The Ecology of Earthworms and their Impact on Carbon Distribution and Chemical Characteristics in Soil

A thesis submitted towards the degree of

Doctor of Philosophy

Hannah O. Bishop

School of Biological and Environmental Sciences
University of Stirling

October 2003

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like to thank the following people for their help and support throughout my PhD:

Firstly, to my main supervisor, Professor David Hopkins, for his invaluable advice, help and support throughout my work. Also to second supervisor Dr. Ian Grieve for comments on my thesis, and to 'adopted' supervisor Sandy Chudek for providing guidance and help on NMR related matters.

To all those who came to help dig the soil and cut the grass I offer my thanks and gratitude, especially to Dr. Emma Tilston who also carried out the identification of the plants at the mesocosm site. Thanks also to Emma for assistance in the lab, and also Lorna English for the same! Thanks are extended to Dr. Sarah Buckland and Dr. Graham Burt-Smith, Sourhope site managers.

Thanks go to Dr. Nick Ostle (CEH) for providing the PAR data, Dr. Nigel Wilby for statistical advice and Bill Jamieson and John McArthur for helping me when I needed it. Also, Dr. Claire Robinson, for help on earthworm identification at the beginning, when all help was greatly appreciated!

I would like to thank my parents, as without their support this would have been a far more arduous task to complete.

Also thanks go to my wonderful boyfriend, Kev Jones, for putting up with me, especially as it got close to the deadline!

Also, to Sue Bowen for being a really great office mate, and Amanda Thomson and Ian Griffin, and the rest of the PhD's.

Finally, the Natural Environment Research Council for funding this project.

Preface

This is a thesis containing no material that has been accepted or previously submitted for the award of any other degree or diploma. It contains no material previously published or written by another person except where due reference is made in the text.

Summary

In many soils earthworms play a major role in the decomposition of organic matter and the recycling of nutrients. Earthworm populations in acidic soils tend to be small and this can result in a breakdown in nutrient cycling processes, and the development of a thick mat of undecomposed plant material.

In this study earthworm communities were modified by the introduction of three species, representing different ecological groups, into enclosed boxes of limed and unlimed soil at a site in the Scottish borders. The survival of the introduced communities in these two soil types was examined and their effects on soil carbon, specifically its chemical characteristics and distribution through the soil profile examined.

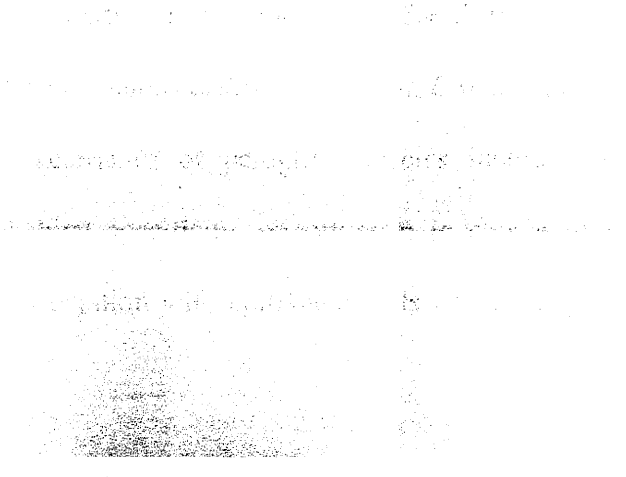
Liming resulted in the increase of one species only, *Allolobophora chlorotica*, and results indicate that this effected a reduction in the thickness of the litter layer present at the soil surface. This is not behaviour associated with this endogeic species in the UK, although similar observations for other endogeic species have been recorded in Australia and the Netherlands.

Cross Polarization Magic Angle Spinning ^{13}C NMR spectroscopy was used on soils collected from this field experiment, and on cast material collected in a laboratory microcosm experiment, to determine the chemical characteristics of carbon. This showed that the direct impact that earthworms have on the decomposition of organic material is small, and their important effects are in the incorporation, comminution and mediating further microbial decomposition of the organic material. Cast material contains a relatively smaller concentration of easily assimilated carbohydrate rich material than the soil, and a concomitant increase in less easily degradable carbon compounds and microbial metabolites. Any small differences between species can be related to the quality of organic

matter the earthworm ingested. Microbial activity in casts was greater when the ingested organic material was of a high quality.

In this study the conclusions are:

- The liming of acidic soil does not result in an increase in the abundance of all earthworm species, and the results are dependant on intraspecific competition;
- There was no significant impact on plant yields of an increased size of earthworm community;
- Large size fractions from limed soil appear more decomposed due to an increase in earthworm, and therefore microbial, decomposition, and;
- There is little difference in the chemical composition of cast material from different earthworm species, and what differences there are can be directly related to the quality of organic material ingested by the earthworm.



Chapter 1 Introduction

1.1 Lumbricid earthworms

Earthworms are widely distributed throughout temperate and tropical regions, and where environmental conditions allow, they dominate the invertebrate biomass (Curry, 1994). The two most important earthworm families ecologically in Europe, North America, Australia and Asia are the Megascolecidae and the Lumbricidae, with the Lumbricidae the dominant endemic family in the Palearctic zone, including Europe. Because of their ability to colonize new soils and become dominant, to the near exclusion of local endemic species, the Lumbricidae have followed the spread of human colonization from the more developed countries around the world. The earthworm populations in crop-growing areas in temperate regions are far more likely to consist of Lumbricidae than of members of any other family (Curry, 1994).

There are about 220 species of lumbricids of which about 19 are common in Europe (Edwards and Bohlen, 1996). Nearly 100 species of earthworms, *circa* 3% of all known species are peregrine species. The most widespread of these species include 20-30 species of the Lumbricidae, particularly of the genera *Lumbricus, Aporrectodea, Allolobophora, Eisenia, Dendrobaena, Dendrodrilus, Octolasion, Eiseniella* and *Bimastos* (Lee, 1985).

Special characteristics of peregrine species include tolerance of environmental variability, low habitat specificity, opportunism in choice of food, ability to withstand chemical stress, association with cultivated soils and ecological plasticity (Edwards and Bohlen, 1996).

1.2 Earthworm ecological groups or vertical distributions

There have been several systems proposed to classify earthworms into major ecological categories (Edwards and Bohlen, 1996 *op cit* Lee, 1959; Piearce, 1972). The basis of these classification systems include differences in food preferences, burrowing behaviour and

reproductive rates which represent functional adaptations which can allow different species of earthworms to coexist in many soil environments, by exploiting different food resources and habitat space (Edwards and Bohlen, 1996). Table 1.1 shows the main diagnostic features of European Lumbricid earthworms as defined by Bouché (1977).

Table 1.1. Diagnostic characteristics of the major ecological groups of European Lumbricid earthworms, from Edwards and Bohlen (1996).

Diagnostic character	**Epigeic species**	**Anecic species**	**Endogeic species**
Food	Decomposing litter on soil surface; little or no soil ingested	Decomposing litter on the soil surface some of which is pulled into burrows; some soil ingested	Mineral soil with preference for material rich in organic matter
Pigmentation	Heavy, usually both ventrally and dorsally	Medium-heavy, usually only dorsally	Unpigmented or lightly pigmented
Size of adults	Small-medium	Large	Medium
Burrows	None; some burrowing in upper few centimetres of soil by intermediate species	Large, permanent, vertical burrows extending into mineral soil horizon	Continuous, extensive, subhorizontal burrows, usually in the upper 10-15 cm of soil
Mobility	Rapid movement in response to disturbance	Rapid withdrawal into burrow but more sluggish than epigeics	Generally sluggish
Longevity	Relatively short-lived	Relatively long-lived	Intermediate
Generation time	Shorter	Longer	Shorter
Drought survival	Survives drought in the cocoon phase	Becomes quiescent during drought	Enters diapause in response to drought
Predation	Very high, particularly from birds, mammals and arthropods	High, especially when they are at the surface; somewhat protected in their burrows	Low, some predation by ground-dwelling birds and predatory arthropods

These ecological groups are broad classifications, and there are numerous species intermediate to these categories, for example, *Aporrectodea longa* (Ude). In Bouché's

(1977) classification system, *A. longa* is classified as an anecic species. However Trigo *et al.* (1999) found that that it ingests a mixture of relatively undecomposed plant litter (grass leaf fragments and seeds) and amorphous humus, i.e. it is intermediate between detritivory and geophagy. However, Bouché's (1977) classification system is the most practical to use in this piece of work, as it groups species on where in the soil they burrow and feed, and their feeding preferences.

Epigeic species live in the litter layer or in the upper few centimetres of organic horizons or in temporary deposits of organic matter, without a permanent burrow system, and feed on relatively undecomposed plant litter. They are heavily pigmented r- strategists, balancing a high mortality rate by feeding on high quality resources which permits high growth rates and high fecundity (Lavelle and Spain, 2001). Examples are *Dendrobaena octaedra* (Savigny), *Lumbricus castaneus* (Savigny) and *Lumbricus rubellus* Hoffmeister.

Anecic species form semi-permanent or permanent burrow systems in the mineral soil which open at the soil surface, which is where the earthworm collects relatively undecomposed plant litter. Feaces are deposited at the opening of the burrow at the soil surface in middens, or within the burrow system. An example is *Lumbricus terrestris* Linnaeus.

Endogeic species form horizontal, continuous burrow systems in the mineral soil which is better buffered and more predictable than the litter environment, and are geophagous, feeding on organic material associated with mineral soil particles and around plant roots. Examples are *Allolobophora chlorotica* (Savigny), *Aporrectodea caliginosa* (Savigny) and *Octolasion cyaneum* (Savigny).

There are however a few species which fall outside these earthworm classification systems and occupy specialised habitats outwith soil.

1.2.1 Specialised habitats

Megascolecid earthworms have been found under the bark of trees, in rotting logs, in swamps, lakes and under stones and debris in the intertidal zone of the sea shore (Lee, 1985). Lumbricid species are also found outwith the soil. *Eiseniella tetraedra* (Savigny) is adapted to aquatic habitats and is commonly found under stones in streams and lakes (Lee, 1985). Many terrestrial species are occasionally found in aquatic habitats as most earthworms can survive for periods in oxygenated fresh water. *A. chlorotica,* for example, has been recorded at a depth of several metres in Lake Windermere (Lee, 1985).

1.3 Earthworm interactions with soil organic matter

Earthworms play a major role in the decomposition of organic matter and the release and recycling of the nutrients (Huhta, 1979). They can influence the spatial variability of resources, altering the availability to micro-organisms, thereby regulating nutrient cycling processes (Schuster *et al.*, 2001). In many soils they are probably the most important invertebrates in the initial stages of organic matter decomposition (Edwards and Bohlen, 1996).

Decomposition is "a cascade process whereby primary dead organic material experiences a succession of physical and chemical transformations in the soil leading to mineralization of part of the resource, and storage of resistant compounds as humus" (Lavelle *et al.,* 1997 *op cit* Swift, Heal and Anderson, 1979). The organic carbon in soils is a complex mixture of decomposing plants, microbial and animal remains, their waste metabolites and newly synthesized compounds, which interact with the inorganic components of soil (Hopkins *et al.*, 2000).

Decomposition is assumed to be mainly mediated by micro-organisms such as bacteria and fungi, but the activity of micro-organisms is accelerated by the activity of soil fauna, for example, earthworms enhancing the access of micro-organisms to leaf litter by

incorporating it into the soil (Edwards and Bohlen, 1996).

While feeding, epigeic and anecic species incorporate large quantities of partially decomposed plant litter and other organic detritus in to the mineral soil (Lee, 1985). During feeding, the organic material is ingested, moistened with intestinal mucus, acted upon by enzymes and comminuted (fragmented), before being egested as cast material. These processes 'prime' the organic matter in casts for further subsequent decomposition by microorganisms (see Section 1.3.2). The cast material is egested either at the soil surface, in existing void spaces in the soil, or within their own burrow system. The drilosphere (burrow lining) also becomes enriched in organic matter relative to the uningested soil material, with epidermal mucus and urine secretions.

1.3.1 Physical incorporation of organic matter

The effects of different worm species on the incorporation of organic material into the mineral soil depends on their feeding and burrowing behaviour. Epigeic species feed on partially decomposed plant litter, and the egested cast material is composed of comminuted, but otherwise relatively unaltered plant material (Ziegler and Zech, 1992). They demonstrated the effects of *Eisenia fetida* (Savigny) gut passage on beech litter. The egested cast consisted of thoroughly comminuted plant residues, but ^{13}C NMR spectroscopy revealed that it was minimally altered compared to the uningested litter with respect to the main carbon functional groups. The age of the beech litter is not stated, but Bonkowski *et al.* (2000) state that leaf litter, like beech, which has a high concentration of secondary metabolites, is only usefully consumed after a year of exposure to microbial decomposition.

The impacts of epigeic earthworms on the redistribution of organic matter deeper in

the mineral soil are not as great as the effects of anecic species (Schuster *et al.*, 2001). The impact of anecic species has been called the 'the anecic effect' (Guggenberger *et al.,* 1996 *op cit* Lavelle, 1988). The litter is mixed with mineral soil during gut passage, deposited either as an accumulation of cast material ('midden') at the soil surface or within their burrow system, or other soil void spaces. Anecis species can have a profound effect on the incorporation of raw and processed organic material into the mineral soil horizons (Edwards and Bohlen, 1996; Lavelle *et al.,* 1997), and also on soil structure, as burrows can be important in moving water and solutes into the lower soil horizons (Blair *et al.*, 1995).

Activity of species such as *L. terrestris* account largely for the overall fragmentation and incorporation of litter in many temperate woodlands, and they are primarily responsible for the formation of mull soils where the surface litter and organic layers are thoroughly mixed with the mineral soil (Edwards and Bohlen, 1996).

Geophagous endogeic species live in burrow systems which only occasionally open at the soil surface. They continuously extend their horizontal burrows whilst feeding selectively on highly decomposed, amorphous organic material associated with the inorganic soil particles (Bonkowski *et al.,* 2000 *op cit* Judas *et al.,* 1997), and decaying roots and leaves (Edwards and Bohlen, 1996). The result of activity by endogeic species can be a more uniform distribution of organic and inorganic soil components (Scheu and Parkinson, 1994). Where endogeic and anecic species occur together they can have a synergystic effect on the redistribution of organic matter within the soil profile, with a more even distribution throughout the soil matrix than when present alone (Shaw and Pawluk, 1986).

1.3.2 Priming effects

'Priming' can be described as accelerated soil organic matter decomposition, caused by an increase in extra-cellular enzymes produced by microorganisms, due to the addition of organic substrates, such as those found in the rhizosphere, or those produced by earthworms (Lavelle and Gilot, 1994; Bohlen *et al.,* 2002). Large amounts of mucus secreted in the anterior of the earthworm gut are mixed with the ingested organic matter. The mucus is a mixture of low-molecular-weight water soluble compounds, including amino acids, sugars and glycoproteins. Concentrations (as a % of the dry weight of soil ingested) vary from 15-18% across pan-tropical species, to 42% in Lumbricidae in northern Spain.

The effect of intestinal mucus extracted from the tropical geophagous earthworm *Millsonia anomala* on soil microorganisms was examined by Lavelle and Gilot (1994) *op cit* Gilot (1990). Microbial activity increased rapidly, reaching a peak after 45 minutes before falling to values similar to control soil within a further 60 minutes. In soils amended with glucose, the respiratory activity increased throughout the 2 h experimental period. The authors suggest that the rapid increase in activity in the presence of the intestinal mucus could be an adaptation to the specific conditions of earthworm digestion. This rapid activation is critical to allow formerly dormant microorganisms to recover their ability to digest soil organic matter, as most microorganisms remain in a relatively inactive state in the soil due to constraints in C availability (Bohlen *et al.,* 2002 *op cit* Paul and Clark, 1989). Organisms which sharply increase their metabolic activity by feeding on mucus in the earthworm intestines are able to digest soil organic matter at an increased rate, up to 30 times higher, compared to conditions in bulk soil (Lavelle *et al.*, 1997). These increases in microbial activity are temperature dependent. At 28°C microbial respiratory activity was 6-10 times higher than in bulk soil, but was limited to 2 times higher than bulk soil at 15°C,

and Lavelle *et al.* (1997) hypothesize that the increased amount of intestinal mucus produced in temperate Lumbricidae could compensate for this temperature mediated limitation. This is corroborated by work done by Trigo *et al.* (1999), who used specimens of the epigeic species, *Eisenia andrei* Bouché, from tropical and temperate conditions and found that in temperate conditions, more mucus was added to the ingested organic material compared to tropical conditions. This was also true for endogeic species. In the middle and posterior gut, mucus is mostly absent, either metabolised by microflora or reabsorbed through the gut wall, as its production represents a high energetic cost to the earthworm, although some mucus does remain in the egested cast.

The impact of gut passage on soil microorganisms is complex, and may depend on the resource quality of the organic matter the earthworm feeds on, and results obtained can vary between species belonging to different ecological groups, both due to different food preferences and gut residence times. Piearce (1978) found that the passage of ingested material is more rapid through the gut of endogeic species' compared to epigeic species'.

1.3.3 Fungi, protozoa and bacteria as food sources

The relationship between earthworms and soil microorganisms is a complex one with many factors impacting on it, including the quality of the organic matter the earthworm feeds on.

Changes in the numbers of bacteria and microfungi during gut transit were investigated in 2 earthworm species from contrasting ecological groups by Kristufek *et al.* (1992). They found that numbers of microorganisms increased during passage through the gut of the epigeic *L. rubellus,* while in contrast, passage through the gut of the endogeic *A. caliginosa* resulted in a decrease. They suggest that this difference may be as a result of the different chemical composition of the ingested food material. The litter consumed by *L.*

rubellus is rich in readily available chemical compounds, resulting in a stimulation of bacterial growth in the earthworm gut. As organic residues decompose, the more readily degradable components such as sugars and proteins are removed leaving cellulose, hemicellulose and newly synthesised, recalcitrant microbial metabolites (Kristufek *et al.*, 1992; Martin and Lavelle, 1992). Therefore, the humified organic material ingested by *A. caliginosa* cannot similarly stimulate gut bacteria, and so they are digested as a nutrient source.

Zhang *et al.* (2000) presented evidence for the tropical earthworm *Metaphire guillelmi* digesting soil microorganisms. *M. guillelmi* is described as an anecic earthworm, but which feeds on partially decomposed organic materials rich in micro-organisms rather than fresh organic residues. This could therefore be considered as a species which does not fit directly into one of Bouché's (1977) three broad ecological classifications.

The low levels of cellulolytic activity and the relatively high concentrations of protease and phosphatases found in the in the gut of *M. guillemi* suggests that that the plant residues are used as a primary resource by soil microorganisms, which are in turn digested in the earthworm gut. This is supported by findings which show a reduction in the total microbial biomass after gut passage, but interestingly, an increased respiratory quotient (qCO_2), indicating a smaller but more metabolically active microbial community. Higher levels of cellulase activity were found in the gut of *Eisenia fetida*, which may indicate that this species uses the plant litter directly as a food source. Zhang *et al.* (2000) also report other authors finding higher levels of cellulases in epigeic species compared to geophagous endogeic species.

Lavelle *et al.* (1980) experimented with different quality litter and found at low resource quality microbial activity appeared stimulated, with an increased respiratory quotient (qCO_2) compared to uningested soil, while at high resource quality, qCO_2 was

lower. These results are supported by work presented by Blair *et al.* (1995) who suggested that if soil contains low quality organic matter, *i.e.* more recalcitrant material, then gut passage, with its supply of readily metabolised mucopolysaccharides will have a large stimulatory effect on the metabolism of ingested microorganisms. When resource quality is high, the passage through the earthworm gut will not have a large effect on microbial activity as the microorganisms are already active.

Bonkowski *et al.* (2000) suggest that the prevalence of earthworms feeding selectively on soil fungi (e.g. Trigo *et al.,* 1999; Wolter and Scheu, 1999; Zhang and Schrader, 1993; Cooke, 1983; Zhang *et al.,* 1993) is not only as a major source of food, but also as a cue for detecting fresh inputs of organic resources in soils. They demonstrated that the fungal species preferentially selected by epigeic and anecic lumbricid species are primarily those which are characteristic of early successional stages of decomposition, and indicate a new resource for litter feeding earthworms to exploit within the heterogeneous soil environment. In comparison, geophagous endogeic species were less selective about the fungal species they consumed. A hypothesis was proposed by Wolter and Scheu (1999) suggesting that along with feeding directly on the bacteria and fungi, the earthworms may select microsites of high microbial activity because they benefit from soluble resources made available by exoenzymes produced by microorganisms.

The evidence for earthworms feeding on bacteria, fungi, algae and protozoa is numerous e.g. (Cooke and Luxton, 1980; Piearce, 1978). The presence of protozoa (mainly flagellates and ciliates) in the diet of *E. fetida* was found to be essential for this species to reach sexual maturity (Bonkowski and Schaefer, 1997 *op cit* Miles, 1963). In a multiple choice feeding experiment, *A. caliginosa* distribution was significantly correlated with numbers of amoebae in soil, indicating a preference and active selection of soil microsites of high protozoan densities. Over the twenty days of the experiment, earthworm weight

gain was increased by 40% relative to earthworms in soil without amoebae (Bonkowski and Schaefer, 1997).

Oligonucleotide probes and epifluorescence microscopy were used by Cai *et al.* (2002) to investigate the impact of *L. terrestris* gut passage on cysts and vegetative cells of *Acanthamoeba sp.*, an ubiquitous soil protozoan. Vegetative cells were disrupted in the anterior part of the earthworm digestive tract, while cysts passed through the digestive tract morphologically intact. This reflects findings by Piearce and Phillips (1980) who examined the fate of a common freshwater ciliate (*Colpidium campylum)* in fluids extracted from different parts of the gut of *L. terrestris*. Pharyngeal and hindgut fluids did not affect the behaviour of the *C. campylum*, but even low concentrations (1.5%) of midgut fluid resulted in abnormal movement, and adding ciliate culture to an equal volume of midgut fluid resulted in immediate immobilization and frequently, the disintegration of ciliate cells.

1.3.4 Gut microflora

1.3.4.1 Indigenous microflora

A study by Parle (1963a) did not find any evidence that earthworms have a specialised gut microbial community quantitatively different to that in the soil. However, more recent work in the area has provided evidence of an indigenous gut microbial community. An examination of the microbial community in the gut of *L. terrestris* by scanning electron microscopy (SEM) revealed filamentous organisms attached to gut epithelium, with 'socket-like' structures, similar to those by which segmented, filamentous bacteria are attached to the epithelium of rats and mice (Jolly *et al.*, 1993). The authors suggest that while other gut-associated microorganisms were found, e.g. cocci and rod-shaped cells, only the attached filamentous forms were likely to be indigenous. Morphologically similar

bacteria were found in the hindgut of *E. fetida* (Vinceslas-Akpa and Loquet, 1995).

Further evidence for an indigenous gut microbial community in *E. fetida* was provided by Toyota and Kimura (2000). *E. fetida* specimens were collected from farmyard manure and casts from these individuals were compared to casts from worms collected and then starved in distilled water for 24 hours. Their results suggested that the microbial community in the unstarved group was similar to that in the farmyard manure, but the community found in the starved group represented a community indigenous to the earthworms, which were all fast growing, Gram-negative fermentative bacteria, which were not detected in the substrate.

It is important to remember however, that many studies in this area are based on growth-dependant analysis approaches, where results may be affected by the difficulty of growing microorganisms, or the differentiation between vegetative and dormant stages (Cai *et al.,* 2002; Winding *et al.,* 1997).

1.3.4.2 Mutualistic relationships between lumbricid earthworms and bacteria

Although numerous enzymes that catalyse the digestion of soil organic matter have been found in lumbricid earthworms, the origin of these enzymes- whether produced by the earthworm or by microorganisms, is not clear (Lattaud *et al.*, 1999).

Urbášek (1990) showed for a range of epigeic and endogeic species including *Dendrodrilus rubidus* (Savigny), *D. octaedra, L. castaneus, L. rubellus, Aporrectodea rosea* (Savigny) and *A. caliginosa*), that all species could break down cellulose, although activity was higher in epigeic species which ingest more cellulose than endogeic species. However, *L. rubellus* had an unusually low cellulase activity which the author presented as evidence of symbiotic cellulolytic microorganisms in the gut of this species. For the other earthworms included in the study, it was suggested that microbial cellulases have only an

accessory role. Unpublished work by the same author was also discussed, which showed higher numbers of cellulolytic microorganisms in fresh casts of *L. rubellus* compared to the epigeic species *D. octaedra.*

Lattaud *et al.* (1999) showed that *E. fetida* produces a cellulase that catalyses the hydrolysis of cellulose to cellobiose, and the ingested soil microflora secreted cellobiase to catalyse the breakdown of cellobiose to *D*-glucose. This is an interesting contrast to the tropical species *M. anomala* and *Hormogaster elisae* (Eudrilidae), where ingested soil microflora degrade cellulose to cellobiose, and the worm then secretes cellobiase to degrade cellobiose to glucose.

1.3.4.3 Mutualistic relationships between tropical earthworms and bacteria

The theory of mutualism between tropical geophagous earthworms and ingested soil microflora has been established (Lavelle and Gilot, 1994 *op cit* Zhang *et al.,* 1992; Lattaud *et al.,* 1997). Zhang *et al.* (1993) showed that cellulase and mannanase activity in the gut of *Pontoscolex corethrurus* (Glossoscolecidae) were not found in cultures of isolated gut tissue, and therefore concluded that they were produced by ingested microflora. This was also demonstrated for *Millsonia anomala* (Lattaud *et al.*, 1999). A mutualistic association allows worms to exist in soils poor in organic matter. Microorganisms are supplied with water and nutrient rich mucus, along with suitable physico-chemical conditions, enabling them to rapidly recover the ability to degrade and render the soil organic matter digestible to the earthworm. It has been estimated that 3-19% of the ingested soil organic matter can be assimilated during a single transit of the gut (Lavelle *et al.*, 1997). The amount of intestinal mucus produced is inversely proportional to the relative organic matter content of the substrate, with a higher substrate organic matter content resulting in a lower relative enrichment with intestinal mucus, as it is not as necessary for the earthworm to induce a

stimulation of the soil microorganisms (Trigo *et al.*, 1999).

1.3.5 Microbial activity in egested casts and the drilosphere

Data collected on microbial activity in earthworm casts are often contradictory, and appear to depend on soil type, initial resource quality and age of casts. It has been reported that earthworms increase the size of the active microbial biomass in casts and drilosphere soil (Scheu, 1992) along with total microbial biomass e.g. (Parle, 1963b; Binet *et al.*, 1998) and as a consequence, bacterivorous soil organisms (Andrén *et al.*, 1988). Where the soil microbial community is enhanced by passage through the earthworm gut, it is biomass of bacteria rather than fungi which is increased e.g. Parle (1963b); Scheu and Parkinson (1994); Winding *et al.* (1997).

Soil bacteria which colonize freshly deposited substrata generally fix a smaller amount of carbon in their cells and have a higher turnover rate, in comparison to fungi which tend to dominate the microbial biomass in undisturbed soils. This initial phase of microbial succession is also accompanied by an intense mineralization of organic compounds. Fischer *et al.* (1997) showed a significant increase in the number of vegetative cells and a concomitant reduction in the number of bacterial spores of the soil bacterium *Bacillus megaterium* after passage through the gut of *L. terrestris,* and concluded that germination is enhanced by gut passage. They suggest that this was due to nutrient release from either the ingested material or intestinal mucopolysaccharide secretions, an alteration in the physical environment, e.g. moisture levels, pH, or simply by mechanical disruption of the spore coats.

Tiunov and Scheu (1999) investigated the effects of *L. terrestris* in a lime-oak-beech forest, and found that microbial biomass and respiration were greater in drilosphere soil compared to control soil. The growth response to nutrient additions was faster in the

drilosphere than the control soil, and the authors suggest that the microbial community contained a larger fraction of metabolically active microorganisms, adapted to continuous resource additions by earthworm faeces and epidermal mucus secretions. This suggests that *L. terrestris* burrow walls are stable microhabitats which sustain a large and active microbial community. Lavelle and Spain (2001) found that while the drilosphere of anecic earthworm species comprises a relatively small part of total soil volume, it may contain 5-25% of the whole soil microbial community, with a predominance of certain functional groups, typically 13% anaerobic N fixers, 16-40% denitrifiers, over 40% proteolytic and aerobic N fixers and more than 60% of the hemicellulolytic and pectinolytic bacteria.

There are conflicting reports where authors show that passage through the earthworm gut reduces the size of the microbial community in the freshly deposited cast (Zhang *et al.*, 2000), or has no impact (Daniel and Anderson, 1992). Haynes *et al.* (1999) report contradicting results for two soils, with *A. caliginosa* casts from a pasture soil showing a reduced microbial biomass, and casts from an arable soil showing an increased microbial biomass, while both have a higher respiratory quotient compared to bulk soil. Many other authors (Zhang *et al.*, 2000; Görres *et al.*, 1997; Wolters and Joergensen, 1992) also report an increase in the respiratory quotient of casts compared to soil, even when there is a reduction in the size of the microbial biomass. Coupled with an increase in microbial respiration, this is indicative of a smaller, more metabolically active community.

Haynes *et al.* (1999) observed a decrease in the amount of readily available C in cast material of *A. caliginosa* from an unamended arable soil. They suggest that the relative shortage of available C led to a microbial community more efficient at immobilizing C, and observed a smaller metabolic quotient compared to the uningested soil. Where the soil was amended with wheat residues they report an increase in the basal respiration rate and a larger respiratory quotient in cast material.

1.3.5.1 Bacterivorous organisms as indicators of bacterial activity

Measurement of the size of the bacterivorous community may be used to provide an estimate of bacterial activity rather than direct measures of bacterial biomass (Andrén *et al.*, 1988). An increase in bacterivorous protozoa abundance was observed by Winding *et al.* (1997) in response to increased microscopic bacterial counts in the presence of the epigeic *L. festivus.* Similarly, Binet *et al.* (1998) found a stimulation of microbial activity in *L. terrestris* casts shown by a significant increase in both soil respiration (around 50% increase), and a 3- 19% increase in the population density of a bacterivorous protozoan. A biomass increase in both bacterial feeding nematodes and collembolas in middens of *L. terrestris* in a beechwood was described by Maraun *et al.* (1999), again indicating an increased bacterial biomass.

1.3.5.2 Ageing casts

Parle (1963b) found that while bacterial counts did not decline within ageing casts, there was a decline in microbial respiration, which may be due to the formation of bacterial resting stages. The growth of fungal hyphae increased as casts aged, and other authors report an increase in the fungal: bacterial biomass ratio in ageing casts e.g. Zhang *et al.* (2000), with the fungal content largely determining the structural stability of cast material (Parle, 1963b; Marinissen and Dexter, 1990).

Ageing casts can contain 7 times more N than bulk soil (Haynes *et al.*, 1999) due to the excretory products, urine from earthworms, and increased rates of mineralization of soil organic N by micro-organisms (Edwards and Bohlen, 1996). There is also up to 5-10 times more extractable phosphorus in ageing casts compared to soil (Lee and Foster, 1991), which can impact on plant growth (see section 1.7.2).

Incubating cast material for even a short period can change the size, activity and

composition of the microbial community, and most authors do not state the age of the casts used in their experiments.

1.4 Earthworm impacts on soil physical properties

Feeding and burrowing by earthworms affects soil structure, and they have, along with termites, been described as ecosystem engineers because of their earth-moving activities (Lavelle *et al.*, 1997). Direct impacts on soil physical properties include improved water infiltration and gas exchange, especially through the activity of anecic species, with their near vertical burrows e.g. (Cannavacciuolo *et al.*, 1998). However, the large macropores which result from the extensive burrowing of endogeic species activity are also important for water movement and gas diffusion (Lee and Foster, 1991) (Figure 1.1).

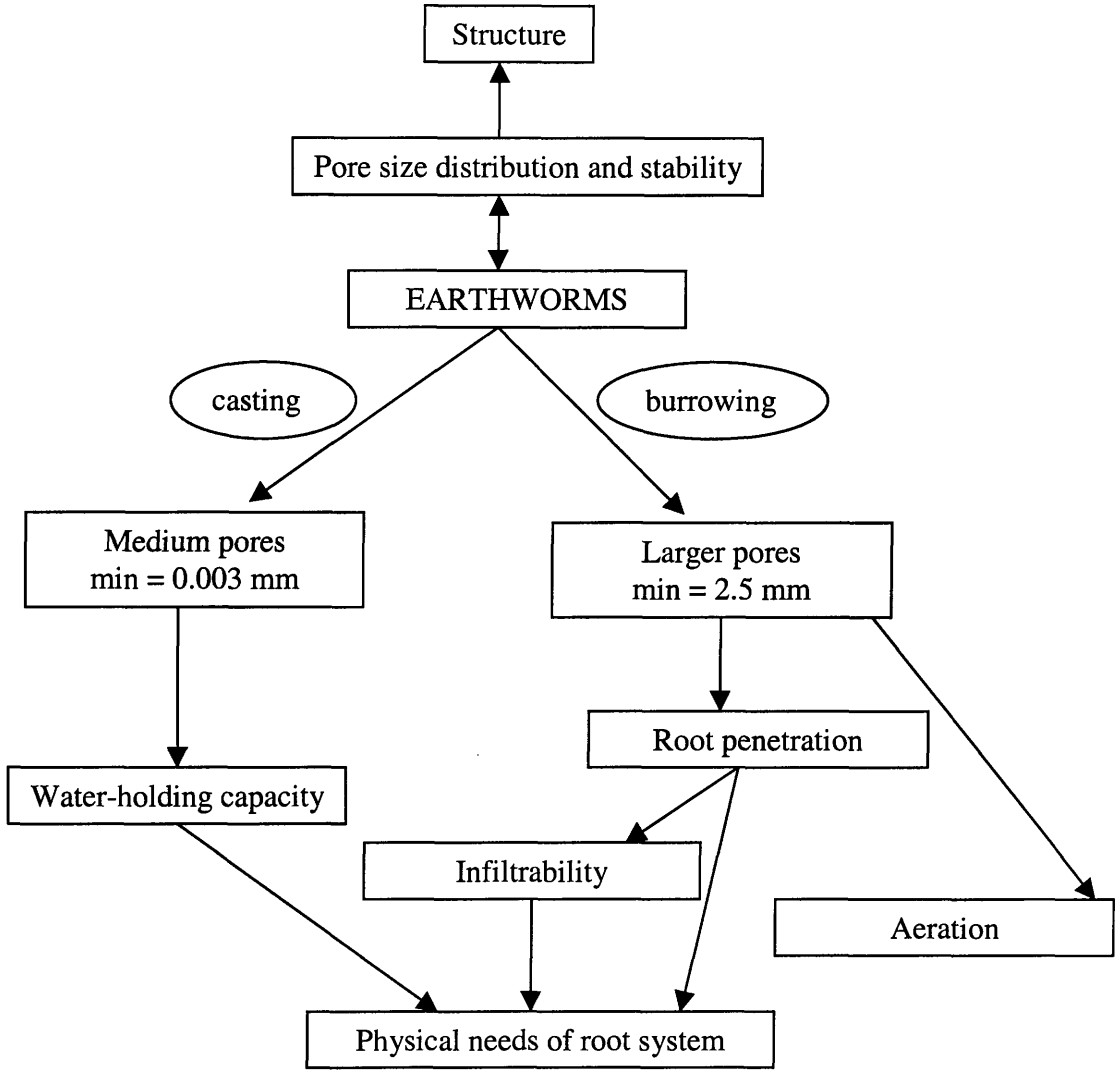


Figure 1.1 Interrelationship of soil physical characteristics as influenced by earthworm activities from Syers and Springett (1983).

Earthworm casts can make an important contribution to soil aggregation, the adhesion of mineral granules and soil organic matter that resist breakdown when exposed to internal or external stresses (wetting, drying, compaction, etc.), and can comprise the majority of structural aggregates in the upper 10-20 cm of soil (Lee and Foster (1991); Tisdall *et al.* (1997). As casts age they become colonised by fungi, which help enmesh microaggregates in the soil in to larger stable aggregates (Tisdall *et al.*, 1997). Earthworms have been described as "the chief agents responsible for the crumb structure and mull formation typical of fertile soils" (Huhta, 1979).

It is outwith the scope of this thesis however, to fully discuss the effects of earthworms on soil structure. A fuller account can be found in Lee and Foster (1991), Edwards and Bohlen (1996) or Lee (1985).

1.5 Earthworms and acidic soil conditions

1.5.1 pH and calcium

A number of earthworm species are intolerant of acidic soil, while others are commonly found in these conditions. Guild (1948) observed that acidic grasslands support small earthworm communities with low species diversity and abundance, but noted that *L. rubellus, D. octaedra* and *D. rubidus* were more tolerant of these conditions than the majority of lumbricid species, and frequently dominated acid soils. In a study examining the distribution and population density of earthworms in Scottish pasture fields, Guild, (1951) found that in highly acidic hill farm pastures, *A. longa* and *A. caliginosa* were usually absent. More recent work has shown that epigeic species absorb calcium efficiently across the walls of the intestine and can therefore inhabit areas with lower calcium concentrations than endogeic species (Persson, 1988) (see later). Earthworms can therefore be grouped according to their tolerance of acidic conditions (Figure 1.2).

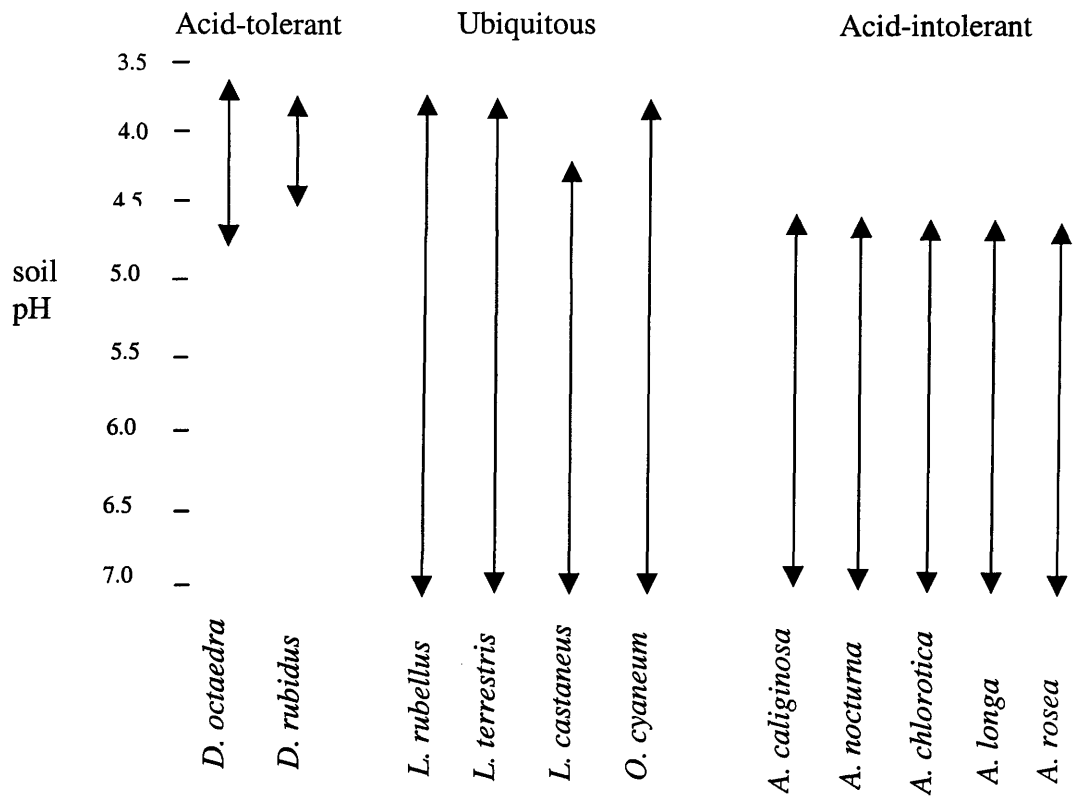


Figure 1.2 Classification of earthworms as a function of the soil pH after Satchell, (1955)

Earthworms classed as acid intolerant are generally found in soils with a pH > 4.5, and include *A. caliginosa, Aporrectodea nocturna* (Evans), *A. chlorotica, A. longa* and *A. rosea.* These are all endogeic earthworms, with the possible exception of *A. longa* which displays intermediate endogeic/anecic behaviour (see section 1.2). Acid tolerant species, which are often found in soils with a pH < 4.5, include *Lumbricus eiseni* Levinsen, *D. octaedra and D. rubidus*, which are all epigeic species which tend to be small and highly mobile species. Earthworms which are found in soil over a wide range of acidic soils (pH 3.5-7) are described as ubiquitous and include *L. rubellus, L. castaneus, O. cyaneum* and *L. terrestris* (Satchell. J.E., 1955).

The ubiquitous group includes earthworms of all three ecological classifications, although with the exception of *O. cyaneum,* all are litter feeding species, which tend to be

small and highly mobile, which may enable them to exploit a range of microhabitats, such as the dung of grazing animals.

Earthworms can influence the pH of their immediate surroundings. Schrader (1994) introduced representatives of acid intolerant, acid tolerant and ubiquitous species, *A. caliginosa, L. rubellus* and *L. terrestris,* into agar media treated with pH indicators and adjusted to different pH conditions. After 90 minutes, the alkaline medium was neutralised by *A. caliginosa*, after 19 h by *L. terrestris* and the acid tolerant *L. rubellus* took 23 h. All species changed the colour of bromothymol blue i.e. from pH 6.0 to pH 7.6 in 17-20 hours. The most acidic medium at pH 4.2, was mixed with methyl red which changes colour at pH 6 and this took the longest time to change. The ubiquitous and acid tolerant species affected this change within 5-6 days but *A. caliginosa* did not change the colour of the medium and died after 4 days. The author reports findings by Günther and Greven (1990) who demonstrated an increase in the number of epidermal mucus secreting gland cells in *L. terrestris* when exposed to acidic conditions. After 5 days at pH 4.0 there was a significant increase in the number of these cells, i.e. the same length of time it took to begin neutralizing the acidic medium.

Satchell (1955) suggested that extreme soil acidity was more important in limiting the distribution of earthworms than deficiencies in calcium supply. However, Piearce (1972) suggested that pH may not be the most important factor. Calcium and pH are highly correlated over a wide range of soil types, and litter feeding species have very active calciferous glands. Proposed functions of the calciferous glands include regulation of blood pH, ionic and osmotic regulation and excretion of excess calcium absorbed from the gut (Lee, 1985). It has been demonstrated (Piearce, 1972) that the litter feeding, ubiquitous *L. rubellus* absorbs more calcium from its food than does the geophage *A. caliginosa. L. rubellus* may therefore require a specialised physiological mechanism i.e. active

calciferous glands to secrete excess calcium from the body tissues to the gut, while this is simply not required in *A. caliginosa*. Active calciferous glands in some species contain large quantities of carbonic anhydrase, an enzyme which is important in acid-base reactions. Metabolic activity produces CO_2 which can increase the acidity of the coelomic fluid. The carbonic anhydrase in the calciferous glands can counteract this by catalysing CO_2 precipitation as calcium carbonate. Experimental removal of these glands resulted in a lowering of coelomic pH (Edwards and Bohlen, 1996).

Stockdill (1982) observed that small populations of earthworms are associated with low calcium levels on grazed pasture land and that applications of lime of 2.5 t ha^{-1} increased worm numbers by 50%.

1.5.2 Effect of lime application

The application of lime to acidic soils can result in increases in cocoon production and hatching success, increased juvenile growth rates and immigration, and reduced mortality rates which result in higher population growth at high pH (Persson, 1988; Robinson *et al.*, 1992a). As well as directly increasing soil pH, the addition of lime can also stimulate microbial growth due to increases in the availability of soluble carbon sources in the upper soil horizons (Kalbitz *et al.*, 2000). At low pH, aluminium which adsorbs to organic matter is mobilized and can flocculate dissolved organic carbon (DOC) into particulate form. Liming can increase DOC concentrations by reducing the solubility of aluminium and therefore the precipitation of DOC (Grieve, 1990). This increase in the microbial community may be exploited directly by the earthworms, or the earthworms may graze on microbivorous protozoa as discussed in Section 1.3.3.

Six years after liming plots in a spruce forest, Huhta (1979) found that inoculated *A. caliginosa* were abundant in limed plots, and in greater numbers than were inoculated. In

comparison, none were found in the unlimed plots. Similarly, Robinson *et al.* (1992a) introduced *A. caliginosa* into limed and unlimed peat monoliths under a stand of *Picea sitchensis.* In the limed monoliths (pH 5.7), earthworm biomass was maintained over a 12 month period and cocoons were produced, compared to only 4% biomass maintained in the unlimed treatments (pH 4.0) and no cocoon production.

Robinson *et al.* (1992b) introduced a mixed species assemblage of earthworms into a limed (pH 6.1) and unlimed (pH 4.0) deep peat soil under a stand of *P. sitchensis.* After one year, only *D. rubidus* was recovered, and only in the limed plots. Inoculated *A. longa, L. rubellus, L. terrestris* and *A. caliginosa* were not recovered. Two uninoculated species were found in the limed plots (*D. octaedra* and *L. eiseni*), with *L. eiseni* the numerically dominant species, accounting for 61% of total earthworm numbers. The earthworms recovered were small acid tolerant or ubiquitous epigeic species. As the pH of the limed stands was only raised in the top 5 cm of soil in this experiment however, the soil at depth may still have been unsuitable for deeper burrowing species. However, under *P. sitchensis* stands in Ireland, the limed plots had a sizeable earthworm community, with 79 individuals m^{-2} in the limed stands, including acid intolerant species rare in coniferous soils, compared to 5 individuals m^{-2} in the unlimed stands.

1.5.3 Lime incorporation

Where lime is applied as a topdressing in soils without worms present, the lime appears as a surface veneer within the surface organic horizon (Stockdill, 1982; Dampney, 1985). Syers and Springett (1983) *op cit* Stockdill and Cossens (1966) found that without earthworms present, at application rates of 5 t lime ha^{-1}, pH and calcium levels were not affected below 2.5 cm, even after 4-5 years. However, in the presence of *A. caliginosa*, the

lime was incorporated through the top 20 cm of soil. Judas *et al.* (1997) observed that by inoculating *L. terrestris* into a limed spruce forest, lime was incorporated into the burrows, raising the pH compared to non-drilosphere soil. The influence of the worms was restricted to drilosphere soil, as no horizontal bioturbation was affected by endogeic species which were absent from the site.

Different species vary in efficiency of incorporating lime into soils. Syers and Springett (1983) demonstrated that *A. caliginosa,* and especially *A. longa,* mixed topdressed lime into soil in a laboratory experiment. *O. cyaneum* activity did not incorporate lime into the soil, and the distribution of lime in the presence of this species was the same as that in the control soil where no worms were added.

In a field experiment where *A. longa* was added to a population of *L. rubellus* and *A. caliginosa* in a New Zealand pasture, within 10 months lime was incorporated to a much greater depth and an increase in pH was observed, along with improved infiltration rates and soil porosity and an increase in yield (Springett, 1984).

1.6 Spatial variation in earthworm populations

Darwin (1881) observed that even within the same field earthworm distribution was not uniform, without any visible differences in the nature of the soil.

Variations in earthworm populations in Scottish hill pastures were recorded by Guild (1952), who suggested that local variations in physico-chemical factors (e.g. soil moisture, temperature, acidity, texture) and food availability (herbage cover and organic matter content) were responsible for the aggregations observed.

Aggregations of earthworms were also reported by Satchell (1955) in more uniform plots at Rothamsted Experimental Station, where variations in the factors suggested by

Guild (1952) appeared too small to account for differences in earthworm aggregations. It is probable that these measurements have failed to reveal some combination of factors in the environment which are important in determining earthworm spatial variation. However, observed aggregated distributions may also arise as a consequence of the relationship between reproductive rate and dispersal rate. If a population reproduces more rapidly than juveniles can disperse, this can lead to 'family groups', which may overlap to produce aggregations. Satchell (1955) examined the non-uniform distribution of adult and juvenile *A. rosea,* and showed that the distribution of adults was random, while the aggregation was accounted for entirely by juvenile earthworms.

With *L. castaneus* this pattern is not seen, but as this species matures more rapidly than *A. rosea* it is probable that individuals identified as adult were newly mature, and had not yet dispersed from the 'family' groupings.

The spatial distribution of the tropical endogeic earthworm, *Polypheretima elongata* was also suggested to be of demographic, rather than environmental origin (Rossi *et al.*, 1997). Various soil variables including the proportion of clay, silt and sand and C and N content) were summarised by PCA (principal components analysis). No association between earthworm density and soil heterogeneity was found. Unlike the data presented by Satchell (1955) however, Rossi *et al* (1997) observed that the high population densities of adults corresponded to areas of lower cocoon and juvenile abundances and where adults were found at lower abundances, this corresponded to the highest numbers of juveniles. The authors suggest that intra-specific competition may lead to reduced fecundity in places where adult numbers are high.

Most studies however, tend to focus on earthworm responses to environmental variables, rather than on earthworm demographic variation. In a survey of 132 pasture fields across Scotland, with 5 samples per field, Boag *et al.* (1997), found that the

occurrence of earthworm species did not vary significantly with pH, % sand, % soil moisture and % organic matter. However, the mean pH of the soils varied only between 5.3- 5.7, and similarly the sites sampled were loamy sand or sandy loam soils. With such a narrow range of values in the variables measurements, it is unlikely that these would account for earthworm species spatial distribution.

Positive relationships between earthworm distributions and soil variables have been reported however. Nuutinen *et al.* (1998) reported wide variations in both soil properties and herbage cover in a grass-clover field in Finland. They present evidence for a relationship between *L. terrestris* and *D. rubidus* distributions and different soil textures. A lack of overlap in the distribution of these two species may also be attributable to competitive interaction as these two species are both litter feeders. However, it is important to note that worms were sampled using both the formalin extraction technique and hand sorting. Not all sample points had soil removed for hand sorting, and this was corrected for by multiplying the formalin extraction value by the mean value of the ratio of number of hand-sorted worms: number of formalin extracted worms. In a study examining the spatial distribution of earthworms, results obtained in this manner must be questioned.

The relationship between 13 species and 20 soil factors were examined by Briones *et al.* (1992), using detrended canonical correspondence (dCCA), to indicate relationships between species and soil factors. They found that species were distributed according to different preferences for organic matter and moisture contents and soil texture. Briones *et al.* (1995) using ecological profiles showed that for the majority of species (*A. caliginosa, A. chlorotica, D. rubidus, L. rubellus* and *O. cyaneum*) pH, exchangeable cations and soil texture can explain their presence in a certain habitat. To a lesser extent, % organic matter and N content are also important in *O. cyaneum* distribution, and moisture content and C:N ratio for *L. rubellus.*

Didden (2001) used CCA to examine variations in earthworm communities according to a range of physico-chemical parameters. Earthworm species richness and diversity were apparently not related to variations in soil characteristics, but variations in the abundance and biomass of individual species could be related to factors including pH, particle size distribution and the bioavailability of metal ions in grassland polluted with heavy metals.

It appears that, as discussed by Sánchez *et al.* (1997) after examining the ecological preferences of some earthworm species in Spain, contradictory results in the literature are due to areas sampled having different biotic and abiotic characteristics, different variables being analysed, and the use of differing statistical techniques. For example, soil humidity in the Sánchez *et al.* (1997) study was a controlling factor in earthworm distribution, as the study site was characterised by dry soil in the summer months, but in contrast, in wetter areas, soil moisture was not a determining factor e.g. Boag *et al.* (1997). The species present will also affect which factors are significant in explaining earthworm population variations. Species which live at the soil surface e.g. *L. rubellus,* can remain highly mobile, even when suffering from a water loss of 30% body mass (Curry, 1994). Deep burrowing species like *A. longa* and *L. terrestris,* showed greatly reduced mobility with increasing water loss. Surface dwelling species have to be more tolerant of water loss than species which can escape by burrowing or aestivating. Sánchez *et al.* (1997) also suggest however, that as local conditions vary, e.g. with altitude or latitude, earthworm species occurrence may be related to different soil factors, which indicates their plasticity and adaptation to different soil conditions.

1.7 Earthworm impact on plant communities

1.7.1 Plant yields

The mechanisms by which earthworm activity can impact on plant growth are the alteration of soil structure, impacting on the rate if nutrient cycling, and mineralization and altering the dynamics of soil microbial communities (Doube *et al.*, 1997b).

In glasshouse trials, the inoculation of *A. trapezoides* (equivalent to 460 worms m^{-2}) increased wheat plant (*Triticum aestivum*) biomass by 39%, grain yield by 35% and grain N content by 14%. After the wheat harvest, clover was grown and showed an increase in biomass of 21%. *A. rosea* had no effect (Baker *et al.*, 1997).

A mixed lumbricid community present in reclaimed peat grassland soil, with abundances of between 318-408 worms m^{-2}, had little effect in the first year, but increased herbage yields of *Lolium perenne* and *Trifolium repens* by 25% in the second year after inoculation and by 49% in the third year, compared to control plots (Curry and Boyle, 1987).

Recently reclaimed land, or grassland without an established earthworm community can show significant increases in productivity where lumbricid earthworms are introduced. Stockdill (1982) found increases in spring pasture yield of 72% four years after the introduction of *A. caliginosa* into grassland with no resident earthworm community. Less dramatic increases were observed by Hoogerkamp *et al.* (1983) and Springett (1985), who observed pasture yield increases of approximately 10%.

However, in a calcareous grassland, Zaller and Arnone (1999) found that the effects of all three earthworm ecological groups, inoculated at 3 different abundances, did not affect the above ground plant community, either in terms of biomass or plant functional type. It is important to note that this experiment was only run over two growing seasons,

and the authors acknowledge that may be too short a period to observe changes. Another important point to note is that plots with inoculated earthworms were isolated below ground to a depth of 45 cm, which in a shallow soil should have strongly limited subsurface lateral movement of earthworms. However, the above ground barrier extended only 15 cm above the soil surface. This is insufficient to prevent earthworm movement (*pers obs*). The artificial manipulation of earthworm population densities is unlikely to have been successful using such a barrier. Despite these reservations, the authors do propose reasons as to why, if successful, the increased size of earthworm communities may have had little impact on the plant communities. They suggest that a) perennial plant species common to native grassland are less responsive, or respond more slowly to earthworm induced enhancement of nutrient availability than agronomic plant species, or b) the increase in earthworm activity created by increasing the size of the earthworm community was insufficient to impact on the above ground biomass. They also suggest that the effect of earthworm activity on plant community structure occurs slowly where earthworm activity may relieve soil constraints on plant growth and perhaps through selective seed burial and dispersal (Zaller and Arnone, 1999; Grant, 1983).

Like the variable effects of earthworms on soil organic matter, the baseline growing conditions are important in determining whether the worms have a positive, negative or no discernible impact on plant growth and productivity (Doube *et al.*, 1997b).

1.7.2 Plant hormones

Decaying organic material contains hormone-like substances as a result of microbial metabolism (Tomati *et al.*, 1988), and it has been suggested that some of the beneficial effects of earthworm casts on plant growth may be due to hormone-like substances

contained in the egested cast. These effects include promotion of rooting, root growth, plant development and improved crop production.

Nielson (1965) hypothesized that the increased productivity in New Zealand pasture, observed where lumbricid earthworms were experimentally introduced, may be due to their secretion of plant growth hormones into egested casts, rather than the breakdown of the pasture root mat and concomitant release of nutrients (see section 1.7.5). Nielson (1965) examined extracts of several lumbricid earthworms for biologically active compounds, and extracts of *A. caliginosa, L. rubellus* and *E. fetida* were found to contain several indole substances, one of which from each species was found to have an impact on plant growth. The effects of these indole compounds on pea shoots were examined and compared to the plant hormone indole-3-acetic-acid (IAA). The cut tips of pea shoots were exposed to the active compounds extracted from the earthworms and the response degree of curvature towards the extract, which is proportional to the concentration of the substance) was measured. The degree of curvature towards the earthworm extracts exceeded the response to IAA between 15° to 43°.

Lee (1985) *op cit* Springett and Syers (1979) observed that the roots of rye grass (*Lolium perenne)* seedlings grew laterally or upwards into *L. rubellus* casts present on the soil surface. They suggested that this response was due to either an increase in nutrient availability, or due to the presence of an auxin-like substance. High concentrations of dissolved organic phosphorus (DIP) were found in the casts (16.7 $\mu g\ g^{-1}$ casts), more than twice the concentration in both in bulk soil (6.7 $\mu g\ g^{-1}$) and *A. caliginosa* casts (7.8 $\mu g\ g^{-1}$), which did not induce the same negative geotropism. The concentration of total and plant available P in earthworm casts, while lower than plant litter, is higher than the surface soil. Concentrations of available P are commonly 5-10 times higher in casts compared to surface soil, with values of 20-70 $\mu g\ g^{-1}$ soil compared to 140-380 $\mu g\ g^{-1}$ casts (Lee, 1985).

However, after adding inorganic phosphorus fertiliser to the soil, they found that at both DIP concentrations (9 and 37 $\mu g\ g^{-1}$ soil) there was a tendency for the plant roots to be negatively geotropic, and grow into the casts of *L. rubellus*, but this did not happen with casts of *A. caliginosa.* Springett and Syers (1979) concluded that *L. rubellus* casts contain an auxin-like substance, or some substance that modifies the effects of the plant's auxins.

There is no evidence that oligochaetes can synthesize hormone-like substances (Tomati *et al.*, 1988), and it seems likely that, if present, these metabolites are produced by micro-organisms whose activity is stimulated in earthworm casts. Bonkowski and Brandt (2002) suggested a model illustrating how soil protozoa (*Acanthamoebae sp.)* enhanced the growth of watercress seedlings (*Lepidium sativum* L.) via grazing-induced hormonal effects on root growth. Grazing on bacteria by protozoa strongly affects plant growth, and can significantly change root morphology towards a finer and more branched root system. Plant root exudates stimulate the growth of a diverse bacterial community and, as a consequence, the growth of protozoan predators. Selective grazing of *Acanthamoebae sp.* shifts the composition of the microbial community towards IAA-producing bacteria and lateral root growth is induced by the release of bacteria-derived hormonal substances. Tryptophan is liberated by protozoan grazing on bacterial cells which can stimulate IAA producing bacteria, as tryptophan is a precursor of IAA. An increase of 37% in IAA-producing bacteria was observed by Bonkowski and Brandt (2002). A stimulation of root growth leads to increased root exudation and subsequent bacterial growth.

