al.* 2003).

There is evidence for high variability within the findings presented above as a large variety of different management practices are employed under the labels of organic and, especially, conventional farming. This makes it problematic to identify trends when comparing management systems, and many benefits of one or the other system will depend on the individual farming practices used by the farmers (Elmholt 1996; Shepherd *et al.* 2003). In addition, most of these findings are site specific, i.e. the system response depends on the particular climate, soil type and management strategies. This causes difficulties to extrapolate results and make statements for other areas of the world, which means it is necessary to validate the conclusions for different environments including New Zealand (Condron *et al.* 2000). Overall, more information and research are needed to resolve outstanding questions, especially regarding the effects of farm management practices on biodiversity in general, the diversity of the soil microbial community and the associated nutrient cycling processes (see Clark *et al.* 1998; Condron *et al.* 2000; Shepherd *et al.* 2000b; Stolze *et al.* 2000; Tinker 2000a; Wells *et al.* 2000; Drinkwater *et al.* 2001; Stockdale *et al.* 2001; Watson *et al.* 2002; Abbott and Murphy 2003b; van Steensel *et al.* 2004).

Green manures and biological processes in organic farming

Leguminous plants, either as pasture components or as green manure crops, are key parts of stockless organic rotations, as they help build and maintain soil fertility by fixing atmospheric C and adding organic matter to the soils. In New Zealand, the overall productivity of organic cropping systems is limited by N supply due to a combination of prohibition of the use of soluble N fertilisers and the unavailability of alternative organic N fertiliser materials, such as farmyard manure for fertilisation. In comparison to European production systems, where livestock are overwintered inside producing large quantities of manure that can be applied to cash crops, New Zealand has year round grazing (Condron *et al.* 2000). Hence, legumes are an important source of nutrients for most organic systems and crops under organic management are almost exclusively dependent on soil biological processes to provide sufficient amounts of N from soil organic matter by mineralisation. This study, consequently, focused on N mineralisation as a key process (or function) because of its particular importance in supplying N to crops in organic farming systems. While mineralisation of N from soil organic matter also plays an important role in conventional cropping systems, the availability of soluble mineral fertilisers reduces the dependence on these soil processes. Nevertheless, the use of green manures is considered good management practice for both conventional and organic systems due to their many positive effects on soil fertility and quality (Doran *et al.* 1988; Greenland 2000; Shepherd *et al.* 2000a; Watson *et al.* 2002).

1.3 Soil microbial ecology

1.3.1 Biodiversity and ecosystem processes

Biodiversity and how it is affected by changes in environmental conditions, including agricultural intensification and climate change, has been of interest to biologists, ecologists and environmentalists for a number of years and is often discussed in the context of sustainability (see Dick 1992; Paoletti and Pimentel 1992; Schulze and Mooney 1993; Tilman 1996; Andrén and Balandreau 1999; Loreau *et al.* 2001; Duffy 2002; Loreau 2004). Biodiversity is being conserved for aesthetic, cultural and economic reasons, but, most importantly, on the ecosystem level biodiversity acts as a buffer against changes in environmental conditions by ensuring the variability/ diversity needed by species to adapt to these changes (insurance hypothesis) (Loreau *et al.* 2001).

With respect to above-ground communities, biodiversity is essential for ecosystem stability, and changes in biodiversity can alter ecosystem functions (Tilman 1996; Naeem 2003). Positive relationships exist between biodiversity and primary (biomass) production and the factors that affect productivity (e.g. soil fertility, climate, disturbance and herbivory). This indicates that a certain number or a particular assemblage of species is necessary to sustain the stability and functioning of ecosystems; however, it remains debatable if it is a relatively

small number of key species or a larger variety of complementary species that drive ecosystem processes (Brussaard *et al.* 2004; Wardle *et al.* 2004). More research is needed to fully understand the relationships between diversity and ecosystem processes. It has been proposed that species composition and types (functional groups or species) have greater influence on ecosystem functioning and stability than species richness (e.g. Bengtsson 1998; Loreau *et al.* 2001) and Wardle and Grime (2003) suggested that the effects of plant species and functional groups on ecosystem processes have to be assessed separately from the effects of species richness to gain better understanding of how diversity influences ecosystem stability.

All levels of biodiversity (above-ground fauna and flora, below-ground soil biota, including microorganisms, earthworms and arthropods, etc.) need to be considered (Shepherd *et al.* 2003). However, links between above- and below-ground communities are neither clear nor consistent (Brussaard *et al.* 2004). Only weak connections exist between plant community diversity and decomposition rates in the soil and individual plant species seem to have bigger influence on the soil biodiversity than overall (plant) diversity (Wardle *et al.* 2004). It is also problematic to make assumptions regarding the role of below-ground diversity for the functioning of the soil system merely based on the knowledge on above-ground biodiversity and its influence on ecosystem stability (Loreau *et al.* 2001). However, it is widely acknowledged that some aspect of microbial diversity (species richness, evenness or composition; see Chapter 1.3.3, this section) is important to sustain soil functioning since the microbial community is responsible for most the ecosystem processes (e.g. organic matter decomposition, nutrient cycling) (Brussaard *et al.* 2004; Coleman *et al.* 2004).

1.3.2 The role of microorganisms in the soil

The soil biota includes macrofauna (e.g. beetles, earthworms), mesofauna (e.g. nematodes, collembolan, mites), microfauna (protozoa) and microflora (bacteria, fungi, algae), which interact in the soil food web through different mechanisms, such as predation, competition (for nutrients and space), symbiosis and commensalism. Soil biota plays a vital role in the maintenance of soil fertility and productivity (Metting 1993; Coleman *et al.* 2004). While all groups and their interactions are important for the functioning of the soil ecosystem, it is soil microorganisms that drive most soil processes, e.g. nutrient cycling, availability and retention, decomposition of organic materials, soil organic matter build-up and stabilisation of soil aggregates (e.g. Ritz *et al.* 1994; Insam and Rangger 1997; Liesack *et al.* 1997; Maier *et al.* 2000; Coleman *et al.* 2004). These processes can affect soil erosion and the sustainability of the ecosystem. Hence, soil biota and its functions are closely linked with issues of soil quality maintenance and ecosystem sustainability (Wardle *et al.* 1999).

Although we are able to appreciate the significance of microorganisms in the soil we have little information on the importance of microbial diversity in the functioning of soil systems

(Ritz *et al.* 1994; Beare 1997; Insam and Rangger 1997; Johnsen *et al.* 2001; Nannipieri *et al.* 2003; Brussaard *et al.* 2004; Coleman *et al.* 2004). Are there links between microbial diversity and soil fertility? If so, how are they affected by management practices and perturbation?

1.3.3 Soil microbial diversity

Microbial diversity in soils can be assessed on different levels. Structural diversity (i.e. taxonomic and genetic diversity) addresses the question 'Who is there?'; functional diversity looks at 'What do they do?'; the soil-process level addresses mineralisation and decomposition processes; and the ecosystem-function level examines soil biodiversity on a broader scale looking at the productivity of the ecosystem and the maintenance of soil fertility (Figure 2).

Three aspects of structural diversity exist:

- species richness, addressing the absolute numbers of species present in the soil;
- species evenness, an indicator for the relative abundance of different species;
- species composition, i.e. which particular types of species are present in the soil (Zak *et al.* 1994; Kennedy and Smith 1995; Griffiths *et al.* 1997).

The methods that can be used to describe the microbial community and its functions include measurements of microbial biomass size, culture dependent approaches such as soil dilution plating or community level physiological profiles, molecular techniques, phospholipid fatty acid (PLFA) analysis, enzyme activities, respiration assays, etc. The methods that were considered for and used in this study to determine soil biological properties are described and discussed in Section 2.

Only a small proportion (1-10%) of all soil microorganisms are culturable (Torsvik *et al.* 1994; Stotzky 1997; Insam 2001), i.e. traditional methods to determine structural diversity (e.g. soil dilution plating) target only a small fraction of the microorganisms present in the soil. Consequently, an accurate identification and determination of functional properties is difficult using these methods and might create an insufficient picture of microbial diversity and its significance in the soil. The application of new, mainly molecular techniques, that do not rely on culturing to identify soil microorganisms, offers more insights into the functional and structural diversity of soil biota and can provide information on the relationship between microbial community structure and function and how it impacts on soil quality, resilience and sustainability (Torsvik *et al.* 1994; O'Donnell and Görres 1999; Insam 2001; O'Donnell *et al.* 2001). To minimise bias and to obtain more complete information a combined approach using different methods should be employed (Atlas 1984; Liesack *et al.* 1997; Insam 2001; Widmer *et al.* 2001).

1.3.4 Diversity-function relationships

The concept of the different levels of diversity does not give any indication as to how the different components affect each other and the question remains if and/ or to what degree functional diversity depends on structural diversity and how both relate to ecosystem functions and stability (Zak *et al.* 1994; Griffiths *et al.* 1997; O'Donnell and Görres 1999; Nannipieri *et al.* 2003). The general perception and most obvious assumption is that function depends on the diversity of the microbial community (Figure 2). However, there are different hypotheses regarding the diversity-function relationships in soil (Ritz and Griffiths 2001); for example, "as long as all functional groups are represented, system functioning is independent of diversity" (Lawton and Brown 1994 cited in Brussard *et al.* 2004) or "all species make a significant contribution to function and a decrease in diversity leads to a progressive decline in function" (Lawton 1993 cited in Brussard *et al.* 2004). This gives indication that functional diversity is not directly linked to microbial community composition in soils and that soil processes do not depend on diversity, suggesting that differently sized and structured communities can fulfil similar functions, that loss of diversity does not necessarily result in changes in ecosystem processes and that presence or absence of particular species is more important than species diversity or abundance (Zak *et al.* 1994; Griffiths *et al.* 1997; Andrén and Balandreau 1999; Griffiths *et al.* 2000; Brussaard *et al.* 2004).

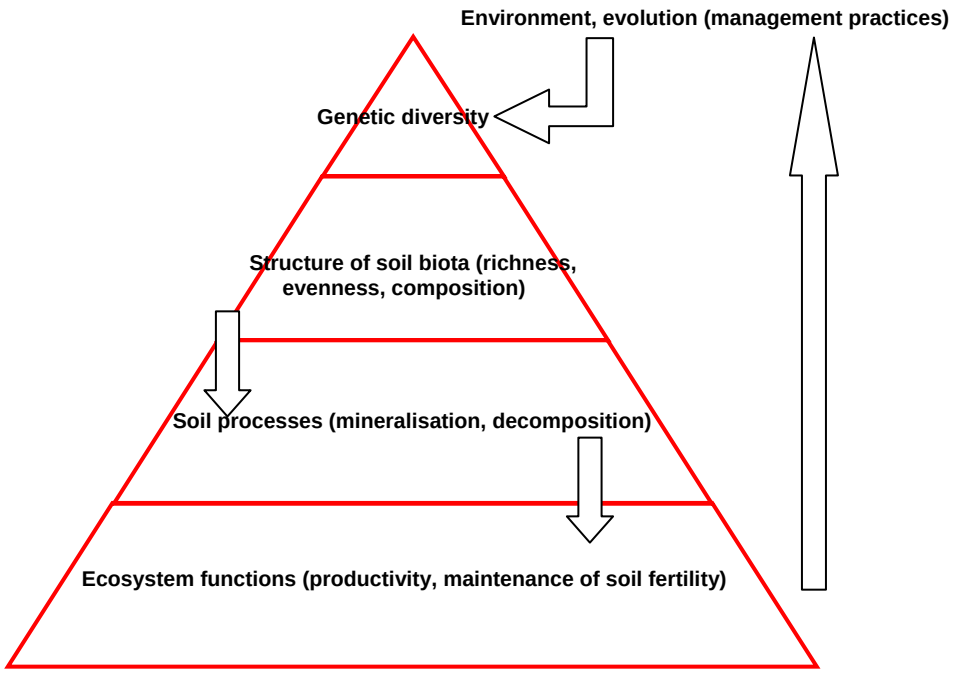

Figure 2: Relationships between microbial structure, soil processes and ecosystem functions in soil (after Mäder *et al.* 1996; O'Donnell *et al.* 2001).

Mikola and Setälä (1998) found that in an experimental food web the response of mineralisation was unrelated to changes in microbial diversity and Müller *et al.* (2002) showed only a weak relationship between microbial function and microbial diversity when

measuring differences in the heat tolerance of the microbial community that were not accompanied by differences in microbial diversity. Correspondingly, Crecchio *et al.* (2001) reported no changes in the structure of bacterial communities 2 years after the addition with municipal solid waste, while the amendment increased enzyme activities (dehydrogenase and nitrate reductase). In a study by Marschner *et al.* (2003) an increase in biomass size and differences in the composition of the bacterial community following long-term amendments with organic materials (30 years) were not accompanied by changes in enzyme activities (protease, alkaline phosphatase, arylsulfatase, urease, xylanase). Griffiths *et al.* found that a reduction in diversity did not affect soil functioning under permanent (copper contamination) and transient perturbation (heat stress) (2001b), and the authors concluded that there was no direct, consistent link between biodiversity and the soil processes measured (e.g. potential nitrification, community level physiological profiles, decomposition). However, other literature suggests that microbial function and structure show closer links under permanent perturbation, such as heavy metal contamination (Müller *et al.* 2002; Griffiths *et al.* 2004). Marschner *et al.* (2005) reported a link between bacterial community composition (assessed by DGGE) and function (assessed by enzyme assays) in rhizosphere soils. Thus, a relationship between microbial diversity and function cannot be entirely ruled out, but it seems that the influence of biodiversity strongly depends on the particular function measured (Griffiths *et al.* 2000). These findings suggest that changes in microbial community structure do not necessarily lead to changes in microbial activity. It is more likely that community composition and biomass size play a greater role in determining microbial functional diversity than biodiversity (i.e. species richness) (e.g. Brussaard *et al.* 2004; Griffiths *et al.* 2004), a concept I personally agree with. Consequently, I mostly avoided the use of the terms "biodiversity" and "microbial diversity" and the calculation of commonly used diversity indices.

An improved understanding of the role, function, and interactions of microbial species is essential to our comprehension of the functioning of soils (cf. Kennedy and Smith 1995; O'Donnell and Görres 1999; Waldrop *et al.* 2000; Griffiths *et al.* 2001a; Insam 2001; O'Donnell *et al.* 2001). Understanding the response of the microbial community to agricultural disturbances is equally important to clarifying how management practices contribute to sustainable fertility and productivity. This can help to develop improved management systems. Eventually, it could open a way to actively and precisely manage the soil microbial community, which in turn would improve our ability to manipulate soil processes like nutrient cycling and retention *in situ* in order to maintain soil quality and ensure sustainability of agroecosystems (Kennedy and Gewin 1997; Wardle *et al.* 1999; Brussaard *et al.* 2004).

1.3.5 Influence of management practices on soil microbial diversity

The effect of different management regimes and perturbations on the soil microbial community has been studied in a wide range of soil environments. Researchers investigated effects of different fertilisation regimes (Hatch *et al.* 2000; O'Donnell *et al.* 2001; Sarathchandra *et al.* 2001; Belay *et al.* 2002), herbicide and pesticide application (Johnsen *et al.* 2001; Seghers *et al.* 2001; Dungan *et al.* 2003), crop rotations (Campbell *et al.* 1991; Campbell *et al.* 1992; Campbell *et al.* 1999), manure applications (Bossio *et al.* 1998; Gunapala and Scow 1998; Girvan *et al.* 2003), heavy metal contamination (Frostegård *et al.* 1993; Brohon *et al.* 2001; Turpeinen *et al.* 2004), wet/ dry cycles (Lundquist *et al.* 1999b) and different tillage systems (Lupwayi *et al.* 1998; Ibekwe *et al.* 2002; Zaitlin *et al.* 2004) on microbial soil properties. Amongst the environments studied were forest soils (Leckie *et al.* 2004; Li *et al.* 2004), grassland and pasture systems (Grayston *et al.* 2004; Hunt *et al.* 2004; Ritz *et al.* 2004; Parfitt *et al.* 2005), arable soils (Breland and Eltun 1999; Haynes 1999; Griffiths *et al.* 2001b; Nsabimana *et al.* 2004), including conventional, low-input and organic systems (Domsch 1986; Lundquist *et al.* 1999b; Fliessbach and Mäder 2000; Schjønning *et al.* 2002). Studies and results relevant to this research project will be reviewed in more detail in the respective discussion sections.

Microbial properties in soils are influenced by several factors, such as inherent soil properties, environmental conditions and anthropogenic activities, including land management systems (Table 1) (O'Donnell *et al.* 2001; Girvan *et al.* 2003). Most research suggests that organic farming practices have a positive, stimulating influence on the soil microbial community by enhancing diversity and improving soil functions like nutrient cycling and antagonistic potential and that soil quality is higher in organically farmed soils (e.g. Yeates *et al.* 1997; Gunapala and Scow 1998; Lundquist *et al.* 1999b; Ryan 1999; Condron *et al.* 2000; Greenland 2000; Shepherd *et al.* 2000a; Shepherd *et al.* 2000b; Mäder *et al.* 2002; Rayns *et al.* 2002; Girvan *et al.* 2003; Bending *et al.* 2004). In comparison, there is little evidence in the literature of negative effects of conventional production practices, such as use of mineral fertilisers and pesticides, on the soil organic matter, microbial diversity and activity (Fraser *et al.* 1988; Fauci and Dick 1994; Gunapala *et al.* 1998; Shepherd *et al.* 2000a; Belay *et al.* 2002). This suggests that individual production techniques (e.g. green manuring, use of catch crops, crop rotations, crop residue management, conservation tillage) impact on the soil microbial community rather than the land-use system itself. Hole *et al.* (2005) made the same observations regarding beneficial effects of farming practices on above- and below-ground biodiversity. This means that these management techniques will have the same beneficial effects on the soil organic matter and the microbial community whether applied in an organic or a conventional system. However, since these practices are commonly linked to organic farming systems, it is reasonable to assume that soils cultivated under long-term organic or conventional management show differences in microbial biomass composition and function (Gunapala and Scow 1998; Lundquist *et al.* 1999b; Ryan 1999). Understanding the effect of management practices on maintaining fertility and productivity of arable soils is a key to

improving sustainability of agroecosystems. This requires an understanding of the structure and function of soil microbial communities and how they are affected by farming practices (Thomas and Kevan 1993; Beare 1997). It might be possible to influence nutrient cycling processes and soil quality by manipulating the microbial community in soils. However, more information is needed on the role of microbial structural diversity in the functioning of the soil ecosystem and on the links between microbial biomass size and activity and soil processes that drive nutrient availability and fertility (Kennedy and Smith 1995).

Table 1: Factors influencing microbial diversity in the soil (O'Donnell *et al.* 2001).

Environmental factors	*Soil factors*	*Anthropogenic factors*
Landscape	Cation exchange capacity	Crop
Soil type	Organic matter	pH
Parent material		Fertilisers
Rainfall		Rotations
Microbes		Pesticides
Vegetation		Tillage

2 Aims, objectives and thesis structure

For this study, soils from organic and conventional farming systems were selected to investigate the relationships between microbial composition, function and environmental impacts caused by different farming practices. In the absence of long-term comparative field experiments in New Zealand, two sites of the same soil type were identified within the Lincoln University cropping farm that had been farmed under contrasting organic and conventional management for at least 25 years at the time this study began. This provided an opportunity to examine the long-term effects of different management regimes on biological soil properties, microbial diversity and soil processes (N mineralisation).

Environmental conditions and management practices (i.e. soil type, inputs, cultivation, weather) have a considerable impact on the soil biota and soil processes. It is important to recognise these influences when studying the impacts of management systems on the soil microbial community. By studying the effects of past and current management on the soil microbial community and on N dynamics under controlled (incubation experiments) and semi-controlled conditions (lysimeter study), the influence of environmental factors could be limited and the effects of individual management practices on the soil microbial community could be determined.

The principal aims of this study were to establish possible relationships between microbial structural diversity and soil processes and to study the effect of organic and conventional farming practices on microbial soil properties and N dynamics.

The following specific objectives were chosen to accomplish these aims:

- to select and evaluate suitable experimental methods to be used throughout the study;
- to assess the effect of long-term and current organic and conventional farming practices on biological properties and N mineralisation (past and current management); and
- to determine the amounts of mineral N lost by leaching from soils under organic and conventional management.

The three main experiments designed to achieve these objectives are described and discussed in detail in Sections 2, 3 and 4 of this thesis. How they relate to the objectives is shown in Figure 3.

To compare the effects of farming history on the microbial community and to establish a baseline for subsequent experiments, soil samples were taken from organically and conventionally managed sites of same soil type within the Lincoln University cropping farm on three occasions throughout the first year of the study. The samples were analysed for biological and biochemical soil properties; results are presented and discussed in Section 2 (farm site comparison). At the same time, available soil biological methods (microbial

biomass C (C_{mic}) and N (N_{mic}), microbial activity [enzymes] and structural diversity [soil dilution plating, extraction of total community DNA, followed by polymerase chain reaction (PCR) amplification and denaturing gradient gel electrophoresis (DGGE) profiling]) were tested to determine their suitability and value for the other experiments.

Section 3, the lysimeter study, details the development of a strategy that allowed for regular soil sampling from intact monolith lysimeters, and describes the field experiment that was set up to determine links between microbial diversity and function *in situ* and to compare effects of prior vs. current management on the microbial community and leaching losses. Lysimeters were taken from the organic and conventional farms used in the farm site comparison and managed under organic and conventional practices (using organic and mineral fertilisers, respectively). The crop rotation was identical for all treatments. Soil and leachate samples were regularly taken and analysed for biological soil properties (C_{mic} and N_{mic}, enzyme activities and structural diversity [DGGE profiling]) and mineral N content, respectively.

The incubation experiments (Section 4) investigated links between microbial diversity and soil processes, and the effects of past and current management practices on biological soil properties and processes under laboratory conditions. This allowed for variables such temperature, soil moisture or spatial variation within the soils to be controlled and reduced, respectively. The soils used in these experiments were the same soils used in the farm site comparison and the lysimeter study. For incubation experiment I, three different amounts of a leguminous green manure were added to the soils. Soils were sampled periodically over 81 days and analysed for microbial biomass size and activity, structural diversity and soil process rates (N mineralisation). Incubation experiment II evolved from experiment I and carried out under the same conditions. Nitrogen was added to the soils in different forms (organic and mineral) but at the same rates (100 kg ha^{-1}). Over a period of 91 days, soil samples were taken at intervals and analysed for microbial biomass size, activity, diversity and N mineralisation. Sampling frequency was increased in the first 2 weeks compared to incubation experiment I.

Each section contains a focused introduction and a detailed discussion of the outcomes of the respective experiments. An overall discussion and conclusions of the entire thesis are presented in Section 5.

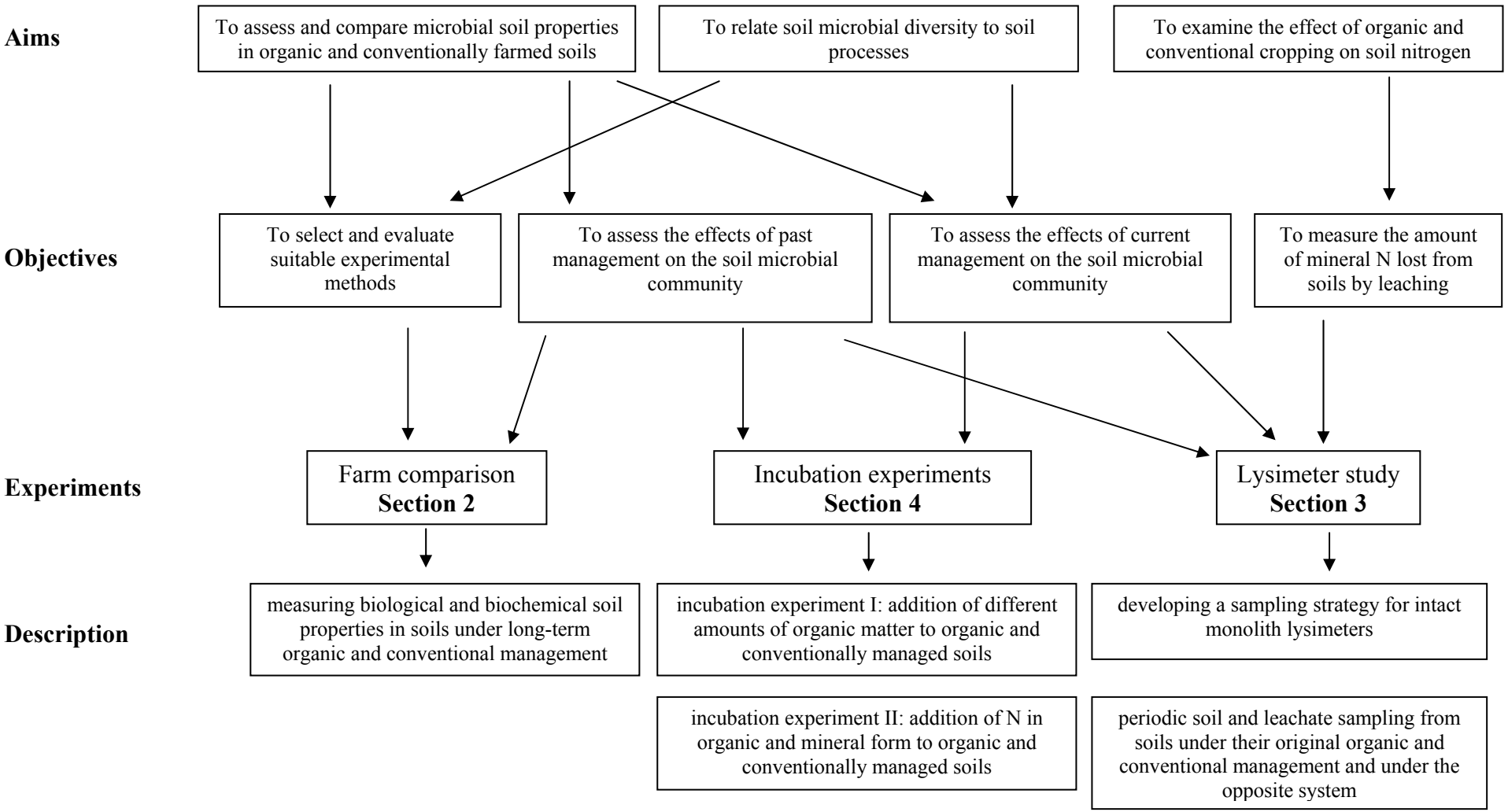

Figure 3: Organisation and structure of experiments and thesis

Section 2 – Method selection and evaluation and farm site comparison

This section covers the process of selecting and assessing methods used in later experiments to determine microbial biomass size, activity and diversity. Available methods are reviewed and a detailed description of the study areas is given. The methods were tested by conducting a farm site comparison, the main results of which are discussed in Chapters 2 and 3. Overall conclusions for this section are presented in Chapter 4.

1 Introduction

1.1 Aims and objectives

One aim of the thesis was to evaluate differences in microbiological soil parameters resulting from past and current management and to establish relationships between soil microbial biomass size, activity, diversity and function. This involved repeated measurements, often in short succession, of biological properties in soil samples taken from lysimeters managed under organic and conventional farming practices and in soils exposed to different treatments in an incubation experiment.

Many different methods are available to investigate microbial soil properties and it was necessary to find a suitable set of methods that addressed different aspects of microbial diversity (biomass size, activity and structure), and could be carried out using existing resources, equipment and expertise in a short time frame, ideally a few days. This short processing time was important as biological soil properties change rapidly once samples have been taken due to changes in soil temperature and moisture. The process of assessing the different methods also provided an opportunity to compare the two soils that were to be used in the following incubation and lysimeter experiments with respect to their initial microbial soil properties and to establish a baseline for later experiments.

In brief, the objectives of this part of the study were:

- to evaluate a range of methods for measuring biological and biochemical soil properties and to select a suite of tests for later experiments; and
- to compare soil samples from an organic (BHU) and a conventional cropping farm (LCF) for differences in microbial biomass, activity and diversity.

1.2 Method selection

Several methods are available to evaluate the different aspects of biological soil properties. It was neither necessary nor feasible, due to resource and time restraints, to use them all. A selection process was therefore undertaken to choose the most appropriate tests for this research, taking into consideration both scientific and practical issues.

While the reasons are discussed, why each of the proposed methods was, or was not, chosen for further use, no attempt has been made to review the advantages and limitations of the methods in great detail as there is an expansive range of literature on these issues (e.g. Tunlid and White 1992; Paul and Clark 1996; Hurst *et al.* 1997; Dalal 1998; O'Donnell and Görres 1999; Insam 2001; Johnsen *et al.* 2001; Widmer *et al.* 2001; Nannipieri *et al.* 2003; Coleman *et al.* 2004; Kirk *et al.* 2004).

The methods that were considered are presented in Table 2.

Table 2: Soil microbial community characteristics and suitable methods.

Characteristic	*Method*
microbial biomass size	microbial biomass carbon and nitrogen (C_{mic}, N_{mic})
	substrate induced respiration (SIR)
microbial activity	enzyme assays: arginine deaminase activity (ADA),
	fluorescein diacetate hydrolysis (FDA)
	basal respiration
microbial diversity	soil dilution plating
	DNA amplification followed by DGGE analysis
	phospholipid fatty acid analysis (PLFA)
microbial function / metabolic potential	Biolog™ (or community level physiological profiles, CLPP)
	catabolic response profile (CRP)

Microbial biomass

The size of the entire microbial biomass in soils can be easily estimated by, for example, fumigation extraction or substrate induced respiration (SIR).

For SIR, the evolution of carbon dioxide (CO_2) is estimated after addition of glucose to the soil samples. The respiration response is measured in intervals over a period of at least 8 hours and the size of the microbial biomass is then calculated by applying a conversion factor (Anderson and Domsch 1978). This method is suitable to determine the amount of carbon (C) held in the metabolically active part of the microbial biomass and does not distinguish between fungal and bacterial biomass size (Kjøller and Struwe 1994). An incubation/ titration approach was used to determine SIR (following the method of Anderson and Domsch (1978)), which unfortunately proved to be time consuming and produced highly

variable results (see Appendix I). The method was therefore not used in subsequent experiments.

Fumigation extraction requires one set of soil samples to be fumigated with chloroform and then to extract fumigated and non-fumigated samples with K_2SO_4. The amount of total C or N is measured in the extracts and converted to biomass C or N, respectively, using a conversion factor (Sparling and West 1988). The levels of microbial biomass C and N are of particular interest in relation to total C (microbial quotient) and total N, respectively, as these ratios show the importance of the microbial community in nutrient cycling processes and carbon dynamics, which are affected, by cultural practices and inputs associated with agronomic and natural systems. The ratios also allow for comparisons across soils. For the fumigation extraction technique the soil microbial community does not need to be in equilibrium (as required for SIR) (Schinner *et al.* 1995), however, this method does not distinguish between active and dormant biomass or between fungal and bacterial communities (Horwarth and Paul 1994).

An objective of this project was to examine N losses from and mineralisation in organic and conventionally farmed soils. Part of the mineral N in the soils is taken up by microorganisms and converted into organic N that is a component of cells and tissues (Haynes 1994). It was, therefore, necessary to determine the amount of N contained in the microbial biomass. Since the fumigation extraction method by Sparling and West (1988) allows for microbial biomass C and N to be determined in the same K_2SO_4 extract, this method was used to measure the size of the microbial biomass size.

Microbial activity

Microbial activity can be estimated by basal respiration (CO_2 evolution), various enzyme assays, substrate induced respiration, ATP (adenosine triphosphate) content, etc. (Alef and Nannipieri 1995).

The amount of CO_2 released by the soil microbial community over time (basal respiration) (Anderson 1982) can give an estimate of the rate of metabolic processes taking place in the microbial community and thus provide a measure of microbial activity in soils. This value is of particular interest when considered in relation with microbial biomass size (metabolic quotient, i.e. microbial respiration to microbial biomass ratio, estimating the proportion of the metabolically active microbial biomass). However, as mentioned for SIR earlier, the incubation/ titration approach used to determine respiration was lengthy and produced inconsistent results and the method was not used in subsequent experiments.

In addition, two enzyme assays were evaluated to determine microbial activity: arginine deaminase activity (ADA) and hydrolysis of fluorescein diacetate (3′, 6′-diacetylfluorescein) (FDA). Both assays measure the potential activity of the soil microbial community. The addition of the amino acid arginine to soil samples results in the release of ammonia

(ammonification or deamination), i.e. ADA is measuring a microbial process involved in the N cycle which is known to take place inside microbial cells and is not initiated by extracellular enzymes (Alef and Kleiner 1986, 1987). The FDA assay is a quick, simple and sensitive method that has been shown to correlate closely with soil basal respiration (Schnürer and Rosswall 1982). Since fluorescein diacetate hydrolysis is carried out by a variety of enzymes (e.g. proteases, lipases and esterases), FDA can be regarded as a bulk enzyme assay measuring a range of biological processes. The ability of the main decomposers, bacteria and fungi, to hydrolyse FDA has been recorded (Schnürer and Rosswall 1982; Dick *et al.* 1996; Adam and Duncan 2001) and the method can be considered a measure of overall microbial activity. However, interpretations of results should be carried out with care as both intra- and extracellular enzyme activities contribute to FDA hydrolysis, i.e. the measured activity cannot inevitably be attributed to microorganisms (Nannipieri *et al.* 2002).

Microbial structural diversity

Microbial structural diversity can be determined by soil dilution plating, molecular techniques based on extraction of DNA from the entire microbial community, and phospholipid fatty acid (PLFA) analysis.

Soil dilution plating is an inexpensive way to assess microbial structural diversity by culturing microorganisms, however, it has a number of limitations. Only a small proportion of soil microorganisms can be cultured (1-10%); there is often bias towards fast growing organisms by the choice of culturing media and incubation conditions; and dislodging bacteria or spores from soil particles can be difficult (Torsvik *et al.* 1994; Pankhurst *et al.* 1996; Tabacchioni *et al.* 2000; Insam 2001). However, dilution plating can show differences in microbial structural diversity in soils and reveal key species indicative of different farming systems. This method was, hence, included in the initial stage of the study to compare the diversity of culturable soil bacteria and fungi in the organically and conventionally farmed soils. However, results presented in Chapter 3 of this section showed that soil dilution plating did not deliver the anticipated outcomes as it was not possible to isolate and identify indicator organisms and differences in bacterial and fungal numbers were small between the two sites. In addition, the method proved to be very time consuming. Consequently, soil dilution plating was considered unsuitable for further use in the main experiments.

Community DNA extractions, polymerase chain reaction (PCR) amplification of 16S rDNA fragments followed by denaturing gradient gel electrophoresis (DGGE)[1] are a common way to determine the genetic (i.e. structural) diversity of microbial communities in environmental samples, e.g. in pasture, forest and arable soils (Girvan *et al.* 2003; Seghers *et al.* 2003;

[1] The process of DNA extraction, PCR amplification and DGGE will in the following simply be referred to as DGGE analysis.

Griffiths *et al.* 2004; Leckie *et al.* 2004), composts (Marshall *et al.* 2003), rhizospheres (Kandeler *et al.* 2002), phyllospheres (Heuer and Smalla 1999) and aquifers (Heuer *et al.* 2001). 16S rRNA genes contain conserved and variable regions and are, therefore, suitable for PCR amplification and the sequence information can be used for phylogenetic markers. During DGGE, DNA fragments of the same length with different sequences are separated over a linearly increasing formamide-urea gradient which causes the fragments to be separated according to their melting behaviour (Heuer *et al.* 2001). DGGE analysis allows researchers to profile complex microbial communities and investigate community structure and dynamics over time. The technique also gives insights into the phylogenetic affiliation of parts of the community when bands of interest are sequenced, and is particularly suited for comparison of bacterial communities in different soils. However, profiling microbial communities by DGGE is a non-quantitative approach, which makes it difficult to correlate banding patterns with quantitative data obtained from soil analysis, and it provides limited information on physiological and ecological traits of organisms. Other limitations include the low resolution of the gel electrophoresis, i.e. DGGE analysis of 16S rDNA fragments is suitable to detect microbial groups rather than species; bias due to PCR amplification and DNA extraction; the interpretation of results can be complicated due to co-migration (1 band = several species) and sequence heterogeneities between different operons (several bands = 1 species); mainly predominant species are detected; and viable as well as non-viable organisms are extracted (von Wintzingerode *et al.* 1997; Tiedje *et al.* 1999; Heuer *et al.* 2001; Nannipieri *et al.* 2003; Kirk *et al.* 2004). Despite these limitations, I considered DGGE analysis a useful and suitable tool to compare environmental samples that were obtained and amplified under similar conditions and, more importantly, technical expertise and equipment was available and accessible which made it feasible to use this method continuously throughout the experiment. Results presented in Subsection 2.3.2 show that DGGE analysis provided reproducible results and that it was possible to distinguish the two soils based on the banding patterns.

Phospholipids are a constant proportion of microbial biomass found in the membranes of living cells that can be analysed by gas chromatography/ mass spectrometry. PLFA analysis is, hence, a broad measure of the size of the viable microbial community (PLFAs rapidly break down when cells are lysed (Tunlid and White 1992)) and can give information on relative structural changes and population dynamics of the whole community rather than on specific groups. It is based on the extraction of the lipid fraction and the analysis for fatty acid patterns. Signature fatty acids can give information about the presence of different microbial groups, however, the method does not provide insight into species evenness (relative number of individuals per species) (Zelles *et al.* 1992; Frostegård *et al.* 1993; Paul and Clark 1996; Bossio *et al.* 1998). PLFA analysis is very labour intensive and I decided that the additional information that this method could provide did not justify the work and time needed. In addition, resources and equipment necessary to use this method were unavailable and/ or inaccessible, so I decided against further assessment and use of PLFA analysis.

Functional diversity

Functional diversity (or metabolic potential) can be determined by measuring community level physiological profiles using Biolog™ or catabolic response profiles (CRP).

Biolog™ profiling (Anonymous 1999) is used to measure the functional diversity or metabolic potential of the fast growing bacterial community in soils by determining C source utilisation patterns which are indicated by colour development in the wells. This simple method allows for large numbers of samples to be processed. However, the method relies on extracting microorganisms from soil samples and is ultimately a culturing approach. This produces bias by favouring culturable bacteria that are able to metabolise under the given conditions (temperature, humidity, pH). Also, changes in the microbial community can occur during incubation. Consequently, the metabolic profiles are not an accurate representation of the structure and function of the microbial community present in the soil. It is also expected that the bacterial community would eventually adapt to the available C compounds and organisms might be able to metabolise exudates of others, which would also result in a measurable change of colour. Interpretation and statistical analysis can be also problematic (Zak *et al.* 1994; Haack *et al.* 1995; Garland 1997; Insam and Rangger 1997; Smalla *et al.* 1998). Considering the limitations of the method and the amount of time needed to obtain and analyse data I decided that Biolog™ would not add value to the experimental data.

Similar to Biolog™, catabolic response profiles (CRP) measure the potential of a soil microbial community to metabolise different C substrates. In contrast to Biolog™, CRP does not rely on soil extractions and determines the response of the entire microbial community rather than bacteria alone. For CRP, CO_2 evolution is measured from soil samples after addition with a range of C compounds (like SIR which is based on the addition of glucose) (Degens 1998). This method has not been widely used (Nannipieri *et al.* 2003) and determining respiration relies on an incubation/ titration approach (similar to SIR and basal respiration) which is lengthy and not practicable in combination with other methods. I did not consider this approach any further.

Summary

The final suite of methods consisted of C_{mic}, N_{mic}, ADA, FDA and DGGE analysis and was chosen based on a combination of factors including sensitivity and consistency of results, and practicality (availability and accessibility of equipment and expertise). I also considered the length of time required for each method (incubation periods, soil and statistical analyses) and how much value it added to the study. The other main deciding factor was the requirement to work on fresh soil and perform all tests at the same time. The chosen methods were complementary and addressed different aspects of microbial diversity. They did not require additional, specialised equipment, could be carried out using existing facilities and all analyses could be completed within a few days after sampling.

1.3 Site description

Two sites under the same environmental conditions (approximately 2 km apart) and the same soil type were identified within the cropping farm at Lincoln University, Canterbury, New Zealand (43°38'S; 172°27'E), that had been farmed under contrasting organic and conventional management systems for a substantial period of time:

(a) Lincoln University Biological Husbandry Unit (BHU), established in 1976 and managed under 'organic' (i.e. non-conventional) (BioGro New Zealand 2001) cropping for 25 years at the time the experiment commenced;

(b) Lincoln University Cropping Farm (LCF), established over 100 years ago and managed conventionally.

The soil at both sites was a Wakanui silt loam (free draining to 75 cm) (Mottled Immature Pallic Soil, NZ classification; Udic Ustochrept, USDA) with broadly comparable chemical and physical topsoil properties (Table 3) (see Appendix I for soil profile descriptions).

The BHU lysimeters were taken from a low input area that had been under a six-year rotation until 1999 and had not been managed between then and the time of sampling. The rotation included a one year mixed herb lay (14 species including lucerne (*Medicago sativa*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*)) was followed by high fertility crops, such as potatoes (*Solanum tuberosum*) or brassicas, followed by mixed grains, onions/ garlic/ leeks (*Allium* spp.) followed by green crops, corn (*Zea mays*) or beans (*Phaseolus vulgaris*) (undersown with green crops) and then squash (*Cucurbita* spp.), beet (*Beta vulgaris*) and lettuce (*Lactuca sativa*). There was no record of any fertiliser inputs or compost applications during this period. After the six-year rotation, a restorative herb-ley consisting of red/ white clover, chicory (*Cichorium intybus*), timothy grass (*Phleum pratense*) and perennial ryegrass (*Lolium perenne*) followed a tomato crop (*Lycopersicon esculentum*) with no fertiliser additions. A rotary hoe was used for preparation of the tomato area to around 10 cm depth. There were no records of any inversion ploughing and most of the residues were left on the surface (Horrocks 2002).

The LCF site had been under pasture for almost 2 years when the samples were taken and prior to that had been under an 8-year rotation including oats (*Avena sativa*) for grazing, wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), ryegrass (*Lolium perenne*), wheat, vining peas (*Pisum sativum*) and pasture. During the rotation, residues were incorporated to a depth of 15 cm by ploughing. The site received a restorative management with 2 years out of 8 in pasture. The total annual amount of N applied during the 8-year rotation was 70 kg N per ha (as urea and Cropmaster 20), while a total of 16 kg phosphorus (P) per ha was applied (mainly as water-soluble monocalciumphosphate).

Table 3: Chemical and physical soil properties of BHU and LCF topsoil samples (0-15 cm).

Soil property	*BHU*	*LCF*
C (%)	2.73	2.91
N (%)	0.24	0.24
C:N ratio	11.4	12.1
S (μg g^{-1})	260	300
pH	6.1	5.7
soil resin P (μg g^{-1})	45	37
total P (μg g^{-1})	813	771
CEC ($cmol_+$ kg^{-1})	14	14
Ca ($cmol_+$ kg^{-1})	7.3	7.0
Mg ($cmol_+$ kg^{-1})	0.79	0.56
K ($cmol_+$ kg^{-1})	0.76	0.39
Na ($cmol_+$ kg^{-1})	0.17	0.19
water holding capacity (%)	27.2	31.6
bulk density (g cm^{-3})	1.44	1.38

It has to be emphasized that the soil sampling for this thesis was carried out over a 2½-year period. The lysimeters (Section 3) were taken from the areas described above while the soil samples for the farm site comparison (this section) and the incubation studies (Section 4) were taken from the areas described above as well as directly adjacent areas. The areas have the same soil type and both BHU areas were under a similar herb-ley while the LCF areas were at varying stages of cultivation at the time of sampling (farm site comparison: January 2002: pasture; March 2002: cultivated; June 2002: barley crop; incubation studies: pasture). Sampling different areas and different stages of cultivation was unavoidable as BHU and LCF are actively managed farms and the management of the areas was independent from this study. All sampled sites at BHU and LCF had the same soil type and similar fertility, however, for the lysimeter collection it was necessary to find areas that were free draining to 70 cm (the usual sampling depths for lysimeters; see 3.2.2, Section 3), which was the main reason for the choosing the sites.

With regard to the farm site comparison, using different areas might have increased variation of microbial biomass, activity and diversity among the sampling dates and made comparing of the three sampling points to each other more difficult. However, the main objective was to evaluate the proposed methods. Comparing the biological soil properties of the sites was more a fortunate consequence. For the incubation studies, it was important to collect soils from areas under similar plant cover. Using soils from different sites than in the other experiments was not a problem, since the incubation studies were independent experiments.

2 Farm site comparison using selected methods

2.1 Introduction

The objective of the farm site comparison was to determine the suitability and usefulness of the proposed methods (C_{mic}, N_{mic}, ADA, FDA and DGGE analysis) by way of comparing the organic and conventionally managed soils for initial differences in microbial biomass size, activity and structure.

2.2 Materials and methods

2.2.1 Soil sampling

Soils from BHU and LCF were analysed on several occasions to determine differences in chemical and biological soil properties that might relate to farm management history and to evaluate the suitability of the proposed methods. In March, April and June 2002, topsoil samples (0-15 cm) were taken from each site by removing blocks of soil (10x10x15 cm) with a spade. Ten samples were bulked and mixed before placing a subsample (approximately a quarter of total) in a plastic bag. The samples were stored in a cooling bin with ice until they were brought back to the lab. Immediately after sampling, all samples were passed through a 4 mm mesh to remove roots and other plant material. Sieved samples were stored at 4ºC in the dark for up to 5 days before analyses took place. All analyses were carried out in triplicate on field moist soils.

2.2.2 Analysis of microbial and biochemical soil properties

Microbial biomass C and N

Microbial biomass C (C_{mic}) and N (N_{mic}) were estimated by fumigation-extraction following the method of Sparling and West (1988) using an extraction ratio of 1:4. Total extractable C was determined on a TOC-5000 A analyser (Shimadzu), and total extractable N was measured by persulphate digestion following the method of Cabrera and Beare (1993). For conversion of total C and N to C_{mic} and N_{mic}, factors of k_{eC} = 0.35 (Sparling *et al.* 1990) and k_{eN} = 0.54 (Brookes *et al.* 1985) were used, respectively.

Arginine deaminase activity (ADA)

The method of Alef and Kleiner (1987) as described in Alef and Nannipieri (1995) was used to measure ADA. Arginine solution (1.25 ml, 0.2% w/v) (sample) or deionised water (blank control) was added to 5 g of soil after 15 min of preincubation at 30°C. After incubation for

3h at 30°C, all samples were immersed in liquid N for 10s to stop the reaction. Following extraction with 2 M KCl (extraction ratio 1:4), samples were filtered (Whatman No. 42) and ammonium-N (NH_4^+-N) concentration in the samples was determined by automated flow injection analysis (Tecator, Sweden).

Fluorescein diacetate (FDA) hydrolysis

In April and June, the rate of FDA hydrolysis in the soils was determined according to Adam and Duncan (2001). Briefly, 15 ml of 60 mM potassium phosphate buffer (pH 7.6) was added to 2 g of field moist soil. FDA stock solution was added to a triplicate set of samples; blank controls were prepared with buffer only. After 20 min of incubation, the reaction was stopped by adding 15 ml of chloroform/ methanol (2:1). After filtering the solutions (Whatman No. 42), absorbance was measured at 490 nm on a UV Visible Spectrophotometer (Cary 50, Varian Australia Pty Ltd.).

Total carbon and nitrogen

Air-dried and sieved samples (2 mm) were analysed for total C (C_{tot}) and N (N_{tot}) on a Leco® CNS-2000 elemental analyser.

2.2.3 Analysis of soil microbial community structure

In June 2002, community DNA was extracted from the soil samples in duplicate using the UltraClean™ Soil DNA kit (MoBio Laboratories, Inc., USA) according to manufacturer's instructions and stored at -20°C until further processing. 16S rDNA fragments of the samples were amplified by PCR using eubacterial primers F984GC (position 968-984 numbered according to the *E. coli* 16S rDNA sequence (Brosius *et al.* 1981)) (CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC) and R1378 (position 1378-1401) (CGG TGT GTA CAA GGC CCG GGA ACG). A GC-rich sequence was attached to the forward primer (sequence underlined) to prevent complete melting during DGGE separation (Muyzer *et al.* 1993). For all amplifications, 25 µl reaction mixtures were used (Table 4) and the following thermal cycling conditions were chosen: initial denaturation for 5 min at 94°C, followed by 35 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 53°C, and primer extension for 2 min at 72°C, ending with a final extension step for 10 min at 72°C.

Table 4: Composition of amplification reaction mixture.

Reagents and concentrations	*Final volume*
HotMaster Taq buffer (10x) (Eppendorf, Germany)	2.5 µl
Deoxynucleotide triphosphate (dNTPs) (2.5 mM)	2 µl
Bovine serum albumin (BSA) (0.01 g ml^{-1}) (in 1st round only)	1 µl
Forward primer (10 µM)	1 µl
Reverse primer (10 µM)	1 µl
HotMaster Taq DNA polymerase (Eppendorf, Germany) (1.25 units)	0.25 µl
Template DNA (1st round) or 1/200 diluted PCR product (2nd round)	1 µl
Total volume	25 µl

Amplified DNA was verified by electrophoresis of aliquots of PCR mixtures (5 µl) in 1% agarose in 1×TAE buffer (0.04 M Tris-acetate, 1 mM EDTA; pH 8.5). The DCode Universal Mutation Detection System (Bio-Rad, Hercules, CA) was used for the DGGE (Denaturing Gradient Gel Electrophoresis). Six microliters of PCR product plus 4 µl of water were loaded onto an 8% (w/v) acrylamide gel (acrylamide/bis solution, 37.5:1) with a linear chemical gradient (7 M urea and 40% (v/v) formamide) of 40-70%. The gels were run in 1×TAE buffer (preheated to 60°C) for 10 min at 200 V followed by 16 h at 80 V. The gels were silver stained to detect DNA using a standard protocol (Sanguinetty *et al.* 1994). The following solutions were used: fixation solution (20% (v/v) ethanol and 1% acetic acid), staining solution (0.2% (w/v) $AgNO_3$), developing solution (1.5% (w/v) NaOH; 0.3% formaldehyde; spatula tip $NaBH_4$) and preservation solution (25% ethanol; 10% glycerol). Gels were dried overnight at 60°C before being scanned using a GS-700 Imaging Densitometer (Bio-Rad, USA).

2.2.4 Statistical analysis

All numerical data was analysed by general linear model analysis of variance using GenStat Release 7.1 (©2003, Laws Agricultural Trust, Rothamsted Experimental Station, UK). Least significant differences ($LSD_{0.05}$) were calculated when samples were significantly different. Cluster analysis following Ward's method (1963) was performed on DGGE profiles using Quantity One 1-D Analysis Software (Version 4.5.2) (Bio-Rad, USA).

2.3 Results

2.3.1 Microbial and biochemical soil properties

Comparing the measured soil properties and the microbial quotient (C_{mic}:C_{tot}) in the two soils showed that microbial activity (ADA) was significantly higher in BHU (Figure 4), while LCF

had significantly higher levels of microbial biomass C (Figure 5), organic C and, consequently, a higher microbial quotient (Table 5) at all sampling dates.

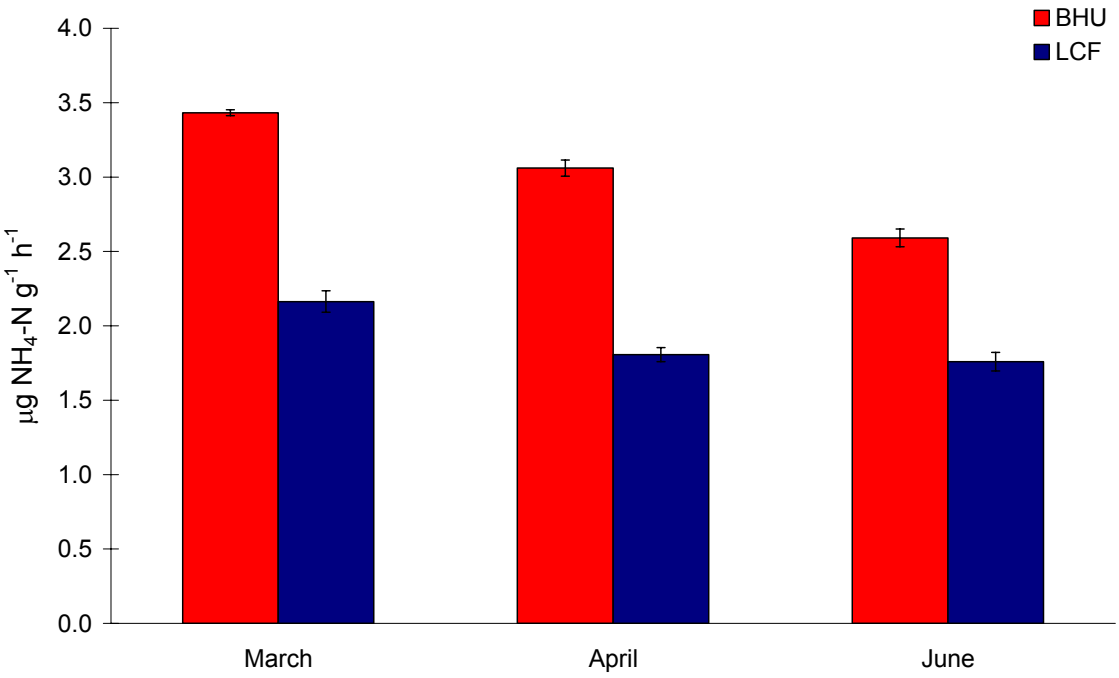

Figure 4: Mean rates (µg g^{-1} h^{-1}) of arginine deaminase activity in BHU and LCF soils determined in March, April and June 2002. Bars indicate standard errors of means. n=3. $LSD_{0.05}$=0.30.

Table 5: Mean values (of three sampling dates) and levels of significance for soil properties measured in BHU and LCF topsoil samples (0-15 cm).

Soil property	*BHU*	*LCF*	*Significance*
C_{mic} (µg C g^{-1})	494 (25.0)	596 (26.4)	***
N_{mic} (µg N g^{-1})	50.1 (3.42)	47.6 (2.49)	NS
ADA (µg NH_4-N g^{-1} h^{-1})	2.86 (0.10)	1.91 (0.08)	***
FDA (µg fluorescein g^{-1} h^{-1})	115 (9.3)	123 (12.1)	NS
C_{mic}:C_{tot} (%)	1.93 (0.05)	2.25 (0.04)	**
C_{tot} (%)	2.77 (0.01)	2.93 (0.04)	**
N_{tot} (%)	0.242 (0.001)	0.243 (0.003)	NS

Standard errors of means in parentheses. ***, p<0.001; **, p<0.01; NS, not significant. n=9 for C_{mic}, N_{mic}, and ADA. n=6 for FDA. n=3 for C_{tot}, N_{tot} and C_{mic}:C_{tot}.

Levels of N_{mic}, N_{tot} and FDA were consistently higher in LCF; however, these differences were not significant overall (Table 5). Seasonal variation was similar for both soils: Microbial activity (ADA and FDA) decreased over time, while microbial biomass (C_{mic}) increased. Levels of N_{mic} were highest in March and lowest in April.

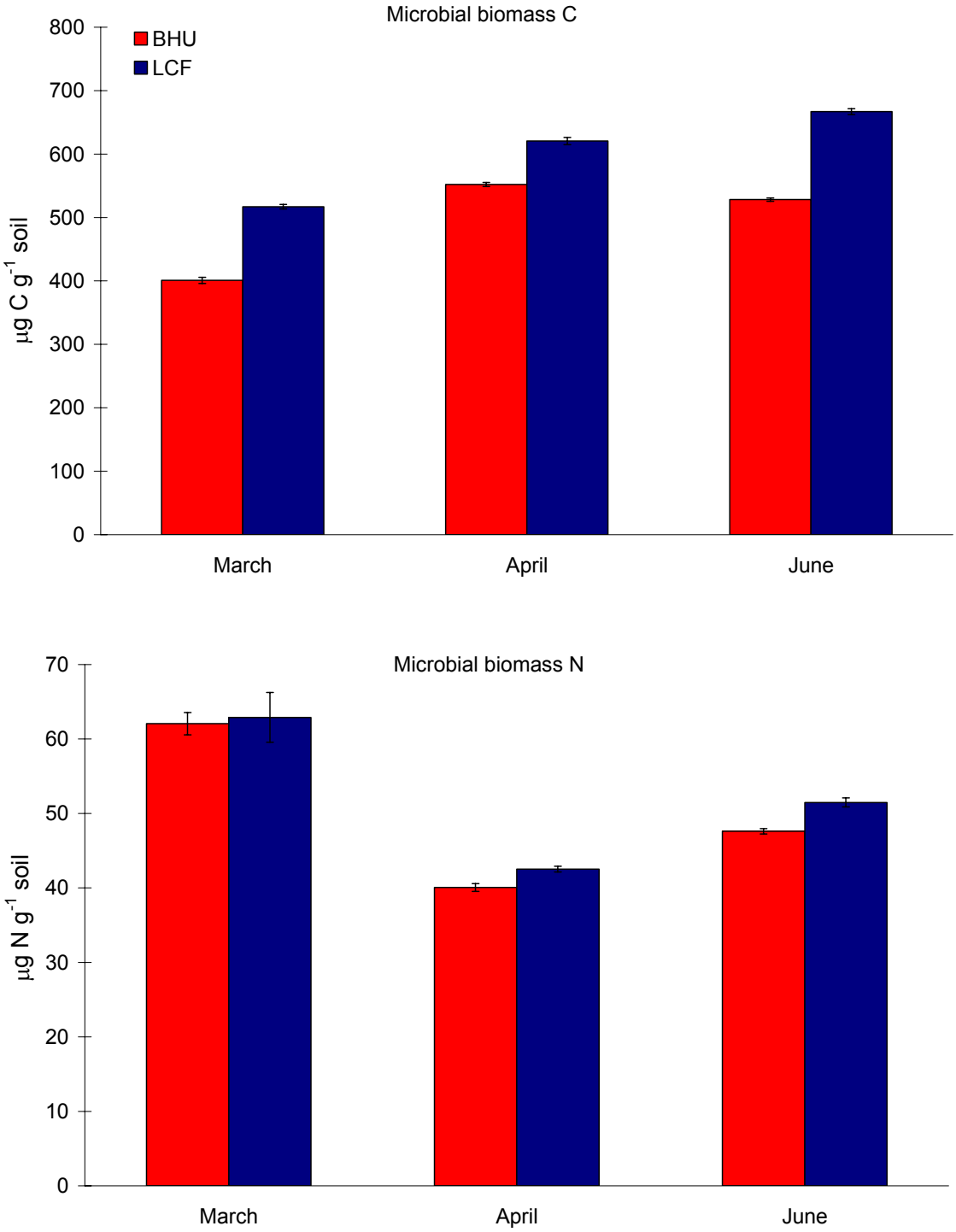

Figure 5: Mean concentrations (µg g^{-1}) of microbial biomass C and N in BHU and LCF soils determined in March, April and June 2002. Bars indicate standard errors of means. n=3. $LSD_{0.05}$=55.8 (C_{mic}) and 9.9 (N_{mic}).

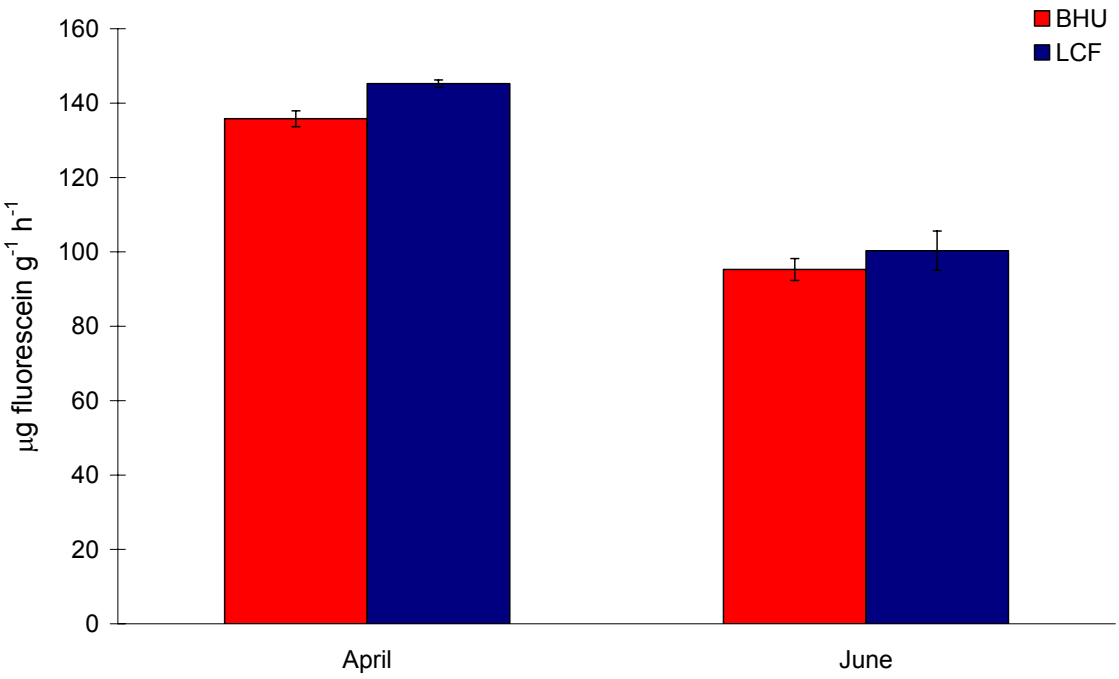

Figure 6: Mean rates (µg g^{-1} h^{-1}) of fluorescein diacetate hydrolysis in BHU and LCF soils determined in April and June 2002. Bars indicate standard errors of means. n=3. $LSD_{0.05}$=25.8.

2.3.2 Soil microbial community structure

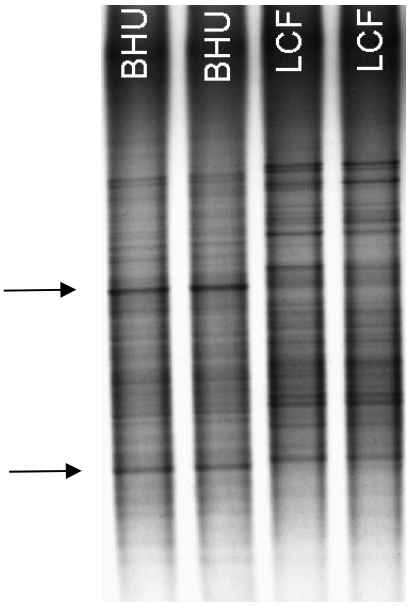

Figure 7: Soil bacterial communities from BHU and LCF after DGGE separation of 16S rDNA fragments in June 2002. Each soil is represented by two lanes corresponding to duplicate DNA extractions. Arrows mark most prominent differences.

Figure 7 shows the DGGE patterns of the bacterial communities that were amplified from the two soils. Both profiles had similar numbers of bands and the DNA extraction method and amplification showed good reproducibility with the duplicate profiles being nearly identical.

In the BHU profile, however, two strong bands stood out while LCF showed a large number of bands of equal intensity.

2.4 Discussion

The farm site comparison was primarily conducted to test the available methods, which is why soils were sampled irregularly throughout the year. Not all assays were performed at all points in time (FDA in March and June, DGGE in June only) and the varying plant covers and cultivation stages at LCF (see Chapter 1.3, this section) were considered to be of no consequence for this study.

Measurements of microbial biomass and activity in soil samples from BHU and LCF showed no significant differences between the two farms for FDA, N_{mic} and N_{tot}. However, significantly higher levels of ADA were measured in BHU and elevated C_{mic} and C_{tot} in LCF. The seasonal variation was similar for the two sites suggesting that differences due to changes in crop cover and cultivation at LCF can be neglected.

The higher levels of microbial biomass (C_{mic}) in LCF were expected, despite the general perception that organic farming systems are more beneficial for the soil microbial community and stimulate microbial diversity (e.g. Mäder *et al.* 2002; Girvan *et al.* 2003; Hole *et al.* 2005). The BHU samples came from an area that had not been managed for approximately 3 years prior to this experiment while the LCF site had been grazed, cultivated and fertilised regularly. The literature suggests that crop rotations and fertilisation have a positive influence on the soil microbial biomass through greater return in crop residues (Campbell *et al.* 1991; Campbell *et al.* 1992; Wander *et al.* 1995). Nsabimana *et al.* (2004) found that enzyme activity (arginine deaminase activity and FDA hydrolysis) per unit of microbial biomass correlated strongly with respiration to microbial biomass ratio (metabolic quotient). Both ratios can serve as indicators of disturbance as well as efficiency of the microbial community with lower levels indicating a less efficient, more stressed community (Miller and Dick 1995; Wardle and Ghani 1995; Bending *et al.* 2004). Based on this assumption, the larger microbial biomass combined with lower levels of ADA in LCF suggest that the microbial community in LCF is more efficient compared to BHU (i.e. less energy is used for maintenance). The different results for FDA as compared to ADA can be explained through the possible involvement of extracellular enzymes in the hydrolysis of FDA (see Chapter 1.2, this section). Dick (1997) indicated that extracellular enzymes add a "historical component" to the assessment of microbial soil properties and soil quality and that they can point towards long-term influences of soil development and management on soil biological properties. The similar levels of FDA observed for the soils in this study (Table 5) are, therefore, not surprising and most likely linked to inherent soil properties like soil type and long-term management practices that might have affected chemical soil properties, which were very similar for the two soils (Table 3).

The DGGE analysis showed clear differences between the profiles for BHU and LCF, indicating that the species composition of the eubacterial communities was distinctly different in the two soils. However, the number of bands (species richness) were similar in both profiles, suggesting a comparable number of different species was present in both soils, despite significant differences in microbial biomass size. This implies differences in species evenness which is also suggested by the intensity of the different bands (BHU: small number of dominant species; LCF: large number of bands of equal intensity (Heuer *et al.* 2001)). The differences observed in ADA activity (BHU>LCF) might be reflected in the banding patterns and could relate to the two strong bands in the BHU profile (Figure 7). DGGE analysis was performed at only one sampling, which was considered sufficient to establish the usefulness of the method for subsequent experiments. However, one data set was not enough to draw conclusions regarding the effect of management history on microbial diversity and to reveal links between microbial diversity and functions (one aim of this research). In addition, correlation of DGGE profiles with the numerical data of the soil analyses is problematic and interpretation remains subject to speculation.

The lack of significant differences in microbial soil properties between the two soils suggests that past management and the more diverse plant cover at the BHU site (mixed herb ley vs. pasture at the time of sampling) did not have a lasting, positive effect on soil microbial biomass and activity. This gave the first indication that the influence of management history on soil biology was less than that of short-term management in the two sites. It is more likely that particular management practices, e.g. cultivation practices, rather than the use (or lack thereof) of pesticides and mineral fertilisers affect microbial biomass and diversity (Fraser *et al.* 1988; Hole *et al.* 2005).

3 Soil microbial diversity as determined by soil dilution plating

3.1 Introduction

A simple, easy way to assess structural diversity of the soil community is soil dilution plating (or plate count technique), which is considered a traditional approach to quantify microbial organisms. Soil extracts are plated on different agars and soil microorganisms are cultivated which allows for enumeration and identification of the organisms present. By using various culture media, specific groups of microorganisms (e.g. fungi, bacteria or individual species) can be directly targeted. However, only culturable soil microorganisms are recovered which may constitute only 1-10% of the entire microbial community (Stotzky 1997; Torsvik and Øvreås 2002). The plate count technique usually suffers from low reproducibility and high variability and correlates poorly with measures of microbial function and activity (Elmholt 1996; Roper and Ophel-Keller 1997). Using dilution plating to evaluate microbial diversity might, nonetheless, produce key species or indicator organisms that can be easily identified and are representative of particular management practices.

The specific objectives of this part of the study were:

- to determine microbial structural diversity in soils from contrasting farming systems by quantifying total numbers of bacteria and fungi;
- to identify one or several key species that are indicative of the management system by subculturing and identifying a subset of the isolated organisms.

3.2 Materials and methods

3.2.1 Soil dilution plating

Fungal, bacterial and actinomycete populations in organic and conventionally managed soils were enumerated using a colony forming unit (cfu) assay. Soil dilution plates were prepared from fresh soil on the day of sampling. On three different occasions (January, March, and June 2002), soil samples were collected from BHU and LCF as described in Chapter 2.2 (this Section) and passed through a 2 mm mesh. Remaining roots and plant parts were removed by hand. From each soil sample, three subsamples were processed by shaking 10 g of soil end-over-end with 90 ml of sterile water agar (0.01% w/v) (WA) and a ten-fold dilution series was prepared in WA up to a dilution of 10^{-6}. Spread plates were prepared in triplicate from every second dilution (10^{-2}; 10^{-4}; 10^{-6}) and on four different media (Staley 1996). All plates were incubated in the dark at 20°C, and fungal, bacterial and actinomycete (June sampling only) colonies were counted after 3-5 days (bacteria) and 2 weeks (fungi), respectively. The number of cfu g^{-1} dry soil was estimated by taking the soil dilution factor and soil moisture content

into account. The following media were used: Czapek Dox agar (CDA) (for fungi/yeasts/actinomycetes) (Oxoid Ltd., England); Nutrient agar (NA) (general medium for bacteria) (Oxoid Ltd., England); *Trichoderma* selective medium (TSM) (from Lincoln University) (McLean 2001); King's medium B (KB) (selective for fluorescent *Pseudomonas*)* (Atlas 2004). Based on the results of the March assay and on the recommendation that about 30 to 300 colonies per plate are required to be able to reliably determine numbers of cfu in the dilution and consequently the soil, the following two assays were carried out with 10^{-2} and 10^{-4} dilutions only.

After enumeration of colonies, the plates were assessed visually and selected fungal colonies were subcultured onto Hay agar (HA), Potato carrot agar (PCA), Glycerol nitrate agar (G25N) and Malt extract agar (MEA)* and colonies were identified to genus level (*Penicillium* and *Trichoderma* according to Pitt (1979) and Rifai (1980), respectively) based on morphological characteristics by a fungal taxonomist.

Because of the results obtained by bacteria counts (see 3.3.1, this section) (no significant differences in numbers in the two soils), bacterial colonies were not subcultured and identified.