A similar mechanism may operate in earthworm casts and drilosphere soil, where bacterial activity and metabolism are stimulated, leading to an increase in protozoan grazing (see Section 1.3.5.1). Concentrations of plant-like hormones have been found in earthworm casts in comparable concentrations to those found in the rhizosphere of many plants (Tomati *et al.*, 1988).

Microbial activity in earthworm casts results in the mineralization of nutrients into plant available form, and as a consequence of their secondary metabolism release plant growth regulating substances. Further work is needed in the area to determine the relative importance of plant hormone-like substances in earthworm casts, compared to the increase in plant growth due to the enhanced availability of often-limiting plant nutrients, like phosphorus, in freshly deposited casts.

1.7.3 Nitrogen

The concentration of plant available inorganic N in fresh earthworm casts and drilosphere is usually much greater than concentrations in bulk soil (Parle,1963b; Parkin and Berry, 1994; Subler and Kirsch, 1998; Whalen *et al.,* 2000). Earthworms consume large volumes of organic matter which contains considerable quantities of nitrogen, and the increase in inorganic nitrogen in earthworm casts is due to microbial mineralizaion of this N rich material and the addition of earthworm excretory products and mucus. The presence of earthworms in well aerated moist soil increases the rate of oxygen consumed and the accumulation of NH_4^+ and NO_3^- during the early stages of decay (Edwards and Bohlen, 1996; Haimi and Einbork, 1992). Epidermal mucus is secreted to prevent earthworm desiccation, to form a protective coat against noxious materials, facilitate respiration and to provide lubrication for movement through the soil. Epidermal mucus secretions and mucus in casts from the endogeic species, *Octolasion lacteum* (Őrley), accounted for 63% of total daily N losses (Scheu S., 1991). Excretory products include ammonia, which is released into the gut to be egested in cast material, and urea, possibly uric acid and allantoin are excreted in urine from the nephridiopores (Edwards and Bohlen, 1996).

Examination of *A. caliginosa* casts showed that when fed on finely ground plant litter, about 6% of the nonavailable N ingested by the worms was egested in plant available

forms (Syers and Springett, 1983, *op cit* Barley and Jennings, 1959). The amount of nitrogen present in freshly deposited earthworm casts appears to be a reflection of the N content of the organic matter used as a food source by the earthworms (Parkin and Berry, 1994). The authors examined nitrification and denitrification rates in *Octolasion tyrtaeum* (Savigny) and *Aporrectodea tuberculata* (Eisen) casts, where the worms were provided with organic residues of different qualities. Nitrification rates were highest in soils amended with organic residues, with a decrease in NH_4^+ over a 2 week period, and a corresponding increase in NO_3^-. Denitrification activity was correspondingly low in cast material where worms were not provided with organic enrichment. When earthworms were provided with N rich organic substrates, N excretion was greater than when fed on N poor substrates, as earthworms reduce N secretion to conserve tissue N when N resources are limited (Whalen *et al.*, 2000).

In the absence of earthworms, there is no transient accumulation of NH_4^+, like that which is found in earthworm casts (Parkin and Berry, 1994). The authors suggest that this indicates that in the absence of earthworms, the rate limiting step in the formation of NO_3^- is the decomposition rate of organic matter. Where earthworms are present, the mineralization rate of organic matter is increased and nitrification is the rate limiting step in NO_3^- production. This suggests that the primary effect of earthworms on nitrogen cycling in soils is to increase the rate of organic-N mineralization.

Earthworms contribute a significant amount of readily assimilable nitrogen to soil. A contribution of 30- 40 kg N $ha^{-1}yr^{-1}$ from *L. terrestris* mucus and urine to a woodland soil N pool has been by estimated (Edwards and Bohlen, 1996 *op cit* Satchell, 1963). Using ^{15}N labelled *L. terrestris, A. tuberculata* and *L. rubellus*, Whalen *et al.* (2000) estimated a value of 41.5 kg N ha^{-1} yr^{-1} as the annual earthworm N excretion rate in an inorganically fertilized maize agroecosystem, representing 22% of crop N uptake. The authors suggest

that this represents an agronomically significant contribution to the N pool. Curry and Byrne (1992) estimated that earthworm excretory products and decomposing tissues of dead worms could supply 30% of winter wheat N requirements (50-70 kg N ha^{-1} yr^{-1}). This again represents a potentially significant contribution to the soil N pool.

A value of 2.68-3.52 kg N ha^{-1} yr^{-1} was the estimated nitrogen excretion rate of a population of *A. caliginosa* by Knight *et al.* (1992). The authors state that this is an agronomically insignificant value, but warn that this is only the direct contribution, and does not include indirect effects on microbial N transformations. However, this does not take into account the redistribution of organic matter in the soil profile, or the instantaneous rate of NH_4^+ and NO_3^- production throughout the year in relation to root distribution and nutrient uptake (Syers and Springett, 1983). Syers and Springett (1983) suggest that the importance of earthworms is not in the total turnover of the nutrient pool, but in producing frequent small increases in enzyme activity and nutrient availability in close proximity to plant roots.

Pot experiments carried out by Doube *et al.* (1997b) showed an increased N content of wheat plants grown in the presence of *Aporrectodea trapezoides* (Dugès) and *A. rosea.* It is suggested that N derived from earthworms is not directly responsible for the increase in shoot N, as increases in shoot N content were 3 times greater than the amount of N lost by the worms during the course of the experiment. One possible source of the extra N is from the release of nitrogenous compounds due to the death of at least a proportion of the earthworms in the experiment. Significant amounts of nitrogen can pass into the soil N pool directly from dead earthworm biomass. Earthworm tissue is about 60-70% dry weight) protein and has a nitrogen content of about 12% (Lee, 1985). The contribution from dead *L. terrestris* in an English woodland was calculated to be 60-70 kg N/ ha/yr, of which a possible 70% is mineralizable in 10- 20 days (Edwards and Bohlen, 1996 *op cit*

Satchell, 1963).

Discrepancies between results of various studies may be due to differences in experimental techniques, but it is also likely that soil type, N content of the organic matter substrate, plant and earthworm species are all important in determining the effects of earthworm activity on N uptake by plants (Parkin and Berry, 1994; Baker *et al.*,1997).

1.7.4 Root growth

Undisturbed soil columns planted with barley inoculated with either *A. caliginosa* and *A. chlorotica* (endogeic) or *L. terrestris* (anecic) showed increased root biomass between 15 and 25 cm depth for *L. terrestris*, compared with the shallow burrowing endogeic species treatment (Logsdon and Linden, 1992 *op cit* Edwards and Lofty, 1978). Both treatments increased shoot growth. Similar results were found for winter wheat, where *A. longa* was added to the *L. terrestris*. As discussed in section 1.7.2, earthworm casts can promote root initiation and increased root biomass (Tomati *et al.*, 1988).

The impact of unmodified earthworm populations on crop plant roots have been examined (Logsdon and Linden, 1992 *op cit* Ehlers *et al.*,1983), and it was shown that oat roots have been found running along earthworm burrow channels, especially at increasing soil depth, and at depths greater that 45 cm all the oat roots were restricted to worm burrows. Similarly, the authors cite work done by Wang *et al.* (1986) who observed that soybean root tips died at a depth of 30-45 cm if they did not encounter an earthworm burrow. If the root entered a burrow, it was followed to the end and these burrows were reused year after year.

1.7.5 Root mat degradation

An accumulation of plant litter and roots occurring at the soil surface can interfere with water infiltration into the soil. Plant productivity is reduced and the plant species composition can be altered. Earthworm populations are typically low or absent in this mor type soil (Lee, 1985).

Earthworms contribute to the removal and incorporation of the root mat into the soil. Where *A. caliginosa* was inoculated into pasture land that previously lacked an earthworm community, it resulted in the development of a mull type soil, with dead plant residues and animal dung intimately incorporated into the soil (Barratt, 1964), and a much deeper organic matter distribution (Stockdill,1982; Hoogerkamp *et al.,* 1983). Similar results were found when *A. longa* was added to a community consisting of lumbricid epigeic and endogeic species (Springett, 1985). Surface applied lime was incorporated into the soil, instead of remaining as a thin veneer, and soil physical properties were improved (structure, infiltration rates, water holding capacity). The removal and subsequent decomposition of the root mat resulted in an increased pasture yield due to the rapid release of nutrients (Stockdill, 1982).

1.8 Investigating the decomposition of soil organic matter using solid-state ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy

^{13}C NMR spectroscopy has been used as a tool for the study of soil organic matter chemistry and dynamics for over 20 years (Skjemstad *et al.*, 1997), providing information on the chemistry of the organic materials present. Early studies used solution-state techniques, but this required chemical extraction of the organic material with concomitant constraints and compromises. Even the best chemical extraction procedures result in

incomplete extractions, with perhaps only 50 % yields of soil C in solution, with possible modifications of molecular structure on dissolution. Insoluble soil C fractions are also omitted from analysis. As the extraction procedures are destructive any subsequent analyses on the same sample are prevented (Hopkins *et al.*, 2000).

The development of solid-state ^{13} C NMR techniques resulted in a non-destructive method for soil organic matter analysis where insoluble soil components, whole soils and fractions derived on physical rather than chemical fractionation schemes could be investigated. Problems associated with solid-state compared to liquid-state NMR include dipolar interactions and chemical shift anisotropy which result in spectral line broadening. Long ^{13}C nuclei relaxation times (T_1) are also problematic, as it is necessary for nuclei to relax back to their equilibrium magnetisation after each radiofrequency pulse, and many thousand scans are needed to obtain a spectrum with an acceptable signal-to-noise ratio.

1.8.1 Elementary theory

1.8.1.1 Nuclear spin

NMR signals are observed by exciting nuclei in a magnetic field with a pulse of energy and measuring the energy re-emitted as radiowaves when the nuclei relax back to equilibrium. This occurs when a nucleus spins, due to an odd number of protons or neutrons and creates a magnetic field. If this spinning nucleus is exposed to an external magnetic field, it will precess and take up a number of orientations with respect to this applied magnetic field. ^{13}C has two possible spin orientations and is therefore detected using NMR spectroscopy, in contrast to the ^{12}C nucleus which has zero spin.

The low natural abundance of ^{13}C (1.1 atom%) can result in weak spectra. The enrichment of experimental soils with ^{13}C labelled plant litter can therefore be useful in decomposition studies as this will aid in the collection of high quality spectra, as labelled

metabolites will stand out above the natural abundance spectrum.

1.8.1.2 Dipolar interactions

Dipolar interactions arise from the polarizing effect of different functional groups in a molecule which result in electrons not being shared equally between nuclei. This generates small differences in the local magnetic fields of the nuclei and means that similar ^{13}C nuclei will have different resonance frequencies due to different local magnetic fields, which results in broad resonance lines (Wilson, 1987).

Dipolar interaction effects can be removed by high powered proton decoupling. A powerful radiofrequency field at the resonance frequency of the abundant nuclei (i.e. ^{1}H) is applied, so all the ^{1}H are in the same energy state and therefore the local magnetic field due to proton nuclei is zero (Abraham, Fisher, and Loftus, 1988).

1.8.1.3 Chemical shift anisotropy and magic angle spinning (MAS)

Chemical shifts arise from the shielding of a nucleus by the interactions of electrons with an applied magnetic field (Homans, 1992). When a molecule is placed in a uniform magnetic field, the electrons of the molecule circulate and produce a secondary magnetic field that contributes to the chemical shift of nuclei in the molecule.

As functional groups in an organic molecule polarise the surrounding electrons, the nuclei of these functional groups are differentially shielded from the applied magnetic field. This means that carbon nuclei in different functional groups resonate at slightly different frequencies due to differences in their local magnetic environment.

In liquid-state NMR, molecular orientations vary rapidly, which results in the averaged contribution of the different magnetic field orientations (chemical shift anisotropy) being equal to zero. In the solid-state however, groups of molecules may bear

different, but fixed orientations with respect to the applied magnetic field, hence the molecular screening is not averaged. This results in a range of contributions of each different nuclear shift and thus broad appearances of spectral lines.

This effect is negated by spinning the sample throughout the experiment at an angle of 54.7° to the applied magnetic field (Abraham *et al.*, 1988).

1.8.1.4 Spinning sidebands

If a sample with a large chemical shift anisotropy is spun at speeds less than required for maximum line narrowing, the broad line resonance breaks up into a number of *spinning sidebands* (Wilson, 1987). These are a series of peaks located around the main resonance peak at multiples of the spinning rate, and can be readily identified. They can lead to quantification problems as the presence of sidebands can detract from the main signal for a particular functional group. Sidebands may also fall outside the shift range of that particular functional group and be superimposed onto other resonances which distorts their intensities (Hopkins *et al.*, 2000). There are methods to suppress sidebands, but which don't necessarily restore the sideband intensities to the main resonance. Acknowledging that sidebands and their effects are present has been the preference of other authors (Hopkins *et al.*, 2000) and this will be followed here.

1.8.1.5 Relaxation and cross polarization

The repetition of the pulse/ acquisition sequence to obtain an accumulation of spectra is dependant on the rate at which nuclear spins can return to their equilibrium state (i.e. with all spins aligned with the applied magnetic field). In liquid state NMR the random motion of molecules provides abundant opportunities for energy to be exchanged and the nuclei to relax. This is known as spin-lattice or T_1 relaxation. In solid state NMR, molecular mobility is decreased and spin-lattice relaxation becomes less effective, and so T_1 is

increased.

Cross polarization allows dilute ^{13}C nuclei to interact energetically with and relax at the same rate as rapidly relaxing proton nuclei. This process results in a much shorter T_1 relaxation time and an increase in the intensity of the dilute ^{13}C signal (Abraham, Fisher, and Loftus, 1988). The length of time that the radiofrequency pulse is applied and the ^{1}H and ^{13}C spins are in phase is defined as the contact time.

The effect of using cross polarization magic angle spinning is to produce high-resolution spectra from solids of comparable quality to solution state NMR spectra. However, cross polarization leads to quantification problems. Carbon nuclei can only cross polarize with protons that are in close proximity, approximately 4-5 bond lengths. Furthermore, the rate at which magnetisation is transferred from the proton to the carbon nucleus is a function of distance. This means that carbons not directly bonded to hydrogens, such as substituted aromatic carbons and carbonyls, will require longer contact times than carbons such as methyl groups which have abundant protons. This means that CP/MAS ^{13}C NMR spectra are distorted with C in highly protonated functional groups being amplified whilst those functional groups without H nuclei or where H nuclei are remote give small signals (Skjemstad *et al.*, 1997).

The presence of paramagnetic species, such as iron, in the soil can also have an impact of quantification. The presence of a paramagnetic species close to a carbon nucleus results in the C being undetectable because the electron spins of the paramagnetic species reduce the proton T_1 and they lose magnetisation too quickly to transfer it to carbon nuclei (Kögel-Knabner, 1997; Skjemstad *et al.*, 1994; Kinchesh *et al.*, 1995).

1.8.2 Interpretation of CP MAS ^{13}C NMR spectra

One of the main limitations of NMR spectroscopy is its inherent lack of sensitivity due to the relatively small magnitude of the energy changes involved in NMR transitions. One of

the simplest ways of overcoming this is to record many thousands of spectra from a sample and add them together. The NMR signals will add coherently, whereas the noise, which is random, will only add as the square root of the number of spectra accumulated. This leads to an improvement in the signal-to-noise ratio (Abraham *et al.*, 1988).

The position in a spectrum at which a nucleus resonates is called the *chemical shift* of the nucleus and this provides information about the chemical environment in which that nucleus exists (Figure 1.3). The main signals of interest occur at shift ranges between 0 and 200 ppm with respect to tetramethylsilane. The broad shift ranges shown in Figure 1.3 can be refined further in to the 6 shift ranges found most often in soil studies (Chemical shift ranges of the six shift ranges commonly used in soil ^{13C} NMR studies Table 1.2).

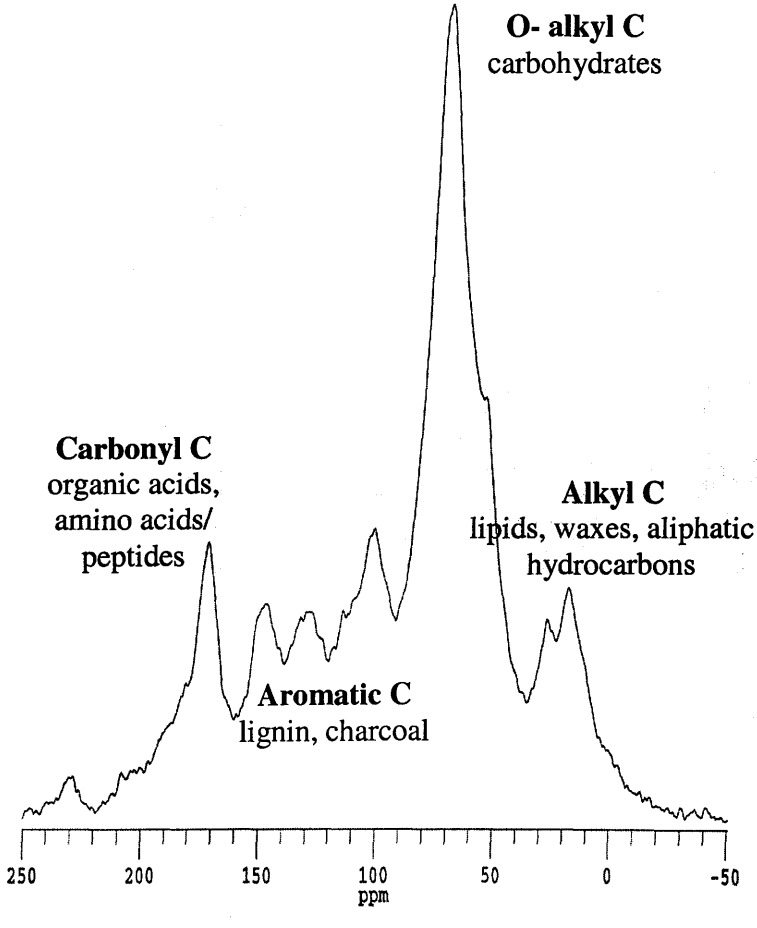


Figure 1.3 ^{13}C CP MAS NMR spectra showing four general chemical regions and typical chemical shift assignments for soil organic matter

Chemical shift ranges of the six shift ranges commonly used in soil ^{13}C NMR studies Table 1.2 (Hopkins *et al.*, 2000)

Shift range (ppm)	**Functional groups**	**Typical classes of biochemical represented**
10-45	Methyl- and alkyl-C	Lipids, waxes and aliphatic hydrocarbons
45-60	O-methyl- and N-alkyl-C	Lignin substituents and amino acids
60-90	O-alkyl-C	Polysaccharides (inc. cellulose and hemicellulose)
90-110	Acetal- and ketal-C di-O-alkyl-C)	Polysaccharides (inc. cellulose)
110-160	Aromatic-C	Lignin and tannins
160-200	Carbonyl-C	Organic acids and peptides

The relative contribution of each type of carbon can be made from the area under individual peaks or regions, rather than peak heights, as peaks vary in both width and shape (Skjemstad *et al.*, 1997).

1.9 Objectives of the thesis and Research design

The objectives of this thesis are to investigate the ecology of earthworms in limed and unlimed upland grassland soil, and their impacts on soil carbon distribution and chemical characteristics. As discussed in section 1.5.2, much of the previous work investigating the effect of lime on the size and diversity of earthworm communities has been done in peat soils, often under coniferous woodland, and the impact of liming may differ in an upland mineral soil. Similarly, there is contrasting evidence on the effects of earthworms on soil organic matter across many soil types and agricultural systems, seen both in the effects on the soil microbial community and C decomposition, and the plant community.

This thesis will therefore address the effects of liming on earthworm ecology and their effects on soil carbon in an upland grassland system. his will be carried out using a field mesocosm experiment, and a laboratory based microcosm experiment.

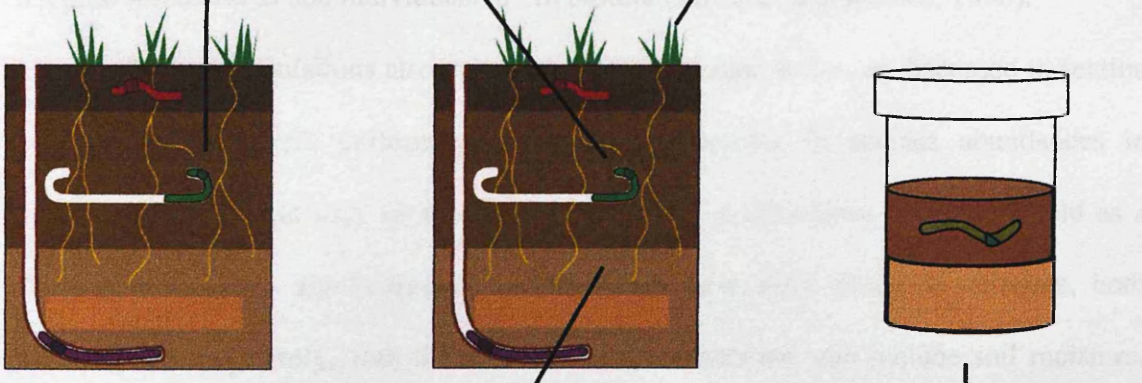
Each of these general objectives is addressed in turn in Chapters 2 to 5 (Figure 1.4), where the specific objectives are presented. A final general discussion draws together the results and conclusions from this work in Chapter 6.

Chapter 2
Objective 1:

To investigate the effects of lime on manipulated earthworm communities, and to determine the environmental and experimental variables which explain individual earthworm species' distribution.

Chapter 3
Objective 2:

To examine the impact of these earthworm communities on plant above-ground biomass harvests and species distributions.



Sourhope field site **Mesocosm experiment** **Microcosm experiment**

Objective 3:
Chapter 4

To investigate the effects of the earthworm communities distribution on soil C through the soil profile and the chemical characteristics of this organic matter using CP MAS ^{13}C NMR spectroscopy.

Objective 4:
Chapter 5

To investigate earthworm and earthworm induced respiration and examine cast material from individual earthworm species using CP MAS ^{13}C NMR spectroscopy.

Figure 1.4 Research design and specific objectives of the thesis

Chapter 2 The effects of lime and soil properties on endemic and manipulated earthworm communities

2.1 Introduction

In most terrestrial ecosystems, earthworms are the dominant soil invertebrates (Lavelle, 1983; Curry, 1994). However the size and composition of earthworm communities vary greatly across different habitats. Acidic soils, coniferous woodland and moorland soils support smaller, less diverse communities compared to deciduous woodland soils, pastures and natural grasslands (Ponge, 2003). In the UK numbers can vary from 40 individuals m^{-2} in a pine woodland to 500 individuals m^{-2} in pasture (Edwards and Bohlen, 1996).

Earthworm populations also vary across much smaller scales, as discussed in section 1.6. Even in relatively uniform pasture fields, variations in species abundances in individual samples can vary up to +/- 50% of the mean abundance across the field as a whole (Guild, 1952). Environmental factors which have been found to correlate, both positively and negatively, with these variations are numerous, and include soil moisture, soil pH, C and N content, soil depth, herbage cover and soil texture (Guild, 1952; Cannavacciuolo *et al.*, 1998; Poier and Richter, 1992; Nuutinen *et al.*, 1998). Apparent contradictions between studies may be due to both the wide variety of sites sampled and the variety of statistical techniques employed. Ordination techniques have been used by a number of authors e.g. Briones *et al.* (1992) and Didden (2001), and are valuable tools in examining the variation in community structure in relation to environmental variables (Lepš and Šmilauer, 2003).

This chapter aims to examine the effects of adding lime to both a species-poor earthworm community typical of acidic upland grassland and experimentally manipulated earthworm communities. The importance of several soil edaphic variables for the distribution of the earthworm species present will also be examined.

2.2 Specific Objectives

The aim of the work in this chapter was to determine:

1. The size and diversity of the earthworm community present at the Sourhope field site.
2. The effect of lime on the size of this community.
3. The persistence of introduced earthworm communities inoculated into limed and unlimed Sourhope soil.
4. The environmental variables which explain the distribution of the earthworm species present.

2.3 Materials and Methods

2.3.1 Site description

The Soil Biodiversity Thematic Programme Rigg Foot field site is situated at Sourhope Research Station, 24 km south of Kelso, at the head of the Bowmont Valley in the Scottish Borders (Figure 2.1). This field site is a north-facing slope of upland grassland on brown forest soils of the Sourhope and Bellshill series. The site is at an altitude of approximately 309 m above sea level, and has a varying slope from 4° at the bottom of the field to 8° at the upper end. The vegetation at the site is dominated by species typical of upland grassland on base poor, damp, mineral soils, e.g. *Agrostis capillaris, Festuca ovina* and *Galium saxatile* (from 'Protocol for Sampling and Recording at the Sourhope Field Experiment Site, 1999).

Pollen investigation at a nearby site at Sourhope indicated that cereal and oat pollen were present from between the 12th century to mid 17th century. This indicates that the experimental site at Sourhope is likely to have been cultivated and manured until the middle of 17th century and possibly even slightly later. This would have homogenised the upper soil horizons and drainage on the site was improved through rig construction.

Following abandonment, the upper litter and peaty horizons would have gradually formed (Davidson, 2002).

Sheep have grazed the site for the past 50 years, but have been excluded since April 1998, when the fieldsite was fenced, to prevent access to grazing animals. It was divided into thirty 20 m x 12 m plots, which were used for 6 treatments, each replicated five times (Figure 2.2). Each of these plots was divided into sub-plots (Figure 2.3). Of the 6 treatments imposed, the ones used in this experiment were the Control 1 unlimed) and Lime plots, where a lime was applied annually from May 1999, at a rate of 6 t $CaCO_3$ ha^{-1}. The site was mown monthly May- September, and the grass cuttings removed.

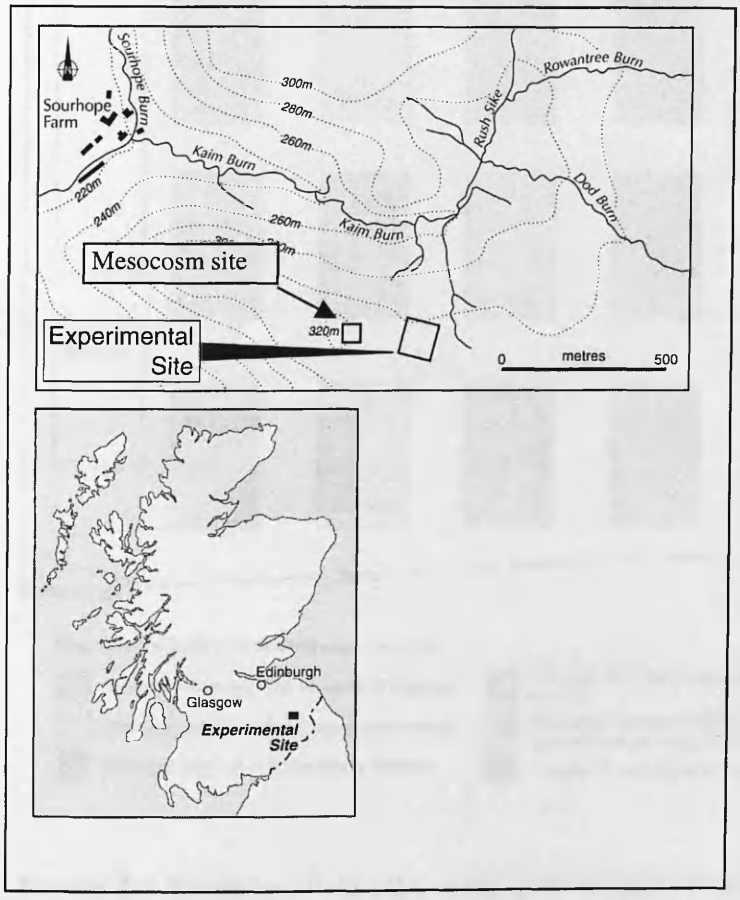


Figure 2.1 Map showing location of Sourhope field site (Davidson *et al.*, 2002), modified to include the location of the mesocosm experiment.

Site plan of Soil Biodiversity Experiment at Sourhope.

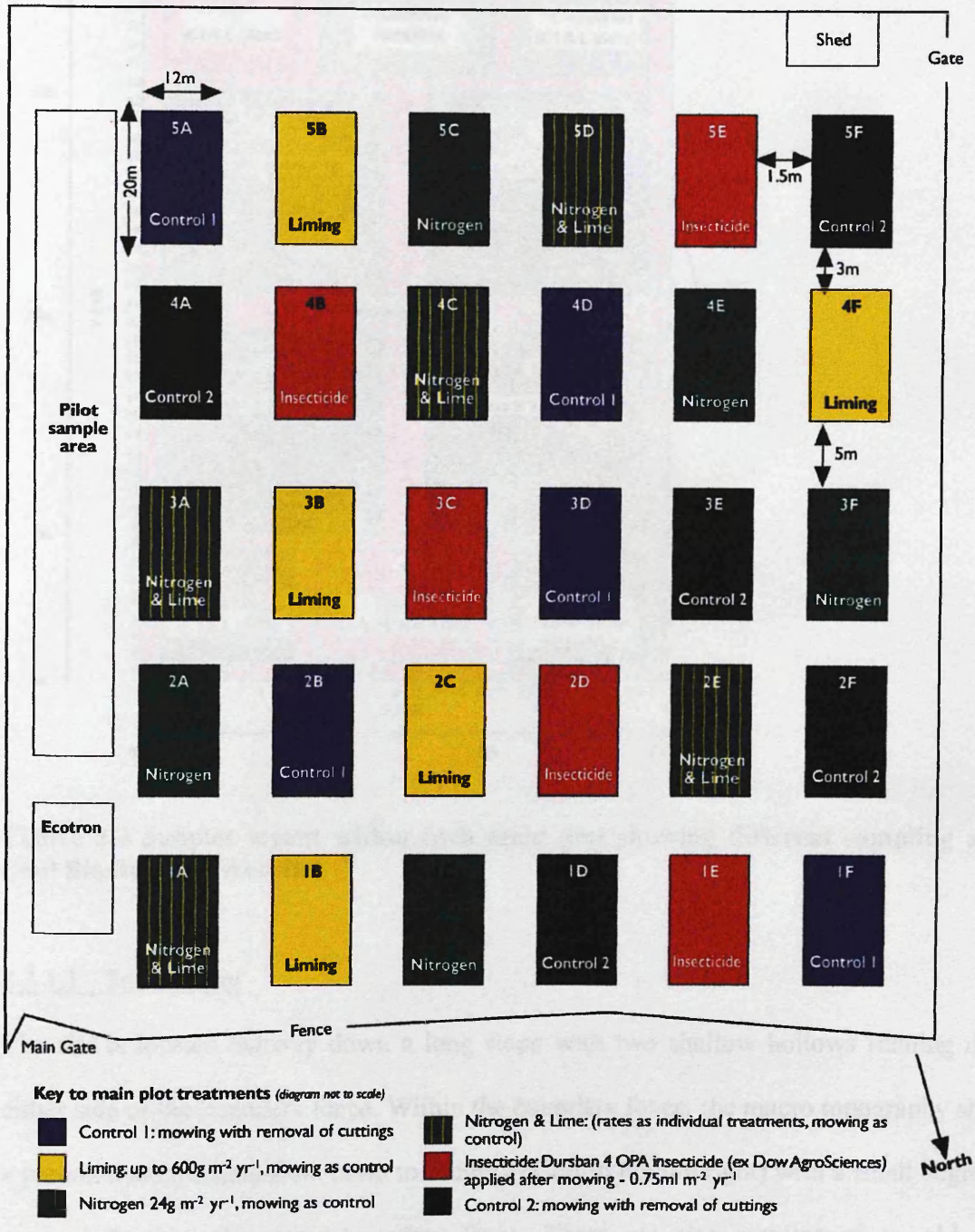


Figure 2.2 Sourhope field site, main plot layout showing 6 soil treatments per row, replicated 5 times (Soil Biodiversity Website)

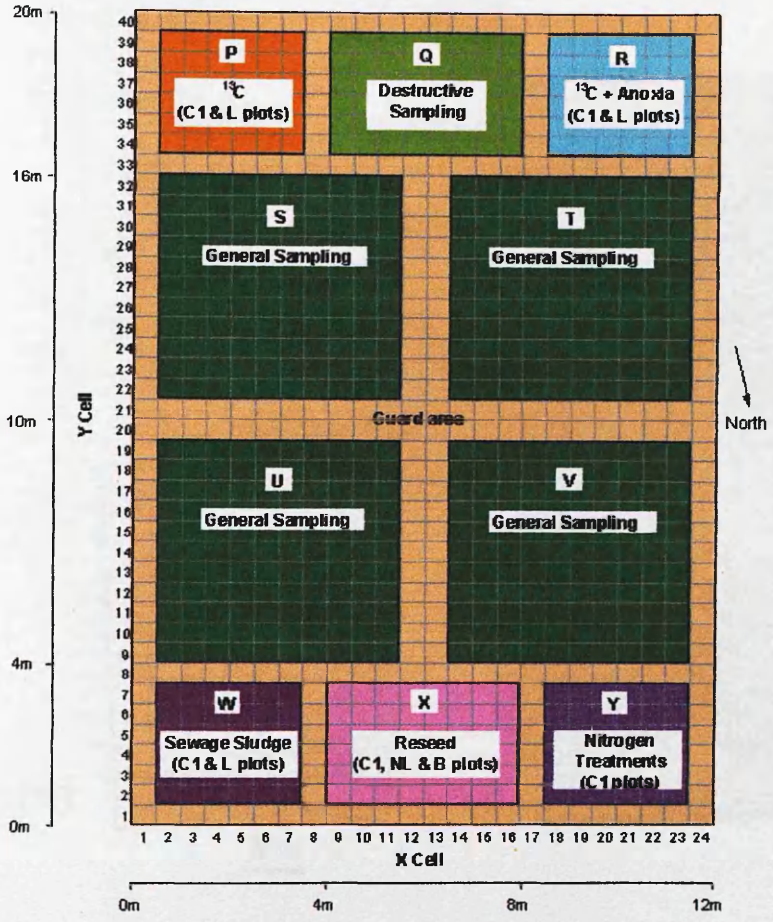


Figure 2.3 Subplot layout within each main plot showing different sampling areas (Soil Biodiversity Website)

2.3.1.1 Topography

The site is located halfway down a long slope with two shallow hollows running down either side of the boundary fence. Within the boundary fence, the macro topography shows a predominant gradient from north to south (top left to bottom right) with a small degree of convexivity near the upper boundary fence. There are also remains of an old field boundary, which appears as a ridge, running down the east side of the field site. Figure 2.4 b shows a number of systematic undulations (ridges and furrows) running from the top of the field to the bottom, which are a legacy of past agricultural practices. Figure 2.4 shows a detailed topographic map of the Sourhope site (Whelan *et al.*, Submitted).

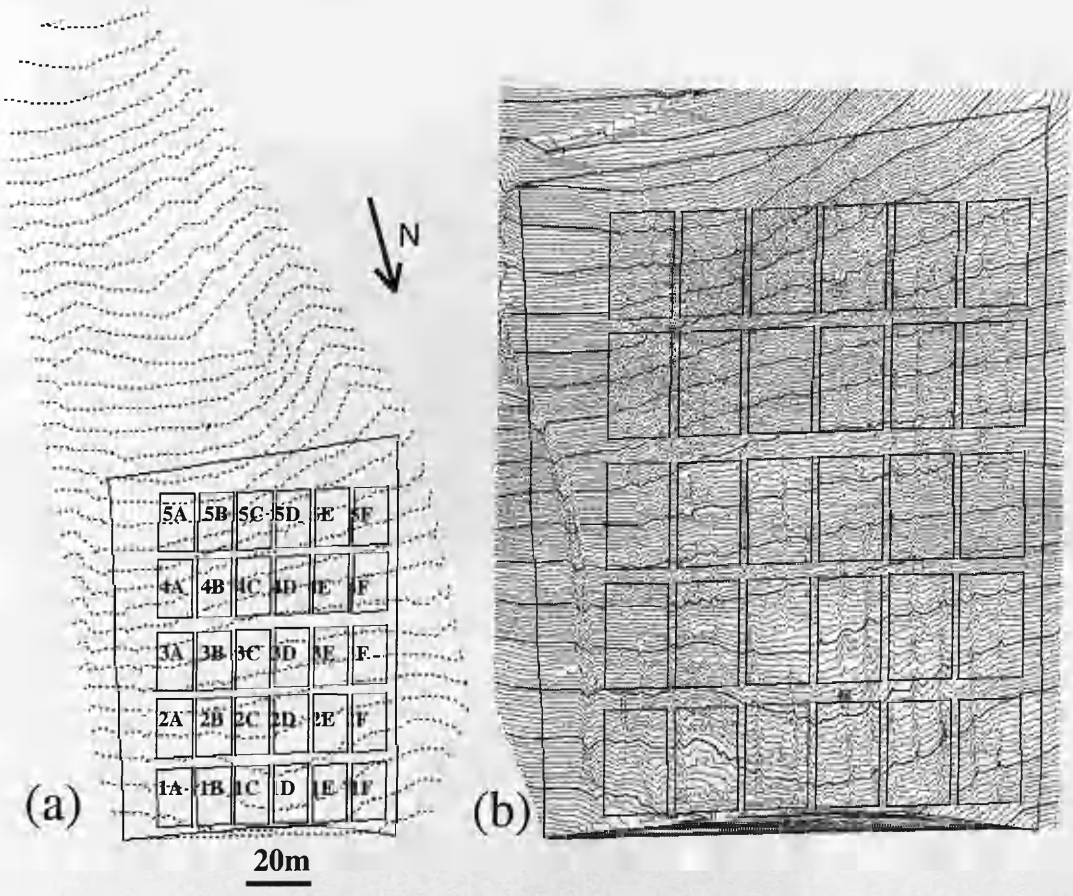


Figure 2.4 Topography of the Sourhope field site showing a) topographic context of the fenced area and b) detailed topography within the fenced area. Major contours 1m, minor contours 0.1m. Major plot boundaries are also shown. (Whelan *et al.*, Submitted).

2.3.1.2 Soil moisture content and soil sub-types

Figure 2.5 shows both spatial and temporal variation in soil moisture content at the Sourhope field site. These measurements were collected using a Theta Probe (ML2), which records a voltage that is converted to a soil moisture content via a calibration curve (Whelan *et al.*, Submitted).

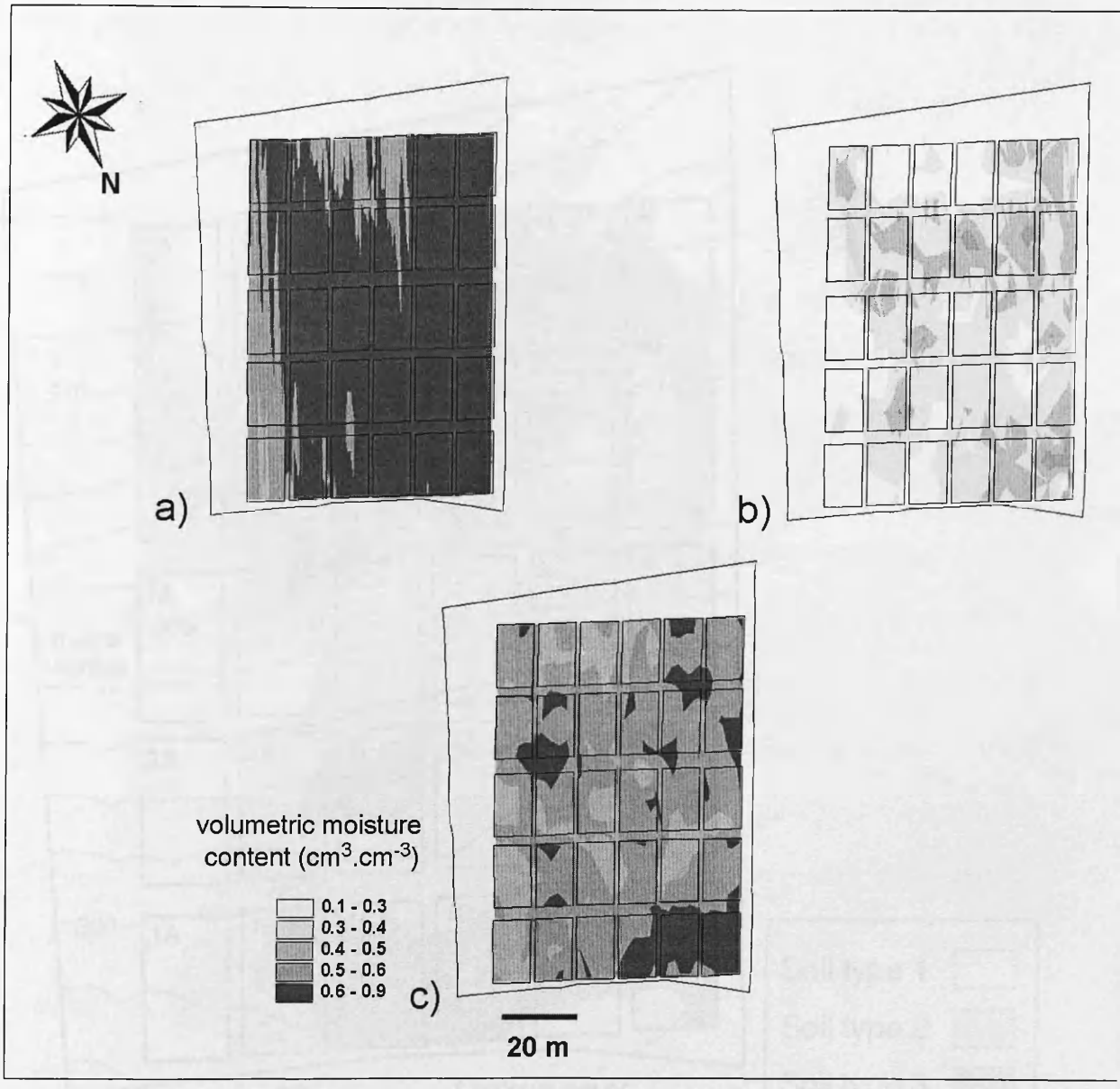


Figure 2.5 Soil moisture content variation across the Sourhope field site a) April 2000; b) July 2000 and c) September 2000 taken from (Whelan *et al.*, Submitted).

From these maps it can be seen that plots 1E and 1F are always relatively wet and similarly, plots 3B, 4B, 5A and 5E have areas which are consistently wet.

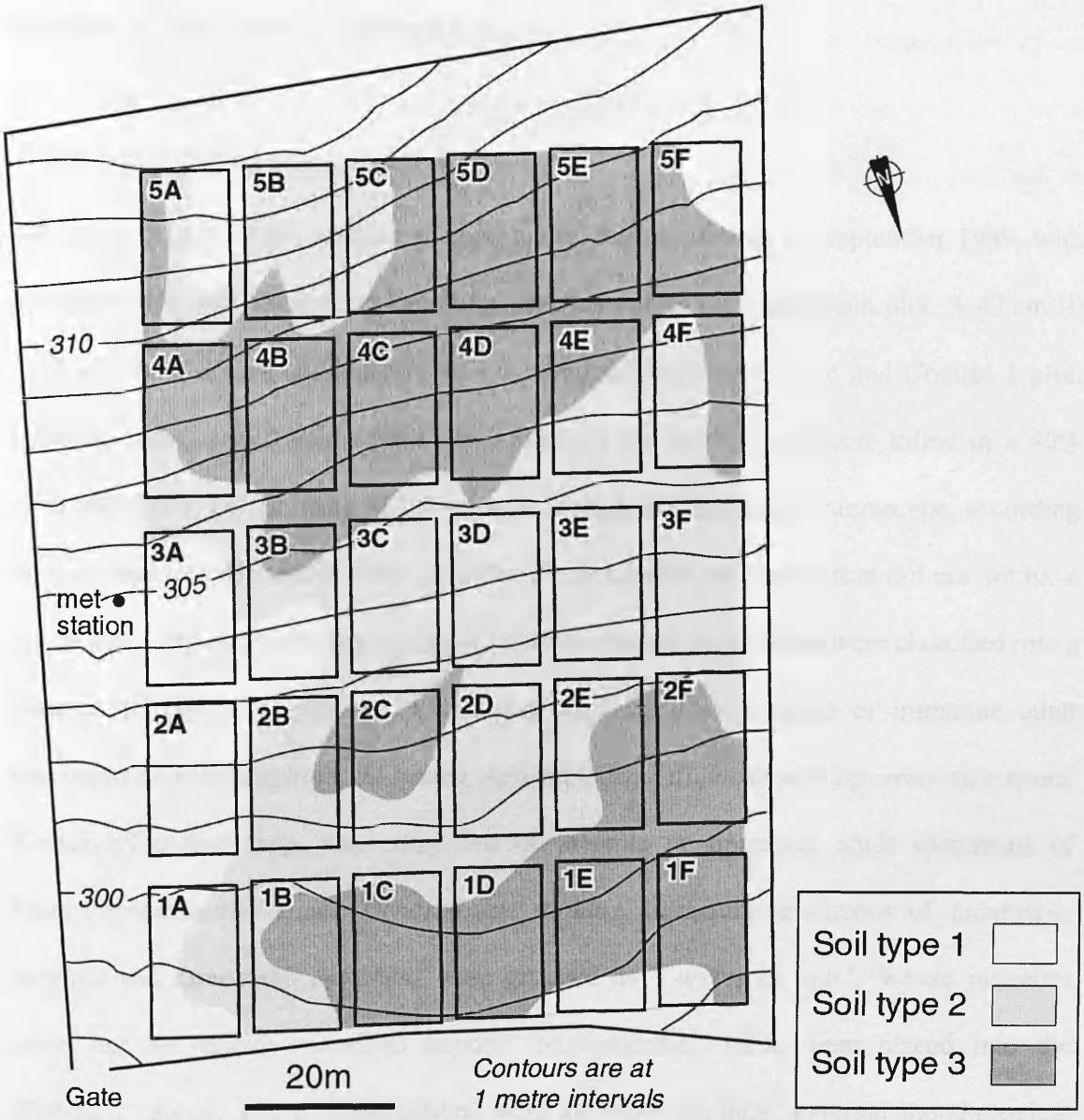


Figure 2.6 Distribution of soil sub types across the experimental Sourhope plots (Davidson *et al.*, 2002).

Three soil sub-types were identified at the Sourhope site (Davidson *et al.*, 2002) with different sequences of upper horizons. Soil sub-type 1 has an LF directly overlying an Ah horizon. This sub-type is more associated with soils on higher, and therefore better-drained, rigs. Sub-type 2 has LF and H horizons overlying the Ah horizon. Soil sub-type 3 is distinguished by the presence of an H_{phy} below the H horizon, which is a narrow horizon with a higher phytolith content (mineral particles formed within plant tissue) than the

upper H horizon. These differences in soil horizon formation may be related to the variation in drainage across the field site.

2.3.2 Initial earthworm census

An initial census of the earthworm community was carried out in September 1999, with soil taken from the 'destructive sampling' area (sub-plot Q) of each main plot. A 42 cm (l) x 30 cm (b) x 25 cm (h) soil sample was removed from each Lime and Control 1 plot, avoiding furrows, and earthworms were removed by hand. These were killed in a 40% ethanol solution before being identified with the aid of a dissecting microscope, according to Sims and Gerard (1985) where possible. For immature specimens that did not yet have the external features necessary to identify them to species level, these were classified into a number of 'catch all' groups. 'Allol/Aporr. spp' included juvenile or immature adult specimens of *Allolobophora chlorotica, Aporrectodea caliginosa* and *Aporrectodea rosea.* 'Dendrob/Dendrod. spp. was composed of juvenile or immature adult specimens of *Dendrobaena octaedra* and *Dendrodrilus rubidus.* Immature specimens of *Lumbricus rubellus* and *Lumbricus castaneus* were grouped as '*Lumbricus* spp.'. Where juveniles could not be reliably classified beyond 'unpigmented', these were placed into the 'Endogeic' group. These classifications were all based on those external morphological features which are present in juvenile earthworms, e.g. morphology of prostomium, setal arrangement in the 'post-clitellar' region and the presence or absence of conspicuous dorsal pores and body colour.

2.3.3 Sourhope endpoint earthworm census

An endpoint census of the Sourhope site was carried out in September 2002, following the same method as given above.

2.3.4 Trial inoculation of supplied earthworm species

In order to modify the earthworm community at the field site, large numbers of earthworms were required. Earthworm species numerous at the field site were purchased from earthworm suppliers (Appendix 1) and inoculated into boxes of Sourhope soil to determine which survived inoculation into the acidic soil.

Four boxes of soil were removed from Control 1 plot 3D in November 1999, and indigenous earthworms removed by hand. The purchased earthworms were inoculated into the soil, and the boxes stored at 5-6 °C in a walk-in cold room. The boxes were examined monthly and any dead worms removed. After 4 months the numbers of live worms were recorded.

The choice of which species to use was based on both the survival of the earthworms in this trial and the reliability of each supplier in delivering different earthworm species requested. For example, in surveys of commercial earthworm farms in the US, Europe and Australia, all of the earthworms sold under the name of *L. rubellus* were all *E. fetida* or *E. andrei* (Edwards and Bohlen, 1996). Similar problems were encountered here for some species.

2.3.5 Field mesocosm experiment

As earthworms were to be inoculated into the soil, a small enclosure on an adjacent hillside was chosen as the site for the field mesocosm experiment. This prevented any possible interference from earthworms with other Soil Biodiversity projects taking place on the main Sourhope plots.

In November 1999, ten soil monoliths were removed from each of the Control 1 and Lime plots, avoiding the furrows. Half of the monoliths were placed undisturbed with indigenous earthworm population intact, in plastic boxes with internal dimensions 42 cm (l) x 30 cm (b) x 25 cm (h) with holes drilled in the bottom and a nylon mesh (approx.

mesh size 0.5mm) placed over the holes.

Indigenous earthworms were left in these boxes as there was no method to remove both worms and their cocoons from the soil without either disturbing the soil structure or killing the worms which would result in a flush of nutrients, both of which were to be avoided. These boxes were inserted into trenches lined with sharp sand to help ensure free drainage in a randomised block pattern.

The soil in the remaining boxes was air dried and homogenised on a horizon by horizon basis to remove both earthworms and existing earthworm derived soil structures. The soil was then repacked into the boxes and these were transported to the field site in early February 2000 and inserted into the trenches (Figure 2.7). Any gaps between the boxes were filled with loose soil. All the boxes were left uncovered, until the earthworms were inoculated in late April 2000, after the risk of frosts had passed.

Figure 2.7 Mesocosm site showing plastic boxes in trenches prior to earthworm inoculation, with Sourhope field site in the background.

The above-ground plant biomass was harvested approximately monthly during the growing season. The mesh covers were removed and the vegetation trimmed with scissors to approximately 1 cm above ground level. The plant material was taken back to the lab, oven dried at 60°C and weighed.

2.3.5.1 Inoculation treatment plan

With both the results from the census (section 2.4.1) and the trial inoculation experiment (section 2.4.2.1) the inoculation treatment plan was devised. The aim was to inoculate soil monoliths with enough earthworms to bias the population of the boxes towards the inoculated species. The 5 earthworm treatments imposed on undisturbed soil collected from each limed and unlimed Sourhope plot were:

1. No inoculated earthworms
2. All three ecological groups (15 x *L. rubellus,* 20 x *A. chlorotica and* 4 x *L. terrestris)*
3. Epigeic species (15 x *L. rubellus,* equivalent to 107 m^{-2})
4. Endogeic species (20 x *A. chlorotica,* equivalent to 143 m^{-2})
5. Anecic species (4 x *L. terrestris,* equivalent to 28 m^{-2})

In addition, the following 'disturbed' soil treatments were decided *:

6. No inoculated earthworms
7. All three ecological groups (as above)
8. No inoculated earthworms (uncovered box)

*These treatments were primarily for use in a related PhD project by C.A. Spring. However, the effects of these treatments on both the earthworm and plant communities (Chapter 3) have been examined here.

Unless stated otherwise, the boxes were covered with nylon mesh held in place with silicone sealant, to prevent earthworms escaping or further natural incursions. Treatment 8 was designed to investigate the differences in earthworm recolonisation of limed and unlimed soil.

2.3.6 Mesocosm endpoint earthworm census

An endpoint census was performed when the mesocosm boxes were removed from the site in October 2001 and destructively sampled. The boxes were handsorted over a period of 2 months. The worms from each box were removed and stored in soil in separate plastic boxes with holes punched in the lid, in a walk-in cold store (5-6°C) prior to identification. Post- identification, the worms were kept for 24 h on damp tissue paper, to void their gut contents, then weighed.

2.3.7 Statistical analysis

Kruskal-Wallis and Mann-Whitney tests were carried out using Minitab statistical software, Release 13.1.

2.3.7.1 Redundancy Analysis of earthworm data

The relationship between earthworm species' abundances and environmental and experimental variables were analysed by Redundancy Analysis (RDA), using CANOCO v.4.0. RDA is a constrained ordination method, which ranks the importance of the individual explanatory variables and gives both a graphic and statistical assessment of the relationship between the earthworm species and the variables that explain their distribution.

In ordination techniques such as principal component analysis (PCA), the 'variables' which represent the best for the values of all the species become the ordination axes, and these are later interpreted with the help of external environmental data.

Canonical ordination is a combination of ordination and multiple regression, which includes data on environmental variables. The regression model is inserted in the

ordination model and the ordination axes appear in the order of variance explained by linear combination of the environmental variables. These axes are given a lambda (λ) value, to denote the percentage variation in species variation they account for. A species-environmental variable biplot gives, in linear methods, a display of approximate values of correlations between species and environmental variables (ter Braak and Smilauer, 1998; Lepš and Šmilauer, 2003)

2.3.7.1.1 Interpreting RDA ordination biplots

By connecting the point of origin with a species point an arrow is obtained that points in the direction in which the species' abundance value increases at the largest rate across the ordination diagram. Arrows for the variables are obtained in a similar way. The closer a species is to the environmental variable arrow, the more positive an effect that variable had on the distribution of that species. If the species is at the end opposite to the arrow, the variable has had a negative effect on its distribution. If the species is at a right-angle to the line, its distribution is independent of the variable. Environmental variables with long arrows are the most important in the analysis. The longer the arrow, the more confidence there is in the inferred correlations, and the larger the effect of the variable on the species (ter Braak and Smilauer, 1998).

Prior to running each analysis, a PCA correlation biplot was run on the environmental variables to determine how closely correlated the environmental variables were to each other. If a group of variables were closely correlated, these appeared as a cluster of arrows. If this occurred, the one with the longest arrow (explaining the most variation) was selected for inclusion in the RDA analysis.

2.3.7.1.2 Permutation tests

Monte-Carlo permutation tests were used to evaluate the statistical significance of the relationship between species and environmental variables. This is a test of statistical significance obtained by repeatedly shuffling (permuting) the samples. No assumptions of normally distributed data are made (Lepš and Šmilauer, 2003).

The data were analysed as split-plot designs. The effect of the environmental variables that vary between whole- plots (blocks) were tested by permuting (or shuffling) whole-plots at random, while keeping the split-plots individual (boxes) of each whole-plot together. The null hypothesis is that the species data were unrelated to the environmental data. The effect of environmental variables that vary within the whole-plots or blocks can be tested by permuting the split-plot factors without permuting whole-plots.

2.3.7.1.2.1 Marginal effects:

The first variable listed in the table of Marginal Effects has the highest explanatory power; the last has the minimum ability to explain patterns in the species data. Here, each variable is judged separately, without considering the effect of the other explanatory variables.

2.3.7.1.2.2 Conditional effects:

The sequence of variables in the Conditional Effects table is obtained by a stepwise selection procedure. The explanatory variable best fitting the species data is selected first, then the next best fitting variable is added, together with the additional variance each variable explains at the time it was included. The basis of the stepwise selection is that the environmental arrow points in the direction that any particular sample point would move if that particular environmental variable increased in value, while the other variables kept their values. If a variable does not contribute to the fit of the species data in addition to the contributions of the other variables in the analysis, it has a $p > 0.05$.

2.4 Results

2.4.1 Sourhope earthworm communities

2.4.1.1 Initial Sourhope census

The results of the September 1999 census are shown in Figure 2.8. The total abundance and diversity of species found at the site was larger than expected for an acidic upland grassland.

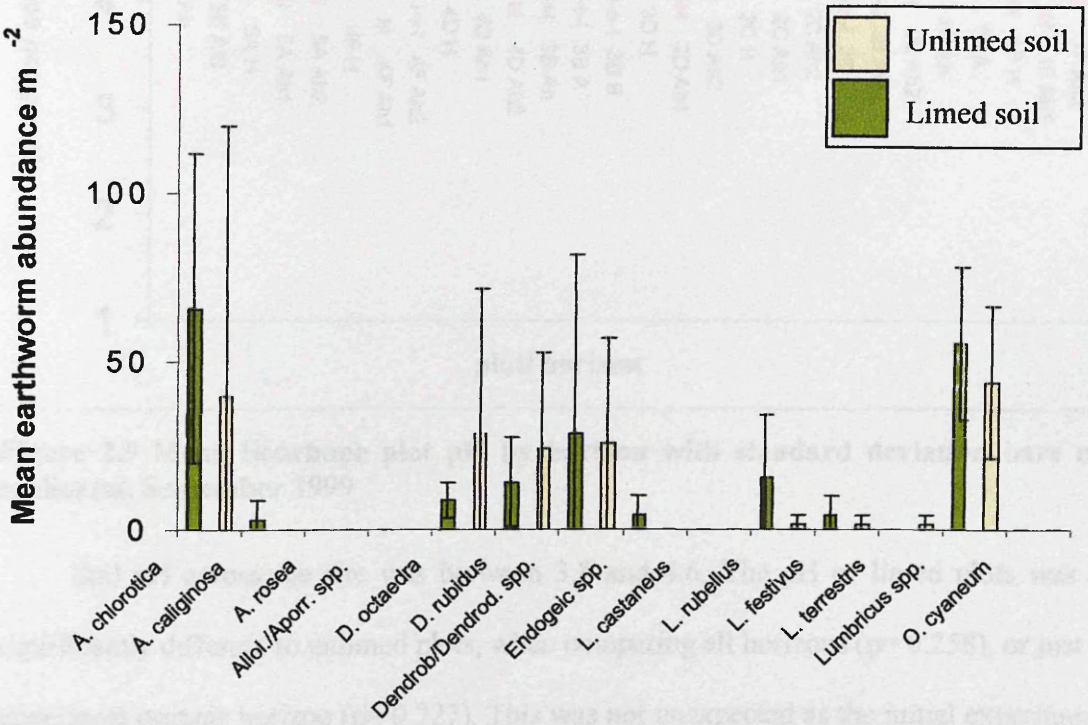


Figure 2.8 Earthworm census data from Sourhope plots, September 1999, with standard deviation bars of 5 replicates

Earthworm species names are included here where no specimens were found in order to make the comparison with the final census data easier (Section 2.4.1.3).

Species richness was greatest for the epigeic group, with 4 species present. *D. octaedra, D. rubidus, L. festivus* and *L. rubellus* were identified to species level, with '*Lumbricus* spp.' comprising of immature individuals, which had not yet developed the

distinguishing characteristics required to identify them further.

The endogeic earthworm, *A. chlorotica,* was the most abundant species identifiable to species level. The anecic *L. terrestris* was found in very low numbers in the survey.

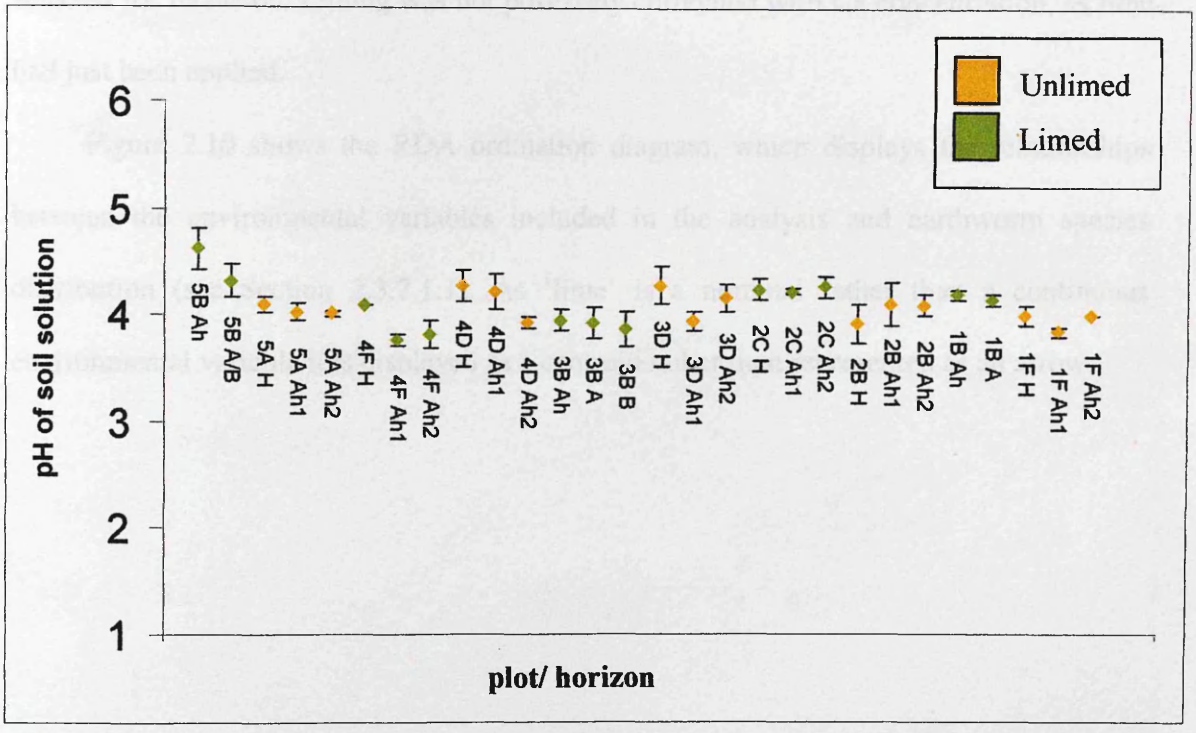


Figure 2.9 Mean Sourhope plot pH by horizon with standard deviation bars of 3 replicates, September 1999

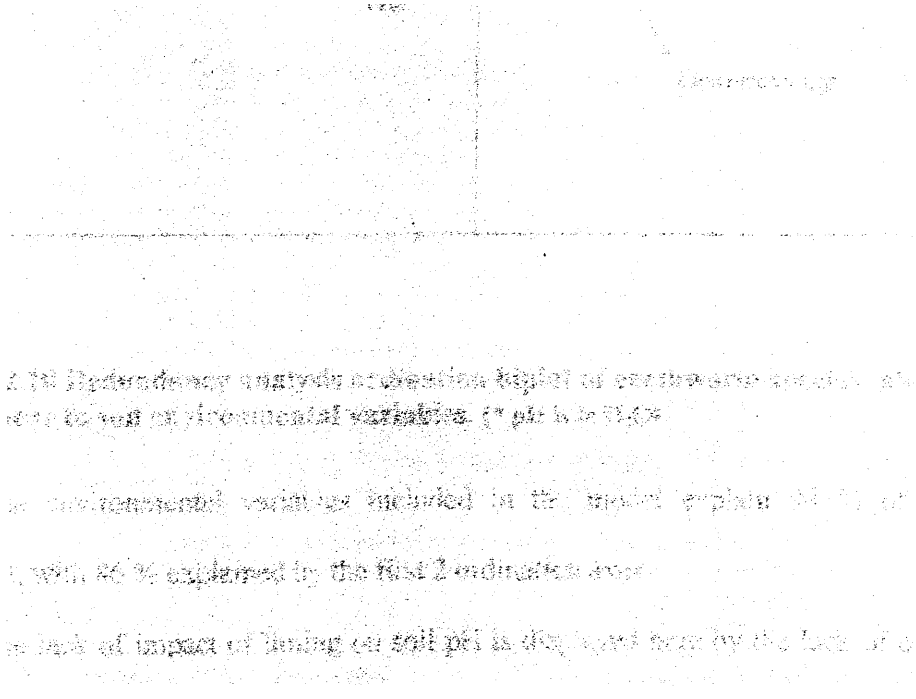
Soil pH across the site was between 3.8 and 4.6. The pH of limed plots was not significantly different to unlimed plots, when comparing all horizons ($p= 0.258$), or just the uppermost organic horizon ($p= 0.227$). This was not unexpected as the initial experimental application of lime took place in May 1999, only 4 months prior to this survey.

2.4.1.2 Spatial variation of earthworms at the Sourhope field site

The environmental data used in this analysis was taken from the 'Soil Biodiversity Programme: Baseline Data 1998: Soil sampling and Analysis Data 1'(Anon, 1998) , collected in August 1998 (See Appendix I). A principal component analysis (PCA) was carried out on this environmental data to determine which variables to include in the RDA

redundancy analysis) analysis. The PCA (Appendix D) showed that '% LOI', '% N', '% C' and '% moisture loss' were positively correlated with each other, with '% LOI' selected for inclusion in the model. '% K' and 'pH' were negatively correlated, and 'pH' was selected for inclusion. Liming was not positively correlated with Ca concentration, as lime had just been applied.

Figure 2.10 shows the RDA ordination diagram, which displays the relationships between the environmental variables included in the analysis and earthworm species distribution (see Section 2.3.7.1.1). As 'lime' is a nominal rather than a continuous environmental variable it is displayed as a centroid rather than represented as an arrow.



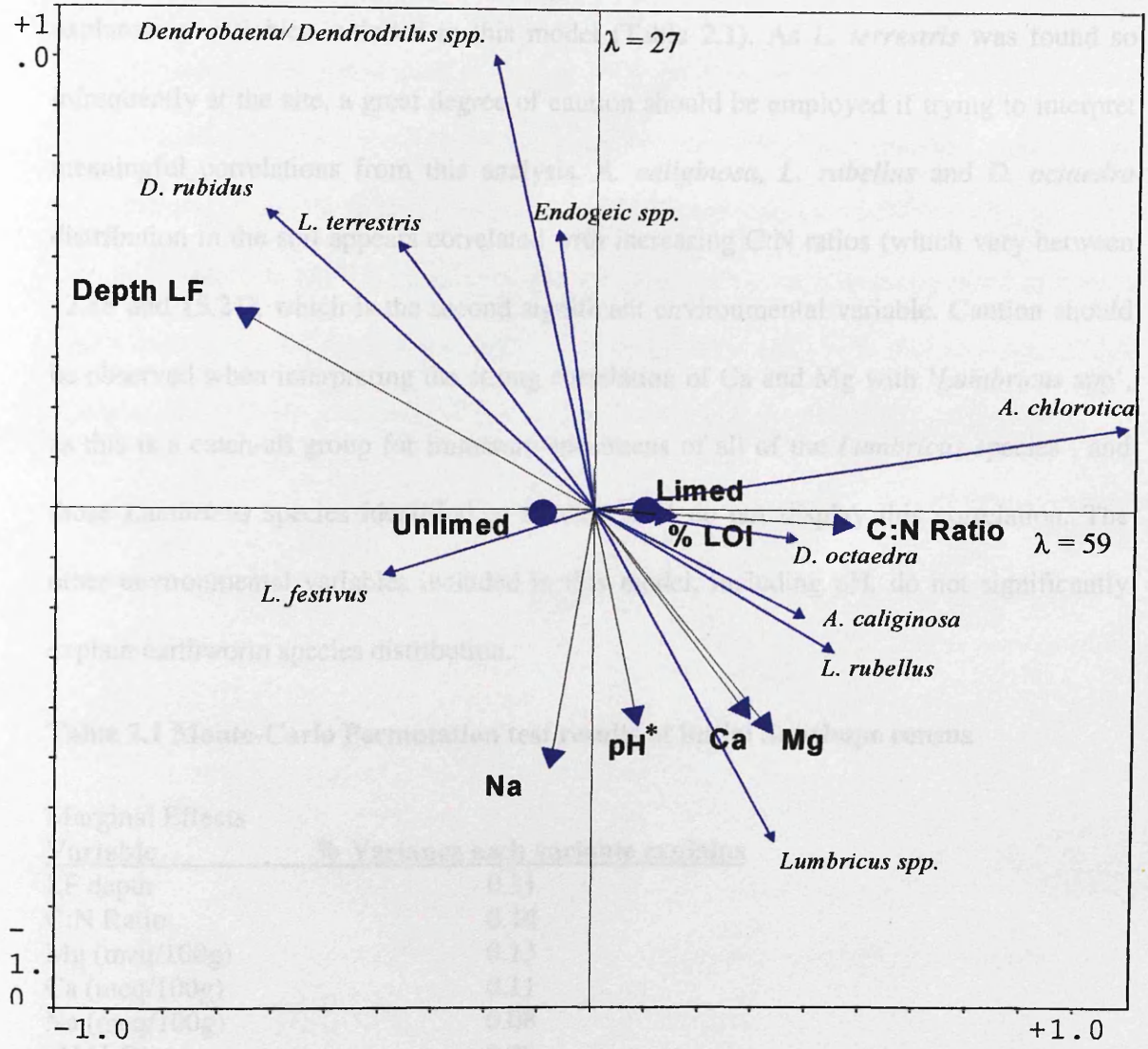


Figure 2.10 Redundancy analysis ordination biplot of earthworm species' abundances in response to soil environmental variables. (* pH is in H_2O)

The environmental variables included in the model explain 94 % of the total variation, with 86 % explained by the first 2 ordination axes.

The lack of impact of liming on soil pH is displayed here by the lack of correlation between the 'liming' and 'pH' variables, with the pH arrow appearing approximately perpendicular to the limed-unlimed gradient. The distribution of *A. chlorotica* should therefore be interpreted as being independent of pH, rather than strongly correlated with liming. *D. rubidus* is negatively correlated with increasing soil pH and Ca concentration

and positively with increasing depth of LF horizon, which is one of the two significant explanatory variables included in this model (Table 2.1). As *L. terrestris* was found so infrequently at the site, a great degree of caution should be employed if trying to interpret meaningful correlations from this analysis. *A. caliginosa, L. rubellus* and *D. octaedra* distribution in the soil appears correlated with increasing C:N ratios (which vary between 12.88 and 15.21), which is the second significant environmental variable. Caution should be observed when interpreting the strong correlation of Ca and Mg with '*Lumbricus* spp', as this is a catch-all group for immature specimens of all of the *Lumbricus* species', and those *Lumbricus* species identified to species level do not display this correlation. The other environmental variables included in this model, including pH, do not significantly explain earthworm species distribution.

Table 2.1 Monte-Carlo Permutation test results of initial Sourhope census

Marginal Effects

Variable	**% Variance each variable explains**
LF depth	0.31
C:N Ratio	0.14
Mg (meq/100g)	0.13
Ca (meq/100g)	0.11
Na (meq/100g)	0.08
pH H_2O)	0.08
Lime	0.05
% LOI	0.03

Conditional Effects

Variable	**% Variance each variable explains**	**P**	**F**
Depth LF	0.31	0.025	3.59
Lime	0.10	0.355	1.20
Na (meq/100g)	0.09	0.365	1.06
Mg (meq/100g)	0.17	0.060	2.58
C:N Ratio	0.17	0.030	4.31
pH (H_2O)	0.06	0.250	1.60
% LOI	0.04	0.315	1.52
Ca (meq/100g)	0.02	0.620	0.59

2.4.1.3 Final Sourhope census

Soil pH measurements collected in August 2002 (by Graham Burt-Smith, Sourhope field site manager) revealed significant differences between limed and unlimed plots. Limed plots had a significantly higher pH compared to unlimed plots ($p = 0.000$), with a mean pH of 6.7 compared to pH 4.5 in unlimed plots (Figure 2.11). An interesting point to note is that a comparison of the unlimed plot pH in September 1999 with that in August 2002 revealed that soil pH in August 2002 was significantly higher ($p = 0.000$).

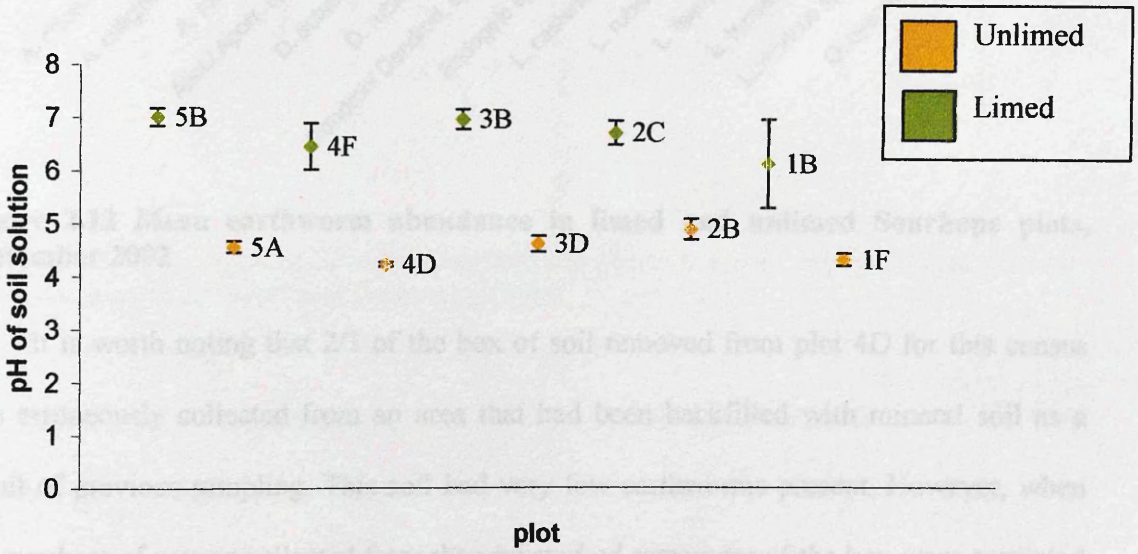


Figure 2.11 Mean Sourhope plot pH at 5 cm depth with standard deviation bars of 4 replicates collected from sub-plots S, T, U and V (Data collected by Graham Burt-Smith)

The final Sourhope earthworm census in September 2002 revealed that there were significantly more earthworms in the limed soil compared to the unlimed soil ($p = 0.021$) (Figure 2.12). However, when each species was analysed separately, there were significantly more specimens in limed soil for only two species, *D. rubidus* ($p = 0.047$) and *A. rosea* ($p = 0.028$). Although not significant, mean abundances of *A. chlorotica* and *L. rubellus* were greater in the limed soil. *O. cyaneum* was found in approximately equal numbers in both limed and unlimed plots.

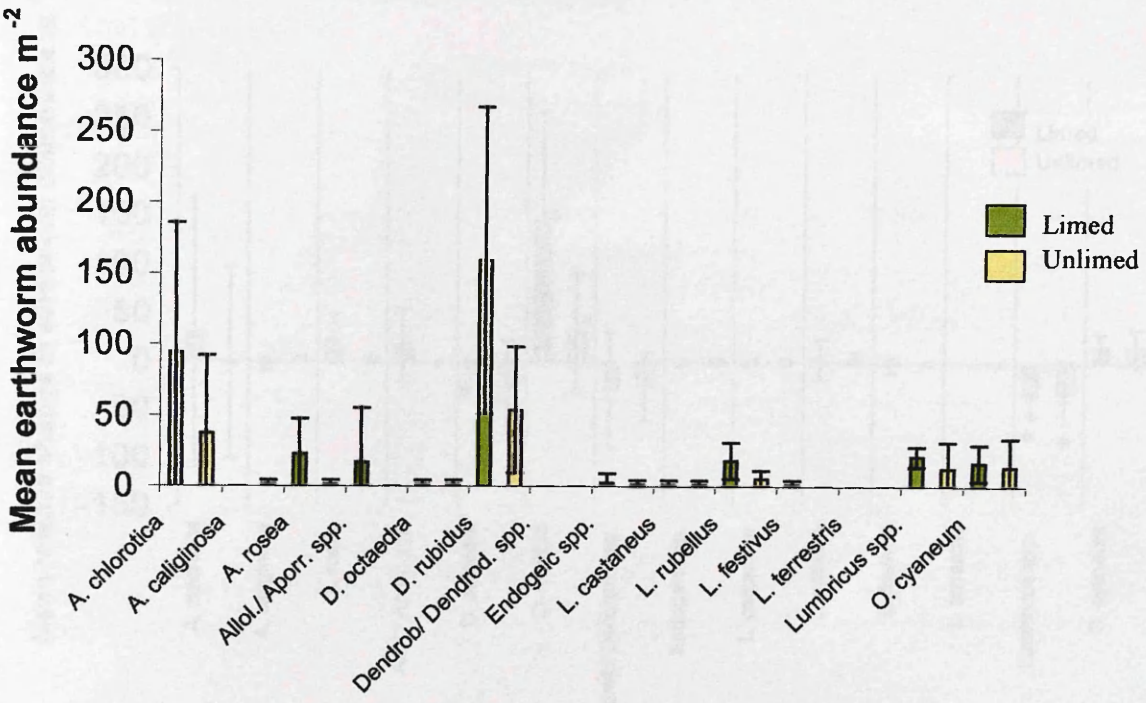


Figure 2.12 Mean earthworm abundance in limed and unlimed Sourhope plots, September 2002

It is worth noting that 2/3 of the box of soil removed from plot 4D for this census was erroneously collected from an area that had been backfilled with mineral soil as a result of previous sampling. This soil had very few earthworms present. However, when the numbers of worms collected from the undisturbed remainder of the box were corrected to take into account the smaller sample size, earthworm abundance was not significantly less than that found in the original census ($p = 0.082$), and community composition was similar.

2.4.1.4 Census comparison

Figure 2.13 shows the relative changes in earthworm abundance at the Sourhope site based on the data collected during the original earthworm survey in September 1999 and the final census taken in September 2002.

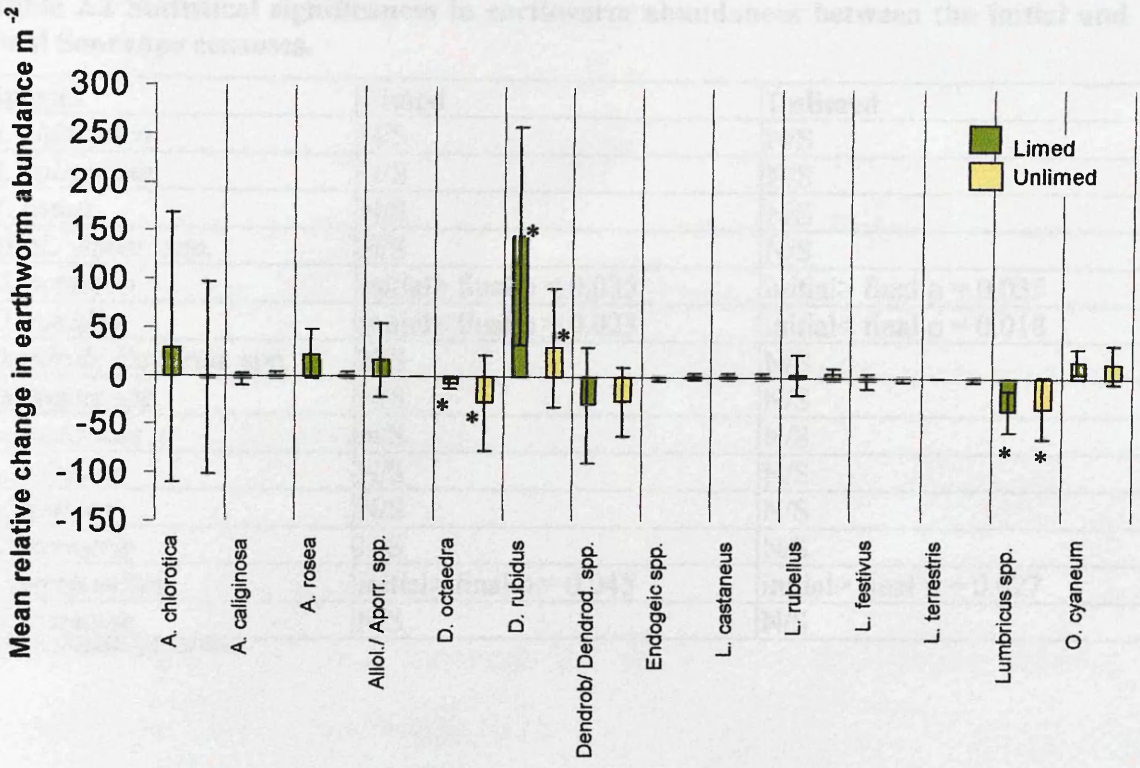


Figure 2.13 Mean relative change in earthworm abundances at the Sourhope field site between September 1999 and September 2002

*** p<0.05**

There was a large variation in *A. chlorotica* abundances in both the initial and final surveys, but no significant changes in abundance in either the limed or unlimed plots. There were significantly more *D. octaedra* in the September 1999 survey in both the limed and unlimed plots than in the 2002 survey. This was also true for the *Lumbricus* Spp. category. However, as this is a 'catch-all' group for juvenile *L. rubellus*, *L. castaneus* and *L. festivus*, caution should be employed in any interpretation of this result. *D. rubidus* was significantly more abundant in the final survey, and the magnitude of this change was larger in the limed soil treatment. *A. rosea* and *O. cyaneum* were recorded only in the second survey, and no *L. terrestris* specimens were found in the second survey.

As can be seen from Table 2.2, where significant differences in earthworm abundances are recorded, these changes occur in the same direction in both limed and unlimed soil, i.e. an increase or decrease in both soil types.

Table 2.2 Statistical significances in earthworm abundances between the initial and final Sourhope censuses.

Species	Limed	Unlimed
A. chlorotica	N/S	N/S
A. caliginosa	N/S	N/S
A. rosea	N/S	N/S
Allol./ Aporr. spp.	N/S	N/S
D. octaedra	initial> final p = 0.035	initial> final p = 0.035
D. rubidus	initial< final p = 0.023	initial< final p = 0.018
Dendrob/ Dendrod. spp.	N/S	N/S
Endogeic spp.	N/S	N/S
L. castaneus	N/S	N/S
L. rubellus	N/S	N/S
L. festivus	N/S	N/S
L. terrestris	N/S	N/S
Lumbricus spp.	initial> final p = 0.045	initial> final p = 0.027
O. cyaneum	N/S	N/S

N/S= not significantly different

2.4.2 *Mesocosm inoculation experiment*

2.4.2.1 Trial inoculation of supplied earthworm species

Figure 2.14 shows the percentage survival of 'supplied' earthworms, 4 months after inoculation into unlimed Sourhope soil.

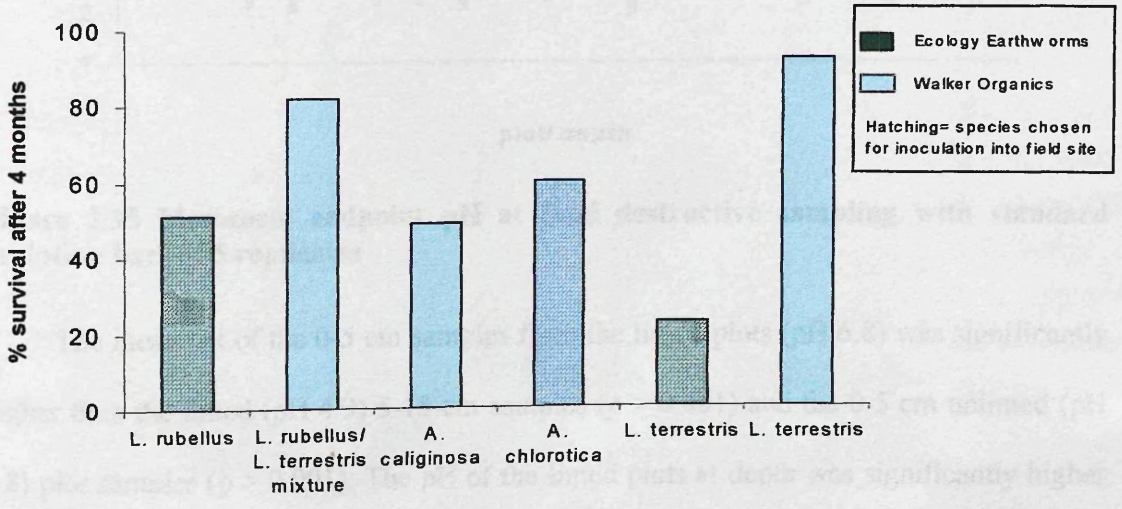


Figure 2.14 Survival of purchased earthworms inoculated into unlimed Sourhope soil.

Survival of *A. chlorotica* and *A. caliginosa* was similar, and *A. chlorotica* was chosen for inoculation, as this was more numerous at the fieldsite. It was decided to use *L. rubellus* and *L. terrestris* supplied by 'Ecology Earthworms', despite the lower survival rate. These worms were supplied as pure cultures, as opposed to a mixture of *Lumbricus* spp., of which the juveniles were difficult to identify. The decision on which species to use was therefore made based upon a) the result of this experiment, and b) the speed and reliability of the suppliers with respect to each species.

2.4.2.2 Effect of lime on soil pH measurements

Figure 2.15 shows the mean mesocosm plot pH at the final destructive sampling (measurements taken by Mark Pawlett, UEL).

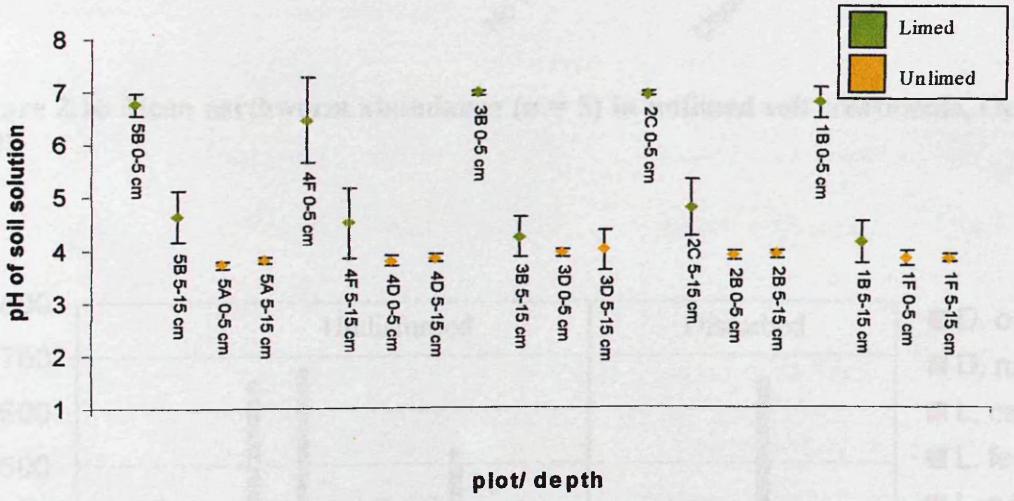


Figure 2.15 Mesocosm endpoint pH at final destructive sampling with standard deviation bars of 5 replicates

The mean pH of the 0-5 cm samples from the limed plots (pH 6.8) was significantly higher than the limed (pH 4.9) 5-15 cm samples ($p > 0.001$) and the 0-5 cm unlimed (pH 3.8) plot samples ($p > 0.001$). The pH of the limed plots at depth was significantly higher than for the corresponding unlimed (pH 3.9) samples ($p > 0.001$). There was no depth effect in the unlimed samples.

2.4.2.3 Mesocosm earthworm communities, inoculated earthworm survival and consequences

Figure 2.16 and Figure 2.17 show the mean earthworm abundance in each treatment 19 months after inoculation, in unlimed and limed soil respectively.

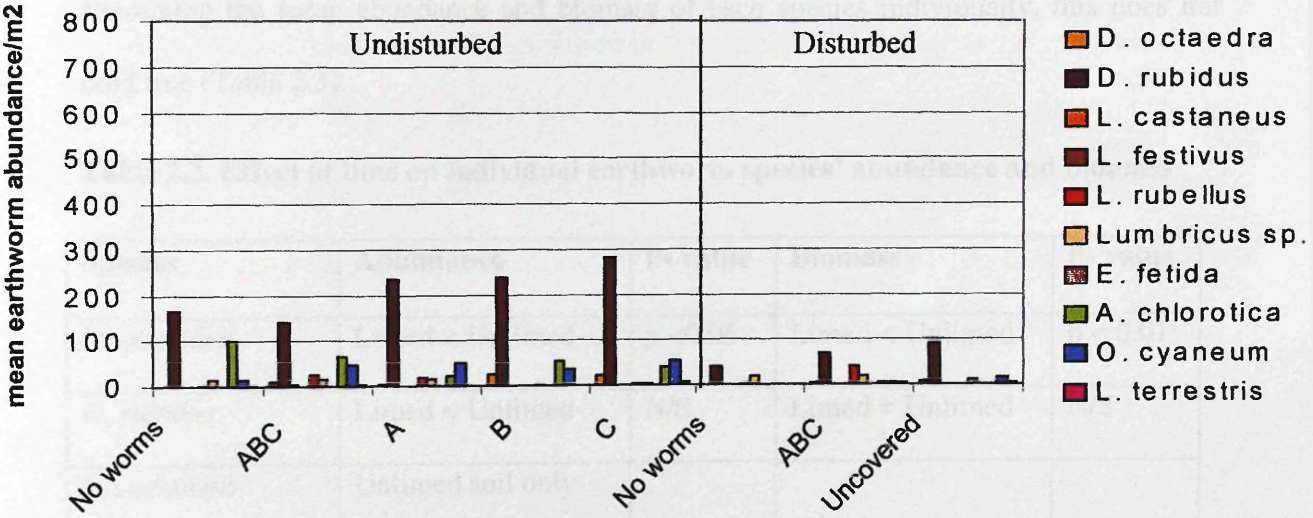


Figure 2.16 Mean earthworm abundance (n = 5) in unlimed soil treatments, October 2001

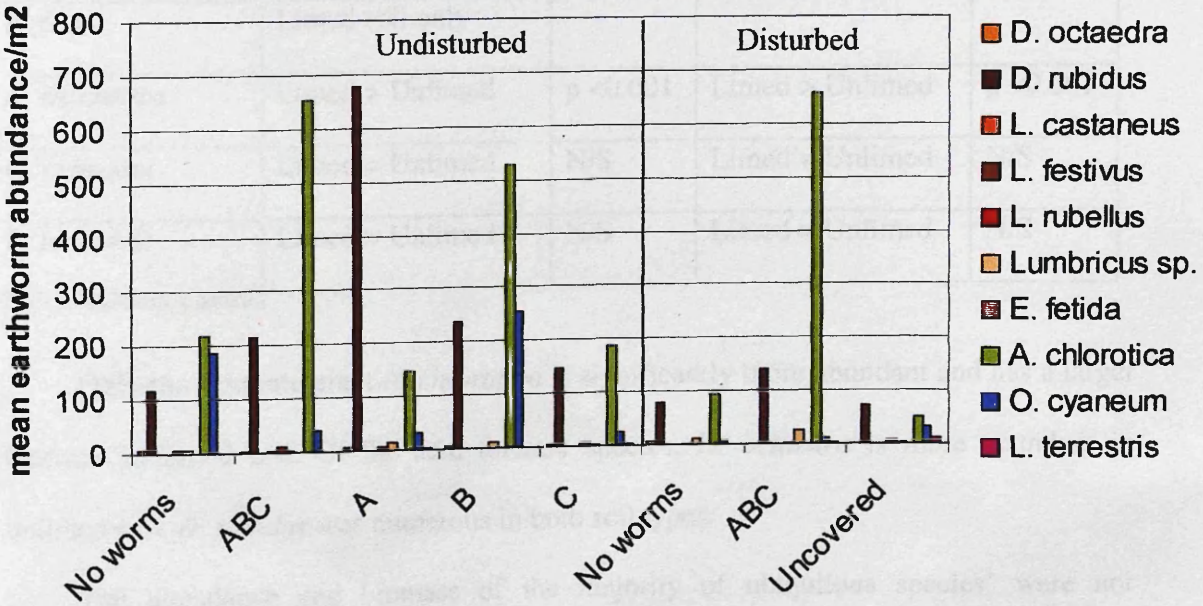


Figure 2.17 Mean earthworm abundance (n = 5) in limed soil treatments, October 2001

KEY: **No worms**- no inoculated earthworms; **ABC**- all three ecological groups inoculated; **A**- *L. rubellus* inoculated; **B**- *A. chlorotica* inoculated; **C**- *L. terrestris* inoculated; **Uncovered**- uninoculated disturbed box, without a mesh cover.

Total mean earthworm abundance and biomass were significantly greater in limed soil, compared to unlimed soil (p=0.0318 and p=0.0471 respectively). However, when examining the mean abundance and biomass of each species individually, this does not hold true (Table 2.3).

Table 2.3. Effect of lime on individual earthworm species' abundance and biomass

Species	Abundance	P- value	Biomass	P- value
D. octaedra	Limed < Unlimed	p <0.05	Limed < Unlimed	p < 0.01
D. rubidus	Limed = Unlimed	N/S	Limed = Unlimed	N/S
L. castaneus	Unlimed soil only			
L. festivus	Limed soil only			
L. rubellus	Limed = Unlimed	N/S	Limed = Unlimed	N/S
Lumbricus spp.	Limed = Unlimed	N/S	Limed = Unlimed	N/S
E. fetida	Limed soil only			
A. chlorotica	Limed > Unlimed	p <0.001	Limed > Unlimed	p <0.001
O. cyaneum	Limed = Unlimed	N/S	Limed = Unlimed	N/S
L. terrestris	Limed = Unlimed	N/S	Limed = Unlimed	N/S

N/S= not significantly different

Only the acid intolerant *A. chlorotica* is significantly more abundant and has a larger biomass in limed soil. Of the acid tolerant species, *D. octaedra* is more abundant in unlimed soil. *D. rubidus* was numerous in both soil types.

The abundance and biomass of the majority of ubiquitous species' were not significantly affected by the application of lime. The exceptions were *L. festivus, E. fetida*

and *L. castaneus*. *L. festivus* and *E. fetida* were both present in limed boxes only, *L. festivus* in small numbers and only a single *E. fetida* specimen. This was found in a limed, disturbed, but uninoculated box which had a mesh cover.

Figure 2.18-Figure 2.27 present both abundance and biomass data for each plot individually. These data can be found in tabulated form in Appendix G. It is possible that some juvenile *A. rosea* were misidentified and incorrectly classified as *O. cyaneum*. It is unlikely that this will cause any serious problems in further interpretation and analysis of these data. Both species belong to the endogeic functional group, and only a small number of samples are possibly affected, those with a high abundance of '*O. cyaneum*' with a lower than expected biomass. These could be juvenile *O. cyaneum* or a mixture of juvenile *O. cyaneum* and *A. rosea*. A summary of the mesocosm earthworm inoculation treatments is provided in Table 2.4.

Table 2.4 Summary of mesocosm treatments

Treatment code	Earthworm inoculation/ soil disturbance/ mesh cover		
1,0	No inoculated earthworms	Undisturbed soil	Covered
2,0	All 3 ecological groups inoculated	Undisturbed soil	Covered
4,0	Epigeic - *L. rubellus*	Undisturbed soil	Covered
5,0	Endogeic - *A. chlorotica*	Undisturbed soil	Covered
6,0	Anecic – *L. terrestris*	Undisturbed soil	Covered
1,1	No inoculated earthworms	Disturbed soil	Covered
2,1	All 3 ecological groups inoculated	Disturbed soil	Covered
3,1	No inoculated earthworms	Disturbed soil	Uncovered

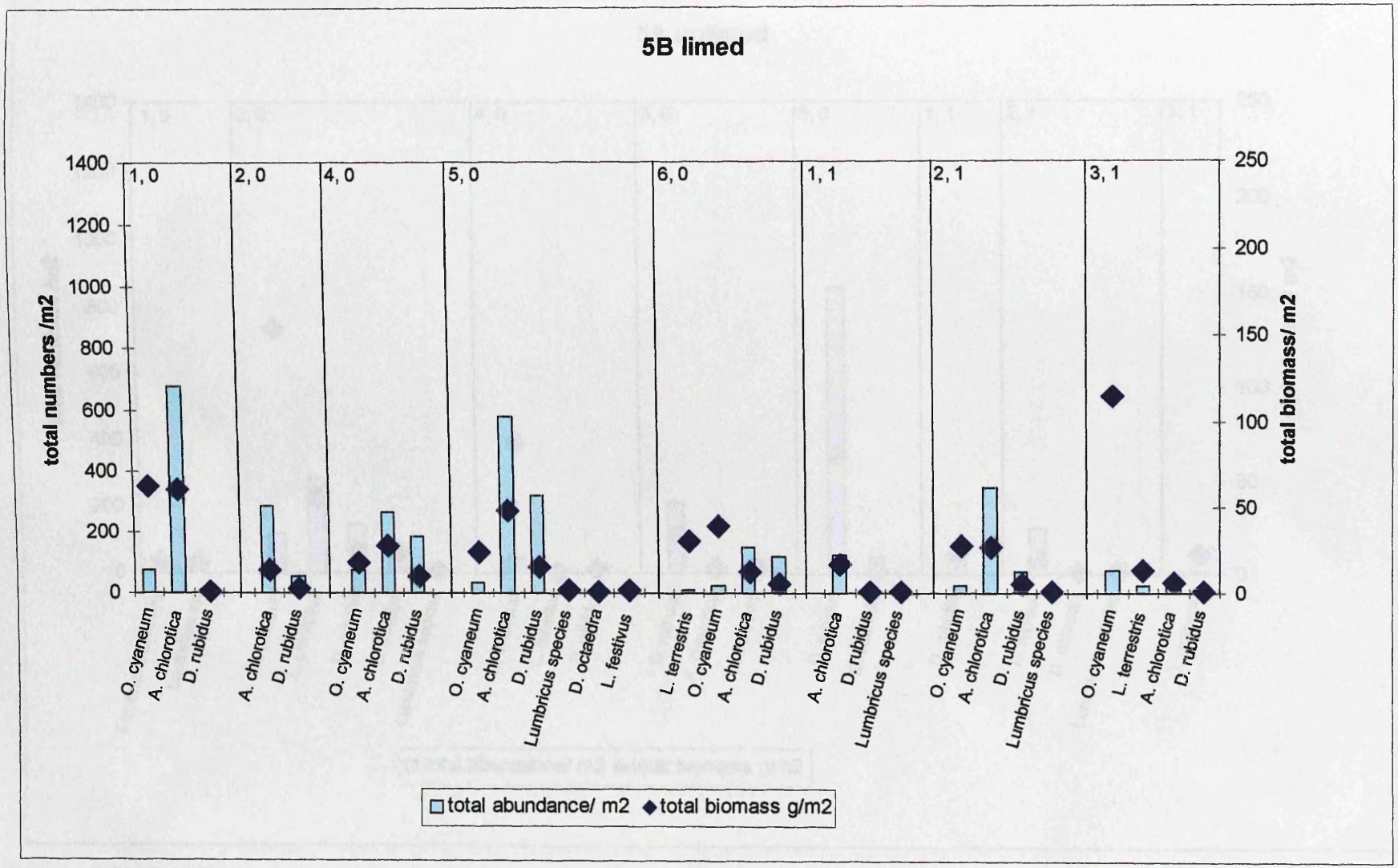


Figure 2.18 Earthworm abundance and biomass found in each inoculation treatment at the conclusion of the field mesocosm

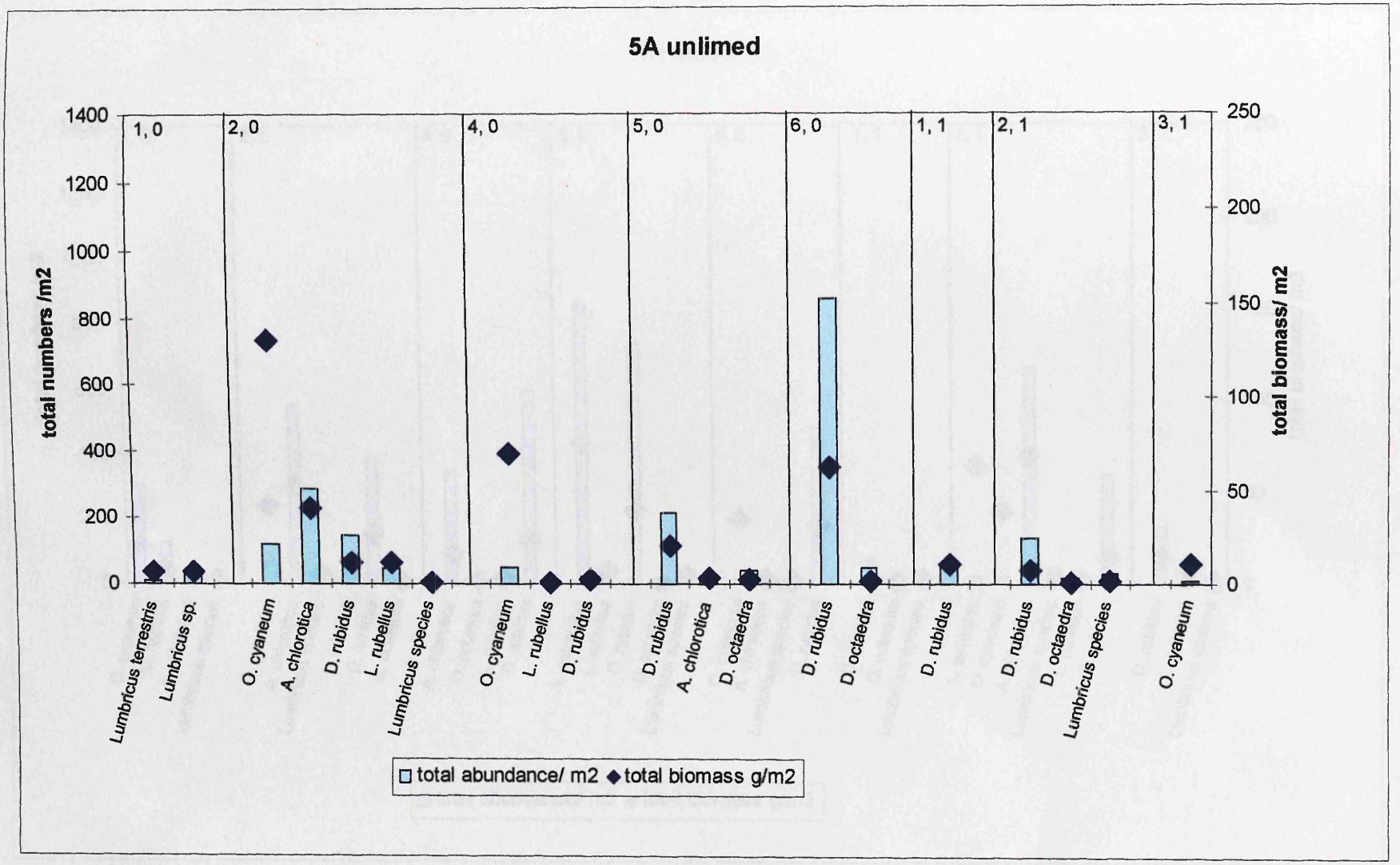


Figure 2.19 Earthworm abundance and biomass found in each inoculation treatment at the conclusion of the field mesocosm experiment, plot 5A unlimed.

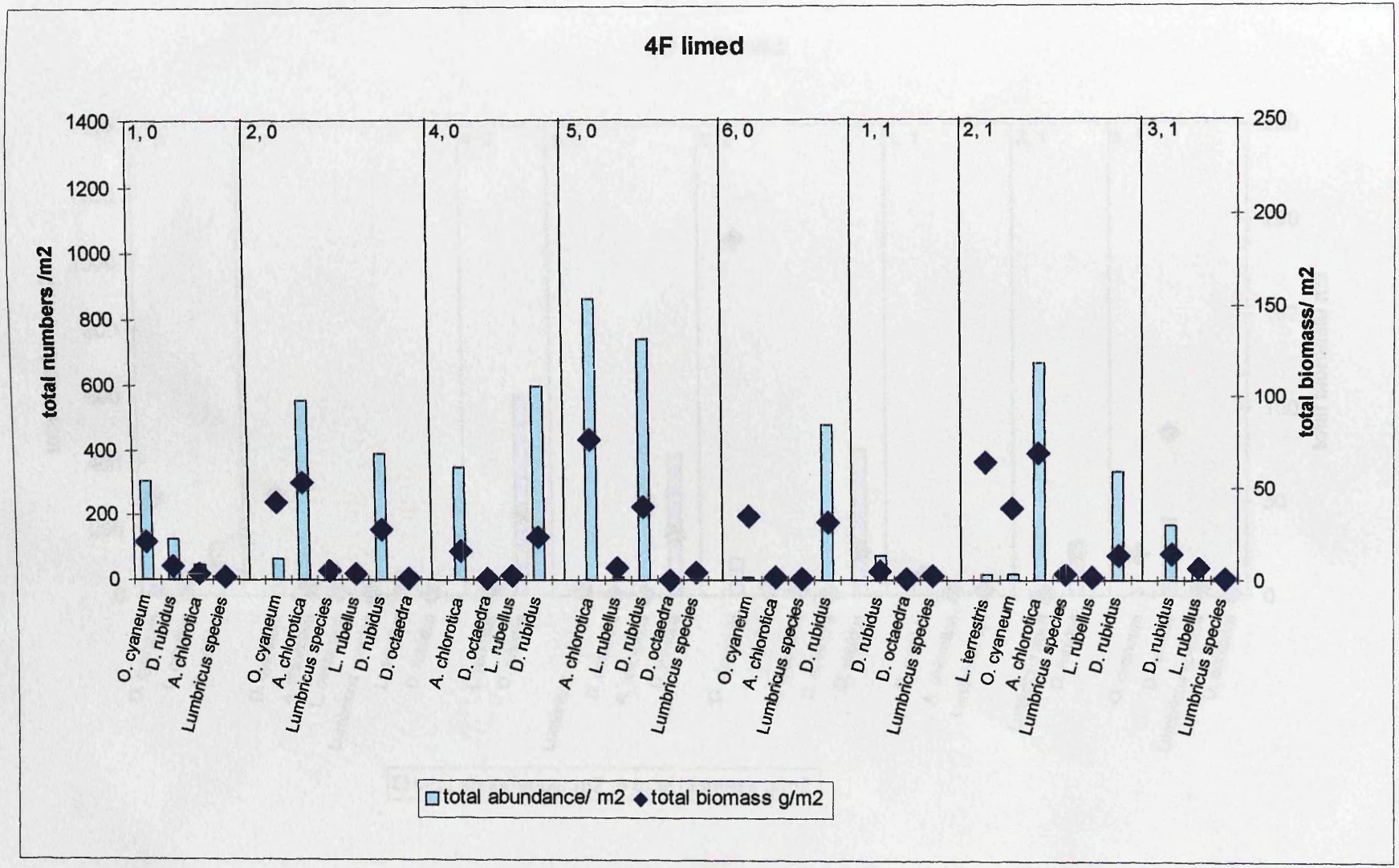


Figure 2.20 Earthworm abundance and biomass found in each inoculation treatment at the conclusion of the field mesocosm experiment, plot 4F limed.

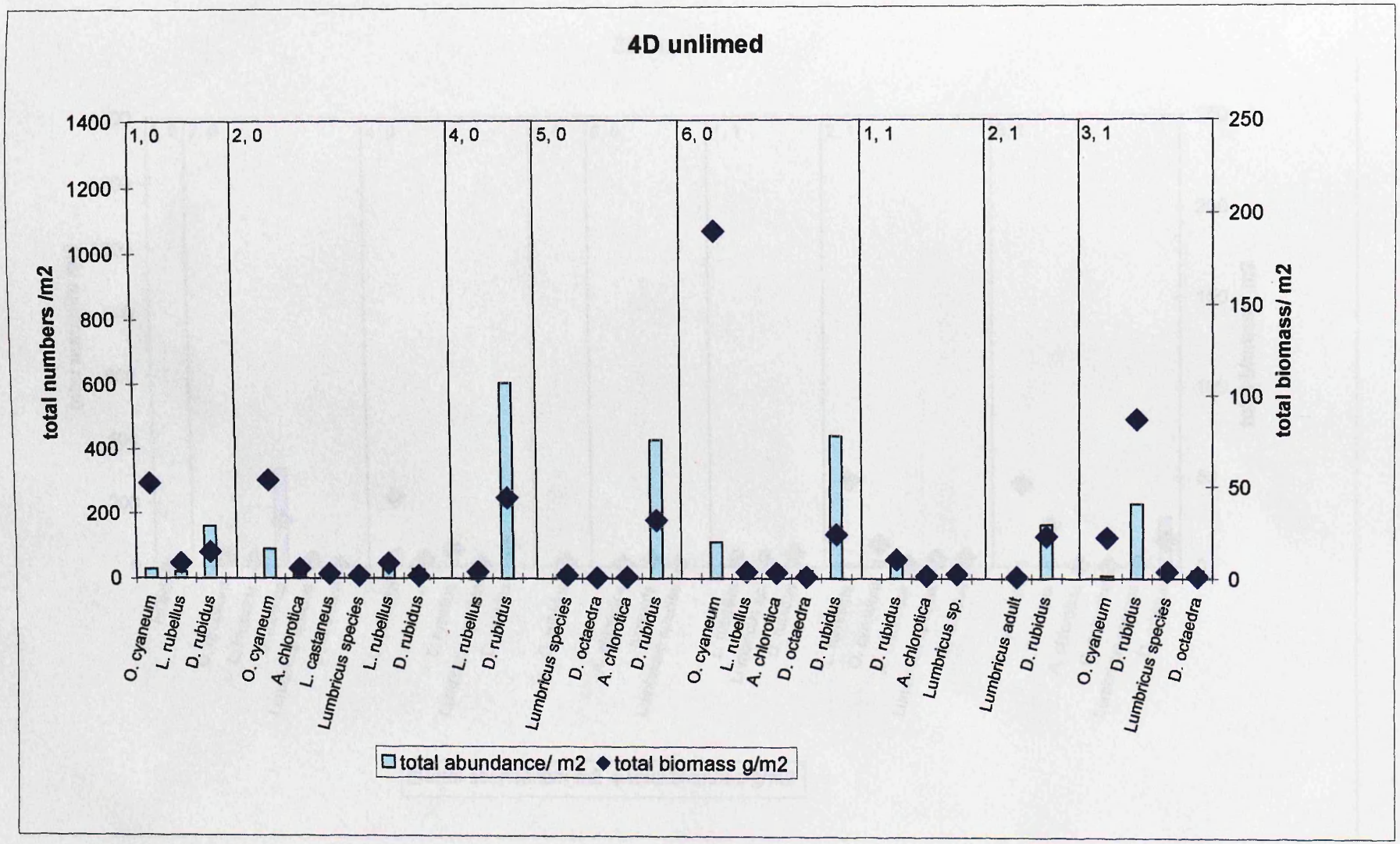


Figure 2.21 Earthworm abundance and biomass found in each inoculation treatment at the conclusion of the field mesocosm experiment, plot 4D unlimed.

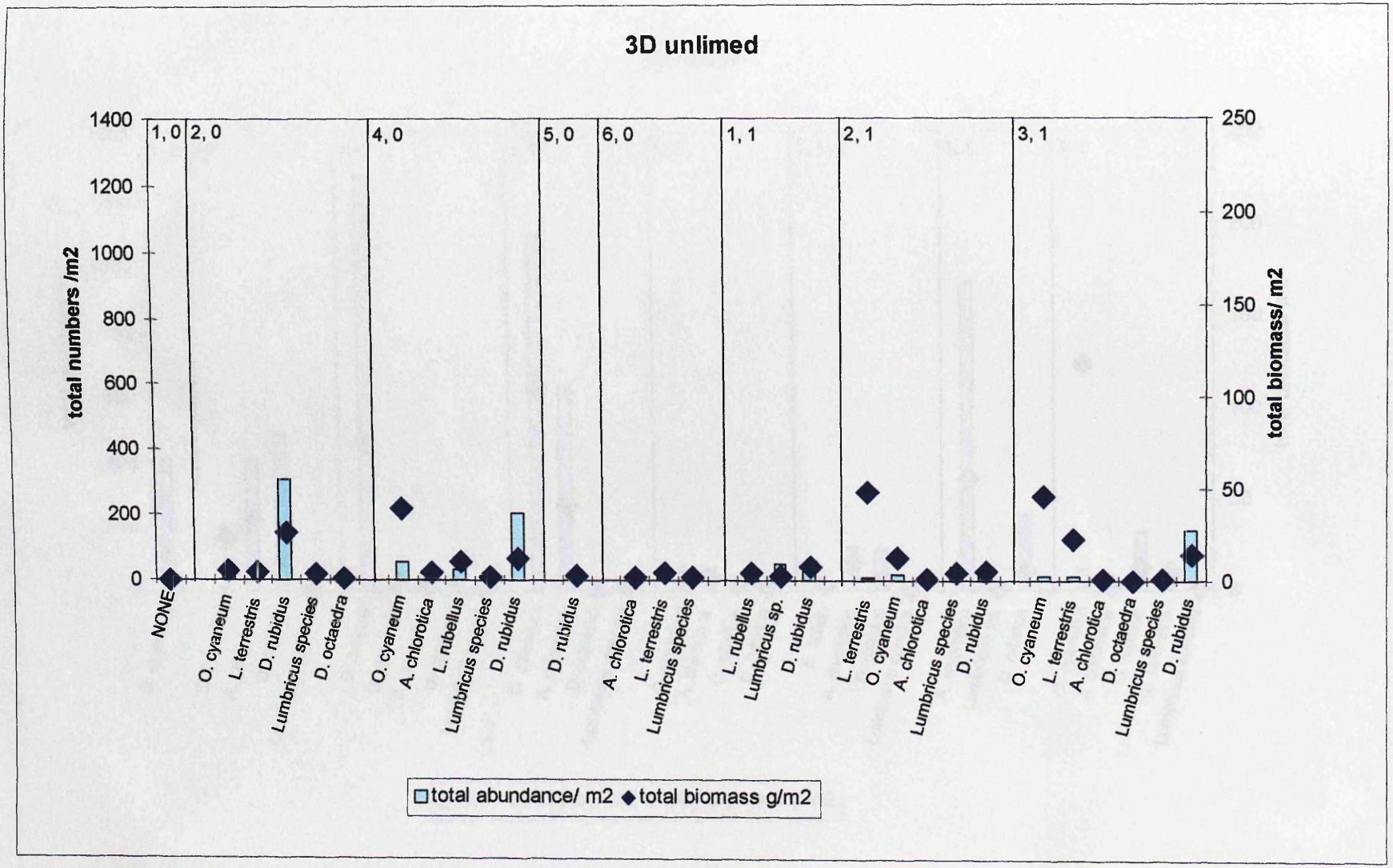


Figure 2.22 Earthworm abundance and biomass found in each inoculation treatment at the conclusion of the field mesocosm experiment, plot 3D unlimed.

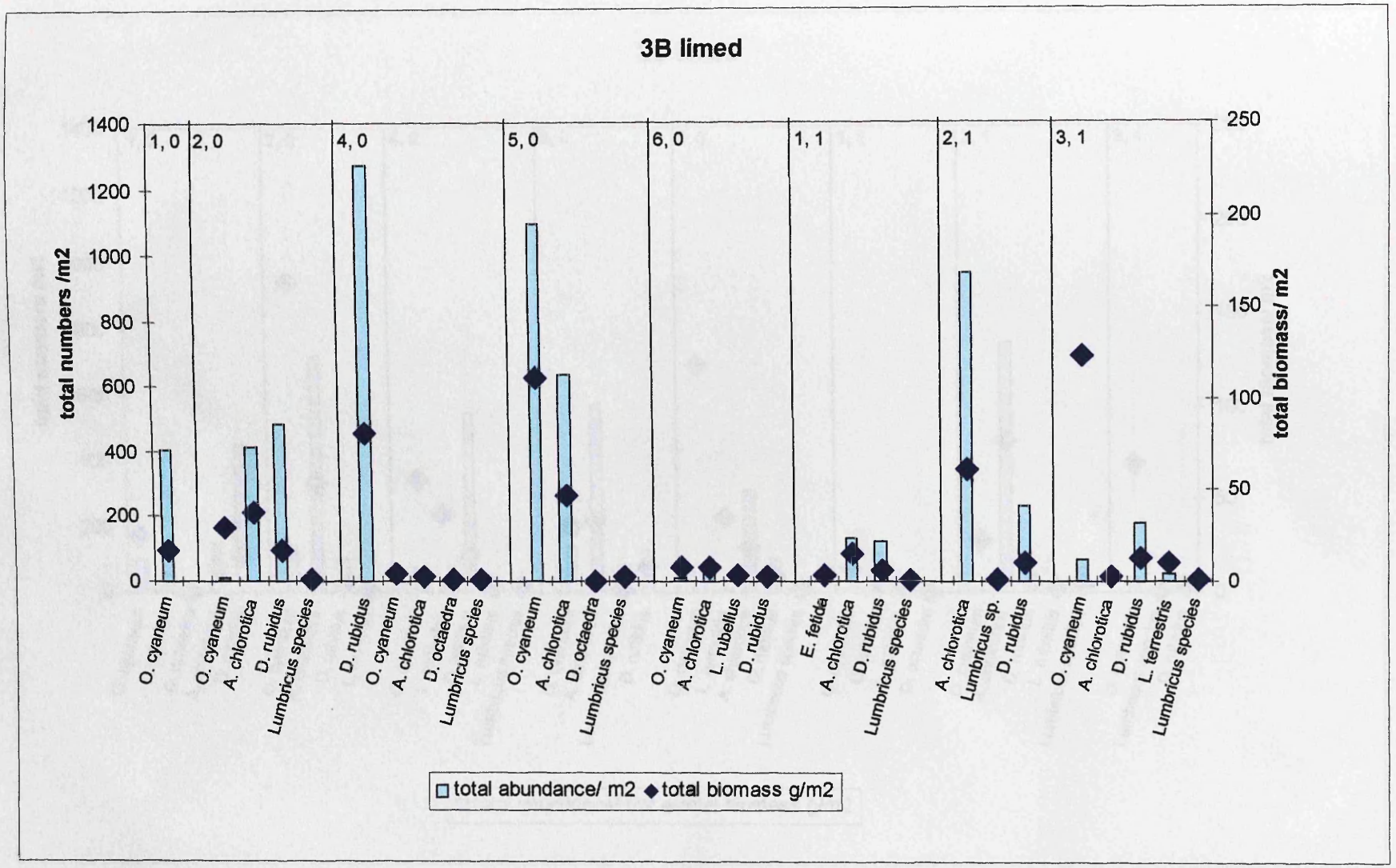


Figure 2.23 Earthworm abundance and biomass found in each inoculation treatment at the conclusion of the field mesocosm experiment, plot 3B limed.