3.2.2 Statistical analysis

All analyses were done by general linear model analysis of variance using Minitab® for Windows Release 14.1 (©2003, Minitab Inc., USA) on total and $\log_{10}$ transformed values where appropriate. Least significant differences ($LSD_{0.05}$) were calculated to indicate significant differences between samples ($p<0.05$).

3.3 Results

3.3.1 Total numbers of microorganisms

Figure 8 shows $\log_{10}$ transformed numbers of bacterial and fungal cfu that were isolated from BHU and LCF on different growth media. Higher numbers of organisms were recovered from LCF on most media (exceptions: bacteria cfu on TSM [TSM b]) and the differences were significant for fungi on CDA ($p<0.001$) and TSM ($p=0.036$). Significantly more bacteria were recovered from BHU compared to LCF only on TSM ($p<0.001$). The bacterial count for January seems unusual, being two orders of magnitude higher than all other counts, however, variance amongst the replicates was high indicating a random effect evident only in a few replicate soil samples or spread plates, and the trends (BHU counts higher than LCF counts) were consistent for all sampling dates (Table 6).

* See Appendix I for detailed description of composition of culture media.

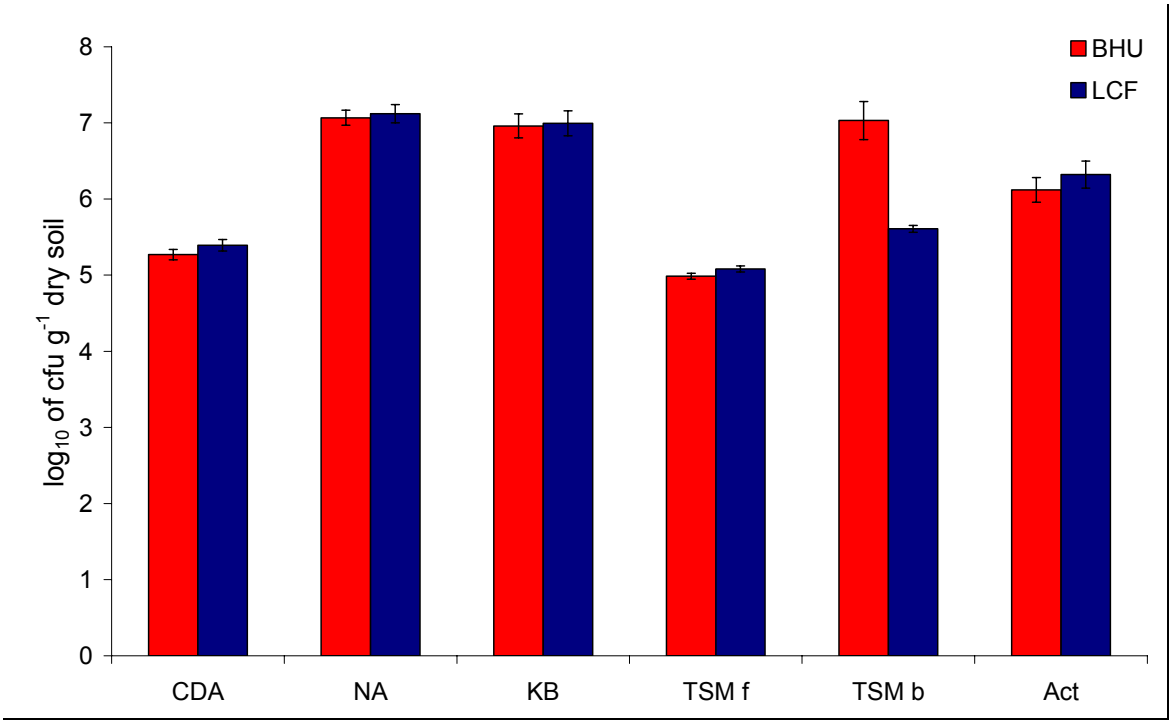

Figure 8: Numbers of bacterial and fungal cfu on four growing media isolated from soils of two sites (LCF and BHU). Numbers are log_{10}-transformed averages of three sampling dates. (CDA, fungi on Czapek Dox Agar; NA, bacteria on Nutrient Agar; KB, bacteria on King's medium B; TSM f, fungi on TSM; TSM b, bacteria on TSM; Act, actinomycetes on King's Medium B). Bars show standard errors of means. n=27.

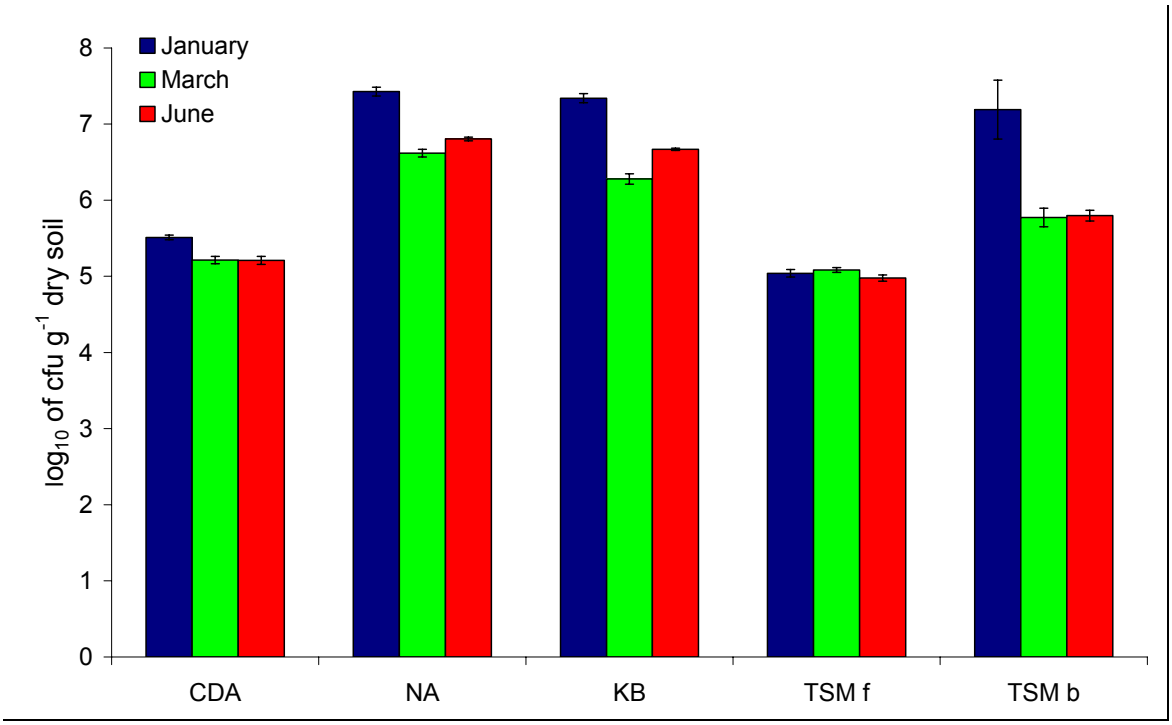

Figure 9: Numbers of bacterial and fungal cfu on four growing media at three sampling dates in 2002. Numbers are log_{10}-transformed averages of two sites. (CDA, fungi on Czapek Dox Agar; NA, bacteria on Nutrient Agar; KB, bacteria on King's medium B; TSM f, fungi on TSM; TSM b, bacteria on TSM). Bars show standard errors of means. n=18.

Table 6: Effect of sampling date (within site) on total bacterial, fungal and actinomycete numbers isolated on different culture media.

Sampling month	*Site*	*Fungi on CDA (10^5)*	*Fungi on TSM (10^4)*	*Bacteria on TSM (10^5)*	*Bacteria on KB (10^6)*	*Bacteria on NA (10^6)*	*Actinomycetes on KB (10^6)*
Jan-02	BHU	3.09 (0.32)[a]	8.96 (1.23)[a]	305 (158.7)[a]	20.7 (0.94)[a]	22.9 (3.93)[a]	
	LCF	3.39 (0.34)[a]	13.0 (1.57)[b]	4.93 (0.16)[b]	23.2 (7.69)[a]	30.7 (5.67)[b]	
Mar-02	BHU	1.27 (0.12)[a]	11.8 (1.27)[a]	8.91 (0.61)[a]	1.92 (0.38)[a]	5.18 (0.10)[a]	
	LCF	2.00 (0.51)[b]	12.5 (6.14)[a]	2.95 (1.60)[a]	1.89 (0.38)[a]	3.12 (0.18)[a]	
Jun-02	BHU	1.22 (0.11)[a]	8.33 (1.28)[a]	8.26 (0.57)[a]	4.72 (0.06)[a]	6.91 (0.44)[a]	1.32 (0.18)[a]
	LCF	2.02 (0.34)[b]	10.7 (5.65)[b]	4.32 (0.46)[a]	4.60 (0.32)[a]	5.82 (0.43)[a]	2.09 (0.15)[b]
$LSD_{0.05}$		0.36	1.93	11.3	5.64	5.0	0.66

Different letters indicate significant differences between sites for each sampling date separately ($p<0.05$). Values are means of total number of cfu g^{-1} dry soil. Standard errors of means in parenthesis. n=9.

The absolute differences between the two sites were minor (except bacteria on TSM which were at least twice as numerous in BHU compared to LCF soils) and might not have practical significance in regard to soil ecology and functioning (Table 6).

The changes in average numbers of microorganisms from both soils were assessed on three occasions (January, March, June). The differences observed between sampling dates were very small for fungi (CDA and TSM f). On CDA, significantly higher numbers were obtained in January, while the numbers did not differ much on TSM for the three sampling dates. Greatest bacterial numbers (TSM b, KB, NA) were recovered in January, followed by June, with lowest numbers recovered in March (Figure 9, Table 6).

3.3.2 Identification of fungal isolates

Characterisation of selected fungal isolates did not show major differences between the two farm soils although seasonal variations could be observed. Table 7 shows a selection of fungal species that were isolated from the two soils and were considered potential key species. Unfortunately, no particular fungal species was prominent in terms of diversity of typed isolates or occurrence and numbers of subcultured isolates were generally small (1 to 38 colonies on a total of 36 plates).

Table 7: Fungal species and number of isolates subcultured from BHU and LCF soils on three sampling dates in 2002.

	January		*March*		*June*	
	BHU	*LCF*	*BHU*	*LCF*	*BHU*	*LCF*
Number of plates fungi were subcultured from*	35	31	13	14	29	26
Penicillium spp.	17	15	4	12	2	13
Cladosporium spp.	4		7		38	21
Gliocladium spp.	1				3	
Trichoderma spp.	10	12	1	4	5	8
Mortierella spp.			1	5	1	1
Coelomycete spp.	1				1	1
Fusarium spp.	1		1			

*36 plates were prepared for each soil at each sampling in total.
Other genera that were identified on single occasions included *Chrysosporium* spp. (1), *Phoma* spp. (3), *Alternaria* spp. (1), *Ulocladium* spp. (1), *Paecilomyces* spp. (1), *Botrytis* spp. (2), *Acremonium* spp. (2), *Gonytrichium* spp. (1) and *Mucor* spp. (1).

Most fungal colonies were present on an equal number of spread plates from each site. *Penicillium* and *Trichoderma* were the most consistently and most frequently isolated types on the three sampling dates in both soils, with LCF showing slightly higher numbers. This

corresponds with LCF showing higher numbers of total fungal counts. At least three different species of *Penicillium* and *Trichoderma* were found resulting in small numbers of isolates per genera. The small and inconsistent numbers (usually only 1 or 2 per plate at 10^{-2} dilution; not consistently on all plates) suggested a random pattern of occurrence; therefore, statistical analyses were not performed.

3.4 Discussion

The first objective ("to determine microbial structural diversity in soils from contrasting farming systems by quantifying total numbers of bacteria and fungi", see Chapter 3.1, this section) has been partly fulfilled. On three occasions, soil samples were taken from BHU and LCF, which had been under long-term organic and conventional management, respectively, and numbers of bacterial and fungal cfu were determined by soil dilution plating.

Only minor differences in total numbers were detected between the two soils. However, consistently higher numbers of fungi were isolated from LCF soils, while more bacteria were found in BHU soils (not significant). This can be explained with the slightly lower pH favouring fungi in LCF soils (5.7 compared to 6.1 for BHU). The seasonal variation was the same for both soils and similar for fungi and bacteria and was caused by shifts in environmental factors, physical and chemical soil properties, as well as the fact that the LCF site had been under changing plant cover. The declining temperatures in autumn and the cultivation of the area (ploughed in March and under barley in June) would have caused lower recovery rates of organisms (especially bacteria; Table 6) in these months. This is consistent with the observation that soil organic matter and microbial biomass C are usually higher in grasslands compared with arable sites (Robertson and Morgan 1996; Grayston *et al.* 2001; Steenwerth *et al.* 2002).

The method proved to be unsuccessful for identifying key species indicative of the system of farm management (Objective 2). A range of fungal colonies that seemed promising at visual assessment (bacteria did not show any promising isolates) were isolated, subcultured and identified based on morphological characteristics, but numbers of re-occurring organisms were too small to represent trends and the same organisms were present in both soils. Despite some differences in total numbers, the microbial community composition in the two soils was very similar and no bacterial or fungal species were repeatedly isolated from either soil in large numbers. These findings are inconsistent with previous studies where organic management resulted in higher bacterial counts in soils and decreased numbers of plant pathogens (e.g. Martyniuk and Wagner 1978; Fraser *et al.* 1988; Bulluck *et al.* 2002). However, it has been reported that the positive effects on microbial counts are mainly due to organic matter amendments as part of the organic farming practices. The results are in accordance with the biological soil properties measured for the farm site comparison (Chapter 2, this section) which did not show large differences between the two soils. As observed for

microbial biomass and activity, it is likely that the lower microbial numbers in BHU and the general lack in significant differences results from the fact that the two sites were under similar plant cover at the time of sampling (pasture [LCF] and herb-ley [BHU], respectively). In addition, the soil samples from BHU came from an area that had not been cultivated for the previous 3 years, while LCF was coming to the end of a 2-year pasture phase after cropping, i.e. the two sites were providing comparable conditions for the microbial populations. This supports the theory that microorganism numbers in soils are mostly influenced by management practices that change the soil environment, such as green manure applications or crop rotations (Fraser *et al.* 1988).

In the present study, only a limited selection of isolates was subcultured after a visual assessment of the plates due to time restraints. This might have unintentionally introduced bias towards certain species and it is reasonable to assume that subculturing and identifying all isolated organisms (fungi, bacteria and actinomycetes) to species level could have shown significant differences between the farms. In addition, not all subcultured fungi could be successfully identified based on morphology alone; the application of molecular techniques might have provided additional information and allowed for a definite statement regarding the presence of indicator species. However, using molecular techniques as well as subculturing and typing all isolated organisms was considered too time consuming and, more importantly, this approach did not address the principal question of this study, i.e. the attempt to relate microbial diversity with function in soils.

Major limitations of the method are that only 1-10% of the total microbial community in the soil is culturable (Stotzky 1997), i.e. plate counts are not a good estimate for the total microbial population, and that the extraction process and the selectivity of culture media introduces bias (Insam 2001). A large part of the microbial community is closely associated with soil particles, like clays and humates, and cannot be dislodged easily and/ or do not grow on commonly used media (Schinner *et al.* 1995; Taylor *et al.* 2002). Numbers of culturable organisms correlate poorly with measures of total microbial biomass, microbial activity and ecosystem functions and viable counts are not a good indicator of soil quality (Pankhurst *et al.* 1997; Roper and Ophel-Keller 1997; Griffiths *et al.* 2001b), partly because individual populations fluctuate and are less stable than communities and community functions. Biodiversity encompasses complex interactions between the different parts of the community which cannot be assessed by studying individuals (Lawlor *et al.* 2000). These interactions are reflected in the ecosystem processes.

As mentioned previously, soil microorganisms are influenced by environmental and climatic factors and soil management, i.e. large fluctuations in numbers and inconsistencies over time and space can be expected. It is, therefore, not advised to use total numbers of certain organism groups (i.e. bacteria, or fungi) as indicators for management-induced differences (Elmholt 1996). The general agreement is that soil dilution plating can provide useful results for determining the effects of perturbations on species numbers and studying distinct groups

of organisms like nitrifiers or decomposers (Roper and Ophel-Keller 1997; Insam 2001). When assessing ecosystem processes and soil fertility, however, quantifying numbers of single species is only of value if the nature of the relationship between diversity and function has been established and we know whether this species is crucial for the functioning of the soil ecosystem. The concept of key species is thus valid; rather than expecting single species to be representative of a farming system, it would be more useful to investigate the occurrence of species that fulfil functions of interest and how their population dynamics relate to ecosystem processes (Bengtsson 1998). Using dilution plating in combination with other methods assessing microbial diversity function, e.g. molecular techniques, PLFA analysis or Biolog™, has been proposed as a useful and valuable approach that addresses different aspects of microbial diversity (Lawlor *et al.* 2000). However, plate counts are laborious and molecular tools can offer new, and more valuable insights (e.g. Insam 2001; Johnsen *et al.* 2001).

The soil dilution plating method was not continued in the subsequent experiments as it was considered too time consuming, did not reveal new information when compared to measurements of biological soil properties and did not deliver the anticipated results in terms of isolating a key species. In addition, it did not contribute to achieving the aim of linking microbial diversity in soils to ecosystem processes.

4 Conclusions

The following conclusions can be drawn from this part of the overall study.

- A variety of methods was tested regarding their suitability for the main experiments and measures of microbial biomass (C_{mic} and N_{mic}), microbial structural diversity (DGGE analysis) and enzyme activities (ADA, FDA) were found to be the most suitable range to be used in subsequent experiments.
- Significantly higher numbers of fungi were isolated from LCF while there were no major differences in bacterial numbers between the two soils. However, differences in absolute numbers were small. The composition of the fungal and bacterial communities was similar for the two sites and no one single type was consistently and repeatedly isolated from one site alone. A key species indicative of one farming system could not be identified.
- Differences in microbial biomass C (LCF higher) and ADA (BHU higher) resulted from more recent management rather than use of mineral fertilisers and pesticides; the other soil properties (N_{mic}, FDA, C_{tot}, N_{tot}) did not vary significantly between sites. Seasonal variation was similar for both soils.

- Management practices such as manure application, crop rotation, tillage system, etc. seem to have a greater influence on the soil microbial community, including microbial biomass size, activity and community structure, than the use (or lack thereof) of mineral fertilisers and pesticides.

Section 3 – Comparison of soil biological properties and leaching losses as affected by past and current management practices in intact monolith lysimeters

This section describes a lysimeter study carried out between November 2001 and October 2004. It is divided into three subsections: Chapter 1 covers the introduction, while Chapter 2 deals with the development of a soil sampling regime for lysimeters that allowed for repeated removal of soil from small areas, including the rationale, methodology, results and discussion. Chapter 3 describes the management of the lysimeters and details how the soil microbial community is affected by past and current management practices.

1 Introduction

A lysimeter experiment was designed to investigate differences in soil biological properties and leaching losses induced by past and current organic and conventional management practices. Like a pot trial, a lysimeter study has a number of advantages when compared with a field experiment. Most importantly, the experimental area is limited in size and in close vicinity to laboratories, which facilitates management, sampling and rapid sample processing. Also factors like irrigation, pest and weed control, etc., can be controlled more efficiently than would be possible in the field. In this study, using lysimeters enabled us to manage organic soils using conventional practices (including application of mineral fertiliser, herbicides, pesticides), which would be difficult in a field situation or in an on-farm comparison. In addition, lysimeters allow the measurement of leaching and/ or gaseous losses. A field study on the other hand, allows the evaluation of a management system with all influencing factors and inter-relationships (holistic approach). However, it only offers limited control over the trial area, can lack practicability (e.g. due to the size of the managed area or when dealing with large distances between field sites) and field sites can be subject to management differences that cannot be controlled. By using lysimeters, I avoided these difficulties and yet could look at the properties of a naturally layered block of soil under cultivation.

In spite of some disadvantages (the limited surface area of 0.2 m^2 might be problematic for regular soil sampling and the design of a suitable cropping sequence), I decided that intact monolith lysimeters were a suitable way to investigate links between microbial diversity and activity under field conditions and to assess the influence of past and current farming

practices on the soil microbial community and on the environment, i.e. nitrogen (N) leaching losses. The experiment aimed to answer the following question:

> What is the influence of farm management history as opposed to current management on soil biological properties, microbial diversity and leaching losses?

I developed a strategy to repeatedly take soil samples from intact monolith lysimeters under cropping and measured soil biological properties and leaching losses in two soils of the same soil type that had been under organic and conventional management for at least 25 years at the time of lysimeter collection and were then subjected to the same crop rotation (including a leguminous green manure) and managed under the original and the opposite farming system, respectively.

2 Soil sampling from intact monolith lysimeters

2.1 Introduction

In order to take soil samples from intact monolith lysimeters an appropriate sampling strategy had to be developed taking into account the spatial variation within the lysimeters (as many samples as possible) as well as the small sampling area (0.2 m^2) and the limited amount of soil that could be removed (as few samples as necessary).

Protocols have been established to take soil from different depths in the lysimeters by destructive sampling (Fraser *et al.* 1994; Cookson *et al.* 2001). However, it is not common practice to repeatedly take *in situ* soil samples from undisturbed monolith lysimeters since the type and size of lysimeters used in this study are more commonly maintained under grassland where sampling would create preferential flow pathways (Bidwell 2000; Di and Cameron 2000; Stout *et al.* 2000; Turner and Haygarth 2000). A thorough review of the literature revealed no published studies on sampling strategies for intact monolith lysimeters of the size used in this study (c. 0.2 m^2). The relatively small surface area and volume of the lysimeters were the main factors limiting the number of samples that could be taken from the lysimeter at any one time without ultimately compromising the experiment.

The objective of the present study was to develop and evaluate a strategy for regular sampling of soil from lysimeters by measuring variance and spatial variability for a range of soil biological parameters and analysing them by means of geostatistics. The results also have implications for field sampling in terms of appropriate distances between sampling points.

2.2 Materials and Methods

2.2.1 Experimental design

The spatial variability study was carried out at BHU and LCF on areas of soil equivalent to the lysimeters used in the main experiment (Chapter 3 in this section). In April 2002, soil samples were taken within 50-cm-diameter areas (surface area of a lysimeter) on each farm in a distinctive pattern (Figure 10) and a range of soil properties was tested to determine spatial variation within each area.

The three sampling areas were placed at centre-to-centre distances of approximately 70 to 90 cm from each other. This corresponded to the distance between the monoliths when the lysimeters were collected. The soil properties were measured in duplicate in each of 60 soil cores (2.5 cm diameter; 15 cm depth) – two sites, three areas per site (replicates), ten sample points per replicate (positions). The design of the sampling pattern was based on two main constraints: the limited area (c. 0.2 m^2) and volume of the lysimeters and the requirement of a relatively large number of samples and distance comparisons to guarantee meaningful

geostatistical analysis. The selected pattern ensured a variety of distances between sampling points resulting in 10 to 40 cm-distance comparisons.

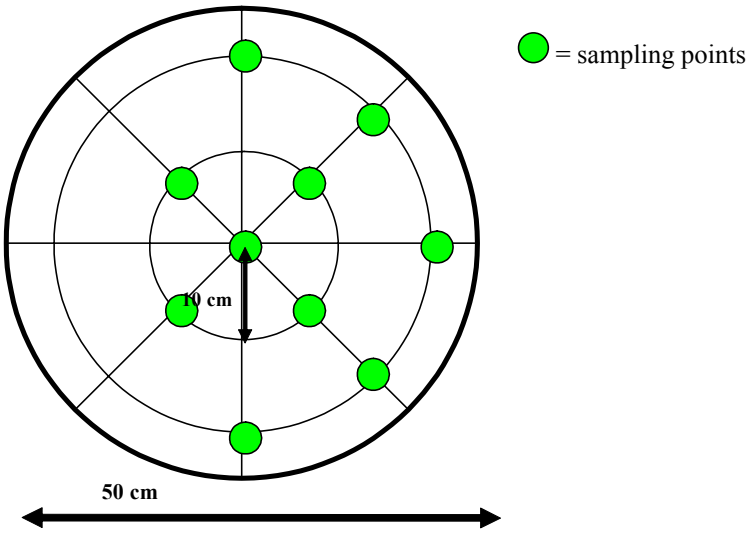

Figure 10: Sampling design tested to determine spatial variability pattern in lysimeters.

2.2.2 Soil analyses

The samples were passed through a 4 mm mesh and plant material and roots were removed. Sieved samples were stored at 4ºC in the dark for up to three weeks until analyses took place. Soils were analysed for microbial biomass carbon (C_{mic}) and N (N_{mic}) (Sparling and West 1988) and arginine deaminase activity (ADA) (Alef and Kleiner 1987). Total carbon (C_{tot}) and N (N_{tot}) were measured in air-dried and sieved samples (2 mm) on a Leco® CNS-2000 elemental analyser. For a detailed description of the methods, see Section 2.

2.2.3 Statistical analysis

Variation in biotic and abiotic soil parameters was determined by calculating coefficients of variance between positions (sampling points within each area), replicates (sampling areas) and sites. These different levels of variance were determined for the soil properties C_{mic}, N_{mic}, ADA, C_{tot}, and N_{tot} and some ratios of these (microbial C: microbial N ratio [CN_{mic}]; total C: total N ratio [C:N]; microbial C: total C ratio [C_{mic}:C_{tot}]).

Geostatistical methods were applied to study spatial variability of the soil properties (Goovaerts 1998; Legendre and Legendre 1998) (Equation 1):

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{n} [z(s_i) - z(s_i + h)]^2$$

Equation 1: Equation to calculate semivariance for soil properties at a given distance h between sampling points, where γ(h) = semivariance at a given distance h; N(h) = number of observation pairs for a given distance h; $z(s_i)$ = value of respective soil property at sampling point s_i; $z(s_i+h)$ = value of soil property at a sampling point with the distance h from s_i.

Based on frequency distributions (see Subsection 2.3.2, this section), C_{mic}, N_{mic} and ADA data were used to measure autocorrelation of positions and estimate semivariograms. For each soil property, semivariance was calculated for BHU, LCF and for the average of the two.

Five distance classes were established, 10 cm (consisting of comparisons where h = 10 cm; N(h)=18), 15 cm (h=14.1-15.3 cm; N(h)=42), 20 cm (h=20 cm; N(h)=33), 30 cm (h=28-30 cm; N(h)=33) and 40 cm (h=37-40 cm; N(h)=9). Due to the limitations in sampling area and removable soil volume, a sampling plan consisting of 10 samples was chosen; as a result the rule of thumb (at least 30 observation pairs per distance class) (Stein and Ettema 2003) could not be fulfilled for all distance classes.

Semivariograms for the three soil properties were fitted with an exponential model (based on Equation 2), which allowed the spatial dependence for the respective soil properties to be determined (Legendre and Legendre 1998).

$$y = C_0 + C_1\left(1 - \exp^{(-3x/a)}\right)$$

Equation 2: Equation to determine exponential model to fit semivariograms where y = semivariance; x = distance between sampling points; C_0 = nugget or intercept, i.e. the level of variability at distance h = 0 cm; C_1 = component related to the spatial pattern of the model; a = range, i.e. distance between sampling points at which asymptote is reached.

If spatial dependence exists, it is possible to fit an exponential model and the data will show low levels of variance for short separation distances (in this case 10 cm), followed by an increase until the asymptote is reached at a distance *a* (range). Beyond the range, the semivariance measured for the data points is independent from the respective separation distance. The sill ($C = C_0 + C_1$ [Equation 1]) indicates the variance of the asymptote and signifies the total variance of the model.

The decreasing number of pairs of observations with increasing distance between sampling points can result in too few comparisons to achieve sound results. In this case, it was possible to fit the model effectively to data derived from sampling points with separation distances from 0 to 40 cm for BHU and average, while for LCF only points from the 30 cm-distance class were included.

The data was analysed using general linear model analysis of variance in Minitab® for Windows Release 13.1 (©2000, Minitab Inc., USA). Regressions were performed in

Sigmaplot for Windows Version 5.00 (© 1986-1999, SPSS, Inc.) and R^2 was used to measure goodness of fit of data to the exponential model.

2.3 Results

2.3.1 Variance between sites, replicates, and positions

For C_{mic}, N_{mic} and ADA, the variability among the 10 positions within each area, expressed as coefficients of variance of the mean value (CV), was highest ($CV_{position}$: 78.6% [C_{mic}]; 56.1% [N_{mic}]; 50.4% [ADA]) (Figure 11). For C_{mic} and N_{mic}, this was followed by the variability between the areas within each site (CV_{area}). ADA showed a similar variability between sites (CV_{site}) as among the 10 positions ($CV_{position}$), while CV_{area} was smallest (<5%). For CN_{mic}, CV_{site} was highest (46.6%), while CV_{area} and $CV_{position}$ were both around 27%. For C_{mic} and N_{mic}, CV_{site} was regarded as zero based on the observation that in both cases the mean squares (MSq) for the sites were smaller than for the replicates.

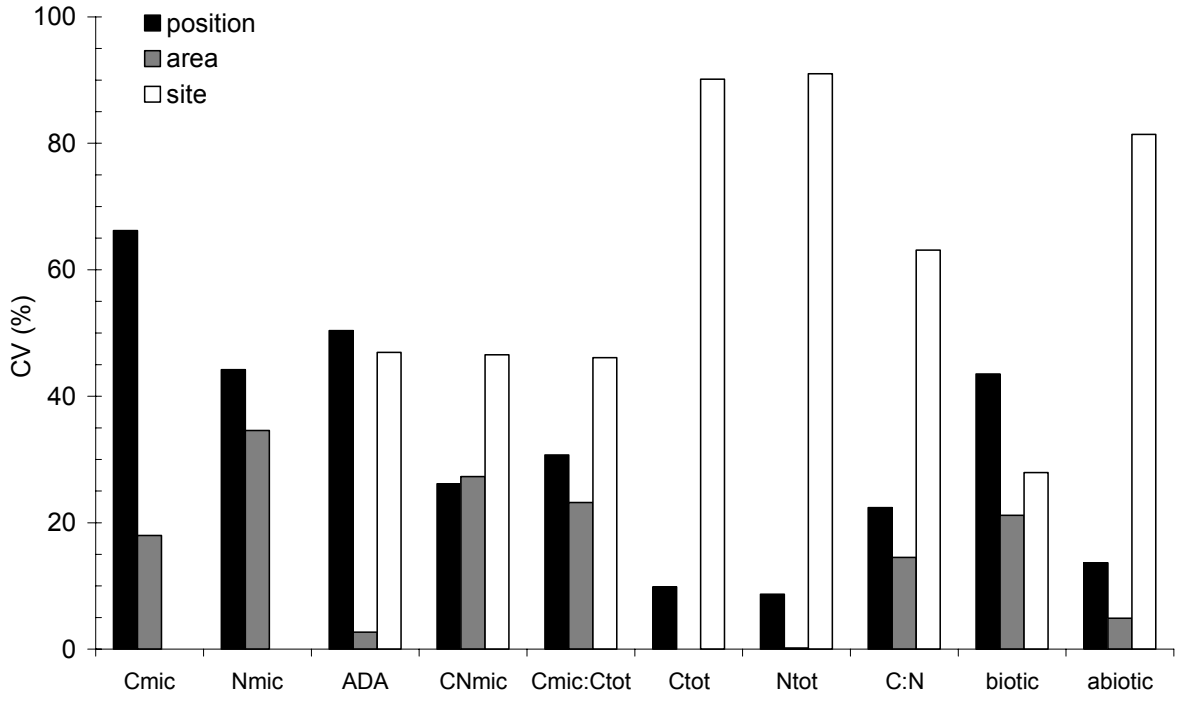

Figure 11: Coefficients of variance at different levels of variability for eight soil properties and their averages to determine spatial variability within lysimeters. position, variance between ten positions within each area; area, variance among three replicates within each site; site, variance between the sites BHU and LCF. biotic, average of biological soil properties (C_{mic}; N_{mic}; ADA; CN_{mic}); abiotic, average of biochemical soil properties (C_{tot}; N_{tot}; C:N).

The patterns of variability were similar for C_{tot} and N_{tot} and, consequently C_{mic}:C_{tot} and C:N. $CV_{position}$ was larger than CV_{area} (tendency towards zero for C_{tot} and N_{tot}), while the highest degree of variance could be observed for *site*. For C_{tot}, the error mean square was bigger than

the mean square for the effect *area (site)* (replicates within each site). Consequently, the calculated CV was negative and was regarded as zero. The average values of the biotic soil properties C_{mic}, N_{mic} and ADA show that CV_{site} was smaller than CV_{area} and $CV_{position}$ (23.4% as compared to 23.8% and 52.8%, respectively) whereas the average CVs of the abiotic soil properties show the differences between sites were larger (81.5%) than between positions (13.7%). For the average of chemical soil properties, CV_{area} was smallest with 4.9%.

Table 8: Results of ANOVA for the soil properties measured to determine spatial variability in lysimeters.

Soil property	*Position (area)*	*Area (site)*	*Site*	*MSq*
C_{mic}	***	**	NS	area (site)>site
N_{mic}	***	***	*	area (site)>site
ADA	***	NS	**	site>area (site)
CN_{mic}	***	***	***	site>area (site)
C_{mic}:C_{tot}	ND	***	***	site>area (site)
C_{tot}	ND	NS	***	site>area (site)
N_{tot}	ND	NS	***	site>area (site)
C:N	ND	***	***	site>area (site)

position (area): positions within each area; area (site): areas within each site; ***, $p<0.001$; **, $p<0.01$; *, $p<0.05$; NS, not significant; ND, not determined; n=60 for area (site) and site; n=120 for position (area)[1]. Displayed are levels of significance for the effects of position (area), area (site) and site, as well as comparisons of mean square values (MSq).

This is supported by the results presented in Table 8. The positions in each replicate could be differentiated on a significant level by measuring biotic soil properties (C_{mic}, N_{mic}, ADA and CN_{mic}). All but one property (C_{mic}) were able to detect significant differences between the sites, and this effect was highly significant for the abiotic soil properties, C_{tot}, N_{tot}, C_{mic}:C_{tot}, and C:N ($p<0.001$). Measuring abiotic rather than biotic soil properties separated the two sites best. Comparing the mean square values for site and area (site) supports these findings. For the soil properties that showed significant differences between sites, the mean square value for site (MSq_{site}) was bigger than the mean square value for areas within sites ($Msq_{area\ (site)}$) (Table 8). On the other hand, $Msq_{area\ (site)}$ is expected to be greater than MSq_{site} when differences between replicates are more pronounced than between sites. In this case, variability between sites was regarded as zero (C_{mic} and N_{mic}), as the main purpose of this study was to evaluate the spatial variability within each area (i.e. between positions).

As mentioned above, *site* had a significant effect on all but one property (C_{mic}). The mean values indicated that a significantly higher ADA activity was measured on BHU. Similarly, there were higher contents of N_{mic}, and, as a consequence thereof, higher microbial quotient

[1] For C_{tot}, N_{tot}, C:N and C_{mic}:C_{tot} N=60 for position (area), which makes statistical analysis impossible.

(C_{mic}:C_{tot}) and C:N ($p<0.001$ in both cases). LCF showed higher levels of C_{tot}, N_{tot} ($p<0.001$ in both cases) and C_{mic} (not significant) (Table 9).

Table 9: Levels and significance of the soil properties in BHU and LCF measured to determine spatial variability in lysimeters.

Soil property	*BHU*	*LCF*	*Significance*
C_{mic} (µg C g^{-1})	460.8 (16.1)	502.6 (16.1)	NS
N_{mic} (µg N g^{-1}	62.7 (2.36)	54.6 (2.36)	*
ADA (µg NH_4-N g^{-1} h^{-1})	0.96 (0.047)	0.74 (0.047)	**
CN_{mic}	7.77 (0.30)	9.61 (0.30)	***
C_{mic}:C_{tot} (%)	2.02 (0.043)	1.77 (0.043)	***
C_{tot} (%)	2.27 (0.042)	2.84 (0.042)	***
N_{tot} (%)	0.19 (0.004)	0.24 (0.004)	***
C:N	12.3 (0.048)	11.5 (0.048)	***

Values are means. Standard errors of means in parentheses. ***, $p<0.001$; **, $p<0.01$; *, $p<0.05$; NS, not significant. n=60.

Looking at the levels of significance of the three effects on average biotic and abiotic soil parameters, respectively, revealed that highly significant differences among the positions within each area and among areas within each site were detected by assessing biotic soil characteristics (Table 10). The abiotic soil properties on the other hand revealed significant differences between sites. However, they did not distinguish between the replicates within each site. These findings are supported by the results displayed in Table 8 and Figure 11.

Table 10: Significance of the effects of position (area), area (site) and site on average log-transformed values of biotic and abiotic soil properties measured to determine spatial variability in lysimeters.

Effects	*Biotic*	*Abiotic*
position (area)	***	ND
area (site)	**	NS
site	NS	***

position (area): positions within each area; area (site): areas within each site. biotic: C_{mic}, N_{mic}, ADA, CN_{mic}; abiotic: C_{tot}, N_{tot}, C:N. ***, $p<0.001$; **, $p<0.01$; NS, not significant; ND, not determined.

2.3.2 Spatial pattern determination

Frequency distribution

Across the 60 sampling points at six sampling locations, ADA varied intensely (data range = 1.6 µg NH_4-N g^{-1} h^{-1}; CV = 22.5%) and displayed a strong positive skew. Tests for normality suggest that the data was randomly distributed; hence, the changes in value seemed

to be independent from the respective sampling locations (Table 11). For C_{mic}, values covered a range of 420 µg g^{-1} soil and showed a coefficient of variance of 0.77%. The frequency distributions for C_{mic} showed a slight positive skew. For N_{mic}, concentrations ranged over 70 µg g^{-1} with a standard variation of 16.4 µg g^{-1}. The coefficient of skewness for the frequency distribution was 0.32. Tests for normality showed that in both cases the frequency distributions could be regarded as being normally distributed (Table 4). This indicates that a dependence exists between change in value and increasing sampling point distance.

For C_{tot} and N_{tot}, the frequency distributions were totally random (results not shown) indicating no correlation exists between sampling location and change in value for these parameters within the scale sampled; hence, only C_{mic}, N_{mic} and ADA were tested for semivariance.

Table 11: Descriptive statistics for frequency distribution of C_{mic}, N_{mic} and ADA measured to determine spatial variability in lysimeters.

Descriptive parameters	*C_{mic} (µg C g^{-1})*	*N_{mic} (µg N g^{-1})*	*ADA (µg NH_4^+-N g^{-1} h^{-1})*
A^2	0.320	0.394	1.14
p	0.524	0.365	0.005
Mean	481.7	58.6	0.851
StDev	97.4	16.4	0.284
Skewness	0.218	0.323	1.49
Median	480.6	59.0	0.799
SE	12.6	2.1	0.037
CV (%)	0.736	2.52	2.5

A^2, p: Anderson-Darling Normality Test.

Spatial variability

Looking at the development of semivariance in C_{mic} and N_{mic} for BHU, LCF and the average of the two with increasing distance between sampling points, it can be noticed that LCF showed much lower levels of semivariance than BHU in both, C_{mic} and N_{mic} (Figure 12). The exponential model, however, suggested a bigger nugget (intercept) for LCF than BHU and average. In all cases, the model represented the observed amounts of variance sufficiently well, although LCF showed low values for spatial dependence and R^2 (Table 12).

In ADA, the exponential models did not match the data as well as they did for the other two soil properties. For BHU, high value for the sill could be observed compared to the initial value. As shown in Figure 12, the curve does not reach its asymptote within the displayed range. For BHU, LCF and average the estimated range was higher in ADA than in C_{mic} and N_{mic}; and while values for spatial dependence were within the same range as for the other two

properties, R^2<0.9 and p>0.05 indicate that the regression was not significant for BHU, LCF and average (Table 12).

Table 12: Parameters of autocorrelation in C_{mic}, N_{mic} and ADA based on best-fit lines derived from exponential model (Equation 1).

Soil property	*Site*	*Nugget (C_0)*	*Sill ($C=C_0+C_1$)*	*Spatial dependence*	*R^2*	*p*	*range (cm)*
C_{mic}	BHU	824	16292	0.95	0.99	0.001	43
	LCF	1110	3075	0.64	0.98	0.018	31
	average	958	8788	0.89	0.98	0.003	13
N_{mic}	BHU	21	333	0.94	0.99	0.002	31
	LCF	27	61	0.56	0.96	0.04	25
	average	23	184	0.88	0.99	<0.001	22
ADA	BHU	0.023	0.126	0.82	0.81	0.082	179
	LCF	0.016	0.028	0.44	0.67	0.327	48
	average	0.019	0.047	0.60	0.81	0.081	36

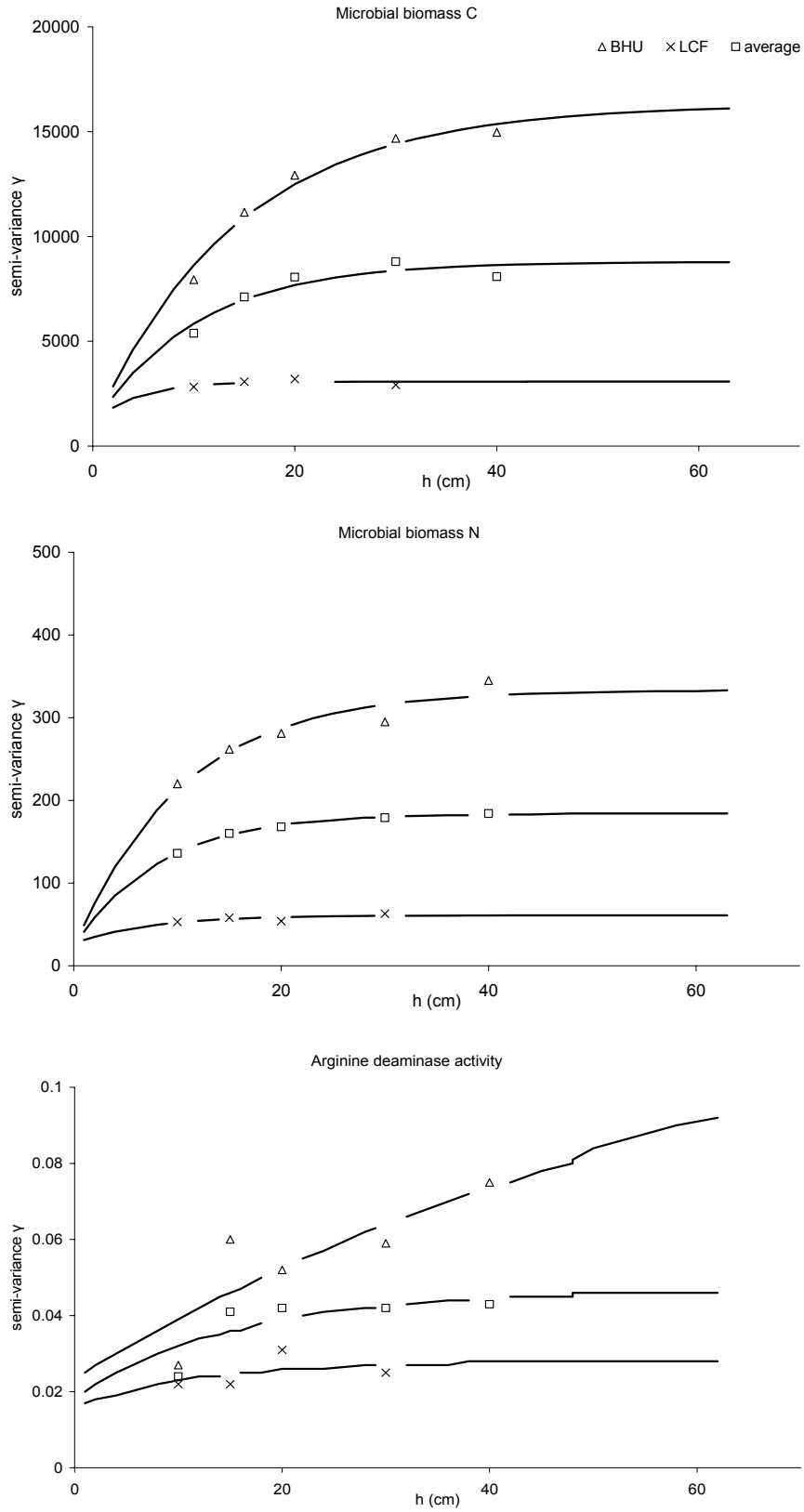

Figure 12: Patterns of semivariance (γ) in C_{mic}, N_{mic} and ADA with increasing distance (h) between sampling points for BHU (distance classes 0-40 cm), LCF (0-30 cm) and average (0-40 cm) to determine spatial variability in lysimeters. Best-fit lines (based on exponential model) are shown for BHU (average of three replicates), LCF (average of three replicates) and average (overall average).

2.4 Discussion

It is well known that soils vary spatially even over short distances. Spatial variability is understood to have an effect on transport processes in soils (Søvik and Aagaard 2003), biomass turnover rate (Harden and Joergensen 2000) and on nutrient cycling processes (de Boer *et al.* 1996; Corre *et al.* 2002). Consequently, assessing the spatial structure of a site will help develop better nutrient management strategies, and can serve as a means of designing suitable sampling patterns (Ettema and Wardle 2002; Franklin *et al.* 2002; Stein and Ettema 2003). Soil spatial structure has been investigated at different scales (cm, m, km) and with different objectives. Most studies on spatial variability have concentrated on above-ground biota and abiotic soil properties, e.g. soil moisture, hydraulic conductivity, temperature (Bruckner *et al.* 1999; Al-Kayssi 2002; Søvik and Aagaard 2003). Nonetheless, an increasing number of studies have begun to examine heterogeneity of biological soil properties in general and the microbial community in particular (Morris 1999; Harden and Joergensen 2000; Corre *et al.* 2002; Franklin *et al.* 2002; Nunan *et al.* 2002; Ettema and Yeates 2003). Depending on the scale, spatial structure in microbial soil properties is influenced by land use, soil C content, topography, plant cover (including form, size, spacing), soil aggregates, fine roots and substrate hotspots (Ettema and Wardle 2002). This implies the importance of determining spatial variability even within small areas in order to obtain meaningful bulk samples. However, the literature has revealed no evidence of the application of spatial variability data to design a soil sampling strategy for lysimeters.

The overall goal of this study was to design a protocol that allows soil samples to be taken from intact monolith lysimeters on a regular basis. Therefore, the degree of variance was determined on different levels (between sites, among replicates, among positions) and the pattern of spatial heterogeneity within the area of a lysimeter was established.

In general, results suggest that the biotic soil properties studied exhibited spatial dependence within the area of a lysimeter. The existence of spatial patterns in biological soil parameters at this scale is consistent with results shown in previous studies (Brockmann and Murray 1997; Bruckner *et al.* 1999; Morris 1999). BHU and LCF showed similar patterns of semivariance. However, spatial dependence was much smaller for LCF than for BHU (44-64% as opposed to 82-95%) (Table 5). This was expected since previous research had found that cultivation decreases spatial variability in soils (Goovaerts 1998; Röver and Kaiser 1999). The LCF soil had been cultivated a short time before sampling took place, whereas BHU had been under herb ley for approximately 18 months. This initial difference did not interfere with sampling during the main experiment given that all lysimeters were cropped and cultivated in the same way. Therefore, an adjustment of the spatial variability in the soils originating from LCF is to be expected over time.

Patterns for semivariance were similar in C_{mic} and N_{mic}, both reaching the sill within the sampled scale, whereas in ADA, semivariance displayed a different trend. This suggests that for C_{mic} and N_{mic}, samples taken within the area of the lysimeters (50 cm diameter) are

independent from each other when their separation distance exceeds 25 to 30 cm, while, according to the exponential model, for ADA, the samples will not be stochastically independent from each other within the area of the lysimeters. The random distribution of the data points indicates a nugget effect, i.e. the variance is not spatially structured at the scale measured (10-40 cm). However, spatial dependence might exist below this scale.

Statistical analysis can be problematic for autocorrelated samples since autocorrelation reduces variance within the measured bulk sample and increases variability among samples (Franklin *et al.* 2002). It is, therefore, important to collect a representative bulk sample that consists of a large number of independent samples in order to make valid statistical analysis possible. Regarding the lysimeter sampling design, this stresses the importance of taking a considerable number of independent subsamples (with separation distances >20 cm). In this study, 10 soil cores were sampled per lysimeter (Figure 1) resulting in 25 pairs with separation distances of 20 cm or more, which was sufficient to achieve meaningful results. It is difficult to largely increase the number of samples that are more than 30 cm apart given the limited area (c. 0.2 m^2). However, in order to increase the number of pairs in the distance classes 20, 30 and 40 cm, I suggest a sampling regime that consists of 11 soil cores and, consequently, 39 observation pairs per bulk sample (Figure 4). It is also important that the samples taken from the different lysimeters are independent to ensure valid statistical analysis. This can be considered the case based on the results displayed in Table 12 and Figure 12, and assuming that the lysimeters have separation distances of more than 50 cm.

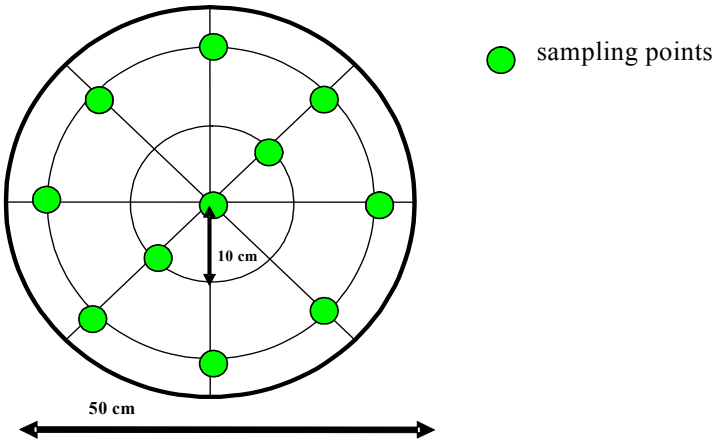

Figure 13: Revised strategy suitable for regular soil sampling from lysimeters.

The relatively higher values of $CV_{position}$ for C_{mic} and N_{mic}, ADA, and consequently for the average for biotic soil properties, imply that these soil properties can be used to recognise differences on a small spatial scale, i.e. among sampling points up to 40 cm apart, while abiotic soil properties (C_{tot} and N_{tot}) are useful for differentiating on a larger scale, in this case between sites 2 km apart (Figure 11). The methods showed good reproducibility, there was little variance between the replicates (data not shown) and for all soil parameters the

measurable differences among replicates expressed as coefficient of variance were smaller than the differences among positions or sites. Thus, one general sampling strategy can be applied for all treatments and replicates. The results suggest that this is particularly true when assessing biotic soil properties as only minor differences between replicates and sites were detected. The potential of the examined abiotic properties to differentiate between sites was of advantage after the four different treatments have been imposed on the lysimeters and treatment related effects have to be determined.

2.5 Conclusions

In conclusion, this study revealed that:

- a spatial structure exists in the biotic soil properties at the scale examined with samples being independent beyond separation distances of 25-30 cm;
- measuring biological soil properties (C_{mic}, N_{mic}, ADA, CN_{mic}) detect differences at a small-scale spatial level (<0.5 m), whereas abiotic soil properties (C_{tot}, N_{tot}, C_{mic}:C_{tot}, and C:N) can be used to distinguish on a larger scale (between sites, separated by several kilometres);
- the differences between replicates can be disregarded when designing a sampling regime for intact monolith lysimeters. Consequently, all lysimeters can be sampled following the same protocol. However, the sampling design should be adjusted and the number of subsamples increased from 10 to 11 to ensure more distance comparisons of 20 cm and over.

3 Lysimeter study

3.1 Introduction

A lysimeter experiment was designed to investigate differences in soil biological properties and leaching losses caused by past and current organic and conventional management practices. Intact monolith lysimeters were taken from areas of the same soil type that had been under either long-term organic or conventional management and, were then managed according to best organic and conventional practice. To minimise differences that influence the soil microbial community and its properties, the crop rotation was the same for all treatments and the lysimeters received the same amounts of mineral and BioGro approved fertilisers (BioGro New Zealand 2001).

The specific objectives of this study were to:

- evaluate the effect of farm management history on the soil microbial community and N leaching losses;
- evaluate the effect of current management practices on the soil microbial community and N leaching losses; and
- establish the extent of links that exist among biological soil properties, i.e. microbial biomass size, activity and diversity, *in situ*.

To achieve these objectives, the size, activity and diversity of the microbial biomass and the amount of mineral N lost were determined by repeatedly collecting leachate and taking soil samples from lysimeters. Microbial biomass C and N, enzyme activities and the genetic diversity of the microbial community were measured in the soil samples and mineral N content in the leachates.

3.2 Materials and Methods

3.2.1 Site description and weather conditions

The lysimeters were taken from the same sites that were used for the farm site comparison (Section 2) and the development of a soil sampling strategy for the lysimeters (Chapter 2, this section). BHU and LCF are two sites within the Lincoln University cropping farm (Canterbury, New Zealand) that have the same soil type and, at the time of lysimeter collection, had been under organic (BHU) and conventional (LCF) management for 25 and over 100 years, respectively. For a detailed site description, refer to Section 2.

By profiling the soils at the collection areas prior to taking the lysimeters, I ensured that the soils were free draining to 70 cm at both sites, which is the depth of the lysimeter used (50 cm diameter by 70 cm deep).

Table 15 shows that for the trial period the weather conditions in Lincoln deviated from the long-term means on several occasions. The winter months, June to August, usually have the highest rainfall. In 2003, however, rainfall was well below average for most of the year and, in 2002 and 2004, precipitation was less than average for the winter period. This would have resulted in unusually low leaching losses from the lysimeters.

3.2.2 Lysimeter collection and experimental design

In November 2001, eight replicate intact monolith lysimeters in steel casings were taken from each site, and installed in the Lincoln University lysimeter laboratory according to the protocol established (Cameron *et al.* 1992; Di and Cameron 2002a). For the months prior to data collection, the lysimeters were maintained under their original plant cover (mixed herb-ley [BHU] and pasture [LCF]) and were irrigated on a regular basis to maintain soil moisture levels adequate for plant growth.

Four lysimeters from BHU and four from LCF were randomly placed on either side of the lysimeter trench (approximately 1.5 m wide), allowing for the two sides to be maintained under different management regimes (organic [ORG] and conventional [CON]) while reducing risk of cross-contamination, spray drift, etc (Figure 14).

Table 13: Details of treatments included in the lysimeter study.

Treatment ID	*Soil origin (past management)*	*Current management*
B org	BHU (organic)	organic
B con	BHU (organic)	conventional
L org	LCF (conventional)	organic
L con	LCF (conventional)	conventional

In April 2002, the original cover was sprayed off with glyphosate (CON) or cut (ORG) and cultivated to 15 cm. For the following three years, four lysimeters from each site were managed under the original production system, while the other four were managed under the alternative management system, resulting in four treatments distinguished by farming history and current management practice (Table 13). Cropping regimes were identical for all treatments. Irrigation was applied according to crop requirements and time of year, usually 20 or 25 mm at a time. Over a 2½-year period, leachate was collected after irrigation or rainfall events (see 3.2.4, this section) and soil samples were taken after crop harvest (see 3.2.5, this section). After each soil sampling, the top 15 cm of each lysimeter was taken out, coarsely

mixed with a spade and returned to emulate cultivation and to prevent preferential flowpaths, which could have formed in the soil core holes.

Due to the limited surface area of the lysimeters (0.2 m^2), I decided that hand weeding was the most appropriate method of weed control for all treatments and application of pesticides proved unnecessary for the duration of the trial. Therefore, the main aspect distinguishing organic from conventional management practices was the types of fertilisers applied. The conventionally managed lysimeter received Cropmaster15 (NPKS 15/10/10/8) (Ravensdown Fertiliser) as well as urea (46% N) (amounts as outlined in Table 14), while the lysimeters under organic management did not receive any additional N and were fertilised with phosphate as reactive rock phosphate (8.7% P), potassium as Patentkali (25K/17S/6Mg) and sulphur as elemental sulphur (100% S) and Patentkali (17% S) (amounts equivalent to those applied to the conventional lysimeters).

The lupin green manure grown between March and September 2003 was incorporated into the soil after the weight of the above ground organic matter was established. A subsample was taken to determine C and N content in the material and the remaining sample was cut into 10 to 20 cm long pieces and mixed with the soil in the manner described above.

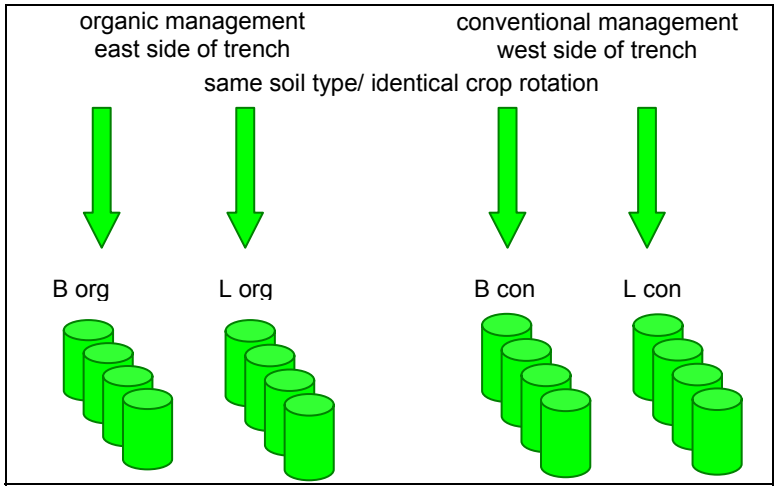

Figure 14: Schematic representation of the lysimeter trial setup.

Table 14: Cultivars, management practices and fertilisation regime for crops planted in the lysimeter study.

	Barley	***Lupin***	***Maize***	***Rape***
	Hordeum vulgare L.	*Lupinus angustifolius L.*	*Zea mays L.*	*Brassica napus L.* ssp. *oleifera*
Cultivar	County	Fest	Elita (hybrid)	Winfred
Sowing rate (kg ha^{-1})	120	200	50	3
Plants per lysimeter	40	19	4	20
Sowing date	23 May 2002	09 March 2003	11 November 2003	03 April 2004
Harvest date	29 January 2003	18 September 2003 (+ incorporation)	08 March 2004	19 October 2004
Fertilisation date	August 2002	not fertilised	November 2003	not fertilised
Amount (NPKS ha^{-1})	50/ 35/ 35/ 35 (+ 70 urea)		40/ 27/ 27/ 21 (+ 40 urea)	
Soil sampling	February 2003	November 2003	March 2004	October 2004

Table 15: Average monthly rainfall and temperature during the course of the lysimeter experiment (April 2002 - October 2004) and long term means.

	2002		***2003***		***2004***		***Long term means (1975-1991)***	
	Rainfall (mm)	*Temperature (°C)*	*Rainfall (mm)*	*Temperature (°C)*	*Rainfall (mm)*	*Temperature (°C)*	*Rainfall (mm)*	*Temperature (°C)*
January			32.4	16.0	21.0	17.9	50.3	17.0
February			15.4	15.9	43.6	15.2	51.3	16.3
March			43.6	15.2	36.8	14	58.9	15.0
April	90.8	11.5	74.6	10.4	60.4	10.6	51.8	12.2
May	26.4	9.5	23.0	10.0	61.0	10.5	50.4	8.7
June	94.6	7.3	15.0	8.7	9.0	8.5	63.0	6.3
July	15.6	5.5	61.4	5.4	40.6	5.1	73.7	6.1
August	30.2	8.1	48.4	7.2	136.8	6.2	68.1	7.6
September	43.4	11.0	86.6	9.3	34.2	9.0	40.1	9.2
October	49.4	9.9	28.8	10.6	25.4	10.8	54.9	11.3
November	86.4	12.2	35.6	12.7			55.7	13.1
December	32.2	15.3	1.2	15.8			61.3	15.7

Source: Meteor Weather Analysis Program for Windows (version 1.0) (©1994 New Zealand Institute for Crop & Food Research Ltd) (Lincoln University network: T:\METDATA\METEOWIN.EXE)

3.2.3 Initial nutrient status

Next to the lysimeter sampling sites, four replicate soil samples were taken from five different depths (0-7.5 cm; 7.5-15 cm; 15-30 cm; 30-50 cm; 50-70 cm), air-dried, sieved (2 mm) and analysed for total C and N (Table 16). Bulk density of the topsoil (0-15 cm) was measured adjacent to the lysimeter sampling areas using a combined Gamma/ Neutron probe (Model-MCS Strata Gauge) (BHU: 1.44 g cm^{-3}; LCF: 1.38 g cm^{-3}).

Table 16: Total C and N content in BHU and LCF soils at different sampling depths (0-70 cm) adjacent to lysimeter sampling site.

Depth in cm	*C (%)*		*N (%)*	
	BHU	LCF	BHU	LCF
7.5	2.91 (0.032)	2.99 (0.069)	0.26 (0.003)	0.25 (0.005)
15	2.55 (0.050)	2.83 (0.045)	0.22 (0.003)	0.23 (0.003)
30	1.73 (0.050)***	1.16 (0.123)	0.16 (0.004)***	0.11 (0.009)
50	0.38 (0.043)***	0.29 (0.009)	0.05 (0.003)	0.04 (0.0003)
70	0.27 (0.023)	0.16 (0.007)	0.04 (0.002)***	0.02 (0.001)

*** indicates significant differences between sites ($p<0.05$). n=4.

3.2.4 Leachate collection

Leachate was collected from the lysimeters after irrigation or significant rainfall events between April 2002 and October 2004. After establishing total leachate volume, a 100 ml subsample was taken, filtered through a 0.45 µm micropore filter to remove suspended particles and stored at -20ºC until analyses took place. Samples were analysed for ammonium (NH_4)-, nitrate (NO_3)- and nitrite (NO_2)-N by Flow Injection Analysis (Tecator, Sweden).

3.2.5 Soil sampling and analyses

On four occasions, soil samples were taken from the lysimeters after crop harvest[1] (Table 14) using the approach developed previously (Figure 13). In brief, 11 soil cores (0-15 cm) were taken from each lysimeter, bulked and sieved (4 mm). All plant material and roots were removed and the samples were stored at 4°C for up to 5 days before analyses took place.

The samples were analysed for total carbon (C_{tot}) and nitrogen (N_{tot}), microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) (Sparling and West 1988), arginine deaminase activity

[1] Taking soil samples during crop growth would have resulted in soil core holes and, therefore, preferential flow paths. Soil samples could only be taken after harvesting as the soils could be turned over and mixed once the crops had been removed.

(ADA) (Alef and Nannipieri 1995) and fluorescein diacetate hydrolysis (FDA) (Adam and Duncan 2001) (refer to Section 2 for detailed method description). The ratios of microbial biomass C to total C (microbial quotient [C_{mic}:C_{tot}]), total C to total N (C:N) and microbial biomass C to N (CN_{mic}) were calculated.

In addition, dehydrogenase hydrolysis activity (DHH) was measured in the soil samples at all sampling dates using tris buffer (pH 7.6) and triphenyltetrazolium chloride as substrate solution (0.6% w/v) (Thalmann 1968). Five grams of field moist soil were incubated in 5 ml of tris buffer with (sample) and without (blank) substrate solution for 16h at 25°C. After extraction with 25 ml of acetone, samples and blanks were filtered and absorbance was measured at 546 nm using a UV Visible Spectrophotometer (Cary 50, Varian Australia Pty Ltd.).

On three occasions (November 2003, March 2004 and October 2004), genetic diversity of the soil microbial community was determined by DNA extraction (UltraClean™ Soil DNA kit; MoBio Laboratories, Inc., USA), followed by polymerase chain reaction (PCR) amplification and denaturing gel electrophoresis (DGGE) as described in detail in Section 2. In November 2003 and March 2004, primers F984GC and R1378 were used to amplify 16S rDNA fragments of the soil bacterial community. In addition, AOB (ammonia oxidising bacteria) were studied in November 2003 and actinomycete communities and 18S rDNA fragments of fungi were assessed in March 2004. In October 2004, a two-step PCR approach was used to amplify 16S rDNA fragments of actinomycetes, α proteobacteria and Pseudomonads (sensu stricto). See Table 18 and Table 17 for detailed primer descriptions, PCR conditions and denaturing gradients. The PCR reaction mixture was identical to that detailed in Table 4 with the exception that no BSA was used in the second round of a nested PCR and for AOB, an equimolar mixture of the three forward primers was added (1 µl).

3.2.6 Statistical analysis

All data was analysed by repeated measures analysis of variance, general linear model analysis of variance and correlation analysis using GenStat Release 7.1 or Minitab® for Windows Release 14.1. Samples were considered significantly different when $p<0.05$ and least significant differences ($LSD_{0.05}$) were calculated. DGGE patterns were analysed by cluster analysis according to Ward (1963) using Quantity One 1-D Analysis Software (Version 4.5.2) (Bio-Rad, USA).

Table 17: Primer sequences used for amplification of 16S rDNA fragments in the lysimeter study.

Primer[a]	*16S rDNA target (positions*[b] *or product size)*	*Sequence (5' to 3')*	*Denaturing gradient*	*Reference*
F243	Actinomycetes (226-243)	GGA TGA GCC CGC GGC CTA	40-55%	Heuer *et al.* (1997)
F203α	α proteobacteria	CCG CAT ACGCCC TAC GGG GGA AAG ATT TAT	40-55%	Gomes *et al.* (2001)
R1494	Bacteria (1492-1513)	CTA CGG YTA CCT TGT TAC GAC		Weisburg *et al.* (1991)
ps for	Pseudomonads (292-311)	GGT CTG AGA GGA TGA TCA GT	40-55%	Clegg *et al.* (2003), Widmer *et al.* (1998)
ps rev	Pseudomonads (1263-1280)	TTA GCT CCA CCT CGC GGC		
F984GC	Bacteria (968-984)	CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC	40-70%	Heuer *et al.* (1997)
R1378	Bacteria (1378-1401)	CGG TGT GTA CAA GGC CCG GGA ACG		Heuer *et al.* (1997)
CTO189fA-GC CTO189fB-GC	AOB (465 bp)	CCG CCG CGC GGC GGG CGG GGC GGG GGC ACG GGG GGA GRA AAG CAG GGG ATC G	30-60%	Kowalchuk *et al.* (1997)
CTO189fC-GC	AOB	CGC CCG CCG CGC GGC GGG CGG GGC GGG GGC ACG GGG GGA GGA AAG TAG GGG ATC G		
CTO654r	AOB	CTA GCY TTG TAG TTT CAA ACG C		
EF4f	Fungi (530 bp)	GGA AGG GRT GTA TTT ATT AG		van Elsas *et al.* (2000), Smit *et al.* (1999)
NS2f	Fungi (230 bp)	GGC TGC TGG CAC CAG ACT TGC	35-65%	
fung5r(-GC)	Fungi	CCG CCG CGC GGC GGG CGG GGC GGG GGC ACG GGG GTA AAA GTC CTG GTT CCC		

[a] F or f, forward primer; R or r, reverse primer. [b] Positions numbered according to the *E. coli* 16S rDNA sequence (Brosius *et al.* 1981).

Table 18: Primers for DNA amplification and denaturing gradients used at different sampling dates in lysimeter study.

Sampling date	*Primer set*	*Target*	
Nov 2003	F984GC-R1378	Bacteria	5 min at 94°C, followed by 35 cycles of 1 min at 95°C, 1 min at 53°C and 2 min at 72°C, followed by a final extension step of 10 min at 72°C
	CTO189fGC-CTO654r	AOB	1 min at 93°C followed by 35 cycles of 30 s at 92°C, 1 min at 57°C and 45 sec at 68°C (+ 1 sec per cycle), followed by a final extension step of 5 min at 68°C
Mar 2004	F984GC-R1378	Bacteria	5 min at 94°C, followed by 35 cycles of 1 min at 95°C, 1 min at 53°C and 2 min at 72°C, followed by a final extension step of 10 min at 72°C
	1st round: F243-R1378	Actinomycetes	5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 63°C, and 2 min at 72°C, followed by a final extension step of 10 min at 72°C
	2nd round: F984GC-R1378		5 min at 94°C, followed by 35 cycles of 1 min at 95°C, 1 min at 53°C and 2 min at 72°C, followed by a final extension step of 10 min at 72°C
	1st round: EF4f-fung5r	Fungi	3 min at 94°C followed by 40 cycles of 1 min at 94°C, 1 min at 48°C and 2 min at 72°C, followed by a final extension step 10 min at 72°C
	2nd round: NS2f-fung5rGC		3 min at 94°C followed by 10 cycles of 1 min at 94°C, 1 min at 60 to 52°C (2° per step, 2 cycles per step) and 1 min at 72°C, followed by 25 cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C, followed by a final extension step of 10 min at 72°C
Oct 2004	1st round: F243-R1494	Actinomycetes	5 min at 94°C; 35 cycles of 30s at 95°C, 30s at 63°C and 1 min at 68°C; 5 min at 68°C
	2nd round: F984GC-R1378		as detailed above for Nov 2003
	1st round: F203-R1494	α proteobacteria	5 min at 94°C; 35 cycles of 30s at 95°C, 30s at 56°C and 1 min at 68°C; 5 min at 68°C
	2nd round: F984GC-R1378		as detailed above for Nov 2003
	1st round: ps for-ps rev	Pseudomonads	5 min at 95°C; 35 cycles of 1 min at 95°C, 1 min at 62°C and 2 min at 72°C; 10 min at 72°C
	2nd round: F984GC-R1378		as detailed above for Nov 2003

3.3 Results

3.3.1 Effect of past and current management on dry matter yield and crop N uptake

The lysimeters originating from LCF (L org and L con) ($p=0.003$) and the lysimeters managed conventionally (B con and L con) ($p<0.001$) showed significantly higher dry matter yields (DMY) for the first crop, barley, compared with the BHU originating and the organically managed lysimeters. For maize and lupin, the LCF lysimeters showed lower yields than those from BHU ($p=0.005$ for maize), and L org showed the lowest yields for both crops; however, most of these differences were not significant. Rape yields did not show any variation among treatments (Table 19). The overall (cumulative) crop yield revealed no significant differences among the four treatments (Table 21), however, yields were lower for crops in the LCF lysimeters (BHU average: 43.2; LCF average: 38.1 t ha^{-1}).

Table 19: Dry matter yield (t ha^{-1}) of crops grown as part of 2½-year crop sequence in the lysimeter study.