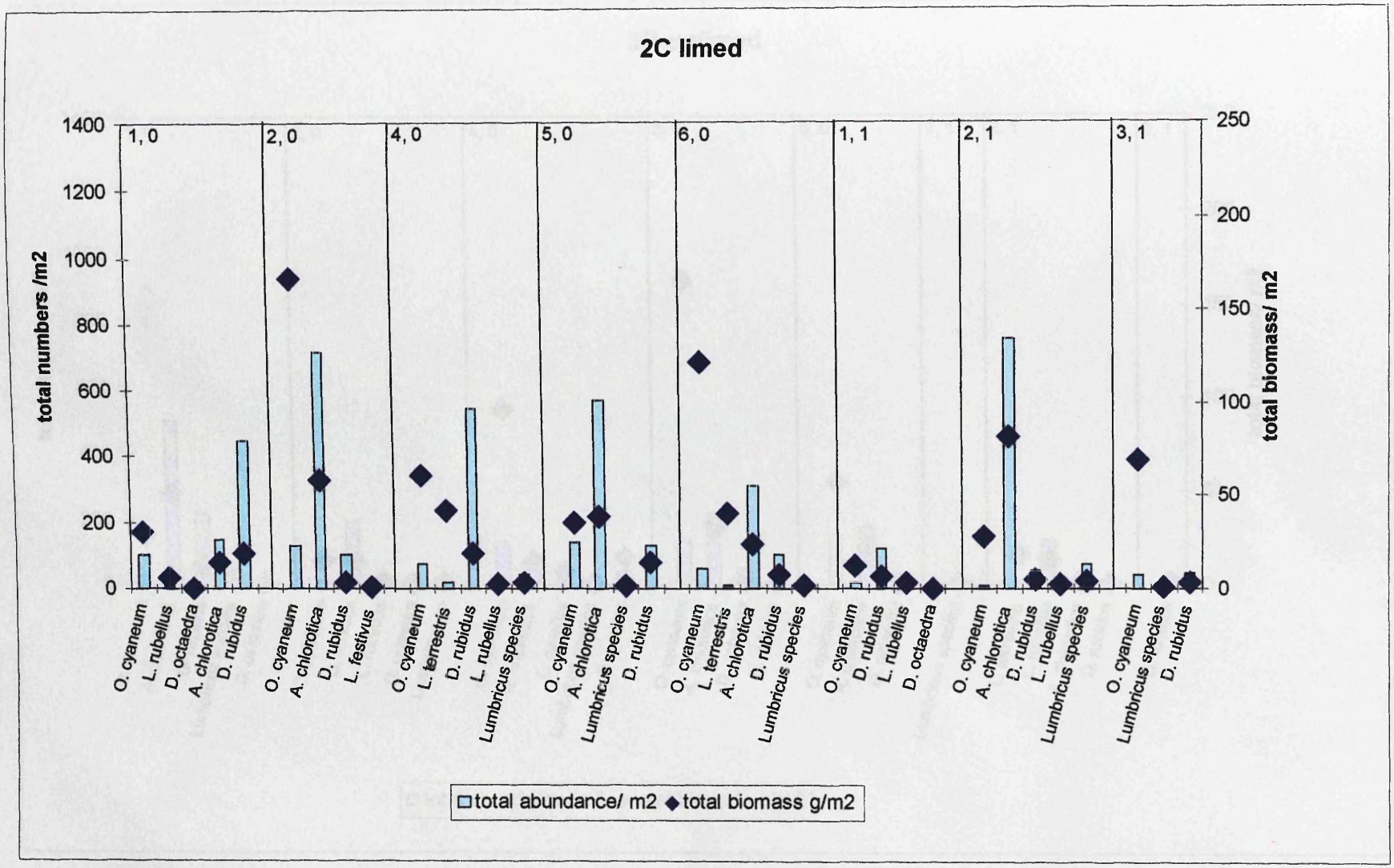


Figure 2.24 Earthworm abundance and biomass found in each inoculation treatment at the conclusion of the field mesocosm experiment, plot 2C limed.

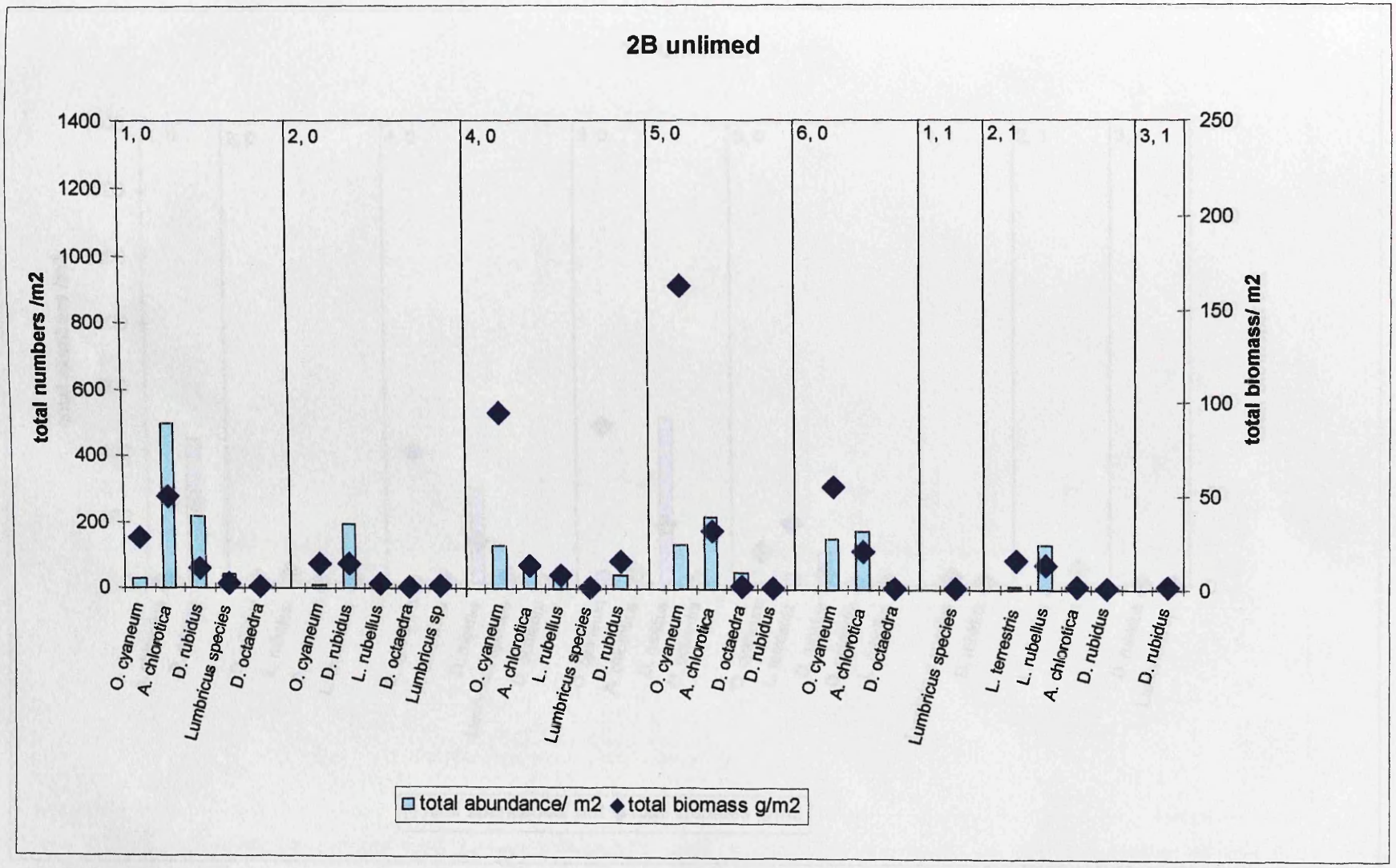


Figure 2.25 Earthworm abundance and biomass found in each inoculation treatment at the conclusion of the field mesocosm experiment, plot 2B unlimed.

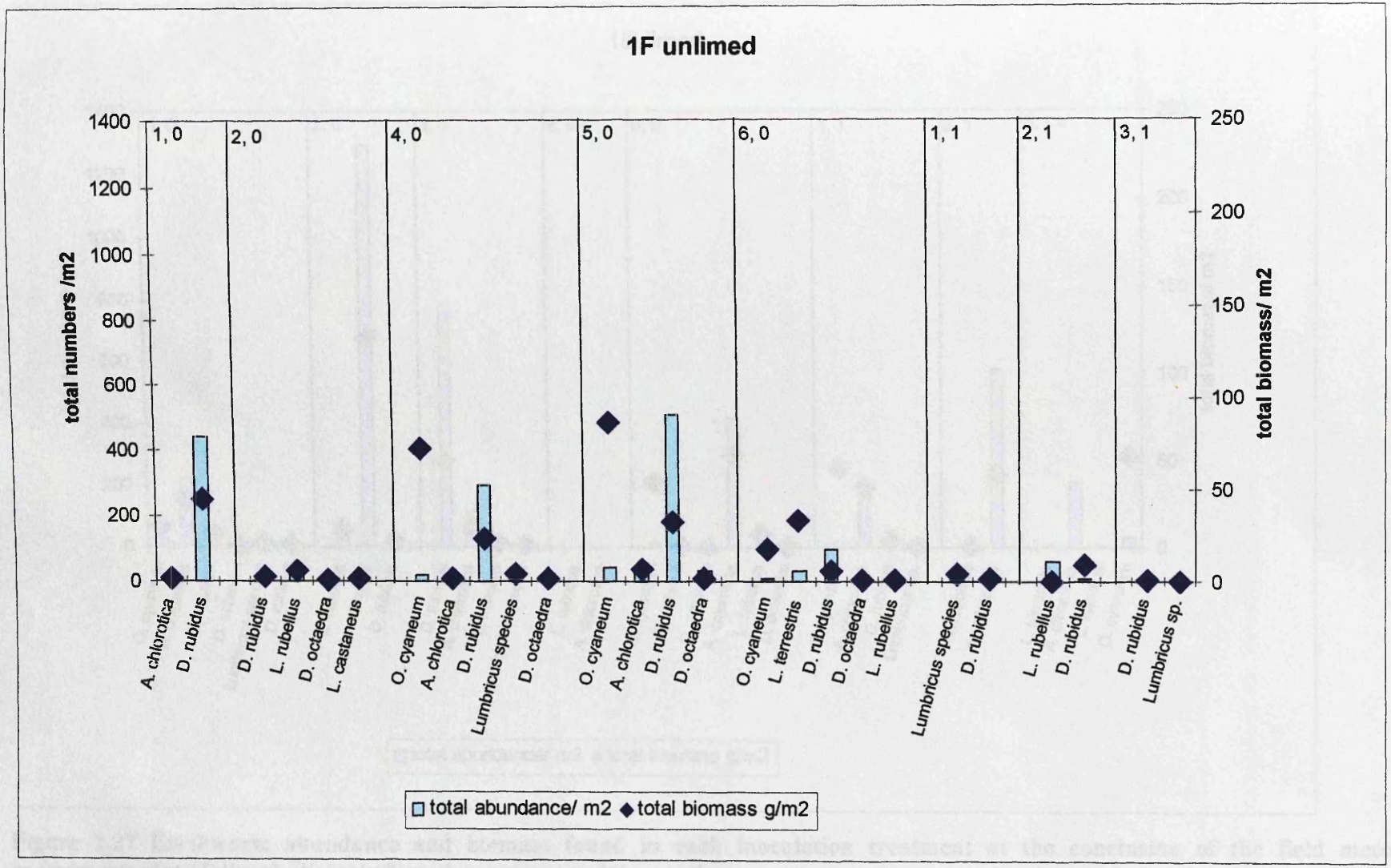


Figure 2.26 Earthworm abundance and biomass found in each inoculation treatment at the conclusion of the field mesocosm experiment, plot 1F unlimed.

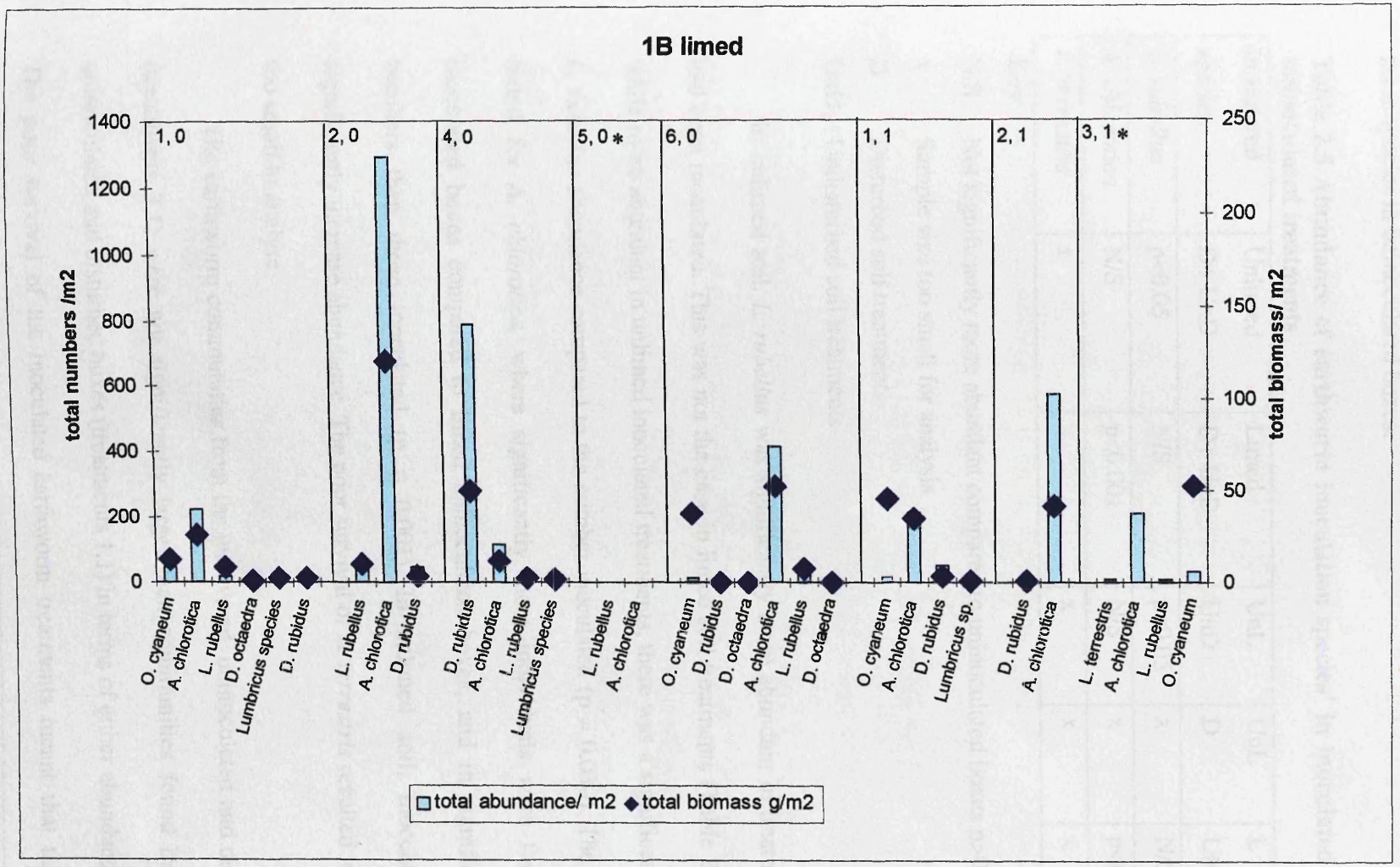


Figure 2.27 Earthworm abundance and biomass found in each inoculation treatment at the conclusion of the field mesocosm experiment, plot 1B limed. *Some/ all earthworms were decomposing so some measurements may be missing

The species used to modify the earthworm community were not consistently more abundant in the boxes they were inoculated into (Table 2.5) compared to the abundance of these species in uninoculated boxes.

Table 2.5 Abundance of earthworm inoculation species' in inoculated compared to uninoculated treatments

Inoculated species	Unlimed D+ UnD	Limed D+ UnD	UnL UnD	UnL D	L UnD	L D
L. rubellus	$p<0.05$	N/S	$p<0.05$	x	N/S	x
A. chlorotica	N/S	$p<0.001$	N/S	x	$p<0.01$	$p<0.01$
L. terrestris	x	x	x	x	x	x

Key:

N/S Not significantly more abundant compared to uninoculated boxes $p>0.05$)

x Sample size too small for analysis

D Disturbed soil treatments

UnD Undisturbed soil treatments

In unlimed soil, *L. rubellus* was significantly more abundant in treatments where it had been inoculated. This was not the case in limed soil treatments (Table 2.5). However, while more abundant in unlimed inoculated treatments, there was a significant reduction in *L. rubellus* abundance compared to the number inoculated ($p = 0.030$). The converse was found for *A. chlorotica,* where significantly more individuals were found in limed inoculated boxes compared to limed uninoculated boxes, and in significantly greater numbers than those inoculated ($p = 0.001$). In unlimed soil, inoculation did not significantly increase abundance. The poor survival of *L. terrestris* resulted in sample sizes too small to analyse.

The earthworm communities from the uncovered, uninoculated and disturbed boxes (treatments 3,1) were not significantly larger than communities found in the covered, uninoculated and disturbed boxes (treatments 1,1) in terms of either abundance or biomass. The poor survival of the inoculated earthworm treatments meant that the comparison

between these treatments was no longer a valid one, as treatments being compared could have very different earthworm communities. It was decided therefore to analyse the distribution of earthworm species using a direct gradient analysis method, to explain the species abundances using environmental variable data.

2.4.2.4 Response of earthworm species' to experimental variables

Figure 2.28 shows the RDA ordination diagram of the distribution of all the earthworm species in response to measured experimental variables. The nominal environmental variables 'lime', 'disturbance' and 'mesh cover' are displayed as centroids, rather than arrows. As a way of incorporating information about the plant species recorded from each of the mesocosm boxes (Appendix D), this analysis included the first 4 axes of the RDA analysis (Figure 3.6) of plant species data included as environmental axes. A PCA of the environmental variables included in this analysis revealed that 'depth LF horizon', 'axis 1' and 'axis 2' were positively correlated, with 'depth LF horizon' selected for inclusion in the model (Appendix D). 'Axis 4' and 'disturbance' were negatively correlated, and 'disturbance' was selected for inclusion, as 'Axis 4' probably reflects the plant species' response to disturbance.

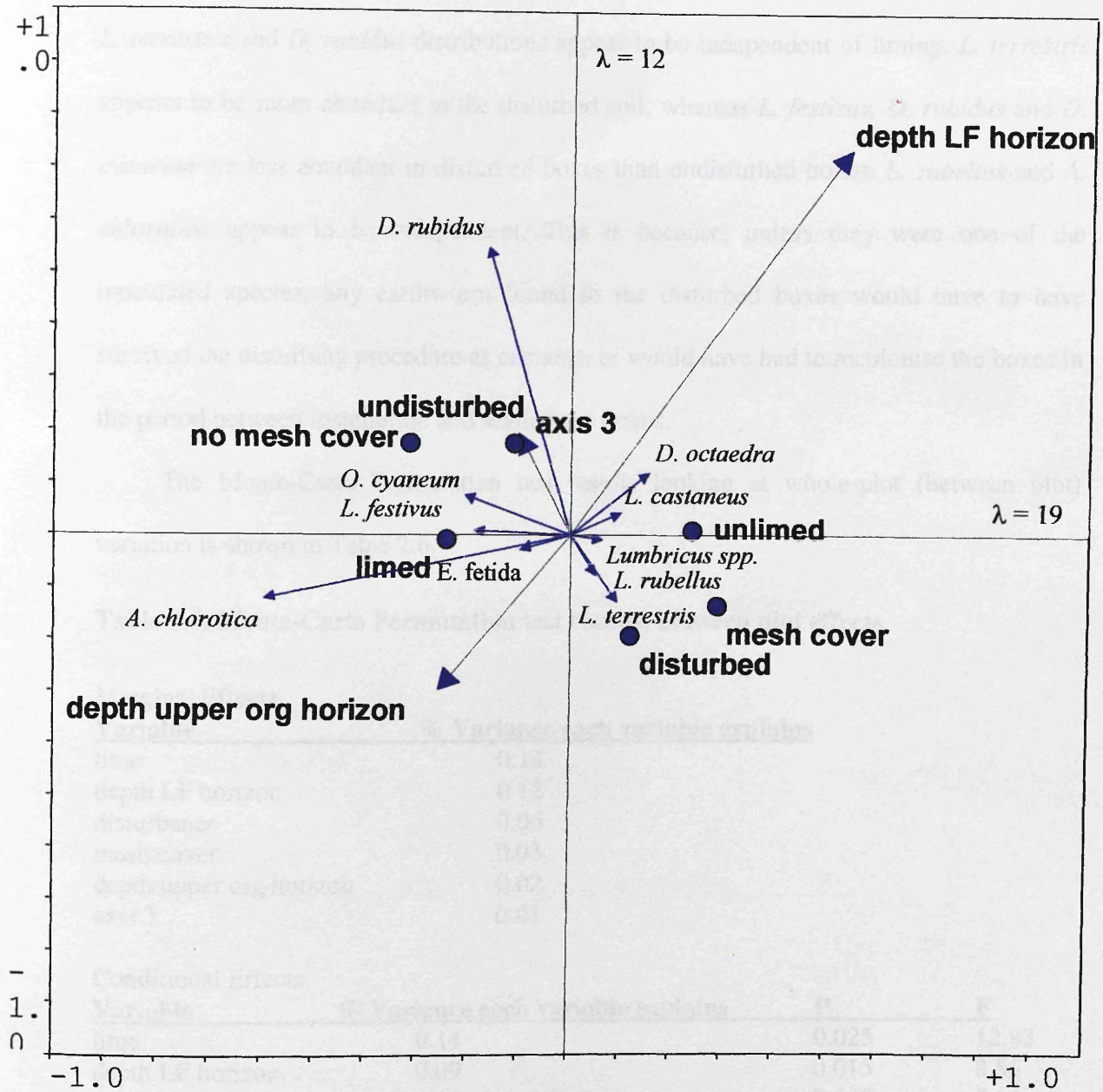


Figure 2.28 Redundancy analysis ordination biplot of earthworm species' abundances in response to experimental variables.

The environmental variables included in the model explain 32% of the total variation, with 31% explained by the first 2 ordination axes.

This analysis indicates that *A. chlorotica* abundance is increased in limed boxes, while *D. octaedra* and *L. castaneus* responded negatively to liming. Again, as *Lumbricus* spp. comprises all the *Lumbricus* species present at the site, caution should be employed in

attaching significance to the placement of this label in the ordination diagram. *O. cyaneum, L. terrestris* and *D. rubidus* distributions appear to be independent of liming. *L. terrestris* appears to be more abundant in the disturbed soil, whereas *L. festivus, D. rubidus* and *O. cyaneum* are less abundant in disturbed boxes than undisturbed boxes. *L. rubellus* and *A. chlorotica* appear to be independent. This is because, unless they were one of the inoculated species, any earthworm found in the disturbed boxes would have to have survived the disturbing procedure as cocoons, or would have had to recolonise the boxes in the period between installation and sealing the boxes.

The Monte-Carlo Permutation test results looking at whole-plot (between plot) variation is shown in Table 2.6.

Table 2.6. Monte-Carlo Permutation test results, between plot effects

Marginal Effects

Variable	% Variance each variable explains
lime	0.14
depth LF horizon	0.12
disturbance	0.06
mesh cover	0.03
depth upper org horizon	0.02
axis 3	0.01

Conditional Effects

Variable	% Variance each variable explains	P	F
lime	0.14	0.025	12.83
depth LF horizon	0.09	0.015	8.56
disturbance	0.05	0.530	5.14
axis 3	0.03	0.030	3.13
mesh cover	0.00	0.405	0.88
depth upper org horizon	0.01	0.350	0.57

When added together sequentially, the environmental variables included in the model account for 34% of the variation in earthworm abundance across the plots. Liming accounted for the greatest between plot variation (14%), followed by the depth of the LF horizon (9%).

Lime, depth of LF horizon and botanical axis 3 significantly influenced earthworm distribution (p=0.030, 0.010 and 0.020 respectively). Disturbance was not significant at the whole plot level, as each whole plot contains both disturbed and undisturbed samples. 'Botanical axis 3' appears to be negatively correlated with disturbance, and may be some aspect of botanical composition that the earthworms are responding to or alternatively, both plants and earthworms may be responding to some other, undefined environmental variable. Table 2.7 shows the results of the Monte-Carlo Permutation test results for split-plot (within-plot) variation.

Table 2.7. Monte-Carlo Permutation test results, within plot effects

Marginal Effects

Variable	**% Variance each variable explains**
lime	0.14
depth LF horizon	0.12
disturbance	0.06
mesh cover	0.03
depth upper org horizon	0.02
axis 3	0.01

Conditional Effects

Variable	**% Variance each variable explains**	**P**	**F**
lime	0.14	1.000	12.83
depth LF horizon	0.09	0.005	8.56
disturbance	0.05	0.010	5.14
axis 3	0.03	0.035	3.13
mesh cover	0.00	0.430	0.88
depth upper org horizon	0.01	0.840	0.57

Here, the variables significantly affecting earthworm distribution within the plots are: depth of LF horizon (p = 0.005), disturbance (p = 0.005) and botanical axis (p = 0.005). In this case, liming is not significant in explaining variation within the plots, as this is a whole plot level variable.

2.5 Discussion

2.5.1 The size and diversity of the earthworm community

The September 1999 census of the Sourhope earthworm community revealed that most abundant ecological group was the epigeic group comprising half of the species present. This is consistent with the findings of Guild (1952) who stated that earthworm communities on upland sites consist of small acid tolerant or ubiquitous surface-living species.

Under conditions where litter decomposition is retarded, a thick litter layer builds up constituting an abundant and reliable food source with a high energy content with hydrosoluble organic compounds and energy substrates that can be transformed into simple substances by low level microbial activity. In contrast, soil humic reserves, though abundant, are rarely as accessible, with both physically and chemically protected organic matter. Therefore, epigeic species tend to dominate, and the endogeic group is represented by species that feed on surface organic concentrations or in the rhizosphere, like *A.chlorotica,* which were also extremely numerous at the site.

The high abundance of *A. chlorotica* found at the site was unexpected, as the soil pH is at the lower limit of this species' tolerance. However, while higher than expected, the abundances of some epigeic species recorded may have been underestimated resulting in the apparent dominance of *A. chlorotica.* Species like *D. rubidus, D. octaedra* and *L. rubellus* may have been underestimated because of the difficulty in identifying immature specimens to species level and the apportioning of immature individuals to genus level 'catch-all' groups. Also, another factor which may have impacted could be the chosen method of earthworm extraction. Handsorting is a widely used extraction method (Lee, 1985), and avoids some of the problems associated with other methods, for example, potassium permanganate extraction does not recover all species equally (Edwards and

Bohlen, 1996), and dyes the earthworms so that later identification is rendered more difficult. Chemical extraction methods mean that subsequent chemical analyses cannot be performed on the same soil sample. Washing and sieving the samples and floating off the earthworms is extremely time consuming, and also results in mechanical damage to the earthworms (Edwards and Bohlen, 1996).

Hand sorting is also a time consuming method of extraction, but considered the most efficient way of detecting and quantifying most earthworm species (Boag *et al.*, 1997). One exception is small, dark coloured earthworms of < 0.2 g (Edwards and Bohlen, 1996 *op cit* Lavelle, 1978). An estimate of the mean mass of an individual *D. octaedra* sampled at the Sourhope site was 0.04 g., and *D. rubidus* was 0.07 g. This may account for the relatively low numbers of these species recorded at the site. Another exception is *L. terrestris* which Callaham and Hendrix (1997) found was most effectively sampled using formalin, as this is a fast moving, deep burrowing species. This may be one reason to account for the low numbers of *L. terrestris* recorded during this survey. The soil at the Sourhope field site is also shallow in comparison to the depth of burrow that *L. terrestris* commonly inhabits, which often extends down to a depth of 1 m, and can penetrate as far as 2.5 m (Edwards and Bohlen, 1996). It could be therefore, that the site was not suitable for this species. However, over the duration of the field experiment, several observations of large mature *L. terrestris* specimens were made, which makes this argument unlikely.

Prior to the soil for the survey being collected, there was a prolonged period of warm, dry weather. It seems likely therefore that this deep burrowing species retreated as far as possible into the soil, and it was simply not collected in the samples removed for sorting.

2.5.2 The effect of lime on earthworm communities

2.5.2.1 Sourhope field site

In September 1999 there was no significant difference in the abundance of earthworms sampled from limed and unlimed plots. In the September 2002 survey, the abundance of earthworms was significantly greater in limed plots compared to unlimed plots. However, when examined individually, only one species, *D. rubidus* was significantly more abundant. Both *A. chlorotica* and *L. rubellus* appeared more abundant in limed soil but this was not significant due to wide variation in abundance across the site.

Three species were recorded in the 2002 survey which were not recorded in the 1999 survey. It is unlikely however that this is as a response to liming, as these species were found in both limed and unlimed soil. *O. cyaneum*, although not recorded in the 1999 survey, was repeatedly observed crawling on the soil surface during subsequent fieldwork. It is likely that this large, highly mobile endogeic species responded in a similar way to *L. terrestris* during the spell of warm dry weather, as discussed in the previous section. Its presence in the 2002 survey is therefore unsurprising. The other two species observed, *A. rosea* and *L. castaneus,* were present in low numbers and their apparent appearance at the site is attributed to improvement in this author's earthworm taxonomic skills.

2.5.2.1.1 Survey comparison 1999-2002

The comparison of 1999 and 2002 surveys revealed a significant increase in *D. rubidus* abundance in both limed and unlimed plots, with a larger increase in limed soil. This was the only species to show a significant increase over the time period. Both *D. octaedra* and *Lumbricus* spp. showed a decline. The same caveats apply to this 'catch all' group as previously, and the apparent decline is likely to be again due to improvement in earthworm taxonomic skills re-allocating this group into actual species'.

As the change in these species' abundances occurred in both limed and unlimed soil treatments, it is likely that the observed result occurred as a result of some edaphic factor as a result of the management of the field site. If this variable favoured *D. rubidus* but had a negative effect on *D. octaedra* it could be directly responsible for the changes observed, or it may have created conditions where *D. rubidus* could outcompete *D. octaedra.*

Such variables may also include differences in plant community composition. The Sourhope site management has included regular mowing, which appears to have altered the plant community at the site. There has been a decline of up to 40% in some species, which has occurred in approximately equal measures across all treatments, which does suggest that the decline is due to a site-level factor (Burt Smith). One potential explanation is an adverse reaction to the homogenous mowing, as it differs considerably from the heterogeneous grazing of livestock.

The exclusion of grazing animals from the site will also lead to a gradual decline in available soil nutrients. When herbage is cut and removed this leads to a higher level of nutrient removal compared to when the nutrients are recycled through grazing animals (Burt Smith; Dampney, 1985). This can also result in the removal of calcium in harvested plant tissue effecting the loss of applied lime (Dampney, 1985).

This alteration of vascular plant species composition has been coupled with an upsurgence in moss abundance at the site. This is likely to be due to the maintenance of a sward height of approximately 6cm by the regular mowing, which coupled with the absence of grazing animals for the past five years has produced conditions enabling a rapid expansion in the relative abundance of bryophytes (Burt Smith). This altered plant community composition will result in a different litter composition, which may have impacted on the earthworm community. Differences in leaf litter are distinguishable by earthworms (Cooke, 1983; Cooke and Luxton, 1980) with factors like N content, soluble

carbohydrate concentrations and microbial innoculum influencing palatability. The increase in moss cover may also have impacted on the moisture content of the soil.

Another site management practice which appears to have inadvertently impacted upon the plots is the designation by site management, of specific days on which the plots were to be limed. This resulted in surface applied lime being added to the Lime and Nitrogen and Lime plots on windy days and blowing across the site (pers obs.). This not only resulted in limed plots receiving less lime than intended, but appears it has also resulted in a significant increase in the pH of the unlimed plots in comparison to measurements taken at the outset of the field experiment.

2.5.2.2 Mesocosm experiment

2.5.2.2.1 Persistence of experimentally inoculated species in limed and unlimed soil

The persistence of experimentally inoculated earthworm communities was largely poor. In the unlimed plots, the dominant earthworm species found was the uninoculated epigeic species, *D. rubidus,* which was abundant in both the uninoculated and a large proportion of the inoculated treatments. *O. cyaneum* dominated the biomass where it was present in even relatively low numbers. The earthworm treatments inoculated into the unlimed boxes to bias the earthworm communities towards these introduced species on the whole did not succeed, as inoculated earthworms failed to establish successfully. *L. rubellus* was the only inoculated species to be significantly more abundant in inoculated boxes, but even then numbers were lower than inoculation level abundances. In treatments where *A. chlorotica* was the dominant species, these boxes were uninoculated boxes and the earthworms present were endemic specimens.

The poor survival of *A. chlorotica* in unlimed soil was not indicated by the > 60 %

survival rate of this species when inoculated into unlimed soil in the short preliminary experiment. However, as this is an acid intolerant species, its failure to establish is not as surprising as that of *L. rubellus*. While this species was present, it was at significantly lower abundances that inoculated at, and this may be explained by the high abundances of *D. rubidus*. There is evidence in the literature of interspecific competition between earthworms where there is a niche overlap (Abbott and Parker, 1981; Lowe and Butt, 2002; Edwards and Lofty, 1982). If a relatively high population of *D. rubidus* were established in the boxes, as indicated by the initial Sourhope survey, this species may have outcompeted the introduced *L. rubellus*. Competition with high numbers of epigeic species has also been indicated for poor growth of *L. terrestris* in laboratory studies. Lowe and Butt (2002) suggested that high numbers of epigeic earthworms feeding at the surface not only compete for food, but physical contact may stimulate the withdrawal of *L. terrestris* into its burrow, a predator defence mechanism. Competing for food, and the persistent interruption of feeding behaviour may have contributed to the poor survival of *L. terrestris* in the experimental boxes.

In the limed soil treatments, *D. rubidus* was again one of the most abundant species, with *O. cyaneum* again making a large contribution to total earthworm biomass. *L. rubellus* was present in low numbers again, but was not significantly more abundant in inoculated boxes. Unlike in the unlimed treatments, *A. chlorotica* was more abundant in inoculated treatments, at abundances greater than those inoculated. This species was also abundant in uninoculated treatments which indicates a positive effect of liming on endemic populations.

The reduction of LF horizon depth as observed in the limed plots may be as a result of increased earthworm activity. It is unlikely however, to be a response to *D. rubidus* populations for two reasons. Firstly, there were not significantly more *D. rubidus* in limed

soil compared to unlimed soil, and secondly, this species is largely restricted to litter horizons and does not incorporate litter into the upper organic horizons, unlike *L. rubellus* which is commonly found within the upper 8 cm of the soil profile, and can increase the mixing between organic and mineral horizons (Blair *et al.*, 1995; Persson, 1988). However, as *L. rubellus* numbers were very low, the high numbers of *A. chlorotica* may be responsible for this reduction in the depth of the litter layer. When the endogeic *A. caliginosa* was inoculated into pasture land without a native earthworm community in New Zealand, the pasture root mat was removed and decomposed, and surface applied lime, plant residues and animal dung were incorporated into the soil (Stockdill, 1982). This is not necessarily the result expected upon inoculation of an endogeic species. This species appears to have exploited a resource that it is not usually associated with, which may be due to a lack of competitive exclusion. At the mesocosm site, the lack of a sizeable *L. rubellus* population may have allowed *A chlorotica* to behave similarly. These examples can be used to demonstrate the imperfect classification of earthworm functional groups (Blair *et al.*, 1995; Curry, 2002; Neilson *et al.*, 2000). Another indication that endogeic species may be excluded from exploiting this rich resource is the observation that compared to Europe, *A. caliginosa*, and to a lesser degree *A. chlorotica* and *A. rosea*, are most commonly found to break down pasture root mats in Australasia (Lee, 1985). This difference in behaviour may be due to the different species assemblages present, as the species associated with efficient litter incorporation, *L. terrestris* and *A. longa* are confined to specific regions, although *L. rubellus* is reasonably widespread.

2.5.2.2.2 Earthworm communities in uncovered boxes and anomalous earthworm species found at the site

Robinson *et al.* (1996) suggested that the increase in the number and size of earthworm

populations after liming occurs through not only increased fecundity, but also by reducing mortality and increasing immigration rates. Whilst in the majority of the mesocosm treatments immigration was prevented by the mesh cover, in the uncovered disturbed treatments (treatment code 3,1), earthworms from the surrounding soil were free to colonize the soil. However, the analysis of these data revealed that the communities in the uncovered boxes were not significantly larger (in terms of abundance or biomass) than those in the covered boxes where the hatching of cocoons and subsequent reproduction were responsible for the presence of earthworms. This suggests that earthworms did not significantly invade the boxes from the surrounding soil.

The presence of an individual specimen of *E. fetida* found in a limed, uninoculated disturbed box (plot 3B treatment 1,1) was anomalous. *E. fetida* is characteristically found in organic rich soils, for example, under damp rotting vegetation and logs, in and under compost and manure heaps, sewage filter beds and rich cultivated soils (Sims and Gerard, 1985). It is unlikely that this species would normally be found at the Sourhope site, or naturally colonise this soil. It is possible that a cocoon was unintentionally incorporated into the soil during the time it was drying or being repacked in to the box, as these were often present in the transporting medium that the 'Walker Organics' worms were supplied in.

2.5.3 Environmental variables explaining the distribution of earthworms present

2.5.3.1 Sourhope earthworm communities

Soil samples on which the chemical analyses for these data were performed were collected from each Limed and Unlimed 1 plot, but from subplots S, T, U and V as described in Appendix I, not subplot Q, the area which the earthworms were collected from. However,

as Charles Darwin noted in 1881, earthworms may completely leave their burrows at night and undertake "a voyage of discovery" (Darwin, 1881). Similarly, Mather and Christenssen (1992) observed species belonging to all three ecological groups making surface migrations at night. It appears entirely reasonable, given the 94% of variation explained by the measured variables that not only are the variables measured in the 4 subplots representative of the entire plot, but that the earthworm community encountered, and responded to, the environmental variables measured.

The 1999 Sourhope earthworm census RDA analysis revealed that only 2 variables significantly explained a proportion of variation in the earthworm community, and these were the depth of the LF horizon and the C:N ratio of the soil.

Results presented by Briones *et al.* (1995) suggest that for the majority of species (*A. caliginosa, A. chlorotica, D. rubidus, L. rubellus* and *O. cyaneum*) variation in soil pH, exchangeable cations and soil texture can explain their presence in a certain habitat. To a lesser extent, % organic matter and N content are also important in *O. cyaneum* distribution, and moisture content and C:N ratio for *L. rubellus.* Some of these variables were excluded from this author's RDA analysis as a PCA revealed strong positive correlations between many of the variables, but the results from the analysis presented here fit well with the results shown in the literature.

2.5.3.2 Mesocosm earthworm communities

In comparison to the Sourhope analysis, the mesocosm RDA revealed that only 32% of the variation in the distribution of earthworm species was accounted for by the included environmental variables. However, in this analysis the objective was not to determine which edaphic variables earthworm species were responding to, but which experimental

variables were significant in explaining earthworm distribution. Therefore it is unsurprising that less variation is accounted for.

The significant experimental factors at the between plot level included lime, which is mainly explained by a large increase in the number of *A. chlorotica,* while *L. rubellus, L. castaneus* and *D. octaedra* appeared associated with unlimed soil, with a significant reduction of *D. octaedra* numbers in limed soil. The depth of the LF horizon also varied between plots, and was significantly reduced in limed plots (see Section 3.4.2.2). The litter feeding species *L. castaneus* and *D. octaedra* appear to be associated with an increased depth of LF horizon.

Botanical axis 3 appears to be negatively correlated with disturbance in the RDA ordination diagram, and may be some aspect of the botanical composition that the earthworms are responding to. Alternatively, both earthworms and plant species may be responding to some other, undefined environmental variable which may have been influenced by the disturbance procedure, for example, soil physical properties, including bulk density and water holding capacity.

The significant within-plot factors included disturbance, which had an obvious impact on earthworm communities. Disregarding the species which were inoculated, earthworms in the mesh covered boxes are those which hatched from cocoons which survived the drying and crushing of the disturbance process, or natural inoculations into the uncovered boxes. A good example of this is *D. rubidus* which was extremely abundant in the undisturbed boxes, but much less so in disturbed boxes indicating that not many cocoons survived the disturbance either intact or in a viable state.

The two remaining within-plot factors were depth LF and Axis 3 again.

2.6 Conclusions

The following conclusions were reached concerning the specific objectives:

- The size of the earthworm community at the Sourhope site was larger than expected (mean abundance = 172 earthworms m^{-2}, std dev = 89), and the site was dominated by epigeic species and endogeic species feeding in the upper organic and organomineral horizons.
- Lime did increase the abundance of the Sourhope earthworm community, but on closer examination this was revealed to be due to a significant increase in only one species, *D. rubidus*. A decline in abundance was noted for *D. octaedra*.
- Overall persistence of inoculated species was poor. *L. rubellus* was more abundant in unlimed soil, compared to *A. chlorotica* which was more abundant in limed soil. *L. terrestris* did not survive well in either soil treatment.
- The environmental variables which accounted for a significant proportion of the variablity of the earthworm community at the Sourhope site (prior to liming) agreed well with results published in the literature and included the depth of the LF horizon, and the C:N ratio of the soil.
- The variables which significantly explained a proportion of the variation in the earthworm community at the mesocosm site included lime, disturbance, and some undefined variable which appears to impact on plant community distribution also.

Chapter 3 The response of plant communities to earthworms and experimental variables

3.1 Introduction

Plant ecological strategies are recognised, and have been defined as 'a grouping of similar or analogous genetic characteristics which recur widely among species or populations and causes them to exhibit similarities in ecology' (Grime, Hodgson, and Hunt, 1990). This means that plant species differ in their tolerances to stress and disturbance. Disturbance has been defined as 'the partial or total destruction of the plant biomass', and stress as 'phenomena which restrict photosynthetic production'. The response of the plant species to both earthworms and experimentally imposed environmental variables (e.g. disturbance, mesh covering and liming), will be examined both autecologically and as individual boxes using the national vegetation classification (NVC) system.

The NVC system categorises vegetation samples into distinct communities and is a comprehensive classification of British vegetation from natural, semi-natural and major artificial habitats (Rodwell, 1992). The Sourhope baseline vegetation survey in 1998 revealed the most closely matching NVC community, which was assigned to each plot, was U4d. This community describes a *F. ovina- A. capillaris- G. saxatile* grassland with a *L. multiflora- Rhytidiaelphus loreus* subcommunity. This type of grassland is widespread on base-poor, well drained, mineral soils with high rainfall (> 800 mm annually) which encourages leaching (Rodwell, 1992). There was a localised alternative NVC classification of U5b in the moister, less fertile plots of the site, where *N. stricta* became a more dominant species (Anon, 1998).

The work in this chapter examines the response of the mesocosm plant communities to liming, disturbance and the inoculated earthworm treatments.

3.2 Specific Objectives

The aim of the work in this chapter was to determine:

1. The impact of liming and disturbance on plant yields,
2. The impact of inoculated earthworm communities on plant yields,
3. The experimental variables which explain the distribution of the plant species present.

3.3 Materials and Methods

3.3.1 Impact of mesh covering

The effects of the nylon mesh covering (pore size <0.5 mm) were determined on a set of 6 boxes buried to the left of the mesocosm experiment. Three of the boxes were covered with mesh, and 3 left uncovered. Temperature and soil moisture measurements were taken from these boxes (C.A. Spring, University of Stirling). Photosynthetically active radiation (PAR) measurements on the amount of light penetrating the mesh were carried out by Dr. N. Ostle (CEH Merlewood).

3.3.2 Above ground biomass harvests

The above-ground plant biomass in the boxes was harvested monthly using scissors during the growing seasons (May to September). This was taken back to the lab, dried for 24 hrs at 105°C and then weighed.

3.3.3 Botanical survey

In October 2001, prior to the destructive sampling of the mesocosm experiment, a botanical survey was carried out by Dr. E. Tilston (University of Stirling), using the Braun-

Blanquet cover scale, providing a semiquantitative measure of species abundance.

3.3.3.1 National Vegetation Classification

The mesocosm botanical composition data was analysed using the programme TABLEFIT, version 1.0 (Hill, 1996).

3.3.4 Statistical analysis

T-tests, analysis of variance (ANOVA), Spearmans rank correlation (earthworm biomass was used rather than earthworm abundance as the impact of the activity of a large earthworm is not comparable to a smaller specimen) and Mann-Whitney tests were carried out using Minitab statistical software, Release 13.1.

As stated in Section 2.4.2.3 the randomised block design of the mesocosm experiment was no longer a suitable basis for further analyses due to the varied survival of the inoculated earthworm species. Therefore, the response of the plant community to environmental variables has been analysed by redundancy analysis.

Prior to running each analysis, as in Chapter 2, a PCA correlation biplot was run on the environmental variables to determine how closely correlated the environmental variables were to each other. If a group of variables were closely correlated, these appeared as a cluster of arrows. If this occurred, the one with the longest arrow (explaining the most variation) was selected for inclusion in the RDA analysis.

3.4 Results

3.4.1 Impacts of mesh covering

3.4.1.1 Light penetration

The effects of the nylon mesh on the amount of light reaching the plant communities was quantified with photosynthetically active radiation (PAR) measurements (see Figure 3.1).

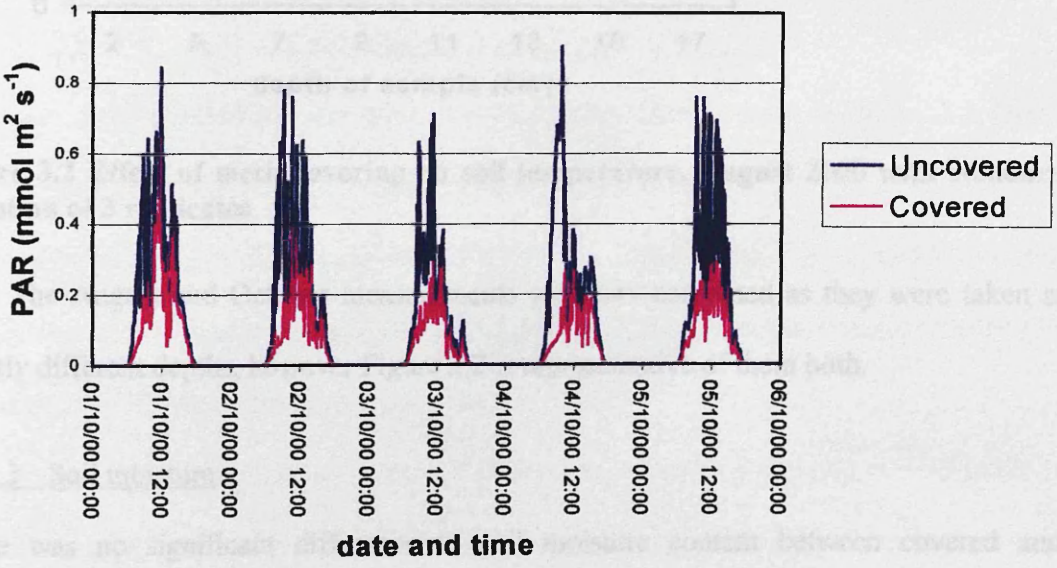


Figure 3.1 Photosynthetically active radiation (PAR) reaching the plant community, October 2000.

Significantly more light reached the plant community in the uncovered boxes, compared to those covered with mesh ($p < 0.001$).

3.4.1.2 Soil temperature

There was no effect of the mesh on the temperature of the soil at various depth measurements in either August (see Figure 3.2) or October 2000 ($p = 0.960$ and $p = 0.412$ respectively).

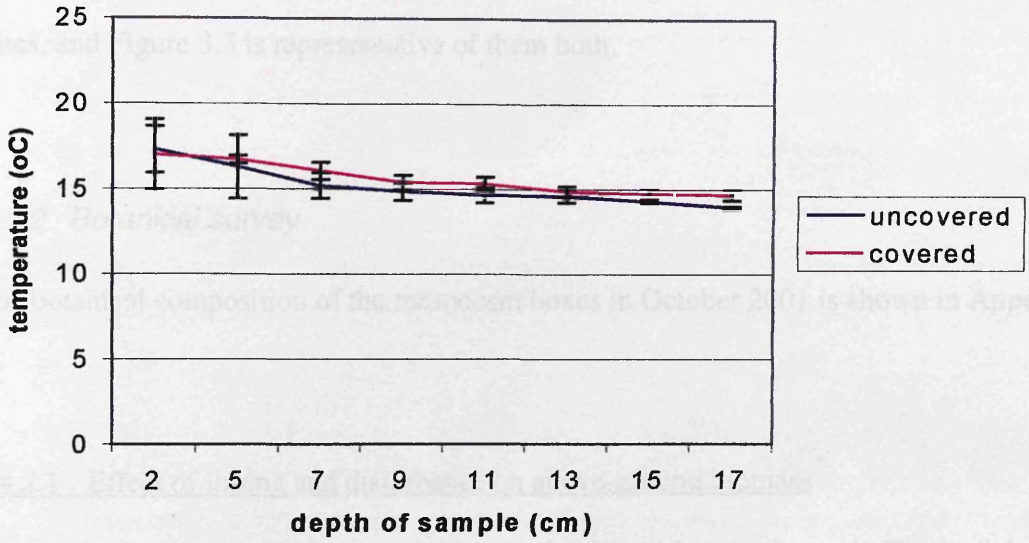


Figure 3.2 Effect of mesh covering on soil temperature, August 2000 with standard deviation of 3 replicates

The August and October measurements were not combined as they were taken at slightly different depths, however Figure 3.2 is representative of them both.

3.4.1.3 Soil moisture

There was no significant difference in soil moisture content between covered and uncovered boxes in either August ($p = 0.288$) (Figure 3.3), or October 2000 ($p = 0.321$).

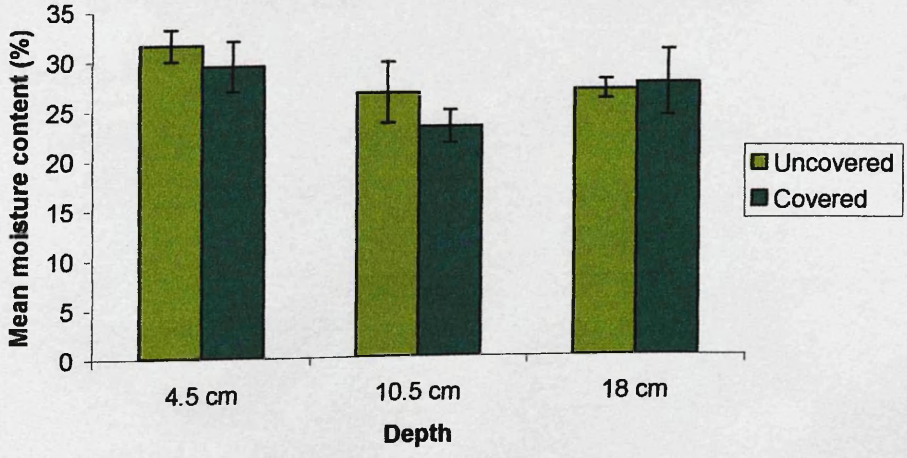


Figure 3.3 Effect of mesh on soil moisture content, August 2000 with standard deviation of 3 replicates

Again, measurements were taken at slightly different depths at the two sampling dates, and Figure 3.3 is representative of them both.

3.4.2 Botanical survey

The botanical composition of the mesocosm boxes in October 2001 is shown in Appendix H.

3.4.2.1 Effect of liming and disturbance on above-ground biomass

The mean above-ground biomass yield at each harvest date is shown in Figure 3.4. In the May harvest, there was no significant difference in yield between the limed and unlimed undisturbed boxes ($p = 0.081$), and similarly the limed and unlimed disturbed boxes ($p = 0.140$). The undisturbed treatments yielded significantly more than the disturbed treatments (limed soil $p = 0.000$; unlimed soil, $p = 0.000$).

However, this large initial difference between the disturbed and undisturbed boxes is not evident at the second harvest date in July, where the yield from the disturbed boxes is not significantly less than the undisturbed boxes. At each of the separate harvest points, neither the liming nor disturbance treatments significantly affect yield.

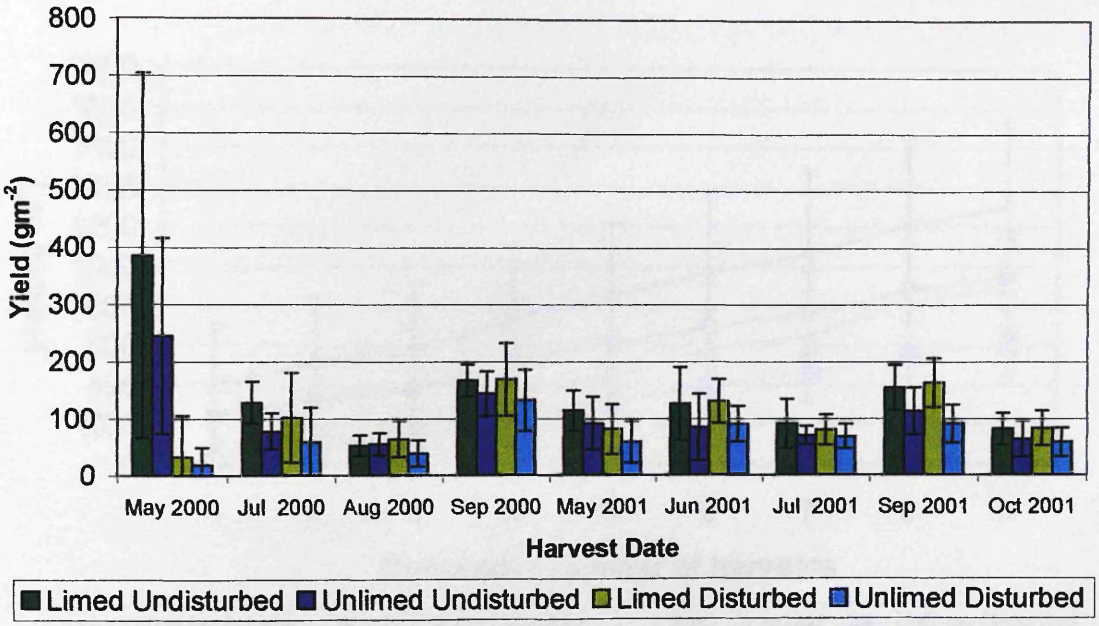


Figure 3.4 Effect of liming and disturbance on above-ground biomass, with standard deviation bars of 5 replicates.

When this data is presented as cumulative data at each harvest date (Figure 3.5) analysis shows that disturbance significantly reduced above-ground biomass yields in both limed and unlimed soil treatments (Appendix F, F.2). However, lime addition increased yields compared to unlimed treatments for both disturbed ($p < 0.001$) and undisturbed boxes ($p < 0.001$), to such an extent that there was no significant difference in the mean above-ground biomass yield between the unlimed undisturbed treatments and the limed disturbed treatments.

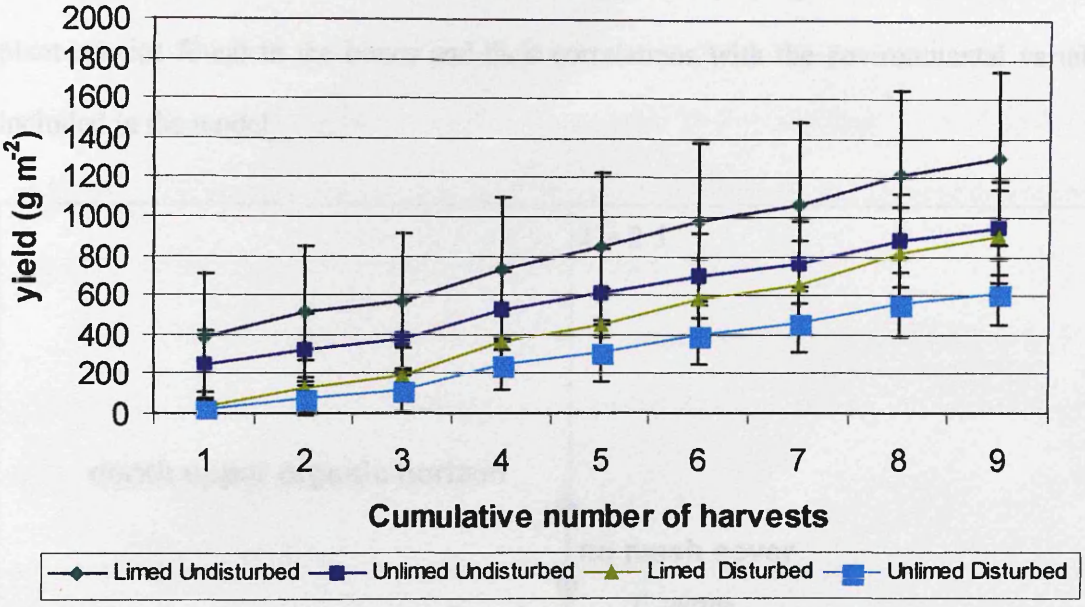


Figure 3.5 Cumulative yield of above-ground biomass with standard deviation bars of 5 replicates

As the mesh cover significantly reduced the PAR reaching the plant communities (Section 3.4.1.1) the total cumulative yields of the disturbed covered boxes were compared to the disturbed uncovered boxes. Mann-Whitney tests (See Appendix F) revealed that there was no significant difference in total cumulative plant biomass harvested between the covered and uncovered boxes ($p > 0.05$).