Treatments	*Barley*	*Lupin*	*Maize*	*Rape*
B con	8.96 (0.27)	17.19 (1.39)	14.40 (0.51)	2.76 (0.26)
B org	5.33 (0.11)	20.74 (3.35)	14.16 (0.39)	2.78 (0.13)
L con	10.35 (0.29)	15.78 (2.27)	12.76 (0.76)	2.76 (0.27)
L org	6.14 (0.43)	14.57 (3.06)	10.77 (1.09)	3.07 (0.21)
$LSD_{0.05}$	0.92	8.11	2.28	0.69

Values are means (Standard errors of means). n=4.

Table 20: C:N ratios of crops grown as part of a 2½-year crop sequence in the lysimeter study.

Treatments	*Barley*	*Lupin*	*Maize*	*Rape*
B con	53.2 (6.79)	17.5 (1.37)	36.1 (1.07)	27.0 (1.21)
B org	46.2 (1.26)	18.5 (0.46)	35.9 (1.57)	26.2 (0.97)
L con	50.9 (2.07)	21.2 (1.67)	41.1 (2.47)	26.8 (1.66)
L org	62.3 (5.59)	20.1 (1.26)	42.4 (2.23)	25.9 (1.26)
CON average	**52.0 (3.31)**	**19.4 (1.22)**	**38.6 (1.57)**	**26.9 (0.95)**
ORG average	**54.3 (4.04)**	**19.3 (0.69)**	**39.1 (1.76)**	**26.0 (0.74)**

Values are means. (Standard errors of means). n=4 or 8 (for averages).

In the four crops, N contents (in %) were comparable; consequently, the patterns of N uptake (in kg ha^{-1}) followed similar trends as dry matter yield. However, it was evident that C:N ratios were on average higher in lysimeters originating from LCF for barley (not significant), lupin (not significant) and maize ($p=0.01$); only barley from the L con treatment had a slightly lower C:N ratio than B con. For rape, C:N ratios were very similar for all four treatments

(between 25.9 and 27). No differences in average C:N ratios were found when comparing ORG to CON treatments (Table 20).

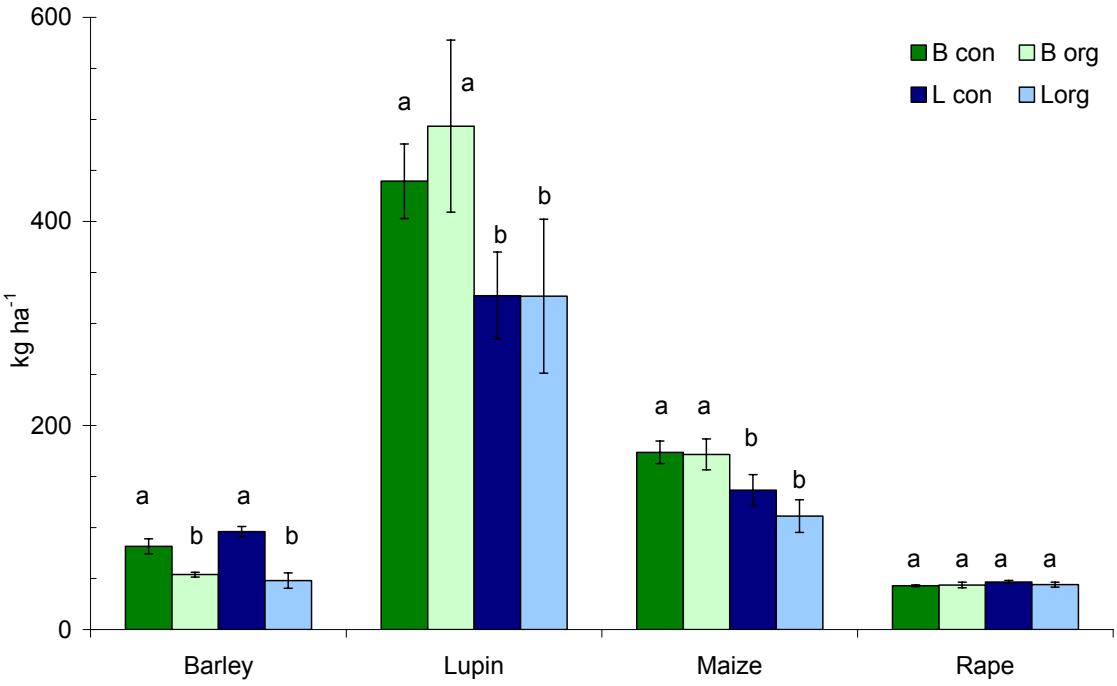

Figure 15: N content (kg ha^{-1}) of crops grown between May 2002 and October 2004 in the lysimeter study. Bars show standard errors of means. Different letters indicate significant differences among treatments at p<0.05. n=4.

In barley, N content was significantly higher under conventional management (B and L con) ($p<0.001$) while no significant differences could be attributed to past management (B vs. L); however, higher N uptake was measured in L lysimeters (72 compared to 68 kg ha^{-1} in B). Lupin and maize, in contrast, showed significantly higher N uptake in the soils from the BHU under both management types (B org and B con) ($p=0.048$ and 0.003 for lupin and maize, respectively). Rape did not show differences in N uptake between current and past farming practice, respectively (Figure 15).

Table 21: Cumulative dry matter yield and N uptake of four crops grown in the lysimeter study.

Treatments	*Yield (t ha^{-1})*	*N uptake (kg ha^{-1})*
B con	43.3 (1.89)	737.4 (41.7)
B org	43.0 (3.53)	762.4 (92.9)
L con	41.6 (3.41)	606.6 (57.1)
L org	34.6 (3.89)	530.1 (91.5)
$LSD_{0.05}$	10.1	228.6

Values are means (standard errors of means).

Table 21 shows that the cumulative N uptake was highest for BHU crops (average of 750 compared to 568 kg ha^{-1} [LCF]), with both treatments showing similar values. Crops subjected to L org had the lowest N content. However, only the difference between B org and L org was significant.

3.3.2 Effect of past and current management on mineral N leaching losses

The graphs in Figure 16 show how the concentration of mineral N in the leachate varied over the course of the experiment. The highest values were measured for B con and the lowest for L org (up to 35 and 23 mg N L^{-1}, respectively). A connection between fertilisation or cultivation events and mineral N concentration can be observed with the general trends of the N concentrations in the leachate decreasing while the lysimeters are under crop, and increasing after soil cultivation (April 2002, February 2003, November 2003, and March 2004) and fertilisation (September and November 2003).

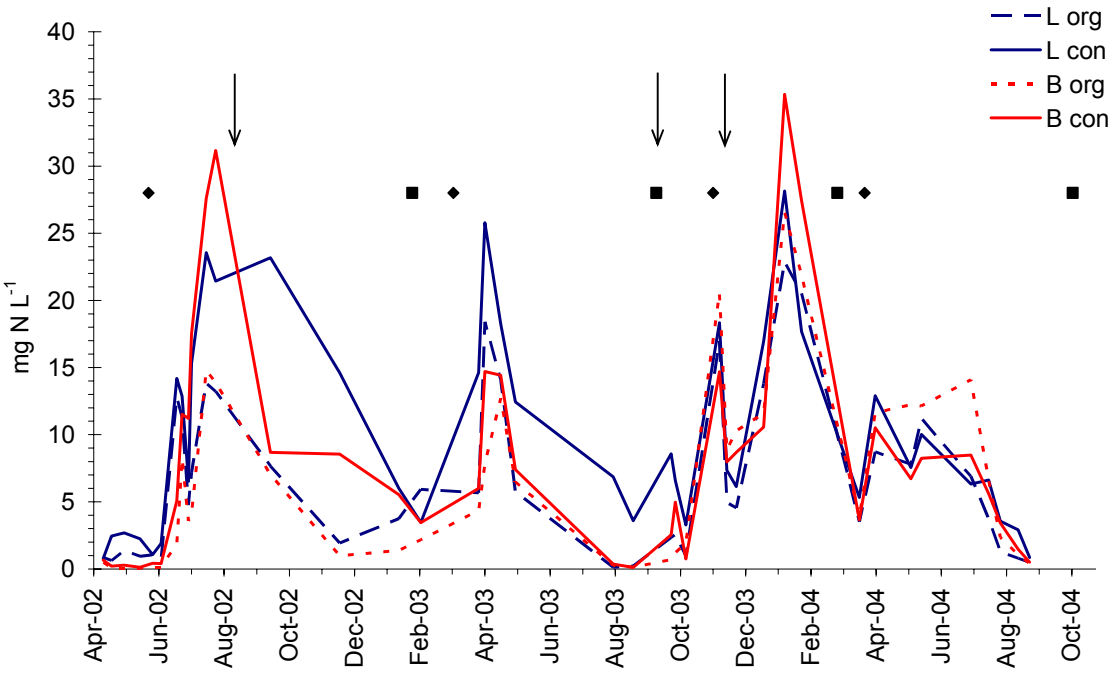

Figure 16: Mean concentration (mg L^{-1}) of mineral N in leachate collected from lysimeters between April 2002 and October 2004. Diamonds, time of crop sowing; Squares, time of crop harvest; arrows represent times of fertilisation and lupin incorporation (see Table 14 for exact dates and amounts). n=4.

Table 22 shows the cumulative amounts of drainage and total mineral N in the leachate that was collected from the lysimeters over 2½ years. While average drainage volume and mineral N content were highest in the first of the three periods (Year 1 > Year 2 > Year 3 for N; Year 1 > Year 3 > Year 2 for drainage), there were no significant differences that can be attributed to past or current management. On average, the BHU as well as the organically fertilised lysimeters had higher drainage in all three periods. Mineral N losses were, however,

higher from LCF (mean of 12.3 compared to 8.5 kg ha^{-1} for BHU) and CON (mean of 12.7 vs. 8.2 kg ha^{-1} for ORG) in Year 1 due to high variability in leaching volume from treatment B org, which showed significantly lower losses than the other treatments. Thereafter, all treatments showed comparable leaching losses (overall mean: 10.2 kg ha^{-1}, range: 8.9-11.7 kg ha^{-1} [Year 2]; mean: 5.7 kg ha^{-1}, range: 5.4-6.3 kg ha^{-1} [Year 3]).

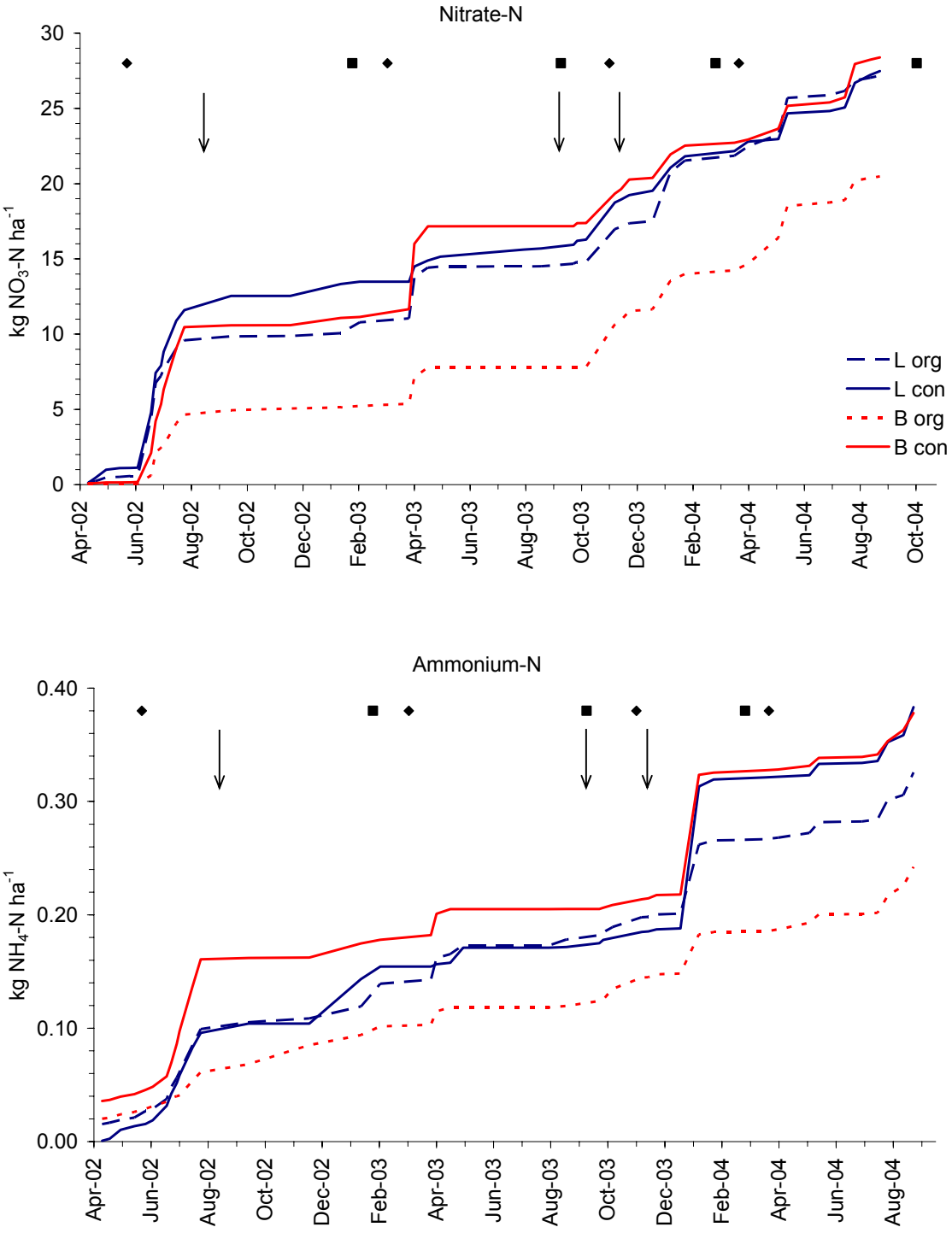

Figure 17: Mean cumulative NO_3-N and NH_4-N leaching losses (kg ha^{-1}) for four treatments between April 2002 and October 2004. Diamonds, time of crop sowing; Squares, time of crop harvest; arrows represent times of fertilisation and lupin incorporation (see Table 14 for exact dates and amounts). n=4.

Table 22: Mean cumulative drainage (mm) and mineral N leached* (kg ha^{-1}) between April 2002 and October 2004 in lysimeter study.

	Year 1 (04/02 – 04/03)		*Year 2 (04/03 – 04/04)*		*Year 3 (04/04 – 10/04)*		*Total*	*Total excl. lysimeter 6*
Treatments	*Mean (sem)*	*Range*	*Mean (sem)*	*Range*	*Mean (sem)*	*Range*	*Mean (sem)*	*Mean (sem)*
drainage (mm)								
B con	167.0 (3.4)	157-172.5	93.8 (18.4)	64.5-170	159.9 (6.9)	148-179	421 (25.6)	421 (25.6)
L con	152.9 (7.9)	142-193	66.1 (3.0)	60-70.8	128.4 (15.2)	85.5-157.5	347 (16.2)	347 (16.2)
B org**	156.1 (33.4)	57.8-200	80.8 (29.5)	16.8-81.5	144.4 (29.6)	56-178.5	444 (84.9)	465 (23.9)
L org	167.1 (11.4)	132-170.3	102.1 (15.6)	70.8-144.5	165.1 (3.3)	158.5-172	434 (17.0)	434 (17.0)
$LSD_{0.05}$	55.9		58.9		52.6		141.3	61.21
mineral N (kg ha^{-1})								
B con	11.6 (0.57)	10.3-12.6	11.7 (3.05)	6.1-19.9	5.7 (2.91)	2.7-14.5	29.1 (2.74)	29.1 (2.74)
L con	13.7 (3.16)	7.0-21.7	8.9 (1.02)	7.3-11.8	5.4 (2.34)	1.3-12.0	28.0 (4.66)	28.0 (4.66)
B org	5.4 (0.93)	3.7-5.9	9.1 (1.95)	4.0-13.2	6.3 (0.22)	2.5-8.1	20.8 (2.75)	23.6 (1.11)
L org	11.0 (1.96)	5.7-15.0	11.2 (1.50)	7.0-14.1	5.4 (1.10)	2.4-7.2	27.6 (3.18)	27.6 (3.18)
$LSD_{0.05}$	6.21		6.24		6.32		10.9	10.5

*NO_3-N constituted 97-98.8%, NH_4-N 0.9-2.2% and NO_2-N 0.16-0.7% of mineral N leached. ** Large standard errors for B org due to low drainage from lysimeter 6. Total presented for data including and without lysimeter 6 (treatment B org). sem, standard errors of means; range, range of values measured. n=4 (n=3 for total without lysimeter 6).

Drainage from lysimeter 6 (B org) was much lower than from the other replicates of the treatment which resulted in large standard errors for the B org drainage means (standard errors for mineral N loss are similar to those observed for the other treatments) (Table 22). Excluding lysimeter 6 from the analysis, emphasized the similarity of the results and the lack of significant differences between the drainage and leaching losses of the four treatments. Over the entire trial period, cumulative NO_3-N losses were similar for B con, L con and L org while B org lysimeters leached lower amounts than the other treatments. In particular, NH_4-N leaching was distinctively lower from B and L org lysimeters compared to the conventional treatments (Figure 17).

3.3.3 Effect of past and current management on biological and biochemical soil properties

To determine the influence of current or past management on selected soil microbial properties, it was more important to evaluate if and how the soil properties were changing in relation to each other, i.e. if the perceived differences or similarities between treatments persisted over the course of the experiment, rather than evaluate the temporal variation. For this reason, the results presented here mainly focus on comparisons between BHU and LCF, and ORG and CON, respectively, over all sampling points and for each date separately, and only very briefly discuss the observed temporal variation within the soil properties measured.

Table 23: Mean soil moisture content (%) determined in the lysimeter study for four different sampling dates.

Treatments	*Feb-03*	*Nov-03*	*Mar-04*	*Oct-04*
B con	22.5 (1.84)	16.5 (0.34)	20.6 (0.15)	22.9 (0.53)
B org	22.0 (0.96)	17.2 (0.38)	21.3 (0.56)	23.0 (1.04)
L con	21.7 (0.36)	19.1 (0.58)	19.5 (0.92)	22.6 (0.91)
L org	23.7 (1.69)	18.8 (0.26)	21.6 (0.52)	24.7 (0.72)

Standard errors of means in parenthesis. n=4.

Influence of time on soil properties

As seen in Figure 18, 19 and 20, the values measured for C_{mic}, ADA, CN_{mic} and microbial quotient in November 2003 appear inconsistent with the results from the other three sampling dates and the other parameters measured. Therefore, the results presented in the tables in this subsection show data with and without the November 2003 sampling. Although removal of the November 2003 data from the analyses lowered $LSD_{0.05}$ and increased p values, it did not change the general trends for most soil properties. For C_{mic} and CN_{mic}, mean differences

between BHU and LCF were not significant when excluding the November 2003 results, while the opposite was true for the microbial quotient (Table 25).

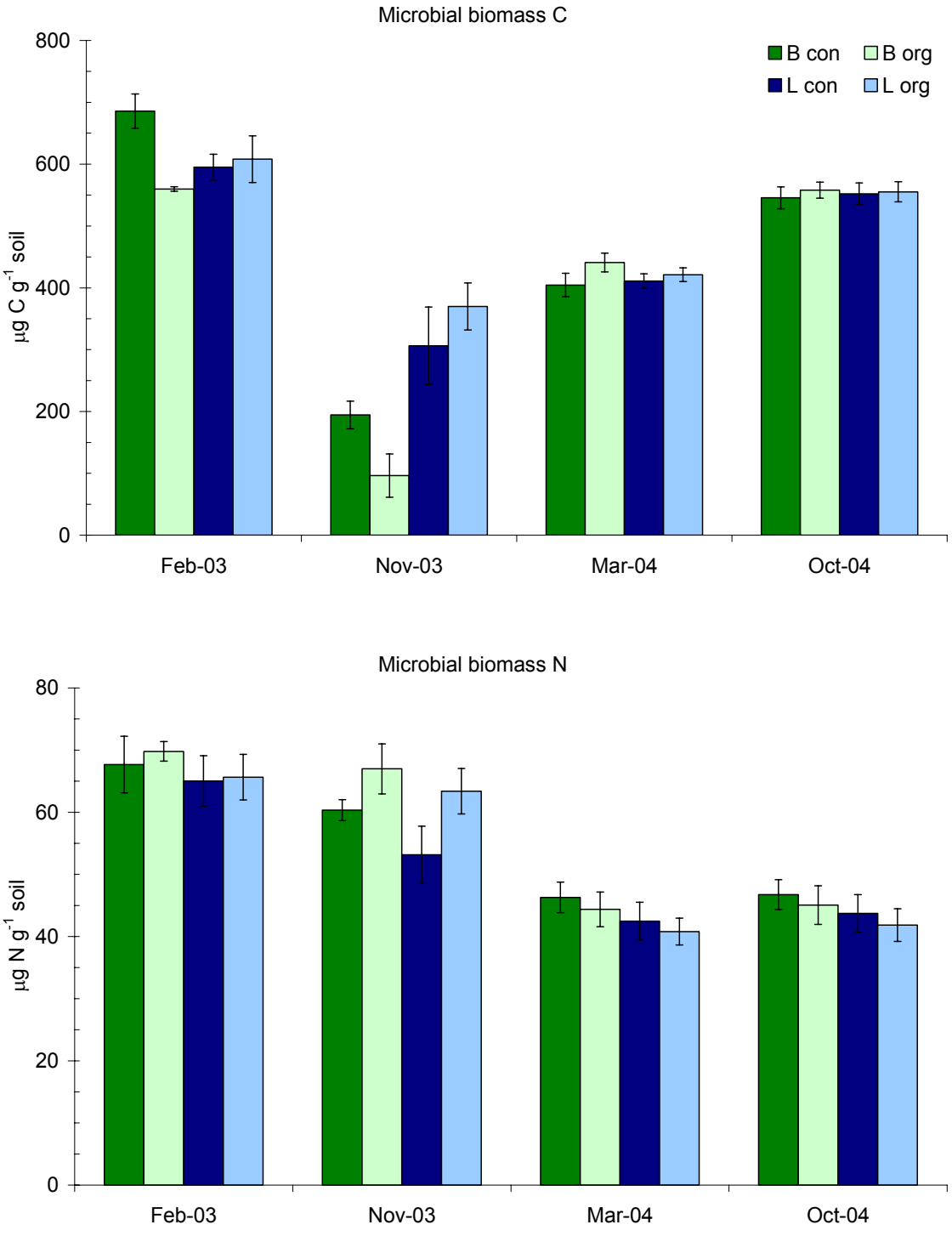

Figure 18: Mean concentrations (µg g^{-1}) of microbial biomass C and N determined in the lysimeter study for four sampling dates. Bars are standard errors of means. n=12.

Repeated measures analysis of variance showed that the differences among the four sampling dates were larger than differences among treatments (current vs. past management) and for most soil properties these differences were significant ($p<0.001$) (except C_{tot} [not

significant]). In most cases, the highest levels of the respective soil parameter were measured at the first or second sampling. FDA and CN_{mic} were exceptions with continuously increasing concentrations over time for all treatments (Figure 19 to Figure 22) and the soil moisture content for all treatments was lowest in November 2003 (Table 23).

The graphs presented in Figures 18-23 indicate that relative differences between BHU and LCF were more pronounced than differences caused by current management practices (ORG vs. CON). Large initial differences between BHU and LCF in microbial biomass levelled out over time (Figure 18) while they persisted in measures of microbial activity (DHH, ADA, and FDA) (Figures 20 and 23) and organic C (Figure 22).

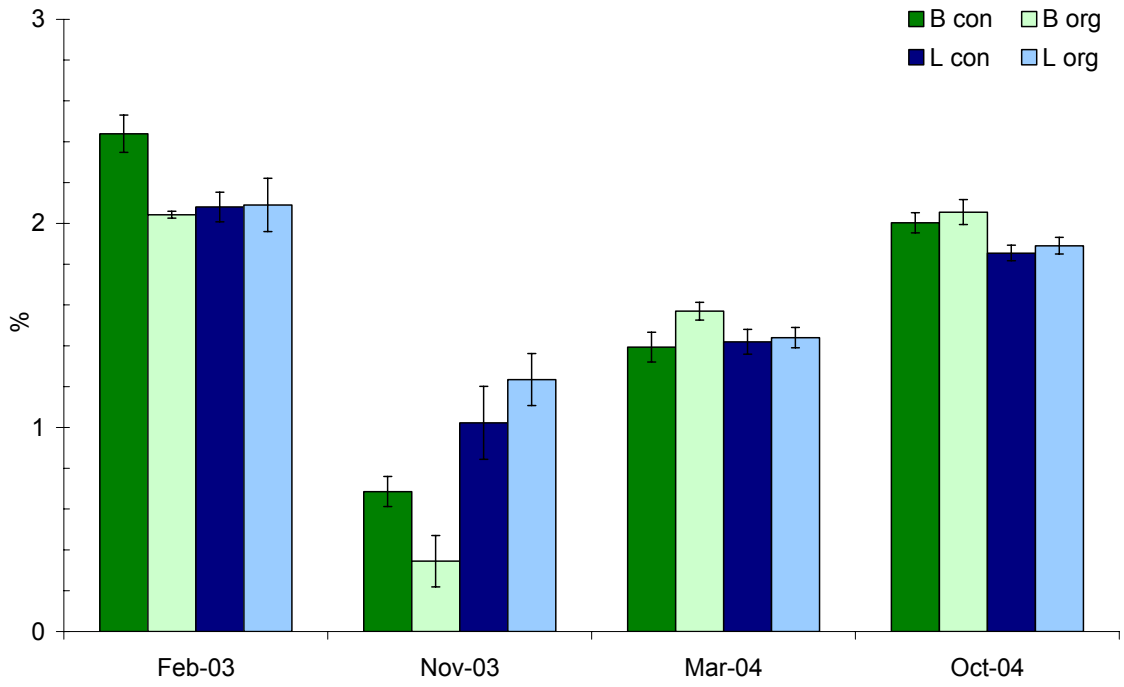

Figure 19: Mean values (%) for microbial quotient (C_{mic}:C_{tot}) determined in the lysimeter study at four sampling dates. Bars are standard errors of means. n=12.

Influence of past management on soil properties

Overall, the soil properties were mainly influenced by past rather than current management (number of significantly affected soil properties in Table 25 is five compared to two in Table 27). Lysimeters from BHU had significantly higher levels of DHH, ADA and microbial quotient, while FDA and C_{tot} were higher in LCF soils.

For DHH, ADA, FDA, C_{tot}, CN_{mic} and microbial quotient, similar trends could be observed on each sampling date (Table 24). Soils from BHU had significantly higher levels of DHH, ADA and microbial quotient while LCF showed increased concentrations of FDA, C_{tot} and N_{tot} compared to BHU. No significant differences were observed for N_{mic}; levels were, however, higher in BHU lysimeters at all sampling dates (Figure 18), while LCF was consistently

higher in CN_{mic} (significant only when November 2003 sampling included [p=0.004]) (Table 24).

Table 24: Effect of past organic (BHU) and conventional (LCF) management on mean concentrations of soil properties at four sampling dates determined in the lysimeter study.

Soil property	*Sampling date*	*BHU*	*LCF*	$LSD_{0.05}$ *(excluding Nov-03)*
DHH	Feb-03	10.63 (0.501)	7.41 (0.431)	0.77 (0.82)
(µg TPF g^{-1} h^{-1})	Nov-03	6.30 (0.206)	3.84 (0.224)	
	Mar-04	5.61 (0.188)	3.97 (0.282)	
	Oct-04	3.99 (0.163)	2.90 (0.172)	
ADA	Feb-03	3.47 (0.095)	2.18 (0.102)	0.45 (0.40)
(µg NH_4-N g^{-1} h^{-1})	Nov-03	4.15 (0.255)	2.53 (0.146)	
	Mar-04	3.31 (0.134)	2.13 (0.212)	
	Oct-04	2.95 (0.276)	1.81 (0.103)	
FDA	Feb-03	133 (4.23)	164 (5.97)	13.4 (12.8)
(µg fluorescein g^{-1} h^{-1})	Nov-03	188 (7.08)	232 (4.97)	
	Mar-04	219 (5.24)	259 (3.80)	
	Oct-04	228 (8.96)	283 (6.95)	
C_{mic}:C_{tot} (%)	Feb-03	2.24 (0.086)	2.09 (0.069)	0.08 (1.14)
	Nov-03	0.52 (0.093)	1.13 (0.109)	
	Mar-04	1.48 (0.052)	1.43 (0.036)	
	Oct-04	2.03 (0.038)	1.87 (0.027)	
CN_{mic}	Feb-03	9.18 (0.636)	9.24 (0.233)	1.44 (1.43)
	Nov-03	2.32 (0.438)	5.88 (0.628)	
	Mar-04	9.39 (0.330)	10.1 (0.375)	
	Oct-04	12.2 (0.641)	13.1 (0.641)	
C_{tot} (%)	Feb-03	2.78 (0.029)	2.89 (0.020)	0.05 (0.09)
	Nov-03	2.77 (0.052)	2.99 (0.028)	
	Mar-04	2.86 (0.031)	2.91 (0.031)	
	Oct-04	2.72 (0.023)	2.96 (0.039)	
N_{tot} (%)	Feb-03	0.23 (0.003)	0.23 (0.002)	0.006 (0.006)
	Nov-03	0.25 (0.005)	0.25 (0.003)	
	Mar-04	0.24 (0.002)	0.24 (0.002)	
	Oct-04	0.22 (0.002)	0.23 (0.002)	

Values are means of two treatments (Standard errors of means). Only properties that show significant differences are displayed. n=8.

Despite significant differences between sampling dates for all soil properties (except C_{tot}), the relative differences remained consistent over time, and C_{mic}, C_{tot} and N_{tot} remained within the

range of 95-105% indicating that the soils were very similar in these properties. The biggest differences were measured in ADA and DHH (LCF levels around 65% of BHU levels for both), N_{mic} (LCF approximately 92% of BHU) and FDA (>120%) (Table 26).

Table 25: Overall mean effect of past management on soil properties determined over 2½ years in the lysimeter study (with and without November 2003 sampling).

Soil property	*BHU*	*LCF*	*p value*
including Nov-03 sampling			
C_{mic} (µg C g^{-1})	436 (34.2)	477 (21.3)	0.022
DHH (µg TPF g^{-1} h^{-1})	6.63 (0.463)	4.53 (0.338)	<0.001
ADA (µg NH_4^+-N g^{-1} h^{-1})	3.34 (0.164)	2.16 (0.084)	<0.001
FDA (µg fluorescein g^{-1} h^{-1})	192 (7.42)	235 (8.44)	<0.001
CN_{mic}	8.27 (0.699)	9.58 (0.521)	0.020
C_{tot} (%)	2.78 (0.019)	2.94 (0.016)	<0.001
excluding Nov-03 sampling			
C_{mic} (µg C g^{-1})	533 (20.02)	524 (18.12)	NS
DHH (µg TPF g^{-1} h^{-1})	6.75 (0.616)	4.76 (0.437)	<0.001
FDA (µg fluorescein g^{-1} h^{-1})	194 (9.67)	236 (11.2)	<0.001
ADA (µg NH_4^+-N g^{-1} h^{-1})	3.19 (0.112)	2.04 (0.089)	<0.001
Microbial quotient (%)	1.92 (0.075)	1.80 (0.063)	0.007
CN_{mic}	10.3 (0.420)	10.8 (0.426)	NS
C_{tot} (%)	2.79 (0.019)	2.92 (0.018)	<0.001

Values are means of four sampling dates and two treatments (standard errors of means). Only properties that show significant differences are displayed. n=32.

Table 26: Relative differences (in percent)* between BHU and LCF for selected soil properties determined at four sampling dates in the lysimeter study.

Soil property	*Feb-03*	*Nov-03*	*Mar-04*	*Oct-04*
C_{mic}	97	232	98	100
N_{mic}	95	91	92	93
DHH	70	61	71	73
FDA	123	123	118	124
ADA	63	61	64	67
C_{mic}:C_{tot}	93	219	96	92
CN_{mic}	101	253	108	108
C_{tot}	104	106	102	109
N_{tot}	100	100	99	104
C:N	104	106	103	105

*LCF expressed as percentage of BHU calculated by LCF/BHU*100%. Values are means of two treatments.

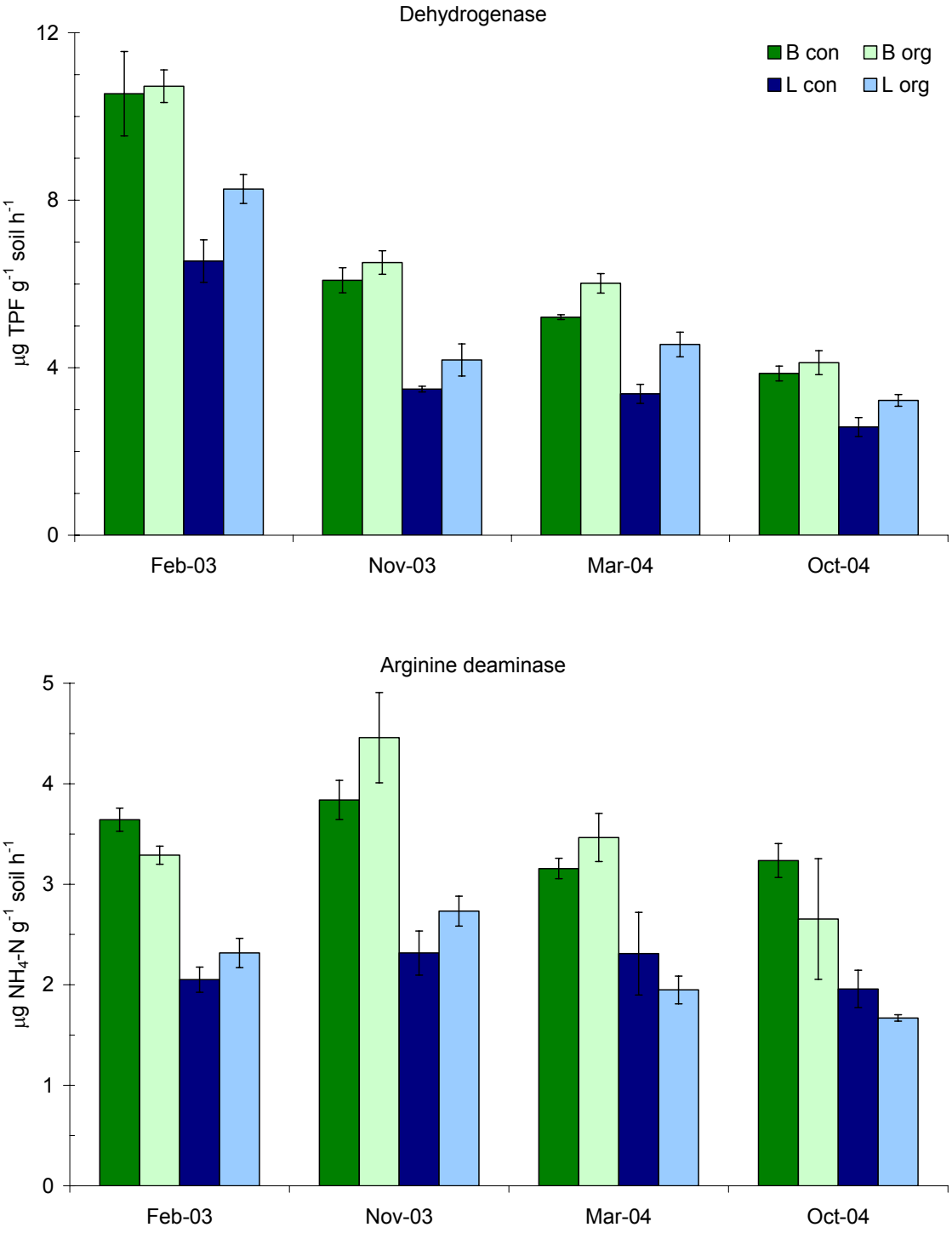

Figure 20: Mean rates (µg g^{-1} h^{-1}) of dehydrogenase hydrolysis and arginine deaminase activity determined in the lysimeter study at four sampling dates. Bars are standard errors of means. n=12.

Influence of current management on soil properties

Comparing the overall effect (average of four sampling dates) of current management practices (ORG vs. CON) on the soil properties showed that microbial activity measured by DHH was significantly higher in ORG while FDA levels were increased in CON (Table 27).

Table 27: Overall mean effect of current organic (ORG) and conventional (CON) management on soil properties determined over 2½ years in the lysimeter study (with and without November 2003 sampling).

Soil property	*ORG*	*CON*	*p value*
including Nov-03			
DHH (µg TPF g^{-1} h^{-1})	5.95 (0.433)	5.21 (0.452)	0.001
FDA (µg fluorescein g^{-1} h^{-1})	204 (8.07)	222 (9.22)	0.001
excluding Nov-03			
DHH (µg TPF g^{-1} h^{-1})	6.15 (0.553)	5.35 (0.580)	0.005
FDA (µg fluorescein g^{-1} h^{-1})	205 (10.26)	224 (12.01)	0.007

Values are means of two soils and four sampling dates (standard errors of means). Only properties that show significant differences are displayed. n=32.

Table 28: Effect of current management (ORG and CON) on mean concentrations of soil properties determined at different sampling dates in the lysimeter study.

Soil property	*Sampling date*	*ORG*	*CON*	$LSD_{0.05}$ *(excluding Nov-03)*
C_{mic} (µg C g^{-1})	Feb-03	584 (19.7)	641 (23.5)	52.0 (39.6)
	Nov-03	233 (56.9)	250 (34.0)	
	Mar-04	431 (9.4)	408 (10.4)	
	Oct-04	557 (9.6)	549 (11.5)	
Microbial quotient (%)	Feb-03	2.07 (0.062)	2.26 (0.087)	0.148 (0.136)
	Nov-03	0.859 (0.192)	0.921 (0.096)	
	Mar-04	1.51 (0.039)	1.41 (0.044)	
	Oct-04	1.97 (0.046)	1.93 (0.040)	
ADA (µg NH_4^+-N g^{-1} h^{-1})	Feb-03	2.80 (0.200)	2.85 (0.311)	0.45 (0.40)
	Nov-03	3.60 (0.393)	3.08 (0.318)	
	Mar-04	2.71 (0.314)	2.73 (0.253)	
	Oct-04	1.65 (0.269)	2.60 (0.268)	
FDA (µg fluorescein g^{-1} h^{-1})	Feb-03	145 (6.2)	152 (9.0)	13.4 (12.8)
	Nov-03	202 (10.8)	218 (8.9)	
	Mar-04	233 (9.3)	246 (7.7)	
	Oct-04	237 (11.6)	274 (10.6)	

Values are means of two sites (standard errors of means). Only properties that show significant differences are displayed. n=8. $LSD_{0.05}$ displayed for analysis including and excluding Nov-03 sampling.

The differences between ORG and CON at each sampling date revealed significantly higher levels of FDA at most sampling dates and increased levels of ADA in October 2004 for CON (Table 28). C_{mic} was significantly higher in CON compared to ORG in February 2003 while ORG had higher levels at the other sampling points (not significant). Higher levels of N_{mic} were measured in CON at all sampling points and ORG had a higher microbial quotient in March and October 2004. These differences, however, were not significant. In 2003, CN_{mic}

was higher in CON while in 2004 higher levels were observed in ORG (not significant). The other soil properties did not show any differences between the management practices.

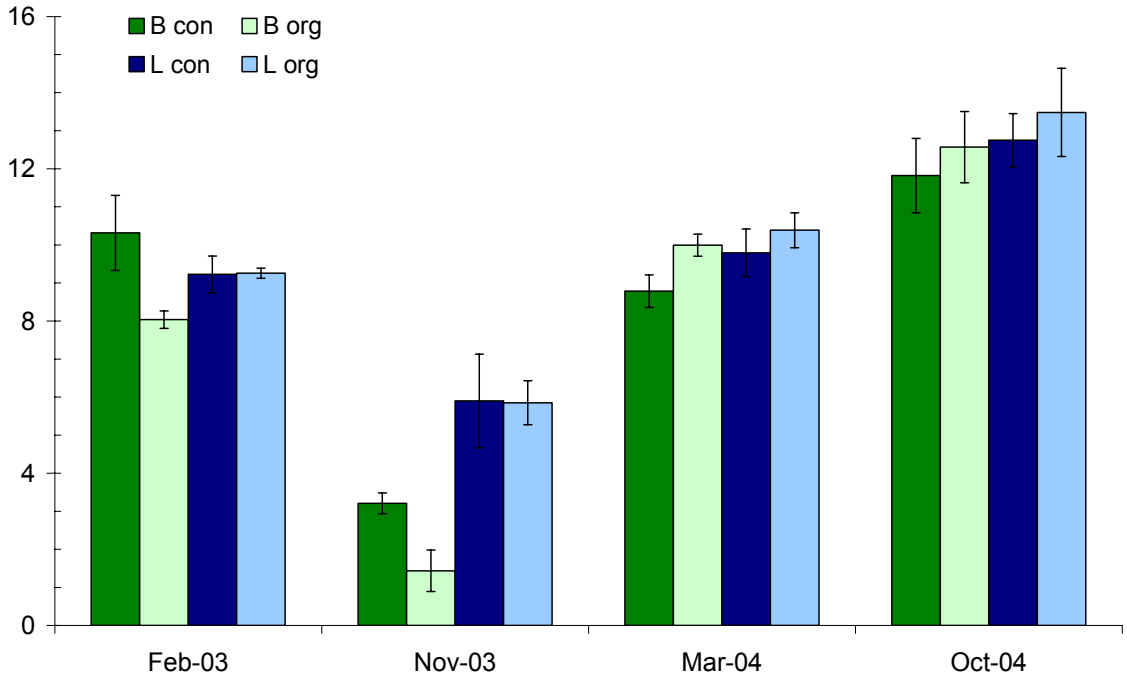

Figure 21: Mean ratio of microbial biomass C to N determined in the lysimeter study at four sampling dates. Bars are standard errors of means. n=12.

Table 29: Relative difference (in percent*) between treatments ORG and CON for soil properties determined over 2½ years in the lysimeter study.

Soil property	*Feb-03*	*Nov-03*	*Mar-04*	*Oct-04*
C_{mic}	91	93	106	101
N_{mic}	102	115	96	96
DHH	111	112	123	114
FDA	95	93	95	87
ADA	99	117	99	74
C_{mic}:C_{tot}	91	92	107	102
CN_{mic}	89	80	110	106
C_{tot}	100	99	99	99
N_{tot}	100	102	100	100
C:N	100	98	99	99

*ORG expressed as percentage of CON calculated by ORG/CON*100%. Values are means of two soils.

While total values changed significantly over time (Figures 19-23), evaluating the levels of the soil properties in ORG and CON relative to each other, showed only minor changes across the four sampling dates and no obvious trends were observed (Table 29). The two management systems showed similar levels of the chemical soil parameters (C_{tot} and N_{tot})

(percentages between 98 and 102%). For ORG, lower levels of C_{mic}, microbial quotient and CN_{mic} were observed in the first year and higher concentrations in the second year. The opposite trends could be measured for N_{mic} but these differences were insignificant (Figures 18, 19 and 21). Differences between ORG and CON in ADA and FDA (CON>ORG) increased over time (cf. Figures 20 and 23).

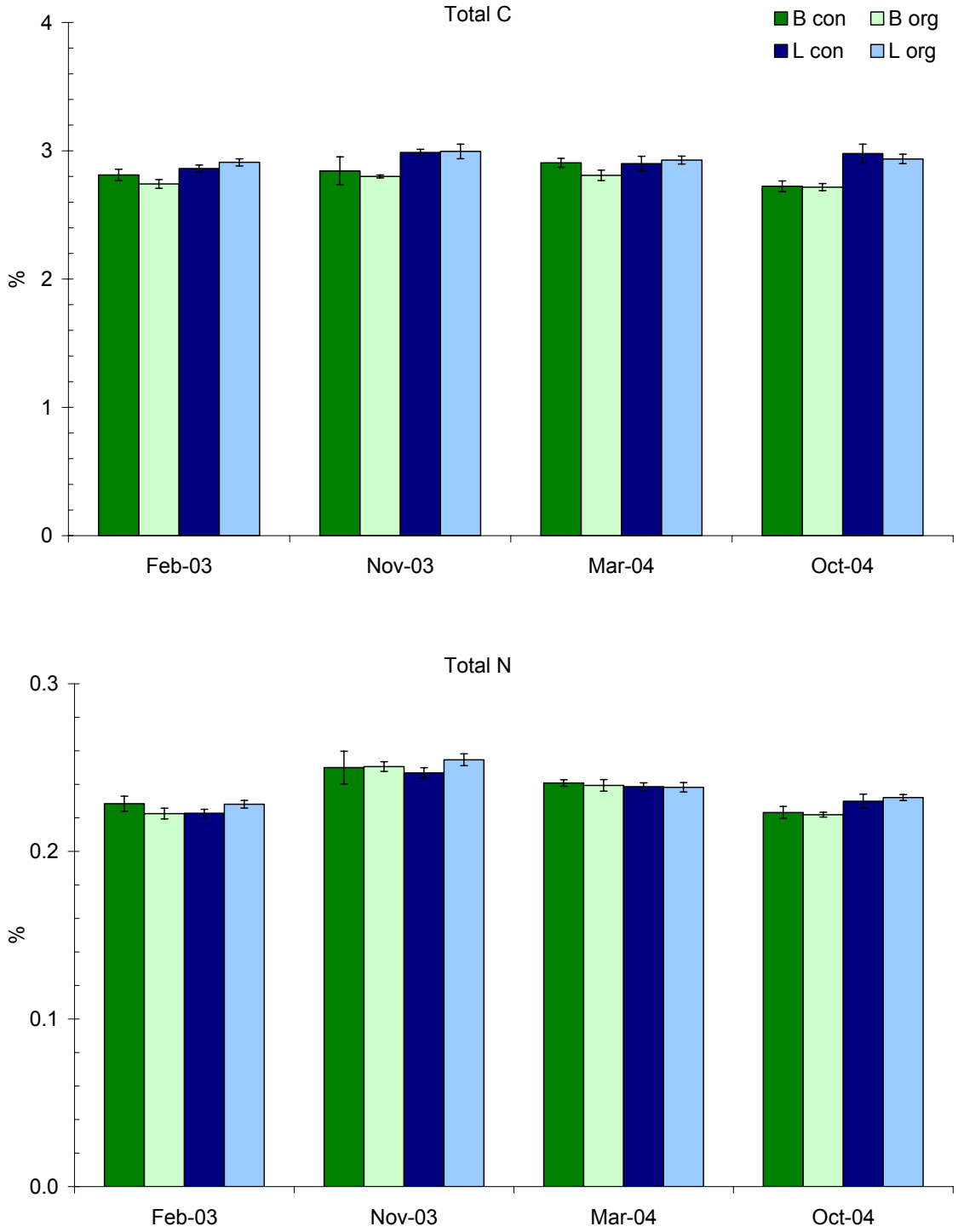

Figure 22: Mean concentration (%) of total C and N determined in the lysimeter study at four sampling dates. Bars are standard errors of means. n=12.

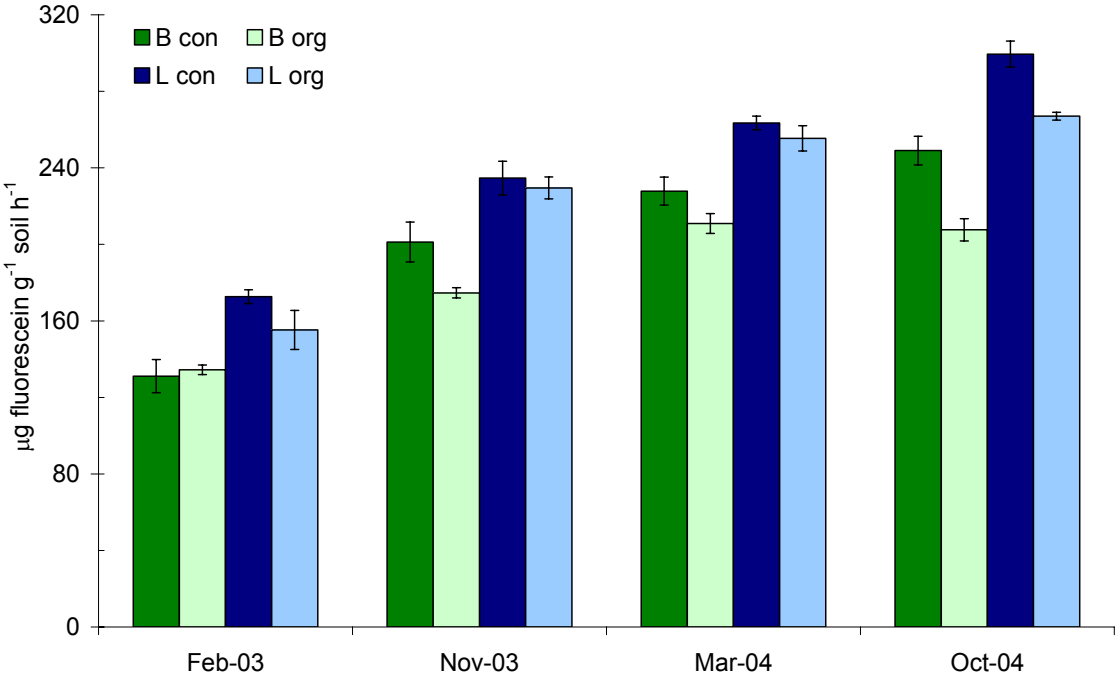

Figure 23: Mean rate (µg g^{-1} h^{-1}) of fluorescein diacetate hydrolysis determined in the lysimeter study at four sampling dates. Bars are standard errors of means. n=12.

Correlations among soil properties (linkages)

The coefficients for correlations among soil properties shown in Table 30 and Table 31 revealed the same trends for the organic and conventional management regimes as for soils originating from BHU and LCF. Highly positive correlations were observed between DHH, ADA and N_{mic}, and C_{tot}, N_{tot} and FDA while negative correlations occurred between C_{mic}–ADA, C_{mic}–N_{tot}, N_{mic}–FDA, DHH–FDA, ADA–FDA, N_{tot}– C_{mic}:C_{tot} and N_{tot}–CN_{mic}.

The biggest differences could be detected for the relationship between C_{mic} and N_{mic}, which was positive for CON and LCF and negative for ORG and BHU. However, the correlations were not very strong. Interestingly, the links of ADA and DHH with C_{mic} showed similar trends for BHU and ORG as well as for LCF and CON, respectively. Although differences were small, it was evident that the negative correlation between ADA and C_{mic} was stronger in BHU and ORG treatments compared to LCF and CON while the opposite could be observed for DHH–C_{mic} (positive correlation stronger in CON and LCF). Stronger negative links were observed for CN_{mic} to ADA and DHH, respectively, in ORG compared to CON while the correlations were of similar strength in LCF and BHU.

Table 30: Correlation coefficients determined among soil properties for organically and conventionally managed soils over 2½ years in the lysimeter study.

	C_{mic}	N_{mic}	*DHH*	*FDA*	*ADA*	C_{tot}	N_{tot}	C_{mic}:C_{tot}
ORG								
N_{mic}	-0.16							
DHH	0.12	0.65***						
FDA	0.01	-0.69***	-0.86***					
ADA	-0.58**	0.54**	0.41*	-0.50**				
C_{tot}	0.03	-0.10	-0.33	0.49**	-0.35*			
N_{tot}	-0.68***	0.13	-0.29	0.29	0.35*	0.59***		
C_{mic}:C_{tot}	0.99***	-0.15	0.16	-0.05	-0.54**	-0.09	-0.75***	
CN_{mic}	0.78***	-0.71***	-0.35*	0.44*	-0.72***	0.07	-0.55**	0.77***
CON								
N_{mic}	0.22							
DHH	0.40*	0.66***						
FDA	-0.29	-0.78***	-0.91***					
ADA	-0.11	0.36*	0.53**	-0.44*				
C_{tot}	-0.18	-0.17	-0.28	0.30	-0.44*			
N_{tot}	-0.66***	-0.06	-0.20	0.16	0.10	0.64***		
C_{mic}:C_{tot}	0.99***	0.24	0.43*	-0.33	-0.05	-0.30	-0.71***	
CN_{mic}	0.76***	-0.45**	-0.08	0.28	-0.32	-0.05	-0.55**	0.74***

Levels of significance: ***, $p<0.001$; **, $p<0.01$; *, $p<0.05$. n=32.

Correlations of microbial biomass C and ADA with soil moisture levels were determined by fitting the data to linear and quadratic regression functions, respectively (Figure 24). The quadratic regression for C_{mic}-soil moisture gave a higher regression coefficient than a linear function (R^2=0.60 compared to 0.48 for a linear regression). Increasing levels of C_{mic} were observed with increasing soil moisture content while there was a weak negative correlation between soil moisture and ADA. The extremely low levels of microbial biomass C measured in November 2003 for treatments B org, B con and L org were outliers and could not be explained with low soil moisture levels.

Table 31: Correlation coefficients determined among soil properties for soils originating from BHU and LCF over 2½ years in the lysimeter study.

	C_{mic}	N_{mic}	*DHH*	*FDA*	*ADA*	C_{tot}	N_{tot}	C_{mic}:C_{tot}
BHU								
N_{mic}	-0.12							
DHH	0.29	0.65***						
FDA	-0.20	-0.72***	-0.89***					
ADA	-0.49**	0.54**	0.24	-0.22				
C_{tot}	-0.22	0.01	0.03	0.15	0.09			
N_{tot}	-0.73***	0.13	-0.15	0.17	0.44**	0.74***		
C_{mic}:C_{tot}	0.99***	-0.12	0.27	-0.20	-0.50**	-0.29	-0.77***	
CN_{mic}	0.86***	-0.59***	-0.13	0.20	-0.67***	-0.22	-0.66***	0.86***
LCF								
N_{mic}	0.28							
DHH	0.49**	0.67***						
FDA	-0.29	-0.75***	-0.88***					
ADA	-0.18	0.52**	0.23	-0.24				
C_{tot}	-0.16	-0.14	-0.27	0.24	0.09			
N_{tot}	-0.62***	-0.04	-0.36*	0.23	0.41*	0.64***		
C_{mic}:C_{tot}	0.99***	0.29	0.52**	-0.32	-0.19	-0.29	-0.68***	
CN_{mic}	0.57***	-0.61***	-0.22	0.44*	-0.54**	0.02	-0.42*	0.55**

Levels of significance: ***, $p<0.001$; **, $p<0.01$; *, $p<0.05$. n=32

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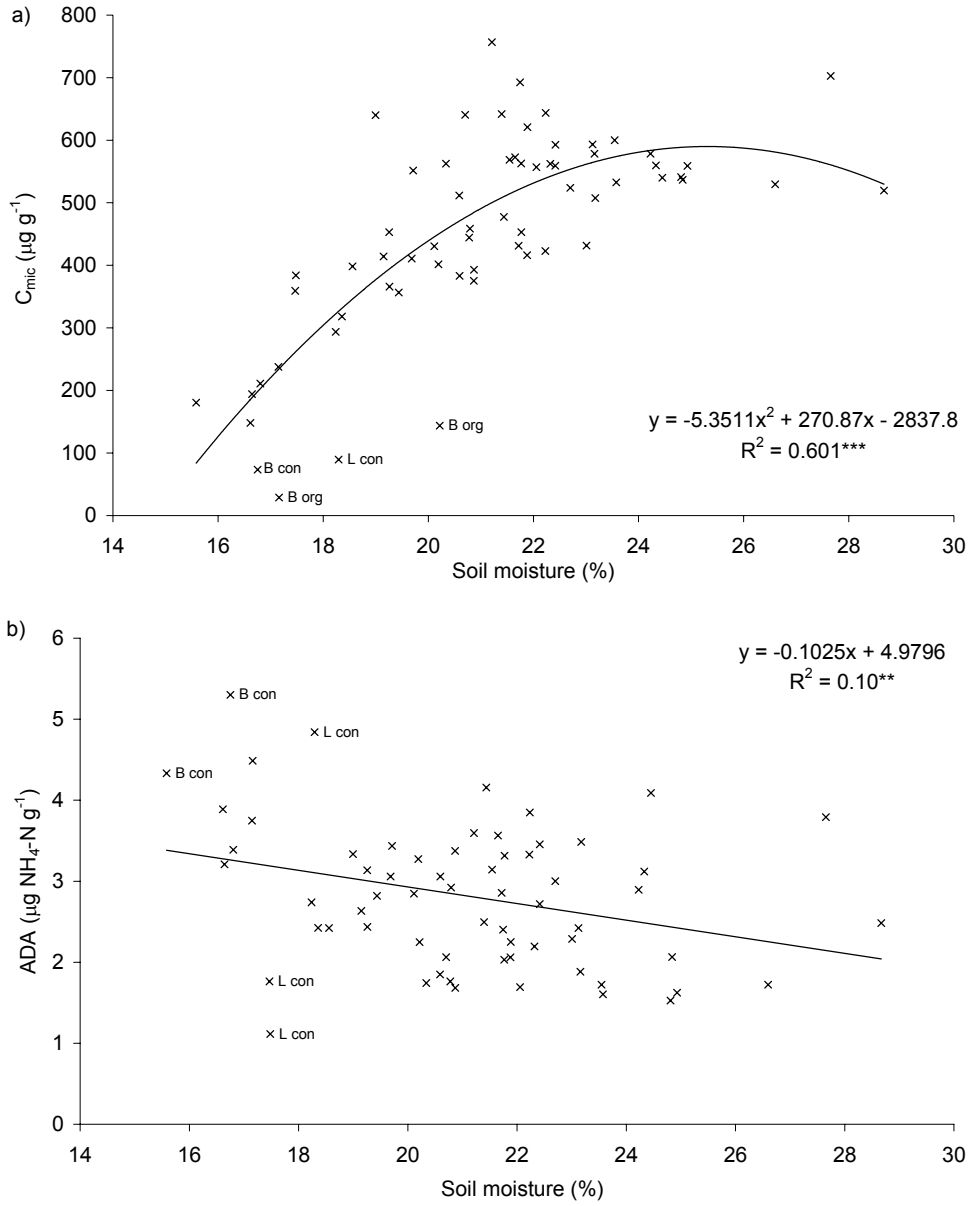


Figure 24: Relationship between soil moisture content, microbial biomass C (a) and arginine deaminase activity (b) for the lysimeter study. Regression equation, line of best fit and statistical significance shown. **, p<0.01; ***, p<0.001. n=64.

3.3.4 Effect of past and current management on microbial community structure

November 2003 sampling

In November 2003, genetic diversity of general bacteria and ammonia oxidisers (AOB) in lysimeter soils were evaluated by DGGE after PCR amplification of 16S rDNA fragments. The DGGE profiles and cluster analyses in Figure 25 show that eubacterial communities were distinctively different in BHU and LCF soils, although the number of bands was similar for both sites. Reproducibility of the method was as high as in the initial sampling (Section 2, 2.3.2) (replicate lanes almost identical) and it was possible to differentiate between the soils by visual assessment alone since BHU and LCF banding patterns showed obvious differences.

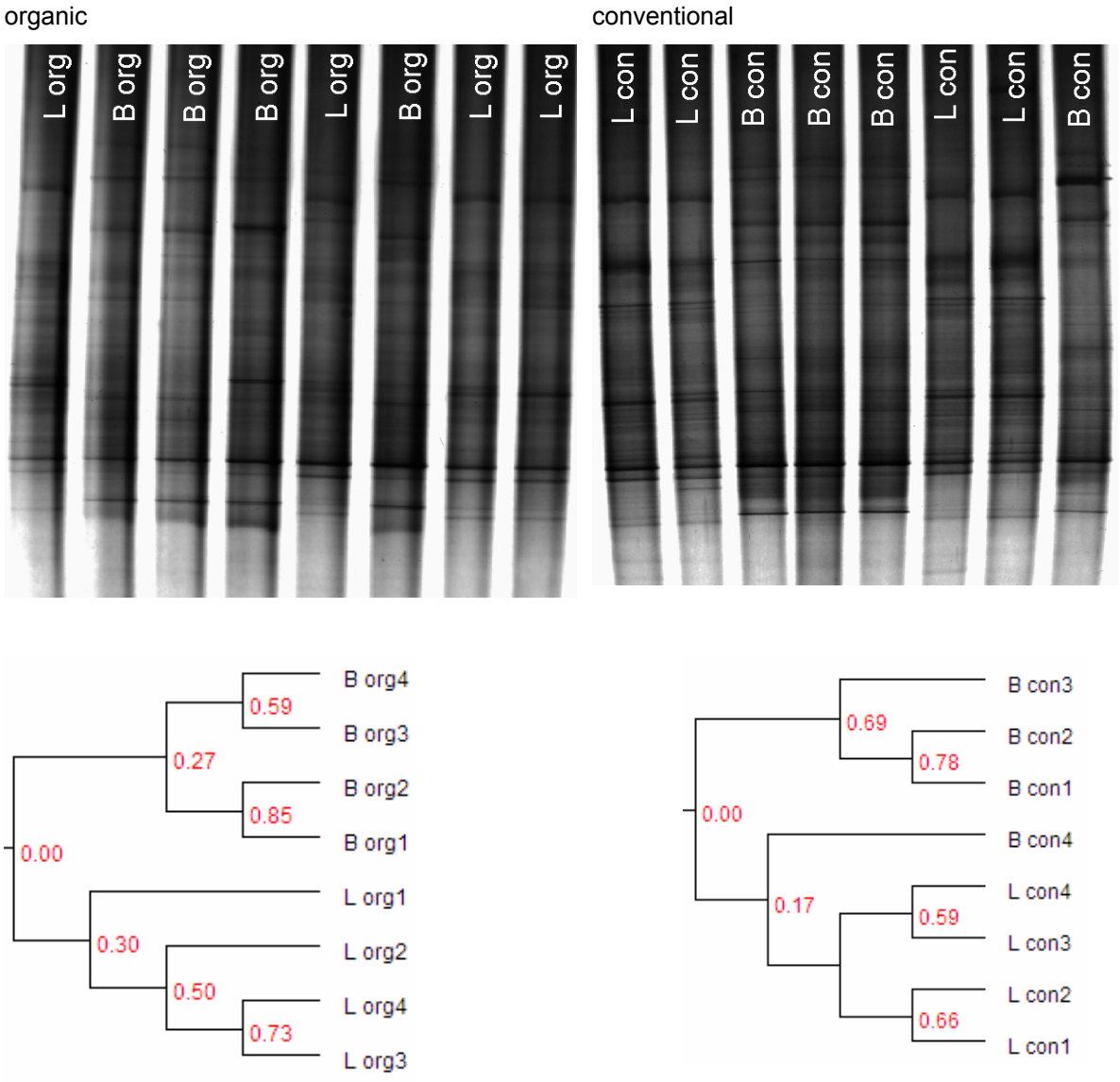


Figure 25: DGGE profiles and cluster analysis for 16S rDNA fragments of eubacterial communities amplified from organic and conventionally managed lysimeter soils taken in November 2003.

These observations are confirmed by the results of the cluster analyses that reveal two separate clusters for BHU and LCF samples and bigger similarities (30-85%) amongst samples of the same soil origin than between soils. Only B con4 (last lane) was grouped with L con rather than B con samples. However, similarity between B con4 and L con samples was only 17%. As PCR products resulting from ORG and CON samples were run on separate gels, a statistical comparison between these treatments was not possible, although visual assessment suggests a high similarity among samples from the same site (i.e. between B org and B con and between L org and L con).

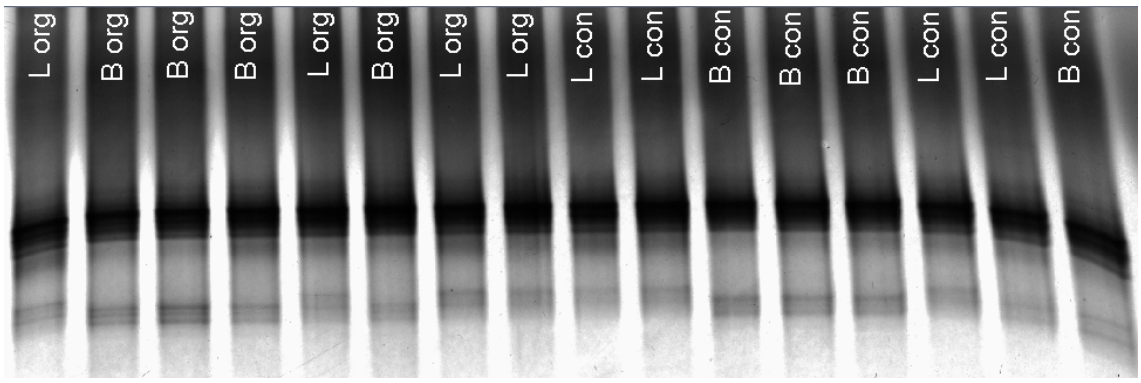


Figure 26: DGGE profiles for 16S rDNA fragments of AOB communities amplified from lysimeter soils taken in November 2003.

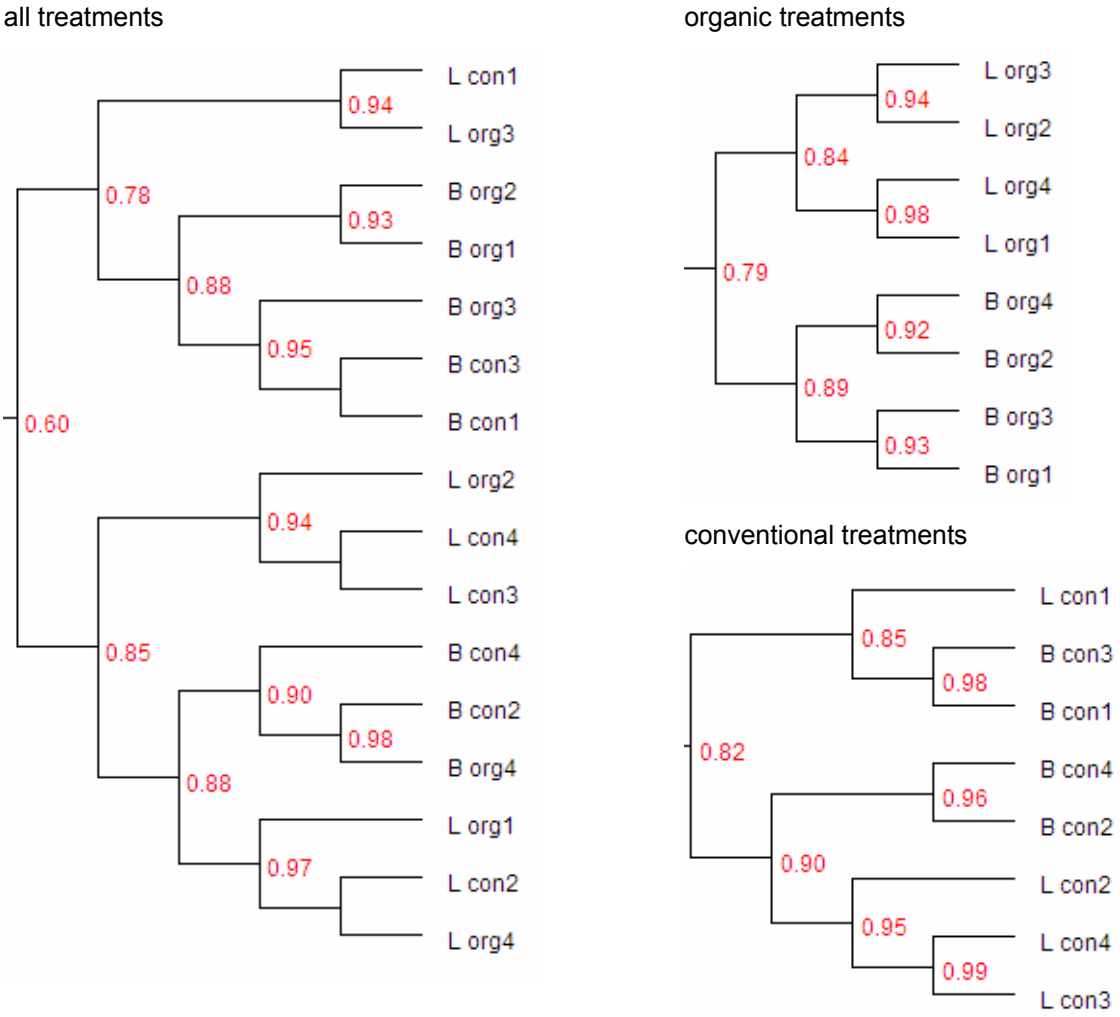


Figure 27: Cluster analysis for 16S rDNA fragments of AOB communities PCR amplified from lysimeter soils taken in November 2003. Shown is the clustering of all treatments together and of the organic and conventional treatments separately.

As expected, DGGE patterns for AOB communities in the lysimeter soils showed fewer bands than observed for general bacteria (Figure 26). The gel resolution was not as high and some bands were not as distinct as seen for eubacteria. DGGE analysis revealed approximately four bands for all treatments, with clear differences observable between BHU and LCF samples.

Figure 27 shows that while cluster analysis could not distinguish between ORG and CON samples of the same soil origin (BHU or LCF), it grouped BHU and LCF in discrete clusters with higher similarities within than between clusters. AOB communities in BHU and LCF soils could be separated even more clearly when analysing the banding patterns from organic and conventional management separately.