Whilst plant yields were not significantly reduced in disturbed boxes compared to undisturbed boxes, the composition of disturbed boxes was significantly altered. This is explored in detail in the following section.

3.4.2.2 Plant response to earthworms and environmental variables

The correlation between plant yield and total earthworm biomass in each box was tested using Spearman' rank correlation. This showed that the two were not significantly

correlated ($R^2 = 0.211$, $p = 0.060$).

Figure 3.6 shows an RDA ordination diagram, displaying the relationship between plant species found in the boxes and their correlations with the environmental variables included in the model.

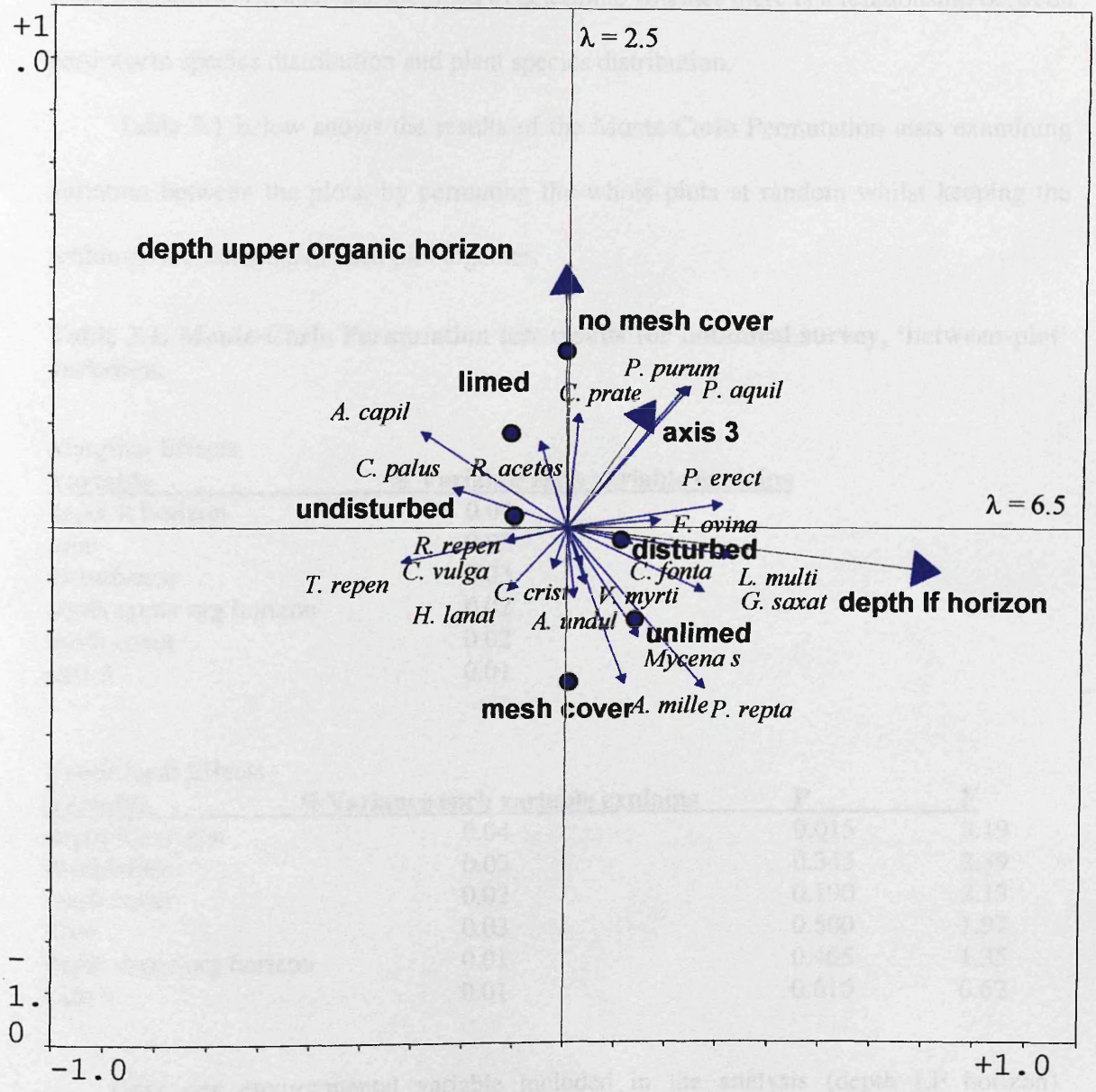


Figure 3.6 Redundancy analysis ordination biplot of plant species' response to environmental variables

Key to plant species: *Achillea millefolium, Agrostis capillaris, Atrichum undulatum, Cardamine pratensis, Cerastium fontanum, Cirsium palustre, Cirsium vulgare, Cynosurus cristatus, Festuca ovina, Galium saxatile, Holcus lanatus, Luzula multiflora, Mycena sp., Potentilla erecta, Potentilla reptans, Pseudoscleropodium purum, Pteridium aquilinium, Ranunculus repens, Rumex acetosa, Trifolium repens, Vaccinium myrtillus.*

The environmental variables included in this analysis explain far less of the observed variation in plant species distribution than the 31% explained in the mesocosm earthworm RDA analysis. In total, 15% of the variation in distribution is explained by the included variables, with just 9% explained by the first 2 axes. In this analysis 'Axis 3' is derived from the earthworm analysis, included to determine whether there is a relationship between earthworm species distribution and plant species distribution.

Table 3.1 below shows the results of the Monte-Carlo Permutation tests examining variation between the plots, by permuting the whole plots at random whilst keeping the within-plot treatments of each plot together.

Table 3.1. Monte-Carlo Permutation test results for botanical survey, 'between-plot' variation.

Marginal Effects

Variable	% Variance each variable explains
depth lf horizon	0.04
lime	0.03
disturbance	0.03
depth upper org horizon	0.02
mesh cover	0.02
axis 3	0.01

Conditional Effects

Variable	% Variance each variable explains	P	F
depth lf horizon	0.04	0.015	3.19
disturbance	0.03	0.345	2.39
mesh cover	0.02	0.190	2.13
lime	0.03	0.500	1.97
depth upper org horizon	0.01	0.465	1.35
axis 3	0.01	0.610	0.62

Only one environmental variable included in the analysis (depth LF horizon) significantly explains a portion of the observed variation in plant species occurrence across the plots (p=0.015). 'Disturbance' and 'mesh cover' are both within-plot variables and are therefore not significant in this analysis. Unexpectedly, liming was not a significant

'between plot' variable. However, a separate analysis showed that lime has a significant effect on the depth of the LF horizon in undisturbed boxes, with a reduced thickness in limed plots (p=0.000), which may be why the depth of the LF horizon is a significant explanatory variable, as measurements taken prior to the establishment of the mesocosm experiment showed no significant difference in LF thickness between the limed and unlimed plots (p = 0.584).

The analysis of 'within-plot' variation in plant species occurrence is shown in Table 3.2, where the individual treatments of a plot are shuffled at random within that plot, without permuting whole-plots.

Table 3.2. Monte-Carlo Permutation test results for botanical survey, 'within-plot' variation.

Marginal Effects

Variable	**% Variance each variable explains**
depth LF horizon	0.04
lime	0.03
disturbance	0.03
depth upper org horizon	0.02
mesh cover	0.02
axis 3	0.01
axis 4	0.01

Conditional Effects

Variable	**% Variance each variable explains**	**P**	**F**
depth LF horizon	0.04	0.015	3.19
disturbance	0.03	0.005	2.39
mesh cover	0.02	0.015	2.13
lime	0.03	0.015	1.97
depth upper org horizon	0.01	0.515	1.35
axis 3	0.01	0.795	0.62

As in the 'between plot' analysis, the variation in the depth of the LF horizon significantly explains the greatest variation in plant species distribution within plots (p = 0.015). This variation in LF horizon depth cannot be explained by an interaction with lime, as this was applied at the whole plot level, nor is it as an effect of disturbance, as a separate

analysis showed no significant difference in LF depth between undisturbed and disturbed boxes in limed ($p = 0.778$) or unlimed ($p = 0.197$) plots.

The effect of liming was a significant within plots variable ($p = 0.015$), which was unexpected as lime was applied as a between plot factor i.e. all samples in a limed plot received the same liming treatment.

The effect of disturbance was a significant explanatory variable ($p = 0.005$), indicating different plant communities present in the disturbed and undisturbed boxes. A separate analysis revealed that *C. fontanum, C. cristatus, P. reptans, G. saxitale* and *R. acetosa* were significantly more abundant in disturbed boxes

Covering the boxes with mesh to prevent earthworm movement also significantly affected plant communities present in those boxes ($p= 0.015$). As shown in Section 3.4, this is likely to be a response to the reduced PAR reaching the plant communities as no impact on temperature or soil moisture levels was recorded.

The earthworm PCA derived variable 'Axis 3' did not significantly add to the model of either Within or Between plot variation.

3.4.2.3 NVC communities of mesocosm experimental boxes

Seventy four of the 80 boxes that comprised the mesocosm botanical survey fit into one of two subcommunities of a perennial open silicaceous grassland, U1b (typical) or U1e. These are described as *F. ovina- A. capillaris- R. acetosella* grasslands. U1e has a *G. saxatile- P. erecta* subcommunity.

3.5 Discussion

3.5.1 Experimental variables and plant ecological preferences

Lime was not a significant variable in accounting for 'between plot' variation in plant yields. However, lime appears to have had an indirect effect, as the depth of the LF horizon was significantly reduced in the limed boxes. This suggests that there was an increase in the rate of litter comminution and incorporation into the H horizon as a result of increased earthworm activity in the limed plots.

While the percentage variation explained by the RDA analysis was low, lime was also a significant 'within plot' factor. This was unexpected as lime was applied at a whole plot factor. The RDA analysis (Figure 3.6) however, indicates that the range of plant species found in the boxes responded differently to liming, with some species preferring the original more acidic conditions. This distribution of plant species coincides well with their known ecological preferences (Grime, Hodgson and Hunt, 1990).

The plant species clustered around the lime centroid; *A. capillaris, R. acetosa, C. pratensis* and *C. palustre,* are plants found on grassland on mildly acidic soils, which, with the exception of *C. palustre* can also extend on to calcareous soils. Those species clustered at the unlimed centroid are characteristic of unimproved grassland. *V. myrtillus* is a strict calcifuge that is often found on peaty soils. *G. saxatile* is also a calcifuge, which is often most abundant in closely cropped sheep pasture, as its low mats tends to be suppressed by grass litter in unmanaged grasslands. Similarly, *L. multiflora* is virtually restricted to acidic soils. *A. millefolium* is most abundant in sites of low productivity and is suppressed by applications of lime (Grime, Hodgson and Hunt, 1990).

Disturbance was a significant 'within-plot' variable and many of the plants which are grouped round this centroid have particular life history traits which make them characteristic species of high disturbance environments, where more potentially productive

species are excluded. Grime, Hodgson and Hunt (1990) defined disturbance as 'the partial or total destruction of the plant biomass, either as a result of the actions of herbivores, pathogens or human activities e.g. mowing, ploughing, trampling) or damage from wind, fire, erosion, frost etc'.

C. fontanum and *G. saxitale* have shoots which readily grow roots when in contact with the soil, allowing them to exploit gaps in ground cover. *P. erecta* can spread vegetatively through the abundant production of runners.

The variation in the depth of the LF horizon also significantly explained the greatest variation in plant species distribution within plots, in addition to contributing to between plot variation. This variation in LF horizon depth cannot be explained by an interaction with lime, as this was applied at the whole plot level. Similarly, the depth of the LF horizon did not vary significantly between disturbed and undisturbed boxes. The conclusion is therefore, that the heterogeneous, natural within plot variation in the depth of the LF horizon affected the plant species distribution within the plots.

3.5.2 NVC

The mesocosm NVC communities identified by TABLEFIT were U1, *F. ovina-A. capillaris-R. acetosella* grasslands. These are defined as perennial open silicaceous grasslands. They are characteristically acidic, nutrient poor soils prone to drought in summer, and are the most widespread calcifuge sward found in the warm and dry lowlands of southern Britain. They typically receive <800 mm rain annually, and experience a mean annual temperature of >27 °C. Disturbance and grazing are important factors in maintaining the typical aspect of this vegetation (Rodwell, 1992).

Although serious disturbance occurred to the vegetation in half the boxes, and all were cut regularly, despite this, it is extremely unlikely that these two factors override the

obvious geographical and climatic features which differ between the warm, dry, drought-prone southern England lowlands and a cool and wet north-facing slope of upland grassland in the Scottish Borders. While lateral water flow through the soil was prevented by the plastic boxes, the vegetation in the boxes appeared as lush as the surrounding vegetation, and it is unlikely that this caused a major shift in the plant communities present.

This anomalous result may well reflect the small surface area of the mesocosm boxes that were sampled. The boxes have a surface are of 0.14 m^2, compared with a quadrat size of 2 x 2 m recommended for NVC community descriptions, where quadrats of 1 m^2 were rejected as inadequate for representative sampling (Rodwell, 1992).

3.6 Conclusions

The following conclusions were reached concerning the specific objectives:

- Liming did significantly increase plant yields compared to unlimed treatments
- Disturbance significantly reduced yields in both limed and unlimed soils
- The impacts of inoculated earthworm communities on plant yields could not be determined due to the failure of inoculated treatments to become established
- The experimental variables that significantly accounted for a portion of the observed variation in plant species distribution included lime, disturbance, the depth of the LF horizon and mesh covering.

Chapter 4 Effects of earthworms on the chemical characteristics and distribution of soil organic matter

4.1 Introduction

As introduced in section 1.3, earthworms play an important role in the decomposition of plant litter and its comminution from large fragments to smaller particles (Killham, 1994). Preferential loss of easily degradable carbohydrate (O-alkyl-C) for example, cellulose and hemicellulose structures, in plant residues is an important process in decomposition. At the same time there is a selective preservation of more recalcitrant plant material and a simultaneous synthesis of microbial metabolites and dead microbial remains, which contributes to the alkyl-C and methyl-C resonance in NMR spectra (Hopkins *et al.*, 1997) (see Figure 1.3). The concomitant increase of alkyl-C and decrease and O-alkyl-C content, suggests that a 'ratio of alkyl- to O-alkyl C may provide a sensitive index of the extent of decomposition where the organic materials are derived from a common starting material' (Baldock *et al.*, 1997; Webster *et al.*, 2001).

With decreasing particle size, the carbon to nitrogen ratio decreases, which indicates a greater degree of humification of the organic material. This is due to relative increases in the concentrations of recalcitrant compounds, for example, lignin-derived compounds (Tiessen *et al.*, 1984; Killham, 1994). The 'random' structure of lignin and its strong linkages make lignin very resistant to microbial and other soil biological decomposition processes. Because of this, the percentage of lignin-C in decomposing plant residues tends to increase with time in the soil (Killham, 1994). The aromatic-C content can vary with particle size, initially increasing, but then decreasing with decreasing particle size, and this limits its usefulness as an index of extent of decomposition (Baldock *et al.*, 1997). The aim of the work in this chapter was to examine the effects of earthworms in limed and unlimed soil, on the decomposition of soil organic matter and its distribution through the soil profile.

4.2 Specific Objectives

The aim of the work in this chapter was to examine and compare:

1. The impact of lime and an altered earthworm community on the chemical characteristics of SOM.
2. The effects of individual species on the distribution of soil C in limed and unlimed soil.
3. The impact of different ecological groups on the distribution of soil C within one soil type.

4.3 Materials and methods

4.3.1 NMR sample preparation, experimental parameters and spectral analysis

The baseline (i.e. initial) samples were taken from soil collected from the main Sourhope plots 2B (unlimed) and 2C (limed) for the September 1999 earthworm census. The final samples were taken in October 2001 at the destructive sampling of the mesocosm field experiment from plots 2B and 2C uninoculated, undisturbed, covered boxes (treatment 1,0).

200 g of soil from each soil horizon were dispersed by shaking end-over-end for 24 h with 500 ml 10% sodium hexametaphosphate (Fisher Scientific) and glass marbles to help disperse aggregates. This was followed by ultrasonication (Decon FS 200 Frequency Sweep) for 5 minutes to disperse any remaining aggregates. The resulting suspensions were wet sieved through Laboratory Test Sieves (Endecotts Ltd). The resulting >2 mm, 2 mm -212 μm, 212 μm -63 μm and <63 μm size fractions were oven dried at 80°C for 12 hours. These samples were then ground using agate chambers, in a Retsch MM200 ball mill to a fine powder. If the sample was very stony, with little organic material present, the organic material was first harvested by density fractionation, using sodium polytungstate adjusted to a density of 3.1 g cm^{-1}. The sodium polytungstate was decanted into a vial and

a portion of the sample was poured in. The vial was agitated gently to help separate the mineral material from the organic material and then left to settle. The organic material was collected using a spatula to remove it from the vial, and the material was placed onto Whatman filter paper (Size 2) and rinsed with distilled water using a Buchner filtration funnel. This process was repeated until the entire sample had been processed and the sample was then dried and ground as described above.

^{13}C NMR spectra were collected under cross-polarization (CP), magic angle spinning MAS) conditions using a Varian/Chemagnetics CMX LITE NMR spectrometer (^{1}H, 300 MHz; ^{13}C, 75 MHz). The operating parameters were 1 ms contact time and 2 s relaxation delay. Baseline samples were run in a 7.5 mm Zirconia Pencil© rotor for between 5000 and 114525 scans, and endpoint samples were run in a 4 mm Zirconia Pencil© rotor for 1000000 scans to achieve optimal signal-to-noise ratios. Baseline scans were due to be re-run with standardized acquisition times, but due to major instrument problems during the second half of this studentship, there was no time to do this. The difference in acquisition times meant that the baseline samples could not be compared semi-quantitatively, i.e. normalised against each other.

Baseline samples run were H and Ah_1 horizon >2 mm and < 63 μm size fractions. For the endpoint samples, H horizon > 2 mm, > 212 μm, > 63 μm and < 63μm samples were run, and Ah_1 horizon > 2 mm and < 63 μm samples.

Spectra collected were scanned using ADOBE Illustrator and had a mm^2 grid superimposed upon them. The spectra were then printed, divided up according to Chemical shift ranges of the six shift ranges commonly used in soil ^{13C} NMR studies Table 1.2 and the area attributable to each chemical shift range was determined by counting the squares.

4.3.2 Organic carbon content determination

Subsamples of the samples prepared for CP MAS ^{13}C NMR spectroscopy were used to determine the organic carbon content, using an ANCA-SL sample converter coupled to a 20-20 isotope ratio mass spectrometer (Europa Scientific, Crewe, UK).

4.3.3 Plot pairings: implications for analyses

A PCA analysis of various physical and biological measurements taken from the main Sourhope plots (Figure 4.1) was done to determine whether the obvious spatial variation across the field site had resulted in limed-unlimed plot pairings of limited comparative use. Variables included in this analysis were soil moisture (April and July 2001), upper organic horizon depth, the Shannon Index of diversity (Begon, Harper and Townsend, 1996) of earthworm samples collected in September 1999, organic matter content, number of plant species present and plot altitude (See Appendix C).

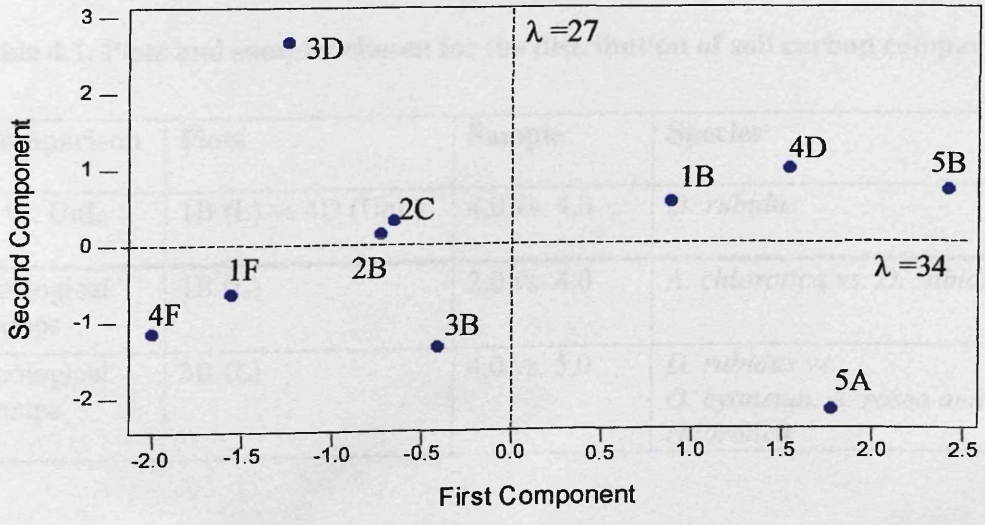


Figure 4.1 Limed and unlimed plot comparison PCA, including various physical and biological variables.

The eigenvalues of the first two PCA axes explain 61% of the variation between plots. It is clear from this analysis that the designated plot pairings were not suitable ones on which to base subsequent analyses. With the exception of plots 2B and 2C which cluster together, the remaining plots are scattered across the two axes. This suggests that if the designated pairings were used as the basis for subsequent individual limed and unlimed plot comparisons, any differences occurring as a result of liming could be masked by inherent between-plot variation.

Using the information from this analysis and the results from the mesocosm endpoint census (Section 2.4.2.2), combinations for subsequent soil analyses were decided. While more plot pairs could have been chosen, these options were limited by the earthworm species present in the plots, and time constraints imposed by the use of the NMR spectrometer. Samples chosen for analysis by CP MAS ^{13}C NMR spectroscopy were from the uninoculated, undisturbed boxes plots 2B and 2C, with populations of *D. rubidus* and *A. chlorotica* respectively. Table 4.1 shows the comparisons being investigated using carbon content data only.

Table 4.1. Plots and samples chosen for the distribution of soil carbon comparison

Comparison	**Plots**	**Sample**	**Species**
L vs. UnL	1B (L) vs 4D (UnL)	4,0 vs. 4,0	*D. rubidus*
Ecological groups	1B (L)	2,0 vs. 4,0	*A. chlorotica vs. D. rubidus*
Ecological groups	3B (L)	4,0 vs. 5,0	*D. rubidus vs. O. cyaneum, A. rosea and A. chlorotica*

4.4 Results

4.4.1 CP MAS ^{13}C NMR spectra for baseline particle size fractions

Figure 4.2 below shows the CP MAS ^{13}C NMR spectra for the particle size fractions of both the limed and unlimed baseline samples.

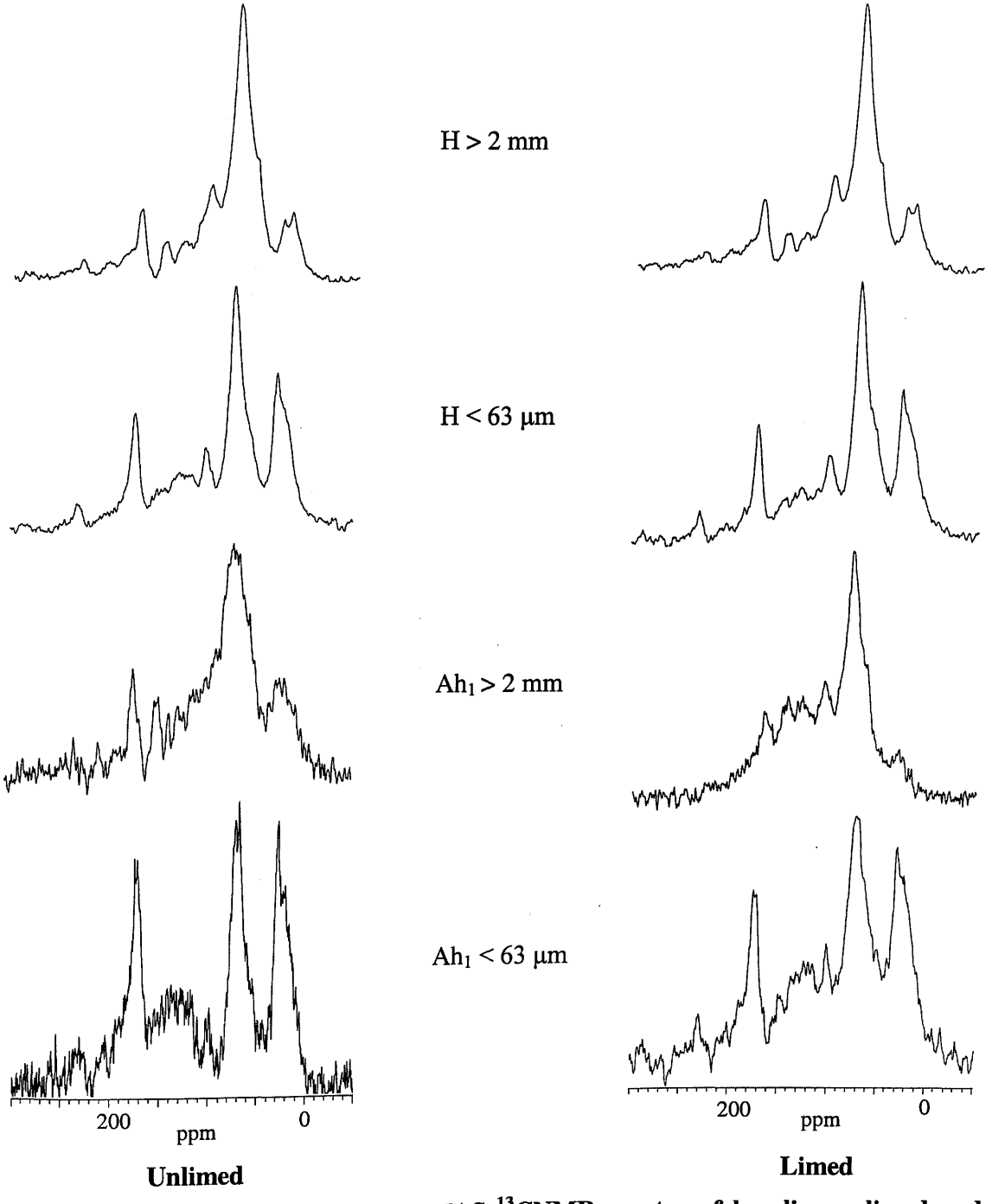


Figure 4.2 Non-intensity-scaled CP MAS ^{13}CNMR spectra of baseline unlimed and limed particle size fractions

The main spectral signals were in the alkyl-C (10-45 ppm), O-alkyl-C (60-90 ppm), acetal-C (and ketal-C) (90-110 ppm) and carbonyl-C (160-200 ppm) regions of both soils.

The alkyl-C resonance is likely to be attributable to aliphatic alkanes, fatty acids and waxes, with small contributions from acetal substituents in lignin (the organic matter input to the soil is from grass litter, and grasses contain relatively little lignin) and hemicellulose. The 60-90 ppm O-alkyl-C peak probably arose from oxygenated C in carbohydrates, with a contribution from hexose monomers in polymers such as cellulose, and pentose monomers in hemicelluloses. The signal between 100-110 ppm is likely to be due to the dioxygenated C (C1) of cellulose. The signal between 160-200 is attributable to carbonyl-C. As the relative intensities of the carbonyl-C signal varied with that of the alkyl-C signal, and there was a lack of signal aromatic-C containing compounds, and this indicated that much of the alkyl- and carbonyl-C were present in the same class of compounds (Hopkins *et al.*, 1993).

4.4.1.1 Unlimed soil

The > 2 mm size fraction from the H horizon had a high relative intensity in the carbohydrate region, comprising 42% of total spectral area. This decreased to 12% for the H < 63 μm fraction. Similarly, the O-alkyl peak area decreased from 31% to 21% between the > 2 mm and < 63 μm for the Ah_1 horizon.

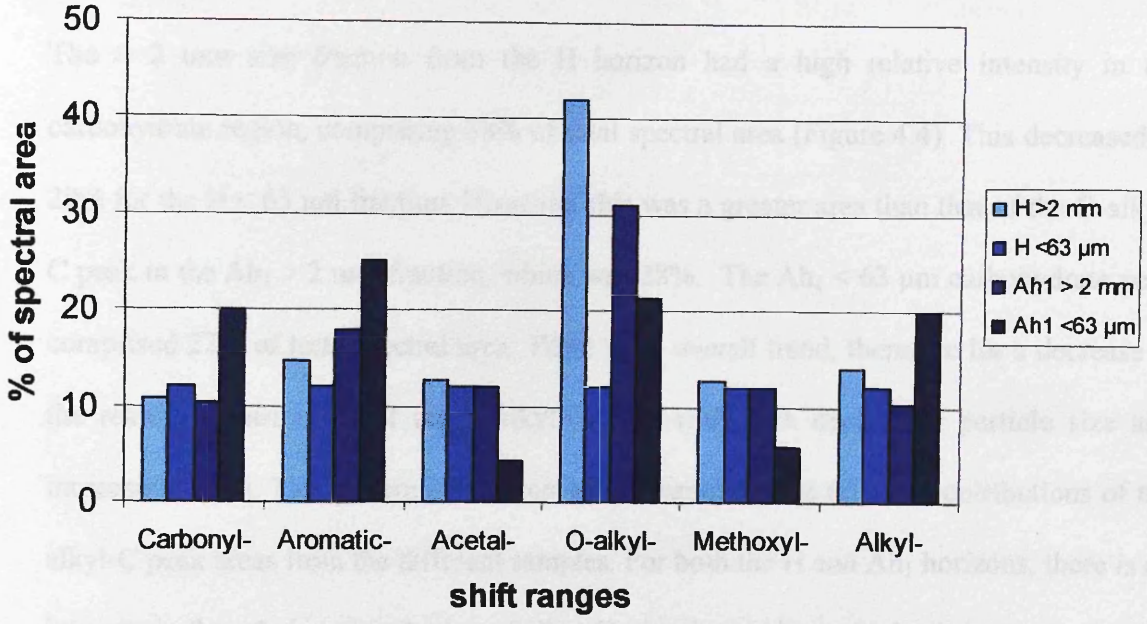


Figure 4.3 Percent of total CP MAS ^{13}C NMR spectra of unlimed soil, represented by spectral shift changes

The alkyl-C peak comprised 10-14% of total spectral area for both H horizon size fractions and the > 2 mm Ah_1 fraction (Figure 4.3). The Ah_1 < 63 µm alkyl-C peak had a greater spectral area, comprising 20% of spectral area.

The organic carbon content (%) and C:N ratio of the samples is shown in Table 4.2. The carbon content decreases with both depth and particle size. The C:N ratio also decreases with particle size, but not uniformly with depth. The C:N ratio of the Ah_1 > 2 mm size fraction is larger than that of the H > 2 mm fraction, while the Ah_1 < 63 µm fraction C:N ration is lower than that of the H horizon equivalent.

Table 4.2 Organic C content (%) for unlimed size fractions

Horizon	Size fraction	Mean % C (+/- std dev)	Mean % N (+/- std dev)	Mean C: N ratio (+/- std dev)
H	> 2 mm	28.91 (0.64)	1.54 (0.00)	18.73 (0.47)
H	< 63 µm	15.15 (0.39)	1.08 (0.15)	14.09 (1.53)
Ah_1	> 2 mm	19.15 (0.08)	0.81 (0.02)	23.56 (0.61)
Ah_1	< 63 µm	8.44 (0.16)	0.70 (0.06)	12.10 (0.77)

4.4.1.2 Limed soil

The > 2 mm size fraction from the H horizon had a high relative intensity in the carbohydrate region, comprising 38% of total spectral area (Figure 4.4). This decreased to 29% for the H < 63 μm fraction. However, this was a greater area than that of the O-alkyl-C peak in the Ah_1 > 2 mm fraction, which was 28%. The Ah_1 < 63 μm carbohydrate peak comprised 22% of total spectral area. There is an overall trend, therefore for a decrease in the relative contribution of the O-alkyl-C peak with both decreasing particle size and increasing depth. This pattern is not seen when examining the relative contributions of the alkyl-C peak areas from the different samples. For both the H and Ah_1 horizons, there is an increase in the relative contribution of alkyl-C with decreasing particle size.

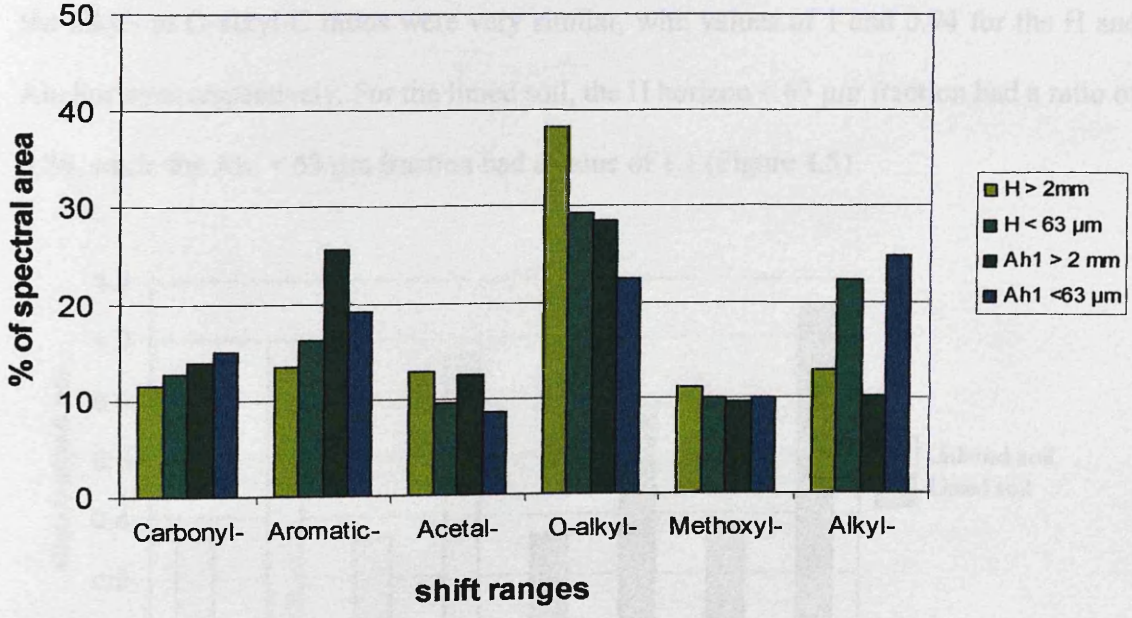


Figure 4.4 Percent of total CP MAS ^{13}C NMR spectra of limed soil, represented by spectral shift changes

As in the unlimed samples, Table 4.3 shows decreasing C content with both depth and particle size. Again, the C:N ratio of the samples decreases with particle size, but there is an increase in the C:N ratio of the > 2 mm size fraction with depth.

Table 4.3 Organic C content (%) for limed size fractions

Horizon	size fraction	Mean % C (+/- std dev)	Mean % N (+/- std dev)	Mean C : N ratio (+/- std dev)
H	> 2 mm	23.49 (0.72)	1.40 (0.03)	16.83 (0.11)
H	< 63 µm	11.62 (0.13)	0.93 (0.06)	12.51 (0.63)
Ah_1	> 2 mm	12.79 (0.33)	0.63 (0.06)	20.32 (1.38)
Ah_1	< 63 µm	6.89 (0.19)	0.62 (0.02)	11.05 (0.12)

4.4.1.3 Alkyl- to O-alkyl-C ratios

The alkyl- to O-alkyl-C ratios for the two soils are very similar, showing that at the outset of the experiment the recently applied lime had not yet impacted on the chemistry of the soil carbon. For both soil types, the alkyl- to O-alkyl-C ratios for the > 2 mm fractions for both horizons vary between 0.33 and 0.36, i.e. the O-alkyl peak occupies 3x greater spectral area compared to the Alkyl-C peak. For the unlimed soil < 63 µm size fractions, the alkyl- to O-alkyl-C ratios were very similar, with values of 1 and 0.94 for the H and Ah_1 horizons respectively. For the limed soil, the H horizon < 63 µm fraction had a ratio of 0.76, while the Ah_1 < 63 µm fraction had a value of 1.1 (Figure 4.5)

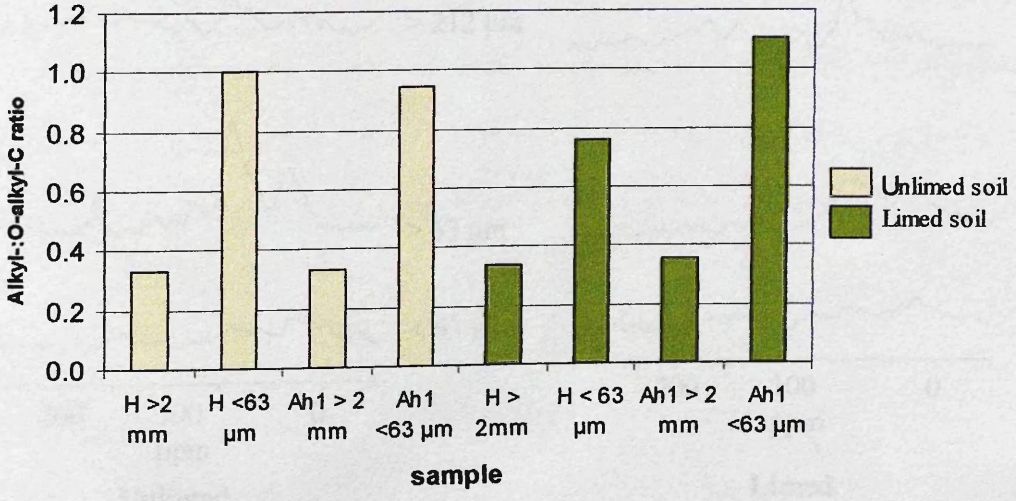


Figure 4.5 Alkyl: O-alkyl-C ratios for unlimed and limed soil size fractions, 1999 samples

4.4.2 CP MAS ^{13}C NMR spectra for particle size fractions after 20 months

As discussed in Section 2.4.2.3, the earthworm communities in the mesocosm boxes varied between plots, and this was true for the uninoculated boxes, as well as for the inoculated treatments. Therefore, the spectra shown in Figure 4.6 from the uninoculated, undisturbed covered boxes, had different earthworm communities both to each other, and the baseline samples. In the limed treatment, *D. rubidus* was the most abundant species, and in the unlimed treatment, *A. chlorotica* dominated. Figure 4.6 shows intensity scaled CP MAS ^{13}C NMR spectra for limed and unlimed particle size fractions from the H horizon.

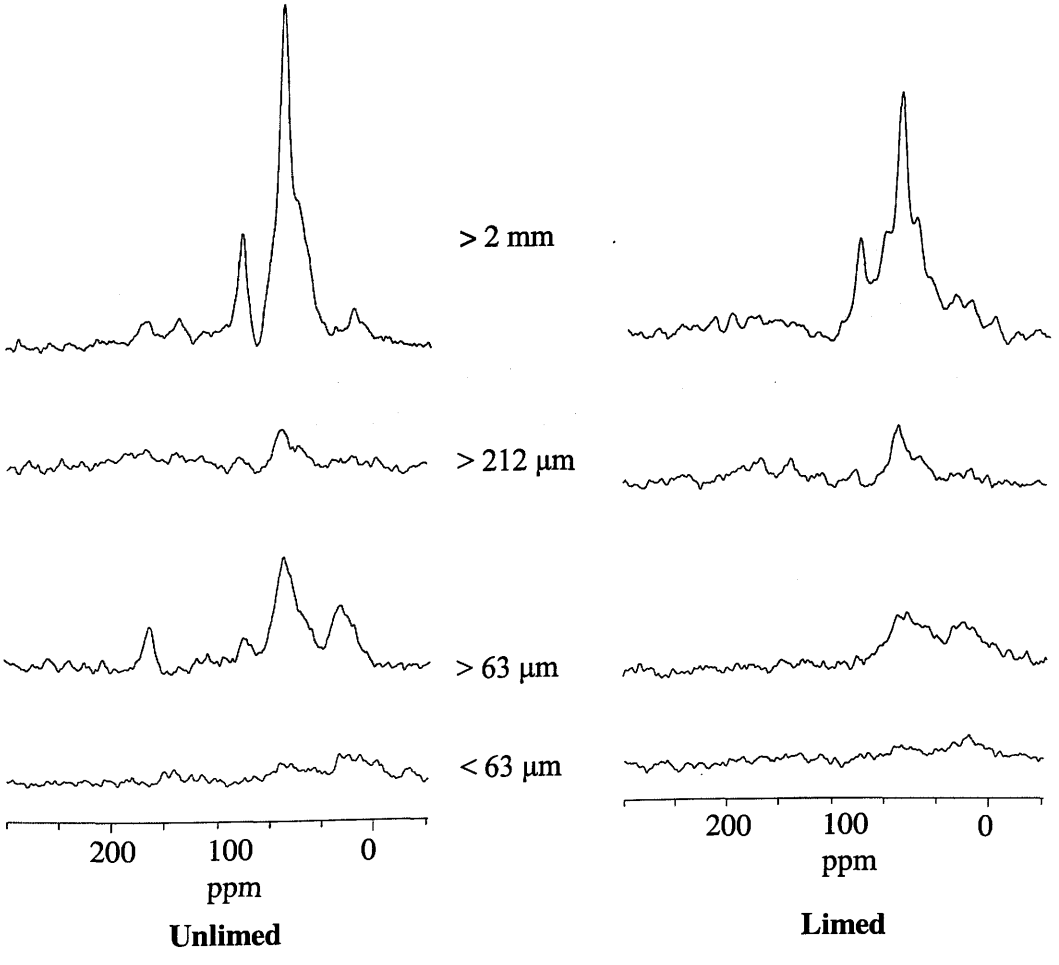


Figure 4.6 Intensity-scaled CP MAS ^{13}CNMR spectra of endpoint unlimed and limed particle size fractions from the H horizon

As in the baseline samples, the predominant NMR signals were in the alkyl-C (10-45 ppm), O-alkyl-C (60-90 ppm), acetal-C (and ketal-C) (90-110 ppm) and carbonyl-C (160-200 ppm) regions of the spectra. However, there are now pronounced differences between the limed and unlimed samples. The limed > 2 mm fraction has 2 distinct peaks off the main 60-90 ppm O-alkyl-C peak. The peak at 50 ppm is likely attributable to alkyl-amino C (Hopkins *et al.*, 1993), and the peak at 80 ppm to C1 from carbohydrate (Haslam, 1998 *op cit* Frűnd *et al.*, 1994). These separate peaks are not seen in the unlimed sample, and only the 50 ppm signal is visible as a shoulder off the main peak.

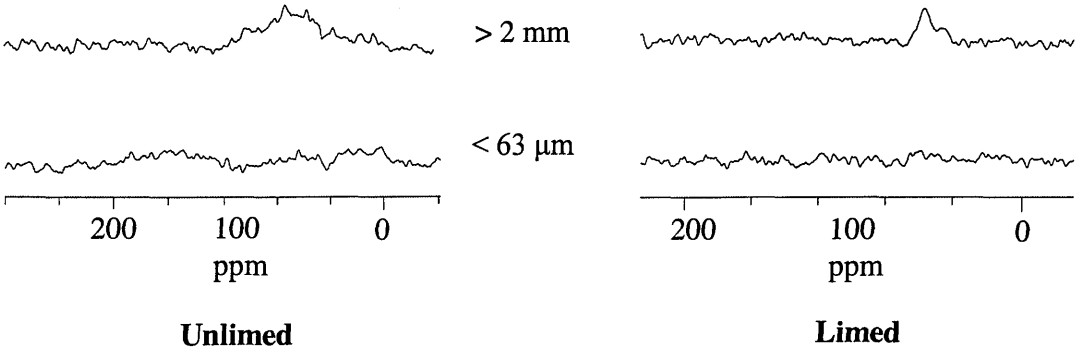


Figure 4.7 Intensity-scaled CP MAS ^{13}C NMR spectra of endpoint unlimed and limed particle size fractions from the Ah_1 horizon

Figure 4.7 shows the spectra from the Ah_1 horizons of the limed and unlimed soils. The Ah_1 > 2mm size fractions have a very weak O-alkyl-C signal only. As in Figure 4.6, there is no signal discernible from the < 63 μm fraction from either the limed or unlimed soil.

4.4.2.1 Unlimed soil

The > 2 mm size fraction from the H horizon had a high relative intensity in the carbohydrate region, comprising 40% of total spectral area (Figure 4.8). This was reduced in the smaller size fractions to between 20 and 27% of the spectral area. The > 63 μm and < 63 μm both had a relatively large spectral area belonging to alkyl-C, at least double that

of the two larger size fractions. This is shown clearly in Figure 4.10. There is not a consistent increase in the percent of spectral area assigned to aromatic-C with decreasing particle size.

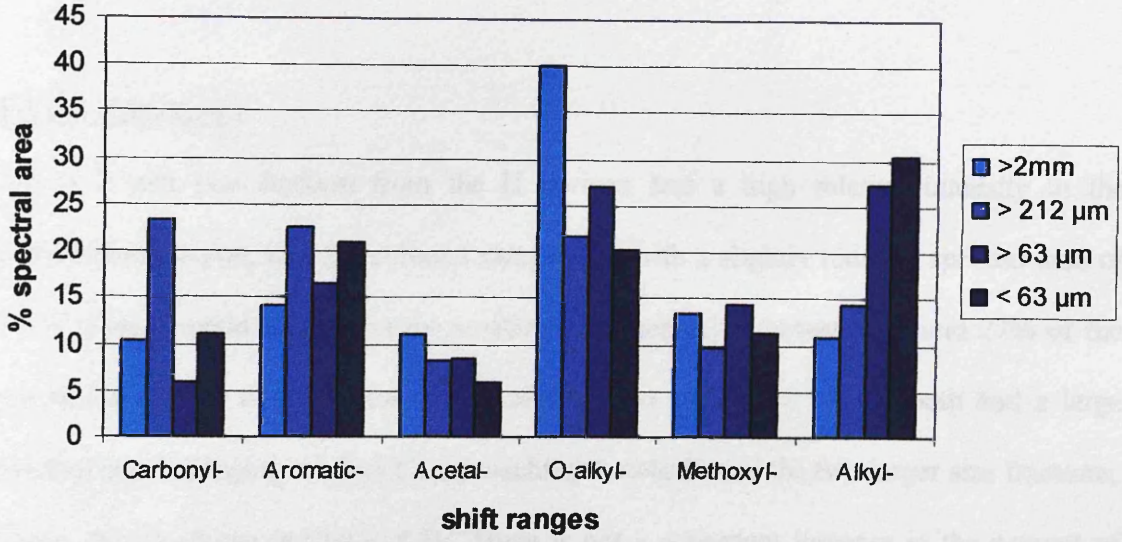


Figure 4.8 Percent of total CP MAS ^{13}C NMR spectra of unlimed H horizon soil size fractions, represented by spectral shift changes

Table 4.4 shows the organic carbon content for the unlimed size fractions from the H and Ah_1 horizons. This shows that, as expected, those spectra with the strongest CP MAS ^{13}C NMR spectra contain the most carbon. Interestingly, the Ah_1 > 63 µm sample which was not selected for NMR analysis had a relatively high C content.

Table 4.4 Organic C content (%) for the endpoint unlimed size fractions of both the H and Ah1 horizons

Horizon	size fraction	Mean % C (+/- std dev)	Mean % N (+/- std dev)	Mean C : N ratio (+/- std dev)
H	> 2 mm	37.10 (0.64)	0.61 (0.03)	60.85 (1.77)
H	> 212 µm	10.54 (0.09)	0.49 (0.01)	21.73 (0.38)
H	> 63 µm	25.94 (0.18)	1.76 (0.06)	14.74 (0.37)
H	< 63 µm	9.85 (1.15)	0.92 (0.02)	10.75 (1.01)
Ah_1	> 2 mm	10.32 (0.14)	0.34 (0.01)	30.39 (1.68)
Ah_1	> 212 µm	5.47 (0.12)	0.33 (0.04)	16.90 (1.47)
Ah_1	> 63 µm	24.62 (0.87)	1.59 (0.02)	15.54 (0.76)
Ah_1	< 63 µm	8.97 (1.09)	0.81 (0.01)	11.02 (1.45)

There is again a reduction in the C:N ratio with particle size, but unlike the baseline samples, the Ah_1 > 2 mm size fraction has a C:N ratio of approximately half that of the H > 2 mm fraction. There is little variability among the C:N ratios of the remaining size fractions.

4.4.2.2 Limed soil

The > 2 mm size fraction from the H horizon had a high relative intensity in the carbohydrate region, like the unlimed sample, but with a slightly reduced spectral area of 36 %. This is again reduced in the smaller size fractions to between 18 and 27% of the spectral area. The two smallest size fractions, > 63 μm and < 63 μm both had a large spectral area belonging to alkyl-C, approaching double that of the two larger size fractions. Again, this is shown in Figure 4.10. There is not a consistent increase in the percent of spectral area assigned to either carbonyl- or aromatic-C with decreasing particle size.

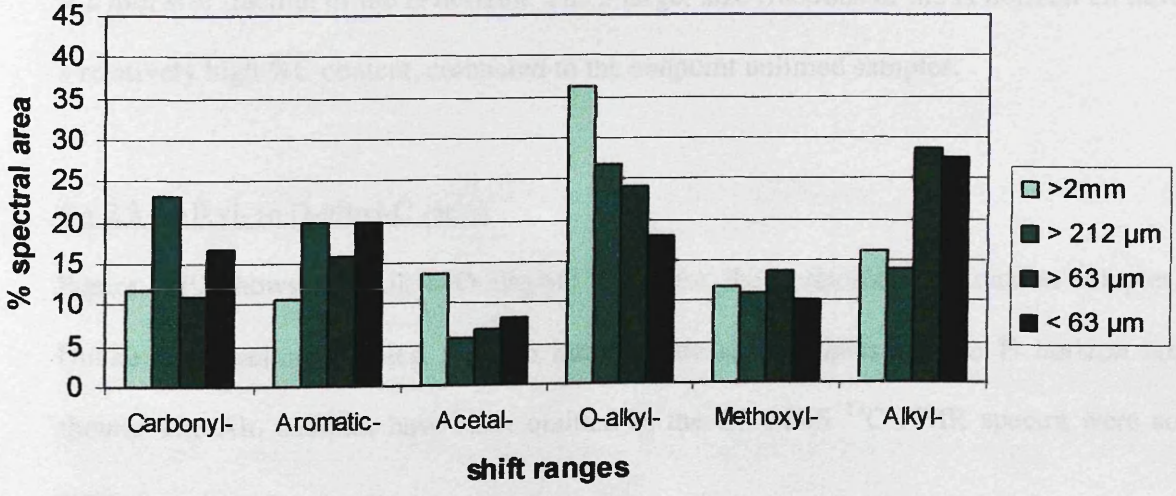


Figure 4.9 Percent of total CP MAS ^{13}C NMR spectra of limed H horizon soil size fractions, represented by spectral shift changes

Table 4.5 shows the organic carbon content for the limed size fractions from the H and Ah_1 horizons. In comparison to the unlimed samples, the 2 largest size fractions of the Ah_1 horizon have a relatively high carbon content.

Table 4.5 Organic C content (%) for the endpoint limed size fractions of both the H and Ah_1 horizons

Horizon	Size fraction	Mean % C (+/- std dev)	Mean % N (+/- std dev)	Mean C : N ratio (+/- std dev)
H	> 2 mm	39.09 (1.99)	1.32 (0.51)	31.78 (10.81)
H	> 212 μm	29.81 (0.69)	1.72 (0.02)	17.30 (0.60)
H	> 63 μm	29.06 (0.58)	1.89 (0.11)	15.93 (0.60)
H	< 63 μm	12.20 (0.03)	0.90 (0.01)	13.59 (0.18)
Ah_1	> 2 mm	31.23 (1.57)	0.56 (0.08)	56.52 (5.77)
Ah_1	> 212 μm	17.29 (0.70)	1.10 (0.04)	15.74 (0.03)
Ah_1	> 63 μm	22.04 (1.18)	1.66 (0.09)	13.31 (0.03)
Ah_1	< 63 μm	11.21 (0.11)	0.89 (0.03)	12.57 (0.30)

Whilst there is again a reduction in the C:N ratio with decreasing particle size, unlike in the unlimed soil, the C:N ratio of the Ah_1 > 2 mm size fraction is larger than that of the > 2 mm size fraction of the H horizon. The 3 larger size fractions of the H horizon all have a relatively high %C content, compared to the endpoint unlimed samples.

4.4.2.3 Alkyl- to O-alkyl-C ratios

Figure 4.10 shows the Alkyl-:O-alkyl-C ratios for the endpoint size fraction samples. Unlike the baseline samples, the two intermediate size samples for the H horizon are shown. The Ah_1 samples have been omitted as the CP MAS ^{13}C NMR spectra were so weak.

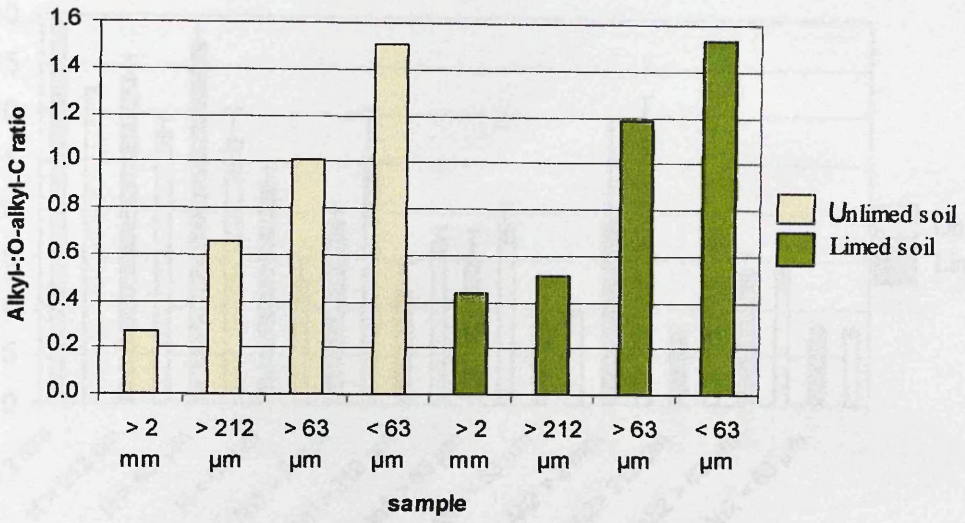


Figure 4.10 Alkyl-: O-alkyl-C ratios for unlimed and limed endpoint H horizon soil size fractions

The limed > 2 mm Alkyl-:O-alkyl-C ratio is greater than that of the unlimed sample. This is reversed however in the > 212 µm sample, where the unlimed sample has a higher ratio. There is little difference between the > 63 µm and < 63 µm samples. The < 63 µm Alkyl-:O-alkyl-C ratios are both greater than the equivalent baseline values, which had values of 1.0 and 1.12 for limed and unlimed soil respectively.

4.4.3 The impact of lime and earthworm ecological groups on carbon distribution through the soil size fractions and with depth

4.4.3.1 The impact of lime on carbon distribution

Figure 4.11 shows the carbon content of limed and unlimed soil, where the earthworm communities were dominated by *D. rubidus*. The carbon content of the limed H horizon samples exceeds those of the unlimed samples for all 4 size fractions. There is more carbon per size fraction for the unlimed soil in the Ah_1 horizon, and little difference between the 2 soil types in the Ah_2 horizon.

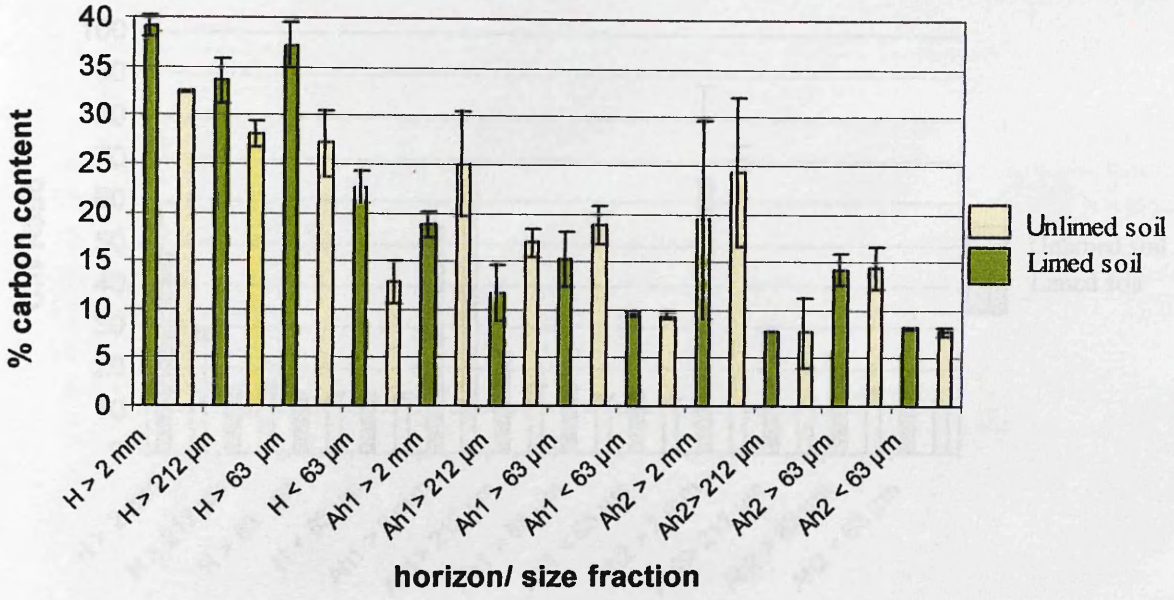


Figure 4.11 Carbon content of particle size fractions from limed and unlimed soil with a *D. rubidus* dominated earthworm community, with std dev bars, n=2

Figure 4.12 shows the C:N ratios of the particle size fractions for each horizon. This shows a similar pattern for each horizon, with the > 2 mm size fractions displaying the highest C:N ratios, with a sharp decline to the > 212 µm and then a gentle decline to the remaining 2 size fractions. The C:N ratio of the H horizon is lower than the Ah_1 horizon, which is lower than the Ah_2.