March 2004 sampling

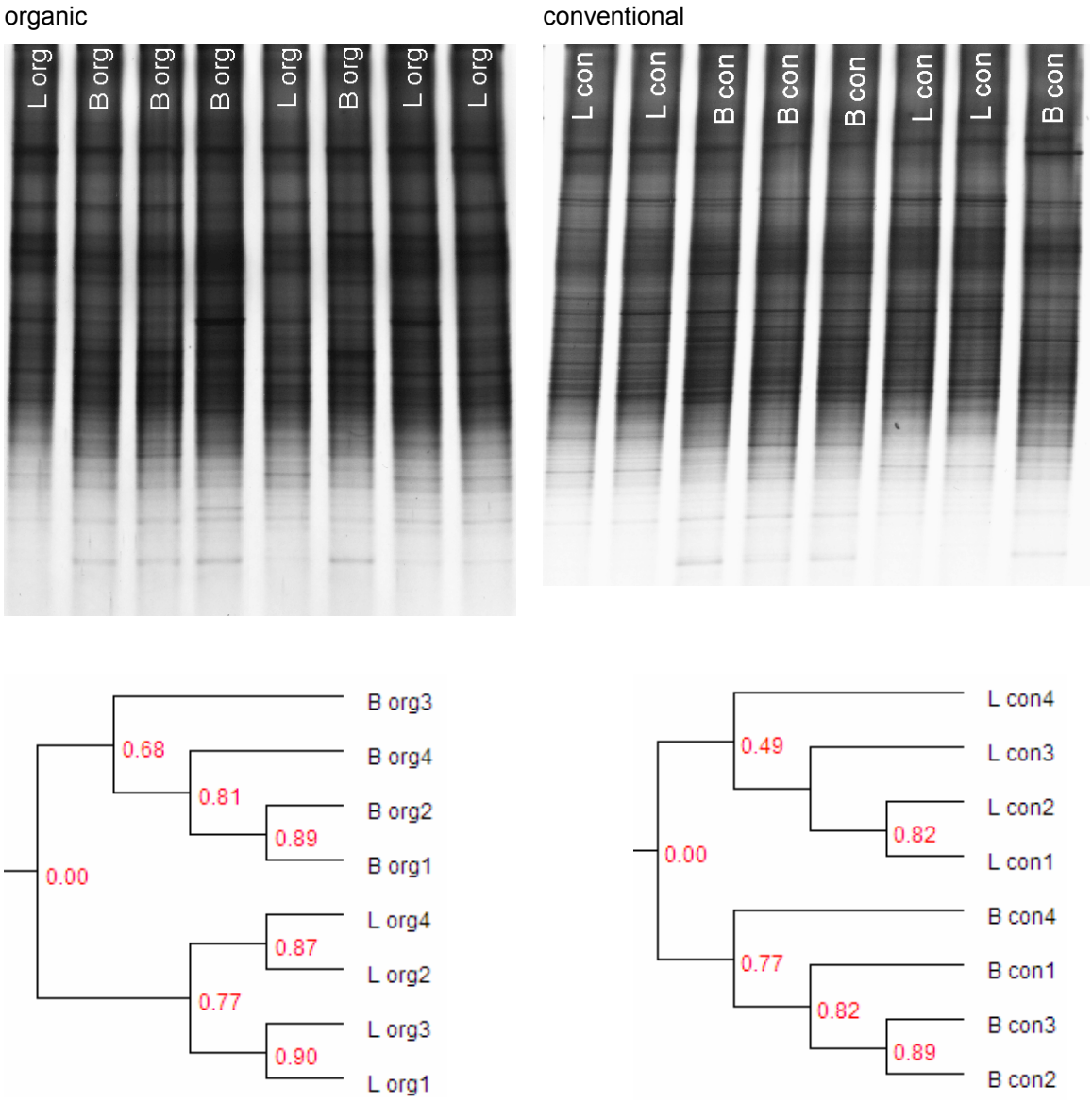


Figure 28: DGGE profiles and cluster analysis for 16S rDNA fragments of eubacterial communities PCR amplified from lysimeter soils taken in March 2004.

In March 2004, in addition to the eubacterial communities, actinomycete and fungal diversities were assessed. The eubacterial communities showed comparable patterns to those observed in the previous sampling. BHU samples could be clearly separated from LCF

samples in ORG and CON treatments visually and by cluster analysis (Figure 28). Similarities within the groups ranged from 49 (L con) to 77% (L org and B con), while it was zero between the clusters separating the treatments. Differences between B org and B con and L org and L con, respectively, were small.

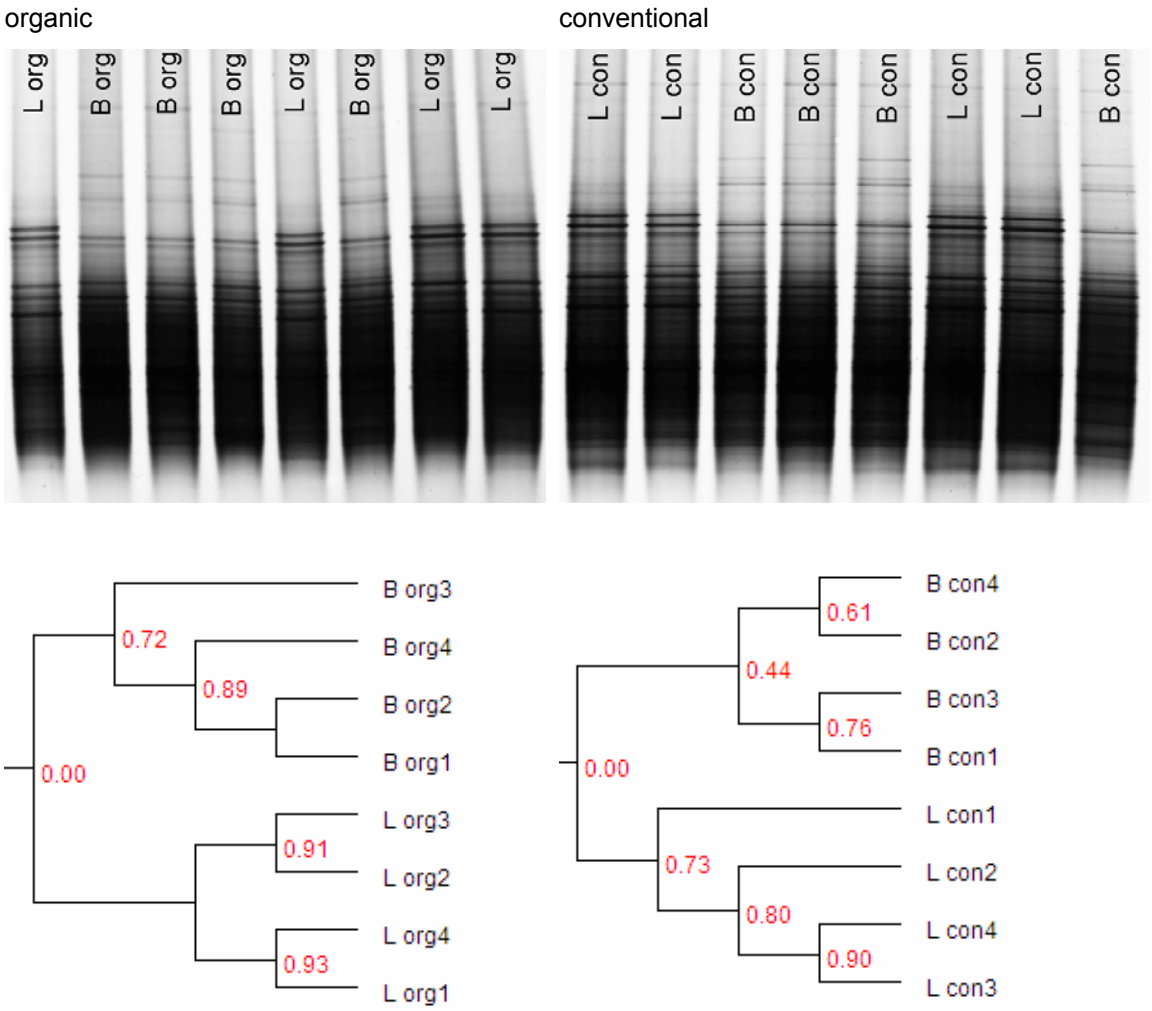


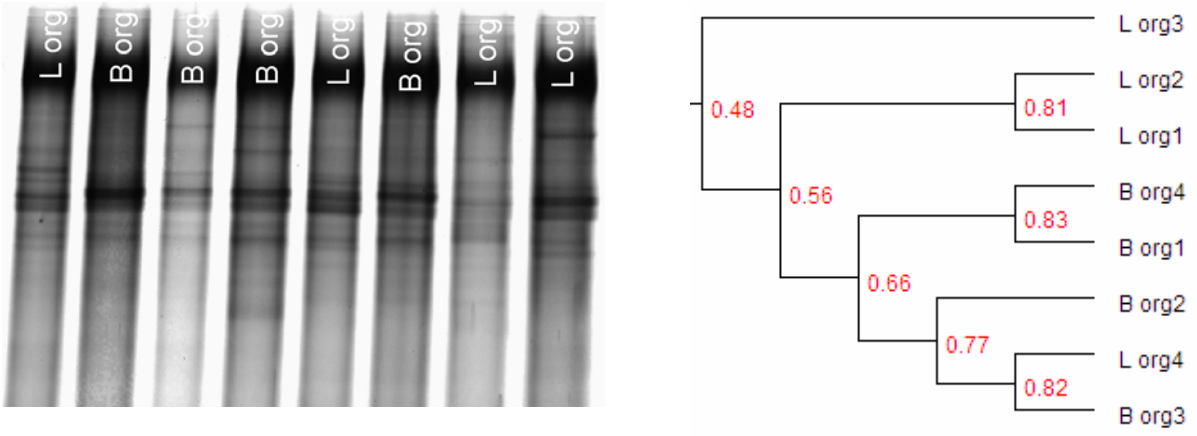
Figure 29: DGGE profiles and cluster analysis for 16S rDNA fragments of actinomycete communities amplified from lysimeter soils taken in March 2004.

For actinomycetes, visual evaluation of the gels showed obvious differences in BHU and LCF banding patterns while the number of bands was similar for all samples (Figure 29). Reproducibility of DGGE analysis and DNA extraction of replicate lysimeter samples was very high with only small differences noticeable between the replicates. The cluster analysis results support these findings. Similarities within each group (BHU vs. LCF) were about >70% in ORG and >40% in CON while no similarity was detected between the two clusters. BHU and LCF DGGE profiles for organic and conventional treatments showed close resemblance.

The DGGE banding patterns of the fungal communities in the lysimeters revealed a different trend (Figure 30). The number of bands was smaller for all four treatments when compared

with bacteria or actinomycetes (Figures 28 and 29) and showed more variability. The profiles for the two soils could not be clearly differentiated. Cluster analysis supported the observations and for ORG, the samples from BHU and LCF could not be sorted into distinct groups by cluster analysis and similarity between the two soils was high.

ORG



CON

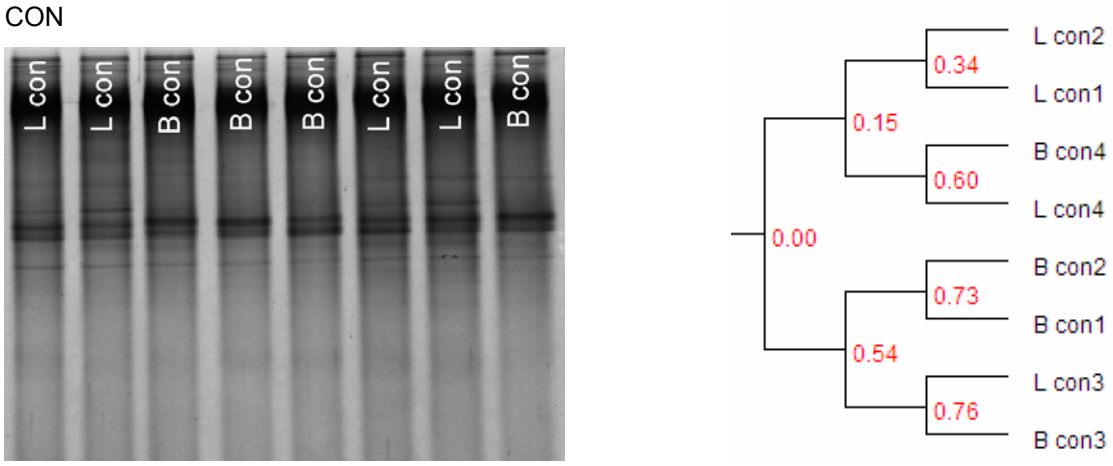


Figure 30: DGGE profiles and cluster analysis for 18S rDNA fragments of fungal communities amplified from lysimeter soils taken in March 2004.

October 2004 sampling

In October 2004, three different primer sets were used to assess diversity of α proteobacterial, actinomycete and pseudomonad communities in the lysimeter soils. In addition, treatments B org and L con, i.e. continuation of the original management regime, and L org and B con, i.e. converted and re-converted soils, were electrophoresed together and compared to see if differences were caused by past or current management practices.

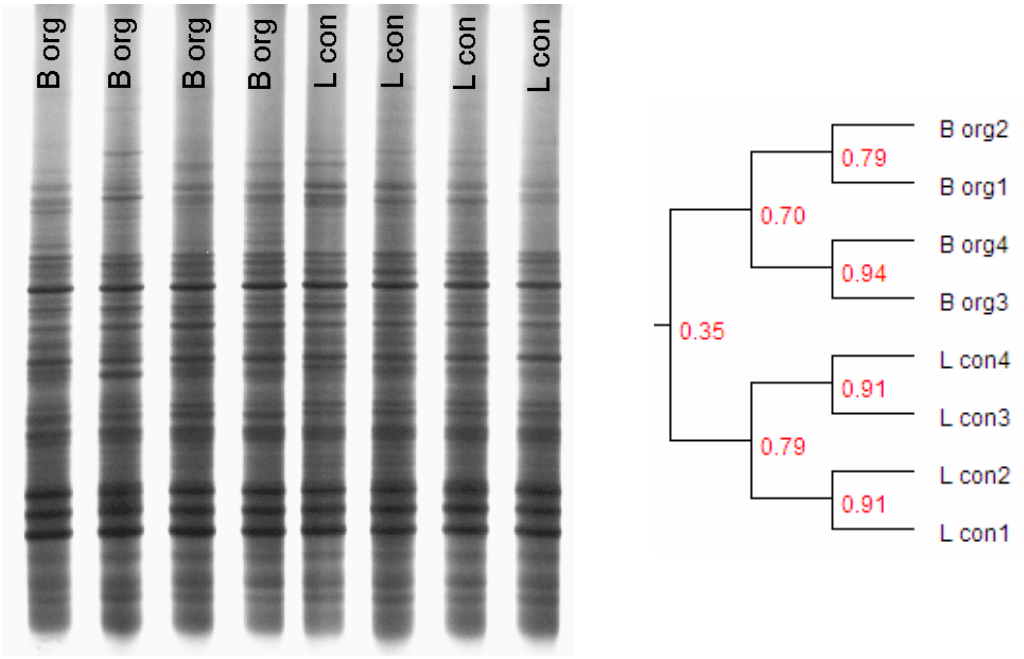


Figure 31: DGGE profiles and cluster analysis for 16S rDNA fragments of α proteobacterial communities amplified from treatments B org and L con in October 2004 in the lysimeter study.

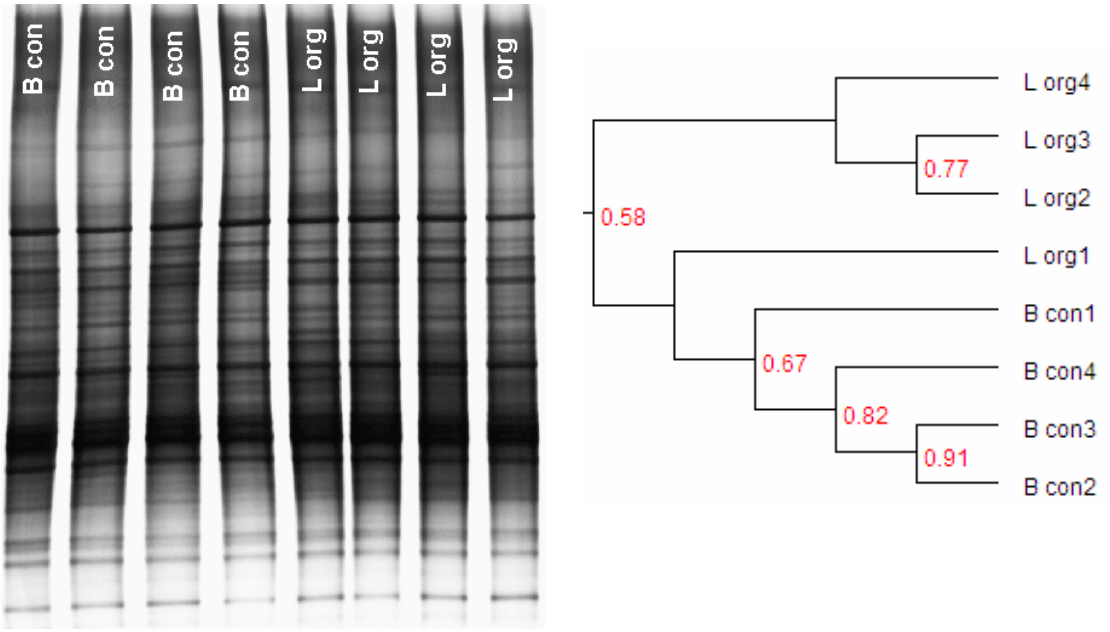


Figure 32: DGGE profiles and cluster analysis for 16S rDNA fragments of α proteobacterial communities amplified from treatments B con and L org in October 2004 in the lysimeter study.

Evaluating the DGGE profiles for α proteobacteria in treatments B org and L con visually showed little difference between the banding patterns (Figure 31). Reproducibility was high for the replicates and even samples from the different treatments seemed to have the same α proteobacteria profiles. Cluster analysis, however, revealed that BHU and LCF samples were grouped into discreet clusters with only 35% similarity compared to >70% within the groups. Comparing the diversity of α proteobacteria in B con and L org to each other showed the same tendency although the clustering was not as robust (Figure 32). L org1 was sorted

into the same cluster with all BHU samples and the similarity between this group and the remaining LCF samples was slightly less than the lowest similarity for the BHU group (58% as compared to 67%).

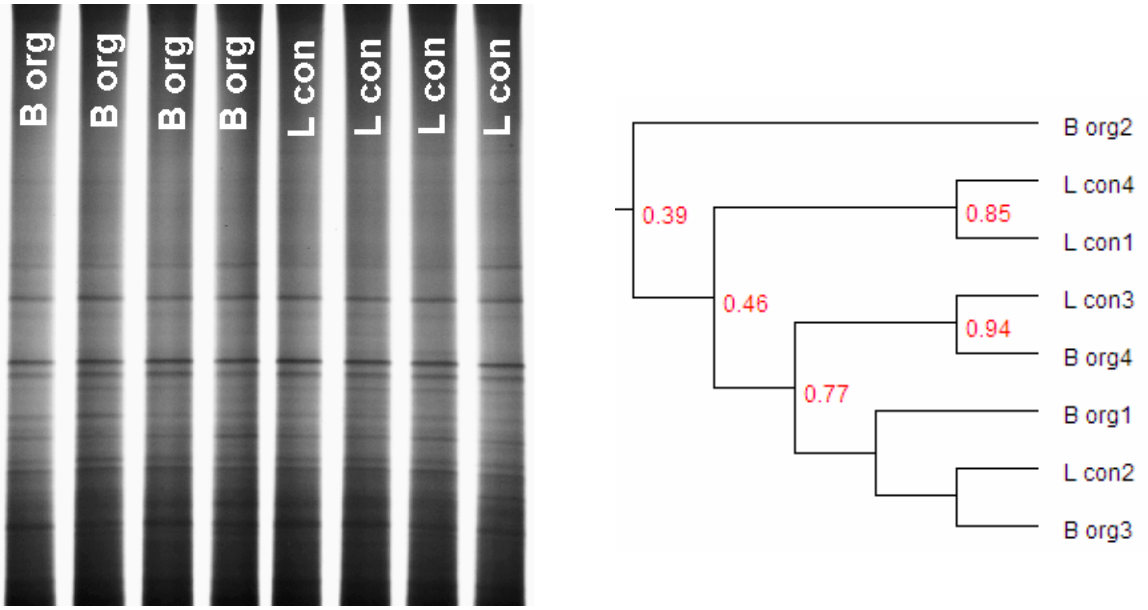


Figure 33: DGGE profiles and cluster analysis for 16S rDNA fragments of actinomycete communities amplified from treatments B org and L con in October 2004 in the lysimeter study.

Actinomycete communities in the B org and L con treatments showed very similar DGGE profiles as seen in Figure 33. The cluster analysis supported this assessment: no distinct clusters were formed for BHU and LCF and high similarities (around 90%) between BHU and LCF samples could be observed.

More distinct differences could be observed between L org and B con samples although DGGE banding patterns seemed to be nearly identical (Figure 34). All but one LCF sample were sorted into a separate cluster with >80% similarity within the group. L org1 was part of the BHU cluster and was more than 80% similar to the B con samples. However, differences between the two treatments were relatively small compared to those observed during the first (November 2003) and second (March 2004) sampling (59% compared to no similarity).

The banding patterns in Figures 33 and 34 suggest that actinomycete communities in the treatments were very dissimilar, however, the fact that the samples were run on different gels makes it difficult to compare them to each other.

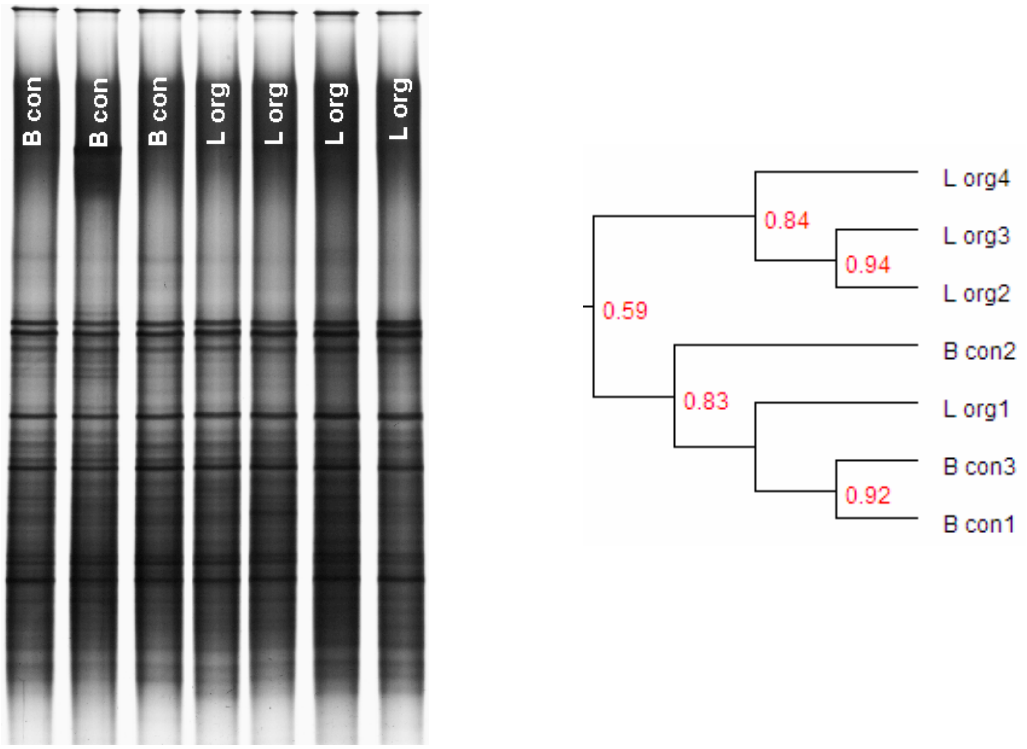


Figure 34: DGGE profiles and cluster analysis for 16S rDNA fragments of actinomycete communities amplified from treatments B con and L org in October 2004 in the lysimeter study.[i]

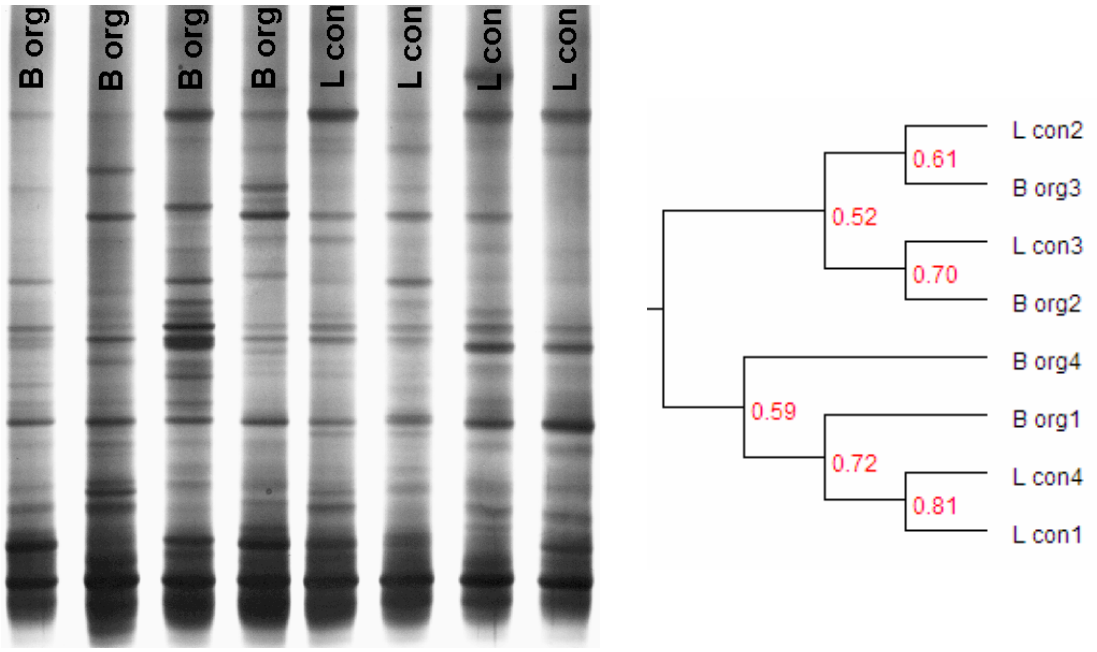


Figure 35: DGGE profiles and cluster analysis for 16S rDNA fragments of pseudomonad communities amplified from treatments B org and L con in October 2004 in the lysimeter study.

[1] One B con sample is missing due to amplification failure. The PCR was repeated and the sample was run on a different gel.

The DGGE profiles for the pseudomonad communities in the lysimeter soils were more variable than actinomycetes and α proteobacteria. Figures 35 and 36 show that no distinct differences or similarities could be observed between BHU and LCF treatments when assessing the gels visually. The same trends were evident when trying to sort the samples by cluster analysis. B org and L con samples could not be clearly distinguished from each other and no discreet clusters were visible. Similarity was relatively low (compared to previous results, e.g. Figures 31 and 32) among all samples (highest of 81% between L con1 and L con4) (Figure 35). Cluster analysis for B con and L org (Figure 36) shows similar results with similarity between samples of the same treatment being as low as between different treatments (73% between B con3 and 4; 68% between B con2 and L org4).

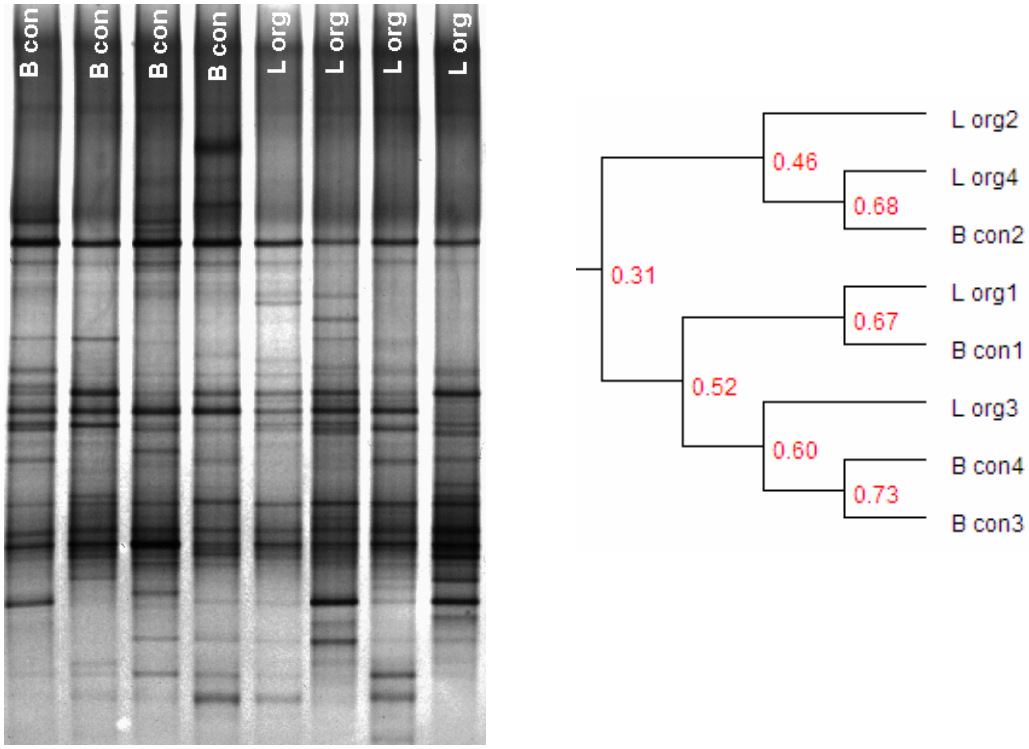


Figure 36: DGGE profiles and cluster analysis for 16S rDNA fragments of pseudomonad communities amplified from treatments B con and L org in October 2004 in lysimeter study.

3.4 Discussion

3.4.1 Achieved objectives

The lysimeter study and the soil analytical methods selected were effective to measure the effects of past and current management on the soil microbial community and N leaching losses, and were used to establish links between microbial biomass size, activity and diversity *in situ*. It was possible to subject the soils to different treatments (organic and conventional management) and to periodically collect leaching samples by using lysimeters. As there was no need to apply pesticides during the trial, treatments differed only in the forms of fertilisers

used. This study was, therefore, not based on a systematic whole-farm approach and was not a comparison of farming systems as such. It was instead focussing on specific farming practices and their effect on the soil microbial community and mineral N losses from soils.

There were also problems associated with the use of lysimeters. No method had previously been established to grow arable crops in lysimeters. In addition, there were no standard techniques to simulate cultivation or take soil samples from lysimeters on a regular basis (as opposed to destructive sampling). Because of the small surface area of the lysimeters, it was difficult to choose a suitable crop rotation for both organic and conventional systems that was representative of farming practices used locally and included crops that would grow in the provided space. Crop growth was strongly affected by the very small growing area and an edge effect was evident as weaker growth in the end lysimeters. This edge effect was minimised by placing one replicate of each treatment at the end of a row and microclimate was created by planting the same crops on a ca. 2 m wide strip next to the lysimeters. However, it was impossible to totally prevent unsheltered areas as the lysimeters were exposed on the trench side. Being on different sides of the trench, conventionally and organically managed lysimeters were differently affected by the weather, especially wind.

By taking soil and leachate samples from the lysimeters at different time points throughout the experiment, the influence of mineral vs. organic fertilisers and including a leguminous green manure in the crop rotation on mineral N leaching and the microbial community were established. Results for most soil analyses did not show any significant differences among the treatments after almost 3 years under the same crop rotation and different management. This shows that the parameters measured were strongly influenced by crop rotation and green manuring, the farming practices that were the same for all treatments. It also indicates that the trial period was too short to make definite statements regarding the influence of the farming practices. Recent research suggests that effects of land-use changes on most soil properties are only detectable after 3 to 5 years (Abbott and Murphy 2003a).

3.4.2 Crop yield and N uptake

The influence of previous (LCF vs. BHU) and current management (CON vs. ORG) was most pronounced for the first crop (barley), which showed significantly higher yields for LCF and CON compared to BHU and ORG treatments. This difference in yield might be a result of the fertilisation that the areas did or did not receive before the lysimeters were taken. While both soils were in a restorative phase in the years leading up to this experiment (pasture and herb-ley, respectively), the LCF site was grazed and had been cultivated and fertilised prior to the pasture phase. In contrast, the BHU area had not been actively managed for about 3 years before the lysimeters were taken and the history prior to that is unclear due to the lack of written records. It is likely that the LCF soils responded better to the conventional fertilisation (L con>B con) because the microbial community was adapted to the form of nutrient input.

The higher yields found under conventional management are not surprising being a likely response to the different fertilisation strategies (no N fertilisation in ORG).

The differences in dry matter yields of the subsequent crops were less pronounced between BHU and LCF, however, lupin (not significant due to large variation within the treatments) and especially maize ($p=0.005$) grown in BHU soils showed higher yields. For lupin, ORG showed slightly higher yields than CON, while for maize, a positive fertilisation effect could be measured (CON>ORG), although the differences were not significant. There was a strong positive correlation between the maize and lupin yields ($R^2=0.742$). This indicates that the maize yield was affected by the addition of the green manure crop, which had slightly higher yields and lower C:N ratios in BHU soils. These soils were, therefore, supplied with more C and N by addition of greater amounts of organic matter, and the lower C:N ratio suggested amendment with higher quality organic material, which made it more easily accessible for the microbial community (Schachtschabel *et al.* 1992). The nutrients in the lupin grown in BHU soils would have been mineralised and made plant available more quickly. The effect was particularly evident for the maize yield in the unfertilised BHU treatment (B org), which was higher than both LCF treatments and similar to B con. Despite differences in fertilisation, rape yields did not differ among the treatments, which suggests that the positive influence of the green manure crop on the unfertilised treatments was still noticeable even a year after incorporation.

Mineral N contents in the crops were similar for all treatments; hence, the differences in N uptake corresponded to the differences in yield of the respective crops. With respect to current management effects, differences in N uptake can also be explained by the higher N availability due to fertilisation in CON. The N uptake of barley was slightly higher in LCF and CON, while lupin showed a significantly higher N uptake from BHU and ORG. Similarly, higher N uptake from BHU and CON soils could be observed for maize, which is due to the higher yields as well as the higher quantity of N available in BHU soils (a result of higher lupin yields [BHU] and fertilisation [CON]). As observed for the dry matter yield data, N uptake by rape did not differ significantly among the four treatments.

The C:N ratios of the four crops were very similar for ORG and CON, while BHU crops had consistently lower C:N ratios indicating higher substrate quality for the crops grown in soils originating from the organic farm. This implies that the application or non-application of mineral fertiliser did not influence the dry matter quality of the crops, and that it was affected by past management for most of the trial period. The exception was rape, which had lower C:N ratios for ORG compared to CON treatments.

Overall, differences in crop yield and N uptake were small, however, they were lower for LCF treatments. While there was clear positive effect of N fertilisation (CON>ORG for both soils), the treatment under continued organic management (B org) responded better to the lack of fertilisation and amendment with organic matter than the treatment that had been converted to organic management as part of the experiment (L org), i.e. conversion to organic farming

practices resulted in lower crop yields. Crop yields may increase only after 3 to 5 years following conversion to organic practices, while similar yields were found when comparing sites under long-term organic or conventional management (Altieri 1995; Drinkwater *et al.* 1995; Reganold *et al.* 2001). This suggests that lower crop yields during the first few years following conversion can be overcome when soils have been under long-term organic management (like B org) (Bulluck *et al.* 2002). This might be related to changes in soil properties resulting from beneficial effects of organic farming practices that allow for better and faster response to organic matter addition and make it possible for the soils to maintain higher fertility despite lack of fertilisation (Robertson and Morgan 1996; Shepherd *et al.* 2000a; Bulluck *et al.* 2002).

3.4.3 Mineral N leaching losses

No significant differences were detected in the amounts of drainage from the four treatments, although the LCF lysimeters showed lower drainage compared to BHU treatments. This indicates slight differences in soil texture and structure between the two sites and is consistent with the higher water holding capacity at LCF (LCF: 31.6%; BHU: 27.2%). Even though the lysimeters were taken from relatively small areas, variations in soil structure and texture can lead to high variability in drainage and nutrient concentration in the leachate (e.g. Toor *et al.* 2005).

There were no significant differences in mineral N losses between treatments, which indicates that the application of inorganic fertilisers did not result in an increase in N leaching. However, mineral N losses from ORG were generally lower than from CON; in particular B org showed lower cumulative leaching losses than the other treatments over the 2½-year period, although leaching from this treatment was highly variable over time and higher losses were recorded from B org than from the other treatments between April and October 2004 (Table 22). Due to the inconsistent and generally lower leaching losses from one replicate in this treatment, the variation was larger for B org than for the other treatments; this can be seen in the large standard errors for drainage as well as mineral N losses (Table 22). Excluding the replicate from the analysis did not change the overall trend that N losses from both ORG treatments were lower than from CON. This suggests that the lack of N fertilisation in the organically managed lysimeters reduced the leaching of mineral N, although not significantly. Several studies have shown that organic farming practices can reduce N leaching losses (e.g. Dalgaard *et al.* 1998; Stolze *et al.* 2000; Shepherd *et al.* 2003), while others stress that mineral N losses can be equal or higher from organic farms. Especially application of manures or incorporation of green manures can result in N flushes due to sudden mineralisation of the organic material (Eriksen *et al.* 1999; Shepherd *et al.* 2003). Fraser *et al.* (1988) reported higher autumn levels of nitrate in soils that received animal manure compared with chemical fertilisers and attributed this difference to increased mineralisation. This can be a problem when cultivation coincides with warm temperatures (stimulating mineralisation) followed by

heavy rain (increasing drainage) or when manures are applied in late autumn when evapotranspiration rates are low and N released from organic matter is lost from the soil by leaching (Di *et al.* 1999; Shepherd *et al.* 2003).

In this study, the amounts of N leaching observed (1.3-20 kg ha^{-1} a^{-1}) are comparable to those measured in other studies (Di and Cameron 2002b), indicating that addition of N in mineral form had no effect on the leaching patterns nor did incorporation of a leguminous green manure increase mineral N losses. This might be a consequence of the lower than average rainfall during the trial period resulting in less drainage than expected for this geographical area (Table 15). However, Drinkwater *et al.* (1998) concluded that the inclusion of leguminous crops in the crop rotation can improve soil N retention and, consequently, reduce losses.

3.4.4 Soil analyses

A number of studies have examined the microbial status of soils managed under organic and conventional management (e.g. Raupp 1995b; Yeates *et al.* 1997; Gunapala and Scow 1998; Lundquist *et al.* 1999b; Ryan 1999; Fliessbach and Mäder 2000; Mäder *et al.* 2002; Schjønning *et al.* 2002). While there is evidence that organic farming practices increase microbial biomass and activity and have a positive effect on soil organic matter, others found that organic practices have no or negative effect on soil microbial communities (Shepherd *et al.* 2000a; Stolze *et al.* 2000). In general, microbial biomass, enzyme activities, soil respiration, earthworm numbers and/or activity were greater in soils under organic management than in those receiving synthetic inorganic fertilizers. Differences in microbial diversity between organically and conventionally managed sites were small (Wander *et al.* 1995; Yeates *et al.* 1997; Shannon *et al.* 2002; Girvan *et al.* 2003), although there is evidence for greater bacterial, actinomycete and fungal abundance and activity under organic management (Mäder *et al.* 1995; Gunapala and Scow 1998; Bulluck *et al.* 2002; Shannon *et al.* 2002).

Different soil microbial properties have been measured as part of the DOC (bioDynamic, Organic, Conventional) trial in Switzerland. Mäder *et al.* (2002) reported higher microbial biomass, microbial quotient, respiration and enzyme activities in organic and biodynamic farms compared to conventional systems with and without farmyard manure. Microbial soil properties (including community composition and microbial activity) and the effects of, for example, seasonal variation or disturbances were assessed in conventional, low-input and organically managed soils as part of the SAFS experiment (Sustainable Agriculture Farming Systems) (e.g. Bossio *et al.* 1998; Gunapala and Scow 1998; Gunapala *et al.* 1998; Lundquist *et al.* 1999b). Higher inputs of organic matter resulted in increased microbial biomass in all management systems irrespective of history (Gunapala *et al.* 1998), however, it was possible to distinguish organic and the conventional systems based on microbial community

compositions (PLFA) (Bossio *et al.* 1998). The studies emphasized the importance of environmental variables affecting microbial communities and ranked their significance as follows: soil type >time > farming practice (e.g. cover crop or mineral fertilisation) > management system > spatial variation in the field (Bossio *et al.* 1998). Girvan *et al.* (2003) found that soil type had a bigger influence on microbial diversity (assessed by DGGE analysis of PCR amplified 16S rDNA fragments and Biolog™) than crop type or management practices (organic vs. conventional; manure vs. no manure). However, study sites with the same soil type were at geographically distinct locations, which would have introduced other environmental variables (climate) affecting the microbial community in the soils, and the sites had been under organic management for 2 years or less, i.e. any management induced effects would not have been detectable yet.

Some studies failed to find significant differences in microbial soil properties between organic and conventional systems or suggested a negative impact of organic practices (Cook *et al.* 1995; Yeates *et al.* 1997; Bardgett and McAlister 1999). However, most of these studies examined grassland systems, and Shepherd *et al.* (2000a) pointed out that differences are expected to be small due to the nature of these systems. Pastures generally have higher a soil organic matter status and microbial biomass than cropping soils and organic matter accumulates in pastoral soils under both management regimes. In addition, there are a number of studies that emphasize the beneficial effects of organic matter amendments on the soil microbial community but did not find any negative impacts of mineral fertilisation (Fraser *et al.* 1988; Söderberg and Bååth 2004; Zaitlin *et al.* 2004). Fauci and Dick (1994) found no effect of short-term mineral N amendments on microbial biomass and enzyme activities, while long-term application seemed to decrease microbial activity. Martyniuk and Wagner (1978) suggested that the input of C sources to soils (either in the form of manures or by increasing plant and root biomass and, consequently, rhizodepositions through inorganic fertilisation) had the strongest positive effect on the soil microbial community when comparing different management regimes. Several other studies concluded that animal and green manures (i.e. the addition of organic matter) are the main factors contributing to higher microbial populations and activity in organically managed soils and can improve soil health within 2 years (e.g. Miller and Dick 1995; Robertson and Morgan 1996; Bossio *et al.* 1998; Gunapala and Scow 1998; Girvan *et al.* 2003). In addition, the influence of plant species and crop rotations on the microbial community have been repeatedly mentioned (Doran *et al.* 1988; Campbell *et al.* 1992; Dick 1992; Grayston *et al.* 2004). Crop rotations (in particular those including leguminous crops) have a positive effect on microbial diversity and activity by increasing the quantity and quality of C inputs (rhizodepositions) and by suppressing organisms that increase under monocultures (Campbell *et al.* 1991; Lupwayi *et al.* 1998; Girvan *et al.* 2003).

It was not the aim of this project to assess the effect of tillage on the microbial community. However, the lysimeters were under a restorative phase (pasture and herb-ley, respectively) when the experiment started, and were subsequently cultivated (by turning the topsoil [0-

15 cm] over to simulate ploughing, i.e. conventional tillage) on a regular basis as part of the arable crop rotation. Conventional tillage systems have been found to decrease microbial activity due to a negative impact on aggregate stability (Dick 1992). Comparing conventional and no-till systems showed a larger microbial biomass (assessed by PLFA) and a more diverse community of ammonia oxidisers under no-till (Ibekwe *et al.* 2002). Similarly, Lupwayi *et al.* (1998) concluded that species richness and evenness (assessed by Biolog™) in soils under wheat were reduced by conventional compared to zero tillage. Consequently, a decrease of microbial activity and diversity was expected to take place in the lysimeter soils.

Effect of time on soil microbial properties

The objective of this study was not to investigate the temporal variation in the soil properties measured, but to determine the effect of past and current management practices. When evaluating the effect of current management practices, it is more important to consider overall trends in microbial biomass levels and microbial activity. The different points in time can mainly be seen as replicate samples. Analyses and discussion, therefore, focused on relative changes in soil microbial properties. The levels of the various soil properties will have been subject to the influence of variations in soil moisture, temperature, crop type, nutrient supply, etc., as well as immediate farming practices, such as fertilisation and cultivation (Campbell *et al.* 1999). Consequently, the differences between the sampling dates were significant for most soil properties (except C_{tot}). Interestingly, the differences between the treatments decreased for microbial biomass C, while they persisted for the three enzyme activities (DHH, ADA, FDA).

For most parameters, the highest values were measured after the first or second crop, i.e. not long after cultivation began. This is consistent with other research showing that cultivation decreases microbial biomass and activity and that lower levels are found under arable crops compared to pasture (Bandick and Dick 1999; Haynes and Tregurtha 1999; Shepherd *et al.* 2000a). The microbial community is also strongly influenced by crop type and rotation (Doran *et al.* 1988; Elmholt 1996), which suggests that more similar communities can be found under the same plant type as was the case in this study where all treatments were subjected to the same crop rotation. Only microbial activity measured by FDA hydrolysis increased during the experiment and was highest at the last sampling point. Although associated with microbial activity, FDA hydrolysis is carried out by a range of enzymes including extracellular enzymes that can persist in the soil as part of inorganic complexes or when associated with organic colloids (Nannipieri *et al.* 2002). The increase in FDA hydrolysis activity may, therefore, indicate changes in soil properties that facilitate and promote activity of extracellular enzymes or changes in the quantity and quality of root exudates. The positive influence of mineral fertilisation on enzyme activity can be attributed to indirect effects of increased plant and root biomass and activity (Doran *et al.* 1988) and has

been observed by Kandeler *et al.* (1999a), who reported doubled and 2.5-times higher xylanase activity under farmyard manure and mineral N fertilisation, respectively.

Microbial biomass C and the associated ratios (CN_{mic} and C_{mic}:C_{tot}) showed uncharacteristically low levels in November 2003, which were difficult to explain. The addition of the lupin green manure 7 weeks prior to sampling should have resulted in an increase in microbial biomass and activity (Bossio *et al.* 1998; O'Donnell *et al.* 2001). Although it has been reported that microbial biomass levels decline after the initial increase following organic matter incorporation (Lundquist *et al.* 1999a), there is no evidence in the literature for a negative effect of organic matter amendments on the soil microbial community. Lundquist *et al.* (1999a) reported that microbial soil properties were highest in the first week after incorporating rye residues to soils and then rapidly decreased, while main differences were sustained over the 6 week trial period. Gunapala *et al.* (1998) also described a 4-day phase of increased activity (measured by SIR) after incorporation of organic material that was followed by a decline to a stable level. In this study, some values measured for C_{mic} in November 2003 were much lower than any other levels measured during the experiment and variation amongst replicates was larger than observed at other times (Figure 18). The lysimeters were irrigated less frequently after the lupin crop had been incorporated in September 2003 and lower soil moisture contents were measured for all treatments in November 2003. This and the high temperature on the sampling date (maximum air temperature: 24°C; soil temperature at 10 cm: 19°C) would have resulted in a smaller microbial biomass (Insam *et al.* 1989; Gunapala and Scow 1998). The relatively higher levels of ADA (when compared with the other sampling dates), especially for B org which had the lowest levels of microbial biomass, might support this theory and indicate a stress response of the microbial community (Wardle and Ghani 1995; Nsabimana *et al.* 2004). However, the lack of a correlation between soil moisture and ADA (R^2<0.1) contradicts this assumption and soil moisture levels could not account for all of the variation (Figure 24). The large variability within replicates for C_{mic}, ADA and soil moisture itself could not be explained.

The overall decrease in microbial biomass N and microbial activity (measured by DHH and ADA) over the trial period is consistent with the negative effects of cropping, especially repeated soil cultivation, on soil organic matter content and microbial soil properties (Dick 1992; Robertson and Morgan 1996; Haynes and Tregurtha 1999). While microbial biomass C levels were, on average, lower at the final sampling than initial levels, they were subject to large variations during the trial period and displayed the unexplained low levels in November 2003 discussed above, i.e. no trend of increase or decrease could be identified for microbial biomass size.

Effect of past management on soil microbial properties

After changing (from organic to conventional management and vice versa) and adjusting management practices across the treatments (same crop rotation, cultivation, irrigation), the initially observed differences in biological soil properties (microbial biomass and activity) between the two sites (Section 2) were expected to decline. Organic matter amendments and plant type have a large influence on the soil microbial community by stimulating microbial growth and activity (Ocio *et al.* 1991; Bossio *et al.* 1998). Different plant species release compounds of varying quantity and composition into the soil (root exudates or rhizodepositions) (Grayston *et al.* 1998; Lupwayi *et al.* 1998; Larkin 2003), which can contain enzymes, form chelates and alter pH and redox potential in the rhizosphere (Amberger 1996). In contrast, total C and N levels were expected to remain at the same levels, as they are considered more stable and less affected by changes in management practices in the short-term. There is evidence that changes in soil organic matter levels that are sufficient to also influence the microbial soil properties might not be detectable earlier than 10 years after new management practices were implemented (Wander *et al.* 1995; Haynes 1999; Ryan 1999).

The effect of past management could not be interpreted without considering the effects of current management, since the values for BHU and LCF were averages of samples taken from lysimeters with continued and changed management. For example, if the fertilisation had a negative effect on the soil properties, a decrease would be expected in the average levels of microbial biomass and activity in BHU soils that did not previously receive soluble mineral fertilisers, while the microbial community in LCF should have been adjusted to the application of synthetic fertilisers. This could result in a decrease and increase of microbial soil properties in BHU and LCF, respectively.

For C_{mic} and N_{mic}, differences between the treatments decreased over time resulting in similar values for BHU and LCF (2003: BHU>LCF; 2004: BHU=LCF). The results suggest that the effect of past management on microbial biomass C decreased over time and that the management practices that were the same for all treatments (i.e. crop rotation) caused differences between BHU and LCF soils in microbial abundance to decline. This observation is in accordance with the findings of Gunapala *et al.* (1998), who reported no differences in microbial biomass C and N after the addition of organic material to soils that had been under long-term organic and conventional management. The microbial C:N ratio is an indicator for the composition of the microbial community as fungi have higher C:N ratios than bacteria (Lovell *et al.* 1995). The differences in microbial C:N ratio in the two soils, therefore, indicate higher bacterial numbers in BHU and higher fungal numbers in LCF, which is supported by the results of the initial soil dilution plating in this study. Gunapala and Scow (1998) also found that bacteria were more dominant under organic management when comparing organic and conventional farming systems. The relative levels of CN_{mic} between the soils remained the same over time, while absolute levels increased in both soils. This indicates changes in the

composition of the microbial community in both soils with a shift towards fungi, which might be a result of the crop type and growth characteristics (rhizosphere microclimate, changes in root exudate and crop residue quality) (Elmholt 1996).

After almost 3 years under the same crop rotation, microbial activity (ADA, DHH: BHU>LCF; FDA: LCF>BHU) and the microbial quotient (BHU>LCF) were still significantly affected by past management. For most soil properties, relative differences between averages of BHU and LCF were consistent over time. Enzyme activities ADA, DHH and FDA and N_{mic} showed the biggest differences between the soils and the levels of the soil properties remained very similar in LCF and BHU soils throughout the experimental period, indicating that the influence of past management persisted over time. This corresponds with the observations by Fauci and Dick (1994), who found that differences in microbial biomass C and N resulting from long-term treatments were still visible after 1 year (four crops of maize) in a greenhouse trial that studied the effect of different organic and mineral amendments. The differences in microbial activity were observable even after addition of mineral fertilisers (as in B con), which indicates some form of "residual activity" that did not respond quickly to changes in management practices, and suggests that the enzymatic activities were not affected by the addition of mineral fertilisers. The findings are supported by other studies that highlight the positive effects of organic matter addition but did not find a particular negative impact of fertilisation on the microbial community (e.g. Martyniuk and Wagner 1978; Bolton *et al.* 1985; Fraser *et al.* 1988; Söderberg and Bååth 2004). Total C and N remained strongly affected by management history and did not show any effects of current management practices, i.e. levels were similar to those measured in the initial study (Table 3). As mentioned before, total C and N levels respond only slowly to changes in management practices (Wander *et al.* 1995; Wander and Traina 1996).

Effect of current management on soil microbial properties

The literature suggests a beneficial effect of organic farming practices on microbial activity and biomass. The amendment with organic materials, in particular, is known to have a stimulating effect on the microbial community (Bolton *et al.* 1985; Bossio *et al.* 1998; Lundquist *et al.* 1999a; Girvan *et al.* 2003). Mäder *et al.* (1996; 2002) found higher microbial activities (measured as enzyme assays [e.g. dehydrogenase, protease, phosphatase] and by respiration) in organic and biodynamic farms compared to conventional systems with and without farmyard manure. Similarly, Gunapala and Scow (1998) measured higher microbial activity (arginine deaminase, SIR) in organically managed soils that had been amended with manures compared to unmanured conventional soils. These results are not surprising as most studies examining the effect of organic and conventional farming practices on microbial soil properties compared the impact of organic matter inputs (e.g. as green or animal manures) in one system with the addition of synthetic fertilisers in the other system. In contrast, in the present study, a green manure crop was incorporated in all treatments and the differences

between the two management systems were the use of mineral and organic fertilisers. Assuming that green manure amendments have a positive effect on all treatments and short-term mineral fertilisation does not affect microbial biomass (Fauci and Dick 1994), microbial abundance and activity should be of similar size in the organically (ORG) and conventionally (CON) managed soils. Results showed that the soils under ORG and CON could be distinguished from each other in terms of microbial activity measured as DHH, ADA and FDA but not by microbial biomass size (C_{mic}, N_{mic}), total C and N or microbial quotient.

Microbial activity measured as DHH was significantly higher in ORG than CON, which is consistent with the higher enzyme activity in the originally organically managed site BHU. This could either indicate a positive effect of certain farming practices, in this case the lack of fertilisation (for the site BHU) and the use of organic fertilisers (for the treatment ORG) compared to mineral fertilisation (for LCF and CON), or a negative impact of mineral fertilisers on this enzyme activity. Measurements of DHH activity under laboratory conditions are thought to underestimate the actual activity of the enzyme in soils (Dick 1997; Nannipieri *et al.* 2002), and Kumar and Tarafdar (2003) suggested that DHH activity is mainly carried out by bacterial and actinomycete populations, while fungal activity only contributes a limited amount to the activity of the enzyme. This does not explain the higher rates of DHH activity in ORG soils where, based on the higher microbial C to N ratio, higher fungal numbers were expected, but the increasing proportion of fungi in the microbial community (increasing CN_{mic} over time; Figure 21) might explain the overall trend of decreasing DHH activity in all treatments (Figure 20).

Hydrolysis of FDA and deamination of arginine (ADA), on the other hand, were higher in CON soils at most sampling points (exception ADA in November 2003) and the differences increased over time. For FDA, this observation is consistent with the higher activity levels in LCF compared to BHU, whereas ADA showed higher levels in BHU soils at all sampling points (Table 24). Other researchers reported increases in FDA hydrolysis due to a positive effect of continuous grassland compared to arable cropping (Bandick and Dick 1999; Haynes 1999) or as a result of organic amendments (e.g. Perucci 1992; Pfotzer and Schüler 1995; Pankhurst *et al.* 2005). However, most of these studies looked at the effect of mineral vs. organic fertilisers on microbial activity and found a stronger positive effect from organic amendments. Pfotzer and Schüler (1995), for example, concluded that microbial activity (measured by FDA) was significantly higher after compost amendments compared to mineral fertilisation. Their results, however, suggest an increase in FDA hydrolysis over time for all treatments and do not indicate a negative or inhibiting effect of mineral fertilisers on FDA hydrolysis. The results presented here indicate that FDA hydrolysis was positively influenced by the conventional practices applied in this study. This is confirmed by the results of Schnürer *et al.* (1985), who observed the highest activities of FDA in soils amended with straw and mineral N. Hydrolysis of FDA might have been indirectly affected by the amendment with synthetic fertilisers and the addition of N to the soils by stimulating crop growth and root activity (Doran *et al.* 1988).

Arginine deamination is a microbial process involved in the N cycle (Schinner *et al.* 1995) that is known to take place inside microbial cells and is closely correlated with microbial biomass (Alef and Kleiner 1986, 1987) and other measures of microbial activity, e.g. SIR, ATP (adenosine triphosphate) content and heat output (Alef *et al.* 1988). The differences between ORG and CON suggest differences in microbial activity and, in particular, the N cycle in the soils subjected to different fertilisation regimes. However, throughout the experiment, BHU soils clearly showed higher levels of ADA compared to LCF soils and ADA was higher in B org than in B con at most sampling points. Only at the last sampling (October 2004), both CON samples had higher rates of ADA than ORG. This indicates that the levels did not follow any obvious trends during the trial period and that ADA activity might suffer from high seasonal variability (Figure 20). It is important to keep in mind that enzyme assays measure potential rather than actual activity and that enzymes can be bound to inorganic complexes or organic colloids and persist in soils for many years (Nannipieri *et al.* 2002).

The other soil properties (C_{mic}, N_{mic}, total C and N) were not significantly different between the treatments at most sampling points, which suggests they were not strongly affected by the application of mineral or organic fertilisers. In the first year, microbial biomass C was lower in ORG, corresponding with the lower yields of barley due to lack of N fertilisation, which would have resulted in less root biomass and lower rhizosphere depositions in the organically managed crops. In second year, C_{mic} levels were very similar for ORG and CON indicating a positive influence of the lupin incorporation and consequent higher maize yields, especially in B org, which initially had the lowest levels of C_{mic} and the highest after maize in March 2004 (Figure 18). Microbial biomass N showed the opposite trends with higher levels for ORG compared to CON in the first year and lower levels overall and for ORG in 2004. The higher microbial biomass N levels in ORG in 2003, especially after lupin incorporation, indicate that the soil environment in ORG was more N-limited with higher immobilisation rates, i.e. the N requirements of the microbial community exceeded the availability of mineral N in the soil solution (Smith *et al.* 1993). The general decrease in biomass N (while C_{mic} remained at similar levels) can be explained with the overall effect of cultivation on microbial soil properties as well as with management-induced changes in the composition of the microbial community. Campbell *et al.* (1991) found that microbial biomass C was less affected by various treatments (fertilisation, cropping frequency, legumes, crop rotation) than microbial biomass N. As observed for C_{mic}, differences in N_{mic} between ORG and CON were not significant in the second year of the experiment. For total C and N, no major differences were observed between ORG and CON throughout the trial period.

3.4.5 Microbial community structure

The structure of the microbial community in the organically and conventionally managed lysimeter soils was studied by DGGE of 16S and 18S rDNA fragments amplified by PCR

using different primer sets. Organic systems do not rely on the input of N in mineral form, but on the use of crop rotations that include leguminous green manures and cover crops to maintain soil fertility and crop production. A major focus is, therefore, the inclusion of farming techniques that stimulate soil processes and improve nutrient cycling and retention in the soils (Lampkin 1994; Condron *et al.* 2000; Shepherd *et al.* 2000a). Differences in nutrient cycling, especially of N as the major contributor to plant biomass production, might be reflected in the diversity of the microbial community involved in these processes. Different primer sets, that have been described in the literature and previously used in soil environments, were used throughout the experiment at the various sampling dates. Different microbial groupings (fungi, eubacteria, actinomycetes, ammonia oxidising bacteria (AOB), pseudomonads and α proteobacteria) were targeted that play important roles in soil organic matter decomposition, nutrient cycling processes, soil aggregation and disease suppression (Eash *et al.* 1994; Beare 1997; Heuer *et al.* 1997; van Elsas *et al.* 2000; Coleman *et al.* 2004; Kennedy *et al.* 2004). DGGE profiles for fungi and AOB produced too few bands, while the banding patterns for eubacterial communities were very complex with a large number of bands, some of which were very faint and hard to detect. Since analysing community structures of eubacteria, AOB and fungi did not give successful results, the use of these primers was abandoned after the first and second sampling, respectively, and the last sampling focused on actinomycetes and two bacterial groups (pseudomonads and α proteobacteria). Although the use of different primers made comparing among sampling dates impossible, I hoped that focussing on these groups for DGGE analysis would produce gels with fewer, more distinct bands that would reveal clear differences between the samples and could be more easily analysed.

Although transformation of banding patterns into numerical data and detailed statistical analysis by, for example, principle component analysis is possible and advisable, it strongly depends on the consistency of experimental conditions for all gels (Fromin *et al.* 2002). In this study, I observed variation in the electrophoretic behaviour of samples on different gels, especially when they were run in separate batches (at different points in time) but also when they were run together in the same buffer solution. The first could be due to variations in buffer concentration, running time, or voltage[1] between batches, while the latter might be caused by inconsistencies in gel composition. Chemical solutions were made up every other week and each gel was prepared at the day of usage from a separate gradient mix; i.e. even gradient solutions for gels of the same gradient were prepared in separate bottles. Minor variations in the chemical composition of the solutions used to prepare the gels and pipetting errors when preparing the gradient solutions could have resulted in different compositions of gels with the same chemical gradient. Fromin *et al.* (2002) pointed out that the low

[1] Problems with fluctuating voltage were encountered on several occasions; as a consequence some gels had to be repeated.

reproducibility between gels is a major disadvantage of the method, which can be overcome by, for example, standardising equipment for each gel that is prepared.

The encountered problems with reproducibility made it impossible to compare across gels despite the use of markers. Therefore, cluster analyses were performed for samples run on the same gels only. In the first two samplings, emphasis was put on comparing the effects of management history. Consequently, PCR products from samples of the same management (ORG and CON) were run together. In the final sampling, samples of the continued management regime (B org and L con) were run on the same gel to determine the effect of past management after 2½ years under the same crop rotation. Samples of the altered management regime (B con and L org) were compared to each other as they were expected to show the strongest differences related to current management.

November 2003 sampling

In November 2003, DGGE banding patterns of eubacterial communities showed clear differences between BHU and LCF samples for ORG and CON treatments (no similarity between LCF and BHU) (Figure 25), while AOB communities did not show as distinct differences (80%) (Figure 27). AOB constitute a subgroup of the general bacterial community and the smaller number of bands indicates a smaller community. The lower quality of AOB gels compared to bacterial gels and the small number of clearly visible bands made confident analysis difficult. However, Kowalchuk *et al.* (2000) found similar-looking banding patterns when assessing AOB communities in grasslands. The number of bands in the bacterial community patterns was similar in BHU and LCF, which could indicate microbial communities of similar diversity assuming that the number of bands can serve as a measure of species richness. As mentioned in Section 2 (review of methods), this interpretation might be compromised by co-migration of bands (1 band = several species) and sequence heterogeneities between different operons (several bands = 1 species) (Heuer *et al.* 2001) and DGGE banding patterns might not reflect actual biodiversity in environmental samples (Kisand and Wikner 2003). Visual assessment did not reveal evidence for differences in eubacterial and AOB community structures caused by organic and conventional management within each soil.

These results indicated that bacterial and AOB community composition were primarily influenced by management history, most likely due to the frequent cultivation and use of mineral fertilisers on LCF. Being subjected to the same crop rotation did not affect the differences observed initially (Figure 7). Approximately 1½ years after organic and conventional management practices were implemented, no influence of mineral N fertilisation on microbial community composition assessed by DGGE analysis was evident. As discussed in relation to microbial soil properties, it can be assumed that the management practices of green manure application and crop rotation had a bigger influence on the microbial

community than the use of mineral or organic fertilisers. This implies that the microbial communities were relatively stable and might need a longer period of influence to be affected by management practices. Correspondingly, Crecchio *et al.* (2001) reported no changes in the structure of bacterial communities 2 years after the addition with municipal solid waste, whereas Marschner *et al.* (2003) investigated the effect of long-term amendment with organic materials (30 years) on the community structure of soil microorganisms and found that amendments increased bacterial biomass size and affected bacterial community composition. As the number of 16S rDNA fragments obtained by DGGE separation might not be representative of the number of genomes present in the soil (Torsvik *et al.* 1994) and DGGE banding patterns might only correspond to the most dominant species (Muyzer *et al.* 1993), it is possible that diversity and community structure of less abundant species were affected by the changes in management practices implemented in this study.

Autotrophic ammonia oxidisers are the main contributors to ammonia oxidation which affects the rate of nitrification, i.e. they play an important part in the N cycle and soil fertility (de Boer and Kowalchuk 2001). The observed differences in the composition of the AOB communities between BHU and LCF, therefore, indicate small differences in the nutrient cycling processes between the two soils. Despite their significance in the N cycle, the analysis of AOB communities was not continued in the subsequent samplings. The lack of differences between the treatments did not address the objective of determining differences in microbial diversity between organic and conventional soils and linking them with microbial activity and function.

March 2004 sampling

In March 2004, clear differences in bacterial and actinomycete community compositions were still apparent between BHU and LCF soils (no similarity). The effect of current management could not be analysed statistically as the samples were run on separate gels, but visual assessment suggested a greater degree of similarity between samples of the same management (ORG and CON) than between samples of the same site (BHU and LCF). This indicates that, after being subjected to the same crop rotation and different fertilisation regimes for 2 years, the influence of past management or inherent soil properties on the structure of bacterial and actinomycete communities was still greater than that of current management practices. It has been shown that soil type and physical soil properties (e.g. soil texture or conductivity) have a large impact on the composition of the microbial community (Doran *et al.* 1988; Gelsomino *et al.* 1999; Marschner *et al.* 2001; Johnson *et al.* 2003).

DGGE analysis of fungal communities revealed very similar communities of low diversity for all treatments, evident in the small number of bands. Cluster analysis did not show any distinct groupings according to management history for organically managed soils, indicating that the fungal communities in the soils under this management regime were very similar,

while cluster analysis of the samples from the conventionally managed soils grouped most LCF and BHU samples into separate clusters (with one exception) (Figure 30). The apparent lack of diversity in the fungal community can be explained by the soil management. It is well known that soil cultivation can result in low fungal biomass due to periodic disruption of hyphal networks (Beare *et al.* 1997). However, it is also possible that the extraction of fungal DNA was not as efficient as for bacteria or that the PCR primers were not optimal (Johnson *et al.* 2003). Due to these limitations and the lack of distinct differences between treatments, the assessment of fungal community structure by DGGE analysis was not used in the last sampling.

October 2004

In October 2004, the effect of management practices on the community structures of pseudomonads, actinomycetes and α proteobacteria was determined. While the banding patterns for the different treatments seemed to be almost identical when evaluated by eye, cluster analysis showed that α proteobacteria communities could be distinguished on the basis of management history, while current management practices did not cause large differences in α proteobacterial community structure (almost 60% similarity between clusters and less accurate clustering). For actinomycete communities, no effect of past management was evident (no clear clustering of replicates), while current management practices resulted in detectable differences between B con and L org. This indicates that, for actinomycetes, effects of management history were smaller than the influence of current management practices (fertilisation and crop rotation); however, high similarities between clusters suggest that neither factor had a strong impact on actinomycete community composition.

Comparing microbial communities in grassland soils, Clegg *et al.* (2003) observed that actinomycete communities were significantly affected by mineral N fertilisation being most abundant in unfertilised soils. In this study, the lack of a fertilisation effect could be due to the short time period of the trial (the sites studied by Clegg *et al.* had been under the respective treatments for 15 years) or the overriding effect of the crop rotation. DGGE profiles of pseudomonad communities were more variable than actinomycetes and α proteobacteria and reproducibility was low. Differences among replicate soil samples were as high as between treatments, resulting in the lack of distinct clusters and the inability to distinguish between treatments. The high variability of pseudomonad communities in soils was expected given that a relatively small subgroup of microorganisms was targeted (Clegg *et al.* 2003). Investigating pseudomonad community compositions in upland grassland soils by plate count, Grayston *et al.* (2001) suggested that differences between sites were strongly influenced by the composition of the vegetation. Subjecting all treatments to the same crops in this study, should have, therefore, decreased variability. However, pseudomonads are nutritionally diverse (Bowen 1980) and respond rapidly to increases in substrate availability (Grayston *et*

al. 2001). This indicates large small-scale variability in pseudomonad communities and the existence of hotspots with a high abundance of pseudomonads.

The results indicate that, in October 2004, the influence of past management on microbial community structure was greater than that of current management practices; however, the large differences initially observed between BHU and LCF decreased with time. After approximately 2½ years of the same crop rotation, fairly uniform microbial communities were found in BHU and LCF soils, indicating the importance of crop type and organic matter additions on the soil microbial community (Doran *et al.* 1988; Johnson *et al.* 2003). Changing management practices, as happened for B con and L org, failed to result in considerable differences in microbial composition between the two treatments. The previously measured differences – a result of differences in past management or soil properties – decreased. This suggests that the application of mineral fertilisers did not cause a change in microbial community composition in the time frame examined in this study and that the overriding effect of crop rotation was stronger than the effect of mineral or organic fertiliser application. The lack of differences shows that the microbial communities in these soils did not change greatly in response to the addition or lack of mineral N fertilisation.

Despite the previously outlined limitations of PCR amplification and DGGE analysis (Section 2), the methods were suitable for determining the effect of past and current management practices on the microbial community composition. The results indicate a strong association of community structure with the management practices of crop rotation, including green manuring, that took effect within 2½ years. The study also showed that the effect of long-term management prevailed in the first 2 years. While identification of microbial species by DNA sequencing of bands might have revealed predominant species and given a better understanding of species richness, application of these techniques was beyond the scope of this study.

3.4.6 Linkages between microbial biomass size, activity and community structure

The correlations between measures of biomass size and activity showed only minor differences when analysing past and current management effects. For all treatments, biomass C and N were positively correlated with dehydrogenase activity. Hydrolysis of FDA, on the other hand, showed negative correlations to biomass C and N, while ADA was negatively correlated to C_{mic} and positively to N_{mic} and N_{tot}. Assuming a close correlation between ADA and basal respiration (Alef *et al.* 1988; Haynes 1999; Lin and Brookes 1999), the stronger negative relationship between biomass size and ADA in BHU and ORG compared to LCF and CON, respectively, suggests differences in substrate use efficiency, and could indicate higher stress levels in the microbial communities in BHU and ORG (Wardle and Ghani 1995; Nsabimana *et al.* 2004). However, the negative relationship between biomass size and arginine deaminase activity is contrary to other studies, which identified strong positive

correlations between ADA and C_{mic} (Alef and Kleiner 1986, 1987; Alef *et al.* 1988; Haynes 1999; Lin and Brookes 1999).

The lack of a correlation might point to a large inactive proportion in the microbial biomass, which is included in the biomass measurements by fumigation-extraction, while the ADA assay assesses the activity of the part of the microbial community that metabolises arginine deaminase. Dilly and Munch (1998) found that positive correlation of ADA to microbial biomass depends on N availability in the soil, which might explain the stronger negative correlation between the parameters in ORG and BHU (which did not receive mineral N fertilisers). Low rates of arginine deaminase activity might also indicate microbial communities of different structure and physiology, e.g. the presence of organisms that use this amino acid to a lesser extent (Dilly and Munch 1998). The positive correlations of ADA with N_{mic} and N_{tot} result from the enzyme's association with the N cycle and its relationship with N mineralisation in soils (Bonde *et al.* 2001).

Assessing the correlations of soil properties under past and current management separately showed there was a negative correlation of ADA to the microbial quotient in BHU and ORG and no link in CON and LCF. A higher microbial quotient indicates increased substrate availability (Böhme *et al.* 2005) and, in this case, is associated with lower microbial activity in the organically managed soils. This could be an indication of lower substrate use efficiency and a stress response in these treatments and could indicate differences in the composition of the microbial community. Since the organic matter additions were the same for all treatments, the application of insoluble organic fertilisers and the lack of N fertilisation could have caused these differences in ORG. The consistency of this effect in BHU and ORG supports this assumption since the BHU site never received mineral fertilisers.

The negative correlations of FDA with most other microbial soil properties (exception CN_{mic}), in particular the other two enzyme assays and N_{mic}, are inconsistent with the observations of Haynes (1999), who found significantly positive relationships amongst all parameters measured (various enzyme activities, including dehydrogenase, arginine deaminase and FDA hydrolysis activities, basal respiration, microbial biomass C and total C). As mentioned before, it might be possible that also activity of extracellular enzymes were measured, which could account for the differences among the three enzyme activities. This supports the suggestion that FDA is not an accurate measure of microbial activity and emphasizes the importance not to rely on a single enzyme assay (Nannipieri *et al.* 2002; Nannipieri *et al.* 2003). The strong negative correlation of FDA and N_{mic} indicates a relationship between this enzyme assay and N dynamics in the soil, as an increase in microbial biomass N is a consequence of biomass growth as well as N immobilisation (Smith 1994). This assumption is also supported by the increase of FDA hydrolysis in the inorganically fertilised treatments.

Although it is problematic to correlate the non-quantitative DGGE results with numerical soil analyses data, it was possible to determine certain trends regarding links between microbial soil properties and community structure. Differences in microbial community structure

decreased over time, as did microbial biomass size, with BHU and LCF banding patterns more similar at the end of the study than in the initial samplings. Only minor differences were observed between the converted treatments B con and L org. It is reasonable to assume that this development is portraying a real trend and is not a result of using different primer sets. At the same time, differences persisted in microbial activity (DHH, ADA, FDA) with bigger differences observable between B org and L con than between B con and L org (Figures 20 and 22). At the first sampling, the microbial communities in the four treatments were of different size (C_{mic}), structure (DGGE) and activity (enzyme activities) with the largest differences caused by soil origin or farm management history (BHU or LCF), while at the final sampling, the four treatments showed similarly sized and structured microbial communities with differing levels of activity due to the persisting influence of management history.

3.5 Conclusions

The following conclusions can be drawn from the lysimeter study.

- Most measured parameters (leaching losses, soil microbial properties, microbial structure) did not show any differences between organically and conventionally fertilised soils, indicating negligible effects of mineral fertilisation on N leaching, microbial biomass, activity and composition. However, the experimental period of 2½ years might have been too short to draw definite conclusions.
- Cumulative crop yields were higher in CON although differences were not significant. Mineral fertilisation significantly increased barley yields in CON treatments due to the addition of N, while green manure incorporation also had a positive effect on yields. There was a positive correlation between lupin and maize yields as could be seen in higher maize yields in BHU treatments. Management history rather than current management influenced C:N ratios of crops resulting in consistently lower C:N ratios of crops grown in BHU soils.
- Over the trial period, leaching patterns were the same for all treatments and mineral N fertilisation or green manure incorporation did not result in increased mineral N losses. Cumulative leaching losses of mineral N were lower from organically managed soils, although differences were not significant.
- Management practices such as crop rotation and green manuring overrode the fertiliser treatment effects and resulted in similar levels of microbial soil properties (microbial biomass C and N, enzyme activities) for ORG and CON treatments.
- The incorporation of a lupin green manure crop stimulated the microbial communities in BHU and LCF soils, i.e. the microbial populations in both soils were sufficient and active enough to respond to the addition of organic materials with increased growth and activity.

Initial differences in microbial biomass declined, while differences caused by long-term management were still evident in enzyme activities and biochemical soil properties.

- Application of a mineral fertiliser did not have any measurable effects on microbial biomass size (C_{mic}, N_{mic}) and microbial activity (measured as ADA), while DHH activity was consistently higher under organic and FDA under conventional practices indicating a positive and negative effect of mineral fertilisation, respectively.
- Correlations among microbial soil properties were not consistent with other researchers' findings. No consistent positive relationships between enzyme activity and microbial biomass size were found which could indicate that varying proportions of the microbial biomass are inactive, show inefficient substrate use or that other soil factors influence the activity of the enzyme measured.
- DGGE profiles were initially different in BHU and LCF soils and the management history effect persisted for approximately 2 years. After 2½ years under the same crop rotation, however, DGGE banding patterns showed no differences between the treatments, indicating comparable microbial abundance and community structures by the end of the experiment.
- The results obtained from measuring microbial biomass and activity and determining microbial community structure in soils under the same crop rotation with differing fertilisation regime imply that similarly sized and structured microbial communities can express varying rates of activity.

Section 4 – Influence of short-term farming practices on organic and conventional soils under laboratory conditions

This section gives detailed descriptions of the two incubation experiments carried out under controlled conditions to determine the effect of organic and mineral amendments on the soil microbial community. The introduction in Chapter 1 puts the studies into context and presents the rationale for the experiments. Objectives, materials and methods, and results of incubation studies I and II are presented and discussed in Chapters 2 and 3. Overall conclusions for the entire section are given in Chapter 4.

1 Introduction

An incubation experiment was considered the most appropriate way to study interactions of microbial soil properties with soil processes. Assessing soil properties under constant conditions allows variables, such as soil moisture levels, temperature, microbial-plant interactions and soil type, to be studied independently of each other, e.g. to examine how changes in soil moisture affect biological soil properties. However, interpretations regarding *in situ* responses of soil microorganisms should be made with caution as incubation studies represent model systems under optimum conditions that rarely occur in the field.

In this study, I was particularly interested in the effects of using a leguminous green manure as an organic nitrogen (N) source and comparing it to the effect mineral N has on the soil microbial community and soil processes.

Often, leguminous plants are part of the crop rotations, either as components of the pasture phase (e.g. clover) or as green manure crops (e.g. lupin), which add labile organic matter to the soil by incorporation. Legumes are an important source of N for most organic systems, especially in New Zealand, where crops under organic management almost exclusively rely on N released from soil organic matter via mineralisation. This highlights the importance of green manure crops and the dependence on biological processes to supply sufficient amounts of N to crops in organic farming systems. However, including green manure in crop rotations is considered good management practice in any agricultural production system due to their many positive effects on soil fertility and quality. By fixing atmospheric N and adding organic material to the soils, legumes help build and maintain soil fertility, improve the soil structure and water retention capacity, help suppress plant diseases and support the soil microbial population (Doran *et al.* 1988; Greenland 2000; Shepherd *et al.* 2000a; Watson *et al.* 2002; Wolf and Snyder 2003). Research indicates that farming practices commonly

associated with organic farming (e.g. green manuring, winter cover crops or crop rotations) have a positive effect on the soil microbial diversity and, consequently, soil processes (Mäder *et al.* 2002; Girvan *et al.* 2003; Hole *et al.* 2005). In comparison, there is little evidence in the literature of negative effects of mineral fertiliser and pesticide use on the soil microbial community (e.g. Fraser *et al.* 1988; Fauci and Dick 1994; Shepherd *et al.* 2000a). It is noted that such practices may have different impacts on other parts of the farm system (Kirchmann and Thorvaldsson 2000; Stolze *et al.* 2000). This suggests that it is not the land-use system itself that impacts on the soil microbial community, but rather individual production techniques (e.g. green manuring, use of catch crops, crop rotations, crop residue management), if it is possible to discuss farming practices separate from management systems. As these practices are commonly linked to organic farming systems, soils cultivated under contrasting management regimes should show differences in biological soil properties and application of such management practices should affect the microbial community (Gunapala and Scow 1998; Lundquist *et al.* 1999b; Ryan 1999).

Two incubation experiments were carried out to study the links between microbial diversity and function and to assess the influence of past and current farming practices on the soil microbial community under controlled conditions.

The key questions that the two experiments addressed were:

- What is the influence of farm management history as opposed to current management on soil biological properties, soil processes and microbial diversity?
- How is the soil microbial community affected by the addition of different forms of N (organic compared to mineral)?

In the first study, the effect on the microbial community (microbial biomass size, activity and diversity) and selected soil processes (N mineralisation) was investigated by adding different amounts of a leguminous green manure (lupin (*Lupinus angustifolius* L.)) to the previously studied two soils of the same soil type. The second experiment was based on the results obtained in the first study and was carried out under the same incubation conditions. It focused on the effect of adding same amounts (100 kg ha^{-1}) but different forms of N (organic: lupin vs. mineral: urea) on microbial biomass size, activity and diversity and N mineralisation.

2 Incubation experiment I

2.1 Introduction

To accomplish the aims outlined in the introduction to this section, the specific objectives of this study were to:

- determine the effect of past land management on the soil microbial community and soil processes (past management);
- determine the effect of adding organic material on the soil microbial community and soil processes (current management); and
- determine relationships amongst biological soil properties, i.e. soil microbial biomass, activity, diversity and function (linkages).

Microbial biomass size and activity, genetic diversity and soil processes (N mineralisation and immobilisation rates) were measured at intervals over a period of 81 days in the two soils with and without the addition of different amounts of a leguminous green manure. Amounts equivalent to 4 and 8 t dry matter per ha were chosen based on data from previous field experiments carried out in Canterbury (Randhawa 2003).

2.2 Materials and methods

2.2.1 Experimental design

Topsoil samples (0-15 cm) were collected from the original sites, BHU (in the following referred to as ORG) and LCF (CON), air dried and sieved (2 mm) (for site description see Section 2). Of each soil, 1.5 kg dry weight equivalent (dwe) were placed in 2 L plastic containers and the water content was adjusted to 70% WHC by adding deionised water. Aeration was provided by two 5 mm diameter holes in the lid. Soil moisture was adjusted every 4 d based on weight loss. After 3 weeks of pre-incubation at 20ºC, ground lupin[1] was incorporated into the soils at rates of 0, 4 (L4) and 8t of dry matter per ha (L8) (Table 32). Each treatment had three replicates.

The soils were sampled after 5, 18, 39, 60 and 81 days by removing approximately 200g of moist soil from the entire depth of each container. After each sampling, the soils in the containers were re-compacted to an approximate bulk density of 1 g cm^{-3}.

Table 32: Details of treatments included in incubation experiment I.

Treatment ID	*Amendment*	*C (%)*	*N (%)*	*C:N*	*Application per kg*	*Application per ha**	*kg N ha^{-1}*
ORG or CON L8	lupin	42.8	2.99	14.3	7.6 g	8t ha^{-1}	239.2
ORG or CON L4	lupin	42.8	2.99	14.3	3.8 g	4t ha^{-1}	119.6
ORG or CON C	(control)	--	--	--	--	--	--

*assuming a bulk density of 1.4 g cm^{-3} and an incorporation depth of 7.5 cm

[1] Lupin, grown to full maturity at a Lincoln University field site prior to the experiment, was dried at 60°C and ground to approximately 2 mm using a Cycltec 1092 mill.

2.2.2 Analysis of soil microbial and biochemical properties and soil processes

Soil biological analyses included measures of microbial biomass C and N (C_{mic} and N_{mic}) (Sparling and West 1988), arginine deaminase activity (ADA) (Alef and Kleiner 1987), fluorescein diacetate hydrolysis (FDA) (Adam and Duncan 2001) and dehydrogenase activity (DHH) (Thalmann 1968). For a detailed method description, see Section 2.

Nitrate-N (NO_3-N), ammonium-N (NH_4-N) and mineral N content (N_{min}= NO_3-N+NH_4-N) were determined according to Blakemore (1987), and total C and N (C_{tot} and N_{tot}) were measured on a Leco® CNS-2000 elemental analyser.

On two occasions (18d and 60d after amendment), gross N mineralisation (MIN) and immobilisation rates (IMM) were estimated using the ^{15}N dilution technique (Zaman *et al.* 1999a; Zaman *et al.* 1999b). In brief, four 20 g subsamples of each soil were weighed into vented plastic containers. Two samples (t1 and t2) were amended with 1 ml ^{15}N-labelled ammonium sulphate (99% enrichment) each (= 2 µg NH_4-N g^{-1} soil) and incubated at 20°C for 24 h (t1) and 4d (t2), respectively. After the incubation period, the samples were extracted with 2 M KCl and analysed for total N content (FIA). The ^{15}N content in the extracts was recovered by the diffusion method described by Stark and Hart (1996) and determined by isotope ratio mass spectrometry (Europa Scientific, UK).

2.2.3 Analysis of the soil microbial community structure

On four occasions (day 5, 18, 39 and 60), microbial community structure was assessed by extraction of community DNA, polymerase chain reaction (PCR) amplification and denaturing gradient gel electrophoresis (DGGE) separation. The methods were the same as described in Section 2 unless specifically mentioned. For the PCR amplification, primers targeting 16S rDNA of bacteria (F984GC-R1378), actinomycetes (applying a nested approach using F243-R1378 for the 1st round and F984GC-R1378 for the 2nd round) and 18S rDNA of fungi (1st round: EF4f-fung5r; 2nd round: NS2f-fung5r GC) were used (for detailed primer description see Table 17).

Reaction mixture composition was identical to that described in Section 2 (Table 4) with the exception that no BSA was added in the second PCR round where a nested PCR approach was used. The respective thermal cycling conditions and denaturing gradients are presented in Table 33.

Table 33: Primers and thermal cycling conditions used for DNA amplification and denaturing gradients used for DGGE in incubation experiment I.

Primer pair	*Target (size of product)*	*Thermocycling program*	*Denaturing gradients*
F984GC – R1378	Bacteria Actinomycetes2*	5 min at 94°C, followed by 35 cycles of 1 min at 95°C, 1 min at 53°C and 2 min at 72 °C, followed by a final extension step of 10 min at 72°C	40-70%
F243 – R1378	Actinomycetes1*	5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 63°C, and 2 min at 72°C, followed by a final extension step of 10 min at 72°C	
EF4f – fung5r	Fungi1*	3 min at 94°C followed by 40 cycles of 1 min at 94°C, 1 min at 48°C and 2 min at 72°C, followed by a final extension step 10 min at 72°C	
NS2f – fung5r-GC	Fungi2*	3 min at 94°C followed by 10 cycles of 1 min at 94°C, 1 min at 60 to 52°C (2° per step, 2 cycles per step) and 1 min at 72°C, followed by 25 cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C, followed by a final extension step of 10 min at 72°C	35-65%

*Numbers indicate first and second round of amplification, respectively, for nested PCR.

2.2.4 Statistical analysis

All statistical analysis was performed using GenStat Release 7.1 (©2003, Laws Agricultural Trust, Rothamsted Experimental Station, UK) or Minitab® for Windows Release 14.1 (©2003, Minitab Inc., USA). The data was analysed by repeated measures analysis of variance, general linear model analysis of variance or correlation analysis where appropriate. A 95% confidence limit ($p<0.05$) was chosen to indicate differences between samples and least significant differences (LSD) were calculated when samples were significantly different. DGGE patterns were analysed by cluster analysis according to Ward (1963) using Quantity One 1-D Analysis Software (Version 4.5.2) (Bio-Rad, USA).

2.3 Results

2.3.1 Effect of long- and short-term management practices on soil properties and processes

The single factors that most influenced the soil properties measured during the 81-day incubation study are presented in Table 34. Most differences were caused by the treatments (addition or no addition of lupin) rather than by soil origin (ORG or CON) or time (five sampling points), indicating that addition of organic material had a larger effect on the soil properties than management history and temporal variation. Only FDA and C_{tot} were more strongly affected by soil origin than treatment.

Depending on sampling date, between 90 and 99% of total mineral N was in the form of NO_3-N. Results of the statistical analyses are, therefore, presented for NH_4-N and N_{min} only. Graphs detailing NH_4-N and NO_3-N levels over time can be found in Appendix II.

Table 34: Main factors (soil origin, treatment or time) influencing soil properties and processes in incubation experiment I.

Soil property	*Factor*	*p value*	*R^2 (%)*
C_{mic}	time	<0.001	83.0
	treatment	<0.001	10.5
N_{mic}	treatment	<0.001	58.8
DHH	treatment	<0.001	76.9
ADA	treatment	<0.001	67.4
FDA	soil origin	<0.001	31.8
C_{mic}:C_{tot}	time	<0.001	86.8
	treatment	<0.001	8.70
NH_4-N	time	<0.001	39.2
	treatment	0.004	7.61
N_{min}	treatment	<0.001	65.8
C_{tot}	soil origin	<0.001	69.2
N_{tot}	treatment	<0.001	54.6

Effect of time on soil properties

Microbial quotient, C_{mic} and NH_4-N were the only soil properties with larger variation among sampling points than among treatments or soil origins, treatment being the second influencing factor for both. The temporal variation in microbial biomass C between days 18 and 81 (second to last sampling) was, however, smaller than the variation among treatments (time averages: between 460 and 680 µg C g^{-1} soil; treatment averages: between 400 and 720 µg C g^{-1} soil) indicating that the increased levels at day 5 are responsible for the high temporal variation. For NH_4-N, the large temporal variation was a result of high levels in L8 at day 18 and the peak in all treatments at day 60.

The three treatments (L8, L4 and control) and two soils (ORG and CON) revealed similar trends for biological and biochemical parameters over time (Figures 37-43). At the first sampling point (day 5), L8 and, to a lesser degree, L4 showed significantly increased levels of enzyme activity (ADA and DHH (Figure 39)), microbial biomass (C_{mic} and N_{mic} (Figure 37)) and microbial quotient when compared with the control. During the incubation period, the initially high amounts in L8 and L4 decreased but stabilised at levels higher than those of the control treatments. Most soil properties did not vary significantly over time in unamended control soils, with the exception of C_{mic} showing elevated levels similar to those observed in the lupin treatments. FDA levels fluctuated over the 81-day incubation for all treatments

(Figure 38). Levels of NO_3-N and N_{min} (Figure 43) showed a continuous increase in all treatments, while NH_4-N content was elevated for L8 in both soils at day 18 and peaked for all treatments at day 60, followed by a decrease back to original levels. Levels of C_{tot} and N_{tot} did not vary over time (Figure 42).

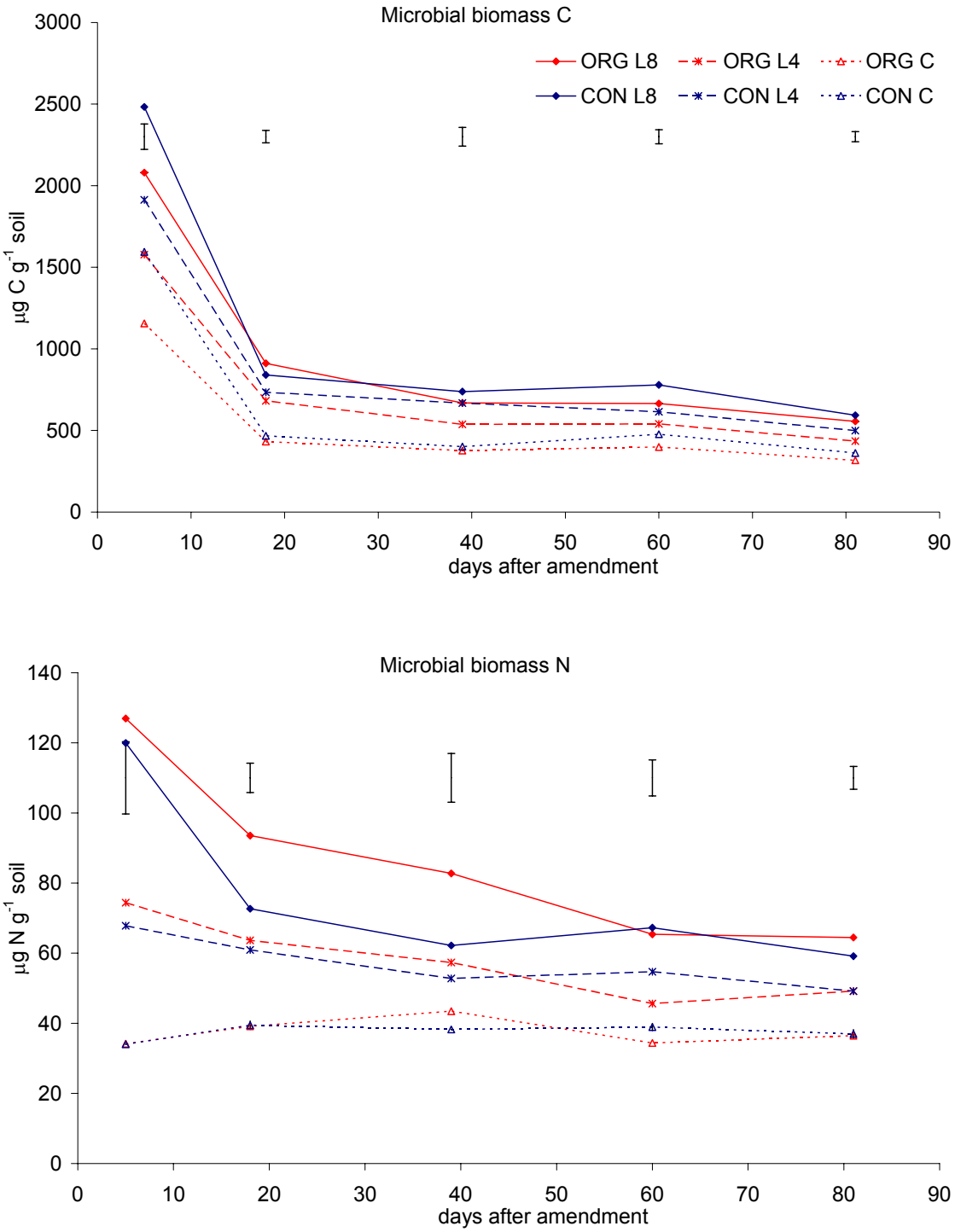


Figure 37: Mean concentrations (µg g^{-1}) in microbial biomass C and N determined over 81 days in incubation experiment I. Bars show $LSD_{0.05}$. n=9.

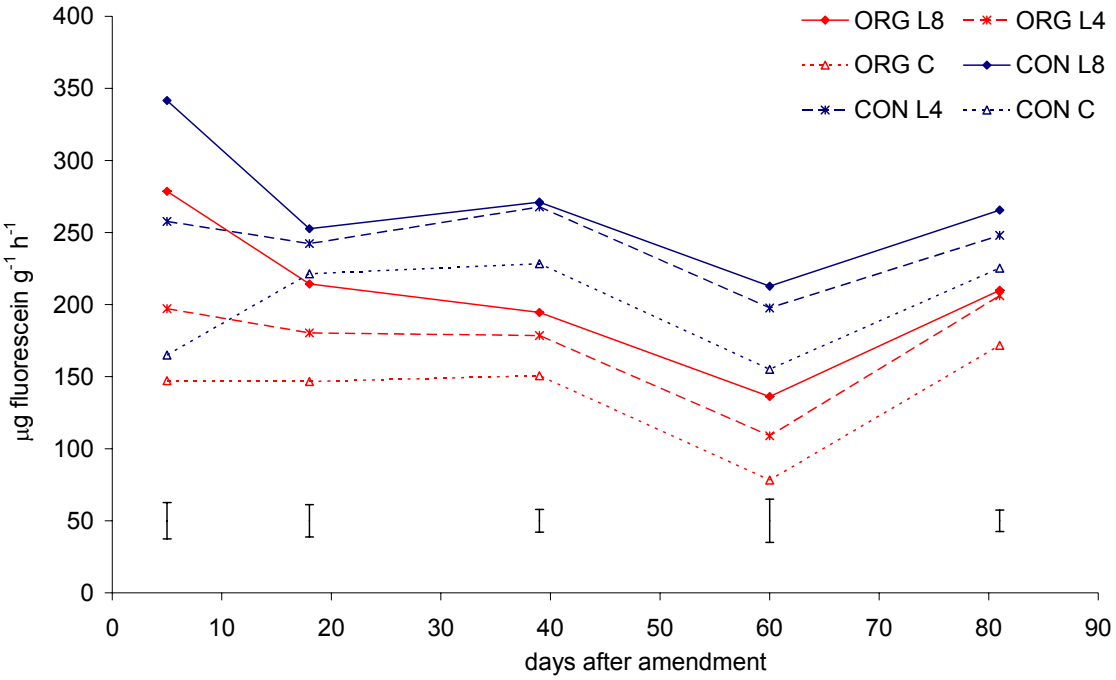


Figure 38: Mean concentrations (µg g^{-1} h^{-1}) in fluorescein diacetate hydrolysis determined over 81 days in incubation experiment I. Bars show $LSD_{0.05}$. n=9.

Effect of past management (soil origin) on soil properties

Statistical analysis indicated that soil origin did not have the greatest influence on soil properties (Table 34: main factor for FDA and C_{tot} only). However, levels of ADA (ORG>CON), DHH (ORG>CON), FDA (CON>ORG), C_{tot} (CON>ORG) and N_{tot} (CON>ORG) were significantly affected by soil origin throughout the experiment (Table 35), while the other soil properties did not show significant differences between ORG and CON. Differences in C_{tot} and N_{tot} were very small between the soils.

Table 35: Overall mean values of selected soil properties determined for the organic and conventional soils over 81 days in incubation experiment I.

Soil property	*Soil origin*		*Significance*
	ORG	*CON*	
DHH (µg TPF g^{-1} h^{-1})	3.01 (0.280)	2.23 (0.206)	***
ADA (µg NH_4^+-N g^{-1} h^{-1})	4.72 (0.481)	3.47 (0.266)	***
FDA (µg fluorescein g^{-1} h^{-1})	173.3 (7.19)	236.8 (6.85)	***
C_{tot} (%)	2.47 (0.014)	2.72 (0.013)	***
N_{tot} (%)	0.218 (0.002)	0.235 (0.002)	***

Values are means of three treatments and five sampling dates (Standard errors of means).***, p<0.001. n=45.

Effect of current management (treatment) on soil properties

Treatment was the most influential factor for most soil properties (Table 34). The addition of either amount of lupin increased microbial biomass size and activity significantly ($p<0.001$), suggesting that amendment with organic material had a positive impact on the biological parameters. On average, L8 values were twice or more than those of the control for most properties (Table 36). Total C and N were significantly different, however, values were very similar for the three treatments. Total mineral N was significantly affected by treatment and highest in L8, followed by L4 and control, while, over all sampling dates, NH_4-N was similar in L4 and control but significantly higher in L8 compared to the other treatments.

Table 36: Overall mean values of selected soil properties determined for the different treatments over 81 days in incubation experiment I.

Soil property	*Treatment*			
	L8	*L4*	*Control*	$LSD_{0.05}$
C_{mic} (µg C g^{-1})	1031.1 (119.0) [a]	820.4 (92.0) [b]	598.0 (75.0) [c]	35.7
N_{mic} (µg N g^{-1})	81.4 (4.44) [a]	57.6 (1.77) [b]	37.5 (0.640) [c]	3.24
C_{mic}:C_{tot} (%)	3.86 (0.444) [a]	3.17 (0.345) [b]	2.37 (0.294) [c]	0.164
DHH (µg TPF g^{-1} h^{-1})	4.15 (0.340) [a]	2.59 (0.124) [b]	1.12 (0.046) [c]	0.162
ADA(µg NH_4-N g^{-1} h^{-1})	5.94 (0.618) [a]	4.15 (0.313) [b]	2.19 (0.070) [c]	0.117
FDA(µg fluorescein g^{-1} h^{-1})	237.7 (10.08) [a]	208.5 (8.77) [b]	169.0 (8.27) [c]	2.06
NH_4-N (µg g^{-1})	3.12 (0.61) [a]	1.87 (0.29) [b]	2.04 (0.30) [b]	0.82
N_{min} (µg g^{-1})	105.3 (6.05) [a]	73.3 (4.55) [b]	41.7 (1.89) [c]	1.25
C_{tot} (%)	2.67 (0.022) [a]	2.59 (0.025) [b]	2.52 (0.029) [c]	0.039
N_{tot} (%)	0.241 (0.002) [a]	0.226 (0.002) [b]	0.212 (0.002) [c]	0.004

Different letters indicate significant differences among treatments at $p<0.05$. Values are means of two soils and five sampling dates (Standard errors of means). n=30 (n=24 for N_{min} and NH_4-N).

Interactions among time, treatment and soil origin

Of all interactions, treatment*time had the strongest influence on the soil properties (Table 37) due to the significantly higher levels of microbial soil properties in L8 compared to L4 and control at day 5. After the first sampling, however, levels of the measured parameters tended to decrease and stabilise for the rest of the trial period (e.g. for DHH in Figure 39).

Interactions did not play a major role in changing the soil properties as they were not significant or had low R^2 values (Table 37). In other words, changes over time were similar for the three treatments (time*treatment) and for the two sites (time*soil origin) and that the two soils were affected by the treatments (soil origin*treatment) to a similar extent. Only NH_4-N showed a pronounced time*treatment effect due to the elevated levels in L8 at day 18.

Table 37: Main interactions influencing soil properties and processes in incubation experiment I.

Soil property	*Factor*	*p value*	*R^2 (%)*
C_{mic}	time*treatment	<0.001	3.5
N_{mic}	time*treatment	<0.001	17.3
DHH	time*treatment	<0.001	18.5
ADA	time*treatment	<0.001	19.2
FDA	time*treatment	<0.001	10.0
C_{mic}:C_{tot}	time*treatment	<0.001	2.9
NH_4-N	time*treatment	<0.001	30.5
N_{min}	time*treatment	<0.001	6.7

Data only displayed for interactions with $p<0.05$.

Table 38 shows that the addition of lupin had a stronger effect on the microbial soil properties in ORG compared CON, resulting in larger increases in microbial biomass and activity in the lupin amended treatments. Comparing the levels of L4 and L8 to each other revealed that not all parameters were affected equally and doubling the amount of lupin resulted in a 1.1 to 1.7-fold increase. Mineral N and NH_4-N levels increased more strongly in CON compared to ORG.

Table 38: Overall factors of change in soil properties following addition of different amounts of lupin in incubation experiment I.

	L8/control		*L4/control*		*L8/L4*	
Soil property	ORG	CON	ORG	CON	ORG	CON
C_{mic}	1.82	1.64	1.41	1.34	1.29	1.23
N_{mic}	2.31	2.03	1.55	1.52	1.49	1.34
DHH	3.99	3.33	2.35	2.25	1.70	1.48
ADA	2.80	2.60	1.84	1.96	1.52	1.33
FDA	1.49	1.35	1.25	1.22	1.19	1.11
NH_4-N	1.45	1.59	0.93	0.96	1.55	1.66
N_{min}	2.46	2.74	1.66	1.70	1.48	1.61

Values are means of five sampling dates. n=15.

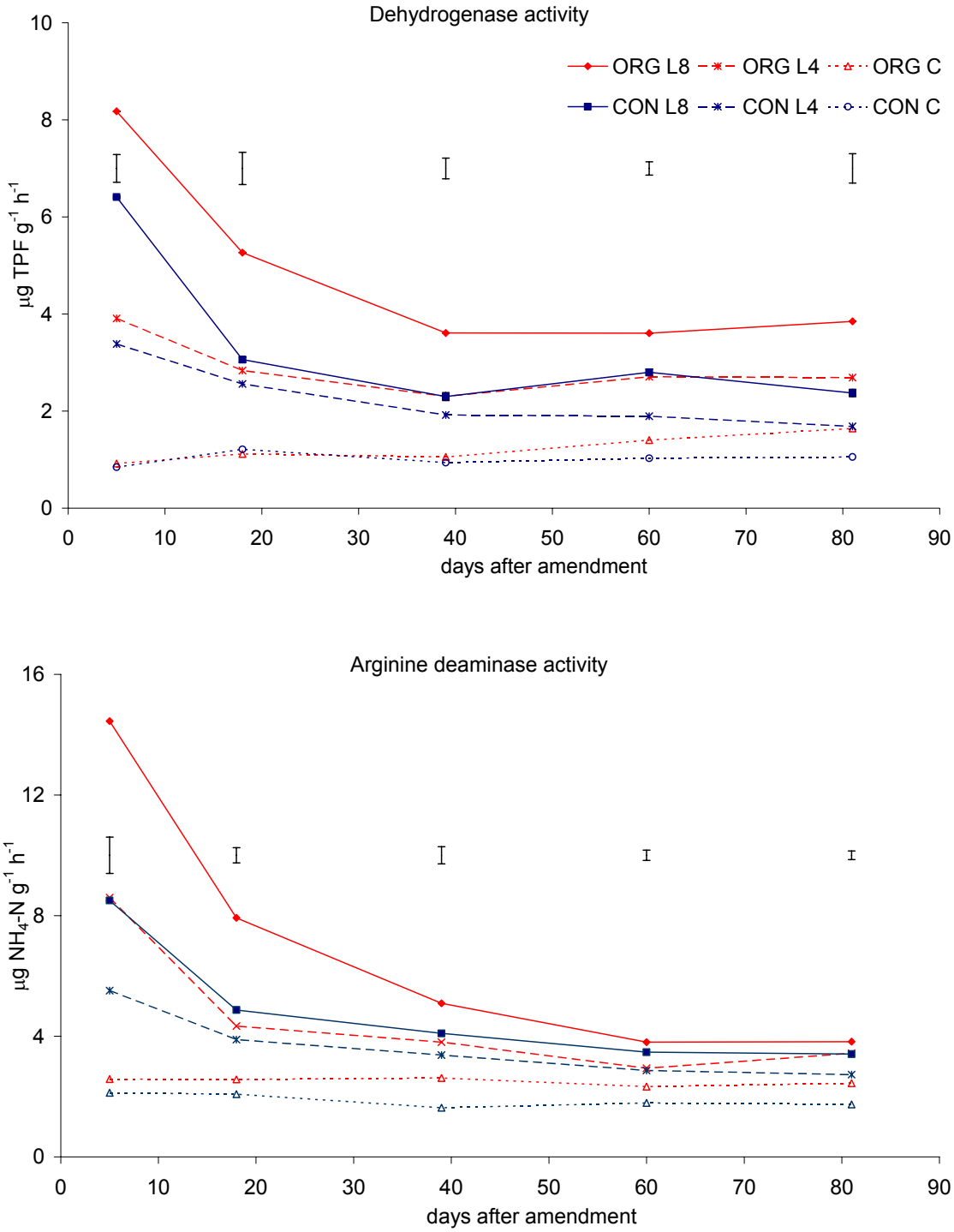


Figure 39: Mean concentrations (µg g^{-1} h^{-1}) in dehydrogenase and arginine deaminase activities determined over 81 days in incubation experiment I. Bars show $LSD_{0.05}$. n=9.

Effect of current and past management on gross N mineralisation and immobilisation rates

Differences in gross mineralisation (MIN) were more likely to be caused by temporal variation and the influence of soil origin than by treatments (Table 39), i.e. differences among treatments were not significant in most cases. Differences in immobilisation rate (IMM), on the other hand, were biggest between soils and among treatments. Differences between the two sampling points were not significant.

Table 39: Analysis of variance of N mineralisation (MIN) and immobilisation (IMM) rates over 81 days in incubation experiment I.

Process	*Factor*	*p value*	*R^2 (%)*
MIN	treatment	0.049	7.27.
	soil origin	<0.001	35.6
	time	<0.001	15.6
	soil origin*time	0.026	5.96
IMM	treatment	0.010	20.2
	soil origin	0.001	25.0
	time	0.703	0.262
	soil origin*time	0.011	13.7

For ORG, the treatments did not show significant differences in MIN at either sampling point, while after 60 days of incubation, MIN was significantly increased in CON for L8 compared to L4 and the control (Figure 40). Mineralisation rates for ORG and CON were higher at day 60 for most treatments (only exception CON C), and IMM was significantly higher at day 60 for ORG, while for CON they were higher at day 18 (not significant) (Figure 41). Overall, average MIN and IMM were significantly higher for CON (MIN: 2.48 and 1.42 µg NH_4^+-N g^{-1} d^{-1} for CON and ORG, respectively; IMM: 2.41 and 1.70 µg NH_4^+-N g^{-1} d^{-1} for CON and ORG, respectively) and L8 had significantly higher rates of gross mineralisation and immobilisation than L4 and the control. Differences between L4 and control were not significant.

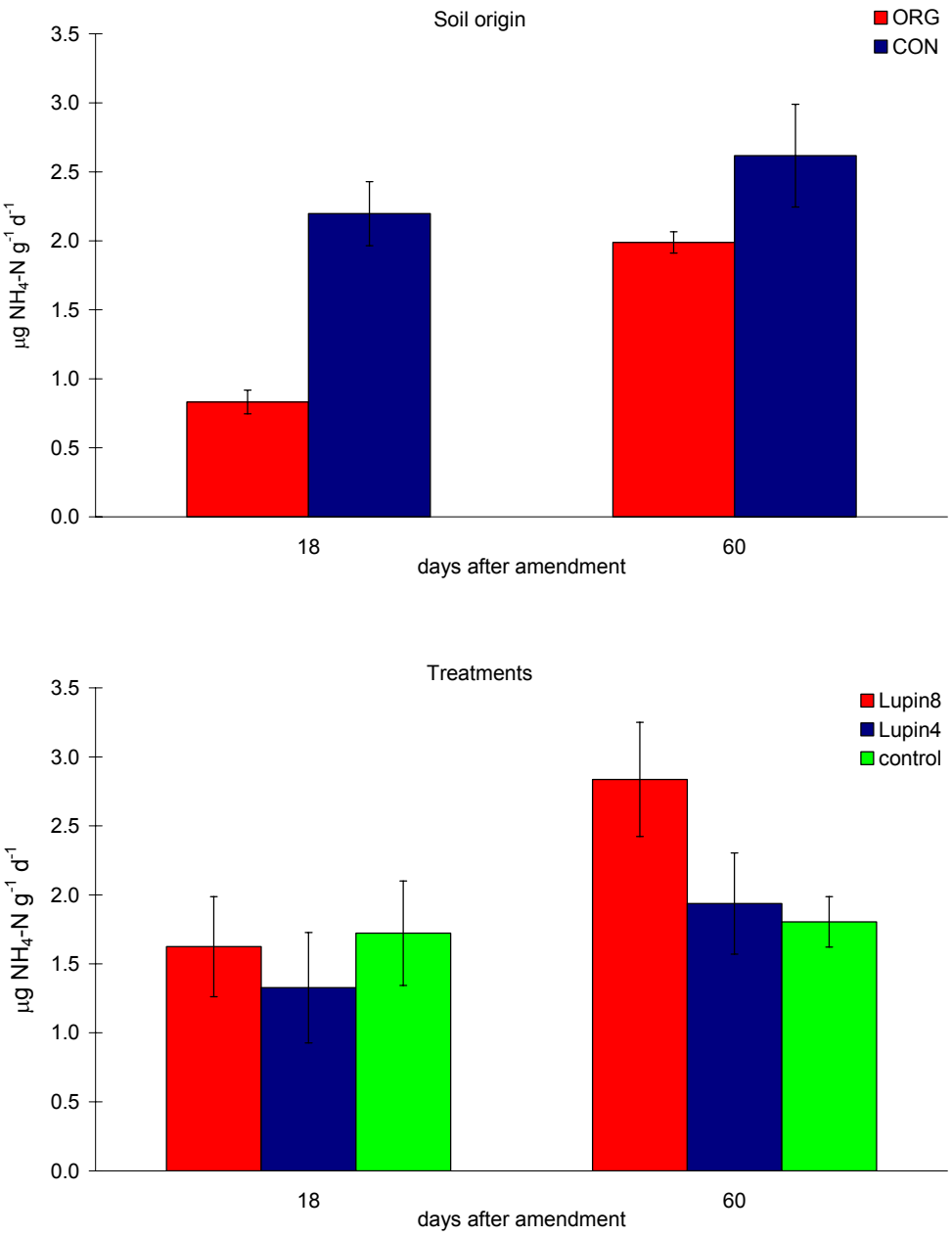


Figure 40: Mean rates (µg g^{-1} d^{-1}) of gross N mineralisation for soil origin (average of three treatments, n=6) and treatments (average of two soils, n=9) at days 18 and 81 in incubation experiment I. Bars show standard errors of means. $LSD_{0.05}$=0.65 for soil origin and treatment.

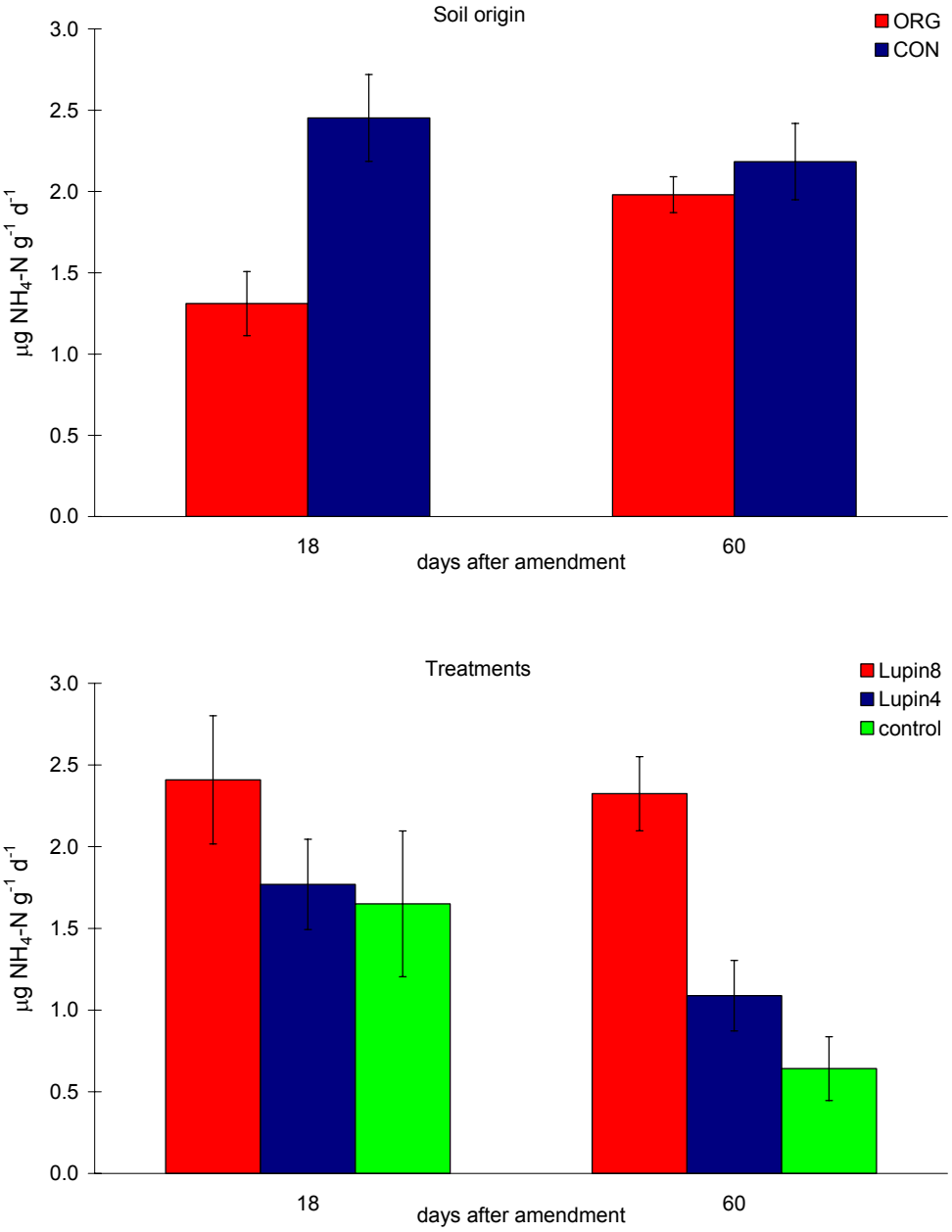


Figure 41: Mean rates (µg g^{-1} d^{-1}) of N immobilisation for soil origin (average of three treatments, n=6) and treatments (average of two soils, n=9) at days 18 and 81 in incubation experiment I. Bars show standard errors of means. $LSD_{0.05}$=0.67 for site and treatment.

Correlations among soil properties and processes (linkages)

Analysing the correlations for each treatment separately revealed similar patterns for the two lupin treatments. They showed strong positive links amongst the biological soil properties (C_{mic}, N_{mic}, DHH, ADA and FDA) and negative correlations to N_{min} (Table 40). The mineralisation rate was negatively correlated with the three enzyme activities ADA, FDA and DHH. The control showed an entirely different pattern with mainly weak correlations. Most noticeably, C_{mic} showed negative correlations with DHH and FDA, and MIN was negatively linked to N_{min}.

Table 40: Correlation coefficients determined among soil properties and processes for the control and lupin treatments over 81 days in incubation experiment I.

	C_{mic}	N_{mic}	*DHH*	*ADA*	*FDA*	N_{min}	C_{tot}	N_{tot}
L8								
N_{mic}	0.70***							
DHH	0.83***	0.36						
ADA	0.79***	0.44*	0.94***					
FDA	0.90***	0.51**	0.91***	0.90***				
N_{min}	-0.81***	-0.32	-0.24	-0.66***	-0.57***			
C_{tot}	0.03	0.40*	-0.48**	-0.43*	-0.26	0.06		
N_{tot}	-0.02	0.21	-0.39*	-0.42*	-0.34	0.39	0.79***	
MIN	0.21	0.14	-0.62***	-0.52**	-0.27	0.35	0.73***	0.23
L4								
N_{mic}	0.29							
DHH	0.75***	-0.15						
ADA	0.79***	0.05	0.84***					
FDA	0.74***	0.21	0.69***	0.81***				
N_{min}	-0.74***	-0.35	-0.15	-0.56**	-0.56**			
C_{tot}	-0.01	0.67***	-0.59**	-0.33	-0.11	-0.21		
N_{tot}	-0.19	0.47**	-0.61***	-0.52**	-0.30	0.11	0.83***	
MIN	-0.43*	-0.039	-0.37	-0.66***	-0.39*	0.13	0.23	0.44
Control								
N_{mic}	-0.12							
DHH	-0.52**	-0.26						
ADA	0.12	-0.58**	0.23					
FDA	-0.46**	0.19	-0.01	-0.01				
N_{min}	-0.29	-0.39	0.37	0.12	-0.44*			
C_{tot}	0.08	0.34	-0.11	-0.02	-0.01	-0.14		
N_{tot}	-0.02	0.58**	-0.25	-0.82***	0.02	-0.01	0.20	
MIN	0.08	0.23	0.26	-0.44*	-0.09	-0.70***	-0.02	0.31

n=30, n=12 for MIN, n=24 for N_{min}; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

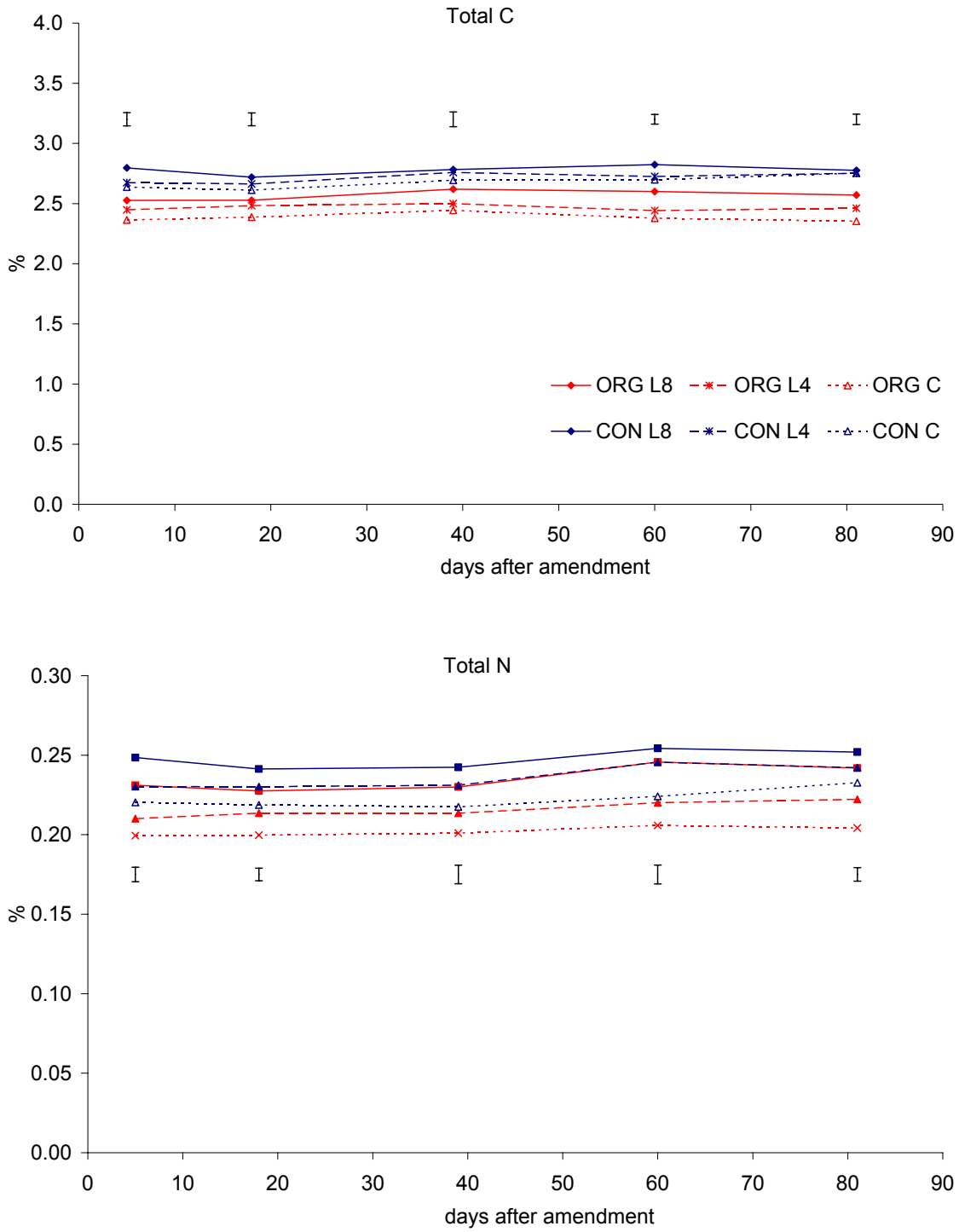


Figure 42: Mean concentrations (%) in total C and N determined over 81 days in incubation experiment I. Bars show $LSD_{0.05}$. n=9.

Correlations for ORG and CON were similar to each other and comparable to the overall correlations (including all treatments and soils) (Table 41), indicating that only minor differences between the two soils existed and that they were responding in similar ways to the amendments. The overall correlations revealed strong positive correlations amongst all measured soil parameters. Only the gross N mineralisation rate showed weak correlations with most other characteristics. However, MIN had a smaller sample size (n=12 due only two

samplings) probably resulting in less reliable values. The observed relationships were nonetheless positive.

Table 41: Overall correlation coefficients determined among soil properties and processes over 81 days in incubation experiment I.

	C_{mic}	N_{mic}	*DHH*	*ADA*	*FDA*	N_{min}	C_{tot}	N_{tot}
N_{mic}	0.47***							
DHH	0.66***	0.47***						
ADA	0.73***	0.45***	0.93***					
FDA	0.68***	0.58***	0.94***	0.90***				
N_{min}	0.33**	0.11	0.57***	0.32**	0.48***			
C_{tot}	0.13	0.42***	0.11	0.06	0.19	0.20		
N_{tot}	0.18	0.60***	0.36**	0.19	0.44***	0.62***	0.44***	
MIN	0.20	0.18	0.15	-0.08	0.15	0.30*	0.17	0.46***

n=90, n=36 for MIN, n=72 for $N_{min;}$ *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

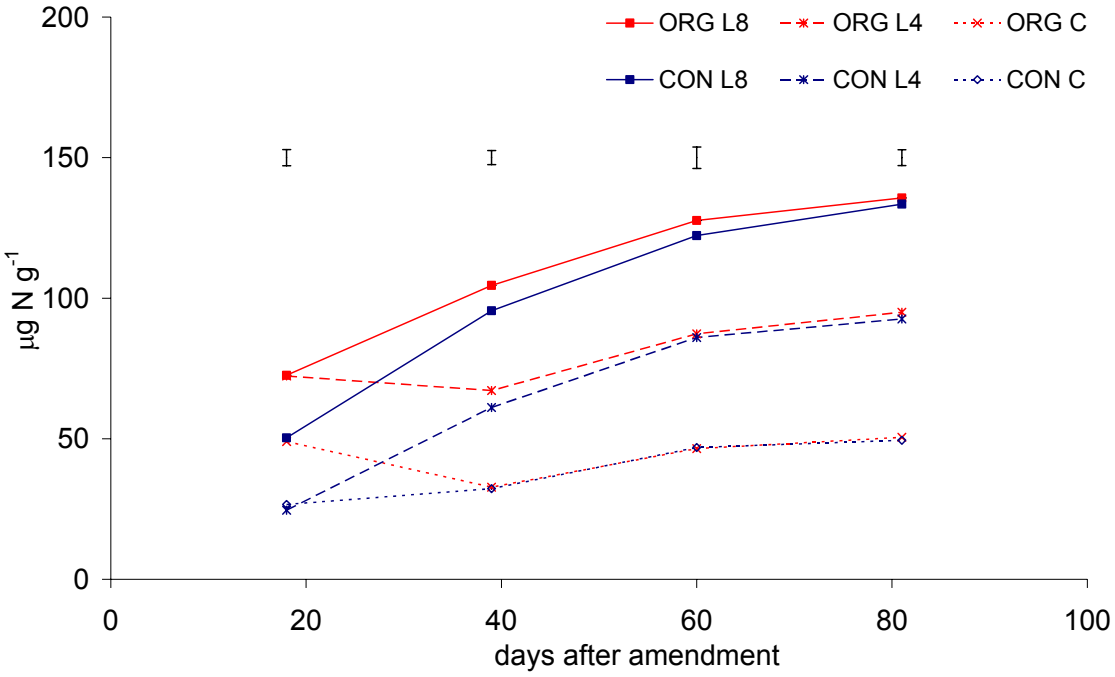


Figure 43: Mean concentrations (µg g^{-1}) of total mineral N determined over 81 days in incubation experiment I. Bars show $LSD_{0.05}$. n=9.

2.3.2 Effect of long- and short-term management practices on soil microbial community structure

DGGE profiles of PCR amplified eubacterial and actinomycete 16S rDNA fragments from ORG and CON showed high reproducibility, i.e. treatment replicates revealed almost identical fingerprints (Figures 44, 45 and 46).

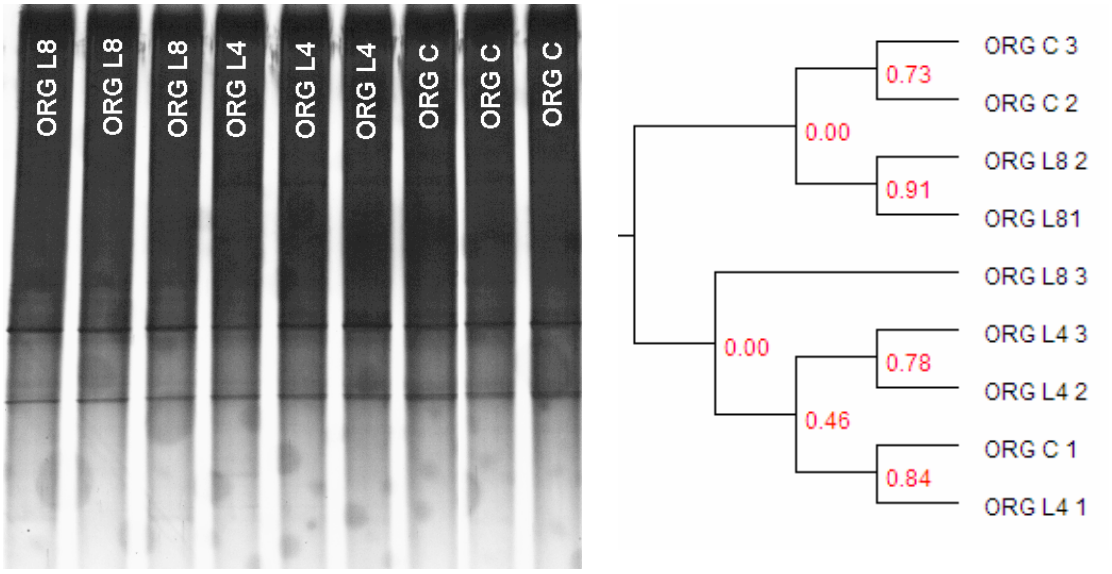


Figure 44: DGGE banding patterns and cluster analysis for replicate extracts of bacteria DNA from ORG at day 5 in incubation experiment I.

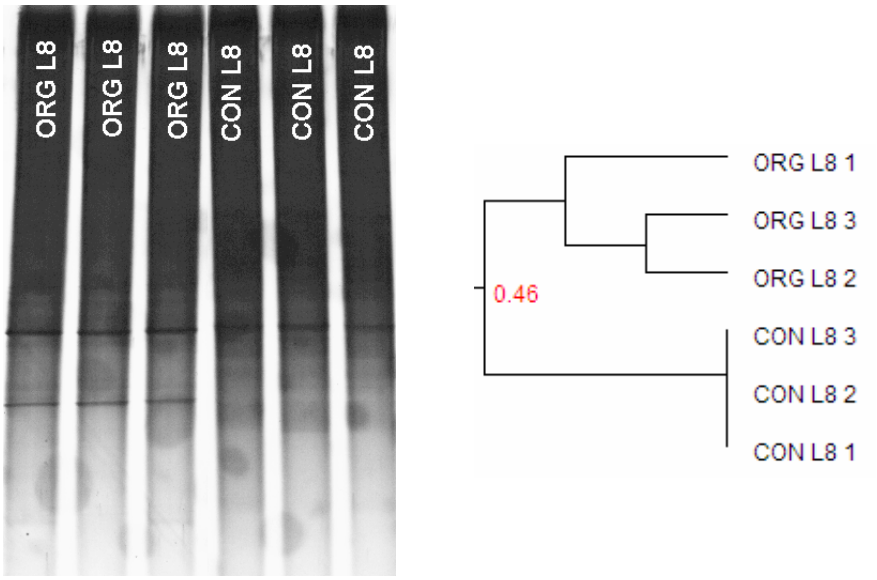


Figure 45: DGGE banding patterns and cluster analysis of bacteria communities in ORG and CON soils amended with 8t lupin ha^{-1} at day 5 in incubation experiment I.

For all organism types, bigger differences could be observed between soils (ORG and CON) than among treatments (L8, L4, C). Cluster analysis indicated that, for eubacteria, the L8 treatment was most different from the other two treatments and that similarities among

replicates were larger than among treatments (Figure 44). However, the treatment replicates were not consistently sorted into the same clusters and assessment of the profiles by eye suggested comparable banding patterns for all treatments at day 5. It was possible to detect differences between soils (Figure 45), but not between treatments (Figure 44). Differences between soils were also observed for actinomycete communities (Figure 46), but not for fungi (see Appendix II). The ORG and CON samples were grouped into separate clusters that showed no similarity to each other for actinomycetes and 46% similarity for eubacteria, which can be attributed to the smaller number of clearly visible bands (Figure 45).

Most bands of the eubacterial profiles were fuzzy, which made it difficult to make a statement regarding number of bands for the different treatments. Actinomycete profiles, however, suggested a similar number of bands for ORG and CON, and community structure was different indicated by the presence of several intense bands in either profile.

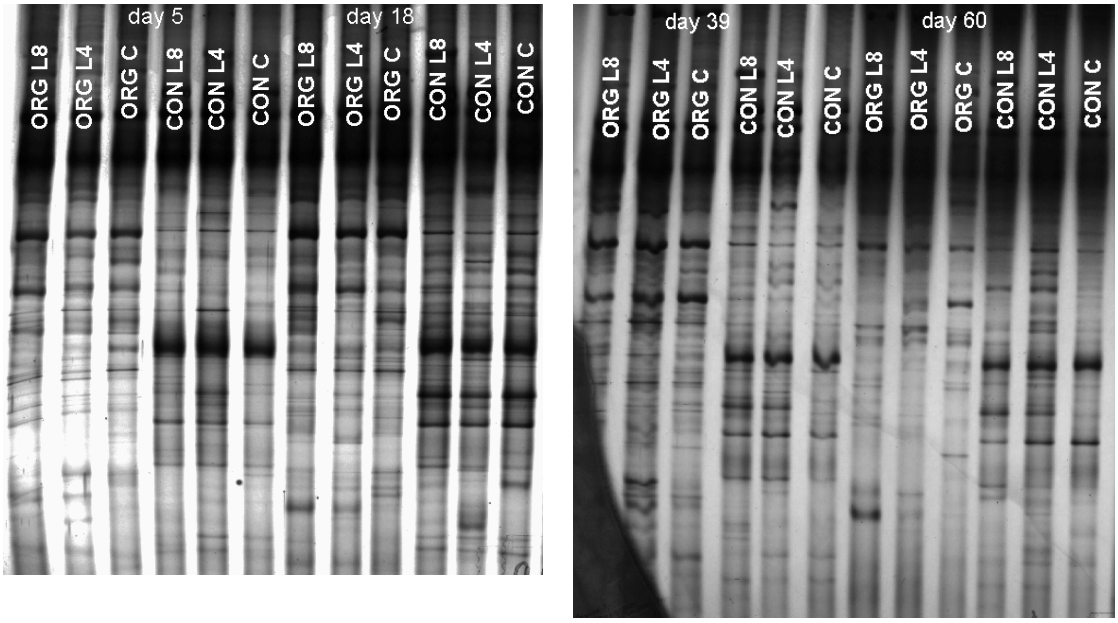


Figure 46: DGGE banding patterns of actinomycete communities in ORG and CON soils at the different sampling dates in incubation experiment I.

No treatment-induced trends were detected in microbial community structure over time, i.e. the differences initially detected between the soils persisted, while no major differences were seen among the treatments. As an example, Figure 46 and Figure 47 show DGGE profiles and the associated cluster analyses, respectively, for actinomycete communities at each sampling point. Similarity within each cluster, i.e. among treatments, was very high (>80%) in most cases. Although cluster analysis across the separate sampling dates is not possible as samples were run on different gels, the gels shown in Figure 46 suggest little changes in actinomycete composition over time.

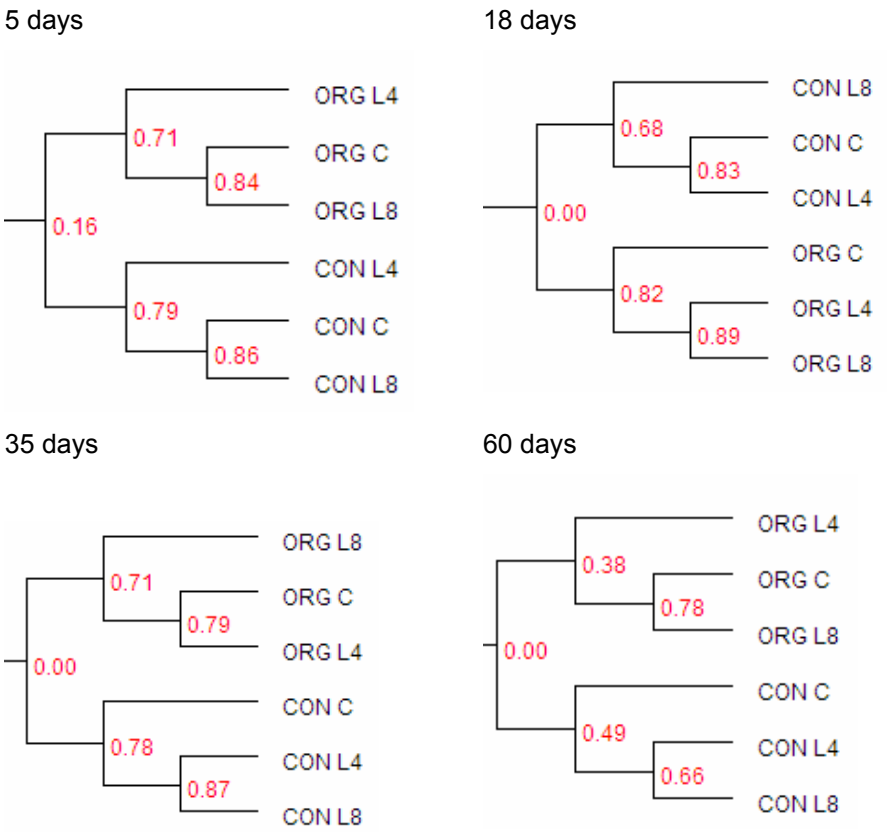


Figure 47: Cluster analyses for actinomycete communities in ORG and CON soils 5, 18, 35 and 60 days after amendment in incubation experiment I.

2.4 Discussion

The influence of various farm management practices on the microbial community is well known (Rovira 1994), and it is widely accepted that microbial biomass size and community composition benefit from organic matter amendments that increase microbial activity and soil processes such as N mineralisation (e.g. Fraser *et al.* 1988; Fauci and Dick 1994; Zaman *et al.* 1999b; Cookson *et al.* 2002; Fontaine *et al.* 2003) (also see discussion of Section 3). Differences in nutrient cycling, in particular N mineralisation, should, thus, be reflected in the structure of the soil microbial community, especially in species diversity and abundance of organisms involved in these processes. However, little is known about the links between microbial activity, function and microbial community structure, and most research suggests that the relationships are neither consistent nor direct (Griffiths *et al.* 2000; O'Donnell *et al.* 2001; Nannipieri *et al.* 2003; Brussaard *et al.* 2004).

An incubation experiment was designed to thoroughly investigate the effect of long- and short-term management on the microbial community. This study was carried out on two soils that had been under different management regimes (organic and conventional) for at least 25 years prior to collecting the soil samples. The response of the microbial community to short-term management practices was studied by amending the soils with organic matter on a single

occasion. This made it possible to establish whether soil microbial communities in the previously organically and conventionally managed soils responded differently to the amendment as a result of management history. Measurements of microbial biomass, activity, function (N mineralisation) and community composition were taken in intervals over an 81-day incubation period to determine the time scale of the microbial response. Thus, the effects of the treatments on the microbial soil properties and community structure could be determined without the interference of environmental factors, such as seasonally varying soil moisture content and temperature and plant growth including root exudation, which are known to influence soil microbial properties (Dalal 1998; Lupwayi *et al.* 1998; Campbell *et al.* 1999; O'Donnell *et al.* 2001). It was possible to determine how different measures of microbial activity relate to each other and to biomass size and microbial community composition. Microbial activity and function were determined by enzyme assays and gross N mineralisation, while the structure of different microbial groups known to play important roles in soil organic matter decomposition and nutrient cycling processes (fungi, eubacteria and actinomycetes), was assessed by DGGE of PCR amplified 16S and 18S rDNA fragments (Eash *et al.* 1994; Beare 1997; Heuer *et al.* 1997; van Elsas *et al.* 2000; Coleman *et al.* 2004; Kennedy *et al.* 2004).

2.4.1 Effect of temporal variation on the soil microbial community

Most soil properties were affected by temporal variation, i.e. significant differences were measured in soil property levels between sampling points. This variation was mainly due to the greatly increased levels in the lupin treatments shortly after amendment compared to subsequent sampling points (for microbial soil properties) or the steady increase with time (for N_{min}).

Despite the rapid decrease of the initially elevated levels, the differences in microbial soil properties persisted over time between the soils and among treatments, indicating that the organic matter priming effect was sustained to a similar extent in ORG and CON and that the microbial communities in both soils responded similarly to the amendments. These results correspond with findings by Lundquist *et al.* (1999a), who observed a decline in microbial soil properties 1 week after adding fresh rye residues to soils, while Gunapala *et al.* (1998) reported that increased activity (measured by SIR) was sustained for 4 days after incorporation of organic material, followed by a decline to a stable level. Compared to the other soil properties, the FDA hydrolysis varied widely over the course of the experiment. These fluctuations could be observed for all treatments and in both soils. Nevertheless, FDA hydrolysis rate was positively correlated with the other enzyme activities and microbial biomass C.

The strong increase in mineral N in the lupin-amended treatments was a result of mineralisation of organic matter and dead microorganisms that was stimulated by microbial

activity and substrate availability (Dalal 1998; Puri and Ashman 1998). The weak rise of mineral N in the unamended control treatment may reflect increased microbial activity and N mineralisation following mixing of the soils after treatment addition (including the control in order to eliminate differences) and sampling. The differences in N dynamics support these findings, as increases in mineralisation rates were measured for all treatments between the two sampling points (Figure 40), while immobilisation rates remained the same for L8 but declined for L4 and control over time (Figure 41). The increase of mineral N in the control treatment also shows that there was little loss of N from the system. Possible *in situ* losses of mineral N (NH_4 and NO_3) include leaching, plant uptake and volatilisation, but these processes do not occur under the laboratory conditions used in this study (Di *et al.* 2000). As mineralisation takes place in any soil system, with and without organic matter addition, an accumulation of mineral N is expected.

Zaman *et al.* (1999a; 1999b) studied gross N mineralisation rates in the field and in a laboratory experiment after application of dairy shed effluent and ammonium chloride fertiliser to soils and reported highest levels of mineralisation at day 16 followed by a decline to or below rates measured initially for the field study. In the laboratory experiment, however, a peak in gross mineralisation at day 8 was followed by a sudden decrease in all treatments. In this study, the declining microbial biomass size coincided with an increase in mineral N in the soils, indicating that more N was released from organic matter and decomposing microbes by mineralisation and nitrification. However, the increase in mineralisation rates and mineral N content was lagging behind the biomass growth: the highest mineralisation rates and N_{min} levels were measured at day 60 and 81, respectively, while biomass C peaked at day 5. This suggests that, despite the relatively low C:N ratio (14) of the lupin material, mineralisation was not initiated instantly. The organic material was not readily accessible. This might have been caused by adding dried plant material that needed to be exposed to soil moisture first.

Mineralisation rates were generally lower than gross immobilisation at the day 18 sampling, while at day 60, the opposite was true, indicating that the microbial communities in the soils were initially N-limited and cell growth and maintenance requirements could not be covered (van Schöll *et al.* 1997). As a consequence, N was immobilised from the soil solution. The decrease in NH_4-N between days 18 and 39 supports this observation. The relatively higher mineralisation rates at day 60 suggest a decrease in microbial biomass size and activity (i.e. less N was needed to maintain microbial growth and metabolism) and a response to the increasing amounts of mineral N in the soil. The absolutely higher rate of gross N mineralisation suggests an increase in microbial activity (Smith *et al.* 1993).