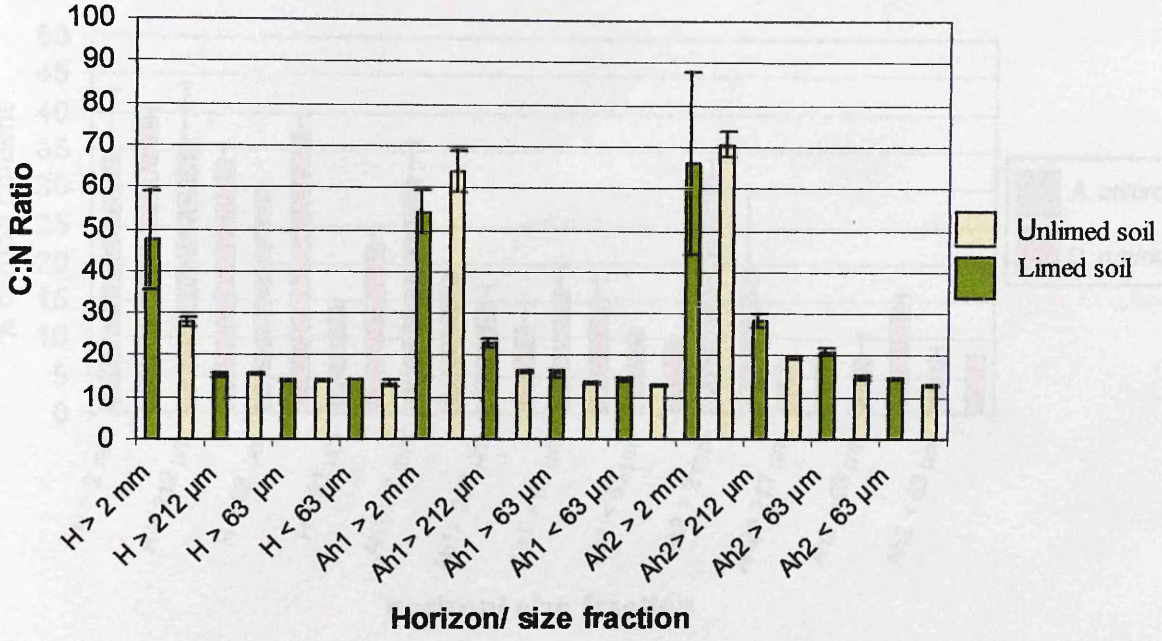


Figure 4.12 Carbon : nitrogen ratios of particle size fractions from limed and unlimed soil with a *D. rubidus* dominated earthworm community, with std dev bars, n=2

4.4.3.2 Effect of ecological groups

Figure 4.13 shows the carbon content of limed soil, with an earthworm community dominated by either *D. rubidus* or *A. chlorotica*. *D. rubidus* samples tend to have a higher carbon content in the H horizon, but this is not true for the lower horizons.

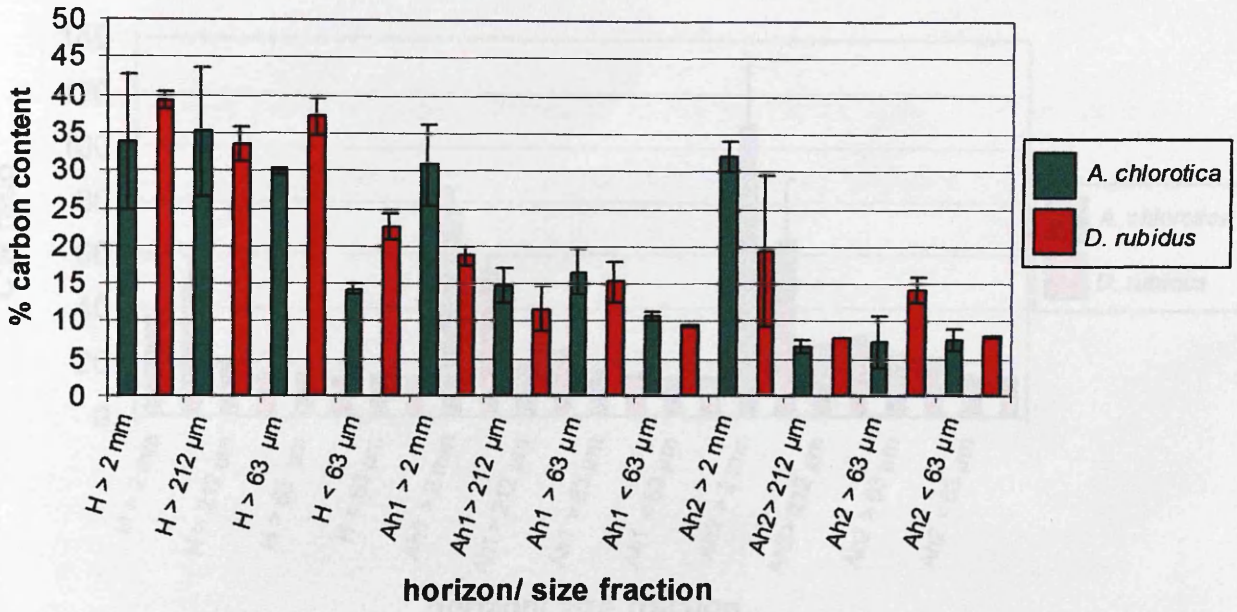


Figure 4.13 Carbon content of particle size fractions from limed soil with an *A. chlorotica* or *D. rubidus* dominated earthworm community, with std dev bars, n=2

The C:N ratios for these samples are shown in Figure 4.14. This shows a similar pattern to the C:N comparison shown in Figure 4.12. Within each horizon there is a decrease in the C:N ratio as particle size decreases. Again, the C:N ratio of the > 2 mm size fraction increases with depth, and the C:N ratios of the *D. rubidus* sample compared to the *A. chlorotica* sample in the H horizon are larger. In the Ah_1 and Ah_2 horizons, the *A. chlorotica* sample has a larger C:N ratio.

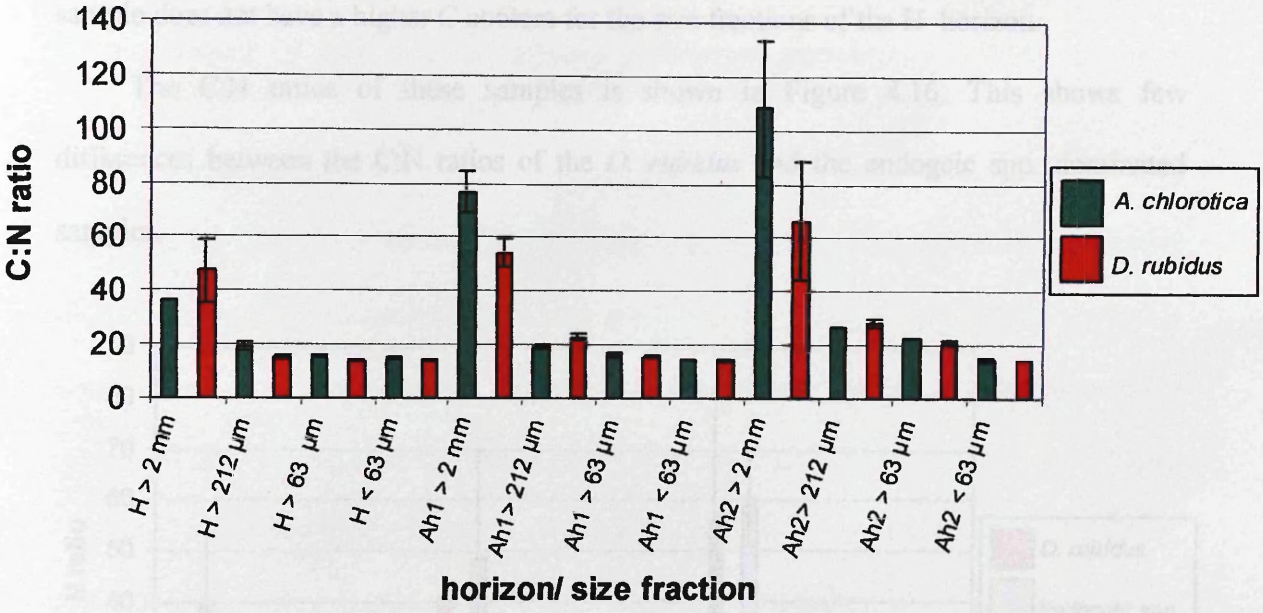


Figure 4.14 Carbon : nitrogen ratios of particle size fractions from limed soil with an *A. chlorotica* or *D. rubidus* dominated earthworm community, with std dev bars, n=2

The comparison between *D. rubidus* and a community of endogeic spp. (*O. cyaneum, A. chlorotica* and *A. rosea*) is shown in Figure 4.15.

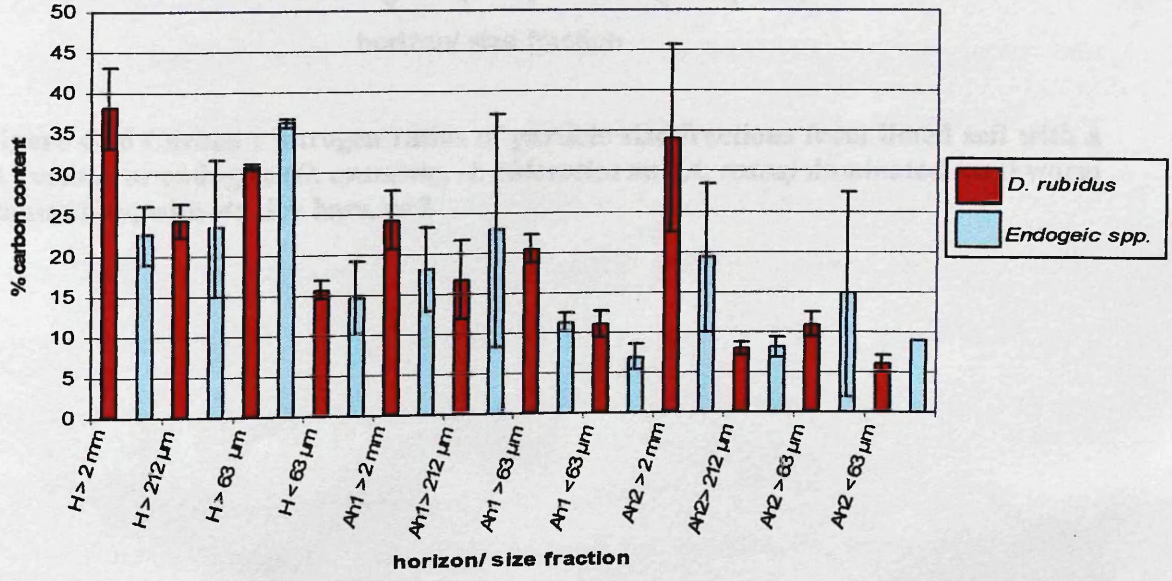


Figure 4.15 Carbon content of particle size fractions from limed soil with a *D. rubidus* or endogeic (*O. cyaneum, A. chlorotica* and *A. rosea)* dominated earthworm community, with std dev bars, n=2

In contrast to the previous 2 graphs of carbon content, here the *D. rubidus* dominated sample does not have a higher C content for the size fractions of the H horizon.

The C:N ratios of these samples is shown in Figure 4.16. This shows few differences between the C:N ratios of the *D. rubidus* and the endogeic spp. dominated samples.

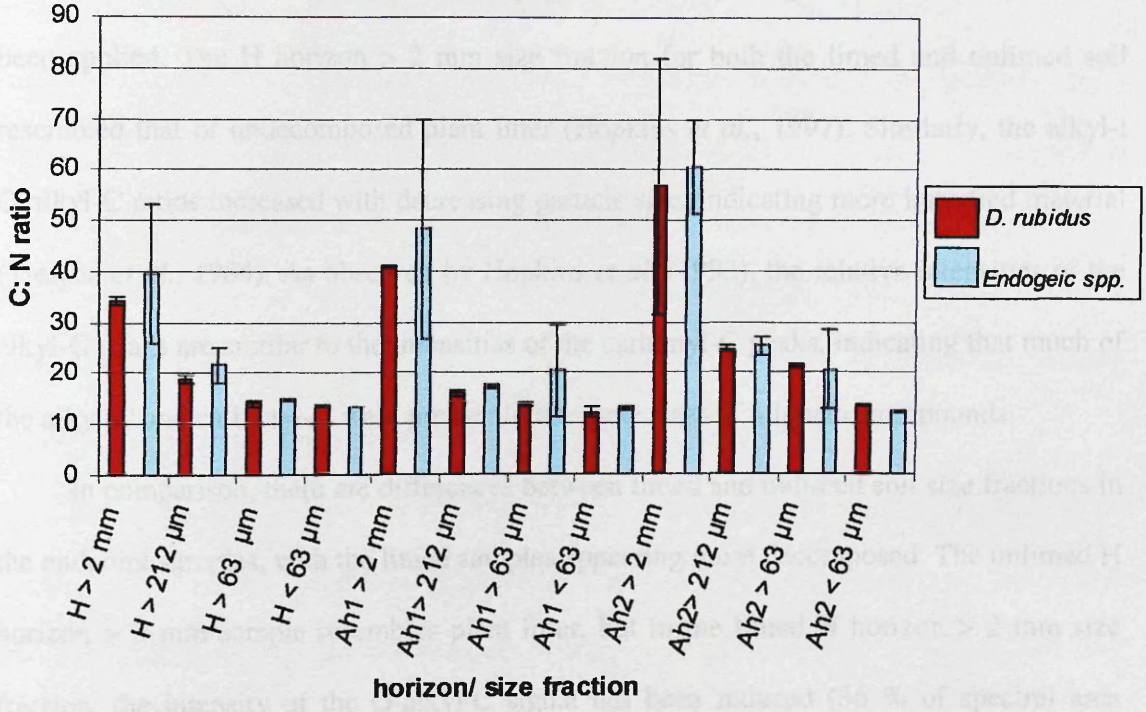


Figure 4.16 Carbon : nitrogen ratios of particle size fractions from limed soil with a *D. rubidus* or endogeic (*O. cyaneum, A. chlorotica* and *A. rosea)* dominated earthworm community, with std dev bars, n=2

4.5 Discussion

4.5.1 Impacts of lime and an altered earthworm community on the chemical characteristics of SOM

4.5.1.1 Impacts of lime

The 1999 baseline NMR spectra of the limed and unlimed soil samples appeared very similar. As for the 1999 earthworm survey, this is not surprising as lime had only recently been applied. The H horizon > 2 mm size fraction for both the limed and unlimed soil resembled that of undecomposed plant litter (Hopkins *et al.*, 1997). Similarly, the alkyl-: O-alkyl-C ratios increased with decreasing particle size, indicating more humified material (Tiessen *et al.*, 1984). As observed by Hopkins *et al.* (1993), the relative intensities of the alkyl-C peaks are similar to the intensities of the carbonyl-C peaks, indicating that much of the alkyl-C and carbonyl-C were present in the same class of aliphatic compounds.

In comparison, there are differences between limed and unlimed soil size fractions in the endpoint samples, with the limed samples appearing more decomposed. The unlimed H horizon > 2 mm sample resembles plant litter, but in the limed H horizon > 2 mm size fraction, the intensity of the O-alkyl-C signal has been reduced (36 % of spectral area compared to 40% in the unlimed sample). This reduction in intensity has resulted in the appearance of peaks at 50 and 80 ppm (attributable to alkyl- amino C and C1 of carbohydrate respectively) off the main O-alkyl- C peak in the limed spectrum. In the unlimed sample where the relative intensity of the peak is greater, only the 50 ppm resonance can be seen, and this appears as a shoulder off the main peak, rather than as a separate peak.

For the remaining size fractions of the endpoint samples, the weakness of the CP MAS ^{13}C NMR spectra is likely to be attributable to interference from paramagnetic species, most likely iron. As discussed in Section 1.8.1.5, this interferance can lead to a

loss of signal, and this becomes more of a problem with decreasing particle size, as organic residues become preferentially associated with iron-rich clay (Hopkins *et al.*, 1997; Arshad *et al.*, 1988)

4.5.1.2 Impacts of an altered earthworm community on the chemical characteristics of SOM

The limed soil which had a reduced intensity of O-alkyl-C in the H horizon >2 mm size fraction had a large population of the epigeic *D. rubidus* in the field. This species is a litter feeder, so the reduction in the large size fraction of carbohydrate-rich material is unsurprising. In comparison, the unlimed > 2 mm size fraction has an O-alkyl-C resonance which resembles that of undecomposed plant litter (Hopkins *et al.*, 1997). This sample was dominated by the geophagous *A. chlorotica* in the field which feeds on amorphous organic material, rather than on large chunks of relatively undecomposed plant litter.

In the limed soil, there was still a high total C content (as opposed to O-alkyl-C) for the H > 2mm fraction, and also for the > 212 μm and > 63 μm fractions. It is suggested that this is as a result of both the incorporation of comminuted material by *D. rubidus* and also as a contribution from plant root exudate. Although the above ground vegetation was harvested regularly, increased plant growth in the limed boxes will have increased carbon flow from plant roots to the soil.

Approximately 50% of the total carbon that plants photoassimilate is translocated below ground, and approx. 7% is lost from roots as exudates (Paul and Clark, 1996). Root exudates comprise of soluble carbohydrates, organic, amino and fatty acids, sterols, vitamins, and enzymes. There is also a contribution from sloughed off cell material which is comprised of readily degradable organic substrates (Killham, 1994). Therefore, the increased growth above ground and litter incorporation by *D. rubidus* resulted in a high C content for the limed soil.

However, these high carbon contents are not reflected by the quality of the ^{13}C NMR spectra collected for the limed soil, nor the > 212 μm fraction of the unlimed soil which also had a high C content. Although the > 212 μm and > 63 μm size fractions were density separated, to increase the C content of the sample in relation to mineral material, there appears to have been an incomplete fractionation of paramagnetic mineral. Interference from paramagnetics, especially Fe- rich material, is characterised by rounded and broad spectral signals (Arshad *et al.*, 1988). The < 63 μm fractions were not density separated, and small mineral particles may have washed through the sieve, resulting in a sample comprised mainly of mineral material, with a low C content. Any future work will aim to incorporate the flotation method of Arshad *et al.* (1988), to try to reduce the content of paramagnetic minerals in samples.

4.5.2 Effects of individual species on the distribution of soil C in limed and unlimed soil

With the results of the earthworm survey of the mesocosm site, and the plot pairing PCA, it became clear that this objective could only be addressed using limited data, and the effects of *D. rubidus* on the distribution of soil C was investigated. The results show that there was more carbon in each size fraction of the H horizon of the limed soil, compared to the unlimed soil. As there were approximately 200 individuals m^{-2} more in the limed soil compared to the unlimed soil, this increase in the carbon content of the soil fits well with increased litter incorporation. As the worms feed and comminute the litter, it becomes available to other soil invertebrates and liable to microbial colonisation. This is shown by the sharp drop in C:N ratios of the litter as the particle size is reduced. There is less carbon in both the Ah_1 and Ah_2 horizons. However, the C:N ratio of the > 2 mm size fractions for both these horizons is greater than that of the H horizon. This material will be comprised

mainly of plant roots, with some contribution from the earthworm incorporated material. However, in contrast to the H horizon, it appears that at depth, the rate of decomposition has been retarded, as the C:N ratios indicate relatively fresh organic material.

4.5.3 Effects of different ecological groups on the distribution of soil C within one soil type

The comparison of *D. rubidus* and *A. chlorotica* in limed soil revealed a greater carbon content for the *D. rubidus* dominated sample, for each size fraction in the H horizon. As discussed in the previous section, this is because *D. rubidus* feeds on and incorporates fresh litter into the upper organic horizon.

With increasing depth, this pattern does not hold and there is more large fraction C in the endogeic *A. chlorotica* sample at depth. This is true also for the large C:N ratios of the > 2 mm size fraction *A. chlorotica* samples in the Ah_1 and Ah_2 horizons. As this species feeds on particulate organic material in the organic and upper organo-mineral horizon the author can add little insight into this result.

In the comparison of *D. rubidus* and an assortment of endogeic species, similar anomalies occur. Whilst the *D. rubidus* sample has the highest C content in the H > 2 mm size fraction, no clear explanation for the comparative increase in the endogeic spp. > 63 μm size fraction can be offered. An interesting point to note however, is that this is the same size fraction which the *A. chlorotica* dominated unlimed CP MAS ^{13}C NMR spectra showed a strong signal. Again, the C:N ratios of the lower horizons reveal the trend of higher values in the endogeic spp. dominated samples.

4.6 Conclusions

The following conclusions were reached concerning the specific objectives:

- As liming resulted in an alteration of the earthworm community it was not possible to separate the effects of lime and the altered earthworm community. However, the limed soil, with a predominance of the epigeic *D. rubidus,* contained more decomposed organic material in the large particle size fraction of the organic horizon. Both limed and unlimed samples showed weaker CP MAS ^{13}C NMR signals in the smaller size fractions of the H and both the largest and smallest fractions of the upper organo-mineral horizon. It is suggested that this is due to intense earthworm activity for 18 months in an enclosed block of soil.
- The impact of an earthworm community dominated by *D. rubidus* in limed soil was to increase the amount of carbon in all size fractions in the organic horizon. The incorporation and comminution of fresh organic matter makes this material available for decomposition by other soil invertebrates and soil microorganisms. This increase in carbon content was limited to the H horizon, where this species is most active.
- Examining the impacts of different ecological groups highlighted the need for future exploration and experimentation. As stated, the impact of *D. rubidus* was to increase the C content of the organic horizon. However, the result of activity of an earthworm community dominated by *A. chlorotica* was to increase the carbon content of the largest size fraction of the upper organo-mineral horizon. For both the *A. chlorotica* and endogeic spp. dominated samples, the C:N ratio of the Ah_1 and Ah_2 samples is greater than for the *D. rubidus* sample. No reasonable explanation can be offered to account for this.

Chapter 5 Chemical characteristics of cast material from different earthworm species and their impact on soil CO_2 evolution

5.1 Introduction

As discussed in sections 1.3 and 1.7, earthworms influence nutrient cycling e.g. (Huhta, 1979; Schuster *et al.*, 2001; Edwards and Bohlen, 1996). The incorporation and turnover of large amounts of soil and organic matter results in an increase in the rate of mineralization of organic matter, by converting organic forms of nutrients into inorganic, plant available forms (Edwards and Bohlen, 1996). Casts tend to be more C rich compared to bulk soil, due to the incomplete re-absorption of mucus in the posterior of the earthworm gut, and the preferential selection of soil fractions enriched in organic material as a food source (Lee, 1985).

Evidence indicates that the chemical processes of decomposition are however mainly microbially-mediated (Edwards and Bohlen, 1996). A ^{13}C NMR study by Ziegler and Zech (1992) showed that *E. fetida* casts collected from specimens fed on beech litter over a three-week period had a slightly reduced C:N ratio, larger relative alkyl-C content, and a relatively lower polysaccharide content than beech litter. The lignin content of casts was also reduced compared to the litter, but this is likely to be due to selective feeding. Changes between casts and litter were not statistically significant. The authors concluded that the cast material was thoroughly comminuted but negligibly chemically altered.

As discussed in section 1.3.5, fresh casts may have a reduced microbial biomass, but an increased microbial respiration, and therefore a higher respiratory quotient (qCO_2) compared to bulk soil. This is indicative of a smaller, more metabolically active community (Zhang *et al*, 2000; Görres *et al* 1997; Wolters and Joergensen, 1992). It is interesting to note that a high qCO_2 is also indicative of a stressed community or adverse environmental conditions (Haynes *et al,* 1999). An increase in qCO_2 can also indicate the

rejuvenation of the microbial community, as the qCO_2 of 'young' microorganisms are often greater than that of 'aged' ones (Zhang *et al.*, 2000 *op cit* Anderson and Domsch, 1978). This data supports Lavelle's 'Sleeping Beauty' paradox, where the earthworm plays the role of Prince Charming in 'reawakening' dormant soil microbes. Earthworms change environmental conditions at the scale of the microorganisms and as a result can stimulate the activity of formerly dormant soil microorganisms (Lavelle and Spain, 2001), and also enhance spore germination (Fischer *et al.*, 1997).

Krištůfek *et al* (1992) also show that the effect of passage through the gut depends on the chemical composition of the food source. Bacteria in the guts of the litter feeding *L. rubellus* multiply rapidly when the earthworm feeds because of the readily available chemical compounds in the fresh plant litter ingested. In the endogeic *A. caliginosa* however, which feeds on more resistant humic substances, similar stimulation of microbial growth does not occur and the worms digest the bacteria in the ingested soil. This is supported by Zhang *et al* (2000), who showed that when the earthworm *M.guillemi* was fed soil with no added organic residues, there was no difference in CO_2 evolution between casts and uningested soil.

The variation in the stimulation of microbial activity is also observed in the effect of passage through the earthworm gut on the C:N ratio of the cast material compared to bulk soil. The C:N ratio can be lowered, due to the assimilation of C by the earthworm, or the excretion of nitrogenous waste in casts. This C:N ratio may alternatively increase. This can occur if the earthworm preferentially ingests relatively C enriched fractions of the soil, or if the worm has a relatively greater assimilation efficiency for N compared to C (Edwards and Bohlen, 1996).

This chapter investigates soil CO_2 output when inoculated with different earthworm species, representing all three ecological groups. The earthworms were incubated in pots in limed and unlimed soil, with ^{13}C enriched plant litter added to the soil surface as a food

source. The chemical composition of the cast material produced by these earthworms was also investigated.

5.2 Specific Objectives

The aim of the work in this chapter was to address the following hypotheses:

1. Where earthworms are inoculated into limed soil, more CO_2 will be evolved compared with the same species inoculated into unlimed soil;
2. Casts from litter feeding species will produce more CO_2 compared to endogeic species because of the differences in quality of the resources they ingest;
3. C content of cast samples from epigeic and anecic treatments will exceed those from endogeic treatments;
4. C: N ratios of cast samples from all earthworm species will be lower than that of bulk soil;
5. Earthworm cast material will have a higher alkyl-:O-alkyl C ratio compared to bulk soil as seen by CP MAS ^{13}C NMR spectroscopy.

5.3 Materials and methods

5.3.1 RESPICOND IV Respirometer

The main microcosm experiment was carried out in a RESPICOND IV respirometer, in which the kinetics and quantity of CO_2 evolution can be followed. A pot containing soil is suspended within a waterbath to ensure a constant temperature. The pot has an open 'conductivity chamber' suspended from the sealed lid of the pot. This conductivity chamber contains 0.6 M KOH solution, and platinum electrodes are submerged in this. As CO_2 is respired from the soil, it is absorbed by the KOH solution, forms carbonate ions,

and the conductivity of the solution decreases. This change in conductivity is calibrated to give a measurement of respiration (Nordgren, 1988).

There were two possible sizes of pots available for the microcosm experiment, standard and large. Standard respirometer pots are 64 mm in diameter and 119 mm high, and large pots are 112 mm in diameter and 145 mm high.

5.3.2 L. terrestris pot size preliminary experiment

An experiment using two different sizes of respirometer pot was carried out to determine whether *L. terrestris* survival was significantly affected by pot size, as this was the largest species to be used in the microcosm experiment. The small pot contained 25% of the soil volume compared to the large pot. This experiment was investigating whether this would result in different levels of mortality between the two pot sizes. Another possible consequence of pot size variation was also investigated, whether pot size affected the amount of CO_2 evolved from limed and unlimed soil, both with and without earthworms inoculated.

Soil collected from plots 2B (unlimed) and 2C (limed) at Sourhope was hand sorted and packed into standard and large respirometer pots, both with a 1:1 ratio of H:Ah_1 horizon, to a total depth of 4 cm and 6 cm for standard and large pots respectively. After settling for 4 weeks at 10°C, earthworm treatments were imposed, as shown in Table 5.1.

Table 5.1. Experimental design in pot size experiment, n=3

Limed soil				Unlimed soil			
Large pot		Standard pot		Large pot		Standard pot	
L. terrestris	control	L. terrestris	control	L. terrestris	control	L. terrestris	control

This experiment used 12 adult specimens (average wet weight 2.4 g +/- 0.41 g). The pots were incubated at 10 °C for 6 weeks, and CO_2 evolution monitored approximately

weekly using a Varian Aerograph gas chromatograph (Model 90-P). The GC was used as there were both relatively few replicates and the CO_2 accumulated was being monitored weekly, as opposed to the microcosm experiment when a measurement was taken every 24 hrs. CO_2 was sampled via a septum in the pot lid, using a syringe and needle. After each CO_2 sample was taken the pots were flushed to remove accumulated CO_2.

5.3.3 Microcosm experiment

Soil was collected from Sourhope plots 2B and 2C and hand sorted to remove large roots and macroinvertebrates. The soil was adjusted to 50 % water holding capacity and packed into standard size respirometer pots inside a fine nylon mesh (the toe of ladies tights), in a 1:1 ratio of H:Ah_1 horizon to a total depth of 4 cm. The nylon mesh was to prevent earthworms from entering the KOH solution chamber, whilst the movement of gases remained unimpeded.

At the end of the experiment, casts collected from the different species were to be analysed using CP MAS ^{13}C NMR spectroscopy. ^{13}C enriched plant litter was therefore added as a food source for litter feeding species to help ensure strong spectral signals. In order to add ^{13}C labelled grass litter to the microcosm experiment, turves were removed from limed and unlimed soil at the Sourhope field site. These were sealed in glass tanks, and kept in a greenhouse and had ^{13}C enriched gas (1.25×10^8 ‰ $\delta^{13}C$) injected into each of the tanks once a week. As necessary, the tanks were unsealed, the turves watered, and the ^{13}C enriched litter (310 ‰ $\delta^{13}C$) harvested, and then dried at 80°C

0.25 g of oven-dried ^{13}C labelled Sourhope plant litter was added to the soil with the earthworms, within the mesh, as food for litter feeding species. The earthworm inoculation plan was: Soil only

Soil plus ^{13}C labelled litter

Soil plus ^{13}C labelled litter + *L. rubellus*

Soil plus ^{13}C labelled litter + *A. chlorotica*

Soil plus ^{13}C labelled litter + *L. terrestris*

Soil plus ^{13}C labelled litter + *O. cyaneum*

Soil plus ^{13}C labelled litter + *E. fetida*

Earthworms were weighed before the start of the incubation and at the end. Treatments were replicated 3 times each in limed and unlimed soil and the experiment ran for 8 weeks, incubated at 15 °C. Accumulated CO_2 (mg) was recorded automatically once every 24 hrs.

5.3.3.1 NMR sample preparation

Cast samples were collected from the respirometer pots, and replicate samples were bulked to provide enough material for analysis. All of the cast material collected from *A. chlorotica* was composed of soil from the H horizon, in all of these samples the soil from the Ah_1 horizon was undisturbed. The cast samples from the remaining 4 species was a mixture of material from the H and Ah_1 horizons.

Samples were oven-dried at 80 °C for 12 hrs. These samples were then ground, using agate chambers, in a Retsch MM200 ball mill to a fine powder. NMR analysis parameters are as described in Section 4.3.1, using the 7 mm rotor, and with an acquisition of 38000 scans per sample.

5.3.4 Statistical analysis

One-tailed Mann Whitney tests determined the significance of the results, using Minitab v. 13.

5.4 Results

5.4.1 Pot size experiment

Earthworm survival was not significantly affected by pot size. Over the six weeks of the experiment, 3 worms died, 2 in large pots and 1 in a small pot.

Figure 5.1 shows the mean total accumulated CO_2 g^{-1} soil for each of the treatments of the *L. terrestris* preliminary pot size experiment.

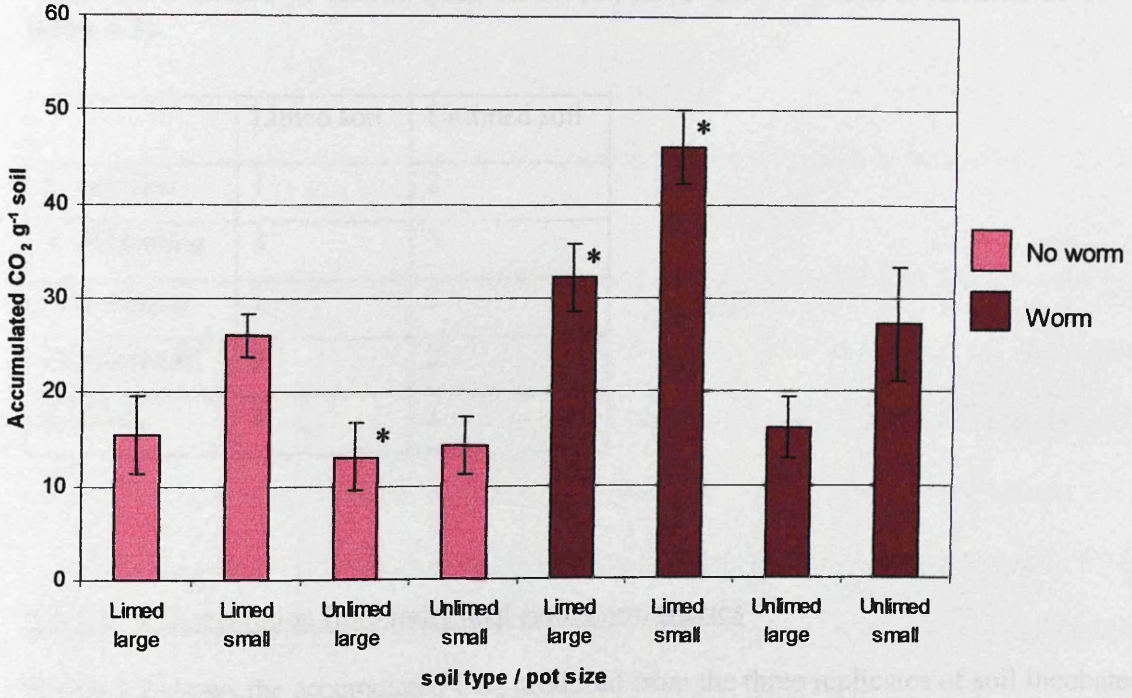


Figure 5.1 Mean total accumulated CO_2 g^{-1} soil for *L. terrestris* preliminary pot size experiment, n=3 (* n=2), with std dev bars.

Analysis of this data showed that in limed soil, with no worms present, soil in small pots evolved significantly more CO_2 compared to large pots ($p = 0.04$). In limed soil with *L. terrestris* present this was not significant ($p = 0.123$). In the unlimed soil treatments, when no worms were inoculated, soil in small pots did not evolve significantly more CO_2 ($p = 0.368$). This difference was significant with earthworms present ($p = 0.040$).

5.4.2 CO_2 produced from limed and unlimed soil inoculated with different earthworm species

In the control treatments, soil and soil + litter, there was no significant difference between CO_2 evolution between limed and unlimed soil ($p = 0.4786$, and $p = 0.4806$ respectively).

The number of specimens of each species recovered alive at the end of the experiment are shown in Table 5.2. Earthworm survival across the species was varied, with only *A. chlorotica* and *E. fetida* survival at 100% in both limed and unlimed soil.

Table 5.2 Number of earthworms recovered alive after 8 weeks incubated at 15 °C (max = 3).

	Limed soil	Unlimed soil
L. rubellus	1	2
A. chlorotica	3	3
L. terrestris	1	2
O. cyaneum	3	2
E. fetida	3	3

5.4.2.1 CO_2 evolution from individual earthworm species

Figure 5.2 shows the accumulated CO_2 produced from the three replicates of soil incubated with individual specimens of *E. fetida*, and the mean of the control treatments, soil only and soil + litter. This figure is representative of the data collected from all the litter feeding species, incubated in both limed and unlimed soil. Whilst the absolute amount of CO_2 accumulated is variable, all of the earthworm replicates evolve more CO_2 than the control treatments.

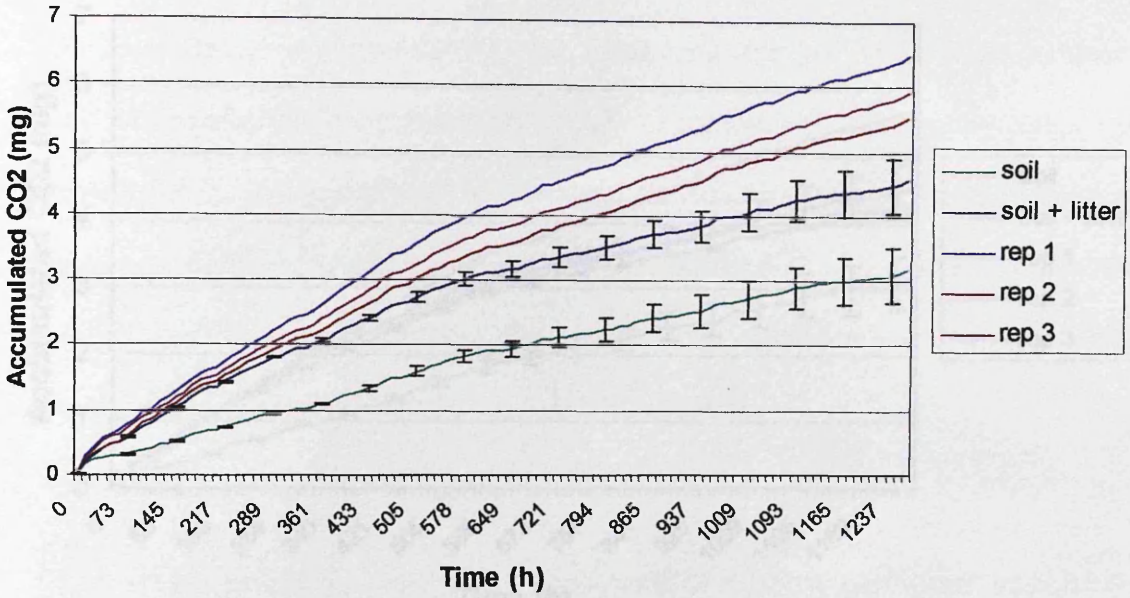


Figure 5.2 Accumulated CO_2 from soil incubated with *E. fetida* in limed soil. Control treatments show std dev bars, n=3

In order to compare between replicates and across earthworm and soil treatments, it would be necessary to standardise the CO_2 output to CO_2 per gram of earthworm. However, the CO_2 evolved was not just as a result of earthworm respiration, but also included earthworm-induced microbial respiration, where microbial activity was stimulated by the C-rich mucus exuded as the earthworm burrows through the soil. While there is a significant correlation between earthworm weight and earthworm respiration, there is no such relationship between the weight of earthworm and the increase in microbial respiration (Binet *et al.*, 1998). As a result it was not possible to standardize and compare these data statistically. However, despite the lack of statistical analysis, interesting observations were noted.

Figure 5.3 shows the CO_2 accumulated during the incubation of *A. chlorotica* in limed soil. Whilst one of the replicates evolved more CO_2 than the soil + litter control, 2 of the earthworm replicates have respired less CO_2 than the control treatments.

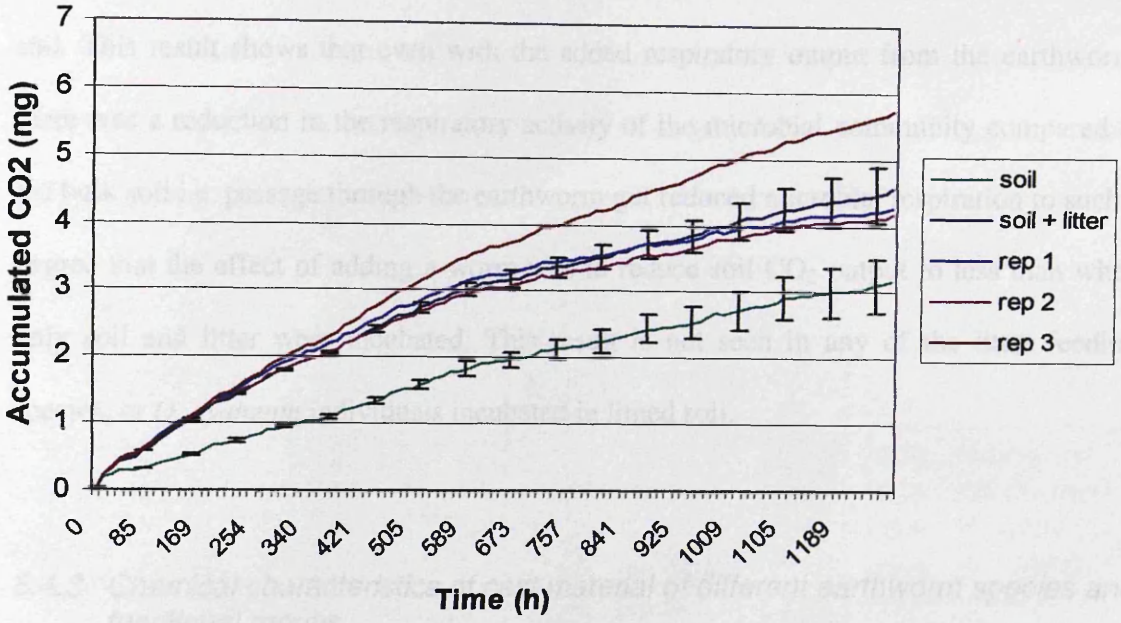


Figure 5.3 Accumulated CO_2 from soil incubated with *A. chlorotica* in limed soil. Control treatments show std dev bars, n=3

A similar result was shown for *A. chlorotica* incubated in unlimed soil (Figure 5.4). The final accumulated CO_2 for 2 of the replicates is within the standard deviation bars of the soil and litter controls.

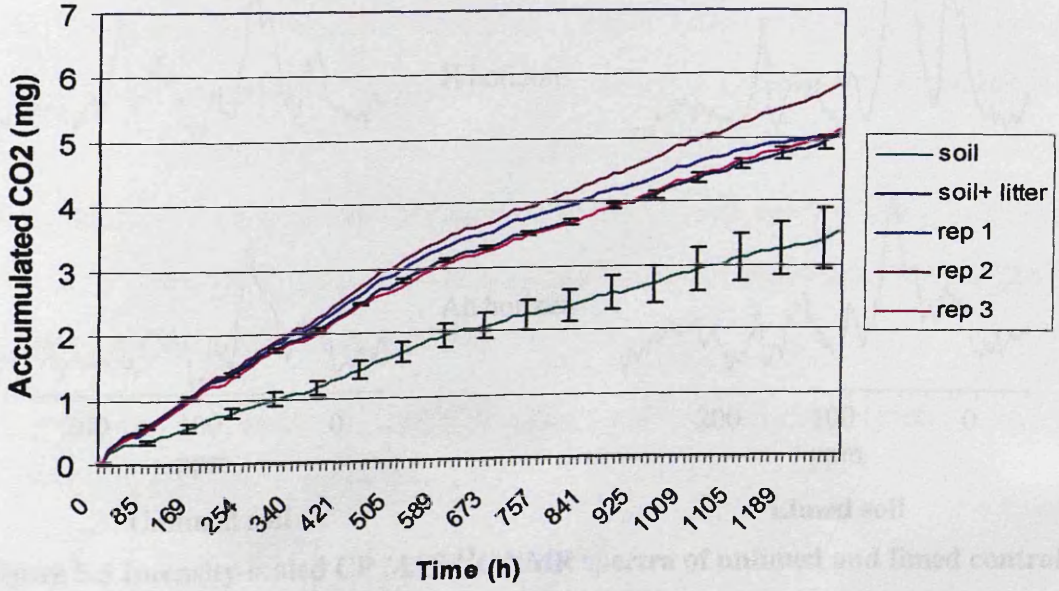


Figure 5.4 Accumulated CO_2 from soil incubated with *A. chlorotica* in unlimed soil. Control treatments show std dev bars, n=3

A similar result is found for 1 of the 2 surviving *O. cyaneum* replicates in unlimed soil. This result shows that even with the added respiratory output from the earthworm, there was a reduction in the respiratory activity of the microbial community compared to the bulk soil i.e. passage through the earthworm gut reduced microbial respiration to such a degree that the effect of adding a worm was to reduce soil CO_2 output to less than when only soil and litter were incubated. This result is not seen in any of the litter feeding species, or *O. cyaneum* individuals incubated in limed soil.

5.4.3 Chemical characteristics of cast material of different earthworm species and functional groups

5.4.3.1 CP MAS ^{13}C NMR spectra of control soil samples

Figure 5.5 below shows the CP MAS ^{13}C NMR spectra of the H and Ah horizon samples from the soil + litter 2 controls for limed and unlimed soil.

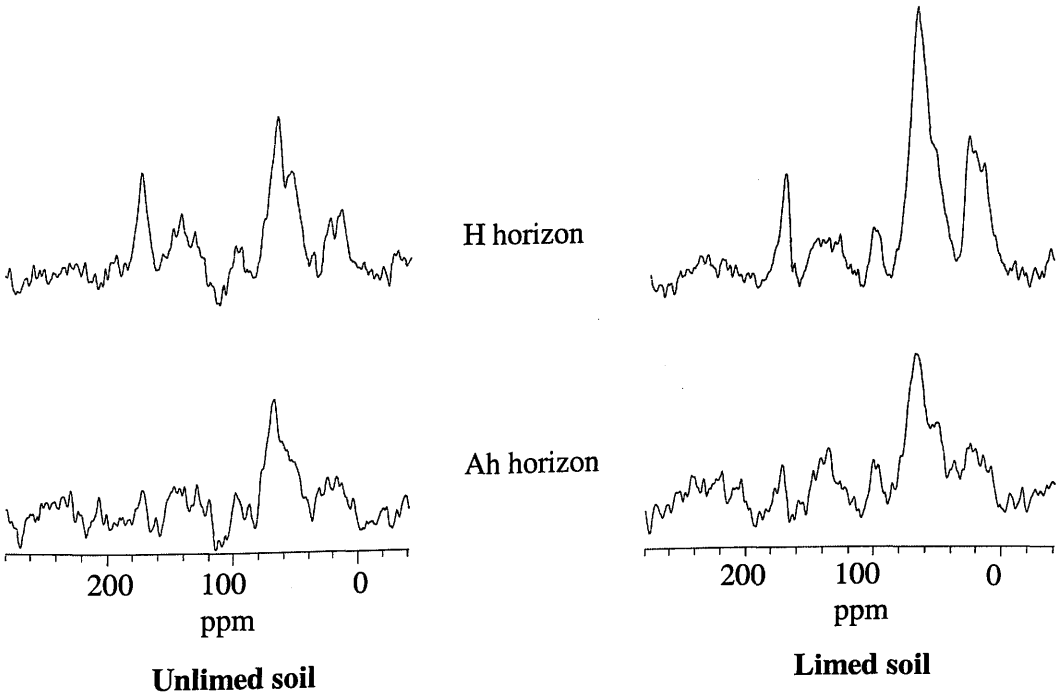


Figure 5.5 Intensity-scaled CP MAS ^{13}C NMR spectra of unlimed and limed controls

The main spectral signals were in the alkyl-C (10-45 ppm), O-alkyl-C (60-90 ppm), aromatic (110-160 ppm) and carbonyl-C (160-200 ppm) regions of both soils. There are differences however, in the spectra collected from limed and unlimed soil, and these differences are most pronounced in the H horizon.

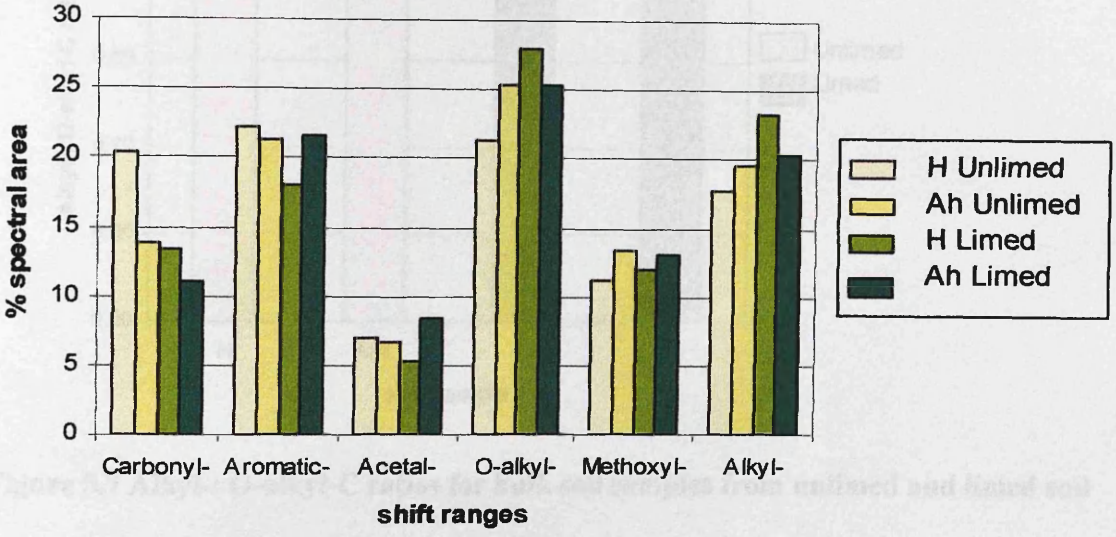


Figure 5.6 Percent of total CP MAS ^{13}C NMR spectra of bulk soil, represented by spectral shift changes

Figure 5.6 shows the data from Figure 5.5 converted into percentages of total spectral area. The spectrum of the limed sample has a higher relative intensity of O-alkyl-C (i.e. carbohydrate) compared to the unlimed sample, which is lower than both the limed and unlimed Ah_1 samples. The H horizon limed sample had the greatest C content (Table 5.3). The unlimed H horizon had the next largest C content, but the smallest O-alkyl-C peak and C:N ratio (Table 5.3). This indicates a greater degree of decomposition, i.e. that this sample contained little fresh organic material.

Table 5.3 Carbon content (%) and C:N ratios for microcosm control soils

Horizon	Soil type	Mean % C (+/- std dev)	Mean % N (+/- std dev)	Mean C: N ratio (+/- std dev)
H	Unlimed	22.07 (0.11)	1.84 (0.03)	11.97 (0.13)
H	Limed	27.42 (0.25)	2.13 (0.04)	12.87 (0.14)
Ah1	Unlimed	10.81 (0.12)	0.87 (0.02)	12.49 (0.17)
Ah1	Limed	12.63 (0.04)	0.97 (0.01)	12.99 (0.11)

The alkyl-C spectral areas show a similar trend to the O-alkyl-C spectral areas, so these differences are not seen in a comparison of the alkyl-: O-alkyl-C ratios (Figure 5.7).

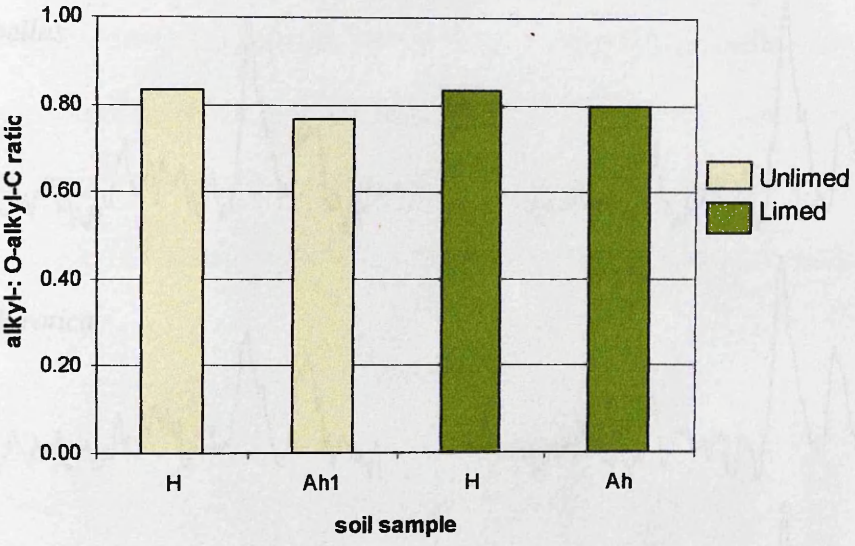


Figure 5.7 Alkyl-: O-alkyl-C ratios for bulk soil samples from unlimed and limed soil

5.4.3.2 CP MAS ^{13}C NMR spectra of cast material

Figure 5.8 shows the CP MAS ^{13}C NMR spectra of cast material from 5 different earthworm species incubated in both unlimed and limed soil.

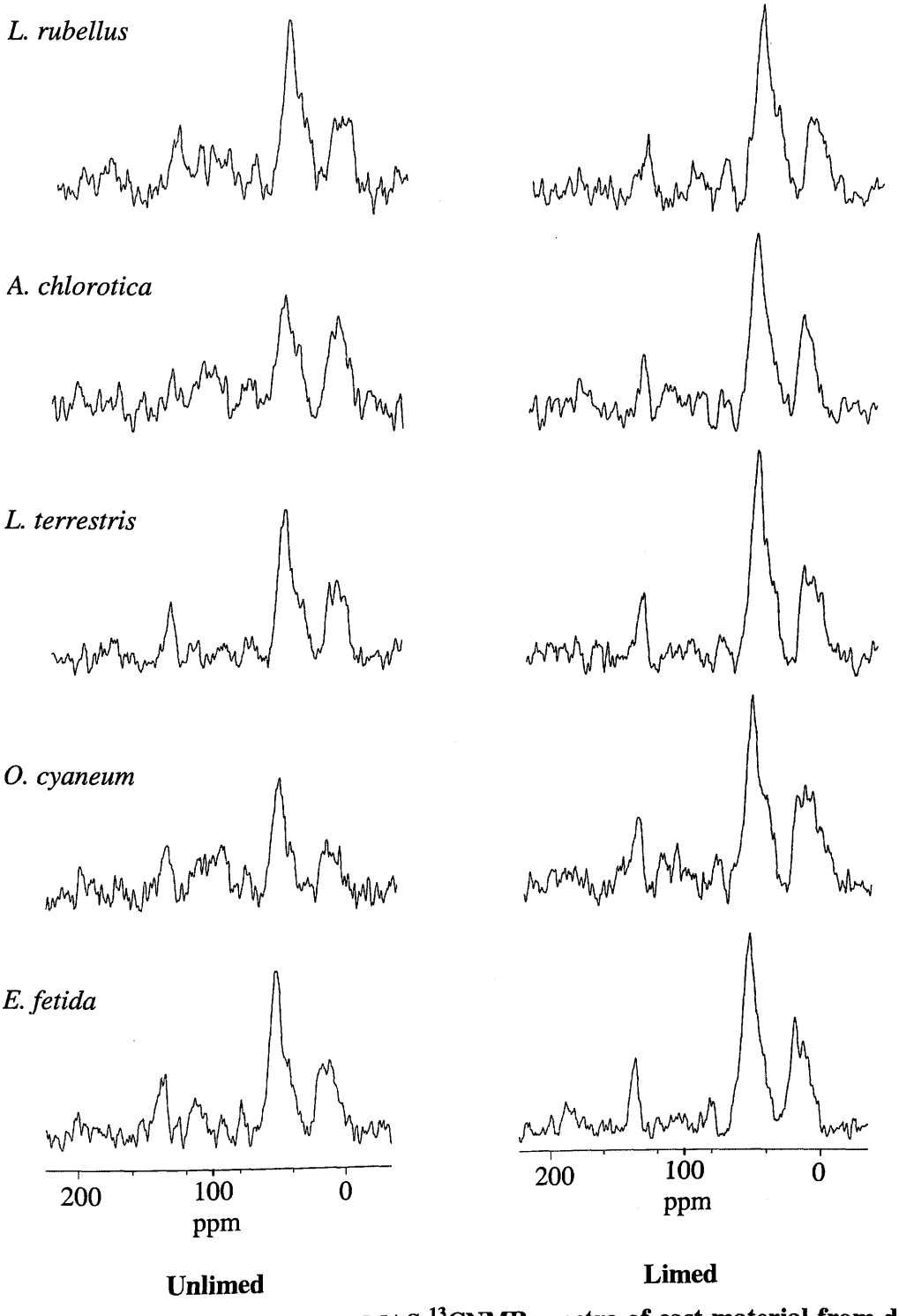


Figure 5.8 Intensity-scaled CP MAS ^{13}CNMR spectra of cast material from different earthworm species collected from unlimed and limed soil

As before, there were strong resonances in the alkyl-C (10-45 ppm), O-alkyl-C (60-90 ppm), aromatic (110-160 ppm) and carbonyl-C (160-200 ppm) regions of both soils.

With the exception of the unlimed *A. chlorotica* and *O. cyaneum* spectra, there were few major differences between cast material from different earthworm species. Spectra collected from limed soil had relatively larger O-alkyl-C peaks compared to their unlimed counterparts The unlimed *A. chlorotica, O. cyaneum* and *L. rubellus* spectra had the largest alkyl-:O-alkyl-C ratios (Figure 5.9), indicating a greater degree of decomposition.

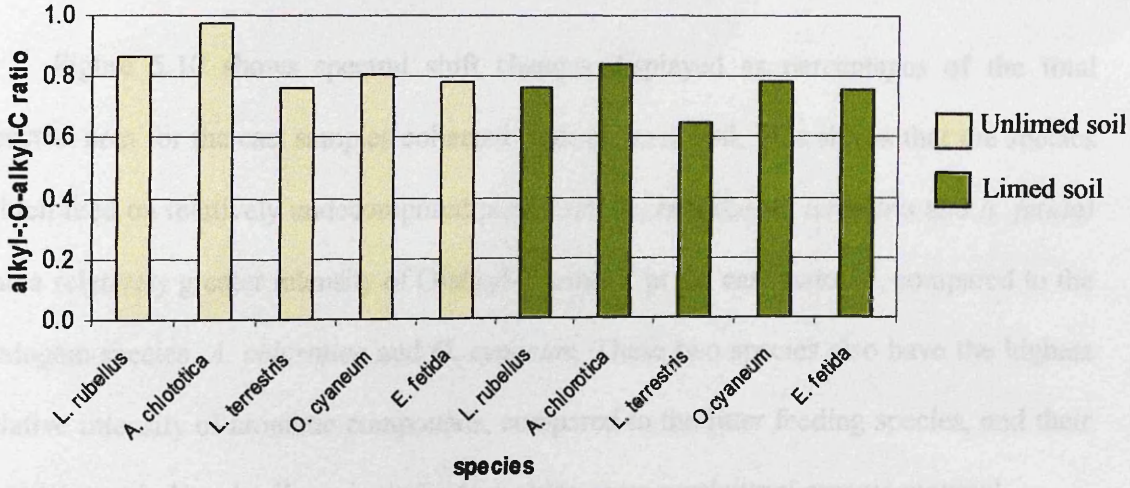


Figure 5.9 Alkyl-: O-alkyl-C ratios for cast material from different earthworm species collected in limed and unlimed soil

The relatively larger O-alkyl-C peaks found in limed soil fitted well with the carbon content of the samples (Table 5.4). All cast samples collected from worms incubated in limed soil have a higher carbon content than samples from unlimed soil. The carbon content of casts from *A. chlorotica* exceed that from *L. rubellus* and *O. cyaneum* casts in both limed and unlimed soil, and the C content of *E. fetida* casts in unlimed soil. However, this is likely to be relatively 'older' carbon.