No changes were observed in microbial community compositions during the incubation period. For each sampling point, cluster analyses and DGGE profiles of eubacterial and actinomycete fingerprints revealed differences between soils (ORG and CON) but not among treatments (L8, L4, control). The microbial communities assessed in this study remained the

same in each soil throughout the experiment and were not influenced by time or treatment. It is, however, possible that fluctuations occurred between sampling dates.

2.4.2 Effect of past management on the soil microbial community

Significant differences between ORG and CON were measured for microbial activity (ADA, DHH: ORG>CON; FDA: CON>ORG) and the chemical soil properties (C_{tot} and N_{tot}: CON>ORG), while differences in microbial biomass C and N and mineral N were not significant between soils. The differences remained at the same levels throughout the experiment, as can be seen in Figure 48 showing dehydrogenase activity representative for the soil properties measured, i.e. the priming effect was sustained to a similar extent in ORG and CON and the microbial communities in both soils responded similarly to the amendments.

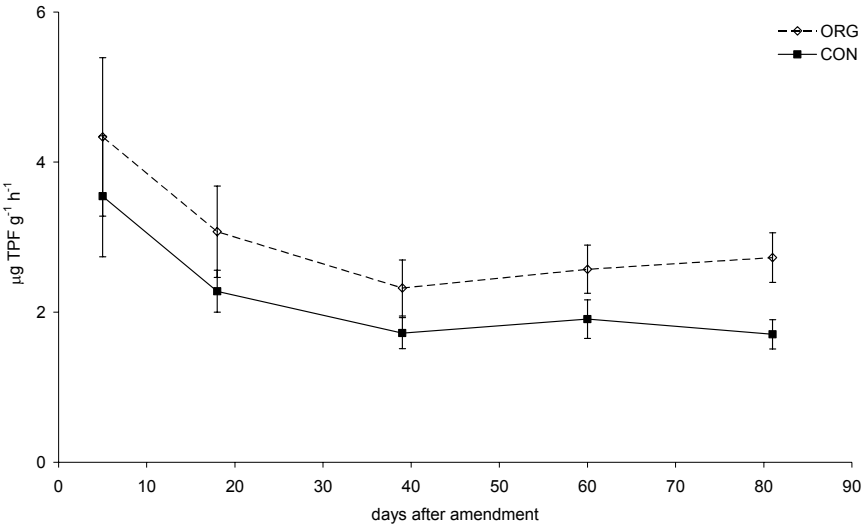


Figure 48: Changes in mean rate (µg g^{-1} h^{-1}) of dehydrogenase activity for two soils over time. Values are means of three treatments. Bars show standard errors of means. n=9.

The results of soil microbial analysis indicate that microbial biomass size, activity and N mineralisation were stimulated by the lupin addition, resulting in elevated levels of microbial soil properties followed by a decline. Comparing microbial biomass and activity in the lupin-amended treatments with the control in each soil, suggested that the addition of organic matter had a more pronounced effect on the microbial community in ORG, e.g. the addition of 8t of lupin per ha resulted in 4-times larger activity of DHH in ORG, while the increase was 3.3-fold in CON (Table 38). This shows that the microbial community in ORG was more responsive to the addition of organic material. This could indicate differences in the microbial community composition in the two soils and suggests that the community in ORG was more adapted to organic matter additions (Barkle *et al.* 2001). However, this is unlikely, considering the management history at the two sites (ORG was a low input area and had been under a herb-ley without any nutrient inputs for approximately 4 years when the soils were

collected, while CON had regularly received mineral fertilisers and had been under varying crops and pasture) (refer to Section 2). The different response could, therefore, be the result of a larger microbial community in ORG that was dormant at the time of amendment but reacted quickly to the addition of nutrients. It also indicates that the microbial population in ORG was more nutrient limited before the amendment compared to CON.

Mineral N content and N immobilisation showed less consistent patterns between the soils with immobilisation rates decreasing in CON and increasing in ORG between days 18 and 60. ORG showed only a slow increase in N_{min} between days 18 and 39, while levels steadily increased in CON. This is consistent with the assumption N limitation in ORG. It indicates that in ORG, microbial N levels were too low for cell synthesis and metabolic functions and N was taken up from the soil solution resulting in a decrease in mineral N levels (Smith *et al.* 1993; van Schöll *et al.* 1997). The higher immobilisation rate suggest that this was the case at day 60, which is in contrast to the increasing mineral N levels in the soil. However, all processes associated with the N cycle influence each other and occur simultaneously (mineralisation, immobilisation, nitrification, microbial growth and decay, etc.). Some effects might override others and result in increasing mineral N levels despite higher immobilisation rates (Dalal 1998; Stevenson and Cole 1999; Arp 2000).

Microbial communities were distinctly different in the two soils indicating that farm management history had a lasting effect on the composition of the soil microbial community, as has been shown previously (Section 2). Crecchio *et al.* (2004) found differences in DGGE fingerprints in soils that were under conventional management with irrigation and organic management, respectively, and Girvan *et al.* (2003) emphasised the discriminatory power of soil type over short-term (up to 2 years) organic management practices with respect to microbial community structure. Differences in DGGE fingerprints of 16S rDNA PCR amplicons were also detected in grassland compared to arable land (van Elsas *et al.* 2002) and in soils after the long-term addition of mineral and organic fertilisers (Marschner *et al.* 2003). In this study, fungal populations did not show any differences as a result of long-term management, which is consistent with the findings of Hagn *et al.* (2003).

2.4.3 Effect of current management on the soil microbial community

As mentioned earlier, organic matter amendments have a positive effect on soil microbial community size, activity and composition (Martyniuk and Wagner 1978; Fraser *et al.* 1988; Fauci and Dick 1994; Robertson and Morgan 1996; Hole *et al.* 2005). Accordingly, the addition of lupin resulted in a significant increase in soil property levels when compared to the control, and higher levels were sustained throughout the experiment (Figure 49). The control showed a similar trend for microbial biomass C as the lupin treatments (initial spike followed by a decline), which was, however, not accompanied by an increase in microbial activity. It might have been in response to mixing the soils when the lupin was incorporated

and when soil moisture levels were adjusted ensuring even organic matter distribution and moistening of the soil. Most of the other soil properties did not vary over time in the control treatment.

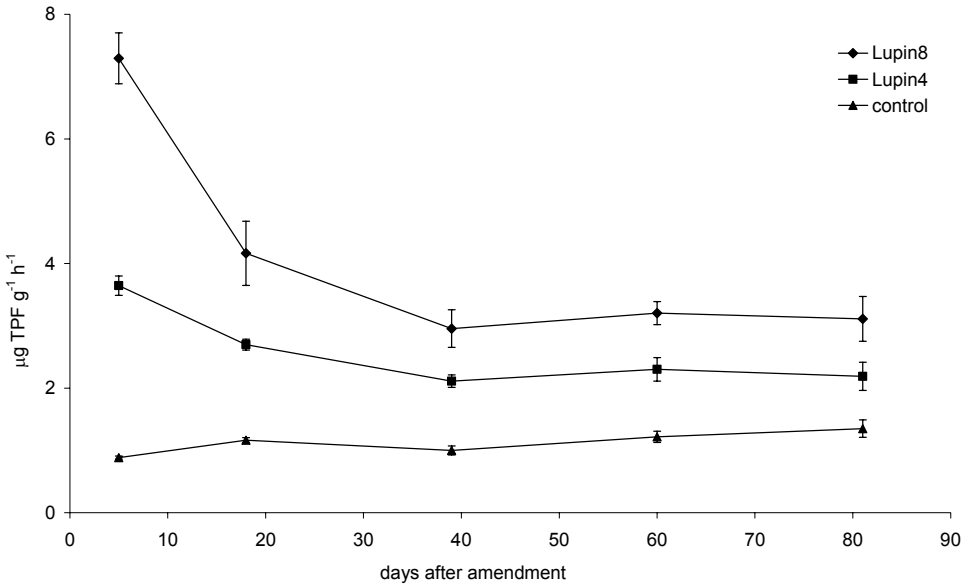


Figure 49: Changes in mean rate (µg g^{-1} h^{-1}) of dehydrogenase activity for three treatments over time. Values are means of two soils. Bars show standard errors of means. n=18.

The addition of organic matter to the soils stimulated the microbial community and resulted in a simultaneous increase of microbial biomass (C and N) and enzyme activities and mineralisation rate. Significant differences were measured among all treatments (Table 36), suggesting that amendment with lupin at both rates had a considerable positive effect on the microbial community. However, adding twice the amount of lupin to the soils resulted in only a 1.1 to 1.7-fold increase in the respective soil properties (Table 38), i.e. the addition of a larger quantity of organic matter did not result in a proportional increase in soil microbial properties. This indicates a non-linear relationship between microbial response and organic matter addition. Griffiths *et al.* (1999) observed similar trends when studying the effect of varying rates of C substrates on the microbial community structure. They also noted that increasing substrate loading rates significantly changed community structure, while the effect on the total microbial biomass size was less pronounced.

The differences in mineralisation and immobilisation rates among the treatments were also closely linked to the addition of organic material (highest amount – highest mineralisation and immobilisation rates) indicating a larger, more active microbial biomass due to the availability of more readily mineralizable material. However, this response was only apparent 60 days after amendment, which indicates that, initially, the soils had similar levels of microbial activity (mineralisation) and that the positive influence of the organic matter amendment was not yet evident. At day 18, however, higher NH_4-N levels in L8 suggest that more NH_4-N was

released from the organic material by mineralisation in this treatment. Although this observation was not reflected in gross mineralisation rates, the differences can be explained by the higher immobilisation rates in L4 and control. While similar amounts of N were mineralised in all treatments, relatively more mineral N was taken up by the microbial communities in L4 and control treatments.

In contrast to the large differences in community composition between ORG and CON, there was no evidence that the microbial community structure was affected by the addition of organic matter within the time frame examined. This shows that in these soils, a one-off amendment with organic matter did not have any lasting impact on the microbial community composition, while biomass size and activity were affected by the treatments. The microbial communities were relatively stable in both soils, and it could be possible that repeated amendments or disturbances result in changes in microbial community structure. It is likely that the treatments had an effect on the structure of less abundant species as only predominant species are targeted by DGGE, and that changes in species evenness (number of species relative to each other) took place that were not detected by DGGE analysis because band intensity is imprecise as a measure of species evenness (Tiedje *et al.* 1999; Heuer *et al.* 2001; Nannipieri *et al.* 2003).

2.4.4 Linkages between microbial biomass size, activity and community structure

Including all treatments in the correlation analysis showed that the enzymes activities (DHH, ADA, FDA) were all positively correlated with C_{mic}, N_{mic} and amongst each other (Table 41), indicating a close relationship between biomass size and microbial activity. This is consistent with the findings by Haynes (1999), who reported highly positive correlations between enzyme activities and biomass C. However, the previously reported significantly positive relationships between C_{tot} and microbial soil properties (Haynes 1999) were not detected in this study. While the (linear) correlation coefficients were mainly positive, the correlations were not significant, which could indicate the presence of relationships other than linear.

Haynes and Tregurtha (1999), for example, suggested that quadratic functions resulted in better fits than linear regressions. It is also likely that a single amendment with organic material was not sufficient to affect total C contents in the soils while it did have an effect on soil microbial properties. As discussed in Section 3, total C and N levels are expected to respond only slowly to organic matter amendments (Wander *et al.* 1995; Haynes 1999; Ryan 1999). There was only a weak linear correlation between microbial biomass C and gross N mineralisation. It is likely that mineralisation activity did not increase as quickly as the enzyme activities that were directly linked to the changes in C_{mic} and N_{mic} and that the response of mineralisation activity lags behind the increase in biomass size. However, it also indicates that microbial activity, in this case mineralisation, can increase independently of microbial biomass, i.e. the size of the microbial population remains the same, while an

increase occurs in microbial activity and most likely, the size of microbial groups involved in N mineralisation. Gross N mineralisation rate was only weakly correlated with the three enzyme activities (ADA, FDA and DHH) and the graphs in Figures 38, 39 and 40 revealed that an increase in one parameter was accompanied by a decrease or no change in the others, although all measurements can be seen as indicators of microbial activity and function (Puri and Ashman 1998; Kandeler *et al.* 1999b). This suggests that different microbial assemblages are involved in the various processes, which makes it possible for the overall microbial activity to remain stable or decrease (assuming enzyme activities are appropriate measures for overall microbial activity), while the activity of particular groups of organisms involved in N mineralisation increases. Similarly, Chen *et al.* (2001) suggested that the lack of correlation between different measures of microbial activity (DHH and SIR) indicated changes in the composition of the microbial community. The positive relationship between mineral N and enzyme activities as well as mineralisation rate suggests that microbial activity influences the soil N content positively and vice versa. Considering the assumption of N-limitation especially in the organically managed soil, a higher mineral N content would be beneficial to the microbial community and stimulate growth and activity.

The positive correlation determined between N_{min} and the enzyme assays when including all treatments, was not reflected in the correlations for the separate treatments (Table 40). Negative correlations were measured between enzymes and N_{min} for the lupin-amended soils. This could indicate that, in these treatments, the mineral N content was not a consequence of increased enzyme activity. The differences in correlations of C_{mic} with N_{mic} and enzyme activities, as well as N mineralisation rate with DHH and FDA between lupin and control treatments suggest differences in microbial community composition or species diversity in the control compared to the lupin treatments. It also indicates that microbial biomass size and activity are not necessarily positively linked to each other, that one might lag behind the other in their response and that, without stimulation (e.g. by organic matter addition), other soil and environmental factors have a large influence on microbial soil properties. Similar outcomes were reported by Böhme *et al.* (2005), who found varying relationships between microbial biomass and activity depending on field site location and climatic conditions.

Although comparison of non-quantitative DGGE results and numerical soil analysis data is problematic (see Sections 2 (1.2) and 3 (2.4)), results of the cluster analyses of DGGE profiles allowed some conclusions to be drawn regarding the linkages between microbial community structure and the activities measured. Community structure of eubacteria and actinomycetes was not influenced by the addition of either amount of lupin and did not change over time, unlike the microbial soil properties that were strongly affected by the lupin amendment and showed temporal variation. However, there were differences in microbial structure between the ORG and CON soils resulting from long-term management history that were also reflected in significant differences in enzyme activities and total C and N (Table 35). Microbial activity increased and decreased independently of noticeable changes in the community composition of the microbial groups assessed, which strongly suggests there is no

direct link between eubacterial and actinomycete community composition and microbial activity, which is in agreement with findings by Marschner *et al.* (2003). The detectable differences due to management history indicate that closer links exist between community composition and inherent soil properties. Soil and environmental factors, such as organic matter content, total N content, crop type, soil type and texture, have previously been identified as having a strong impact on microbial community composition and diversity (Gelsomino *et al.* 1999; Buckley and Schmidt 2001; Johnson *et al.* 2003; Marschner *et al.* 2003). Nonetheless, studying microbial communities following long-term exposure to disturbances or environmental stresses, e.g. heavy metal contamination, has shown links between community structure and function (Griffiths *et al.* 2001b; Müller *et al.* 2002; Griffiths *et al.* 2004). Identification of microbial species by DNA sequencing of bands could have revealed treatment-related differences in predominant species and given more insight into the species richness in the treatments. It might have been possible to link differences in species richness to the observed differences in microbial processes. Similarly, analysis of rRNA abundance in the soils, which is influenced by number of cells and their metabolic activity (Ward *et al.* 1992), might have revealed differences in active microbial communities among the treatments. However, applying these techniques was beyond the scope of this study.

2.5 Conclusions

The following conclusions can be drawn from incubation experiment I:

- As expected, the amendment with lupin stimulated the microbial communities increasing microbial biomass and activity in both soils. However, the high levels recorded 5 days after amendment were followed by an immediate decline in microbial biomass C and N levels and enzyme activity rates, reaching stable levels after 20 to 40 days. The most pronounced changes in soil microbial properties appear to occur only shortly after amendment with organic matter.
- The addition of 4 t ha^{-1} of lupin was sufficient to stimulate the microbial community, resulting in microbial biomass growth and an increase in activity and N mineralisation. While the addition of 8t ha^{-1} caused a greater response, the effect was not proportional to the additional amount added. In L8, levels of soil microbial properties were only 1.1 to 1.7-times higher than in L4.
- Microbial communities in the soils with organic and conventional management histories were adequate to respond to the organic matter amendment with increased growth and activity. The differences in microbial biomass C and N and enzyme activities persisted over time at similar levels. However, the higher lupin amendment had a greater effect on microbial soil properties in the organically managed soil, and differences in N

mineralisation rates suggest that the microbial community in this soil was more N limited compared to the conventional soil.

- DGGE analysis showed that microbial community structure was not affected by lupin amendment and did not change over time. The differences in eubacterial and actinomycete community composition between soils with different management histories persisted, which indicates a closer link of microbial community structure with inherent soil factors that change slowly following organic matter amendment. Under the experimental conditions chosen for this study, no relationship existed between microbial structure and enzyme activities and soil processes. However, the application of techniques that measure other aspects of microbial diversity and the repeated amendment with organic materials might have revealed treatment-induced differences in microbial community structure or abundance of dominant species.
- Correlation analysis for all treatments combined showed strong links between microbial biomass size and activity. Differences in correlation coefficients of soil properties among the three treatments suggest the presence of differences in microbial structural diversity (species richness or evenness) and physiological properties of the microbial community that were not determined by the methods used.

3 Incubation experiment II

3.1 Introduction

A second experiment was designed to address the following issues that arose from incubation experiment I.

(1) The hugely elevated levels and following rapid decline of microbial biomass C and enzyme activities observed in incubation experiment I suggest that the effect of organic matter addition on microbial soil properties is strongest shortly after amendment. The sampling strategy with the first sampling point 5 days after addition was, therefore, not suitable to fully appreciate the influence of organic matter amendments on the soil microbial community. Consequently, an adjusted sampling routine was adopted including more intense sampling in the first 14 days after amendment.

(2) Microbial community structures assessed by DGGE analysis did not reveal significant differences between soils (actinomycetes, eubacteria, fungi) or among treatments (fungi). The results of the soil analyses (treatment-related differences in enzyme activities and soil processes; no differences in community structure) also suggested that it is likely that smaller microbial groups are affected by the treatments. Thus, incubation experiment II focused on the community structures of pseudomonads, α proteobacteria and actinomycetes.

For this study, soils with organic and conventional management histories were amended with equal amounts of N (100 kg ha^{-1}) in organic and mineral form (as lupin and urea, respectively) and microbial biomass size, activity, community structure and soil processes (N mineralisation) were measured in intervals over 91 days. The sampling strategy was adjusted by increasing the number of sampling points in the first two weeks of the incubation period and different primers were used to determine microbial community structure. Addition of 100 kg N ha^{-1} was chosen as this is an amount commonly used for fertilisation of arable crops. For the lupin, 100 kg ha^{-1} was equivalent to 4.3t of dry matter yield per ha (Table 42), which was similar to the amount used in incubation experiment I.

3.2 Materials and methods

3.2.1 Experimental design

Sample preparation took place as described previously (see Chapter 2.2, this section) (1.5 kg dwe of topsoil samples from BHU and LCF[1], air dried and sieved (2 mm) and then incubated in 2 L plastic containers at 70% WHC and 20ºC). Treatments were applied to both soils after 3 weeks of pre-incubation. Equivalent to 100 kg N ha^{-1} was added to the soils as urea (analytical grade) and ground lupin. The control treatment did not receive any amendments. Each treatment had three replicates (for detailed treatment descriptions refer to Table 42).

The soils were sampled after 0, 3, 6, 10, 14, 35 and 91 days by removing approximately 200 g of moist soil from the entire depth of each container. After each sampling, the soils in the containers were re-compacted to an approximate bulk density of 1 g cm^{-3}.

Table 42: Details of treatments included in incubation experiment II.

Treatment ID	*Amendment*	*N content*	*C content*	*Application per ha* *
ORG or CON L	lupin	2.3%	44%	4.3t ha^{-1}
ORG or CON U	urea	46%	20%	217 kg ha^{-1}
ORG or CON C	control	--	--	--

*assuming a bulk density of 1.4 g cm^{-3} and a depth of 7.5 cm

3.2.2 Analyses of soil microbial and biochemical properties and soil processes

Soil analyses included measures of mineral N (NO_3-N, NH_4-N and N_{min}) (Blakemore *et al.* 1987), C_{mic} and N_{mic} (Sparling and West 1988) and DHH (Thalmann 1968). On four occasions (days 0, 10, 35, and 91) gross N mineralisation and immobilisation rates were estimated using the ^{15}N dilution technique (Zaman *et al.* 1999a; Zaman *et al.* 1999b). C_{tot} and N_{tot} were determined on a Leco® CNS-2000 elemental analyser.

3.2.3 Analysis of soil microbial community structure

The methods for DNA extraction, PCR amplification and DGGE were the same as described in Section 2 unless mentioned. On three occasions (days 0, 10 and 91) community DNA was extracted and 16S rDNA fragments were amplified in a two step PCR using primers targeting actinomycetes (F243-R1494) , α proteobacteria (F203α-R1494) and pseudomonads (sensu stricto) (ps for-ps rev) for the first amplification round and eubacterial primers F984GC and R1378 for the second round (Table 17). Diluted (1/200) PCR product from the first round was

[1] in the following referred to as ORG and CON.

used as template. The reaction mixtures were identical to those described previously (Table 4), although no BSA was used in the second round of the nested PCR. Thermal cycling conditions for the respective primer pairs were as described in Table 17. A linear denaturing gradient of 40-55% was used for all DGGE gels.

3.3 Results

3.3.1 Effect of long- and short-term management practices on soil properties and processes

Repeated measures analyses of variance showed which of the factors (time, treatment and soil origin) had the greatest effect on the measured soil properties (Table 43). The chemical soil parameters C_{tot} and N_{tot} were mainly affected by differences resulting from soil origin, while N_{min} and the biological parameters (C_{mic}, N_{mic}, DHH and microbial quotient) showed significant variation caused by treatment and time.

Temporal variation was mainly a result of the lupin treatment significantly increasing microbial soil properties directly after the amendment, and both soils showed similar trends (Figures 50, 51 and 53). After the initial rise and the following decline, the curves did not show much change with time.

As NO_3-N made up 90-99% of total mineral N most of the time (higher proportions of NH_4-N were measured between days 3 and 10 only for the urea treatment [NO_3-N between 45 and 75% of N_{min}]), results are presented for the statistical analyses of N_{min} and NH_4-N, while graphs detailing NO_3- and NH_4-N levels over time can be found in Appendix II.

Table 43: Main factors (soil origin, treatment, time) influencing soil properties and processes in incubation experiment II.

Soil property	*Factor*	*p value*	*R^2 (%)*
C_{mic}	treatment	<0.001	73.4
N_{mic}	treatment	<0.001	65.2
DHH	treatment	<0.001	66.6
C_{mic}:C_{tot}	treatment	<0.001	67.8
MIN	time	0.001	15.7
IMM	time	<0.001	33.1
NH_4-N	treatment	<0.001	38.7
N_{min}	treatment	<0.001	79.3
C_{tot}	soil origin	<0.001	83.5
N_{tot}	soil origin	<0.001	66.9
C:N ratio	treatment	<0.001	33.8

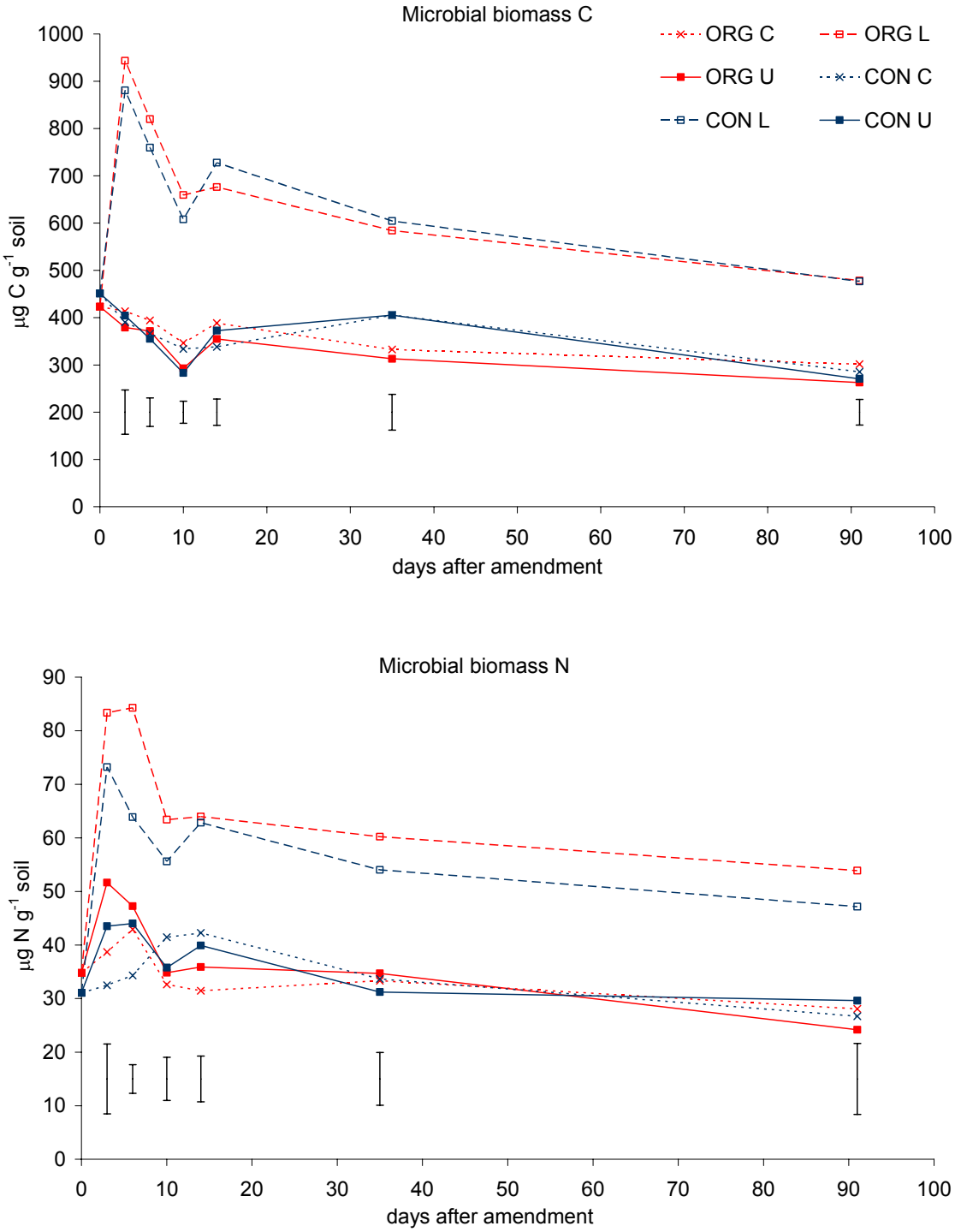


Figure 50: Mean concentrations (µg g^{-1}) in microbial biomass C and N determined over 91 days in incubation experiment II. Bars show $LSD_{0.05}$. n=9.

Effect of time on soil properties and processes

Gross N mineralisation and immobilisation rates were the only parameters strongly influenced by time indicating that, especially for mineralisation, the differences among soils and treatments were negligible. However, temporal variation did not account for a large percentage of the total variation (R^2 values = 12.3 and 31.7% for MIN and IMM, respectively), which suggests random variation (Table 43). Figure 52 shows how soils and

treatments varied in gross N mineralisation and immobilisation rates at the different sampling dates. The initial mineralisation rates were significantly higher than those measured throughout the incubation, while the highest immobilisation rate was measured at day 10 for the urea treatment.

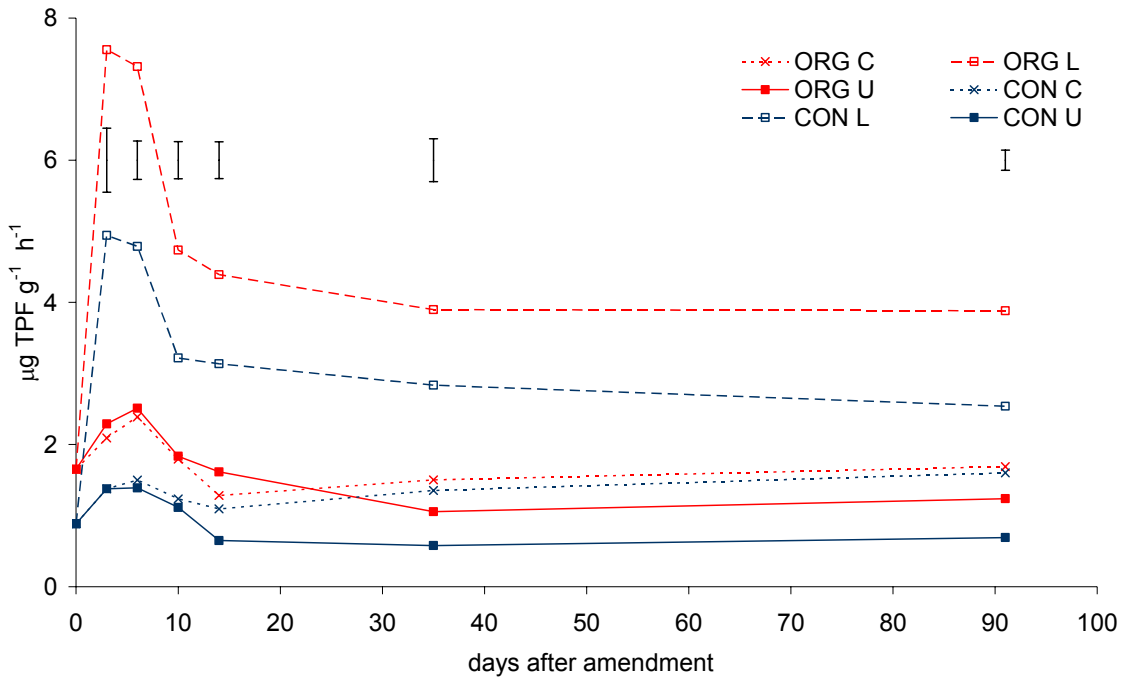


Figure 51: Mean rates (µg TPF g^{-1} h^{-1}) in dehydrogenase hydrolysis determined over 91 days in incubation experiment II. Bars show $LSD_{0.05}$. n=9.

Effect of past management (soil origin) on soil properties and processes

The origin of the soils (ORG and CON) was most strongly influencing chemical parameters (C_{tot}, N_{tot}), with CON having higher levels than ORG. However, significant differences between the soils could be also measured in DHH (ORG>CON), microbial quotient (ORG>CON) and N_{min} (CON>ORG) (Table 44).

Table 44: Overall mean values of selected soil properties determined for the organic and conventional soils over 91 days in incubation experiment II.

Soil property	*Soil origin*		*Significance*
	ORG	*CON*	
DHH (µg TPF g^{-1} h^{-1})	2.88 (0.26)	1.91 (0.18)	***
C_{mic}:C_{tot} (%)	1.95 (0.104)	1.67 (0.082)	***
N_{min} (µg g^{-1})	91.4 (6.38)	127.5 (6.49)	***
C_{tot} (%)	2.35 (0.010)	2.73 (0.012)	***
N_{tot} (%)	0.199 (0.001)	0.229 (0.002)	***

Values are means of three treatments and six sampling dates (Standard errors of means). n=54. ***, p<0.001

ORG showed higher levels of C_{mic}, N_{mic}, N mineralisation and immobilisation, although these differences were not significant (Figures 50 and 52).

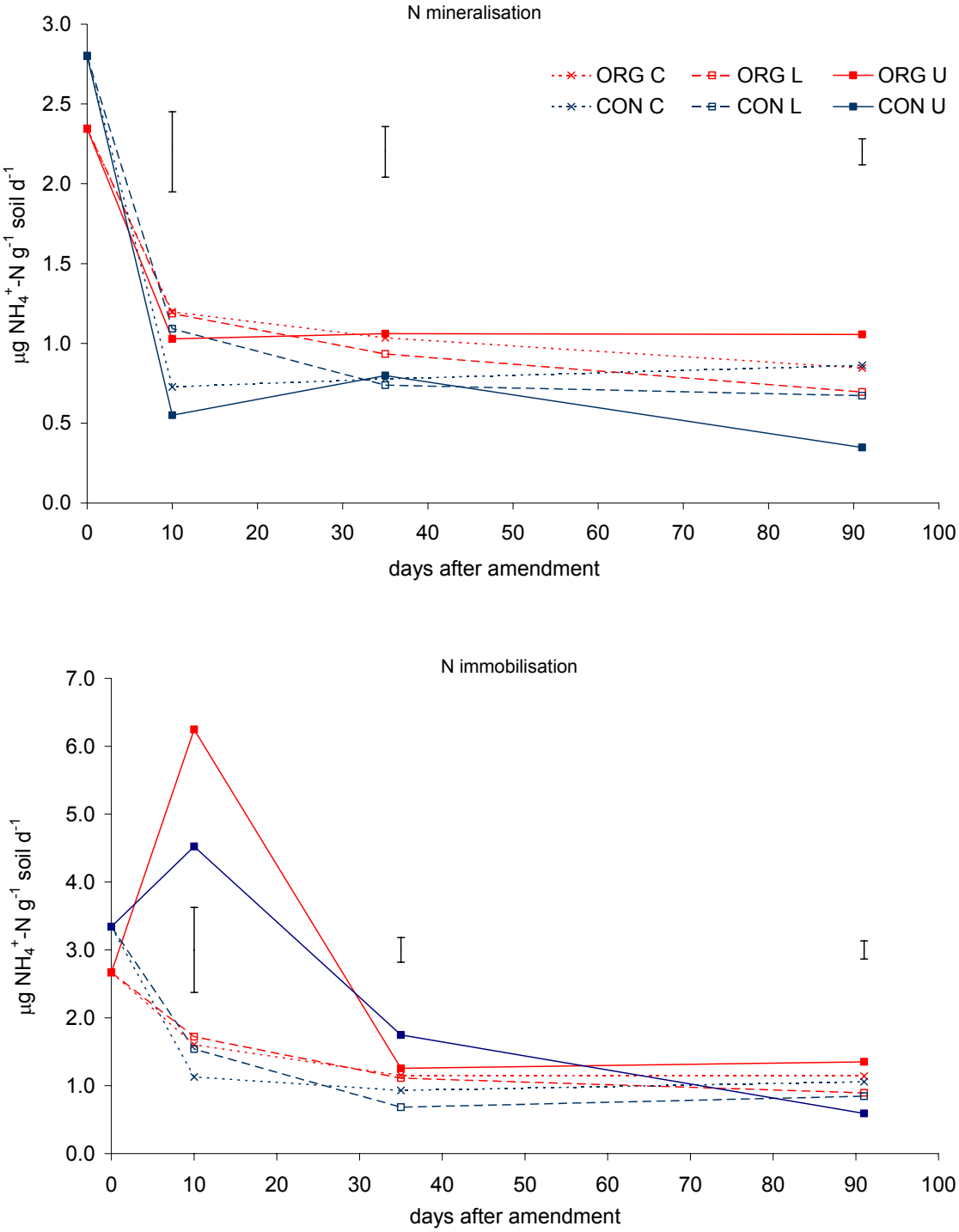


Figure 52: Mean rates (µg NH_4^+-N g^{-1} d^{-1}) of N mineralisation and immobilisation determined over 91 days in incubation experiment II. Bars show $LSD_{0.05}$. n=9.

Comparing microbial soil properties at day 0 and day 3 showed that C_{mic}, N_{mic}, N_{min}, MIN and IMM changed relatively more in ORG than in CON after treatments application. DHH activity, on the other hand, was more strongly affected in CON by the addition of lupin and

urea (Table 45). After 91 days of incubation, relative differences in N_{mic} and DHH activity were larger in CON than in ORG, while N_{min} and the process rates remained more strongly influenced in ORG. Microbial biomass C changed at similar rates for ORG and CON. This indicates that the various soil properties were differently affected by the treatments in the two soils and did not change at the same proportions.

Table 45: Relative increases in levels and rates of soil properties levels and soil processes 3 and 91 days after amendment compared to day 0.

Sampling	*Treatment*	*C_{mic}*	*N_{mic}*	*DHH*	*N_{min}*	*MIN*	*IMM*
day 3	ORG C	1.0	1.1	1.3	0.8	0.5	0.7
	CON C	0.9	1.0	1.6	0.8	0.3	0.3
	ORG L	2.2	2.4	4.6	0.9	0.5	0.7
	CON L	2.0	2.4	5.6	0.9	0.4	0.5
	ORG U	0.9	1.5	1.4	2.0	0.4	2.7
	CON U	0.9	1.4	1.6	1.8	0.2	1.4
day 91	ORG C	0.7	0.8	1.0	1.2	0.4	0.4
	CON C	0.6	0.9	1.8	1.1	0.3	0.3
	ORG L	1.1	1.5	2.4	1.6	0.3	0.3
	CON L	1.1	1.5	2.9	1.4	0.2	0.3
	ORG U	0.6	0.7	0.7	2.5	0.5	0.5
	CON U	0.6	1.0	0.8	2.1	0.1	0.2

Effect of current management (treatment) on soil properties and processes

Treatment was the most important factor affecting the biochemical soil properties C_{mic}, N_{mic}, DHH and microbial quotient (C_{mic}:C_{tot}) as well as N_{min} and C:N ratio. The effect on the C:N ratio, however, was not consistent; the small R^2 value indicates that the variation between replicates was random (Table 43). This indicates that the treatments – control, lupin and urea amendments – had a greater influence on the biological parameters than soil origin. The lupin treatment caused significantly higher values in the biological properties, while N_{min} and NH_4-N were higher in the urea treatment (Table 46).

Shortly after the lupin amendment (day 3), C_{mic} and N_{mic} values were doubled and DHH values increased to approximately 5 times that of the control and urea treatments. They remained elevated until the end of the experiment. This effect was the same for ORG and CON. For most soil properties, the urea treatment did not show significantly different values when compared to the control.

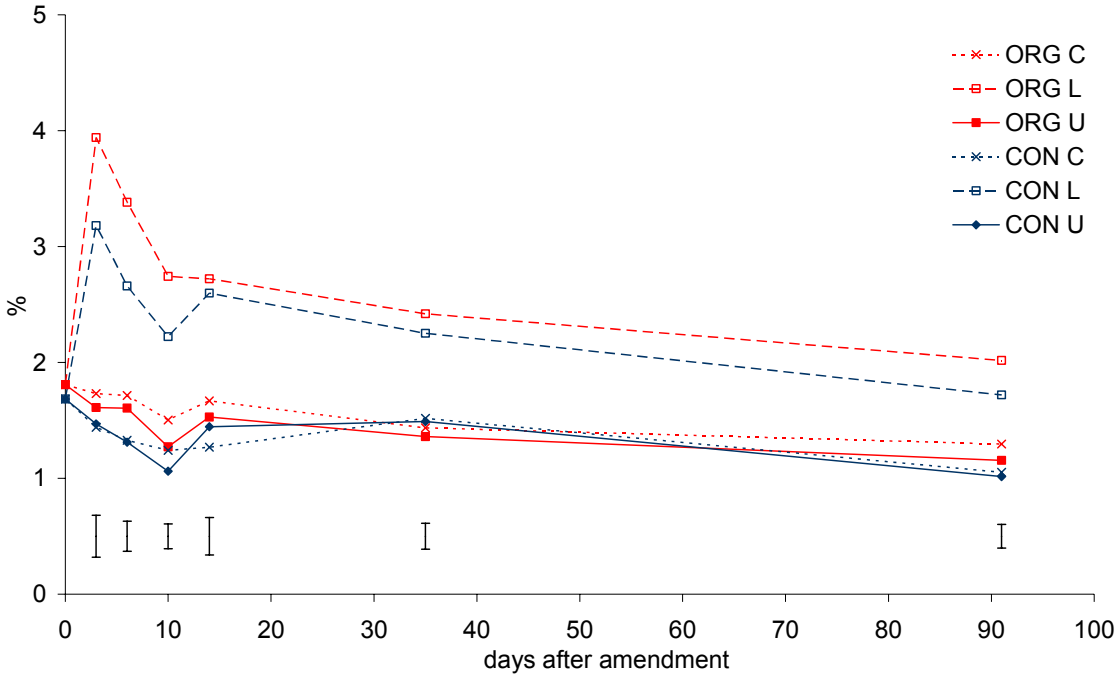


Figure 53: Mean values for microbial quotient (C_{mic}:C_{tot}) (%) determined over 91 days in incubation experiment II. Bars show $LSD_{0.05}$. n=9.

Table 46: Overall mean values of selected soil properties determined for the different treatments over 91 days in incubation experiment II.

Soil property	*Treatment*			*$LSD_{0.05}$*
	Control	Lupin	Urea	
C_{mic} (µg C g^{-1})	362.2 (8.00) [a]	670.2 (25.4) [b]	343.9 (9.54) [a]	19.9
N_{mic} (µg N g^{-1})	34.5 (1.02) [a]	62.3 (2.21) [b]	37.3 (1.56) [a]	3.17
DHH (µg TPF g^{-1} h^{-1})	1.56 (0.065) [a]	4.28 (0.274) [b]	1.36 (0.097) [c]	0.15
C_{mic}:C_{tot} (%)	1.45 (0.037) [a]	2.59 (0.105) [b]	1.38 (0.039) [a]	0.09
N_{min} (µg g^{-1})	78.6 (2.82) [a]	85.7 (3.63) [a]	164.0 (8.21) [b]	7.33
NH_4-N (µg g^{-1})	1.13 (0.12) [a]	3.11 (0.53) [a]	34.9 (5.31) [b]	3.16
C:N ratio	12.1 (0.038) [a]	11.8 (0.066) [b]	11.6 (0.045) [c]	0.11

Values are means of two soils and six sampling dates. (Standard errors of means). n=36. Different letters indicate significant differences among treatments at $p<0.05$.

Interactions between time, treatment, and soil origin affecting soil properties and processes

Of all interactions, treatment*time and soil origin*time interactions had the biggest influence on the soil properties (Table 47). As with the single effects, the changes in the biological properties and N_{min} were driven by treatments, whereas soil origin had a stronger effect on N_{tot} (effects on C_{tot} and C:N ratio were not significant). However, the data suggests that the interactions of treatment and soil origin with time were not affecting the soil properties to a large extent (R^2 values smaller than those of single effects [Table 43]). This indicates that the different treatments and soil origins exhibited similar trends over time. Comparing the

different sampling points within treatment and soil origin did not reveal big differences for the soil parameters. Only NH_4-N was strongly influenced by time*treatment interactions due to the dramatic increase in the urea treatment shortly after addition.

All treatments had comparable mineralisation patterns over the course of the incubation and the control and lupin treatments showed similar trends for immobilisation in both soils, while the urea treatments showed a significant increase at day 10 followed by a decrease to the level of the other treatments (Figure 52). ORG and CON averages revealed similar mineralisation and immobilisation trends for the two soils throughout the incubation.

Table 47: Main interactions influencing soil properties and processes in incubation experiment II.

Soil property	*Factor*	*p value*	*R^2 (%)*
C_{mic}	time*treatment	<0.001	7.77
N_{mic}	time*treatment	0.013	3.53
DHH	time*treatment	<0.001	7.52
C_{mic}:C_{tot}	time*treatment	<0.001	8.16
NH_4-N	time*treatment	<0.001	33.7
N_{min}	time*treatment	0.036	1.17
MIN	time*soil origin	0.008	9.76
IMM	time*soil origin	<0.001	30.9
N_{tot}	time*soil origin	0.046	2.84

Data only displayed for interactions with $p<0.05$.

Correlations among soil properties and processes (linkages)

The correlations among the soil properties showed similar tendencies for ORG and CON. This is consistent with previous results (only minor differences between the two soils were detected). The biological soil properties (C_{mic}, N_{mic}, DHH) showed strong positive correlations to each other ($R^2>0.88$) and were negatively correlated to N_{min} (see Appendix II). C_{tot} and N_{tot} were positively correlated to each other ($R^2=0.92$) and to biomass C, while they did not show any correlations with microbial activity (DHH). Gross mineralisation rate correlated positively with the biological properties and negatively with N_{min}, C_{tot} and N_{tot}.

Correlation analyses for each treatment separately (Table 48) revealed generally weaker correlations for urea and control, i.e. the measured properties were not as closely linked to each other as for the lupin treatment. Correlations between mineralisation rate and mineral N and total C and N were, however, stronger in the urea and control treatment, compared to the lupin-amended soil. The general trends for correlations of the three treatments were similar to the overall correlations.

Table 48: Correlation coefficients determined among soil properties and processes for the control, lupin and urea treatments over 91 days in incubation experiment II.

	C_{mic}	N_{mic}	*DHH*	N_{min}	C_{tot}	N_{tot}
Control						
N_{mic}	0.45**					
DHH	0.23	0.13				
N_{min}	-0.41*	-0.33*	-0.53**			
C_{tot}	-0.05	-0.004	-0.54**	0.80***		
N_{tot}	0.07	0.002	-0.47**	0.73***	0.97***	
MIN	-0.03	-0.04	0.20	-0.45**	-0.43*	-0.44**
Lupin						
N_{mic}	0.88***					
DHH	0.76***	0.87***				
N_{min}	-0.63***	-0.74***	-0.76***			
C_{tot}	-0.02	-0.32*	-0.52**	0.57**		
N_{tot}	0.11	-0.18	-0.37*	0.46**	0.90***	
MIN	0.50**	0.32*	0.37*	-0.64***	-0.08	-0.18
Urea						
N_{mic}	0.49**					
DHH	0.11	0.54**				
N_{min}	-0.04	-0.18	-0.60**			
C_{tot}	0.22	0.04	-0.54**	0.48**		
N_{tot}	0.31*	0.10	-0.48**	0.46**	0.97***	
MIN	0.09	0.07	0.60**	-0.64***	-0.82***	-0.72***

n=36. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

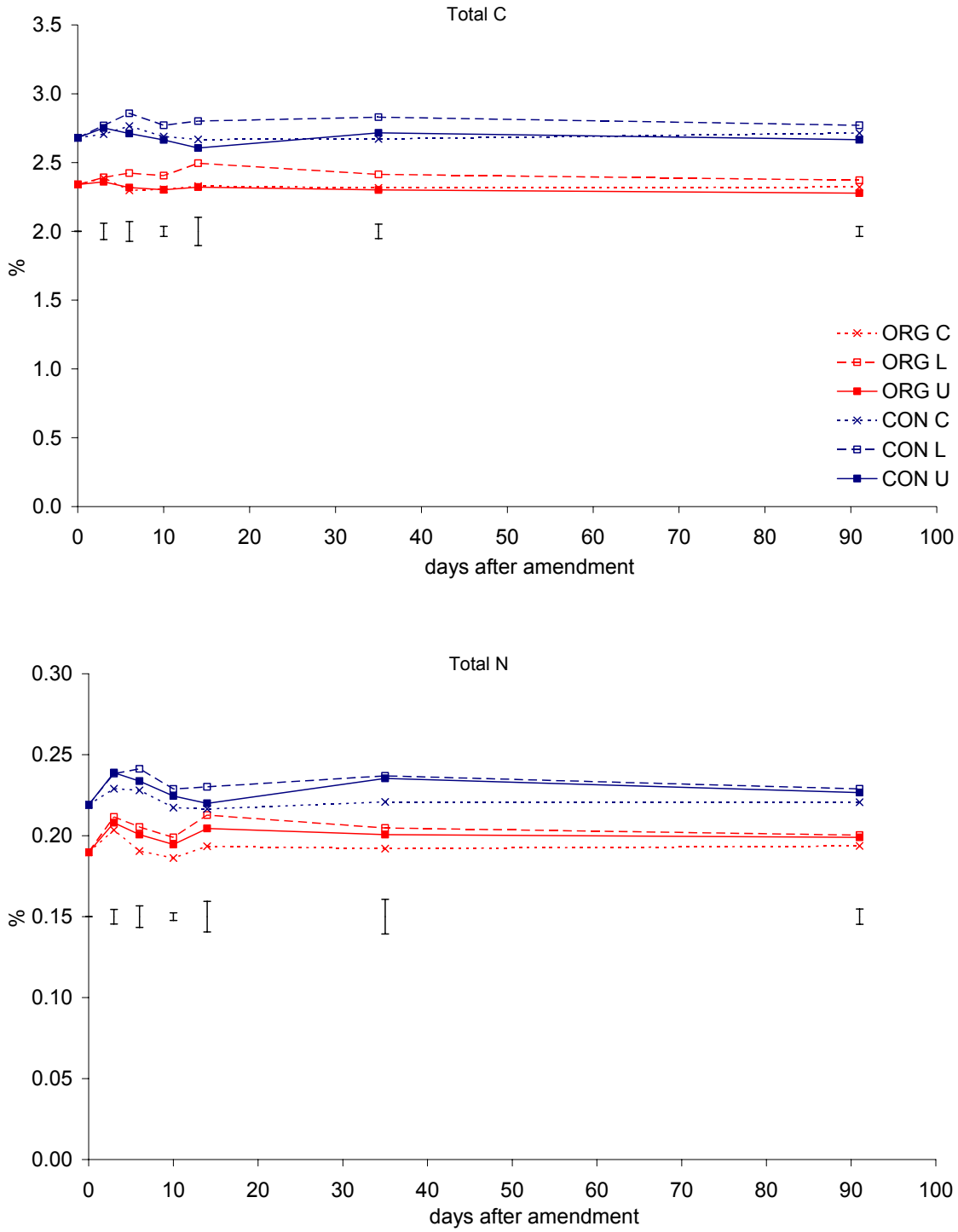


Figure 54: Mean concentrations (%) in total C and N determined over 91 days in incubation experiment II. Bars show $LSD_{0.05}$. n=9.

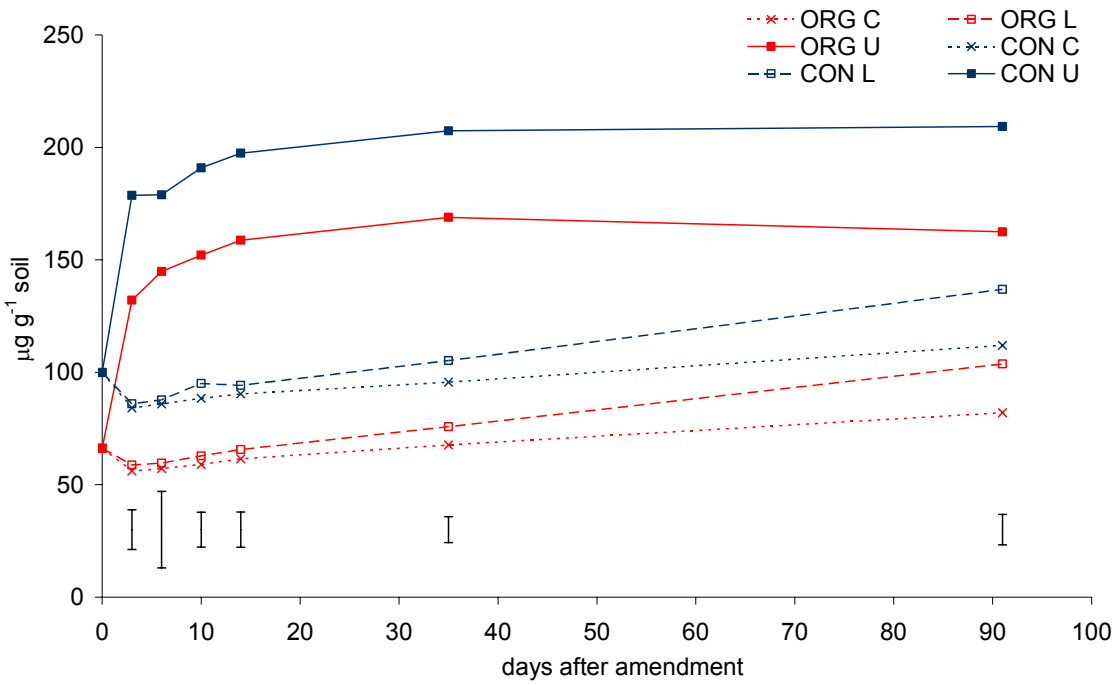


Figure 55: Mean concentrations (µg g^{-1}) of soil mineral N determined over 91 days in incubation experiment II. Bars show $LSD_{0.05}$. n=9.

3.3.2 Effect of long- and short-term management practices on soil microbial community structure

Figure 56 shows the initial DGGE patterns (day 0, DNA extracted before amendment with lupin or urea) of α proteobacteria, actinomycetes and pseudomonad 16S rDNA amplicons. The duplicate samples were very similar for α proteobacteria and actinomycetes, while pseudomonads seemed to be more variable and differences between replicate banding patterns appeared as big as between the soils. Cluster analysis revealed that ORG samples could not be grouped into a discrete cluster and CON samples were more similar to each other than to the ORG samples (Figure 57). In contrast, α proteobacteria and actinomycetes showed evident differences in DGGE profiles between the two soils. For the three primer sets, number of bands was similar for both sites.

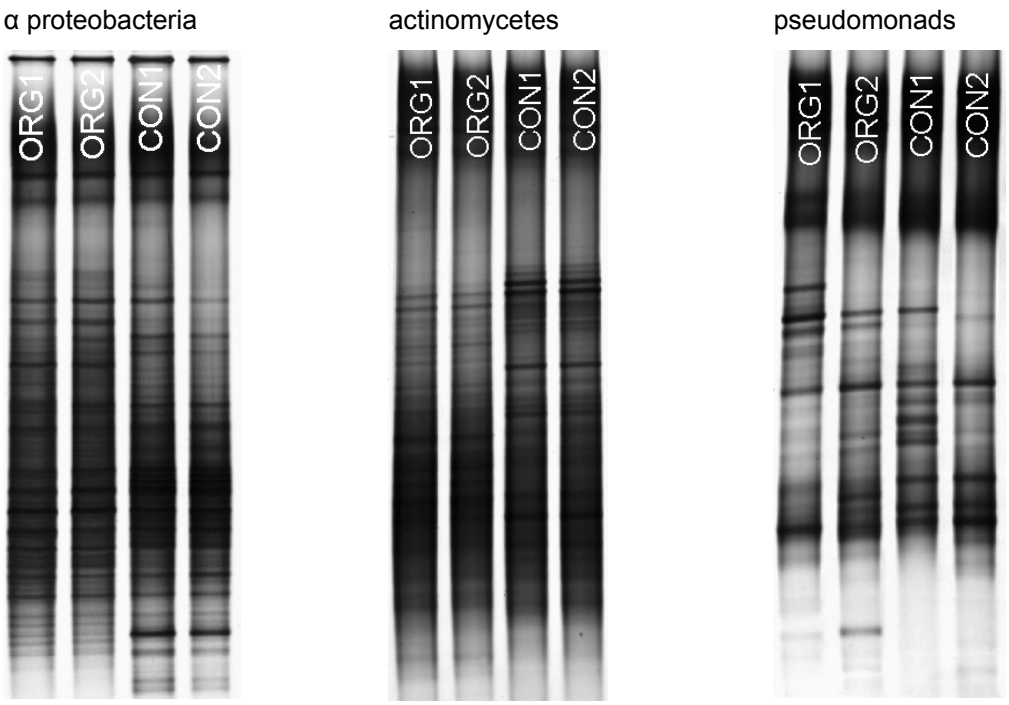


Figure 56: DGGE profiles for duplicate DNA extracts of 16S rDNA fragments amplified using α proteobacterial, actinomycete and pseudomonad primers at day 0 in incubation experiment II.

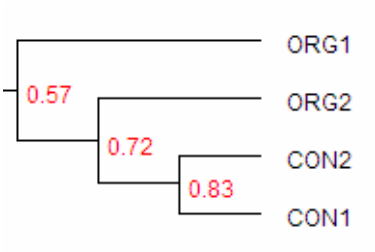


Figure 57: Cluster analysis for ORG and CON samples amplified with pseudomonad primers at day 0 of incubation experiment II.

For α proteobacteria, the three treatments (lupin, urea, control) showed similar DGGE patterns, while differences could be detected between DGGE profiles for ORG and CON (Figure 58). Cluster analyses revealed that in both soils the treatments could be distinguished from each other and the three treatment replicates were sorted into the same clusters. In both soils, the control was most different from the other two treatments, i.e. lupin and urea clusters were more alike.

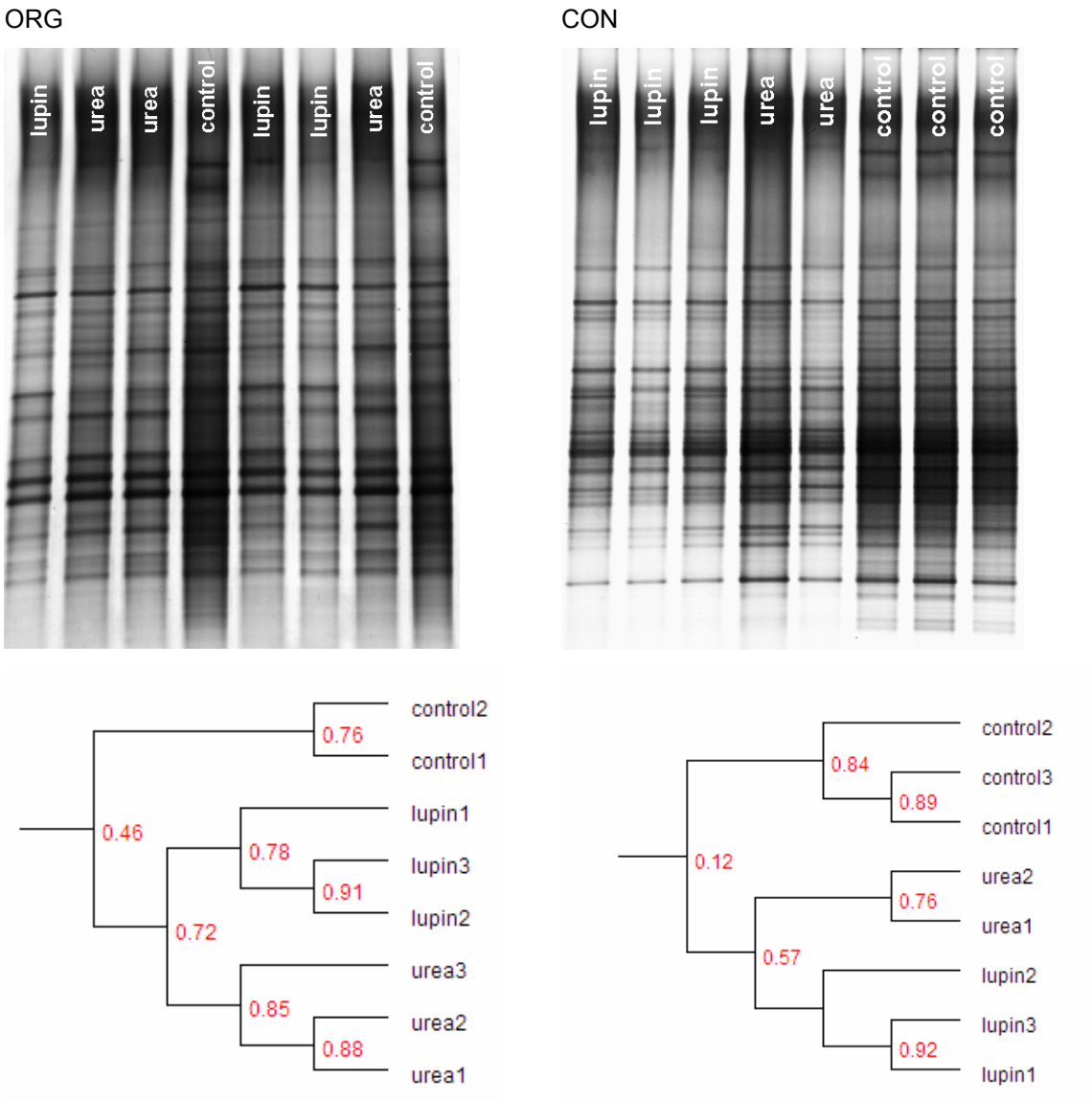


Figure 58: DGGE profiles and cluster analysis for α proteobacterial communities in ORG and CON samples at day 10 of incubation experiment II.

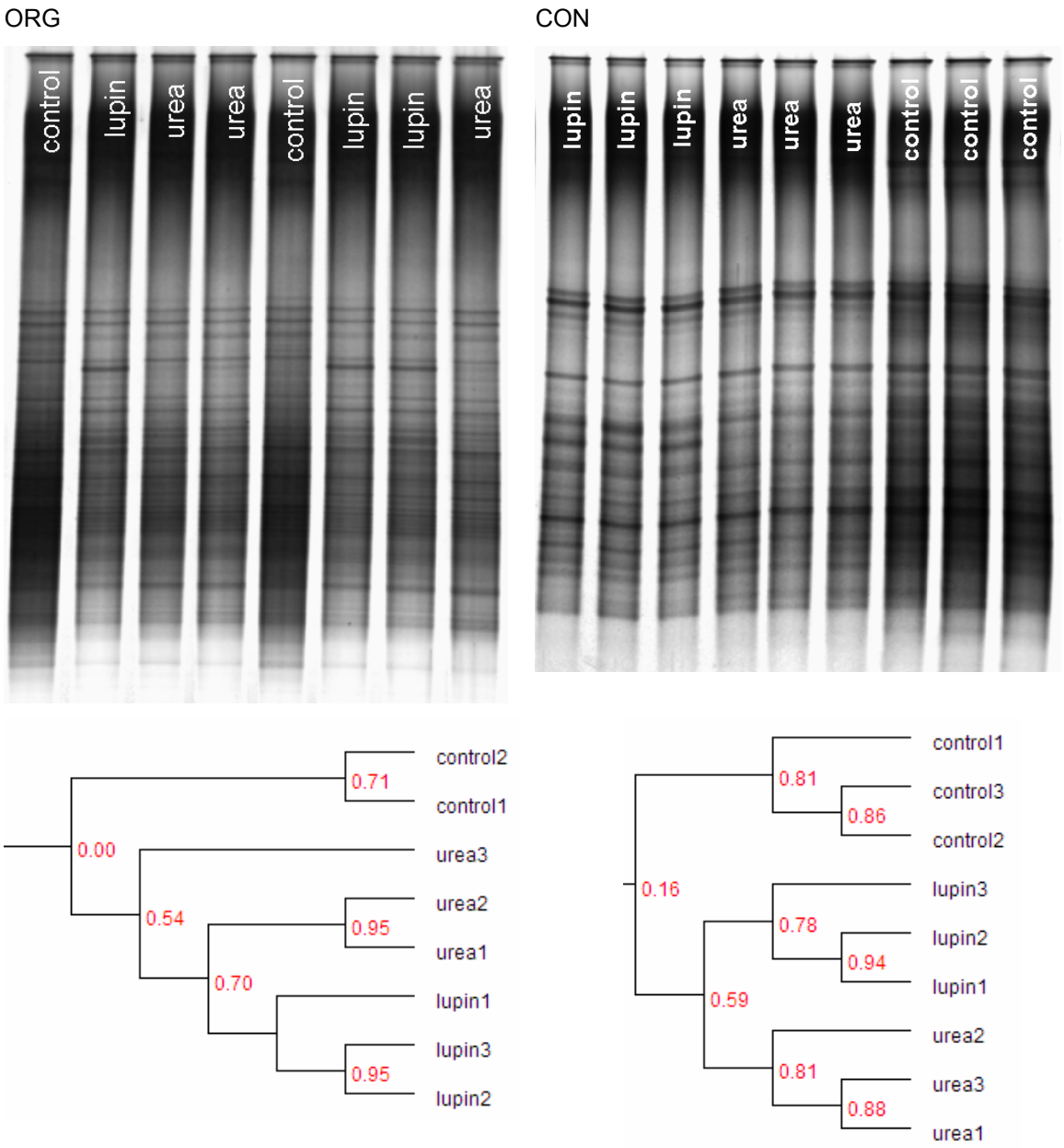


Figure 59: DGGE profiles and cluster analysis for actinomycete communities in ORG and CON samples at day 10 of incubation experiment II.

The trends were the same for samples amplified with actinomycete primers. ORG and CON could be distinguished by their DGGE banding patterns and in both soils differences between treatments could be identified by visual assessment (Figure 59). As for α proteobacteria, banding patterns of replicate samples were very similar for all treatments in ORG and CON and the three treatments were sorted into distinct clusters. The control treatments were least similar to the other treatments. These findings were supported by the results of the cluster analysis.

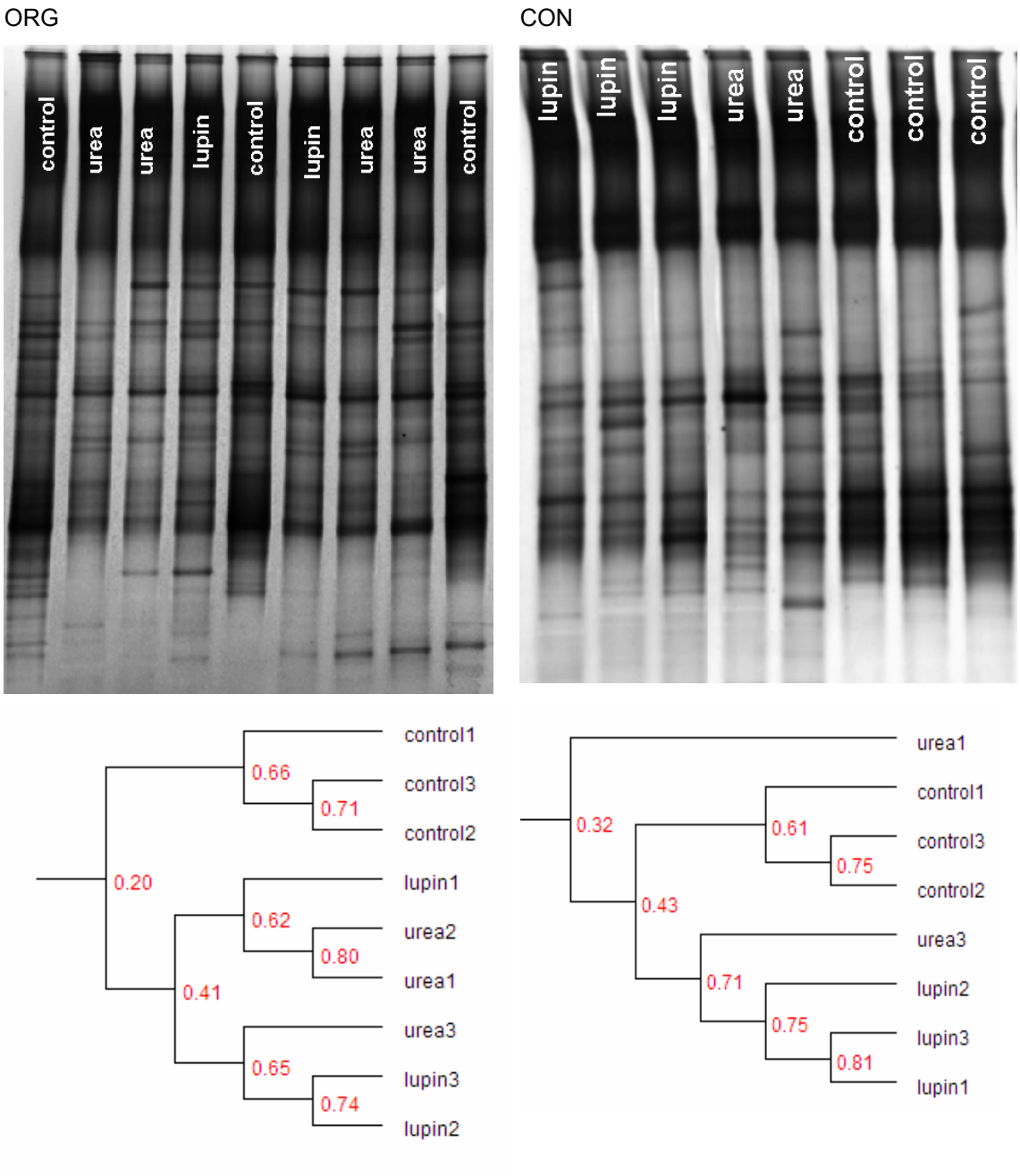


Figure 60: DGGE profiles and cluster analysis for pseudomonad communities in ORG and CON soils at day 10 of incubation experiment II.

As observed in the initial sampling (Figure 56), reproducibility of DGGE profiles was not as high for pseudomonads, i.e. variability between replicates was larger than for the other two community groups (Figure 60). It was, however, possible to detect differences between the two soils. The control samples proved to be most similar to each other and could be visually distinguished from urea and lupin treatments in both soils, which was confirmed by the cluster analyses .

The differences in DGGE profiles of the two soils detected 10 days after amendment persisted over the course of the incubation experiment and were still apparent after 91 days.

Reproducibility remained high with no differences detectable among replicates, however, treatments showed no differences in banding patterns for α proteobacteria and actinomycetes (Figures 61 and 62). This was verified by the cluster analyses that showed more variable and less consistent clusters. The replicate samples were not sorted into discrete groups.