Table 5.4 Carbon content (%) and Carbon: Nitrogen ratios for cast material from 5 different earthworm species incubated in limed and unlimed soil, n=2

Species	soil type	Mean % C (+/- std dev)	Mean % N (+/- std dev)	Mean C: N ratio (+/- std dev)
L. rubellus	Unlimed	21.60 (0.24)	2.05 (0.03)	10.51 (0.06)
	Limed	26.75 (0.36)	2.02 (0.03)	13.26 (0.04)
A. chlorotica	Unlimed	22.26 (0.05)	1.79 (0.01)	12.46 (0.01)
	Limed	25.67 (0.08)	1.88 (0.03)	13.66 (0.25)
L. terrestris	Unlimed	20.02 (0.04)	1.90 (0.01)	16.54 (0.05)
	Limed	24.06 (0.07)	1.79 (0.03)	13.41 (0.29)
O. cyaneum	Unlimed	16.33 (1.11)	1.48 (0.11)	11.01 (0.07)
	Limed	23.58 (0.44)	1.74 (0.01)	13.54 (0.16)
E. fetida	Unlimed	21.06 (0.23)	1.89 (0.05)	11.12 (0.17)
	Limed	25.83 (0.25)	1.86 (0.01)	13.92 (0.08)

Figure 5.10 shows spectral shift changes displayed as percentages of the total spectral area for the cast samples collected from unlimed soil. This shows that the species which feed on relatively undecomposed plant litter (*L. rubellus*, *L. terrestris* and *E. fetida)* had a relatively greater intensity of O-alkyl-C content in the cast material, compared to the endogeic species, *A. chlorotica* and *O. cyaneum*. These two species also have the highest relative intensity of aromatic compounds, compared to the litter feeding species, and their counterparts in limed soil, again, indicating older, more recalcitrant organic material.

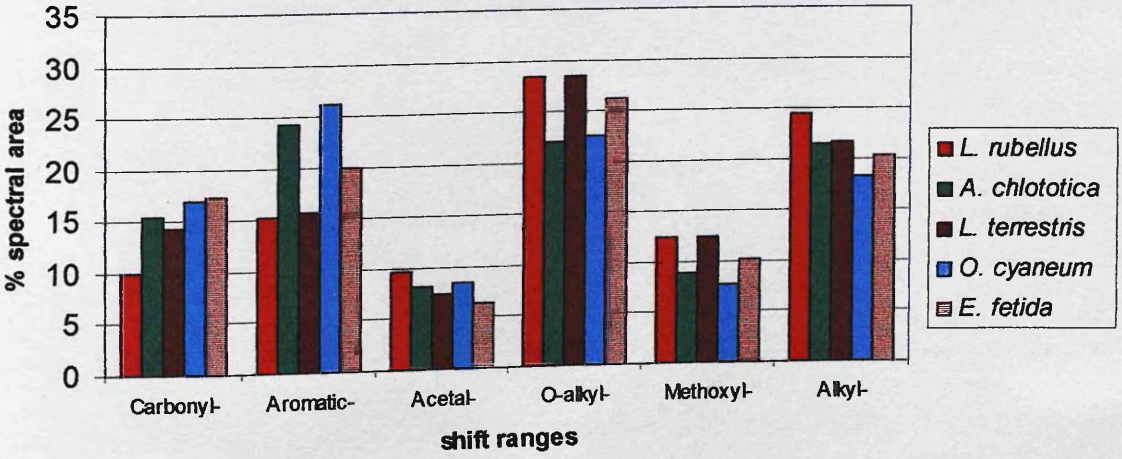
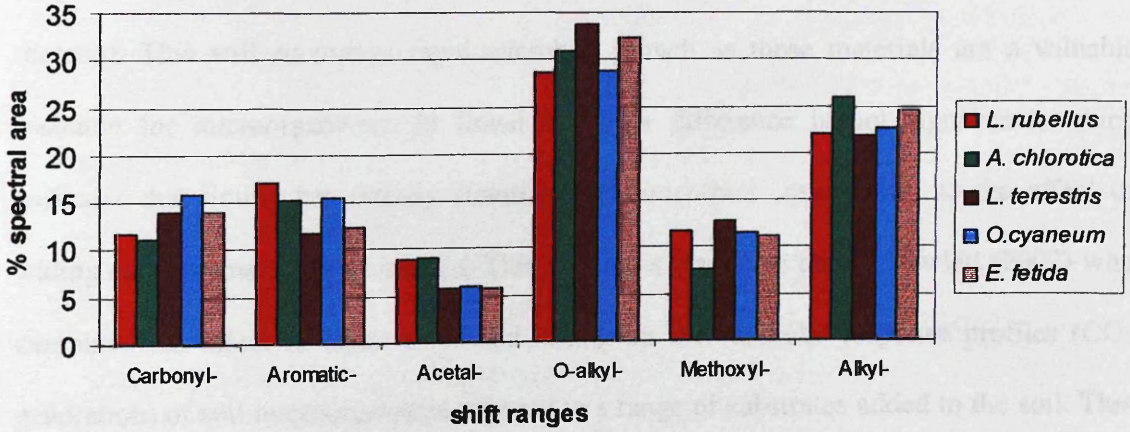


Figure 5.10 Percent of total CP MAS ^{13}C NMR spectra of cast material collected from unlimed soil, represented by spectral shift changes

Figure 5.11 shows the spectral shift changes for the cast material collected from earthworms incubated in limed soil. Unlike cast material collected from unlimed soil, the highest relative intensity of O-alkyl-C peaks was not restricted to litter feeding species, with both endogeic species showing a greater spectral area dominated by O-alkyl- than *L. rubellus.* The relative contribution of aromatics from *O. cyaneum* and *A. chlorotica* was reduced in the cast spectra from limed soil.

Figure 5.11 Percent of total CP MAS ^{13}C NMR spectra of cast material collected from limed soil, represented by spectral shift changes



5.5 Discussion

5.5.1 L. terrestris pot size preliminary experiment

Results show that when earthworms were present, more CO_2 was evolved per g of unlimed soil in small pots than in large pots. For the limed soil, there was no effect of pot size on CO_2 evolution. This suggests that the presence of the earthworm in the unlimed small pots stimulated the microbial activity more than that in the large pots. With 25% of the soil volume compared to the large pots, the earthworm will contact the same soil particles more frequently, and these will become more heavily enriched with epidermal exudate and cast material. This will encourage rapid microbial growth as these materials are a valuable resource for microorganisms. In limed soil, this difference is not significant, which indicates that liming has already stimulated the microbial community, so the effect of adding earthworms is not as marked. This finding is similar to that of Pawlett (2003) who examined the effect of earthworms and liming on the catabolic response profiles (CO_2 generation) of soil microorganisms exposed to a range of substrates added to the soil. This showed that changes in CO_2 generation as a result of earthworm additions were minor in comparison to liming, and were limited to unlimed soil. However, Pawlett (2003) suggests that increased earthworm biomass led to a microbial community with greater capacity to degrade complex substrates.

When no earthworms were present, soil in small pots evolved more than large pots for limed soil only. This suggests an increase in the activity of the soil microbial community as a response to liming. Another contributing factor to the higher CO_2 emissions from small pots could be the larger surface area: volume ratio. This suggests that CO_2 should be able to diffuse out of the soil more easily.

5.5.2 CO_2 produced from limed and unlimed soil inoculated with different earthworm species

As stated in the results section, it was not possible to compare the results from this experiment statistically, as the results combined the respiratory output of the earthworm and any stimulatory effect on microbial respiration. There is a correlation between the weight of a worm and its respiratory output given by: $R=aP^{1-b}$, where R is respiration (µl CO_2 g^{-1} live worm h^{-1}), P is worm live weight (mg) and a and b are constants (Binet *et al.*, 1998). However, Binet *et al.* (1998) tested the hypothesis that there was no relationship between microbial CO_2 output from the soil and worm weight, and concluded that while such a relationship is likely to exist, they were unable to quantify the nature of that relationship. This is likely to be because the amount of mucus and urine exuded into the drilosphere may not be proportional to the size of the individual worm, and will depend on the activity of the worm. If a worm is actively burrowing, it will need to exude more mucus to help lubricate its burrowing through the soil.

The degree of microbial stimulation in cast material is also not size dependant. The resource quality of food ingested by the earthworm will also be important. With a resource of low quality, gut passage, with its supply of readily metabolised mucopolysaccharides may have a large stimulatory effect on the metabolism of ingested microorganisms (Lavelle *et al.*, 1980; Blair *et al.* 1995). Alternatively, the earthworm may digest ingested microorganisms as a nutrient source (Kristufek *et al.*,1992). Similarly, when resource quality is high, the passage through the earthworm gut may not have a large stimulatory effect, as the microorganisms are already active (Blair *et al.* 1995). Conversely, it has been suggested that litter consumed by epigeic and anecic species, which are rich in readily available compounds may result in a stimulation of bacterial growth in the earthworm gut (Kristufek *et al.*,1992). The interaction between earthworms and soil microorganisms therefore depends on many factors and is not easily quantifiable.

Despite this, the results from this experiment show that all the litter feeding species evolved more CO_2 than the soil + litter controls, for both the limed and unlimed soil. However, for 2 out of the 3 *A. chlorotica* replicates incubated in both limed and unlimed soil, there were no differences in total CO_2 accumulated between earthworm treatments and the soil + litter controls. A similar result was shown for 1 of the 2 surviving *O. cyaneum* specimens in unlimed soil. This result fits well with data collected by Zhang *et al.* (2000) on the anecic earthworm *M. guillemi*. These authors showed that when *M. guillemi* was fed soil with no added organic residues, there was no difference in CO_2 evolution between casts and uningested soil. Although this is described as an anecic species, the authors present evidence for this earthworm feeding on partially decomposed organic material rich in microorganisms, indicating that like the endogeic Lumbricidae, this earthworm digests microorganisms.

The litter provided on the soil surface in this experiment would not be consumed by *A. chlorotica*, and in the unlimed soil there would have been no increase in C transfer below ground as there would be in the limed soil, due to stimulated plant growth, as discussed in Chapter 4. The digestion of ingested microorganisms could therefore account for the low value of total accumulated CO_2, to such a degree that the equivalent of the earthworm's own respiratory output was suppressed. A similar line of reasoning is proposed for the *O. cyaneum* replicate in unlimed soil.

Although the increase in plant growth should have increased the concentration of 'young' C below ground in the limed soil, the graph shown in Section 5.4.2.1 shows that a similar observation was recorded for 2 out of the 3 limed *A. chlorotica* replicates. For these and the unlimed *A. chlorotica* and *O. cyaneum* replicate which respired more CO_2 than the control treatments, it appears that while the soil was mixed thoroughly before filling the pots, the carbon resources were obviously not spread uniformly through the pots.

5.5.3 CP MAS ^{13}NMR analyses

5.5.3.1 CP MAS ^{13}C NMR analysis of control material

The H horizon limed sample had a greater intensity of O-alkyl-C signal compared to the unlimed sample. As both soils had ^{13}C enriched plant material added to the surface of the soil this is unlikely to account for the difference. Similarly, large plant roots were also removed from both samples. What seems likely is that the increased plant growth in limed plots increased the flow of carbon below ground (as discussed in Chapter 4), and perhaps small root fragments, contributed to the amount of young, fresh carbon, and therefore the relatively greater O-alkyl-C peak intensity observed in the limed sample. The differences between the Ah_1 samples were much less marked.

5.5.3.2 CP MAS ^{13}C NMR analysis of casts from different earthworm species incubated in unlimed and limed soil

Prior to the discussion of the results of the differences between the casts from different earthworm species, a caveat needs to be introduced. As CP MAS ^{13}C NMR spectra are often unreplicated, their interpretation is usually justified by the agreement of results from all other measurements taken on the sample. However, for the spectra presented here, the agreement of other results cannot be unequivocally stated. The data collected on the CO_2 output of soils inoculated with different earthworm species were not analysed statistically, and so therefore, while those results may agree with those of the CP MAS ^{13}C NMR spectra, they may not, and the interpretation of these spectra must be done with some degree of caution.

So, whilst acknowledging this caveat, there were relatively few major differences between cast material collected from different earthworm species. All of the CP MAS ^{13}C NMR spectra were strongest in cast material collected from limed soil, with similar carbon

contents and alkyl-C: O-alkyl-C ratios. This is despite the different feeding behaviour of the species. The cast material of the litter feeding species resembles that of plant litter, with a slightly greater relative intensity of alkyl-C compared to uningested plant litter. What was unexpected was the similarity of the casts collected from the two endogeic species, *A. chlorotica* and *O. cyaneum* to the litter feeding species' spectra. However, as discussed in the previous section, this is likely to be due to the relatively O-alkyl-C enriched starting material.

The similarity of casts from the endogeic species' to the litter feeding species' casts is not seen in the unlimed soil, where there was no stimulation of plant growth, and the control sample has a much weaker O-alkyl-C signal. This means that there was less 'young' organic material present in the soil, and as a result, the spectra obtained from the unlimed endogeic casts do not have a strong polysaccharide peak, and are characteristic of more decomposed organic material. However, while the *A. chlorotica* sample has an alkyl-:O-alkyl-C ratio of 1, the carbon content is not lower than the litter feeding species, simply in a different chemical form. In comparison, the *O. cyaneum* cast material has the lowest organic matter content of the series, probably due to the mixture of H and Ah1 soil material which the worms ingested.

As a result, the hypothesis that cast material would have a higher alkyl-: O-alkyl-C ratio was proven incorrect.

5.5.3.3 C:N ratios of cast material

The hypothesis that the C : N ratios of cast samples would be lower than that of bulk soil was not found in the limed soil, and was not universal among the unlimed samples. The limed samples all had a greater C:N ratio compared to bulk soil. C:N ratios decrease as decomposition proceeds due to relative increases in the concentration of lignin and aromatic compounds. However, as the limed soil was relatively enriched in fresh,

relatively undecomposed carbon, it seems likely that in addition to any feeding on the plant litter provided, that the worms were selectively feeding on this fresh organic material. In comparison, the spectra of the unlimed soil was characteristic of older, more decomposed organic material. As a result, the C:N ratios of all except *A. chlorotica* were lower than that of the bulk soil. An explanation for this is presented in the following section.

5.5.3.4 Cast carbon content

The hypothesis that the C content of cast samples from litter feeding species would exceed those from endogeic species was proven incorrect. The carbon content of casts from *A. chlorotica* exceeded that of *L. rubellus, L. terrestris, O. cyaneum* in both limed and unlimed soil, and the C content of *E. fetida* casts in unlimed soil. There are 2 possible explanations for this. The first is that *A. chlorotica* is showing strong food selection behaviour, more so than *O. cyaneum*, and is consuming more C and the casts as a result have a higher C content. A more likely explanation is however, that the litter feeding species, which are ingesting relatively undecomposed organic material with more readily degradable C have higher assimilation efficiencies. This means that they assimilate more C per unit of organic matter ingested. This would therefore result in a lower carbon content in cast material.

O. cyaneum probably has a lower C content of cast material compared to *A. chlorotica* as while both are endogeic species, all the casts from *A. chlorotica* were derived from H horizon soil, unlike *O. cyaneum* where casts were collected from both the H and Ah_1. This means that *A. chlorotica* was feeding on a substrate with a higher C content than *O. cyaneum,* but a more recalcitrant material than the litter the epigeic and anecic species were consuming.

5.5.4 Suggested improvements to experimental protocol and future work

As became evident at the end of this experiment, there were several alterations to the experimental protocol which could have improved the interpretability and quality of results collected.

The simplest improvement to experimental protocol would have been to incubate only cast material. This would have allowed a direct comparison of the impact of gut passage of different earthworm species on microbial respiration. As microbial biomass and activity changes as casts age (Parle, 1963b), the collection of fresh cast material from each of the species included in the experiment could have yielded interesting data not only on initial differences, but also any which become different as casts age. However, this would mean discounting the contribution of the drilosphere on microbial activity.

Therefore, the amendment to experimental protocol which would be recommended, is to follow that described in Binet *et al.* (1998). This paper describes a methodology which allows the overall respiratory activity in the presence of an earthworm to be distinguished from earthworm respiration and respiration from the microbial biomass as a result of earthworm activity. This protocol means that the effects of the earthworm drilosphere on microbial respiration do not have to be excluded, as the drilosphere can also be an important site of microbial activity. As the original paper used only *L. terrestris* the expansion of this experiment to include other species, with different feeding preferences could answer the hypotheses set out at the beginning of this chapter.

5.6 Conclusions

The following conclusions were reached concerning the specific objectives:

- Due to the problems in standardising the CO_2 output g^{-1} earthworm, it was not possible to compare the CO_2 output from individual species in limed and unlimed soil. However, as several samples containing endogeic species had a total CO_2 output lower than that of the soil + litter control, it is possible to state that soil containing litter feeding species does evolve more CO_2 than that containing endogeic species.
- The C content of casts from litter feeding species did not exceed the C content of casts from the endogeic *A. chlorotica* but did exceed that of casts from *O. cyaneum.* These differences in C content can be explained by the difference in substrates consumed. *O. cyaneum* casts were collected from both the organic and organo-mineral horizons, and therefore of all the species, this was consuming the lowest quality organic material. While *A. chlorotica* feeds in the upper organic horizons, the litter feeding species feed on the highest quality of organic matter which is most readily degradable. These species therefore have a higher C assimilation efficiency and therefore a lower C content that the soil feeding *A. chlorotica.*
- There appear to be relatively few differences between cast material from different earthworm species, or between cast material and bulk soil. Cast material from all 5 earthworm species incubated in limed soil showed no differences in carbon content and C:N ratio, and alkyl-:O-alkyl-C ratios of the cast material either between species or in comparison to bulk soil. This appears to be due to the greater concentration of 'young' carbon as a result of increased plant growth in the limed soil and therefore increase in carbon flow below ground.
- In unlimed soil there are differences between endogeic and litter feeding species' cast material as the stimulation of plant growth did not occur, and so there is less of this

polysaccharide rich C present in the soil. As a result, endogeic species have cast material which show characteristics of more decomposed material.

The conclusion is however, that these changes appear relatively minor, and that the positive effects on nutrient cycling and plant growth often observed in the presence of earthworm and cast material is due largely to facilitation. Earthworms thoroughly comminute and wet the organic material with mucus, and incorporate it into the soil, for further decomposition by the soil microbial community.

Chapter 6 General discussion

6.1 Introduction

This chapter brings together the results and conclusions from the experimental Chapters 2, 3, 4 and 5, and to place them in the context of the overall aims of this thesis which were presented in Chapter 1.

6.2 Objectives of the thesis

6.2.1 To investigate the effects of lime on manipulated earthworm communities, and to determine the environmental and experimental variables which explain individual earthworm species' distribution

The effect of lime on earthworm communities was investigated in both an unmodified community and in the modified earthworm community of the mesocosm experiment. These earthworm communities gave different results.

At the unmanipulated Sourhope site, liming resulted in the increase in one species, the acid tolerant epigeic species, *D. rubidus*. However, this species significantly increased in unlimed soil too, although the magnitude of the change was larger in the limed soil, indicating some aspect of field site management was also impacting on earthworm abundance. The increase in *D. rubidus* coincided with a significant decrease in another acid tolerant epigeic species, *D. octaedra* in both soil types. This indicates an interspecific competitive interaction between these species, with *D. rubidus* outcompeting the other species.

Further interspecific interactions were found in the manipulated earthworm communities. In the unlimed soil treatments, *D. rubidus* appears to have outcompeted the inoculated *L. rubellus*. However, in the limed soil where *A. chlorotica* was inoculated, this species was more abundant than both *D. rubidus* and *L. rubellus*. In addition to this, analyses

suggest that a high abundance of *A. chlorotica* was associated with a shallow depth of LF horizon. This reduction of accumulated LF horizon at the soil surface has often been observed in Australasia and also in the Netherlands, when endogeic Lumbricidae (usually *A. caliginosa*) are inoculated into pasture without an established native earthworm community or without Lumbricidae present e.g. (Barratt, 1964; Stockdill, 1982; Hoogerkamp *et al.*, 1983). The data presented here supports that finding, which has not been reported previously in UK soils. These results suggest that with the increase in the size of the *A. chlorotica* population, due to liming and inoculated specimens, this species has outcompeted the epigeic species which usually occupy the LF horizon. This would allow *A. chlorotica* access to the microbially–rich rhizosphere on which to feed. The action of burrowing through the LF horizon may have had a similar impact as that of epigeic species, and loosened and incorporated the litter into the upper organic horizon. This result contributes to the growing stock of literature questioning the classification of earthworms into these fixed ecological groups, e.g. Neilson *et al.* (2000).

The environmental variables shown to significantly explain some of the observed variation in the distribution of the earthworm species at the Sourhope field site fit well with those reported in the literature e.g. (Briones *et al.*, 1992; Briones *et al.*, 1995). The significant variables were both related to earthworm food availability (depth LF horizon) and resource quality (soil C:N ratio).

Of the experimental factors found to significantly affect earthworm abundance in the mesocosm experiment, the most interesting was the significance of one of the axes from the RDA on botanical data presented in Chapter 3. This indicates that the distribution of plant species has impacted on the earthworm community or alternatively, some undefined variable is impacting on both the plant and earthworm species. Further experimental work in needed to elucidate this point.

6.2.2 To examine the impact of these earthworm communities on plant above-ground biomass harvests and species distributions

The examination of the effects of specific earthworm communities on plant yield was not possible due to the variable survival of inoculated earthworm species. However, while the introduction of earthworms has been shown to increase yields of crop plants in pot experiments (e.g. Baker *et al.*, 1997) and pasture yields (e.g. Curry and Boyle, 1987; Stockdill, 1982; Hoogerkamp *et al.*, 1983; Springett, 1985) no significant correlation between plant yield and increased earthworm biomass was found in this study.

Similarly, no effects of the earthworm communities was discernible on the occurence of plant species. However, there is a suggestion in the literature that perennial plant species respond more slowly to earthworm mediated enhancement of nutrient availability than crop plants (Zaller and Arnone, 1999). As this experiment had a duration of 20 months, with just 2 growing seasons, a similar argument is extended in this case. Perhaps with an experiment of longer duration, effects of changes in the earthworm community would be observed.

6.2.3 To investigate the effects of the earthworm communities distribution of soil C through the soil profile and the chemical characteristics of this organic matter using CP MAS ^{13}C NMR spectroscopy

The impacts of unamended populations of *D. rubidus* and *A. chlorotica* on soil chemical characteristics and distribution are largely interpreted in terms of ecological groups. At the time of the initial lime application, ^{13}C NMR spectroscopy showed the chemical characteristics of limed and unlimed soil, with similar earthworm communities, to be similar. In contrast, at the end of the field mesocosm experiment, there were notable differences, but these were restricted to the H horizon. Because of the different earthworm communities, it is not possible to separate the effects of earthworms and the effects of liming, as these different factors are likely to be synergistic.

The major chemical difference was in the H horizon > 2 mm size fraction. In comparison to the baseline samples and the unlimed endpoint sample, the limed soil had two separate peaks off the main O-alkyl-C peak. It is suggested that while the total carbon content of this sample was larger, these peaks have become visible because there has been a reduction in the amount of carbohydrate-C present in the sample. This is likely to be a combination of the feeding activity of *D. rubidus* and subsequent enhanced microbial decomposition of comminuted litter in the limed soil.

The other difference between these two soils is a stronger signal in the > 63 μm size fraction of the unlimed soil compared to the limed soil. This may be due to the feeding behaviour of *A. chlorotica,* but this author can offer no clear explanation.

6.2.4 To investigate earthworm and earthworm induced respiration and to collect cast material from individual earthworm species for analysis by CP MAS ^{13}C NMR spectroscopy

Whilst the limitations of this study have been fully discussed in Chapter 5, this-+ should not preclude cautious interpretation of those results which were obtained. It is interesting to note that the apparent change in the behaviour of *A. chlorotica* suggested in the field study is not indicated by the results of the soil CO_2 evolution experiment. These results strongly suggest that in both limed and unlimed soil *A. chlorotica* is digesting soil microorganisms ingested with the soil as it feeds. This experiment was obviously carried out in highly artifical conditions, so care must be taken when comparing these to results from the field, but in this case it is the field experiment which has produced the unexpected result. It would be interesting to repeat part of the field experiment, and inoculate large numbers of *A. chlorotica* into the soil again to determine whether this result is repeated.

6.2.4.1 Implications of this study on future work on soil 'health'

There has been much interest in recent years in trying to define a measurement which could be used to indicate soil health (Webster *et al.,* 2001)., which has been defined as "the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health" (Doran and Safley, 1997). Various biological indicators have been proposed, but the microbial biomass measurements are often favoured as they can be applied across a wide range of soils and are relatively easy to determine.

Webster *et al.* (2001) tested the hypothesis that the alkyl-C: O-alkyl-C ratio of a soil was related to the soil microbial biomass, that is whether the resource quality of the soil could be used to predict the soil microbial biomass. A significant negative relationship was found between the microbial biomass C: total soil C and the alkyl-C: O-alkyl-C ratio (Figure 6.1).

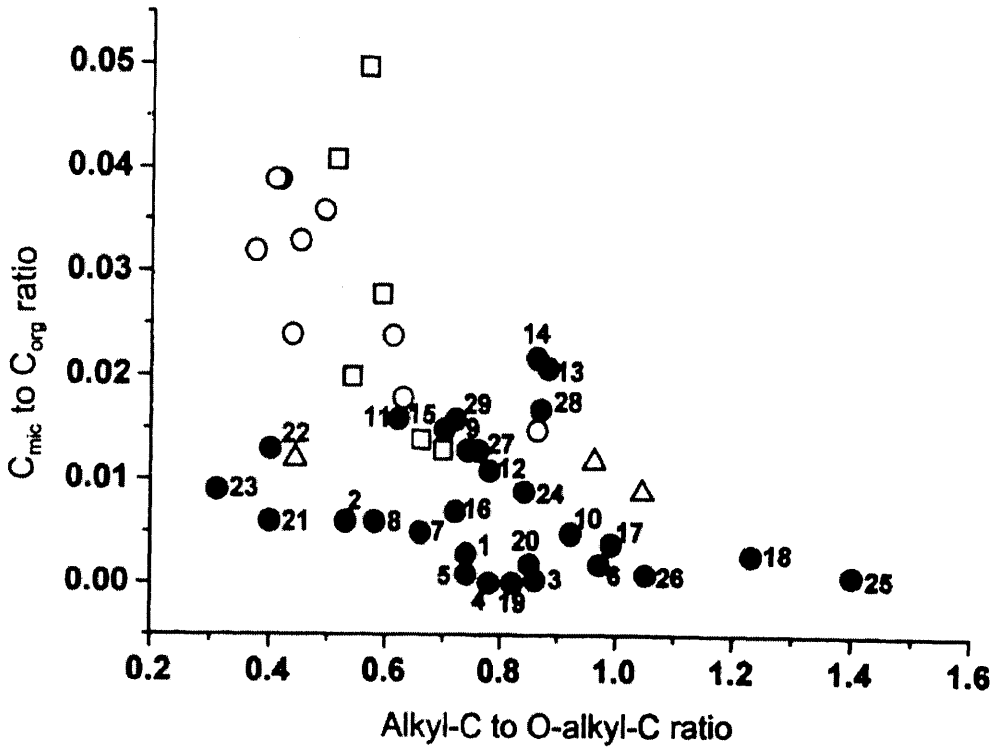


Figure 6.1 Relationship between the biomass C to total C ratio and alkyl-C to O-alkyl-C ratio for a range of soils. Taken from Webster *et al.*, 2001

□ **Urban soils;** ○ **mineral soils;** △ **organic soils**
Data taken from Beyer (1995) and Beyer *et al.*, 1995

● Data collected by Webster *et al.*, 2001

However, when the range of alkyl-C:O-alkyl-C ratios from cast materials of different earthworm species (values between 0.63 and 0.9) is examined, it becomes clear that this is the area of the graph which shows the most variability.

While the authors acknowledge the limitations of their study, due to many of the edaphic factors which can impact on the soil alkyl-C: O-alkyl-C ratio, the influence that the variation in composition of earthworm communities may have on this relationship should not be ignored.

6.2.5 Concluding Remarks

The work presented in this thesis has covered several aspects of earthworm ecology and their impacts on soil carbon, specifically impacts on the chemical characteristics of soil organic matter and its distribution through the soil profile.

The application of lime to acidic soil containing inoculated earthworm communities, did not result in an increase in abundance of all species present, and may have affected the competitive exclusion of some species. A key finding from this research has been the incorporation of plant litter as a result of *A. chlorotica* activity observed for the first time in UK soils. Similar behaviour has been reported in Australia and the Netherlands, for endogeic lumbricidae introduced into soils with no existing lumbricid earthworm community present,. This is an interesting contribution to the ongoing debate over the classification of earthworm species into fixed ecological groups.

Relatively novel techniques were used to show that for a range of species, the main impact of earthworms on soil C decomposition was that of the incorporation of organic material and its priming for microbial decomposition. The use of CP MAS ^{13}C NMR spectroscopy revealed that the relative concentration of carbohydrate was reduced in cast material, with a concomitant increase in the concentration of lipids and waxes, which includes byproducts of microbial growth and metabolism. Any small differences in the chemical composition of cast material between different species can be directly related to the resource quality of the material the earthworms ingested.

6.2.5.1 Further Work

In addition to the suggestions for further work in sections 6.2.1 to 6.2.4, the following areas of research are suggested for further investigation.

1. Interspecific competition between epigeic species

The decrease in the abundance in *D. octaedra* and *L. rubellus* in the presence of *D. rubidus* as observed in Chapter 2 was suggested to be due to intraspecific competition over food between these species which have similar ecological niches. This could be further investigated using an experimental protocol used by Abbott (1980) which was used to determine whether the biomass and cocoon production is affected by intra- or interspecific competition. The reproductive rates of the two earthworm species involved in competition may also be important, and Elvira *et al.* (1996) showed that *D. rubidus* reaches sexual maturity earlier than *L. rubellus* under laboratory conditions, at 54 days compared to 74 days. There was also a higher rate of cocoon production with a mean 2.31 cocoons week $^{-1}$ compared to 0.54 cocoons week $^{-1}$ for *L. rubellus*.

Intraspecific competition between earthworms is an area which has been receiving increasing research interest e.g. Lowe and Butt (1999), Capoweiz *et al.* (2001) and Garvin *et al.* (2002), and this proposed research would be a useful contribution.

2. The reduction in depth of LF horizon associated with high numbers of *A. chlorotica*

As discussed in section 6.2.1, the observation of the reduction in the depth of the LF horizon in the presence of high numbers of *A. chlorotica* is a novel result in soil with an established lumbricid epigeic population. As stated, this result contributes to the number of findings questioning the classification of earthworm species into fixed ecological classifications.

Further work should be carried out to determine under what circumstances *A. chlorotica* will exploit the resources of the LF horizon. Variables which need to be included are the quality and quantity of organic matter resources in the organomineral soil, the epigeic species present and the abundance of *A. chlorotica* present.

3. Chemical characteristics of ageing casts.

As discussed in section 1.3.5.2, the microbial composition and nutrient content of earthworm casts change as the casts age, but this is often ignored in experimental protocol, and casts of different ages have not been examined using CP MAS ^{13}C NMR spectroscopy.

The CP MAS ^{13}C NMR spectra of cast material collected from a range of earthworm species in Chapter 5 revealed that in soils of high resource quality there was little difference in the chemical characteristics between different species, but in low resource quality soil there were more pronounced differences. A worthwhile piece of future research would be to examine the ageing casts of a range of earthworm species, following the protocol of Scheu (1987) to obtain casts of specific age. This may reveal differences in the effect of gut passage between different species which are masked when using bulked samples of casts of different ages.

Appendix A References

References

Abbott, I. (1980) Do earthworms compete for food? *Soil Biol. Biochem.*, **12:** 523-530

Abbott, I. and Parker, C. A. (1981) Interactions between earthworms and their soil environment. *Soil Biol. Biochem.*, **13:** 191-197

Abraham, R. J., Fisher, J., and Loftus, P. (1988) *Introduction to NMR spectroscopy.* John Wiley & Sons Ltd.

Anderson, J. P. E. and Domsch, K. H. (1978) A physiological method for the quantitative measurement of microbial biomass in soil. *Soil Biol. Biochem.*, **10:** 215-221

Andrén, O., Paustian, K., and Rosswall, T. (1988) Soil Biotic interactons in the functioning of agroecosystems. *Agriculture, Ecosystems and Environment,* **24:** 57-67

Anon .(1998) *Baseline data 1998 Vegetation Survey,* Soil Biodiversity Programme

Arshad, M. A., Ripmeester, J. A., and Schnitzer, M. (1988) Attempts to improve solid state ^{13}C NMR spectra of whole mineral soils. *Canadian Journal of Soil Science,* **68:** 593-602

Baker, G. H., Williams, P. M. L., Carter, P. J., and Long, N. R. (1997) Influence of lumbricid earthworms on yield and quality of wheat and clover in glasshouse trials . *Soil Biol. Biochem.*, **29:** 599-602

Baldock, J. A., Oades, J. M., Nelson, P. N., Skene, T. M., Golchin, A., and Clarke, P. (1997) Assessing the extent of decomposition of natural organic materials using solid-state ^{13}C NMR spectroscopy. *Aust. J. Soil Res.*, **35:** 1061-1083

Barley, K. P. and Jennings, A. C. (1959) Earthworms and soil fertility III. The influence of earthworms on the availability of nitrogen. *Aust. J. Agric. Res.*, **10:** 364-370

Barratt, B. C. (1964) A classification of humus forms and micro-fabrics of temperate grasslands. *Jornal of Soil Science,* **15:** 342-356

Begon, M., Harper, J. L., and Townsend, C. R. (1996) *Ecology: individuals, populations and communities.* Blackwell Science Ltd. Oxford

Beyer, L. (1995) Soil microbial biomass and organic matter composition in soils under cultivation. *Biol. Fertil. Soils,* **19:** 197-202

Beyer, L., H.-P. Blume, D.-C. Elsner and Willnow A. (1995) Soil organic matter composition and microbial activity in urban soils. *Sci. Total Environ.* **168:** 267-278

Binet, F., Fayolle, L., and Pussard, M. (1998) Significance of earthworms in stimulating soil microbial activity. *Biol Fertil Soils,* **27:** 79-84

Blair, J. M., Parmelee, R. W., and Lavelle, P. (1995) Influences of Earthworms on Biogeochemistry. In: *Earthworm ecology and biogeograyphy in North America* (ed. Hendrix, P. F.) pp. 127-158. CRC Press, Inc.

Boag, B., Palmer, L. F., Neilson, R., Legg, R., and Chambers, S. J. (1997) Distribution, prevalence and intensity of earthworm populations in arable land and grassland in Scotland. *Ann. appl. Biol,* **130:** 153-165

Bohlen, P. J., Edwards, C. A., Zhang, Q., Parmelee, R. W., and Allen, M. (2002) Indirect effects of earthworms on microbial assimilation of labile carbon. *Applied Soil Ecology,* **20:** 255-261

Bonkowski, M. and Brandt, F. (2002) Do soil protozoa enhance plant growth by hormonal effects? *Soil Biol. Biochem.,* **34:** 1709-1715

Bonkowski, M., Griffiths, B. S., and Ritz, K. (2000) Food preferences of earthworms for soil fungi. *Pedobiologia,* **44:** 666-676

Bonkowski, M. and Schaefer, M. (1997) Interactions between earthworms and soil protozoa: a trophic component in the soil food web. *Soil Biol. Biochem.,* **29:** 499-502

Bouché, M. B. (1977) Stratégies lombriciennes. In: *Ecol. Bull* (eds. Lohm, U. and Perrson, T.) pp. 122-32. Stockholm

Briones, M. J. I., Mascato, R., and Mato, S. (1992) Relationships of earthworms with environmental factors studied by means of detrended canonical correspondance analysis. *Acta Œcologica,* **13:** 617-626

Briones, M. J. I., Mascato, R., and Mato, S. (1995) Autecological study of some earthworm species (oligochaeta) by means of ecological profiles. *Pedobiologia,* **39:** 97-106

Burt Smith, G. *Soil Biodiversity Report IV.* http://www.nmw.ac.uk/soilbio/Download/ReportIV.pdf

Cai, H. J., Zarda, B., Mattison, G. R., Schonholzer, F., and Hahn, D. (2002) Fate of Protozoa transiting the digestive tract of the earthworm *Lumbricus terrestris* L. *Pedobiologia,* **46:** 161-175

Callaham Jr. M. A. and Hendrix, P. F. (1997) Relative abundance and seasonal activity of earthworms (Lumbricidae and Megascolecidae) as determined by hand-sorting and formalin extraction in forest soils on the southern Appalachian Piedmont. *Soil Biol. Biochem.,* **29:** 317-321

Cannavacciuolo, M., Bellido, A., Cluzeau, D., Gascuel, C., and Trehen, P. (1998) A geostatistical approach to the study of earthworm distribution in grassland. *Applied Soil Ecology,* **9:** 345-349

Capoweiz, Y., Montestiez, P, and Belzunces, L. (2001) Burrow systems made by *Apporectodea nocturna* and *Allolobophora chlorotica* in artificial cores: morphological differences and effects of interspecific interactions. *Applied Soil Ecology,* **16:** 109-120

Cooke, A. (1983) The effects of fungi on food selection by *Lumbricus terrestris* L. In: *Earthworm Ecology: From Darwin to Vermiculture* (ed. Satchell, J. E.) Chapman and Hall. London

Cooke, A. and Luxton, M. (1980) Effect of microbes on food selection by *Lumbricus terrestris*. *Rev. Écol. Biol. Sol.*, **17:** 365-370

Curry, J. P.(2002) *Discussion* 7 th International Symposium of Earthworm Ecology. Cardiff University, Cardiff

Curry, J. P. (1994) *Grassland Invertebrates: Ecology, influence on soil fertility and effects on plant growth*. Chapman & Hall. London.

Curry, J. P. and Boyle, K. E. (1987) Growth rates, establishment, and effects on herbage yield of introduced earthworms in grassland on reclaimed cutover peat. *Biol Fertil Soils,* **3:** 95-98

Curry, J. P. and Byrne, D. (1992) The role of earthworms in straw decomposition and nitrogen turnover in arable land in Ireland. *Soil Biol. Biochem.*, **24:** 1409-1412

Dampney, P. M. R. (1985) A trial to determine the lime requirement for reseeded grassland on a peaty hill soil. *Soil Use and Management,* **1:** 95-100

Daniel, O. and Anderson, J. M. (1992) Microbial biomass and activity in contrasting soil materials after passage through the gut of the earthworm *Lumbricus rubellus* Hoffmeister. *Soil Biol. Biochem.*, **24:** 465-470

Darwin, C. R. (1881) *The formation of vegetable mould through the action of worms, with observations on their habits.* Murray. London.

Davidson, D. A. (2002) Bioturbation in old arable soils: quantitative evidence from soil micromorphology. *Journal of Archeological Science,* In Press.

Davidson, D. A., Bruneau, P. M. C., Grieve, I. C., and Young, I. M. (2002) Impacts of fauna on an upland grassland soil as determined by micromorphological analysis. *Applied Soil Ecology,* **20:** 133-143

Didden, W. A. M. (2001) Earthworm communities in grassland and horticultural soils. *Biol Fertil Soils,* **33:** 111-117

Doran, J.W. and Safley, M. (1997) Defining and assesing soil health and sustainable productivity. In: *Biological Indicators of Soil Health* (ed. Pankhurst, C.E., Doube, B.M. and Gupta, V.V.S.R) CAB International, Wallingford

Doube, B. M., Williams, P. M. L., and Willmott, P. J. (1997b) The influence of two species of earthworm (*Aporrectodea trapeziodes* and *Aporrectodea rosea*) on the growth of wheat, barley and faba beans in three soil types in the greenhouse. *Soil Biol. Biochem.*, **29:** 503-509

Edwards, C. A and Bohlen, P. J. (1996) *Biology and Ecology of Earthworms.* Chapman & Hall. London

Edwards, C. A. and Lofty, J. R. (1978) The influence of arthropods and earthworms upon root growth of direct drilled cereals. *Journal of Applied Ecology,* **15:** 789-795

Edwards, C. A. and Lofty, J. R. (1982) The effect of direct drilling and minimal cultivation on earthworm populations. *Journal of Applied Ecology,* **19:** 723-734

Ehlers, W., Kopke, U., Hesse, F., and Bohm, W. (1983) Penetration resistance and root growth of oats in tilled and untilled loess soil. *Soil & Tillage Research,* **3:** 261-275

Elvira, C. Domínguez, J. and Mato, S. (1996) The growth and reproduction of *Lumbricus rubellus* and *Dendrobaena rubida* in cow manure mixed cultures with *Eisenia andrei. Applied Soil Ecology,* ***5:*** 97-103

Fischer, K., Hahn, D., Honerlage, W., and Zeyer, J. (1997) Effect of passage through the gut of the earthworm *Lumbricus terrestris* L. on *Bacillus megaterium* studied by whole cell hybridization. *Soil Biol. Biochem.,* **29:** 1149-1152

Frűnd, R., Haider, K. and Lűdemann, H-D. (1994) Impact of soil amnagement practices on the organic matter structure- investigations cy CPMAS ^{13}C NMR spectroscopy. *Zeitschrift fur Pfanzenernaehrung und Bodenkunde*, **157:** 29-35

Garvin, M. H., trigo, D. Hernandez, P,. Ruiz, M. P. and Cosin, D. J. D. (2002) Interactions of *Hormogaster elisae* (Oligochaete, Hormogastridae) with other earthworm species from Reduena (Madrid, Spain). *Applied Soil Ecology,* **20:** 163-169

Gilot, C. (1990) Bilan de Carbone du Ver de Terre Geophage Tropical *Millsonia anomala* (Megascolecidae). *Mémoire de DEA 'Production végétale et écologie générale.* Institut National Agronomique. Paris-Grignon, France

Görres, J. H., Savin, M. C., and Amador, J. A. (1997) Dynamics of carbon and nitrogen mineralization, microbial biomass, and nematode abundance within and outside the burrow walls of anecic earthworms (*Lumbricus terrestris*). *Soil Science,* **162:** 1629-1666

Grant, J. D. (1983) The activities of earthworms and the fates of seeds. In: *Earthworm Ecology: From Darwin to Vermiculture* ed. Satchell, J. E.) Chapman and Hall. London

Grieve, I. C. (1990) Seasonal hydrological and land management factors controlling dissolved organic carbon concentrations in the Loch Fleet catchments, southwest Scotland. *Hydrological Processes,* **4:** 231-239

Grime, J. P., Hodgson, J. G., and Hunt, R. (1990) *The Abridged Comparative Plant Ecology.* Unwin Hyman Ltd. London.

Guggenberger, G., Thomas, R. J., and Zech, W. (1996) Soil organic matter within earthworm casts of an anecic-endogeic tropical pasture community, Columbia. *Applied Soil Ecology,* **3:** 263-274

Guild, W. J. McL. (1948) Studies on the relationship between earthworms and soil fertility. III. The effect of soil type on the structure of earthworm populations. *Ann. appl. Biol,* **35:** 181-192

Guild, W. J. McL. (1951) The distribution and population density of earthworms (Lumbricidae) in Scottish pasture fields . *Journal of Animal Ecology,* **20:** 88-97

Guild, W. J. McL. (1952) Variation in earthworm numbers within field populations. *Journal of Animal Ecology,* **21:** 169-181

Günther, A. and Greven, H. (1990) Increase in the number of epidermal gland cells- An unspecific response of *Lumbricus terrestris* L. (Lumbricidae: Oligocheata) to different environmental stressors . *Zool. Anz.,* **225:** 278-286

Haimi, J. and Einbork, M. (1992) Effects of endogeic earthworms on soil processes and plant growth in coniferous forest soil. *Biol Fertil Soils,* **13:** 6-10

Haslam, S.F.I. (1998) Carbon Dynamics in Moorland Soils. PhD Thesis, University of Dundee

Haynes, R. J., Fraser, P. M., Tregurtha, R. J., and Piercy, J. E. (1999) Size and activity of the microbial biomass and N, S and P availability in earthworm casts derived from arable and pastoral soil and arable soil amended with plant residues. *Pedobiologia,* **43:** 568-573

Hill, M. O. (96) *TABLEFIT version 1.0, for identification of vegetation types.* Huntingdon: Institute of Terrestrial Ecology

Homans. S.W. (1992) *A Dictionary of Concepts in NMR.* Crarendon Press. Oxford.

Hoogerkamp, M., Rogaar, H., and Eijsackers, H. J. P. (1983) Effect of earthworms on grassland on recently reclaimed polder soils in the Netherlands. In: *Earthworm Ecology: From Darwin to Vermiculture* ed. Satchell, J. E.) Chapman and Hall. London

Hopkins, D. W., Chudek, J. A., Haslam, S. F. I., and Webster, E. A. (2000) Application of solid-state NMR to investigate organic biogeochemistry in soils. In : *NMR in Microbiology: Theory and Applications* pp. 435-56.

Hopkins, D. W., Chudek, J. A., and Shiels, R. S. (1993) Chemical characterization and decomposition of organic matter from two contrasting grassland soil profiles. *Jornal of Soil Science,* **44:** 147-157

Hopkins, D. W., Chudek, J. A., Webster, E. A., and Barraclough, D. (1997) Following the decomposition of ryegrass labelled with ^{13}C and ^{15}N in soil by solid-state nuclear magnetic resonance spectroscopy. *European Journal of Soil Science,* **48:** 623-631

Huhta, V. (1979) Effects of liming and deciduous litter on earthworm (Lumbricidae) populations of a spruce forest, with an inoculation experiment on *Allolobophora caliginosa. Pedobiologia,* **19:** 340-345

Jolly, J. M., Lappin-Scott, H. M., Anderson, J. M., and Clegg, C. D. (1993) Scanning electron microscopy of the gut microflora of two earthworms: *Lumbricus terrestris* and *Octolasion cyaneum. Microb Ecol,* **26:** 235-245

Judas, M., Schauermann, J., and Meiwes, K. (1997) The inoculation of *Lumbricus terrestris* L. in an acidic spruce forest after liming and its influence on soil properties. *Soil Biol. Biochem.,* **29:** 677-679

Kalbitz, K., Solinger, S., Park, J. H., Michalzik, B., and Matzner, E. (2000) Controls on the dynamics of dissolved organic matter in soils: A review. *Soil Science,* **165:** 277-304

Killham, K. (1994) *Soil ecology.* Cambridge University Press. Cambridge

Kinchesh, P., Powlson, D. S., and Randall, E. W. (1995) ^{13}C NMR studies of organic matter in whole soils: I. Quantitation possibilities. *European Journal of Soil Science,* **46:** 125-138

Knight, D., Elliott, P. W., Anderson, J. M., and Scholefield, D. (1992) The role of earthworms in managed, permanent pastures in Devon, England. *Soil Biol. Biochem.,* **24:** 1511-1517

Kögel-Knabner, I. (1997) ^{13}C and ^{15}N NMR spectroscopy as a tool in soil organic matter studies. *Geoderma,* **80:** 243-270

Kristufek, V., Ravasz, K., and Pizl, V. (1992) Changes in densities of bacteria and microfungi during gut transit in *Lumbricus rubellus* and *Aporrectodea caliginosa* (Oligocheata: Lumbricidae). *Soil Biol. Biochem.,* **24:** 1499-1500

Lattaud, C., Mora, P., Garvín, P., Locati, S., and Rouland, C. (1999) Enzymatic digestive capabilities in geophagous earthworms- origin and activities of cellulolytic enzymes. *Pedobiologia,* **43:** 842-850

Lattaud, C., Zhang, B. G., Locati, S., Rouland, C., and Lavelle, P. (1997) Activities of the digestive enzymes in the gut and tissue culture of a tropical geophagous earthworm, *Polypheretima elongata* (Megascolecidae). *Soil Biol. Biochem.,* **29:** 335-339

Lavelle, P. (1978) Les vers de terre de la savanne de Lamto (Côte d'Ivorie). Peuplements, populations et fonctions de l'écosystème. *Publ. Lab. Zool. E.N.S.,* **12:** 1-301

Lavelle, P. (1983) The structure of earthworm communities. In: *Earthworm Ecology: From Darwin to Vermiculture* (ed. Satchell, J. E.) Chapman and Hall. London

Lavelle, P. (1988) Earthworm activities and the soil system. *Biol Fertil Soils,* **6:** 237-251

Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P., Heal, O. W., and Dhillion, S. (1997) Soil function in a changing world: the role of invertebrate ecosystem engineers. *Eur. J. Soil Biol.,* **33:** 159-193

Lavelle, P. and Gilot, C. (1994) Priming effects of soil macroorganisms on microflora: A key process of soil function? In: *Beyond the Biomass* (eds. Ritz, K., Dighton, J., and Giller, K. E.) pp. 173-180. John Wiley & Sons. UK

Lavelle, P., Sow, B., and Schaefer, R. (1980) The geophagous earthworm community in the Lamto savanna (Ivory Coast): niche partitioning and utilization of soil nutritive resources. in : *Soil biology as related to land use practices* (ed. Dindal, D.) EPA. Washington, D.C.

Lavelle, P. and Spain, A. V. (2001) *Soil Ecology.* Kluwer Academic Publishers. Netherlands.

Lee, K. E. (1959) A key for the identification of New Zealand earthworms. *Tuatara,* **8:** 13-60

Lee, K. E. (1985) *Earthworms, their ecology and relationships with soils and land use.* Academic Press. Australia

Lee, K. E. and Foster, R. C. (1991) Soil Fauna and Soil Structure. *Aust J. Soil Res.,* **29:** 745-775

Lepš, J. and Šmilauer, P. (2003) *Multivariate Analysis of Ecological Data using CANOCO.* Cambridge University Press. Cambridge.

Logsdon, S. D. and Linden, D. R. (1992) Interactions of earthworms with soil physical conditions influencing plant growth. *Soil Science,* **154:** 330-337

Lowe, C. N. and Butt, K. R. (1999) Interspecific interactions between earthworms: A laboratory- based investigation. *Pedobiologia,* **43:** 808-817

Lowe, C. N. and Butt, K. R. (2002) Growth of hatchling earthworms in the presence of adults: interactions in laboratory culture. *Biol Fertil Soils,* **35:** 204-219

Maraun, M., Alphei, J., Bonkowski, M., Buryn, R., Migge, S., Peter, M., Schaefer, M., and Scheu S. (1999) Middens of the earthworm *Lumbricus terrestris* (Lumbricidae): microhabitats for micro- and mesofauna in forest soil. *Pedobiologia,* **43:** 276-287

Marinissen, J. C. Y. and Dexter, A. R. (1990) Mechanisms of stabilization of earthworm casts and artificial casts. *Biol Fertil Soils,* **9:** 169-167

Martin, A. and Lavelle, P. (1992) Effect of soil organic matter quality on its assimilation by *Millsonia anomala,* a tropical geophagous earthworm. *Soil Biology and Biochemisrty,* **24:** 1535-1538

Mather, J. G. and Christenssen, O. (1992) Surface migration of earthworms in grassland. *Pedobiologia,* **36:** 51-57

Miles, H. B. (1963) Soil Protozoa and earthworm nutrition. *Soil Science,* **95:** 407-409

Neilson, R., Boag, B., and Smith, M. (2000) Earthworm $\delta^{13}C$ and $\delta^{15}N$ analyses suggest that putative functional classifications of earthworms are site-specific and may also indicate habitat diversity. *Soil Biol. Biochem.,* **32:** 1053-1061

Nielson, R. L. (1965) Presence of plant growth substances in earthworms demonstrated by paper chromatography and the Went pea test. *Nature,* **208:** 1113-1114

Nordgren, A. (1988) Apparatus for the continuous, long-term monitoring of soil respiration rate in large numbers of samples. *Soil Biology and Biochemisrty,* **20:** 955-957

Nuutinen, V., Pitkänen, J., Kuusela, E., Widbom, T., and Lohilahita, H. (1998) Spatial variation of an earthworm community related to soil properties and yield in a grass- clover field. *Applied Soil Ecology,* **8:** 85-94

Parkin, T. B. and Berry, A. M. (1994) Nitrogen transformations associated with earthworm casts. *Soil Biol. Biochem.*, **26:** 1233-1238

Parle, J. N. (1963a) Micro-organisms in the intestines of earthworms. *J. gen. Microbiol.*, **31:** 1-11

Parle, J. N. (1963b) A microbiological study of earthworm casts. *J. gen. Microbiol.*, **31:** 13-22

Paul, E. A. and Clark, F. E. (1989) *Soil Microbiology and Biochemistry.* Academic Press. San Diego.

Paul, E. A. and Clark, F. E. (1996) *Soil Microbiology and Biochemistry.* Academic Press. USA

Pawlett, M. (2003) The interaction between earthworms, liming and soil microbial community diversity and function in upland grassland. PhD Thesis, University of East London

Persson, T. (1988) *Effects of Liming on the Soil Fauna in Forests: A Literature Review.* National Swedish Environment Protection Board Report.

Piearce, T. G. (1972) The calcium relations of selected Lumbricidae. *Journal of Animal Ecology,* **41:** 167-88

Piearce, T. G. (1978) Gut contents of some lumbricid earthworms. *Pedobiologia,* **18:** 153-157

Piearce, T. G. and Phillips, M. J. (1980) The fate of ciliates in the earthworm gut: An *in vitro* study. *Microbial Ecology,* **5:** 313-319

Poier, K. R. and Richter, J. (1992) Spatial distribution of earthworms and soil properties in an arable loess soil. *Soil Biol. Biochem.*, **24:** 1601-1608

Ponge, J. F. (2003) Humus forms in terrestrial ecosystems: a framework to biodiversity. *Soil Biology and Biochemisrty,* **35:** 935-945

Robinson, C. H., Ineson, P., Piearce, T. G., and Parrington, J. (1996) Effects of earthworms on cation and phosphate mobilisation in limed peat soils under *Picea sitchensis. Forest Ecology and Management,* **86:** 253-258

Robinson, C. H., Ineson, P., Piearce, T. G, and Rowland, A. P. (1992a) Nitrogen mobilization by earthworms in limed peat soils under *Picea sitchensis. Journal of Applied Ecology,* **29:** 226-237

Robinson, C. H., Piearce, T. G., Ineson, P., Dickson, D. A., and Nys, C. (1992b) Earthworm communities of limed coniferous soils: field observations and implications for forest management. *Forest Ecology and Management,* **55:** 117-134

Rodwell, J. S. (1992) *British Plant Communities. Volume 3 Grasslands and montane communities.* Cambridge University Press. Great Britain

Rossi, J., Lavelle, P., and Albrecht, A. (1997) Relationships between spatial pattern of the endogeic earthworm *Polypheretima elongata* and soil heterogeneity. *Soil Biol. Biochem.*, **29:** 485-488

Sánchez, E. G., Muñoz, B., Garvín, M. H., Jesús, J. B., and Dìaz Cosìn, D. J. (1997) Ecological preferences of some earthworm species in southwest Spain. *Soil Biol. Biochem.*, **29:** 313-316

Satchell. J.E. (1955) *Some aspects of earthworm ecology.* In: Soil Zoology (ed. Kevan. D.K.McE.) Proceedings of the University of Nottingham Second Easter School in Agricultural Science. Butterworths Scientific Publications, London

Satchell, J. E. (1963) Nitrogen turnover by a woodland population of *Lumbricus terrestris.* in: *Soil Organisms* (eds. Doesken, J. and van der Drift, J) pp. 60-66. North Holland, Amsterdam

Scheu S. (1987) Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae) *Biol Fertil Soils, 5:* 230-234

Scheu S. (1991) Mucus excretion and carbon turnover of endogeic earthworms. *Biol Fertil Soils,* **12:** 217-220

Scheu, S. (1992) Automated measurement of the respiratory response of soil microcompartments: active microbial biomass in earthworm faeces. *Soil Biol. Biochem.*, **24:** 1113-1118

Scheu S. and Parkinson, D. (1994) Effects of earthworms on nutrient dynamics, carbon turnover and microorganisms in soils from cool temerate forests of the Canadian Rocky Mountains- laboratory studies. *Applied Soil Ecology,* **1:** 113-125

Schrader, S. (1994) Influence of earthworms on the pH conditions of their environment by cutaneous mucus secretions. *Zool. Anz.*, **233:** 211-219

Schuster.W.D., Subler, S., and McCoy, E. L. (2001) Deep- burrowing earthworm aditions changed the distribution of soil organic carbon in a chisel-till soil. *Soil Biol. Biochem.*, **33:** 983-996

Shaw, C. and Pawluk, S. (1986) The development of soil structure by *Octolasion tyrtaeum*, *Apporectodea turgida* and *Lumbricus terrestris* in parent materials belonging to different textural classes. *Pedobiologia,* **29:** 327-339

Sims, R.W. and Gerard, B.M. (1985) *Earthworms. Keys and notes for the identification and study of the species.* Synopses of the British Fauna (New Series) No. 31. London: E.J.Brill/ Dr. W. Backhuys (for the Linnean Society of London and the Esturine and Brackish- Water Sciences Association).

Skjemstad, J. O., Clarke, P., Golchin, A., and Oades.J.M. (1997) Characterization of soil organic matter by solid-state ^{13}C NMR spectroscopy. In: *Driven by Nature: Plant Litter Quality and Decomposition* (eds. Cadisch, G. and Giller, K. E.) CAB International. University Press, Cambridge

Skjemstad, J. O., Clarke, P., Taylor, J. A., Oades, J. M., and Newman, R. H. (1994) The removal of magnetic materials from surface soils. A solid state ^{13}C CP/MAS n.m.r study. *Aust. J. Soil Res.*, **32:** 1215-1229

Soil Biodiversity Website. *http.//mwnta.nmw.ac.uk/soilbio?Sourhope_Design.htm.*

Springett, J. A. (1984) Earthworm introduction to improve pasture soils. *NZ Agricultural Science,* **18:** 61-64

Springett, J. A. (1985) Effects of introducing *Allolobophora longa* Ude on root distribution and some soil properties in New Zealand pastures. In: *Ecological interactions in soil: plants, microbes and animals* (eds. Fitter, A. H., Atkinson, D., Read, D. J., and Usher, M. B.) Blackwell Scientific Publications. Oxford

Springett, J. A. and Syers, J. K. (1979) Effect of earthworm casts on ryegrass seedlings. *Proc. 2nd Australasian Conf. Grassld. Invertebr. Ecol.,* 47-49

Stockdill, S. M. J. (1982) Effects of introduced earthworms on the productivity of New Zealand pastures. *Pedobiologia,* **24:** 29-35

Stockdill, S. M. J. and Cossens, G. G. (1966) The role of earthworms in pasture production and moisture conservation . *Proc. N.Z. Grassld. Assoc.,* **13:** 68-83

Subler, S. and Kirsch, A. S. (1998) Spring dynamics of soil carbon, nitrogen, and microbial activity in earthworm middens in a no-till cornfield. *Biol Fertil Soils,* **26:** 243-249

Swift, M. J., Heal, O. W., and Anderson, J. M. (1979) *Decomposition in terrestrial ecosystems.* Blackwell Scientific. Oxford.