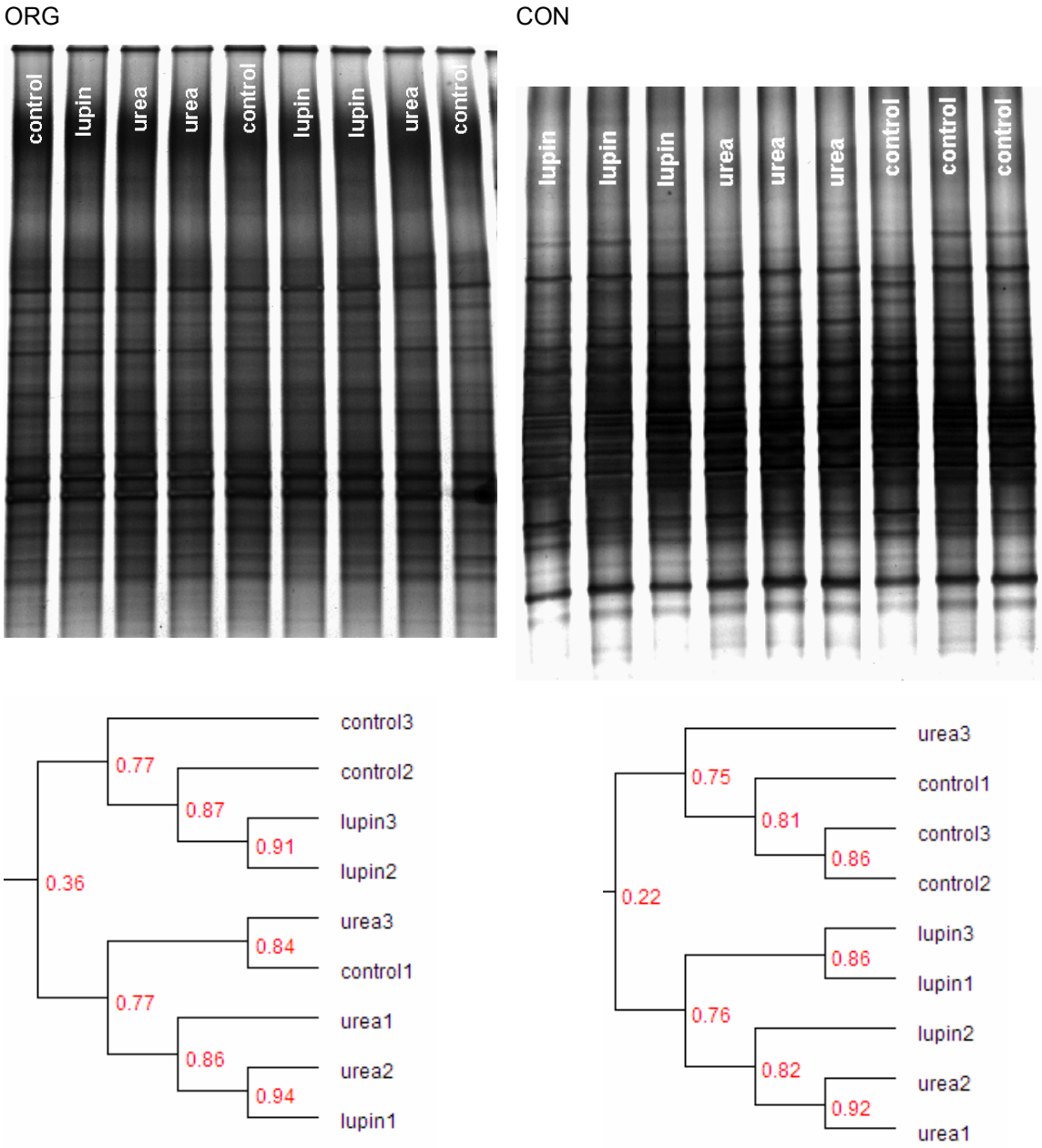


Figure 61: DGGE profiles and cluster analysis for α proteobacterial communities in ORG and CON soils at day 91 of incubation experiment II.

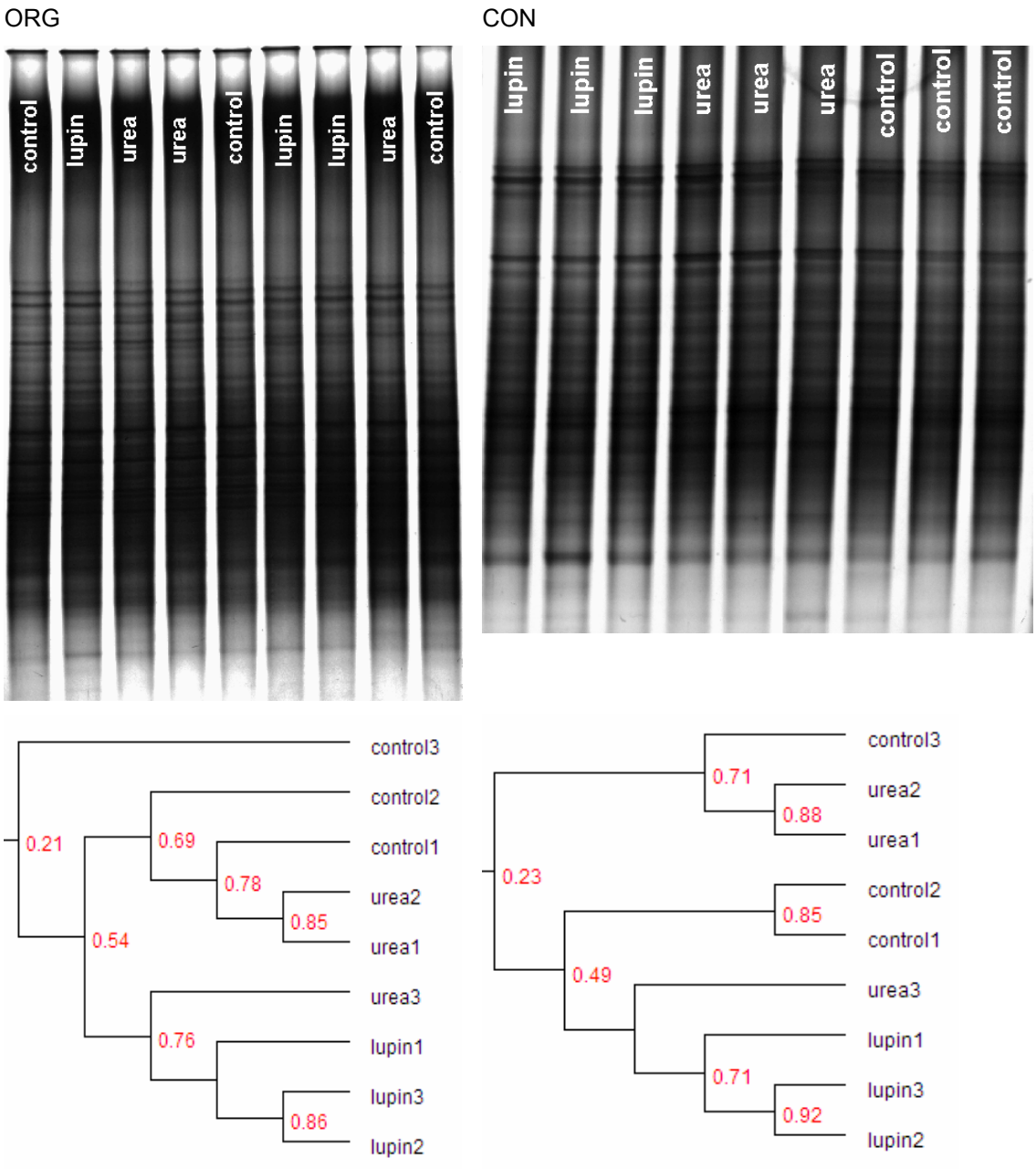


Figure 62: DGGE profiles and cluster analysis for actinomycete communities in ORG and CON soils at day 91 of incubation experiment II.

After 91 days, variability among replicates was as high as in the previous samplings for pseudomonads (Figure 63), however, as for the other two primer sets, less distinct differences were observed between the treatments. The samples of the different treatments could not be grouped into separate clusters and the degree of similarity within each cluster was lower than for previous analyses (around 60% compared to 80%, e.g. in Figure 59).

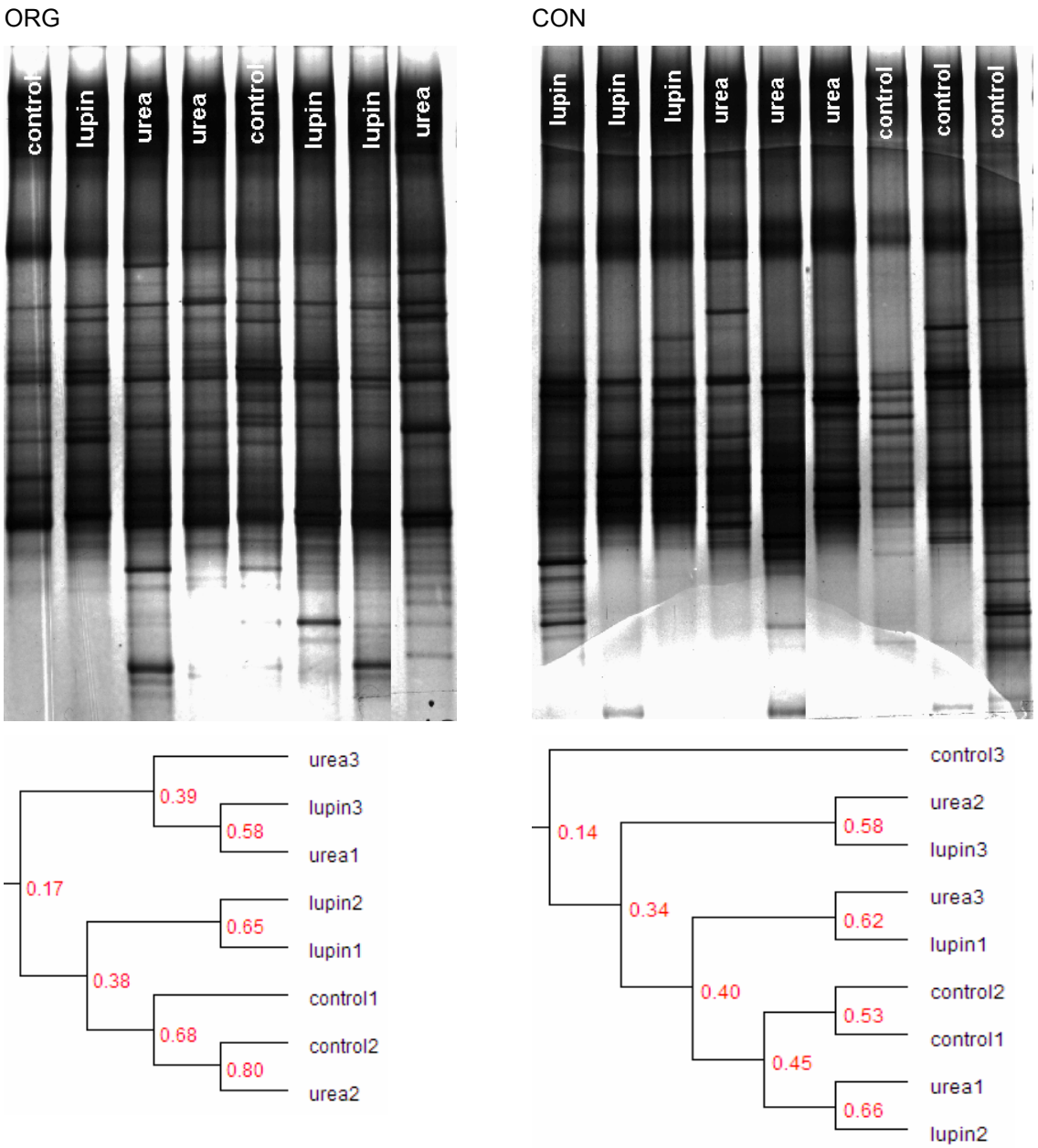


Figure 63: DGGE profiles and cluster analysis for pseudomonad communities in ORG and CON soils at day 91 of incubation experiment II.[1]

3.4 Discussion

While it is well established that organic matter amendments are beneficial for the microbial community in soils (see Section 1, Section 3 and Incubation experiment I, this section), there is little evidence that conventional farming practices, in particular the use of mineral fertilisers, have a negative effect on microbial growth and activity.

[1] One control sample is missing due to amplification failure. The PCR was repeated and the sample was run on a different gel.

Microbial biomass size, activity, function (N mineralisation) and microbial community composition were measured in intervals over a 91-day incubation period to determine differences in the microbial soil properties following organic (lupin) and mineral amendments (urea) and to establish links between the different parameters. By using soils from a long-term organic and conventional farm the response of initially different microbial communities to amendments could be determined as well as the impact of long- and short-term management practices.

3.4.1 Effect of temporal variation on the soil microbial community

As expected, the addition of lupin significantly increased soil microbial biomass, activity, biochemical soil properties and soil processes in the first few days after amendment, which resulted in large temporal variation in most soil parameters (except C_{tot}). The initial peak in C_{mic}, N_{mic}, DHH and microbial quotient was followed by a rapid decrease (especially for DHH), as also observed in incubation experiment I and described by Lundquist *et al.* (1999a) and Gunapala *et al.* (1998). The fluctuations in microbial biomass C and N indicate a natural cycling of microbial biomass over time as microorganisms die off when nutrient sources are exhausted, releasing nutrients and, thus promoting further microbial growth (e.g. Bottomley 1998).

At the second and third sampling point (day 3 and 6), increased levels of DHH and N_{mic} could also be observed for the control, which were most likely a consequence of disturbance and aeration caused by repeated mixing of the soils throughout the experiment (after treatment addition and after each sampling). Although the application of organic material and urea were expected to increase microbial activity and mineralisation rate, gross N mineralisation rates decreased between day 0 and day 10 for all treatments. Soil process rates were not determined at all sampling dates and might have shown higher levels in-between sampling points, e.g. at day 3, as seen in microbial biomass and DHH activity. This assumption is supported by the NH_4-N data, which revealed increased levels 3 and 6 days after lupin and urea amendment, respectively, indicating higher mineralisation rates at these points in time. The overall decline in N mineralisation is consistent with the assumption that the quality of the added substrate changes over time (Andersen and Jensen 2001).

Mineral N levels strongly responded to the amendment with urea (immediate increase in the urea treatment) due to the addition of easily accessible N, which was transformed into NH_4-N within a few days after amendment (Omar and Ismail 1999; Arp 2000). In the lupin treatment, availability of mineralisable substrate and stimulated microbial activity resulted in higher mineralisation rates compared to the control. Mineral N levels, hence, increased more rapidly. Total C and N showed only a weak response to the urea and lupin amendments.

3.4.2 Effect of past management on the soil microbial community

As seen in incubation experiment I, the influence of farm management history was most evident on biochemical soil properties (total C and N), which showed greater variation between soils than among treatments. However, absolute differences between ORG and CON were small (C_{tot}=2.4 and 2.7% for ORG and CON, respectively; N_{tot}=0.2% for both soils) and results were consistent with the expectation that total C and N change slowly under the influence of soil amendments and are usually unaffected by short-term management practices (Wander *et al.* 1994; Wander and Traina 1996).

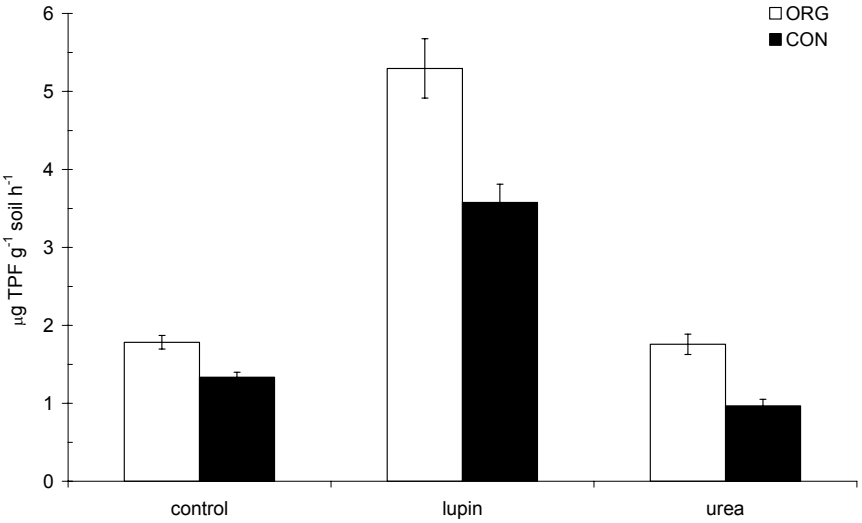


Figure 64: Overall mean dehydrogenase activity rates for ORG and CON soils amended with lupin or urea in incubation experiment II. Bars show standard errors of means. n=54.

Overall means of DHH activity (ORG>CON), mineral N (CON>ORG) and microbial quotient (ORG>CON) and gross mineralisation rates were also significantly different in ORG and CON, while microbial biomass C and N and gross immobilisation rates were not significantly affected but higher in ORG. Considering that levels of C_{mic} and mineralisation rates were initially lower in ORG, the increase in microbial biomass and activity following the amendments is more pronounced, which suggests a more responsive community in ORG (e.g. overall larger increase in DHH due to lupin amendment in ORG [see Figure 64]). The higher mineralisation rates and mineral N levels in ORG also suggest a stronger response to the lupin and urea addition compared to CON; while levels in CON were approximately twice (urea) and 1.4 times (lupin) as high at the last sampling compared to day 0, N_{min} levels increased 2.5-fold for urea and 1.6-fold for lupin between the first and last sampling in ORG (Table 45). The stronger increase in gross immobilisation in ORG between day 0 and day 10 also indicates a larger, more active microbial community in this soil, which confirms the observations that the microbial population in ORG was more responsive to the amendments (Vinten *et al.* 2002). Overall, the differences and similarities between ORG and CON persisted over the incubation period at similar levels (e.g. Figure 65). This indicates that the

incubation (with or without amendment) had the same effect on the two soils and that both soils had microbial communities that were adequate to respond to the addition of organic or mineral N, respectively.

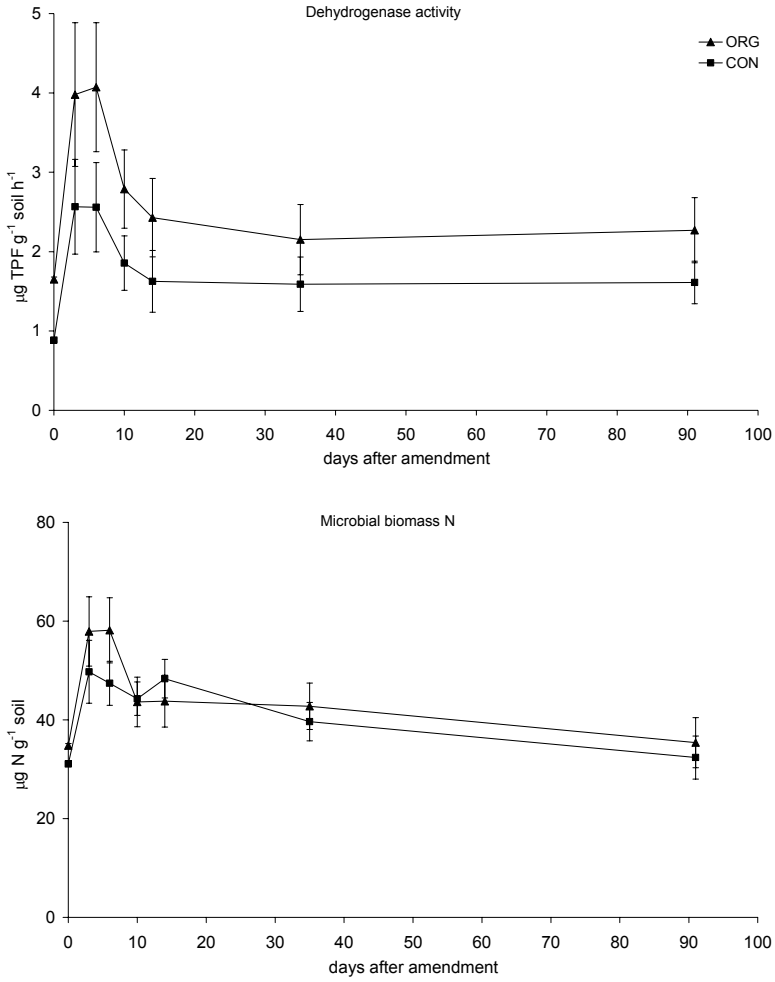


Figure 65: Mean dehydrogenase activity rates and mean microbial biomass N levels for ORG and CON over 91 days in incubation experiment II. Bars show standard errors of means. n=27.

DGGE analysis following PCR amplification of 16S rDNA fragments of actinomycetes, α proteobacteria and pseudomonads revealed that soils from ORG and CON could clearly be distinguished based on microbial community structure at the initial sampling (Figure 56). This indicates that distinctly different communities were present in ORG and CON, which is consistent with observations made during the farm site comparison (Section 2) and in incubation experiment I. Due to the sample arrangement on the gels (i.e. ORG and CON samples were run on different gels), comparison between soils was problematic for the following sample points; however, visual assessment suggested that obvious differences were sustained over the course of the experiment and that the microbial communities in each soil did not change much over time. The results suggest that the microbial communities in both soils were stable and unaffected by the incubation process. However, it was not possible to

measured differences in microbial diversity or species richness, which could have changed over time.

3.4.3 Effect of current management on the soil microbial community

This study, in agreement with other research, showed that the addition of lupin had a priming effect on community size and activity (Fraser *et al.* 1988; Robertson and Morgan 1996; Fontaine *et al.* 2003). It resulted in an increase in microbial soil properties (C_{mic}, N_{mic} and DHH), and levels remained significantly higher than those of other treatments for the rest of the study. The lupin amendment did not significantly affect the biochemical soil properties (C_{tot}, N_{tot}) and/ or levels were very similar among all treatments (e.g. levels of N_{tot} levels within the same range for all treatments). This again shows that these properties do not change rapidly, while the microbial soil properties were influenced by a one-off organic matter amendment.

The addition of urea, on the other hand, did not result in significantly different levels of microbial biomass C and N, total C and N and microbial activity compared to the control soil. This suggests that the addition of urea did not have an effect on soil microbial properties, while N_{min} approximately doubled following the addition of urea (resulting from the large increase in NH_4-N), and levels remained significantly higher compared to the lupin and control treatments. The data shows that the urea amendment initially increased activity but reduced growth (seen in a decrease in microbial biomass C) (Figure 50 and Figure 51). Correspondingly, Omar and Ismail (1999) found that the addition of urea at two rates (0.2 and 0.5 mg N g^{-1}) caused a rise in pH and decreased certain microbial populations (assessed by soil dilution plating), while increasing others and, therefore, changing species composition in the treated soils. Mahmood *et al.* (1997) also reported a decrease in microbial biomass after the addition of urea at a rate of 200 kg ha^{-1} under wheat but not under maize. However, this rate is twice as high as that applied in this study and the presence of plants meant factors other than fertilisation influenced the microbial communities. The rise in C_{mic} at day 14 suggests that adding urea had a delayed positive effect on microbial growth.

Enzymatic activity (DHH) was similar for the urea and control treatments until day 10, after which the enzyme activity declined in the urea treatment below rates measured for the control and levels remained significantly lower for the rest of the incubation period. This suggests that the addition of urea hindered DHH activity without affecting biomass size (microbial biomass C was comparable for the control and urea treatments). The decrease in DHH activity may have resulted from urea inhibiting certain metabolic processes or microbial groups rather than reducing the size of the entire microbial community. The findings of Omar and Ismail (1999) support this observation, but it was not confirmed by other researchers' results that reported no effects of urea on enzyme activities and microbial biomass size or only at rates much higher than those applied here (e.g. Biederbeck *et al.* 1996; Banerjee *et al.* 1999;

Diosma *et al.* 2003). However, these studies investigating the effects of urea fertilisation on microbial soil properties were all field studies, i.e. environmental factors and plant growth had a greater impact on the soil microbial community than fertilisation. A long-term negative influence of the urea amendment on microbial activity cannot be ruled out. However, most results obtained under laboratory conditions cannot be used to predict microbial responses to amendments *in situ* (Madsen 1996). For example, Thirukkumaran *et al.* (2002) reported a negative effect of N fertilisation on microbial biomass and respiration and litter decomposition that was not reproduced under field conditions. They concluded that the observed differences in microbial response between results from field and laboratory studies are due to the lack of plant and root growth activity and leaching in the laboratory experiment.

At day 3 and 6, microbial N levels were higher in the urea treatment compared to the control, indicating a larger microbial biomass or higher immobilisation activity after urea amendment. The assumption of a larger microbial biomass is not supported by the C_{mic} data. Gross immobilisation, however, was significantly higher in the urea treatment at day 10 and the microbial biomass N levels at day 3 and 6 indicate that immobilisation was already elevated before the day 10 measurement. Acquaye and Inubushi (2004) reported higher immobilisation rates and a larger increase in microbial N after fertilisation with urea compared with a slow-release fertiliser in the field. However, in their study microbial biomass size was more influenced by soil type than fertilisation. The differences in gross immobilisation rates between urea and the other two treatments indicate a difference in microbial community composition, as immobilisation is known to be more pronounced when bacteria dominate in the soil (Vinten *et al.* 2002). This corresponds with the drop in microbial C:N ratio directly after urea addition (see Appendix II). The microbial C:N ratio also decreased for the other treatments between days 0 and 10 suggesting a shift towards bacteria dominated communities that sustain higher immobilisation rates. This is not supported by the immobilisation data, although the decrease in N_{min} levels between days 0 and 3 suggests higher immobilisation rates in the lupin and control treatments than measured at day 10. This is consistent with the elevated microbial biomass N levels at day 3.

The treatments caused distinct differences in microbial community structure of α proteobacterial, actinomycete and pseudomonad communities as assessed by DGGE analysis. Ten days after the amendment, microbial communities were most different in the control compared to the urea and lupin-amended soils (0-46% similarity between clusters depending on community type analysed), while these treatments were more similar to each other (41-72%). At the end of the experiment, the trends were less clear and similarities among treatments had increased to a degree that grouping into distinct clusters proved impossible (e.g. Figure 62). These results indicate that addition or non-addition of N had an effect on the community composition of the groups examined and that microbial communities changed due to the availability of additional substrate. The influence of fertiliser type was, however, less pronounced and differences in the community structure of lupin and urea amended soils were smaller than when compared to the control. The fertilisation effect was short-lived, as the

communities were very similar after 91 days indicating that the stimulating influence of additional N was diminished.

3.4.4 Linkages between microbial biomass size, activity and community structure

Correlation analysis including all treatments supports most of the results presented in incubation experiment I: positive, statistically significant correlations were observed among C_{mic}, N_{mic}, DHH, C_{tot}, N_{tot} and gross mineralisation rate. This indicates that microbial biomass size and activity were closely linked to each other and shows that related processes and microbial groups are involved in DHH activity and N mineralisation. In contrast, mineral N content was negatively linked with the microbial soil properties (C_{mic}, N_{mic}, DHH) and N mineralisation. This reflects the role microorganisms play in the N cycle: as microbes die off (and activity decreases) they become decomposable matter and thereby a source of mineral N (Smith 1994; Puri and Ashman 1998). It also shows how immobilisation rates increase with growth of microbial biomass and activity resulting in a decrease in N_{min}.

The strong correlation of C_{mic} to gross N mineralisation rate (R^2=0.50) and DHH (R^2=0.76) in the lupin treatment indicated a direct positive link between microbial growth and activity, while in the other treatments the correlations were not as pronounced (R^2 values between 0.23 and -0.03); C_{mic} decreased up to day 10 and DHH activity increased between days 0 and 6 (Figures 49 and 50). Thus, adding lupin to the soils stimulated growth and activity of the microbial community simultaneously, while in the control and urea treatments changes in biomass size did not occur at the same point in time as changes in activity. This could indicate that the microbial community was more adapted to the urea amendment (increase in activity without growth response) (Barkle *et al.* 2001). The lack of correlation between C_{mic} and gross mineralisation in the control and urea treatments could also suggest a limitation in decomposable compounds other than N in these treatments (most likely C), resulting in nutrient supplies too low to sustain microbial cell synthesis and high activity levels. Similarly, Zaman *et al.* (1999b) observed weak correlations of microbial biomass size and N mineralisation in incubated soils after the addition of ammonium chloride fertiliser.

The changes in microbial community composition following the amendments corresponded to those observed for microbial soil properties. While clear differences were visible in DGGE banding patterns 10 days after the addition of lupin and urea and it was possible to distinguish amended from non-amended soils, differences had disappeared by the end of the experiment. Differences in microbial community structure were smaller between lupin and urea amended soils than between urea and control treatments. Microbial soil properties and total C and N, on the other hand, were mainly influenced by the lupin addition and immobilisation and mineral N by the amendment with urea. Mineralisation rates were similar for all treatments. This indicates that no direct relationship exists between these soil properties and microbial community structure. However, the differences in response to the amendments, i.e. one type

of measurement was more influenced by the form of amendment (mineral or organic), while the other responded more strongly to the presence or absence of N, suggest that the parameters (soil microbial properties, function and community structure) focus on different parts of the microbial community. The differences in the microbial activity or function might be reflected in changes in community structure of even smaller subgroups of microorganisms or in variations in species richness or dominant species.

3.5 Conclusions

The following conclusions can be drawn from incubation experiment II.

- Comparing the soils with organic and conventional management histories showed that the microbial communities in both soils were sufficient to respond to the addition of organic matter and mineral N. Initially observed differences between soils in microbial and biochemical soil properties persisted over time. Gross mineralisation and immobilisation were higher in the organically managed soil indicating N-limitation in the soil environment.
- Consistent with incubation experiment I, amendment with lupin stimulated the microbial communities resulting in a 2 to 5-fold increase in microbial biomass and activity (C_{mic}, N_{mic} and DHH) in both soils. The effect was instantaneous and the increased levels at day 3 were followed by a decline in microbial biomass C and N and DHH activity.
- The effect of urea on soil microbial properties was not significantly different from the control: biomass size was unaffected, and only a weak increase in activity was observed. DHH activity decreased below control levels 10 days after amendment, which was likely to be a consequence of urea decreasing microbial populations and changing community composition under laboratory conditions.
- Differences between lupin and urea treatments in microbial biomass C and N, DHH activity, gross mineralisation and immobilisation rates suggest that the amendments changed the microbial community composition in the soils.
- DGGE analysis showed that the microbial community structure was strongly affected by addition or non-addition of N, while the influence of fertiliser type (mineral or organic) was less pronounced. The fertilisation effect decreased over time and was less evident 91 days compared to 10 days after amendment. The differences in α proteobacterial, actinomycete and pseudomonad community compositions between soils with different management histories persisted over time.
- Correlation analysis for the treatments separately showed differences in links between microbial biomass size and activity indicating differences in nutrient availability (mainly C), and microbial structural diversity (species richness or evenness) and physiological properties as a result of the amendments.

- The addition of mineral and organic amendments had an influence on the microbial community (biomass size, activity and community structure) in the short-term. While the form of N fertiliser (mineral or organic) had an evident effect on microbial soil properties (C_{mic}, N_{mic}, DHH), microbial community structure (assessed by DGGE) was more strongly influenced by the addition or lack of substrate (N and C). The effect was not sustained over the 91 day incubation experiment.

4 Overall conclusions for the incubation studies

Herewith the conclusions derived from incubation experiments I and II.

- The addition of organic material to soils with organic and conventional management histories resulted in an increase in biomass size and enzyme activities in both soils, indicating that both soils contained microbial communities sufficient to respond to the amendments. Higher immobilisation and the stronger response to amendments in the organically managed soil suggest that this soil was more nutrient limited at the time of amendment.
- Biomass growth and increased enzyme activities after addition of lupin indicate a priming effect, i.e. the soil microbial communities were stimulated by the amendment with organic matter.
- The effect of adding urea on microbial biomass C and N was not significantly different from the unamended control, while DHH activity was decreased under the influence of urea.
- Microbial community structure was mainly influenced by long-term management, i.e. differences between soils persisted over the incubation period, while treatment effects were less clear. Incubation experiment I did not reveal any effect of organic amendments on the microbial community structure, while in incubation experiment II, DGGE banding patterns were significantly different in the lupin and urea amended soils compared to the control.
- Correlation analyses of microbial soil properties and processes for each treatment separately suggested differences in nutrient availability, microbial diversity and physiological properties among the treatments. No relationship between microbial community structure and activity or soil processes existed under the experimental conditions.

Section 5 – Overall conclusions and future research recommendations

1 Conclusions

It is important to note that this study did not compare organic and conventional farming systems as such, nor did it attempt to study the effect of management systems on the microbial community or leaching losses, which would have entailed a whole-farm approach involving a large-scale experiment. Instead, this study focused on the influence of specific farming practices on microbial biomass, activity, community structure and N mineralisation. Therefore, no definite conclusions can be drawn regarding the beneficial or negative effects of organic or conventional farming systems on microbial soil properties and the environment, as this would have required measuring many other factors, e.g. pesticides, climate, nutrient budgets and economic viability. However, this study made it possible to determine and quantify the influence of organic and mineral fertilisers on the soil microbial community and N dynamics in a field and laboratory situation. Most importantly, this study highlights the benefits of including a green manure in the crop rotation on microbial soil properties and N retention for either farming system.

It has to be stressed that the management history of the organically managed site (BHU) was not well documented. The main known differences in management history were the absence of animals, soluble fertilisers and pesticides at the BHU site and the fact that the site had not been cultivated for almost 3 years and had been under a herb-ley prior to this experiment, which can be seen as a restorative phase. The conventional site (LCF) was also at the end of a 2-year pasture phase at the time of sampling, but had been under a crop rotation previously. The two sites were chosen for this study partly for their soil type (Wakanui silt loam at both sites), their comparable soil fertility and because microbial soil properties were expected to differ as a result of the respective previous management practices. The similar levels of soil chemical properties suggest that low-input organic farming did not deplete the soil system nor decrease soil fertility. The 3-year period under herb-ley would have positively contributed to restoring soil fertility. This indicates that with careful management organic farming practices can sustain the productivity of the soil systems at levels adequate for cropping.

The results presented in this thesis indicate no direct link between microbial community structure and microbial activity or function, which does not exclude that microbial diversity can affect soil functioning. It does, however, imply that differently structured and sized communities can express similar levels of activity. It seems reasonable to assume that certain microbial groups (or even species) have a larger influence on particular activities and soil processes than the number of species present in the soil. Similarly, adequate rates of microbial activity and soil processes are more important to ensure soil functioning and maintain soil quality. To determine the nature of the relationships between microbial structure, microbial

activity and soil functioning, it is necessary to define what function the ecosystem should fulfil (e.g. high productivity) and determine which factors and soil processes are essential to maintain this function (e.g. N mineralisation). The investigated functions and related soil processes will vary for different soil environments, depending on soil properties, environmental factors and land-use systems.

The differences between the treatments in correlation analyses implied the presence of treatment-induced differences in structural diversity and physiological properties (e.g. metabolic rates) of the microbial communities. This highlights the importance of focussing on small, variable parts of the microbial populations when relating microbial community structure to functional diversity and emphasises the need to apply techniques that measure other aspects of microbial diversity in order to reveal treatment-related differences in microbial community structure or abundance of dominant species. The identification of microbial species by DNA sequencing of predominant bands could identify key species that are affected by the treatments, and give a better understanding of species richness. Analysis of rRNA abundance in soils is influenced by the number and metabolic activity of cells (Ward *et al.* 1992) and could, thus, reveal differences in active microbial communities among the treatments. The application of these techniques exceeded the scope of this study; however, they are good options for future research into the links between microbial community structure and functions.

In the short-term (up to 2 years in the lysimeter study and in the 3-month incubation experiments), microbial community composition was strongly influenced by management history rather than current management practices. These results indicate that microbial community structure is resilient (reverted to initial structure shortly after one-off disturbance [incubation experiments]; affected by changes in management practices only after 2½ years [lysimeter study]) and, therefore, not a suitable tool to reveal short-term changes in the soil environment. It also shows that microbial community structure is closely associated with inherent, slowly changing soil factors. Similarly, microbial activity (enzyme assays) data indicate that management history has a lasting effect on enzyme activities even under changed management. This observation of "residual activity" suggests that despite being measures of microbial activity other soil factors also influence the activity of the enzyme measured and it questions the suitability of enzyme activities as an early indicator for changes in soil quality. The lysimeter study also emphasised that similarly sized and structured microbial communities can express varying rates of activity. Results could also indicate that varying proportions of the microbial biomass are inactive, show inefficient substrate use or that other soil factors influence the activity of the enzymes measured. This highlights the limitations of extrapolating results obtained under laboratory conditions and draw conclusions regarding *in situ* responses of microbial communities.

In the incubation studies, microbial communities were stimulated by the organic matter amendments with the most relevant changes occurring in soil microbial properties shortly

after addition of organic material. The addition of 4t of lupin was sufficient to promote microbial biomass growth and increase microbial activity and had a relatively larger impact on the microbial community compared to the 8t amendment. Both are relatively small amounts compared to the quantities incorporated in the lysimeter experiment, which ranged from 14 to 20 t dry matter ha^{-1}. Including a lupin green manure in the crop rotation, therefore, has a definite positive effect on soil biology, even at low yields and when only small amounts of substrate (C and N) are added to the soil. The results from the incubation experiment suggest that the microbial community response to organic matter amendments is limited (relatively stronger response for smaller amount), which makes the incorporation of smaller quantities more efficient. However, this cannot be taken as a rule as the microbial response is unique to the experimental conditions applied in this study. The findings will have to be confirmed (a) *in situ* and (b) for different soil types and under varying environmental conditions.

In the lysimeter experiment, neither continued mineral fertilisation nor the lack thereof had a long-term negative effect on the biological potential. In fact, most parameters (leaching losses, soil microbial properties, microbial community structure, crop yields) did not show any significant differences between organically and conventionally fertilised soils. However, the experimental period might have been too short to allow definite conclusions regarding the impact of using mineral vs. organic fertilisers on the soil microbial community. An extended study period is necessary to determine how long-term mineral fertilisation affects soil biology and mineral N leaching losses.

Herewith the major specific conclusions derived from this study.

- No definite conclusions could be drawn regarding the relationship of microbial community structure with microbial activity and N mineralisation, however, different treatments (e.g. disturbances rather than amendments) and/ or focussing on smaller microbial assemblages or individual species might reveal treatment-induced differences and show closer links of community composition with activity and function (incubation experiment I and II; lysimeter study).

- Past management caused differences in microbial community structure, biomass size and microbial activity (farm site comparison). The microbial communities in both soils were sufficient to respond to the amendments and neither management system (mineral fertilisation vs. low input) had lasting negative effects on the microbial community; however, the organically managed soil appeared to be more nutrient limited compared to the conventional soil as seen in the stronger response to amendments and higher immobilisation rates (incubation experiments I and II).

- Microbial community structure was strongly affected by management history and changed only slowly (or not permanently) under the influence of specific soil amendments (incubation experiment I and II).
- Enzyme activities were equally affected by short-term and long-term management practices, i.e. despite responding to soil amendments the influence of management history remained significant (incubation experiment I and II; lysimeter study).

    Both parameters seem closely associated with inherent soil properties and factors that are not influenced by organic matter amendments, such as a leguminous green manure, which makes them less useful as early indicators for changes in soil quality.

- Crop rotation and plant type had a stronger influence on the soil biological properties than fertilisation (lysimeter study).
- The addition of mineral and organic amendments influenced the microbial community (biomass size, activity and community structure) in the short-term at the rates supplied, while no long-term effects were measured (incubation experiment I and II; lysimeter study).

    Sound soil management (e.g. green manuring, crop rotations) and plant type have a much larger influence on soil biological properties and community composition than the type of fertiliser applied. This strongly emphasises the importance of using green manures and crop rotations in any production system to improve soil biology and, consequently, soil quality. Including green manures in the crop rotation can sufficiently enhance soil fertility to substitute for the lack of mineral N additions and small amounts show a large effect.

- In the short-term, the form of N fertiliser (mineral or organic) had an evident effect on microbial soil properties, but microbial community structure was more strongly influenced by the addition or lack of substrate (C and N) (incubation experiment II).
- The microbial community structure in these soils was influenced by adding C and N substrates; however, no direct relationships existed between microbial community structure and microbial functions measured in this study.

    To enhance microbial activity and function amendment with organic (high C) compounds is more important than addition of N.

2 Priorities for future research

The findings of this study indicate that future research should:

- focus more on essential soil processes rather than microbial diversity and to relate microbial community composition to key processes essential for functioning and productivity of agroecosystems;
- determine the relevance of specific species or small microbial groups for soil processes and their response to disturbances and amendments;
- determine threshold abundance levels for these organisms below which the functioning is permanently affected;
- verify the findings for other soil environments (soil types, environmental conditions, land-use systems) and other disturbances (e.g. pesticide application) *in vitro* and *in situ*

References

Anonymous 1999. GN2 MicroPlate™: Instruction for use (Sept. 1999). Biolog. Inc., Haywayd, Ca, 8 p.

Abbott, L.K. and Murphy, D.V. 2003a. What is soil biological fertility? In: Abbott, L.K. and Murphy, D.V. (Eds.). Soil biological fertility. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 1-15.

Abbott, L.K. and Murphy, D.V. (Eds.) 2003b. Soil biological fertility. Kluwer Academic Publishers, Dordrecht, The Netherlands. 264 p.

Acquaye, S. and Inubushi, K. 2004. Comparative effects of application of coated and non-coated urea in clayey and sandy paddy soil microcosms examined by the ^{15}N tracer technique. II. Effects on soil microbial biomass N and microbial ^{15}N immobilization. Soil Science & Plant Nutrition 50, 215-223.

Adam, G. and Duncan, H. 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biology & Biochemistry 33, 943-951.

Albiach, R.; Canet, R.; Pomares, F. and Ingelmo, F. 1999. Structure, organic components and biological activity in citrus soils under organic and conventional management. Agrochimica 43, 235-242.

Alef, K. and Kleiner, D. 1986. Arginine ammonification, a simple method to estimate microbial activity potentials in soils. Soil Biology & Biochemistry 18, 233-235.

Alef, K. and Kleiner, D. 1987. Applicability of arginine ammonification as indicator of microbial activity in different soils. Biology & Fertility of Soils 5, 148-151.

Alef, K.; Beck, T.; Zelles, L. and Kleiner, D. 1988. A comparison of methods to estimate microbial biomass and N-mineralization in agricultural and grassland soils. Soil Biology & Biochemistry 20, 561-565.

Alef, K. and Nannipieri, P. (Eds.) 1995. Methods in applied soil microbiology and biochemistry. Academic Press Limited, London, UK. 576 p.

Alföldi, T.; Mäder, P.; Besson, J.-M. and Niggli, U. 1995. DOC-trial: Long-term effects of bio-dynamic, bio-organic and conventional farming systems on soil conditions, yield and product quality In: Raupp, J. (ed.). Main effects of various organic and mineral

fertilization on soil organic matter turnover and plant growth (Fertilization systems in organic farming [concerted action AIR3-CT94-1940]). Darmstadt, Germany. Institute for Biodynamic Research, pp. 3-15.

Al-Kayssi, A.W. 2002. Spatial variability of soil temperature under greenhouse conditions. Renewable Energy 27, 453-462.

Altieri, M.A. 1995. Agroecology. The Science of sustainable agriculture. Westview Press, Boulder, USA. 433 p. 2nd ed.

Amberger, A. 1996. Pflanzenernährung. Verlag Eugen Ulmer, Stuttgart, Germany. 319 p. 4th ed.

Andersen, M.K. and Jensen, L.S. 2001. Low soil temperature effects on short-term gross N mineralisation-immobilisation turnover after incorporation of a green manure. Soil Biology & Biochemistry 33, 511-521.

Anderson, J.P.E. and Domsch, K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10, 215-221.

Anderson, J.P.E. 1982. Soil respiration. In: Page, A.L.; Miller, R.H. and Keeney, D.R. (Eds.). Methods of soil analysis. Part 2, Chemical and microbiological properties. American Society of Agronomy, Inc. and Soil Science Society of America, Inc., Madison, USA. pp. 831-871.

Andrén, O. and Balandreau, J. 1999. Biodiversity and soil functioning--from black box to can of worms? Applied Soil Ecology 13, 105-108.

Arp, D.J. 2000. The nitrogen cycle. In: Triplett, E.W. (Ed.). Prokaryotic nitrogen fixation: A model system for the analysis of a biological process. Horizon Scientific Press, Wymondham, UK. pp. 1-14.

Atlas, R.M. 1984. Diversity in microbial communities. In: Marshall, K.C. (Ed.). Advances in Microbial Ecology. Plenum Press, New York, USA. Vol. 7, pp. 1-47.

Atlas, R.M. 2004. Handbook of microbiological media. CRC Press, Boca Raton, USA. 2051 p. 3rd ed.

Bandick, A.K. and Dick, R.P. 1999. Field management effects on soil enzyme activities. Soil Biology & Biochemistry 31, 1471-1479.

Banerjee, M.R.; Burton, D.L. and Grant, C.A. 1999. Influence of urea fertilization and urease inhibitor on the size and activity of the soil microbial biomass under conventional and zero tillage at two sites. Canadian Journal of Soil Science 79, 255-263.

Bardgett, R.D. and McAlister, E. 1999. The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biology & Fertility of Soils 29, 282-290.

Barkle, G.F.; Stenger, R.; Sparling, G.P. and Painter, D.J. 2001. Immobilisation and mineralisation of carbon and nitrogen from dairy farm effluent during laboratory soil incubations. Australian Journal of Soil Research 39, 1407-1417.

Beare, M.H. 1997. Fungal and bacterial pathways of organic matter decomposition and nitrogen mineralization in arable soils. In: Brussaard, L. and Ferrera-Cerrato, R. (Eds.). Soil ecology in sustainable agricultural systems. CRC Press, Lewis Publishers, Boca Raton, USA. pp. 37-70.

Beare, M.H.; Reddy, M.V.; Tian, G. and Srivastava, S.C. 1997. Agricultural intensification, soil biodiversity and agroecosystem function in the tropics: the role of decomposer biota. Applied Soil Ecology 6, 87-108.

Belay, A.; Claassens, A.S. and Wehner, F.C. 2002. Effect of direct nitrogen and potassium and residual phosphorus fertilizers on soil chemical properties, microbial components and maize yield under long-term crop rotation. Biology & Fertility of Soils 35, 420-427.

Bending, G.D.; Turner, M.K.; Rayns, F.; Marx, M.-C. and Wood, M. 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. Soil Biology & Biochemistry 36, 1785-1792.

Bengtsson, J. 1998. Which species? What kind of diversity? Which ecosystem function? Some problems in studies of relations between biodiversity and ecosystem function. Applied Soil Ecology 10, 191-199.

Bidwell, V.J. 2000. Decision support for protecting groundwater from land treatment of wastewater. Environmetrics 11, 553-562.

Biederbeck, V.O.; Campbell, C.A.; Ukrainetz, H.; Curtin, D. and Bouman, O.T. 1996. Soil microbial and biochemical properties after ten years of fertilization with urea and anhydrous ammonia. Canadian Journal of Soil Science 76, 7-14.

BioGro New Zealand 2001. BioGro New Zealand Organic Standards. Wellington, NZ: New Zealand Biological Producers & Consumers Council. Retrieved April 2002 from http://www.biogro.co.nz/main.php?page=170 (last accessed 26 April 2005).

Blakemore, L.C.; Searle, P.L. and Daly, B.K. 1987. Methods for chemical analysis of soils. NZ Soil Bureau Scientific Report 80, 103.

Blum, W.H.E. and Santelises, A.A. 1994. A concept of sustainability and resilience based on soil functions: the role of ISSS in promoting sustainable land use. In: Greenland, D.J. and Szabolcs, I. (Eds.). Soil resilience and sustainable land use. CAB International, Wallingford, UK. pp. 535-542.

Blum, W.H.E. 1998. Basic concepts: degradation, resilience, and rehabilitation. In: Lal, R.; Blum, W.H.E.; Valentine, C. and Stewart, B.A. (Eds.). Methods for assessment of soil degradation. CRC Press, Boca Raton, USA. pp. 1-16.

Böhme, L.; Langer, U. and Böhme, F. 2005. Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. Agriculture, Ecosystems & Environment 109, 141-152.

Bolton, H., Jr.; Elliott, L.F.; Papendick, R.I. and Bezdicek, D.F. 1985. Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. Soil Biology & Biochemistry 17, 297-302.

Bonde, T.A.; Nielsen, T.H.; Miller, M. and Sørensen, J. 2001. Arginine ammonification assay as a rapid index of gross N mineralization in agricultural soils. Biology & Fertility of Soils 34, 179-184.

Bossio, D.A.; Scow, K.M.; Gunapala, N. and Graham, K.J. 1998. Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecology 36, 1-12.

Bottomley, P.J. 1998. Microbial ecology. In: Sylvia, D.M.; Fuhrmann, J.J.; Hartel, P.G. and Zuberer, D.A. (Eds.). Principles and applications of soil microbiology. Prentice-Hall, Inc., Upper Saddle River, New Jersey, USA. pp. 149-167.

Bowen, G.D. 1980. Misconceptions, concepts and approaches in rhizosphere biology. In: Ellwood, D.C.; Hedger, J.W.; Latham, M.J.; Lynch, J.M. and Slater, J.H. (Eds.). Contemporary microbial ecology. Academic Press, New York, USA. pp. 283-304.

Breland, T.A. and Eltun, R. 1999. Soil microbial biomass and mineralization of carbon and nitrogen in ecological, integrated and conventional forage and arable cropping systems. Biology & Fertility of Soils 30, 193-201.

Brockmann, F.J. and Murray, C.J. 1997. Subsurface microbiological heterogeneity: current knowledge, descriptive approaches and applications. FEMS Microbiology Reviews 20, 231-247.

Brohon, B.; Delolme, C. and Gourdon, R. 2001. Complementarity of bioassays and microbial activity measurements for the evaluation of hydrocarbon-contaminated soils quality. Soil Biology & Biochemistry 33, 883-891.

Brookes, P.C.; Landman, A.; Pruden, G. and Jenkinson, D.S. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid extraction method to measure microbial biomass nitrogen in soil. Soil Biology & Biochemistry 17, 837-842.

Brosius, J.; Dull, T.J.; Sleeter, D.D. and Noller, H.F. 1981. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. Journal of Molecular Biology 148, 107-127.

Bruckner, A.; Kandeler, E. and Kampichler, C. 1999. Plot-scale spatial patterns of soil water content, pH, substrate-induced respiration and N mineralization in a temperate coniferous forest. Geoderma 93, 207-223.

Brussaard, L.; Kuyper, T.W.; Didden, W.A.M.; de Goede, R.G.M. and Bloem, J. 2004. Biological soil quality from biomass to biodiversity - importance and resilience to management stress and disturbance. In: Schjønning, P.; Elmholt, S. and Christensen, B.T. (Eds.). Managing soil quality: Challenges in modern agriculture. CAB International, Wallingford, UK. pp. 139-161.

Buckley, D.H. and Schmidt, T.M. 2001. The structure of microbial communities in soil and the lasting impact of cultivation. Microbial Ecology 42, 11-21.

Bulluck, L.R., III; Brosius, M.; Evanylo, G.K. and Ristaino, J.B. 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. Applied Soil Ecology 19, 147-160.

Cabrera, M.L. and Beare, M.H. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Science Society of America Journal 57, 1007-1012.

Cameron, K.C.; Smith, N.P.; McLay, C.D.A.; Fraser, P.M.; McPherson, P.J.; Harrison, D.F. and Harbottle, P. 1992. Lysimeters without edge-flow: an improved design and sampling procedure. Soil Science Society of America Journal 56, 1625-1628.

Cameron, K.C.; Cornforth, I.S.; McLaren, R.G.; Beare, M.H.; Basher, L.R.; Metherell, A.K. and Kerr, L.E. (Eds.) 1996. Soil quality indicators for sustainable agriculture in New Zealand: Proceedings of a workshop. Lincoln Soil Quality Research Centre, Lincoln University, Lincoln, NZ. 71 p.

Campbell, C.A.; Biederbeck, V.O.; Zentner, R.P. and Lafond, G.P. 1991. Effect of crop rotations and cultural practices on soil organic matter, microbial biomass and respiration in a thin black chernozem. Canadian Journal of Soil Science 71, 363-376.

Campbell, C.A.; Moulin, A.P.; Bowren, K.E.; Janzen, H.H.; Townley-Smith, L. and Biederbeck, V.O. 1992. Effect of crop rotations on microbial biomass, specific respiratory activity and mineralizable nitrogen in a black chernozemic soil. Canadian Journal of Soil Science 72, 417-427.

Campbell, C.A.; Biederbeck, V.O.; Wen, G.; Zentner, R.P.; Schoenau, J. and Hahn, D. 1999. Seasonal trends in soil biochemical attributes: effects of crop rotation in the semiarid prairie. Canadian Journal of Soil Science 79, 73-84.

Carter, M.R.; Gregorich, E.G.; Anderson, D.W.; Doran, J.W.; Janzen, H.H. and Pierce, F.J. 1997. Concepts of soil quality and their significance. In: Gregorich, E.G. and Carter, M.R. (Eds.). Soil quality for crop production and ecosystem health. Elsevier Science Publishers B.V., Amsterdam, The Netherlands. pp. 1-19.

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

Christen, O. 1996. Sustainable agriculture - history, concept and consequences for research, education and extension. Berichte über Landwirtschaft 74, 66-86.

Clark, M.S.; Horwarth, W.R.; Shennan, C. and Scow, K.M. 1998. Changes in soil chemical properties resulting from organic and low-input farming practices. Agronomy Journal 90, 662-371.

Clegg, C.D.; Lovell, R.D. and Hobbs, P.J. 2003. The impact of grassland management regime on the community structure of selected bacterial groups in soils. FEMS Microbiology Ecology 43, 263-270.

Coleman, D.C.; Crossley, D.A., Jr. and Hendrix, P.F. 2004. Fundamentals of soil ecology. Elsevier Academic Press, Inc., Amsterdam, The Netherlands. 386 p. 2nd ed.

Condron, L.M.; Cameron, K.C.; Di, H.J.; Clough, T.J.; Forbes, E.A.; McLaren, R.G. and Silva, R.G. 2000. A comparison of soil and environmental quality under organic and conventional farming systems in New Zealand. New Zealand Journal of Agricultural Research 43, 443-466.

Cook, H.F. and Lee, H.C. 1995. Editors' preface. In: Cook, H.F. and Lee, H.C. (Eds.). Soil management in sustainable agriculture. Wye College Press, Wye, UK. pp. i.

Cook, R.; Bardgett, R.D.; Denton, C.S.; Donnison, L.; Hobbs, P.J.; Leemans, D.K. and Yeates, G.W. 1995. Interactions of grassland managements, soil fauna and microbial populations and activities. In: Grassland into the 21st century: challenges and opportunities (50th Anniversary Meeting, 4-6 December 1995). British Grassland Society, Harrogate, UK. pp. 159-160.

Cookson, W.R.; Rowarth, J.S. and Cameron, K.C. 2001. The fate of autumn-, late winter- and spring-applied nitrogen fertilizer in a perennial ryegrass (*Lolium perenne* L.) seed crop on a silt loam soil in Canterbury, New Zealand. Agriculture, Ecosystems & Environment 84, 67-77.

Cookson, W.R.; Cornforth, I.S. and Rowarth, J.S. 2002. Winter soil temperature (2-15 °C) effects on nitrogen transformations in clover green manure amended or unamended soils: a laboratory and field study. Soil Biology & Biochemistry 34, 1401-1415.

Corre, M.D.; Schnabel, R.R. and Stout, W.L. 2002. Spatial and seasonal variation of gross nitrogen transformations and microbial biomass in a Northeastern US grassland. Soil Biology & Biochemistry 34, 445-457.

Crecchio, C.; Curci, M.; Mininni, R.; Ricciuti, P. and Ruggiero, P. 2001. Short-term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity. Biology & Fertility of Soils 34, 311-318.

Crecchio, C.; Gelsomino, A.; Ambrosoli, R.; Minati, J.L. and Ruggiero, P. 2004. Functional and molecular responses of soil microbial communities under differing soil management practices. Soil Biology & Biochemistry 36, 1873–1883.

Dalal, R.C. 1998. Soil microbial biomass - what do the numbers really mean? Australian Journal of experimental agriculture 38, 649-665.

Dalgaard, T.; Halberg, N. and Kristensen, I.S. 1998. Can organic farming help to reduce N-losses? Nutrient Cycling in Agroecosystems 52, 277-287.

de Boer, W.; Klein Gunnewiek, P.J.A. and Parkinson, D. 1996. Variability of N mineralization and nitrification in a simple, simulated microbial forest soil community. Soil Biology & Biochemistry 28, 203-211.

de Boer, W. and Kowalchuk, G.A. 2001. Nitrification in acid soils: micro-organisms and mechanisms. Soil Biology & Biochemistry 33, 853-866.

Degens, B.P. 1998. Microbial functional diversity can be influenced by the addition of simple organic substrates to soil. Soil Biology & Biochemistry 30, 1981-1988.

Di, H.J.; Cameron, K.C.; Moore, S. and Smith, N.P. 1999. Contributions to nitrogen leaching and pasture uptake by autumn-applied dairy effluent and ammonium fertilizer labeled with ^{15}N isotope. Plant & Soil 210, 189-198.

Di, H.J. and Cameron, K.C. 2000. Calculating nitrogen leaching losses and critical application rates in dairy pasture systems using a semi-empirical model. New Zealand Journal of Agricultural Research 43, 139-147.

Di, H.J.; Cameron, K.C. and McLaren, R.G. 2000. Isotopic dilution methods to determine the gross transformation rates of nitrogen, phosphorus, and sulfur in soil: a review of the theory, methodologies, and limitations. Australian Journal of Soil Research 38, 213-230.

Di, H.J. and Cameron, K.C. 2002a. The use of nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. Soil Use & Management 18, 395-403.

Di, H.J. and Cameron, K.C. 2002b. Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. Nutrient Cycling in Agroecosystems 64, 237-256.

Dick, R.P. 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. Agriculture, Ecosystems & Environment 40, 25-36.

Dick, R.P.; Breakwell, D.P. and Turco, R.F. 1996. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In: Doran, J.W. and Jones, A.J. (Eds.). Methods for assessing soil quality. Soil Science Society of America, Madison, USA. SSSA Special Publication. pp. 247-271.

Dick, R.P. 1997. Soil enzyme activities as integrative indicators. In: Pankhurst, C.E.; Doube, B.M. and Gupta, V.V.S.R. (Eds.). Biological indicators of soil health. CAB International, Wallingford, UK. pp. 144-156.

Dilly, O. and Munch, J.-C. 1998. Ratios between estimates of microbial biomass content and microbial activity in soils. Biology & Fertility of Soils 27, 374-379.

Diosma, G.; Golik, S.I.; Chidichimo, H.O. and Balatti, P.A. 2003. Nitrification potential, dehydrogenase activity and microbial biomass in an argiudol soil cultivated with wheat under two tillering methods. Spanish Journal of Agricultural Research 1, 111-119.

Domsch, K.H. 1986. Influence of management on microbial communities in soil. In: Jensen, V.; Kjøller, A. and Sørensen, L.H. (Eds.). Microbial Communities in Soil. Elsevier Applied Sciences Publishers, London, UK. pp. 355-367.

Doran, J.W.; Fraser, D.G.; Culik, M.N. and Liebhardt, W.C. 1988. Influence of alternative and conventional agricultural management on soil microbial processes and nitrogen availability. American Journal of Alternative Agriculture 2, 99-106.

Doran, J.W. 1996. The international situation and criteria for indicators. In: Cameron, K.C.; Cornforth, I.S.; McLaren, R.G.; Beare, M.H.; Basher, L.R.; Metherell, A.K. and Kerr, L.E. (Eds.). Soil quality indicators for sustainable agriculture in New Zealand: Proceedings of a workshop held for MAF Policy by the Lincoln Soil Quality Research Centre at Lincoln University, 8-9 February 1996. Lincoln Soil Quality Research Centre, Lincoln University, Lincoln, NZ. pp. 20-39.

Doran, J.W. and Jones, A.J. (Eds.) 1996. Methods for assessing soil quality. SSSA Special Publication No. 49. Soil Science Society of America, American Society of Agronomy, Madison, USA. 410 p.

Douglass, G.K. 1985. When is agriculture "sustainable"? In: Edens, T.C.; Fridgen, C. and Battenfield, S.L. (Eds.). Sustainable agriculture and integrated farming systems. Michigan State University Press, East Lansing, USA. pp. 10-21.

Drinkwater, L.E.; Letourneau, D.K.; Workneh, F.; van Bruggen, A.H.C. and Shennan, C. 1995. Fundamental differences between conventional and organic tomato agroecosystems in California. Ecological Applications 5, 1098-1112.

Drinkwater, L.E.; Wagoner, P. and Sarrantonio, M. 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396, 262-265.

Drinkwater, L.E.; Wagoner, P. and Sarrantonio, M. 2001. Regenerative science: Legume-based cropping systems store carbon & nitrogen. The Rodale Institute. Retrieved

November 2001 from http://www.rodaleinstitute.org/science/fst1.html (last accessed 26/04/2005).

Duffy, J.E. 2002. Biodiversity and ecosystem functioning: the consumer connection. Oikos 99, 201-219.

Dungan, R.S.; Ibekwe, A.M. and Yates, S.R. 2003. Effect of propagul bromide and 1,3-dichloropropene on microbial communities in an organically amended soil. FEMS Microbiology Ecology 43, 75-87.

Eash, N.S.; Karlen, D.L. and Parkin, T.B. 1994. Fungal contributions to soil aggregation and soil quality. In: Doran, J.W.; Coleman, D.C.; Bezdicek, D.F. and Stewart, B.A. (Eds.). Defining soil quality for a sustainable environment. Soil Science of America, Inc., Madison, USA. SSSA Special Publication. pp. 221-228.

Elliott, L.F.; Lynch, J.M. and Papendick, R.I. 1996. The microbial component of soil quality. In: Stotzky, G. and Bollag, J.-M. (Eds.). Soil Biochemistry. Marcel Dekker, Inc., New York, USA. Vol. 9, pp. 1-21.

Elmholt, S. 1996. Microbial activity, fungal abundance, and distribution of *Penicillium* and *Fusarium* as bioindicators of a temporal development of organically cultivated soils. Biological Agriculture & Horticulture 13, 123-140.

Eriksen, J.; Askegaard, M. and Kristensen, K. 1999. Nitrate leaching in an organic dairy/ crop rotation as affected by organic manure type, livestock density and crop. Soil Use & Management 15, 176-182.

Ettema, C.H. and Wardle, D.A. 2002. Spatial soil ecology. Trends in Ecology & Evolution 17, 177-183.

Ettema, C.H. and Yeates, G.W. 2003. Nested spatial biodiversity patterns of nematode genera in a New Zealand forest and pasture soil. Soil Biology & Biochemistry 35, 339-342.

Fauci, M.F. and Dick, R.P. 1994. Soil microbial dynamics: short- and long-term effects of inorganic and organic nitrogen. Soil Science Society of America Journal 58, 801-806.

Filip, Z.K. 1998. Soil quality assessment: an ecological attempt using microbiological and biochemical procedures. In: Blume, H.-P.; Eger, H.; Fleischhauer, E.; Hebel, A.; Reij, C. and Steiner, K.G. (Eds.). Towards sustainable land use. Furthering cooperation between people and institutions. Catena Verlag, Reiskirchen, Germany. Advances in GeoEcology. Vol. 31, pp. 21-27.

Fliessbach, A. 1995. Methods to characterise microbial community structure in soil In: Mäder, P. and Raupp, J. (eds.). Effects of low and high external input agriculture on soil microbial biomass and activities in view of sustainable agriculture (Fertilization systems in organic farming [concerted action AIR3-CT94-1940]) - Proceedings of the second meeting in Oberwil (Switzerland). Oberwil (Switzerland). Institute for Biodynamic Research, pp. 39-51.

Fliessbach, A. and Mäder, P. 1997. Carbon utilization by microbial communities in soils under organic and conventional farming practice. In: Insam, H. and Rangger, A. (Eds.). Microbial communities: functional vs. structural approaches. Springer Verlag, Berlin, Germany. pp. 109-120.

Fliessbach, A. and Mäder, P. 2000. Microbial biomass and size-density fractions differ between soils of organic and conventional agricultural systems. Soil Biology & Biochemistry 32, 757-768.

Fontaine, S.; Mariotti, A. and Abbadie, L. 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology & Biochemistry 35, 837-843.

Franklin, R.B.; Blum, L.K.; McComb, A.C. and Mills, A.L. 2002. A geostatistical analysis of small-scale spatial variability in bacterial abundance and community structure in salt marsh creek bank sediments. FEMS Microbiology Ecology 42, 71-80.

Fraser, D.G.; Doran, J.W.; Sahs, W.W. and Lesoing, G.W. 1988. Soil microbial populations and activities under conventional and organic management. Journal of Environmental Quality 17, 585-590.

Fraser, P.M.; Cameron, K.C. and Sherlock, R.R. 1994. Lysimeter study of the fate of nitrogen in animal urine returns to irrigated pasture. European Journal of Soil Science 45, 439-447.

Fromin, N.; Hamelin, J.; Tarnawski, S.; Roesti, D.; Jourdain-Miserez, K.; Forestier, N.; Teyssier-Cuvelle, S.; Gillet, F.; Aragno, M. and Rossi, P. 2002. Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. Environmental Microbiology 4, 634-643.

Frostegård, A.; Tunlid, A. and Bååth, E. 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Applied & Environmental Microbiology 59, 3605-3617.

Garland, J.L. 1997. Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiology Ecology 24, 289-300.

Gelsomino, A.; Keijzer-Wolters, A.C.; Cacco, G. and van Elsas, J.D. 1999. Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. Journal of Microbiological Methods 38, 1-15.

Girvan, M.S.; Bullimore, J.; Pretty, J.N.; Osborn, A.M. and Ball, A.S. 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Applied & Environmental Microbiology 69, 1800-1809.

Gomes, N.C.M.; Heuer, H.; Schönfeld, J.; Costa, R.; Mendoça-Hagler, L. and Smalla, K. 2001. Bacterial diversity of the rhizosphere of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant & Soil 232, 167-180.

Goovaerts, P. 1998. Geostatistical tools for characterizing the spatial variability of microbiological and physico-chemical soil properties. Biology & Fertility of Soils 27, 315-334.

Grayston, S.J.; Wang, S.; Campbell, C.D. and Edwards, C.A. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology & Biochemistry 30, 369-378.

Grayston, S.J.; Griffith, G.S.; Mawdsley, J.L.; Campbell, C.D. and Bardgett, R.D. 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biology & Biochemistry 33, 533-551.

Grayston, S.J.; Campbell, C.D.; Bardgett, R.D.; Mawdsley, J.L.; Clegg, C.D.; Ritz, K.; Griffiths, B.S.; Rodwell, J.S.; Edwards, S.J. and Davies, W.J. 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. Applied Soil Ecology 25, 63-84.

Greenland, D. 2000. Effects on soils and plant nutrition. In: Tinker, P.B. (Ed.). Shades of green - a review of UK farming systems. Royal Agricultural Society of England, Stoneleigh Park, UK. pp. 6-20.

Greenwood, J.J.D. 2000. Biodiversity and environment. In: Tinker, P.B. (Ed.). Shades of green - a review of UK farming systems. Royal Agricultural Society of England, Stoneleigh Park, UK. pp. 59-72.

Gregorich, E.G. 1996. Soil quality: A Canadian perspective. In: Cameron, K.C.; Cornforth, I.S.; McLaren, R.G.; Beare, M.H.; Basher, L.R.; Metherell, A.K. and Kerr, L.E. (Eds.). Soil quality indicators for sustainable agriculture in New Zealand: Proceedings of a workshop held for MAF Policy by the Lincoln Soil Quality Research Centre at Lincoln University, 8-9 February 1996. Lincoln Soil Quality Research Centre, Lincoln University, Lincoln, NZ. pp. 40-52.

Gregorich, E.G. and Carter, M.R. (Eds.) 1997. Soil quality for crop production and ecosystem health. Elsevier Science Publishers B.V., Amsterdam, The Netherlands, Developments in Soil Science. Vol. 25. 448 p.

Griffiths, B.S.; Ritz, K. and Wheatley, R.E. 1997. Relationships between functional diversity and genetic diversity in complex microbial communities. In: Insam, H. and Rangger, A. (Eds.). Microbial Communities: functional versus structural approaches. Springer Verlag, Berlin, Germany. pp. 1-9.

Griffiths, B.S.; Ritz, K.; Ebblewhite, N. and Dobson, G. 1999. Soil microbial community structure: Effects of substrate loading rates. Soil Biology & Biochemistry 31, 145-153.

Griffiths, B.S.; Ritz, K.; Bardgett, R.D.; Cook, R.; Christensen, S.; Ekelund, F.; Sørensen, S.J.; Bååth, E.; Bloem, J.; de Ruiter, P.C.; Dolfing, J. and Nicolardot, B. 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. Oikos 90, 279-294.

Griffiths, B.S.; Bonkowski, M.; Roy, J. and Ritz, K. 2001a. Functional stability, substrate utilisation and biological indicators of soils following environmental impacts. Applied Soil Ecology 16, 49-61.

Griffiths, B.S.; Ritz, K.; Wheatley, R.; Kuan, H.L.; Boag, B.; Christensen, S.; Ekelund, F.; Sørensen, L.H.; Muller, S. and Bloem, J. 2001b. An examination of the biodiversity-ecosystem function relationship in arable soil microbial communities. Soil Biology & Biochemistry 33, 1713-1722.

Griffiths, B.S.; Kuan, H.L.; Ritz, K.; Glover, L.A.; McCaig, A.E. and Fenwick, C. 2004. The relationship between microbial community structure and functional stability, tested experimentally in an upland pasture soil. Microbial Ecology 47, 104-113.

Gunapala, N. and Scow, K.M. 1998. Dynamics of soil microbial biomass and activity in conventional and organic farming systems. Soil Biology & Biochemistry 30, 805-816.

Gunapala, N.; Venette, R.C.; Ferris, H. and Scow, K.M. 1998. Effects of soil management history on the rate of organic matter decomposition. Soil Biology & Biochemistry 30, 1917-1927.

Haack, S.K.; Garchow, H.; Klug, M.J. and Forney, L.J. 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Applied & Environmental Microbiology 61, 1458-1468.

Hagn, A.; Pritsch, K.; Schloter, M. and Munch, J.C. 2003. Fungal diversity in agricultural soil under different farming management systems, with special reference to biocontrol strains of *Trichoderma* spp. Biology & Fertility of Soils 38, 236-244.

Hansen, B.; Alroe, H.F. and Kristensen, E.S. 2001. Approaches to assess the environmental impact of organic farming with particular regard to Denmark. Agriculture, Ecosystems & Environment 83, 11-26.

Harden, T. and Joergensen, R.G. 2000. Relationships between simulated spatial variability and some estimates of microbial biomass turnover. Soil Biology & Biochemistry 32, 139-142.

Hatch, D.J.; Lovell, R.D.; Antil, R.S.; Jarvis, S.C. and Owen, P.M. 2000. Nitrogen mineralization and microbial activity in permanent pastures amended with nitrogen fertilizer or dung. Biology & Fertility of Soils 30, 288-293.

Hatfield, J.L. and Stewart, B.A. (Eds.) 1994. Soil biology: Effects on soil quality. Lewis Publishers, CRC Press, Boca Raton, USA, Advances in Soil Science. 169 p.

Haynes, R.J. 1994. Impact of management practices on nutrient cycling. In: Pankhurst, C.E.; Doube, B.M.; Gupta, V.V.S.R. and Grace, P.R. (Eds.). Soil biota - Management in sustainable farming systems. CSIRO Australia, East Melbourne, Australia. pp. 172-181.

Haynes, R.J. 1999. Size and activity of the soil microbial biomass under grass and arable management. Biology & Fertility of Soils 30, 210-216.

Haynes, R.J. and Tregurtha, R. 1999. Effects of increasing periods under intensive arable vegetable production on biological, chemical and physical indices of soil quality. Biology & Fertility of Soils 28, 259-266.

Hendrix, P.F.; Crossley, D.A., Jr.; Blair, J.M. and Coleman, D.C. 1990. Soil biota as components of sustainable agroecosystems. In: Edwards, C.A.; Lal, R.; Madden, P.;

Miller, R.H. and House, G. (Eds.). Sustainable agricultural systems. Soil and Water Conservation Society, Ankeny, USA. pp. 637-654.

Herman, D.C. and Maier, R.M. 2000. Consequences of biogeochemical cycles gone wild. In: Maier, R.M.; Pepper, I.L. and Gerba, C.P. (Eds.). Environmental Microbiology. Academic Press, San Diego, USA. pp. 347-361.

Heuer, H.; Krsek, M.; Baker, P.; Smalla, K. and Wellington, E.M.H. 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Applied & Environmental Microbiology 63, 3233-3241.

Heuer, H. and Smalla, K. 1999. Bacterial phyllosphere communities of *Solanum tuberosum* L. and T4-lysozyme-producing transgenic variants. FEMS Microbiology Ecology 28, 357-371.

Heuer, H.; Wieland, G.; Schönfeld, J.; Schönwälder, A.; Gomes, N.C.M. and Smalla, K. 2001. Bacterial community profiling using DGGE or TGGE analysis. In: Rochelle, P.A. (Ed.). Environmental molecular microbiology: Protocols and applications. Horizon Scientific Press, Wymondham, UK. pp. 177-190.

Hole, D.G.; Perkins, A.J.; Wilson, J.D.; Alexander, I.H.; Grice, P.V. and Evans, A.D. 2005. Does organic farming benefit biodiversity? Biological Conservation 122, 113-130.

Horrocks, A.J. 2002. Amounts, forms and availability of nitrogen and phosphorus in soil under conventional and organic cropping. Honours dissertation. Soil, Plant & Ecological Sciences Division. Lincoln University, Lincoln, NZ. 51 p.

Horwarth, W.R. and Paul, E.A. 1994. Microbial biomass. In: Weaver, R.W.; Angle, S.; Bottomley, P.; Bezdicek, D.; Smith, S.; Tabatabai, A. and Wollum, A. (Eds.). Methods of soil analysis. Part 2, Microbiological and biochemical properties. Soil Science Society of America, Madison, USA. Soil Science Society of America Book Series no. 5. pp. 753-773.

Hunt, J.; Boddy, L.; Randerson, P.F. and Rogers, H.J. 2004. An evaluation of 18S rDNA approaches for the study of fungal diversity in grassland soils. Microbial Ecology 47, 385-395.

Hurst, C.J.; Knudsen, G.R.; McInerney, M.J.; Stetzenbach, L.D. and Walter, M.V. (Eds.) 1997. Manual of environmental microbiology. ASM Press, Washington DC, USA. 894 p.

Ibekwe, A.M.; Kennedy, A.C.; Frohne, P.S.; Papiernik, S.K.; Yang, C.H. and Crowley, D.E. 2002. Microbial diversity along a transect of agronomic zones. FEMS Microbiology Ecology 39, 183-191.

IFOAM 1997. Organic farming: sustainable agriculture put into practice. IFOAM, Theley, Germany. 19 p.

Insam, H.; Parkinson, D. and Domsch, K.H. 1989. Influence of macroclimate on soil microbial biomass. Soil Biology & Biochemistry 21, 211-221.

Insam, H. and Rangger, A. (Eds.) 1997. Microbial communities: functional vs. structural approaches. Springer Verlag, Berlin, Germany. 263 p.

Insam, H. 2001. Developments in soil microbiology since the mid 1960s. Geoderma 100, 389-402.

Johnsen, K.; Jacobson, C.S. and Torsvik, V. 2001. Pesticide effects on bacterial diversity in agricultural systems - a review. Biology & Fertility of Soils 33, 443-453.

Johnson, M.J.; Lee, K.Y. and Scow, K.M. 2003. DNA fingerprinting reveals links among agricultural crops, soil properties, and the composition of soil microbial communities. Geoderma 114, 279-303.

Jordan, D.; Miles, R.J.; Hubbard, V.C. and Lorenz, T. 2004. Effect of management practices and cropping systems on earthworm abundance and microbial activity in Sanborn Field: a 115-year-old agricultural field. Pedobiologia 48, 99-110.

Kandeler, E.; Stemmer, M. and Klimanek, E.-M. 1999a. Response of soil microbial biomass, urease and xylanase within particle size fractions to long-term soil management. Soil Biology & Biochemistry 31, 261-273.

Kandeler, E.; Tscherko, D. and Spiegel, H. 1999b. Long-term monitoring of microbial biomass, N mineralisation and enzyme activities of a Chernozem under different tillage management. Biology & Fertility of Soils 28, 343-351.

Kandeler, E.; Marschner, P.; Tscherko, D.; Gahoonia, T.S. and Nielsen, N.E. 2002. Microbial community composition and functional diversity in the rhizosphere of maize. Plant & Soil 238, 301-312.

Kennedy, A.C. and Smith, K.L. 1995. Soil microbial diversity and the sustainability of agricultural soils. Plant & Soil 170, 75-86.

Kennedy, A.C. and Gewin, V.L. 1997. Soil microbial diversity: Present and future considerations. Soil Science 162, 607-617.

Kennedy, A.C.; Stubbs, T.L. and Schillinger, W.F. 2004. Soil and crop management effects on soil microbiology. In: Magdoff, F. and Weil, R.R. (Eds.). Soil organic matter in sustainable agriculture. CRC Press, Boca Raton, USA. pp. 295-326.

Kirchmann, H. and Thorvaldsson, G. 2000. Challenging targets for future agriculture. European Journal of Agronomy 12, 145-161.

Kirk, J.L.; Beaudette, L.A.; Hart, M.; Moutoglis, P.; Klironomos, J.N.; Lee, H. and Trevors, J.T. 2004. Methods of studying soil microbial diversity. Journal of Microbiological Methods 58, 169-188.

Kisand, V. and Wikner, J. 2003. Limited resolution of 16S rDNA DGGE caused by melting properties and closely related DNA sequences. Journal of Microbiological Methods 54, 183-191.

Kjøller, A. and Struwe, S. 1994. Analysis of fungal communities on decomposing beech litter. In: Ritz, K.; Dighton, J. and Giller, K.E. (Eds.). Beyond the Biomass: compositional and functional analysis of soil microbial communities. John Wiley & Sons, Inc., Chichester, UK. pp. 191-199.

Kowalchuk, G.A.; Gerards, S. and Woldendorp, J.W. 1997. Detection and characterization of fungal infections of *Ammophila arenaria* (Marram Grass) roots by denaturing gradient gel electrophoresis of specifically amplified 18S rDNA. Applied & Environmental Microbiology 63, 3858-3865.

Kowalchuk, G.A.; Stienstra, A.W.; Heilig, G.H.J.; Stephen, J.R. and Woldendorp, J.W. 2000. Changes in the community structure of ammonia-oxidizing bacteria during secondary succession of calcareous grasslands. Environmental Microbiology 2, 99-110.

Kristensen, L.; Stopes, C.; Kølster, P. and Granstedt, A. 1995. Nitrogen leaching in ecological agriculture: summary and recommendations. In: Kristensen, L. (Ed.). Nitrogen leaching in ecological agriculture (Proceedings of an International Workshop). AB Academic Publishers, Bicester, UK. pp. 331-340.

Kumar, P. and Tarafdar, J.C. 2003. 2,3,5-Triphenyltetrazolium chloride (TTC) as electron acceptor of culturable soil bacteria, fungi and actinomycetes. Biology & Fertility of Soils 38, 186-189.

Lal, R. 1994. Sustainable land use systems and soil resilience. In: Greenland, D.J. and Szabolcs, I. (Eds.). Soil resilience and sustainable land use. CAB International, Wallingford, UK. pp. 41-67.

Lal, R. 1998. Soil quality and agricultural sustainability. In: Lal, R. (Ed.). Soil quality and agricultural sustainability. Ann Arbor Press, Chelsea, USA. pp. 3-12.

Lal, R. (Ed.) 1999. Soil quality and soil erosion. CRC Press, Boca Raton, USA. 329 p.

Lampkin, N. 1994. Organic farming. Farming Press Books, Ipswich, UK. 715 p.

Larkin, R.P. 2003. Characterization of soil microbial communities under different potato cropping systems by microbial population dynamics, substrate utilization, and fatty acid profiles. Soil Biology & Biochemistry 35, 1451-1466.

Lawlor, K.; Knight, B.P.; Barbosa-Jefferson, V.L.; Lane, P.W.; Lilley, A.K.; Paton, G.I.; McGrath, S.P.; O'Flaherty, S.M. and Hirsch, P.R. 2000. Comparison of methods to investigate microbial populations in soils under different agricultural management. FEMS Microbiology Ecology 33, 129-137.

Lawton, J.H. 1993. What do species do in ecosystems? Oikos 71, 367-374.

Lawton, J.H. and Brown, V.K. 1994. Redundancy of ecosystems. In: Schultze, E.D. and Mooney, H.A. (Eds.). Biodiversity and ecosystem function. Springer Verlag, Heidelberg, Germany. pp. 255-270.

Leckie, S.E.; Prescott, C.E.; Grayston, S.J.; Neufeld, J.D. and Mohn, W.W. 2004. Characterization of humus microbial communities in adjacent forest types that differ in nitrogen availability. Microbial Ecology 48, 28-40.

Legendre, P. and Legendre, L. 1998. Numerical Ecology. Elsevier, Amsterdam, The Netherlands. 870 p.

Li, Q.; Lee Allen, H. and Wollum, I., Arthur G. 2004. Microbial biomass and bacterial functional diversity in forest soils: effects of organic matter removal, compaction, and vegetation control. Soil Biology & Biochemistry 36, 571-579.

Liesack, W.; Janssen, P.H.; Rainey, F.A.; Ward-Rainey, N. and Stackebrandt, E. 1997. Microbial diversity in soil: The need for a combined approach using molecular and cultivation techniques. In: van Elsas, J.D.; Trevors, J.T. and Wellington, E.M.H. (Eds.). Modern Soil Microbiology. Marcel Dekker, New York, USA. pp. 375-427.

Lin, Q. and Brookes, P.C. 1999. Arginine ammonification as a method to estimate soil microbial biomass and microbial community structure. Soil Biology & Biochemistry 31, 1985-1997.

Loreau, M.; Naeem, S.; Inchausti, P.; Bengtsson, J.; Grime, J.P.; Hector, A.; Hooper, D.U.; Huston, M.A.; Raffaelli, D.; Schmid, B.; Tilman, D. and Wardle, D.A. 2001.

Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294, 804-808.

Loreau, M. 2004. Does functional redundancy exist? Oikos 104, 606-611.

Lovell, R.D.; Jarvis, S.C. and Bardgett, R.D. 1995. Soil microbial biomass and activity in long-term grassland: Effects of management changes. Soil Biology & Biochemistry 27, 969-975.

Lundquist, E.J.; Jackson, L.E.; Scow, K.M. and Hsu, C. 1999a. Changes in microbial biomass and community composition, and soil carbon and nitrogen pools after incorporation of rye into three California agricultural soils. Soil Biology & Biochemistry 31, 221-236.

Lundquist, E.J.; Scow, K.M.; Jackson, L.E.; Uesugi, S.L. and Johnson, C.R. 1999b. Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/ dry cycle. Soil Biology & Biochemistry 31, 1661-1675.

Lupwayi, N.Z.; Rice, W.A. and Clayton, G.W. 1998. Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. Soil Biology & Biochemistry 30, 1733-1741.

Mäder, P.; Fliessbach, A.; Wiemken, A. and Niggli, U. 1995. Assessment of soil microbial status under long-term low input (biological) and high input (conventional) agriculture In: Mäder, P. and Raupp, J. (eds.). Effects of low and high external input agriculture on soil microbial biomass and activities in view of sustainable agriculture (Fertilization systems in organic farming [concerted action AIR3-CT94-1940]) - Proceedings of the second meeting in Oberwil (Switzerland). Oberwil (Switzerland). Institute for Biodynamic Research, pp. 24-38.

Mäder, P.; Pfiffner, L.; Fliessbach, A.; von Lützow, M. and Munch, J.C. 1996. Soil ecology - the impact of organic and conventional agriculture on soil biota and its significance for soil fertility. 11th Scientific Conference, 11-15 August 1996. Copenhagen, Denmark. IFOAM, pp. 24-46.

Mäder, P.; Alföldi, T.; Fliessbach, A.; Pfiffner, L. and Niggli, U. 1999. Agricultural and ecological performance of cropping systems compared in a long-term field trial. In: Smaling, E.M.A.; Oenema, O. and Fresco, L.O. (Eds.). Nutrient disequilibria in agroecosystems. CAB International, Wallingford, UK. pp. 247-264.

Mäder, P.; Fliessbach, A.; Dubois, D.; Gunst, L.; Fried, P. and Niggli, U. 2002. Soil fertility and biodiversity in organic farming. Science 296, 1694-1697.

Madsen, E.L. 1996. A critical analysis of methods for determining the composition and biogeochemical activities of soil microbial communities *in situ*. In: Bollag, J.-M. and Stotzky, G. (Eds.). Soil biochemistry. Marcel Dekker, New York, USA. Vol. 9, pp. 287-370.

Mahmood, T.; Azam, F.; Hussain, F. and Malik, K.A. 1997. Carbon availability and microbial biomass in soil under an irrigated wheat-maize cropping system receiving different fertilizer treatments. Biology & Fertility of Soils 25, 63-68.

Maier, R.M.; Pepper, I.L. and Gerba, C.P. 2000. Environmental Microbiology. Academic Press, San Diego, USA. 585 p.

Marschner, P.; Yang, C.H.; Lieberei, R. and Crowley, D.E. 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biology & Biochemistry 33, 1437-1445.

Marschner, P.; Kandeler, E. and Marschner, B. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biology & Biochemistry 35, 453-461.

Marschner, P.; Grierson, P.F. and Rengel, Z. 2005. Microbial community composition and functioning in the rhizosphere of three *Banksia* species in native woodland in Western Australia. Applied Soil Ecology 28, 191-201.

Marshall, M.N.; Cocolin, L.; Mills, D.A. and van der Gheynst, J.S. 2003. Evaluation of PCR primers for denaturing gradient gel electrophoresis analysis of fungal communities in compost. Journal of Applied Microbiology 95, 934-948.

Martyniuk, S. and Wagner, G.H. 1978. Quantitative and qualitative examination of soil microflora associated with different management systems. Soil Science 125, 343-350.

Mausbach, M.J. and Seybold, C.A. 1998. Assessment of soil quality. In: Lal, R. (Ed.). Soil quality and agricultural sustainability. Ann Arbor Press, Chelsea, USA. pp. 33-43.

McLean, K.L. 2001. Biological control of onion white rot using *Trichoderma harzianum*. PhD thesis. Soil, Plant & Ecological Sciences Division. Lincoln University, Lincoln, NZ. 193 p.

Metting, F.B., Jr. (Ed.) 1993. Soil microbial ecology. Marcel Dekker, Inc., New York, USA. 646 p.

Mikola, J. and Setälä, H. 1998. Relating species diversity to ecosystem functioning: mechanistic backgrounds and experimental approach with a decomposer food web. Oikos 83, 180-194.

Miller, M. and Dick, R.P. 1995. Dynamics of soil C and microbial biomass in whole soil and aggregates in two cropping systems. Applied Soil Ecology 2, 253-261.

Morris, S.J. 1999. Spatial distribution of fungal and bacterial biomass in southern Ohio hardwood forest soils: fine scale variability and microscale patterns. Soil Biology & Biochemistry 31, 1375-1386.

Müller, A.K.; Westergaard, K.; Christensen, S. and Sørensen, L.H. 2002. The diversity and function of soil microbial communities exposed to different disturbances. Microbial Ecology 44, 49-58.

Muyzer, G.; de Waal, E. and Uitterlinden, A. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied & Environmental Microbiology 59, 695-700.

Naeem, S. 2003. Continuing debate in the face of biodiversity loss. Oikos 100, 619.

Nannipieri, P.; Kandeler, E. and Ruggiero, P. 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Burns, R.G. and Dick, R.P. (Eds.). Enzymes in the environment. Marcel Dekker, Inc., New York, USA. pp. 1-33.

Nannipieri, P.; Ascher, J.; Ceccherini, M.T.; Landi, L.; Pietramellara, G. and Renella, G. 2003. Microbial diversity and functions. European Journal of Soil Science 54, 655-670.

Nsabimana, D.; Haynes, R.J. and Wallis, F.M. 2004. Size, activity and catabolic diversity of the soil microbial biomass as affected by land use. Applied Soil Ecology 26, 81-92.

Nunan, N.; Wu, K.; Young, I.M.; Crawford, J.W. and Ritz, K. 2002. *In situ* spatial patterns of soil bacterial populations, mapped at multiple scales, in arable soil. Microbial Ecology 44, 296-305.

Ocio, J.A.; Brookes, P.C. and Jenkinson, D.S. 1991. Field incorporation of straw and its effects on soil microbial biomass and soil inorganic N. Soil Biology & Biochemistry 23, 171-176.

O'Donnell, A.G. and Görres, H.E. 1999. 16S rDNA methods in soil microbiology. Current Opinion in Biotechnology 10, 225-229.

O'Donnell, A.G.; Seasman, M.; Macrae, A.; Waite, I. and Davies, J.T. 2001. Plants and fertilisers as drivers of change in microbial community structure and function in soils. Plant & Soil 232, 135-145.

Oehlaf, R.C. 1978. Organic agriculture: Economics and ecological comparisons with conventional methods. Allanheld, Osmun & Co., Montclair, USA. 271 p.

Omar, S.A. and Ismail, M.A. 1999. Microbial populations, ammonification and nitrification in soil treated with urea and inorganic salts. Folia Microbiologica 44, 205-212.

Pankhurst, C.E.; Ophel-Keller, K.M.; Doube, B.M. and Gupta, V.V.S.R. 1996. Biodiversity of soil microbial communities in agricultural systems. Biodiversity & Conservation 5, 197-209.

Pankhurst, C.E.; Doube, B.M. and Gupta, V.V.S.R. 1997. Biological indicators of soil health: synthesis. In: Pankhurst, C.E.; Doube, B.M. and Gupta, V.V.S.R. (Eds.). Biological indicators of soil health. CAB International, Wallingford, UK. pp. 419-435.

Pankhurst, C.E.; Blair, B.L.; Magarey, R.C.; Stirling, G.R.; Bell, M.J. and Garside, A.L. 2005. Effect of rotation breaks and organic matter amendments on the capacity of soils to develop biological suppression towards soil organisms associated with yield decline of sugarcane. Applied Soil Ecology 28, 271-282.

Paoletti, M.G. and Pimentel, D. 1992. Introduction. Agriculture, Ecosystems & Environment 40, 1-2.

Parfitt, R.L.; Yeates, G.W.; Ross, D.J.; Mackay, A.D. and Budding, P.J. 2005. Relationships between soil biota, nitrogen and phosphorus availability, and pasture growth under organic and conventional management. Applied Soil Ecology 28, 1-13.

Parr, J.F.; Papendick, R.I.; Hornick, S.B. and Meyer, R.E. 1992. Soil quality: attributes and relationship to alternative and sustainable agriculture. American Journal of Alternative Agriculture 7, 5-11.

Paul, E.A. and Clark, F.E. 1996. Soil microbiology and biochemistry. Academic Press, Inc., San Diego, USA. 340 p. 2nd ed.

Perucci, P. 1992. Enzyme activity and microbial biomass in a field soil amended with municipal refuse. Biology & Fertility of Soils 14, 54-60.

Pfotzer, G.H. and Schüler, C. 1995. Effects of different compost amendment on soil biotic and faunal feeding activity in an organic farming system In: Raupp, J. (ed.). Main effects of various organic and mineral fertilization on soil organic matter turnover and

plant growth (Fertilization systems in organic farming [concerted action AIR3-CT94-1940]). Darmstadt, Germany. Institute for Biodynamic Research, pp. 37-41.

Pitt, J.I. 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, London, UK. 643 p.

Ponting, C. 1992. Foreword. In: Conford, P. (Ed.). A future for the land - organic practice from a global perspective. Green Books, Bideford, UK. pp. ix-xii.

Poudel, D.D.; Ferris, H.; Klonsky, K.; Horwath, W.R.; Scow, K.M.; van Bruggen, A.H.C.; Lanini, W.T.; Mitchell, J.P. and Temple, S.R. 2001. The sustainable agriculture farming system project in California's Sacramento Valley. Outlook on Agriculture 30, 109-116.

Puri, G. and Ashman, M.R. 1998. Relationship between soil microbial biomass and gross N mineralisation. Soil Biology & Biochemistry 30, 251-256.

Randhawa, P.S. 2003. Influence of green manuring and phosphate rock inputs on soil phosphorus cycling and availability. PhD thesis. Soil, Plant & Ecological Sciences Division. Lincoln University, Lincoln, NZ. 151 p.

Raupp, J. 1995a. Long term field experiment in Sweden: Effects of organic and inorganic fertilizers on soils and crops (K-trial) - Soil parameters of the K-trial In: Raupp, J. (ed.). Main effects of various organic and mineral fertilization on soil organic matter turnover and plant growth (Fertilization systems in organic farming [concerted action AIR3-CT94-1940]). Darmstadt, Germany. Institute for Biodynamic Research, pp. 22-27.

Raupp, J. 1995b. The long term trial in Darmstadt: Mineral fertilizer, composted manure and composted manure plus all biodynamic preparations In: Raupp, J. (ed.). Main effects of various organic and mineral fertilization on soil organic matter turnover and plant growth (Fertilization systems in organic farming [concerted action AIR3-CT94-1940]). Darmstadt, Germany. Institute for Biodynamic Research, pp. 28-36.

Rayns, F.; Jackson, L.R.; Bending, G.D.; Turner, M.K.; Wood, M. and Marx, M.-C. 2002. The effect of organic management on soil quality indicators. In: Powell (Ed.). UK Organic Research 2002: Proceedings of the COR Conference 26-28th March 2002. Aberystwyth, UK. pp. 139-140.

Reeve, I. 1990. Sustainable agriculture: ecological imperative or economic impossibility? - A review of overseas government policy and implications for Australian agriculture

(September 1990). The Rural Development Centre, University of New England, Armidale, NSW, 95 p.

Reganold, J.P. 1995. Soil quality and farm profitability studies of biodynamic and conventional farming systems. In: Cook, H.F. and Lee, H.C. (Eds.). Soil management in sustainable agriculture. Wye College Press, Wye, UK. pp. 1-11.

Reganold, J.P.; Glover, J.D.; Andrews, P.K. and Hinman, H.R. 2001. Sustainability of three apple production systems. Nature 410, 926-930.

Rifai, M.A. 1980. A revision of the genus *Trichoderma*. In: Domsch, K.H.; Gams, W. and Anderson, T.-H. (Eds.). Compendium of soil fungi. Academic Press, London, UK. Vol. 1, pp. 1-56.

Ritz, K.; Dighton, J. and Giller, K.E. (Eds.) 1994. Beyond the Biomass: compositional and functional analysis of soil microbial communities. John Wiley & Sons, Inc., Chichester, UK. 275 p.

Ritz, K. and Griffiths, B.S. 2001. Implications of soil biodiversity for sustainable organic matter management. In: Rees, R.M.; Ball, B.C.; Campbell, C.D. and Watson, C.A. (Eds.). Sustainable management of soil organic matter. CAB International, Wallingford, UK. pp. 343-356.

Ritz, K.; McNicol, J.W.; Nunan, N.; Grayston, S.; Millard, P.; Atkinson, D.; Gollotte, A.; Habeshaw, D.; Boag, B. and Clegg, C.D. 2004. Spatial structure in soil chemical and microbiological properties in an upland grassland. FEMS Microbiology Ecology 49, 191-205.

Robertson, F.A. and Morgan, W.C. 1996. Effects of management history and legume green manure on soil microorganisms under 'organic' vegetable production. Australian Journal of Soil Research 34, 427-440.

Robson, M.C.; Fowler, S.M.; Lampkin, N.H.; Leifert, C.; Leitch, M.; Robinson, D.; Watson, C.A. and Litterick, A.M. 2002. The agronomic and economic potential of break crops for ley/ arable rotations in temperate organic agriculture. Advances in Agronomy 77, 369-427.

Roper, M.M. and Ophel-Keller, K.M. 1997. Soil microflora as bioindicators of soil health. In: Pankhurst, C.E.; Doube, B.M. and Gupta, V.V.S.R. (Eds.). Biological indicators of soil health. CAB International, Wallingford, UK. pp. 157-177.

Röver, M. and Kaiser, E.-A. 1999. Spatial heterogeneity within the plough layer: low and moderate variability of soil properties. Soil Biology & Biochemistry 31, 175-187.

Rovira, A.D. 1994. The effect of farming practices on the soil biota. In: Pankhurst, C.E.; Doube, B.M.; Gupta, V.V.S.R. and Grace, P.R. (Eds.). Soil biota: Management in sustainable farming systems. CSIRO Australia, East Melbourne, Australia. pp. 81-87.

Ryan, M. 1999. Is an enhanced soil biological community, relative to conventional neighbours, a consistent feature of alternative (organic or biodynamic) agricultural systems? Biological Agriculture & Horticulture 17, 131-144.

Sanguinetty, C.J.; Dias Neto, E. and Simpson, A.J.G. 1994. Rapid silver staining and recovery of PCR products on acrylamide gels. Biotechniques 17, 915-919.

Sarathchandra, S.U.; Ghani, A.; Yeates, G.W.; Burch, G. and Cox, N.R. 2001. Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils. Soil Biology & Biochemistry 33, 953-964.

Saunders, C.; Manhire, J.; Campbell, H. and Fairweather, J. 1997. Organic farming in New Zealand: an evaluation of the current and future prospects including an assessment of research needs. Wellington: MAF New Zealand. Retrieved 22 April 2001 from http://www.maf.govt.nz/mafnet/rural-nz/sustainable-resource-use/organic-production/organic-farming-in-nz/httoc.htm (last accessed 26/04/2005).

Schachtschabel, P.; Blume, H.-P.; Brümmer, G.; Hartge, K.-H. and Schwertmann, U. (Eds.) 1992. Scheffer/ Schachtschabel - Lehrbuch der Bodenkunde. Ferdinand Enke Verlag, Stuttgart, Germany. 490 p.

Schinner, F.; Öhlinger, R.; Kandeler, E. and Margesin, R. (Eds.) 1995. Methods in soil biology. Springer Verlag, Berlin, Germany. 426 p.

Schjønning, P.; Elmholt, S.; Munkholm, L.J. and Debosz, K. 2002. Soil quality aspects of humid sandy loams as influenced by organic and conventional long-term management. Agriculture, Ecosystems & Environment 88, 195-214.

Schjønning, P.; Elmholt, S. and Christensen, B.T. 2004a. Soil quality management - concepts and terms. In: Schjønning, P.; Christensen, B.T. and Elmholt, S. (Eds.). Managing soil quality: challenges in modern agriculture. CAB International, Wallingford, UK. pp. 1-15.

Schjønning, P.; Elmholt, S. and Christensen, B.T. (Eds.) 2004b. Managing soil quality: Challenges in modern agriculture. CAB International, Wallingford, UK. 344 p.

Schjønning, P.; Munkholm, L.J. and Elmholt, S. 2004c. Soil quality in organic farming - effects of crop rotation, animal manure and soil compaction (poster paper). Euro Soil, 4-12 September 2004. Freiburg, Germany.

Schloter, M.; Dilly, O. and Munch, J.C. 2003. Indicators for evaluating soil quality. Agriculture, Ecosystems & Environment 98, 255-262.

Schnürer, J. and Rosswall, T. 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Applied & Environmental Microbiology 43, 1256-1261.

Schnürer, J.; Clarholm, M. and Rosswall, T. 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. Soil Biology & Biochemistry 17, 611-618.

Schulze, E.-D. and Mooney, H.A. 1993. Biodiversity and ecosystem function. Springer Verlag, Berlin, Germany. 525 p.

Seghers, D.; Reheul, D.; Bulcke, R.; Verstraete, W. and Top, E.M. 2001. Do conventionally and biologically cultivated soils differ in bacterial diversity and community structure? Medelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent (Proceedings of the Fifteenth Forum for Applied Biotechnology, Gent, Belgium, 24-25 September 2001) 66, 381-388.

Seghers, D.; Verthé, K.; Reheul, D.; Bulcke, R.; Siciliano, S.D.; Verstraete, W. and Top, E.M. 2003. Effect of long-term herbicide applications on the bacterial community structure and function in an agricultural soil. FEMS Microbiology Ecology 46, 139-146.

Shannon, D.; Sen, A.M. and Johnson, D.B. 2002. A comparative study of the microbiology of soils managed under organic and conventional regimes. Soil Use & Management 18, 274-283.

Shepherd, M.; Harrison, R.; Cuttle, S.; Johnson, B.; Shannon, D.; Gosling, P. and Rayns, F. 2000a. Understanding of soil fertility in organically farmed soils. Produced for UK Ministry of Agriculture, Fisheries and Food. Retrieved April 2000 from http://www.adas.co.uk/soilfertility/download.html?topid=7 (last accessed 26/04/2005).

Shepherd, M.; Pearce, B.; Cormack, B.; Phillipps, L.; Cuttle, S.; Bhogal, A.; Costagin, P. and Unwin, R. 2003. An assessment of the environmental impacts of organic farming. Retrieved 01/03/2005 from

http://www.defra.gov.uk/science/project_data/DocumentLibrary/OF0405/OF0405_909_TRP.doc (last accessed 26/04/2005).

Shepherd, M.A.; Hatley, D.; Gosling, P.; Rayns, F.; Cuttle, S.P. and Hatch, D. 2000b. Soil fertility in organic farming systems - are there fundamental differences between organic and conventional farms? Aspects of Applied Biology 62, 113-118.

Smalla, K.; Wachtendorf, U.; Heuer, H.; Liu, W.T. and Forney, L.J. 1998. Analysis of BIOLOG GN substrate utilization patterns by microbial communities. Applied & Environmental Microbiology 64, 1220-1225.

Smit, E.; Leeflang, P.; Glandorf, B.; van Elsas, J.D. and Wernars, K. 1999. Analysis of fungal diversity in the wheat rhizosphere by sequencing of cloned PCR-amplified genes encoding 18S rRNA and temperature gradient gel electrophoresis. Applied & Environmental Microbiology 65, 2614-2621.

Smith, J.L.; Papendick, R.I.; Bezdicek, D.F. and Lynch, J.M. 1993. Soil organic matter dynamics and crop residue management. In: Metting, F.B., Jr. (Ed.). Soil microbial ecology. Marcel Dekker, Inc., New York, USA. pp. 65-94.

Smith, J.L. 1994. Cycling of nitrogen through microbial activity. In: Hatfield, J.L. and Stewart, B.A. (Eds.). Soil Biology: Effects on Soil Quality. Lewis Publishers, Boca Raton, USA. pp. 91-120.

Smyk, B.; Rózycki, E. and Barabasz, W. 1986. The effect of fertilization with N and NPK on the microbiocenoses and the ecological stability of selected mountain grassy ecosystems. In: Jensen, V.; Kjøller, A. and Sørensen, L.H. (Eds.). Microbial Communities in Soil. Elsevier Applied Science Publishers, London, UK. pp. 229-243.

Söderberg, K.H. and Bååth, E. 2004. The influence of nitrogen fertilisation on bacterial activity in the rhizosphere of barley. Soil Biology & Biochemistry 36, 195-198.

Søvik, A.K. and Aagaard, P. 2003. Spatial variability of a solid porous framework with regard to chemical and physical properties. Geoderma 113, 47-76.

Sparling, G.P. and West, A.W. 1988. Modifications to the fumigation extraction technique to permit simultaneous extraction and estimation of soil microbial C and N. Communications in Soil Science & Plant Analysis 19, 327-344.

Sparling, G.P.; Feltham, C.W.; Reynolds, J.; West, A.W. and Singleton, P. 1990. Estimation of soil microbial C by a fumigation-extraction method: use on soils of high organic

matter content, and a reassessment of the k_{ec}-factor. Soil Biology & Biochemistry 22, 301-307.

Staley, J.T. 1996. Heterotrophic bacteria: the cultivation approach. In: Hall, G.S. (Ed.). Methods for the examination of organismal diversity in soils and sediments. CAB International, Wallingford, UK. pp. 1-10.

Stark, J.M. and Hart, S.C. 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Science Society of America Journal 60, 1846-1855.

Steenwerth, K.L.; Jackson, L.E.; Calderón, F.J.; Stromberg, M.R. and Scow, K.M. 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biology & Biochemistry 34, 1599-1611.

Stein, A. and Ettema, C.H. 2003. An overview of spatial sampling procedures and experimental design of spatial studies for ecosystem comparisons. Agriculture, Ecosystems & Environment 94, 31-47.

Stevenson, F.J. and Cole, M.A. 1999. Cycles of soil: carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley & Sons, Inc., New York, USA. 427 p. 2nd ed.

Stockdale, E.A.; Lampkin, N.H.; Hovi, M.; Keatinge, R.; Lennartsson, E.K.M.; Macdonald, D.W.; Padel, S.; Tattersall, F.H.; Wolfe, M.S. and Watson, C.A. 2001. Agronomic and environmental implications of organic farming systems. Advances in Agronomy 70, 261-327.

Stolze, M.; Piorr, A.; Häring, A. and Dabbert, S. 2000. Environmental impacts of organic farming in Europe. University of Hohenheim, Stuttgart, Germany. Organic farming in Europe: Economics and policy. Vol. 6. 127 p.

Stotzky, G. 1997. Soil as an environment for microbial life. In: van Elsas, J.D.; Trevors, J.T. and Wellington, E.M.H. (Eds.). Modern Soil Microbiology. Marcel Dekker, New York, USA. pp. 1-20.

Stout, W.L.; Fales, S.L.; Muller, L.D.; Schnabel, R.R. and Weaver, S.R. 2000. Water quality implications of nitrate leaching from intensively grazed pasture swards in the northeast US. Agriculture, Ecosystems & Environment 77, 203-210.

Swift, M.J. 1994. Maintaining the biological status of soil: a key to sustainable land management? In: Greenland, D.J. and Szabolcs, I. (Eds.). Soil resilience and sustainable land use. CAB International, Wallingford, UK. pp. 235-247.

Tabacchioni, S.; Chiarini, L.; Bevivino, A.; Cantale, C. and Dalmastri, C. 2000. Bias caused by using different isolation media for assessing genetic diversity of a natural microbial population. Microbial Ecology 40, 169-176.

Taylor, J.P.; Wilson, B.; Mills, M.S. and Burns, R.G. 2002. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biology & Biochemistry 34, 387-401.

Thalmann, A. 1968. Zur Methodik der Bestimmung der Dehydrogenaseaktivität im Boden mittels Triphenyltetrazoliumchlorid (TTC). Landwirtschaftliche Forschung 21, 249-250.

Thirukkumaran, C.M. and Parkinson, D. 2002. Microbial activity, nutrient dynamics and litter decomposition in a Canadian Rocky Mountain pine forest as affected by N and P fertilizers. Forest Ecology & Management 159, 187-201.

Thomas, V.G. and Kevan, P.G. 1993. Basic principle of agroecology and sustainable agriculture. Journal of Agricultural & Environmental Ethics 5, 1-18.

Tiedje, J.M.; Asuming-Brempong, S.; Nusslein, K.; Marsh, T.L. and Flynn, S.J. 1999. Opening the black box of soil microbial diversity. Applied Soil Ecology 13, 109-122.

Tilman, D. 1996. Biodiversity: population versus ecosystem stability. Ecology 77, 350-363.

Tinker, P.B. (Ed.) 2000a. Shades of green - a review of UK farming systems. Royal Agricultural Society of England, Stoneleigh Park, UK. 100 p.

Tinker, P.B. 2000b. Introduction. In: Tinker, P.B. (Ed.). Shades of green - a review of UK farming systems. Royal Agricultural Society of England, Stoneleigh Park, UK. pp. 1-5.

Toor, G.S.; Condron, L.M.; Cade-Menun, B.J.; Di, H.J. and Cameron, K.C. 2005. Preferential phosphorus leaching from an irrigated grassland soil. European Journal of Soil Science 56, 155-167.

Torsvik, V.; Goksøyr, J.; Daae, F.L.; Sørheim, R.; Michalsen, J. and Salte, K. 1994. Use of DNA analysis to determine the diversity of microbial communities. In: Ritz, K.; Dighton, J. and Giller, K.E. (Eds.). Beyond the biomass. John Wiley & Sons, Inc., Chichester, UK. pp. 39-48.

Torsvik, V. and Øvreås, L. 2002. Microbial diversity and function in soil: from genes to ecosystems. Current Opinion in Microbiology 5, 240-245.

Tunlid, A. and White, D.C. 1992. Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. In: Stotzky, G. and Bollag, J.-M. (Eds.). Soil biochemistry. Marcel Dekker, New York, USA. Vol. 7, pp. 229-262.

Turner, B.L. and Haygarth, P.M. 2000. Phosphorus forms and concentration in leachate under four grassland soil types. Soil Science Society of America Journal 64, 1090-1099.

Turpeinen, R.; Kairesalo, T. and Haggblom, M.M. 2004. Microbial community structure and activity in arsenic-, chromium- and copper-contaminated soils. FEMS Microbiology Ecology 47, 39-50.

Unwin, R.; Bell, B.; Shepherd, M.; Webb, J.; Keatinge, R. and Bailey, S. 1995. The effect of organic farming systems on aspects of the environment (August 1995). Agricultural Resources Policy Division of the Ministry of Agriculture, Fisheries and Food, 115 p.

van Elsas, J.D.; Frois Duarte, G.; Keijzer-Wolter, A. and Smit, E. 2000. Analysis of the dynamics of fungal communities in soil via fungal-specific PCR of soil DNA followed by denaturing gradient gel electrophoresis. Journal of Microbiological Methods 43, 133-151.

van Elsas, J.D.; Garbeva, P.; Salles, J. and van Verseveld, H.W. 2002. Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. Biodegradation 13, 29-40.

van Schöll, L.; van Dam, A.M. and Leffelaar, P.A. 1997. Mineralisation of nitrogen from an incorporated catch crop at low temperatures: experiment and simulation. Plant & Soil 188, 211-219.

van Steensel, F.; Nicholas, P.; Bauer-Eden, H.; Kenny, G.; Campbell, H.; Ritchie, M.; Macgregor, A.N.; Koppenol, M.; Blake, G. and Bacchus, P. 2004. A review of NZ and international organic land management research. The Research and Development Group of the BioDynamic Farming and Gardening Association in New Zealand, Wellington, NZ. 189 p.

Vinten, A.J.A.; Whitmore, A.P.; Bloem, J.; Howard, R. and Wright, F. 2002. Factors affecting N immobilisation/mineralisation kinetics for cellulose-, glucose- and straw-amended sandy soils. Biology & Fertility of Soils 36, 190-199.

von Wintzingerode, F.; Göbel, U.B. and Stackebrandt, E. 1997. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiology Reviews 21, 213-229.

Waldrop, M.P.; Balser, T.C. and Firestone, M.K. 2000. Linking microbial community composition to function in a tropical soil. Soil Biology & Biochemistry 32, 1837-1846.

Wander, M.M.; Traina, S.J.; Stinner, B.R. and Peters, S.E. 1994. Organic and conventional management effects on biologically active soil organic matter pools. Soil Science Society of America Journal 58, 1130-1139.

Wander, M.M.; Hedrick, D.S.; Kaufman, D.; Traina, S.J.; Stinner, B.R.; Kehrmeyer, S.R. and White, D.C. 1995. The functional significance of the microbial biomass in organic and conventionally managed soils. Plant & Soil 170, 87-97.

Wander, M.M. and Traina, S.J. 1996. Organic matter fractions from organically and conventionally managed soils: II. Characterization and composition. Soil Science Society of America Journal 60, 1087-1094.

Ward, D.M.; Bateson, M.M.; Weller, R. and Ruff-Roberts, A.L. 1992. Ribosomal RNA analysis of microorganisms as they occur in nature. Advances in Microbial Ecology 12, 219-268.

Ward, J.H. 1963. Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association 58, 236-244.

Wardle, D.A. and Ghani, A. 1995. A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. Soil Biology & Biochemistry 27, 1601-1610.

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

Wardle, D.A. and Grime, J.P. 2003. Biodiversity and stability of grassland ecosystem functioning. Oikos 100, 622-623.

Wardle, D.A.; Bardgett, R.D.; Klironomos, J.N.; Setälä, H.; van der Putten, W.H. and Wall, D.H. 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1621633.

Watson, C.A.; Atkinson, D.; Gosling, P.; Jackson, L.R. and Rayns, F.W. 2002. Managing soil fertility in organic farming systems. Soil Use & Management 18, 239-247.

Weisburg, W.G.; Barns, S.M.; Pelletier, D.A. and Lane, D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology 173, 697-703.

Wells, A.T.; Chan, K.Y. and Cornish, P.S. 2000. Comparison of conventional and alternative vegetable farming systems on the properties of a yellow earth in New South Wales. Agriculture, Ecosystems & Environment 80, 47-60.

Widmer, F.; Seidler, R.J.; Gillevet, P.M.; Watrud, L.S. and di Giovanni, G.D. 1998. A highly selective PCR protocol for detecting 16S rRNA genes of the genus *Pseudomonas* (sensu stricto) in environmental samples. Applied & Environmental Microbiology 64, 2545-2553.

Widmer, F.; Fliessbach, A.; Laczkó, E.; Schulze-Aurich, J. and Zeyer, J. 2001. Assessing biological characteristics: a comparison of bulk soil community DNA-, PLFA-, and Biolog™-analysis. Soil Biology & Biochemistry 33, 1029-1036.

Wolf, B. and Snyder, G.H. 2003. Sustainable soils: The place of organic matter in sustaining soils and their productivity. Food Products Press, New York, USA. 352 p.

Yeates, G.W.; Bardgett, R.D.; Cook, R.; Hobbs, P.J.; Bowling, P.J. and Potter, J.F. 1997. Faunal and microbial diversity in three Welsh grassland soils under conventional and organic management regimes. Journal of Applied Ecology 34, 453-470.

Zaitlin, B.; Turkington, K.; Parkinson, D. and Clayton, G. 2004. Effects of tillage and inorganic fertilizers on culturable soil actinomycete communities and inhibition of fungi by specific actinomycetes. Applied Soil Ecology 26, 53-62.

Zak, J.C.; Willig, M.R.; Moorhead, D.L. and Wildman, H.G. 1994. Functional diversity of microbial communities: A quantitative approach. Soil Biology & Biochemistry 26, 1101-1108.

Zaman, M.; Di, H.J. and Cameron, K.C. 1999a. A field study of gross rates of N mineralization and nitrification and their relationships to microbial biomass and enzyme activities in soils treated with dairy effluent and ammonium fertilizer. Soil Use & Management 15, 188-194.

Zaman, M.; Di, H.J.; Cameron, K.C. and Frampton, C.M. 1999b. Gross nitrogen mineralization and nitrification rates and their relationships to enzyme activities and

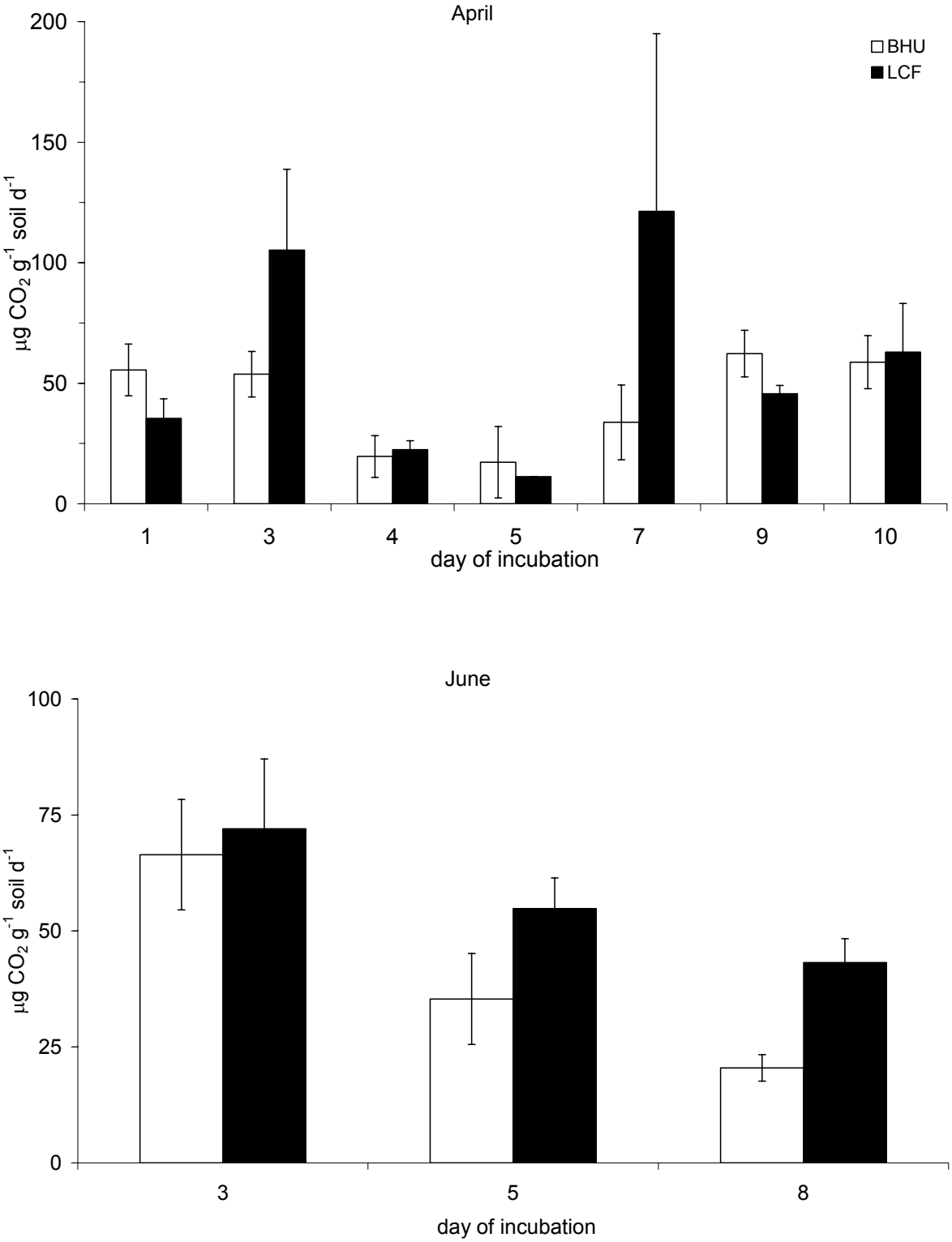
the soil microbial biomass in soils treated with dairy shed effluent and ammonium fertilizer at different water potentials. Biology & Fertility of Soils 29, 178-186.

Zelles, L.; Bai, Q.Y.; Baeck, T. and Beese, F. 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biology & Biochemistry 24, 317-323.

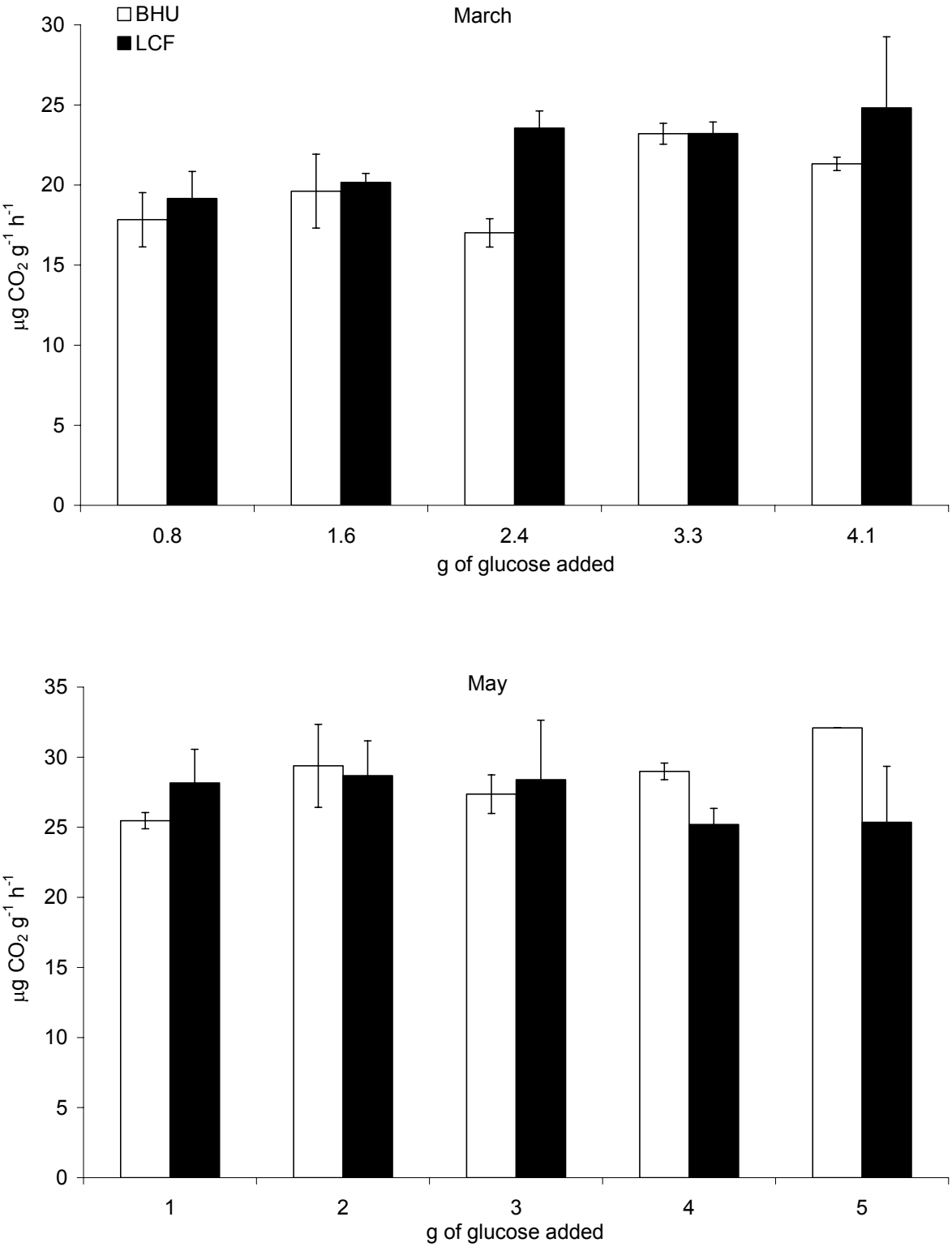
Appendix I

Soil profile descriptions for soils at LCF and BHU.

Horizon	*Depth*	*Description*
LCF		
Ap	0-19 cm	brownish black (10YR3/2) fine sandy loam; weak, brittle; weak to moderately developed fine to medium nutty with fine cast granular structure; few fine roots; indistinct boundary
A/B	19-25 cm	earthworm mixed fine sandy loam; weak, brittle; weakly developed, fine nutty cast granular structure; few fine roots; distinct boundary
Bwf_1	25-58 cm	dull yellow orange (10YR6/4) fine sandy loam; very firm, brittle; structurless, massive; few fine bright brown (7.5YR5/6) mottles; very few fine roots; indistinct boundary
Bwf_2	58-61 cm	dull yellow (2.5Y6/3) fine sandy loam; moderately firm, brittle; structureless, massive; few greyish olive (5Y6/2) veins associated with many yellowish-brown (10YR5/6) mottles; distinct boundary
C	61 + cm	yellowish brown (2.5Y5/3) sandy loam; semi deformable, weak; structureless massive; coarse indistinct yellowish-brown (10YR5/6) mottles
BHU		
Ap	0-21 cm	brownish-black (10YR3/2) fine sandy loam; very weak, semi deformable; moderately developed very fine to medium granular fine to medium nut structure; few fine soft reddish brown (5YR4/6) concretions in lower 8 cm; few fine roots; gradual boundary
AB	21-35 cm	yellowish-brown (2.5YR5/3) fine sandy loam; moderately weak, brittle; weakly developed, fine nutty cast granular structure; few fine soft concretions and few fine distinct bright brown (7.5YR5/6) mottles; few fine roots; gradual transition
Bwf	35-56 cm	yellowish-brown (2.5YR5/3) fine sandy loam; moderatly firm, brittle; structureless, massive; common fine and distinct yellowish-brown (10YR5/8) mottles; very few fine roots; distinct boundary
Bwg	56-70 cm	yellowish-brown (2.5Y5/3) fine sandy loam; firm; structureless, massive; some greyish-yellow (2.5Y6/2) veins and many distinct coarse bright brown (7.5YR5/8) mottles



Basal respiration in BHU and LCF soils assessed after varying days of incubation as part of the method selection process in April and June 2002. n=3. Bars represent standard errors of means.



Substrate induced respiration (SIR) in BHU and LCF soils assessed with different glucose amendments as part of the method selection process in March and May 2002. n=3. Bars represent standard errors of means.

Composition of growth media used for soil dilution plating and fungal identification as part of the farm site comparison:

Trichoderma selective medium (TSM from Lincoln University) (McLean 2001)

Agar	20 g
Glucose	3 g
NH_4NO_3	1 g
$K_2HPO_4 \cdot 3H_2O$	0.9 g
$MgSO_4 \cdot 7H_2O$	0.2 g
KCl	0.15 g
Tetraclor® 75 WP	0.2 g
Rose bengal	0.15 g
1 ml of a mixture of	
$FeSO_4 \cdot 7H_2O$	0.1%
$MnSO_4 \cdot 4H_2O$	0.065%
$ZnSO_4 \cdot 7H_2O$	0.09%

King's medium B (KB) (Atlas 2004)

Agar	15 g
Glycerol	15 ml
$MgSO_4 \cdot 7H_2O$	3 g
K_2HPO_4	2 g
Proteose Peptone	20 g

Hay agar (HA)

finely chopped hay pieces	1 g
Agar	15 g

Potato carrot agar (PCA)

Potato carrot extract (see below)	0.5 l
Agar	15 g

Potato carrot extract

scraped potatoes	40 g
scraped carrots	40 g

Cut potatoes and carrots in small pieces, boil until soft in tap water, filter through cloth, make up to 1L with tap water). Sterilise for 15 min at 121°C.

Malt extract agar (MEA) (Atlas 2004)

Powdered malt extract	20 g
Peptone	1 g
Glucose	20 g
Agar	15 g

Composition of growth media used for soil dilution plating and fungal identification as part of the farm site comparison (continued):

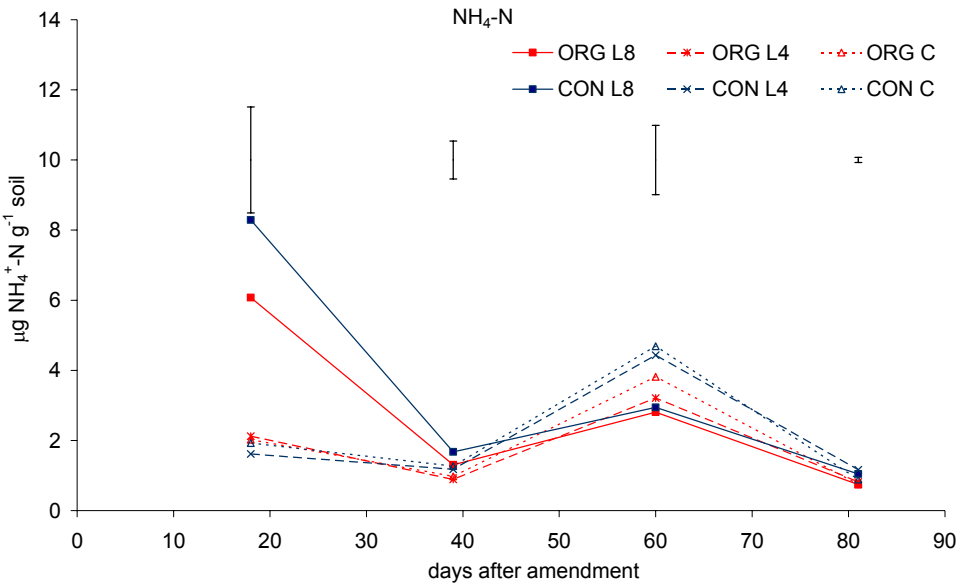
25% Glycerol Nitrate Agar (G25N) (Atlas 2004)

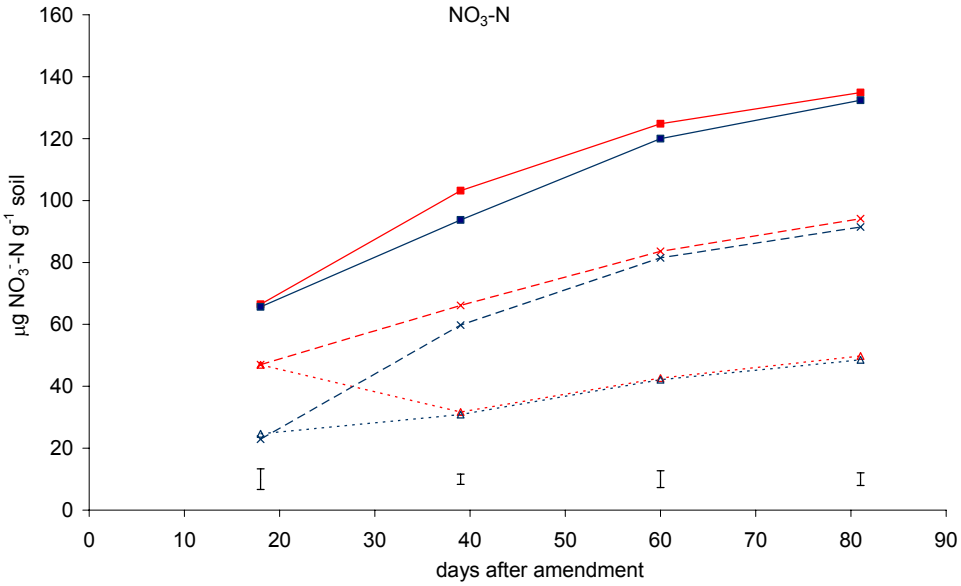
Agar	15 g
K_2HPO_4	0.75 g
Czapek concentrate (see below)	7.5 ml
Yeast extract	3.7 g
Glycerol	250 g

Czapek concentrate

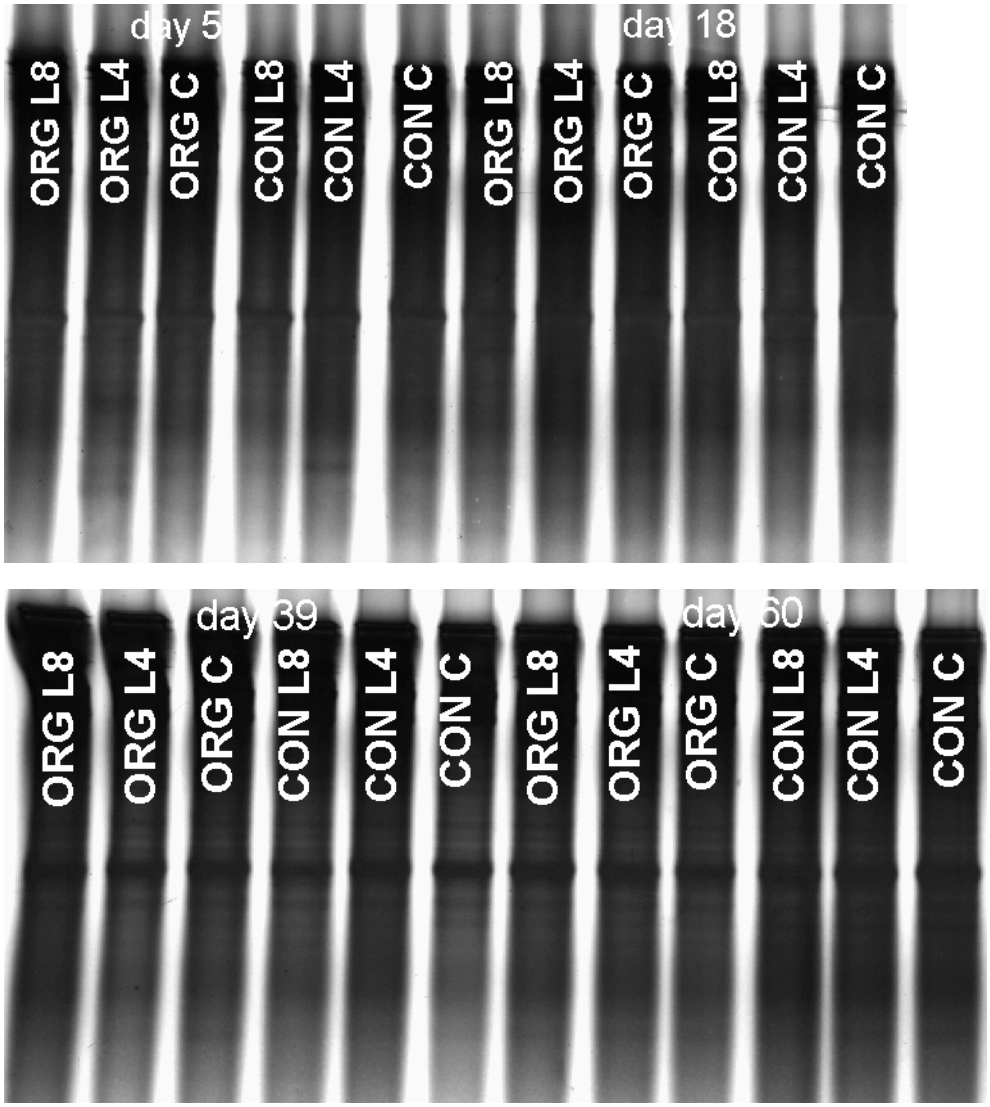
$NaNO_3$	3%
KCl	0.5%
$MgSO_4 \cdot 7H_2O$	0.5%
$FeSO_4 \cdot 7H_2O$	0.02%

Appendix II

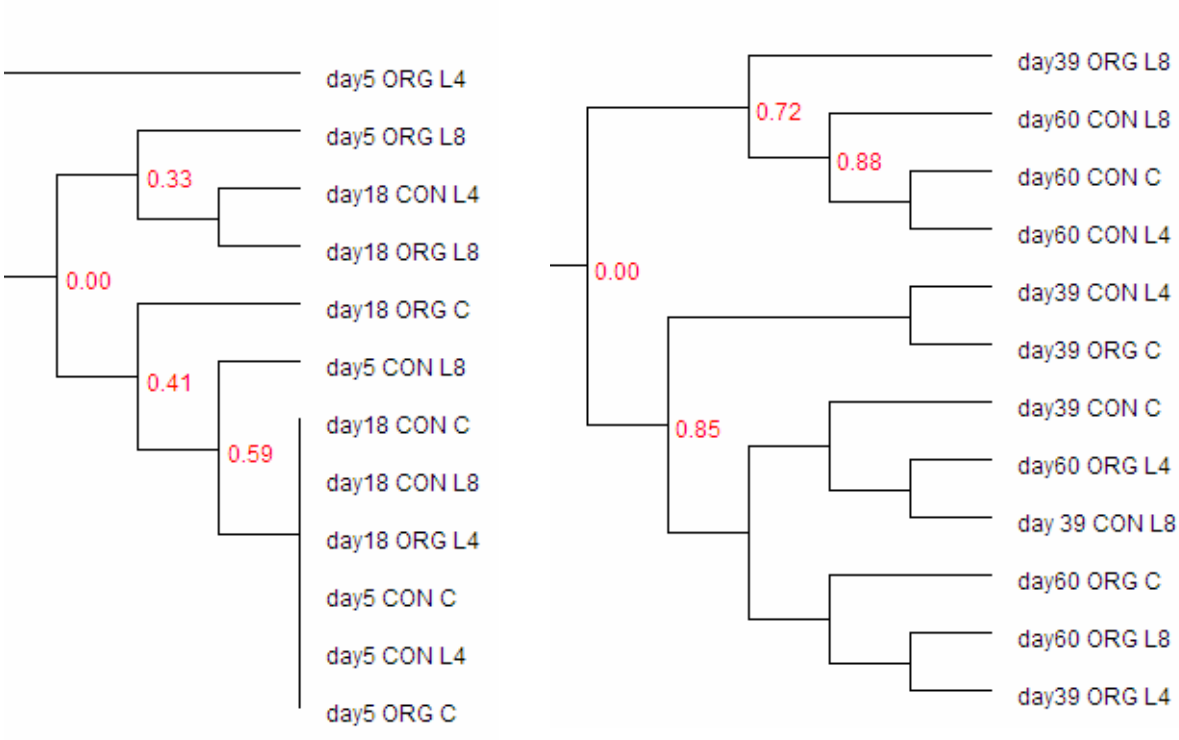




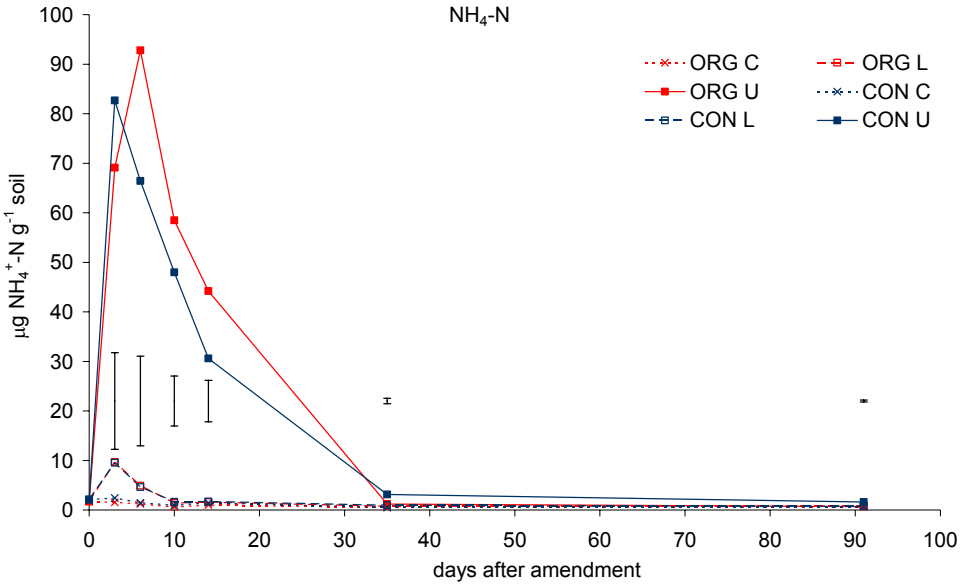
Mean concentrations (µg g^{-1}) of NH_4- and NO_3-N determined over 81 days in incubation experiment I. n=3. Bars show $LSD_{0.05}$.

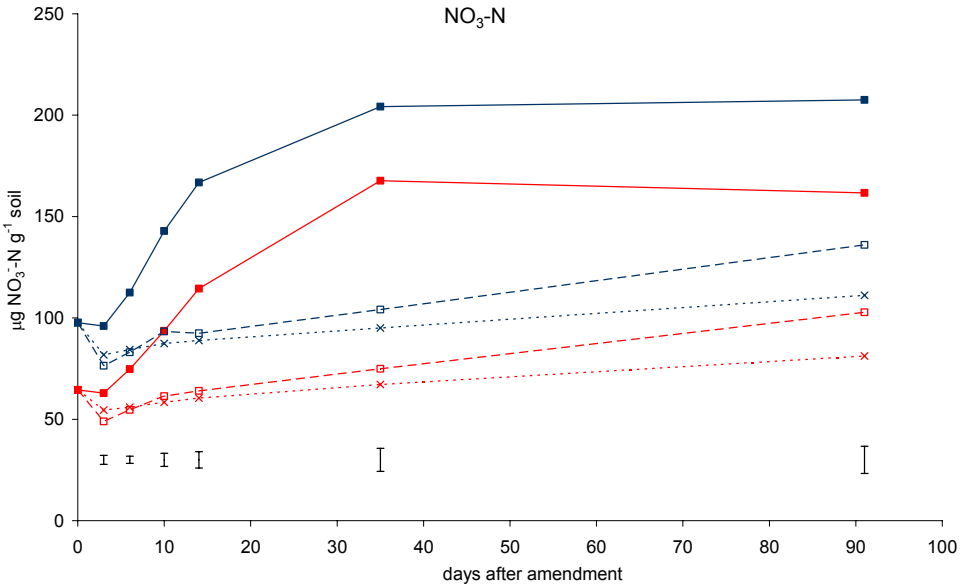


DGGE banding patterns of fungal communities in ORG and CON soils at different sampling dates in incubation experiment I



Cluster analyses for fungal communities in ORG and CON soils 5, 18, 35 and 60 days after amendment with lupin in incubation experiment I.





Mean concentrations (µg g^{-1}) of NH_4- and NO_3-N determined over 91 days in incubation experiment II. n=3. Bars show $LSD_{0.05}$.

Overall correlation coefficients determined among soil properties and processes over 91 days in incubation experiment II.

	C_{mic}	N_{mic}	*DHH*	N_{min}	C_{tot}	N_{tot}
N_{mic}	0.901***					
DHH	0.894***	0.887***				
N_{min}	-0.414***	-0.334***	-0.482***			
C_{tot}	0.211*	0.114	-0.065	0.199*		
N_{tot}	0.272**	0.186	-0.007	0.298**	0.922***	
MIN	0.186	0.130	0.258**	-0.412**	-0.399**	-0.395**

n=110. *, p<0.05; **, p<0.01; ***, p<0.001