Syers, J. K. and Springett, J. A. (1983) Earthworm ecology in grassland soils. In: *Earthworm Ecology: From Darwin to Vermiculture* (ed. Satchell, J. E.) pp. 67-83. Chapman and Hall. London

ter Braak, C. J. F. and Smilauer, P. (1998) *CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4).* Microcomputer Power. Ithica, NY, USA.

Tiessen, H., Stewart, J. W. B., and Hunt, H. W. (1984) Concepts of soil organic matter transformations in relation to organo-mineral particle size fractions. *Plant and Soil,* **76:** 287-295

Tisdall, J. M., Smith, S. E., and Rengasamy, P. (1997) Aggregation of soil by fungal hyphae. *Aust J. Soil Res.,* **35:** 55-60

Tiunov, A. V. and Scheu S. (1999) Microbial respiration, biomass, biovolume and nutrient status in burrow walls of *Lumbricus terrestris* L. (Lumbricidae). *Soil Biol. Biochem.,* **31:** 2039-2048

Tomati, U., Grappelli, A., and Galli, E. (1988) The hormone-like effect of earthworm casts on plant growth. *Biol Fertil Soils,* **5:** 288-294

Toyota, K. and Kimura, M. (2000) Microbial community indigenous to the earthworm *Eisenia Foetida. Biol Fertil Soils,* **31:** 187-190

Trigo, D., Barois, I., Garvín, M. H., Huerta, E., Irisson, S., and Lavelle, P. (1999) Mutualism between earthworms and soil microflora. *Pedobiologia,* **43:** 866-873

Urbášek, F. (1990) Cellulase activity in the gut of some earthworms. *Rev. Écol. Biol. Sol.,* **27:** 21-28

Vinceslas-Akpa, M. and Loquet, M. (1995) Observation *in situ* de la microflore liée au tube digestif de *Eisenia fetida andrei* (Lumbricidae). *Eur. J. Soil Biol.,* **31:** 101-110

Wang, J., Hesketh, J. S., and Wooley, J. T. (1986) Pre-existing channels and soybean rooting patterns. *Soil Science,* **141:** 432-437

Webster, E. A., Hopkins, D. W., Chudek, J. A., Haslam, S. F. I., Šimek, M., and Pîcek, T. (2001) The relationship between microbial carbon and the resource quality of soil carbon. *Journal of Environmental Quality,* **30:** 147-150

Whalen, J. K., Parmelee, R. W., and Subler, S. (2000) Quantification of nitrogen excretion rates for three lumbricid eartthworms using ^{15}N. *Biol Fertil Soils,* **32:** 347-352

Whelan, M. J., Bruneau, P. M. C., Grieve, I. C., and Davidson, D. A. (Submitted) Relationships between soil moisture content and soil development at an upland grassland site. *Geoderma,*

Wilson, M. A. (1987) *N.M.R Techniques and Applications in Geochemistry and Soil Chemistry.* Pergamon Press. Oxford.

Winding, A., Ronn, R., and Hendrikson, N. B. (1997) Bacteria and protozoa in soil microhabitats as affected by earthworms. *Biol Fertil Soils,* **24:** 133-140

Wolter, C. and Scheu, S. (1999) Changes in bacterial numbers and hyphal lengths during gut passage through *Lumbricus terrestris* (Lumbricidae, Oligochaeta). *Pedobiologia,* **43:** 891-900

Wolters, V. and Joergensen, R. G. (1992) Microbial carbon turnover in beech forest soils worked by *Aporrectodea caliginosa* (Savigny) (Oligocheata: Lumbricicidae). *Soil Biol. Biochem.,* **24:** 171-177

Zaller, J. G. and Arnone III, J. A. (1999) Earthworm and soil moisture effects on the productivity and structure of grassland communities. *Soil Biol. Biochem.,* **31:** 517-523

Zhang, B. G., Rouland, C., Lattaud, C., and Lavelle, P. (1992) Origin and activity of enzymes found in the gut contents of the tropical earthworm *Pontoscolex corethrurus* Müller. *Eur. J. Soil Biol.,* **29:** 7-11

Zhang, B. G., Rouland, C., Lattaud, C., and Lavelle, P. (1993) Activity and origin of digestive enzymes in the gut of the tropical earthworm *Pontoscolex corethrurus. Eur. J. Soil Biol.,* **29:** 7-11

Zhang, B., Li, G., Shen, T., Wang, J., and Sun, Z. (2000) Changes in microbial biomass C, N and P and enzyme activities in soil incubated with the earthworms *Metaphire guillelmi* or *Eisenia fetida. Soil Biol. Biochem.*, **32:** 2055-2062

Zhang, H. and Schrader, S. (1993) Earthworm effects on selected physical and chemical properties of soil aggregates. *Biol Fertil Soils,* **15:** 229-234

Ziegler, F. and Zech, W. (1992) Chemical changes of beech litter during passage through the gut of the lumbricid earthworm *Eisenia fetida* (SAV.). *Z. Pflanzenernahr. Bodenk.,* **155:** 69-70

Appendix B Earthworm Suppliers

Walker Organics
Mr Dave Walker,
West Craig Lee Farm,
Roddymoor,
Crook,
Co. Durham.
DL15 9QG

Tel: (01388) 731129

Ecology Earthworms
Mr Dave Hallows,
Hubbards Hall farm,
Bentley,
Ipswich,
Suffolk.
IP9 2LS

Tel: (01473) 327062

Appendix C Plot Comparison PCA data

Plot	Shannon Index	Depth H horizon (cm)	% Org C	% Moisture Loss (July)	% Moisture Loss (April)	No. plant species	Altitude (m) from lowest part of site
5B	1.1	4.0	6.5	0.30	0.62	4	27.0
5A	1.4	3.0	6.0	0.35	0.65	6	27.0
4D	1.0	3.5	6.5	0.34	0.60	4	17.5
4F	0.8	2.0	5.5	0.39	0.67	3	16.5
3B	0.9	3.0	6.0	0.44	0.66	4	12.5
3D	0.4	3.0	5.5	0.29	0.60	3	11.5
2B	1.0	3.0	5.0	0.26	0.64	5	7.5
2C	1.1	2.5	5.5	0.33	0.61	4	7.0
1B	1.0	4.0	6.0	0.34	0.62	5	2.0
1F	1.0	2.5	5.0	0.34	0.64	5	1.0

Appendix D PCA of Environmental variables for Chapters 2 and 3

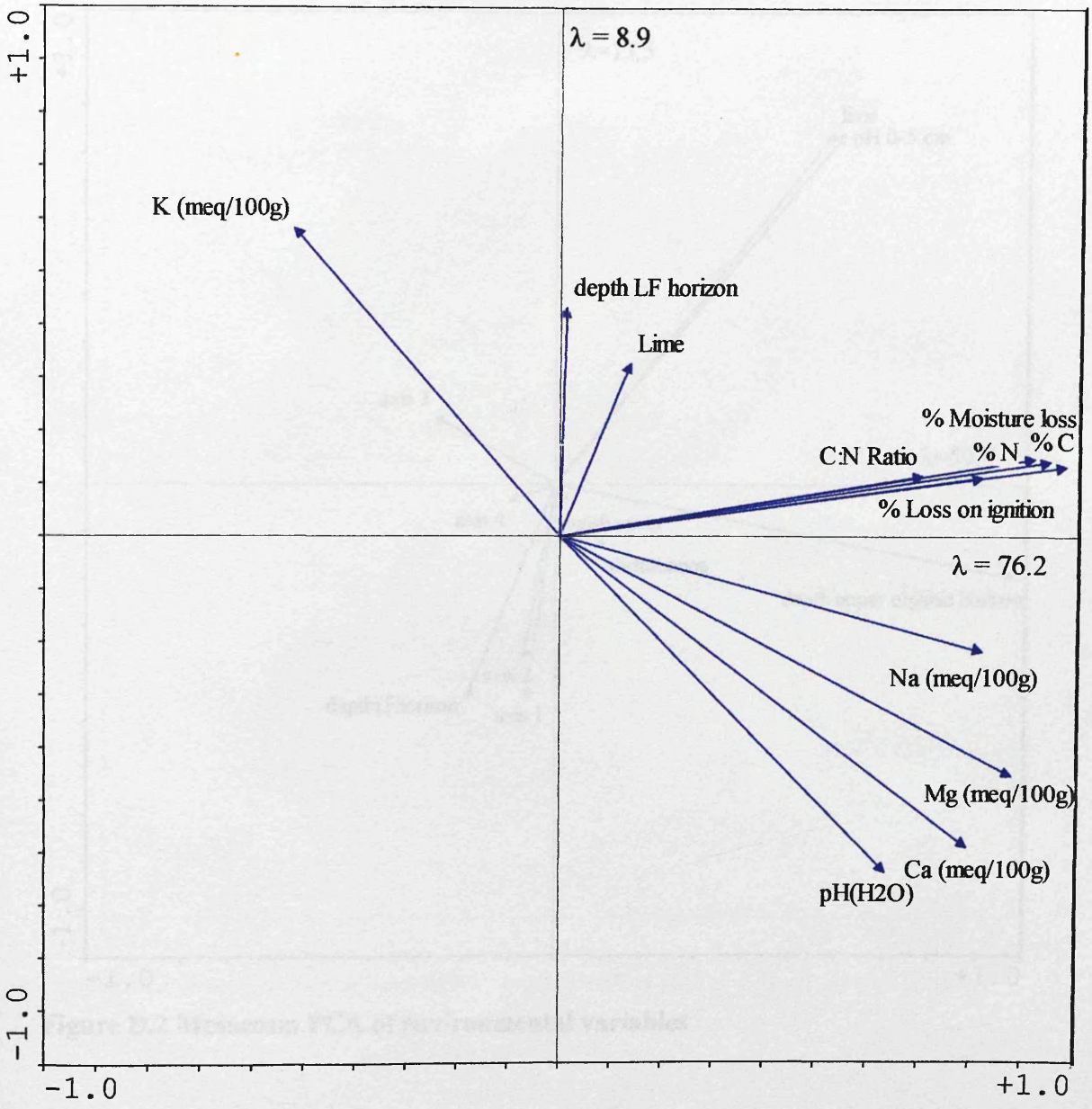


Figure D.1 Sourhope environmental variable PCA

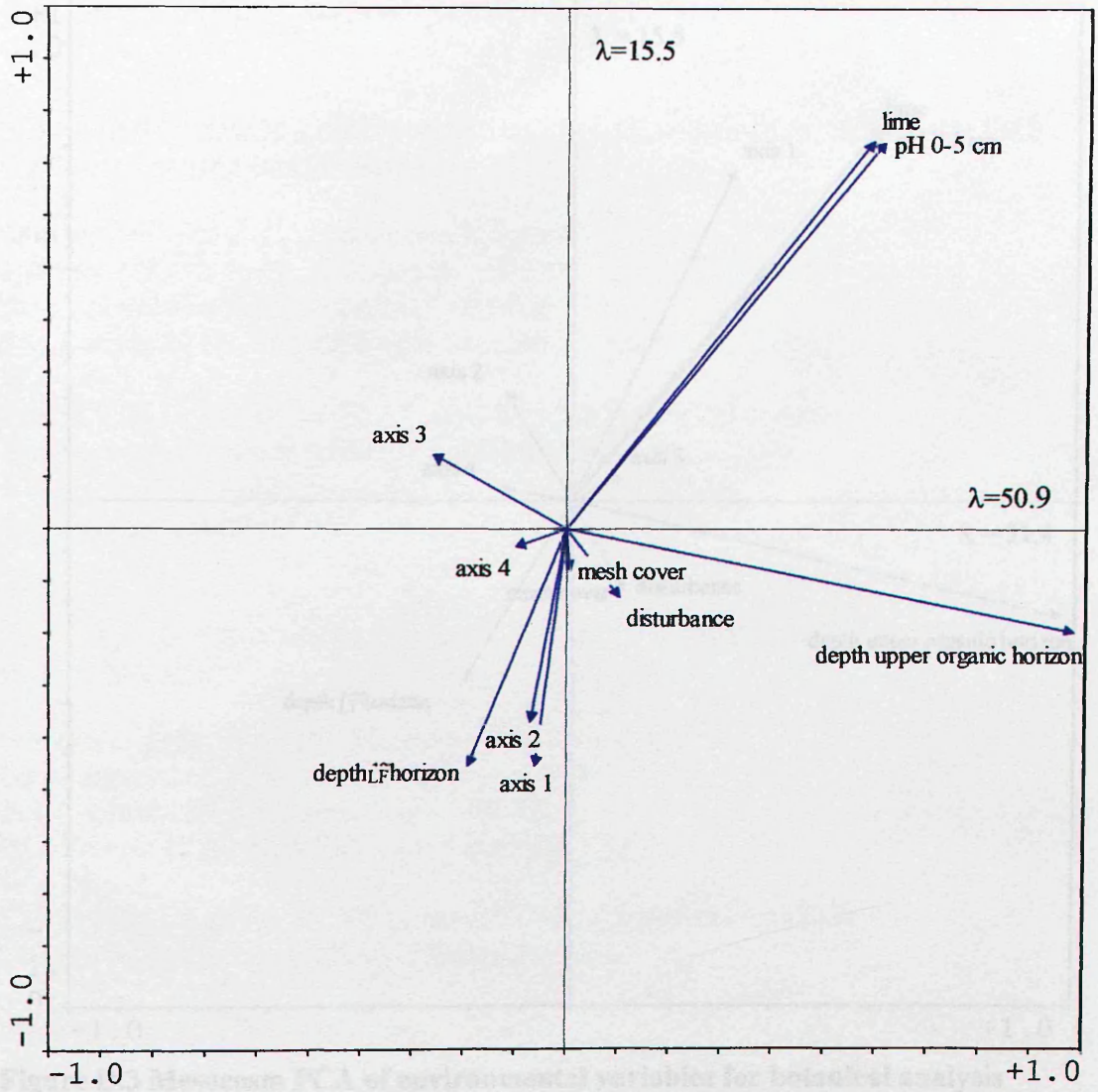


Figure D.2 Mesocosm PCA of environmental variables

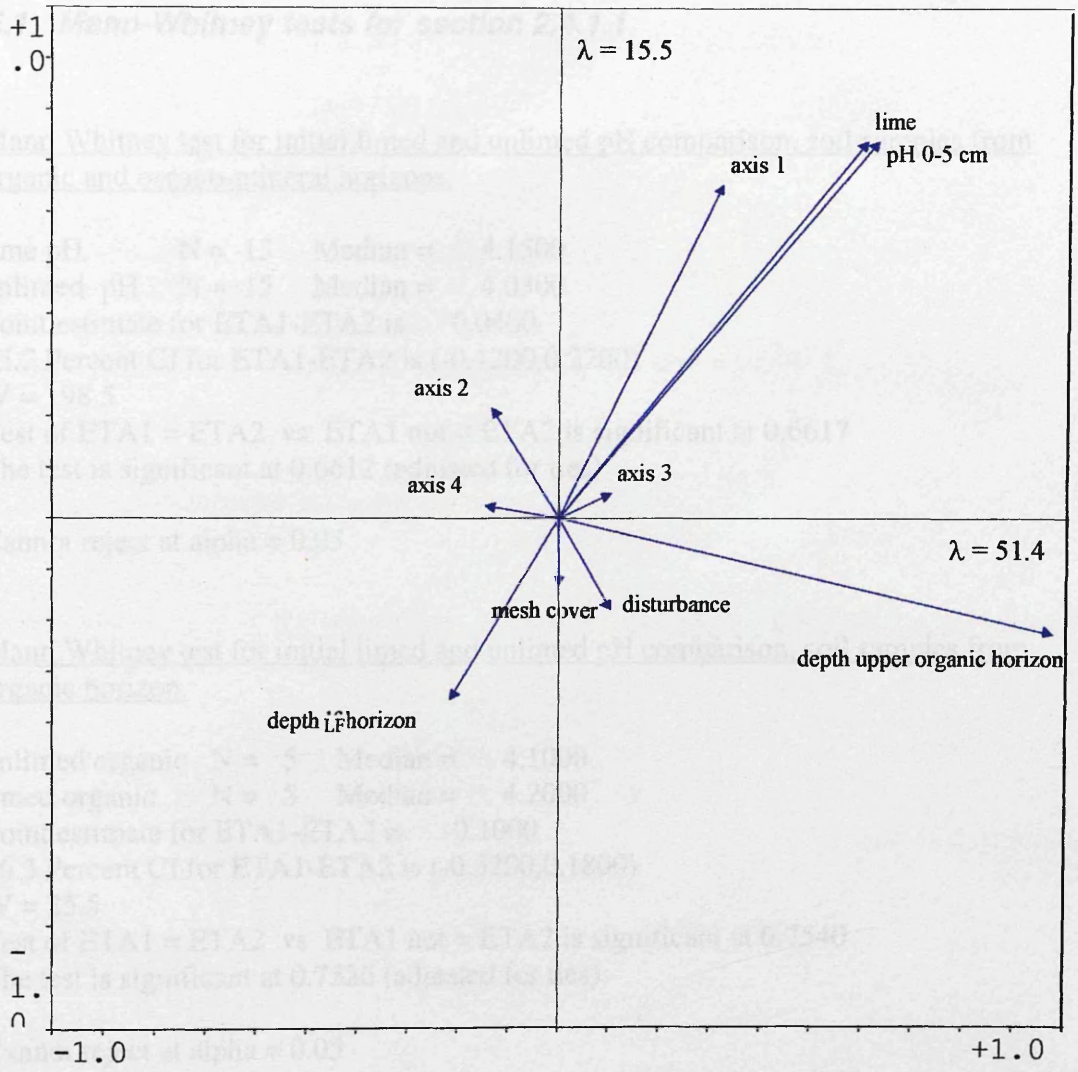


Figure D.3 Mesocosm PCA of environmental variables for botanical analysis

Appendix E Statistical analyses from Chapter 2

E.1 Mann-Whitney tests for section 2.4.1.1

Mann Whitney test for initial limed and unlimed pH comparison, soil samples from organic and organo-mineral horizons.

lime pH N = 13 Median = 4.1500
unlimed pH N = 15 Median = 4.0300
Point estimate for ETA1-ETA2 is 0.0400
95.2 Percent CI for ETA1-ETA2 is (-0.1200,0.2200)
W = 198.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6617
The test is significant at 0.6612 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann Whitney test for initial limed and unlimed pH comparison, soil samples from organic horizon.

unlimed organic N = 5 Median = 4.1000
limed organic N = 5 Median = 4.2000
Point estimate for ETA1-ETA2 is -0.1000
96.3 Percent CI for ETA1-ETA2 is (-0.5200,0.1800)
W = 25.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.7540
The test is significant at 0.7526 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann Whitney test for final limed and unlimed pH comparison, soil samples collected by G. Burt-Smith

Soil pH N = 20 Median = 6.8300
Soil pH N = 20 Median = 4.5200
Point estimate for ETA1-ETA2 is 2.2600
95.0 Percent CI for ETA1-ETA2 is (2.0300,2.4700)
W = 609.0
Test of ETA1 = ETA2 vs ETA1 > ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)

E.2 Two-sample T-tests for section 2.4.1.1

Two-sample T-teast of initial unlimed pH compared to final unlimed pH.

	N	Mean	StDev	SE Mean
initial	15	4.127	0.189	0.049
final	20	4.538	0.269	0.060

Difference = mu initial unlimed surface - mu final_1
Estimate for difference: -0.4113
95% upper bound for difference: -0.2802
T-Test of difference = 0 (vs <): T-Value = -5.31 P-Value = 0.000 DF = 32

E.3 Mann-Whitney tests for section 2.4.2.3

Mann-Whitney test of the comparison of earthworm abundance in uninoculated limed soil compared to uninoculated unlimed soil

Unlimed soil N = 10 Median = 22.12
Limed soil N = 18 Median = 17.49
Point estimate for ETA1-ETA2 is -8.08
95.3 Percent CI for ETA1-ETA2 is (-59.13,11.20)
W = 135.0
Test of ETA1 = ETA2 vs ETA1 < ETA2 is significant at 0.3244
The test is significant at 0.3243 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann-Whitney test of the comparison of earthworm biomass in uninoculated limed soil compared to uninoculated unlimed soil

Unlimed soil N = 10 Median = 3.17
Limed soil N = 18 Median = 3.40
Point estimate for ETA1-ETA2 is -0.10
95.3 Percent CI for ETA1-ETA2 is (-4.99,2.64)
W = 143.5
Test of ETA1 = ETA2 vs ETA1 < ETA2 is significant at 0.4809
The test is significant at 0.4809 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann-Whitney test of the comparison of earthworm abundance in uninoculated uncovered limed soil compared to uninoculated uncovered unlimed soil

Unlimed soil N = 17 Median = 16.08
Limed soil N = 16 Median = 28.23
Point estimate for ETA1-ETA2 is -8.14
95.0 Percent CI for ETA1-ETA2 is (-24.87,7.67)
W = 261.0
Test of ETA1 = ETA2 vs ETA1 < ETA2 is significant at 0.1609
The test is significant at 0.1608 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann-Whitney test of the comparison of earthworm biomass in uninoculated uncovered limed soil compared to uninoculated uncovered unlimed soil

Unlimed soil N = 17 Median = 3.28
Limed soil N = 13 Median = 10.30
Point estimate for ETA1-ETA2 is -2.35
95.1 Percent CI for ETA1-ETA2 is (-12.20,4.28)
W = 241.0
Test of ETA1 = ETA2 vs ETA1 < ETA2 is significant at 0.1786
The test is significant at 0.1786 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann-Whitney test of the comparison of earthworm abundance in uninoculated covered unlimed soil compared to uninoculated uncovered unlimed soil

Covered, uninoculated N = 10 Median = 22.12
Unocvered, uninoculated N = 17 Median = 16.08
Point estimate for ETA1-ETA2 is 2.04
95.3 Percent CI for ETA1-ETA2 is (-7.25,27.27)
W = 170.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1386
The test is significant at 0.1381 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann-Whitney test of the comparison of earthworm biomass in uninoculated covered unlimed soil compared to uninoculated uncovered unlimed soil

Covered, uninoculated N = 10 Median = 3.17
Uncovered, uninoculated N = 17 Median = 3.28
Point estimate for ETA1-ETA2 is 0.13
95.3 Percent CI for ETA1-ETA2 is (-14.71,2.46)
W = 141.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.9800
The test is significant at 0.9800 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann-Whitney test of the comparison of earthworm abundance in uninoculated covered limed soil compared to uninoculated uncovered limed soil

1,1 A L N = 18 Median = 17.49
3,1 A L N = 16 Median = 28.23
Point estimate for ETA1-ETA2 is 0.76
95.3 Percent CI for ETA1-ETA2 is (-16.20,19.12)
W = 328.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.6663
The test is significant at 0.6662 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann-Whitney test of the comparison of earthworm biomass in uninoculated covered limed soil compared to uninoculated uncovered limed soil

1,1 B L N = 18 Median = 3.40
3,1 B L N = 13 Median = 10.30
Point estimate for ETA1-ETA2 is -4.13
95.2 Percent CI for ETA1-ETA2 is (-12.10,1.91)
W = 254.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.1799

Cannot reject at alpha = 0.05

E.4 Wilcoxon Signed Rank Tests: Were inoculated species more abundant in the boxes they were inoclueted into compared to uninoculated boxes?

A. chlorotica Limed soil

Test of median = 143.0 versus median > 143.0

N for	Wilcoxon		Estimated
N	Test Statistic	P	Median
A. chlorotica	101.0	0.001	500.4

A. chlorotica Unlimed soil

Test of median = 143.0 versus median > 143.0

N for	Wilcoxon		Estimated
N	Test Statistic	P	Median
A. chlorotica	7.0	0.898	24.33

L. rubellus Limed soil

Test of median = 107.0 versus median > 107.0

N for		Wilcoxon		Estimated
N		Test Statistic	P	Median
L. rubellus	0.0		0.989	20.72

L. rubellus Unlimed soil

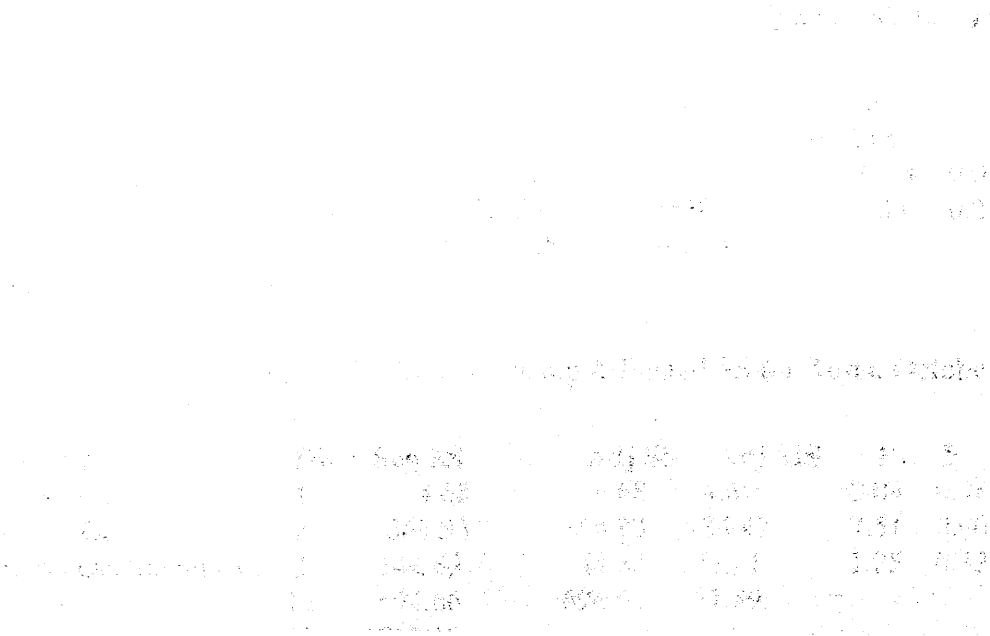
Test of median = 107.0 versus median > 107.0

N for		Wilcoxon		Estimated
N		Test Statistic	P	Median
L. rubellus	1.0		0.982	45.03

L. terrestris (Limed and unlimed soil)

Test of median = 28.00 versus median > 28.00

N for		Wilcoxon		Estimated
N		Test Statistic	P	Median
L. terrestris	0.0		0.978	9.405



Appendix F Statistical analyses from Chapter 3

F.1 Analysis of Variance Tables of the effects of mesh on soil variables at depth

Analysis of Variance for temperature, using Adjusted SS for Tests, August 2000

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Mesh	1	2.5669	2.5669	2.5669	3.55	0.069
depth	7	42.475	42.4748	6.0678	8.39	0.000
mesh*depth	7	1.3848	1.3848	0.1978	0.27	0.960
Error	32	23.1467	23.1467	0.7233		
Total	47	69.5731				

Analysis of Variance for temperature, using Adjusted SS for Tests, October 2000

Source	DF	Seq SS	Adj SS	Adj MS	F	P
mesh	1	0.0003	0.0003	0.0003	0.00	0.948
Depth	4	13.9620	13.9620	3.4905	46.54	0.000
mesh*Depth	4	0.3113	0.3113	0.0778	1.04	0.412
Error	20	1.5000	1.5000	0.0750		
Total	29	15.7737				

Analysis of Variance for % Moisture Content, using Adjusted SS for Tests, August 2000

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Mesh	1	12.255	12.255	12.255	2.66	0.129
Depth	2	93.515	93.515	46.758	10.14	0.003
mesh*Depth	2	12.761	12.761	6.381	1.38	0.288
Error	12	55.325	55.325	4.610		
Total	17	173.857				

Analysis of Variance for % Moisture, using Adjusted SS for Tests, October 2000

Source	DF	Seq SS	Adj SS	Adj MS	F	P
mesh Oct	1	4.68	4.68	4.68	0.08	0.781
depth Oc	2	868.93	868.93	434.47	7.51	0.008
mesh Oct*depth Oct	2	144.83	144.83	72.41	1.25	0.321
Error	12	694.66	694.66	57.89		
Total	17	1713.10				

F.2 Mann-Whitney Tests on the impact of reduced PAR (due to mesh covering) on plant yields

Mann-Whitney Test on the effect of mesh covering on plant yield in unlimed soil

Covered N = 10 Median = 644.7
Uncovered N = 5 Median = 631.3
Point estimate for ETA1-ETA2 is 13.1
95.7 Percent CI for ETA1-ETA2 is (-220.3,210.9)
W = 80.0
Test of ETA1 = ETA2 vs ETA1 < ETA2 is significant at 0.5000

Cannot reject at alpha = 0.05

Mann-Whitney Test on the effect of mesh covering on plant yield in limed soil

Covered N = 10 Median = 1045.7
Uncovered N = 5 Median = 706.6
Point estimate for ETA1-ETA2 is 340.7
95.7 Percent CI for ETA1-ETA2 is (89.2,549.5)
W = 100.0
Test of ETA1 = ETA2 vs ETA1 < ETA2
Cannot reject since W is > 80.0

Mann-Whitney Test on the effect of mesh covering on plant yield in both limed and unlimed soil

Covered N = 20 Median = 791.3
Uncovered N = 10 Median = 653.7
Point estimate for ETA1-ETA2 is 176.0
95.5 Percent CI for ETA1-ETA2 is (-19.9,370.3)
W = 349.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0903

Cannot reject at alpha = 0.05

F.3 Repeated measures ANOVA with Tukey's Post Hoc test, comparing pairs of group means.

Repeated measures ANOVA examining the effects of lime and disturbance on cumulative plant harvest data

ANOVA Table	SS	df	MS
Treatment between columns)	1539000	3	513100
Individual between rows)	2335000	8	291800
Residual random)	96560	24	4023
Total	3970000	35	

Tukey's Multiple Comparison Test	Mean Diff.	P value
L UnD vs UnL UnD	292.1	$P < 0.001$
L UnD vs L D	357.6	$P < 0.001$
L UnD vs UnL D	579.0	$P < 0.001$
UnL UnD vs L D	65.52	$P > 0.05$
UnL UnD vs UnL D	286.9	$P < 0.001$
L D vs UnL D	221.4	$P < 0.001$

F.4 Mann-Whitney Tests of the effect of liming on plant yields

Mann-Whitney test of the effect of liming on plant yields in undistrubed soil treatments

Unlimed N = 25 Median = 2.5000
Limed N = 25 Median = 1.6000
Point estimate for ETA1-ETA2 is 0.8000
95.2 Percent CI for ETA1-ETA2 is (0.4000,1.1003)
W = 810.5
Test of ETA1 = ETA2 vs ETA1 > ETA2 is significant at 0.0004
The test is significant at 0.0004 (adjusted for ties)

Mann-Whitney test of the effect of liming on plant yields in distrubed soil treatments

Unlimed N = 15 Median = 2.000
Limed N = 15 Median = 1.500
Point estimate for ETA1-ETA2 is 0.500
95.4 Percent CI for ETA1-ETA2 is (0.000,1.000)
W = 265.5
Test of ETA1 = ETA2 vs ETA1 > ETA2 is significant at 0.0888
The test is significant at 0.0853 (adjusted for ties)

Cannot reject at alpha = 0.05

Appendix G Tabulated mesocosm earthworm data

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
5A	1	0	0	*A. chlorotica*	0	0
5A	1	0	0	*D. octaedra*	0	0
5A	1	0	0	*D. rubidus*	0	0
5A	1	0	0	*E. fetida*	0	0
5A	1	0	0	*L. castaneus*	0	0
5A	1	0	0	*L. festivus*	0	0
5A	1	0	0	*L. rubellus*	0	0
5A	1	0	0	*L. terrestris*	9.19	6.62
5A	1	0	0	*Lumbricus* sp.	36.76	6.34
5A	1	0	0	*O. cyaneum*	0	0
5A	2	0	0	*A. chlorotica*	289.28	40.32
5A	2	0	0	*D. octaedra*	0	0
5A	2	0	0	*D. rubidus*	144.64	11.48
5A	2	0	0	*E. fetida*	0	0
5A	2	0	0	*L. castaneus*	0	0
5A	2	0	0	*L. festivus*	0	0
5A	2	0	0	*L. rubellus*	45.2	11.30
5A	2	0	0	*L. terrestris*	0	0
5A	2	0	0	*Lumbricus* sp.	9.04	1.08
5A	2	0	0	*O. cyaneum*	117.52	131.17
5A	4	0	0	*A. chlorotica*	0	0
5A	4	0	0	*D. octaedra*	0	0
5A	4	0	0	*D. rubidus*	24.54	2.45
5A	4	0	0	*E. fetida*	0	0
5A	4	0	0	*L. castaneus*	0	0
5A	4	0	0	*L. festivus*	0	0
5A	4	0	0	*L. rubellus*	8.18	0.57
5A	4	0	0	*L. terrestris*	0	0
5A	4	0	0	*Lumbricus* sp.	0	0
5A	4	0	0	*O. cyaneum*	49.08	69.69
5A	5	0	0	*A. chlorotica*	16.62	2.99
5A	5	0	0	*D. octaedra*	41.55	2.41
5A	5	0	0	*D. rubidus*	216.06	20.03
5A	5	0	0	*E. fetida*	0	0
5A	5	0	0	*L. castaneus*	0	0
5A	5	0	0	*L. festivus*	0	0
5A	5	0	0	*L. rubellus*	0	0
5A	5	0	0	*L. terrestris*	0	0
5A	5	0	0	*Lumbricus* sp.	0	0
5A	5	0	0	*O. cyaneum*	0	0
5A	6	0	0	*A. chlorotica*	0	0
5A	6	0	0	*D. octaedra*	49.08	1.80
5A	6	0	0	*D. rubidus*	858.9	62.41

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
5A	6	0	0	*E. fetida*	0	0
5A	6	0	0	*L. castaneus*	0	0
5A	6	0	0	*L. rubellus*	0	0
5A	6	0	0	*L. terrestris*	0	0
5A	6	0	0	*Lumbricus* sp.	0	0
5A	6	0	0	*O. cyaneum*	0	0
5A	1	1	0	*A. chlorotica*	0	0
5A	1	1	0	*D. octaedra*	0	0
5A	1	1	0	*D. rubidus*	62.44	10.79
5A	1	1	0	*E. fetida*	0	0
5A	1	1	0	*L. castaneus*	0	0
5A	1	1	0	*L. festivus*	0	0
5A	1	1	0	*L. rubellus*	0	0
5A	1	1	0	*L. terrestris*	0	0
5A	1	1	0	*Lumbricus* sp.	0	0
5A	2	1	0	*O. cyaneum*	0	0
5A	2	1	0	*A. chlorotica*	0	0
5A	2	1	0	*D. octaedra*	16.98	0.93
5A	2	1	0	*D. rubidus*	135.84	7.56
5A	2	1	0	*E. fetida*	0	0
5A	2	1	0	*L. castaneus*	0	0
5A	2	1	0	*L. festivus*	0	0
5A	2	1	0	*L. rubellus*	0	0
5A	2	1	0	*L. terrestris*	0	0
5A	2	1	0	*Lumbricus* sp.	33.96	1.87
5A	2	1	0	*O. cyaneum*	0	0
5A	3	1	0	*A. chlorotica*	0	0
5A	3	1	0	*D. octaedra*	0	0
5A	3	1	0	*D. rubidus*	0	0
5A	3	1	0	*E. fetida*	0	0
5A	3	1	0	*L. castaneus*	0	0
5A	3	1	0	*L. festivus*	0	0
5A	3	1	0	*L. rubellus*	0	0
5A	3	1	0	*L. terrestris*	0	0
5A	3	1	0	*Lumbricus* sp.	0	0
5A	3	1	0	*O. cyaneum*	8.13	10.65
5B	1	0	1	*A. chlorotica*	679.63	60.52
5B	1	0	1	*D. octaedra*	0	0
5B	1	0	1	*D. rubidus*	9.31	0
5B	1	0	1	*E. fetida*	0	0
5B	1	0	1	*L. castaneus*	0	0
5B	1	0	1	*L. festivus*	0	0
5B	1	0	1	*L. rubellus*	0	0
5B	1	0	1	*L. terrestris*	0	0
5B	1	0	1	*Lumbricus* sp.	0	0

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
5B	1	0	1	*O. cyaneum*	74.48	62.00
5B	2	0	1	*A. chlorotica*	285.51	13.63
5B	2	0	1	*D. rubidus*	55.26	2.49
5B	2	0	1	*E. fetida*	0	0
5B	2	0	1	*L. castaneus*	0	0
5B	2	0	1	*L. festivus*	0	0
5B	2	0	1	*L. rubellus*	0	0
5B	2	0	1	*L. terrestris*	0	0
5B	2	0	1	*Lumbricus* sp.	0	0
5B	2	0	1	*O. cyaneum*	0	0
5B	4	0	1	*A. chlorotica*	265.28	27.27
5B	4	0	1	*D. octaedra*	0	0
5B	4	0	1	*D. rubidus*	182.38	9.53
5B	4	0	1	*E. fetida*	0	0
5B	4	0	1	*L. castaneus*	0	0
5B	4	0	1	*L. festivus*	0	0
5B	4	0	1	*L. rubellus*	0	0
5B	4	0	1	*L. terrestris*	0	0
5B	4	0	1	*Lumbricus* sp.	0	0
5B	4	0	1	*O. cyaneum*	74.61	17.49
5B	5	0	1	*A. chlorotica*	578.22	47.93
5B	5	0	1	*D. octaedra*	8.38	0.75
5B	5	0	1	*D. rubidus*	318.44	15.50
5B	5	0	1	*E. fetida*	0	0
5B	5	0	1	*L. castaneus*	0	0
5B	5	0	1	*L. festivus*	8.38	2.01
5B	5	0	1	*L. rubellus*	0	0
5B	5	0	1	*L. terrestris*	0	0
5B	5	0	1	*Lumbricus* sp.	16.76	1.42
5B	5	0	1	*O. cyaneum*	33.52	24.39
5B	6	0	1	*A. chlorotica*	151.02	12.67
5B	6	0	1	*D. octaedra*	0	0
5B	6	0	1	*D. rubidus*	117.46	5.54
5B	6	0	1	*E. fetida*	0	0
5B	6	0	1	*L. castaneus*	0	0
5B	6	0	1	*L. festivus*	0	0
5B	6	0	1	*L. rubellus*	0	0
5B	6	0	1	*L. terrestris*	8.39	30.04
5B	6	0	1	*Lumbricus* sp.	0	0
5B	6	0	1	*O. cyaneum*	25.17	38.93
5B	1	1	1	*A. chlorotica*	123.9	16.73
5B	1	1	1	*D. octaedra*	0	0
5B	1	1	1	*D. rubidus*	17.7	0.71
5B	1	1	1	*E. fetida*	0	0
5B	1	1	1	*L. castaneus*	0	0

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
5B	1	1	1	*L. festivus*	0	0
5B	1	1	1	*L. rubellus*	0	0
5B	1	1	1	*Lumbricus* sp.	8.85	1.15
5B	2	1	1	*O. cyaneum*	0	0
5B	2	1	1	*A. chlorotica*	343.2	26.94
5B	2	1	1	*D. octaedra*	0	0
5B	2	1	1	*D. rubidus*	68.64	5.66
5B	2	1	1	*E. fetida*	0	0
5B	2	1	1	*L. castaneus*	0	0
5B	2	1	1	*L. festivus*	0	0
5B	2	1	1	*L. rubellus*	0	0
5B	2	1	1	*L. terrestris*	0	0
5B	2	1	1	*Lumbricus* sp.	17.16	0.86
5B	2	1	1	*O. cyaneum*	25.74	27.71
5B	3	1	1	*A. chlorotica*	32.32	5.90
5B	3	1	1	*D. octaedra*	0	0
5B	3	1	1	*D. rubidus*	8.08	0.89
5B	3	1	1	*E. fetida*	0	0
5B	3	1	1	*L. castaneus*	0	0
5B	3	1	1	*L. festivus*	0	0
5B	3	1	1	*L. rubellus*	0	0
5B	3	1	1	*L. terrestris*	24.24	13.25
5B	3	1	1	*Lumbricus* sp.	0	0
5B	3	1	1	*O. cyaneum*	72.72	115.14
4F	1	0	1	*A. chlorotica*	45.8	3.57
4F	1	0	1	*D. octaedra*	0	0
4F	1	0	1	*D. rubidus*	128.24	7.51
4F	1	0	1	*E. fetida*	0	0
4F	1	0	1	*L. castaneus*	0	0
4F	1	0	1	*L. festivus*	0	0
4F	1	0	1	*L. rubellus*	0	0
4F	1	0	1	*L. terrestris*	0	0
4F	1	0	1	*Lumbricus* sp.	18.32	1.47
4F	1	0	1	*O. cyaneum*	302.28	21.16
4F	2	0	1	*A. chlorotica*	553.2	53.66
4F	2	0	1	*D. octaedra*	9.22	0.55
4F	2	0	1	*D. rubidus*	387.24	27.75
4F	2	0	1	*E. fetida*	0	0
4F	2	0	1	*L. castaneus*	0	0
4F	2	0	1	*L. festivus*	0	0
4F	2	0	1	*L. rubellus*	9.22	3.13
4F	2	0	1	*L. terrestris*	0	0
4F	2	0	1	*Lumbricus* sp.	0	4.61
4F	2	0	1	*O. cyaneum*	64.54	42.50
4F	4	0	1	*A. chlorotica*	344.43	15.70

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
4F	4	0	1	*D. octaedra*	16.02	1.04
4F	4	0	1	*D. rubidus*	592.74	23.71
4F	4	0	1	*L. castaneus*	0	0
4F	4	0	1	*L. festivus*	0	0
4F	4	0	1	*L. rubellus*	16.02	2.40
4F	4	0	1	*L. terrestris*	0	0
4F	4	0	1	*Lumbricus* sp.	0	0
4F	4	0	1	*O. cyaneum*	0	0
4F	5	0	1	*A. chlorotica*	859.66	77.21
4F	5	0	1	*D. octaedra*	16.22	0.41
4F	5	0	1	*D. rubidus*	738.01	39.90
4F	5	0	1	*E. fetida*	0	0
4F	5	0	1	*L. castaneus*	0	0
4F	5	0	1	*L. festivus*	0	0
4F	5	0	1	*L. rubellus*	8.11	6.33
4F	5	0	1	*L. terrestris*	0	0
4F	5	0	1	*Lumbricus* sp.	16.22	3.97
4F	5	0	1	*O. cyaneum*	0	0
4F	6	0	1	*A. chlorotica*	7.97	1.67
4F	6	0	1	*D. octaedra*	0	0
4F	6	0	1	*D. rubidus*	478.2	32.04
4F	6	0	1	*E. fetida*	0	0
4F	6	0	1	*L. castaneus*	0	0
4F	6	0	1	*L. festivus*	0	0
4F	6	0	1	*L. rubellus*	0	0
4F	6	0	1	*L. terrestris*	0	0
4F	6	0	1	*Lumbricus* sp.	7.97	0.56
4F	6	0	1	*O. cyaneum*	7.97	35.31
4F	1	1	1	*A. chlorotica*	0	0
4F	1	1	1	*D. octaedra*	16.98	0.59
4F	1	1	1	*D. rubidus*	76.41	4.92
4F	1	1	1	*E. fetida*	0	0
4F	1	1	1	*L. castaneus*	0	0
4F	1	1	1	*L. festivus*	0	0
4F	1	1	1	*L. rubellus*	0	0
4F	1	1	1	*L. terrestris*	0	0
4F	1	1	1	*Lumbricus* sp.	16.98	2.55
4F	2	1	1	*O. cyaneum*	0	0
4F	2	1	1	*A. chlorotica*	662.72	69.15
4F	2	1	1	*D. octaedra*	0	0
4F	2	1	1	*D. rubidus*	331.36	13.52
4F	2	1	1	*E. fetida*	0	0
4F	2	1	1	*L. castaneus*	0	0
4F	2	1	1	*L. festivus*	0	0
4F	2	1	1	*L. rubellus*	8.72	1.66

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
4F	2	1	1	*L. terrestris*	17.44	64.44
4F	2	1	1	*Lumbricus* sp.	34.88	3.58
4F	3	1	1	*A. chlorotica*	0	0
4F	3	1	1	*D. octaedra*	0	0
4F	3	1	1	*D. rubidus*	167	13.86
4F	3	1	1	*E. fetida*	0	0
4F	3	1	1	*L. castaneus*	0	0
4F	3	1	1	*L. festivus*	0	0
4F	3	1	1	*L. rubellus*	33.4	6.35
4F	3	1	1	*L. terrestris*	0	0
4F	3	1	1	*Lumbricus* sp.	8.35	0.84
4F	3	1	1	*O. cyaneum*	0	0
4D	1	0	0	*A. chlorotica*	0	0
4D	1	0	0	*D. octaedra*	0	0
4D	1	0	0	*D. rubidus*	159.97	14.49
4D	1	0	0	*E. fetida*	0	0
4D	1	0	0	*L. castaneus*	0	0
4D	1	0	0	*L. festivus*	0	0
4D	1	0	0	*L. rubellus*	18.82	8.37
4D	1	0	0	*L. terrestris*	0	0
4D	1	0	0	*Lumbricus* sp.	0	0
4D	1	0	0	*O. cyaneum*	28.23	52.51
4D	2	0	0	*A. chlorotica*	26.91	5.20
4D	2	0	0	*D. octaedra*	0	0
4D	2	0	0	*D. rubidus*	26.91	1.35
4D	2	0	0	*E. fetida*	0	0
4D	2	0	0	*L. castaneus*	17.94	2.51
4D	2	0	0	*L. festivus*	0	0
4D	2	0	0	*L. rubellus*	44.85	8.43
4D	2	0	0	*L. terrestris*	0	0
4D	2	0	0	*Lumbricus* sp.	8.97	1.61
4D	2	0	0	*O. cyaneum*	89.7	54.27
4D	4	0	0	*A. chlorotica*	0	0
4D	4	0	0	*D. octaedra*	0	0
4D	4	0	0	*D. rubidus*	603	44.38
4D	4	0	0	*E. fetida*	0	0
4D	4	0	0	*L. castaneus*	0	0
4D	4	0	0	*L. festivus*	0	0
4D	4	0	0	*L. rubellus*	24.12	3.62
4D	4	0	0	*L. terrestris*	0	0
4D	4	0	0	*Lumbricus* sp.	0	0
4D	4	0	0	*O. cyaneum*	0	0
4D	5	0	0	*A. chlorotica*	8.35	0.84
4D	5	0	0	*D. octaedra*	8.35	0.25
4D	5	0	0	*D. rubidus*	425.85	31.90

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
4D	5	0	0	*E. fetida*	0	0
4D	5	0	0	*L. castaneus*	0	0
4D	5	0	0	*L. rubellus*	0	0
4D	5	0	0	*L. terrestris*	0	0
4D	5	0	0	*Lumbricus* sp.	25.05	1.92
4D	5	0	0	*O. cyaneum*	0	0
4D	6	0	0	*A. chlorotica*	7.96	3.18
4D	6	0	0	*D. octaedra*	23.88	1.11
4D	6	0	0	*D. rubidus*	437.8	24.12
4D	6	0	0	*E. fetida*	0	0
4D	6	0	0	*L. castaneus*	0	0
4D	6	0	0	*L. festivus*	0	0
4D	6	0	0	*L. rubellus*	7.96	3.50
4D	6	0	0	*L. terrestris*	0	0
4D	6	0	0	*Lumbricus* sp.	0	0
4D	6	0	0	*O. cyaneum*	111.44	190.48
4D	1	1	0	*A. chlorotica*	8.81	1.59
4D	1	1	0	*D. octaedra*	0	0
4D	1	1	0	*D. rubidus*	70.48	10.22
4D	1	1	0	*E. fetida*	0	0
4D	1	1	0	*L. castaneus*	0	0
4D	1	1	0	*L. festivus*	0	0
4D	1	1	0	*L. rubellus*	0	0
4D	1	1	0	*L. terrestris*	0	0
4D	1	1	0	*Lumbricus* sp.	26.43	2.55
4D	2	1	0	*O. cyaneum*	0	0
4D	2	1	0	*A. chlorotica*	0	0
4D	2	1	0	*D. octaedra*	0	0
4D	2	1	0	*D. rubidus*	163.97	23.04
4D	2	1	0	*E. fetida*	0	0
4D	2	1	0	*L. castaneus*	0	0
4D	2	1	0	*L. festivus*	0	0
4D	2	1	0	*L. rubellus*	0	0
4D	2	1	0	*L. terrestris*	0	0
4D	2	1	0	*Lumbricus* sp.	8.63	0.78
4D	2	1	0	*O. cyaneum*	0	0
4D	3	1	0	*A. chlorotica*	0	0
4D	3	1	0	*D. octaedra*	24.39	1.06
4D	3	1	0	*D. rubidus*	227.64	87.15
4D	3	1	0	*E. fetida*	0	0
4D	3	1	0	*L. castaneus*	0	0
4D	3	1	0	*L. festivus*	0	0
4D	3	1	0	*L. rubellus*	0	0
4D	3	1	0	*L. terrestris*	0	0
4D	3	1	0	*Lumbricus* sp.	24.39	3.82

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
4D	3	1	0	*O. cyaneum*	8.13	22.28
3D	1	0	0	*A. chlorotica*	0	0
3D	1	0	0	*D. rubidus*	0	0
3D	1	0	0	*E. fetida*	0	0
3D	1	0	0	*L. castaneus*	0	0
3D	1	0	0	*L. festivus*	0	0
3D	1	0	0	*L. rubellus*	0	0
3D	1	0	0	*L. terrestris*	0	0
3D	1	0	0	*Lumbricus* sp.	0	0
3D	1	0	0	*O. cyaneum*	0	0
3D	2	0	0	*A. chlorotica*	0	0
3D	2	0	0	*D. octaedra*	9.58	0.57
3D	2	0	0	*D. rubidus*	306.56	25.48
3D	2	0	0	*E. fetida*	0	0
3D	2	0	0	*L. castaneus*	0	0
3D	2	0	0	*L. festivus*	0	0
3D	2	0	0	*L. rubellus*	0	0
3D	2	0	0	*L. terrestris*	9.58	4.50
3D	2	0	0	*Lumbricus* sp.	28.74	3.45
3D	2	0	0	*O. cyaneum*	9.58	4.60
3D	4	0	0	*A. chlorotica*	16.58	3.90
3D	4	0	0	*D. octaedra*	0	0
3D	4	0	0	*D. rubidus*	207.25	11.85
3D	4	0	0	*E. fetida*	0	0
3D	4	0	0	*L. castaneus*	0	0
3D	4	0	0	*L. festivus*	0	0
3D	4	0	0	*L. rubellus*	33.16	9.87
3D	4	0	0	*L. terrestris*	0	0
3D	4	0	0	*Lumbricus* sp.	24.87	1.33
3D	4	0	0	*O. cyaneum*	58.03	38.80
3D	5	0	0	*A. chlorotica*	0	0
3D	5	0	0	*D. octaedra*	0	0
3D	5	0	0	*D. rubidus*	23.7	2.61
3D	5	0	0	*E. fetida*	0	0
3D	5	0	0	*L. castaneus*	0	0
3D	5	0	0	*L. festivus*	0	0
3D	5	0	0	*L. rubellus*	0	0
3D	5	0	0	*L. terrestris*	0	0
3D	5	0	0	*Lumbricus* sp.	0	0
3D	5	0	0	*O. cyaneum*	0	0
3D	6	0	0	*A. chlorotica*	7.9	1.88
3D	6	0	0	*D. octaedra*	0	0
3D	6	0	0	*D. rubidus*	0	0
3D	6	0	0	*E. fetida*	0	0
3D	6	0	0	*L. castaneus*	0	0

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
3D	6	0	0	*L. festivus*	0	0
3D	6	0	0	*L. rubellus*	0	0
3D	6	0	0	*Lumbricus* sp.	15.8	1.55
3D	6	0	0	*O. cyaneum*	0	0
3D	1	1	0	*A. chlorotica*	0	0
3D	1	1	0	*D. octaedra*	0	0
3D	1	1	0	*D. rubidus*	43.05	7.58
3D	1	1	0	*E. fetida*	0	0
3D	1	1	0	*L. castaneus*	0	0
3D	1	1	0	*L. festivus*	0	0
3D	1	1	0	*L. rubellus*	17.22	3.79
3D	1	1	0	*L. terrestris*	0	0
3D	1	1	0	*Lumbricus* sp.	51.66	2.50
3D	2	1	0	*O. cyaneum*	0	0
3D	2	1	0	*A. chlorotica*	8.66	1.13
3D	2	1	0	*D. octaedra*	0	0
3D	2	1	0	*D. rubidus*	25.98	4.59
3D	2	1	0	*E. fetida*	0	0
3D	2	1	0	*L. castaneus*	0	0
3D	2	1	0	*L. festivus*	0	0
3D	2	1	0	*L. rubellus*	0	0
3D	2	1	0	*L. terrestris*	8.66	48.24
3D	2	1	0	*Lumbricus* sp.	43.3	4.16
3D	2	1	0	*O. cyaneum*	17.32	12.21
3D	3	1	0	*A. chlorotica*	8.04	0.56
3D	3	1	0	*D. octaedra*	8.04	0.32
3D	3	1	0	*D. rubidus*	152.76	14.07
3D	3	1	0	*E. fetida*	0	0
3D	3	1	0	*L. castaneus*	0	0
3D	3	1	0	*L. festivus*	0	0
3D	3	1	0	*L. rubellus*	0	0
3D	3	1	0	*L. terrestris*	16.08	22.91
3D	3	1	0	*Lumbricus* sp.	8.04	0.56
3D	3	1	0	*O. cyaneum*	16.08	46.15
3B	1	0	1	*A. chlorotica*	0	0
3B	1	0	1	*D. octaedra*	0	0
3B	1	0	1	*D. rubidus*	0	0
3B	1	0	1	*E. fetida*	0	0
3B	1	0	1	*L. castaneus*	0	0
3B	1	0	1	*L. festivus*	0	0
3B	1	0	1	*L. rubellus*	0	0
3B	1	0	1	*L. terrestris*	0	0
3B	1	0	1	*Lumbricus* sp.	0	0
3B	1	0	1	*O. cyaneum*	402.3	16.54
3B	2	0	1	*A. chlorotica*	413.54	37.40

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
3B	2	0	1	*D. octaedra*	0	0
3B	2	0	1	*D. rubidus*	485.46	16.63
3B	2	0	1	*L. castaneus*	0	0
3B	2	0	1	*L. festivus*	0	0
3B	2	0	1	*L. rubellus*	0	0
3B	2	0	1	*L. terrestris*	0	0
3B	2	0	1	*Lumbricus* sp.	8.99	0.81
3B	2	0	1	*O. cyaneum*	8.99	29.22
3B	4	0	1	*A. chlorotica*	16.14	2.10
3B	4	0	1	*D. octaedra*	16.14	0.81
3B	4	0	1	*D. rubidus*	1275.06	81.35
3B	4	0	1	*E. fetida*	0	0
3B	4	0	1	*L. castaneus*	0	0
3B	4	0	1	*L. festivus*	0	0
3B	4	0	1	*L. rubellus*	0	0
3B	4	0	1	*L. terrestris*	0	0
3B	4	0	1	*Lumbricus* sp.	8.07	1.21
3B	4	0	1	*O. cyaneum*	8.07	3.79
3B	5	0	1	*A. chlorotica*	633.36	46.77
3B	5	0	1	*D. octaedra*	8.12	0.32
3B	5	0	1	*D. rubidus*	0	0
3B	5	0	1	*E. fetida*	0	0
3B	5	0	1	*L. castaneus*	0	0
3B	5	0	1	*L. festivus*	0	0
3B	5	0	1	*L. rubellus*	0	0
3B	5	0	1	*L. terrestris*	0	0
3B	5	0	1	*Lumbricus* sp.	16.24	2.84
3B	5	0	1	*O. cyaneum*	1096.2	111.97
3B	6	0	1	*A. chlorotica*	55.72	7.72
3B	6	0	1	*D. octaedra*	0	0
3B	6	0	1	*D. rubidus*	23.88	2.31
3B	6	0	1	*E. fetida*	0	0
3B	6	0	1	*L. castaneus*	0	0
3B	6	0	1	*L. festivus*	0	0
3B	6	0	1	*L. rubellus*	7.96	3.02
3B	6	0	1	*L. terrestris*	0	0
3B	6	0	1	*Lumbricus* sp.	0	0
3B	6	0	1	*O. cyaneum*	7.96	7.40
3B	1	1	1	*A. chlorotica*	129.6	15.21
3B	1	1	1	*D. octaedra*	0	0
3B	1	1	1	*D. rubidus*	120.96	5.96
3B	1	1	1	*E. fetida*	8.64	3.20
3B	1	1	1	*L. castaneus*	0	0
3B	1	1	1	*L. festivus*	0	0
3B	1	1	1	*L. rubellus*	0	0

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
3B	1	1	1	*L. terrestris*	0	0
3B	1	1	1	*Lumbricus* sp.	17.28	0.78
3B	2	1	1	*A. chlorotica*	945.88	61.08
3B	2	1	1	*D. octaedra*	0	0
3B	2	1	1	*D. rubidus*	229.84	10.08
3B	2	1	1	*E. fetida*	0	0
3B	2	1	1	*L. castaneus*	0	0
3B	2	1	1	*L. festivus*	0	0
3B	2	1	1	*L. rubellus*	0	0
3B	2	1	1	*L. terrestris*	0	0
3B	2	1	1	*Lumbricus* sp.	8.84	0.88
3B	2	1	1	*O. cyaneum*	0	0
3B	3	1	1	*A. chlorotica*	8.11	2.43
3B	3	1	1	*D. octaedra*	0	0
3B	3	1	1	*D. rubidus*	178.42	12.73
3B	3	1	1	*E. fetida*	0	0
3B	3	1	1	*L. castaneus*	0	0
3B	3	1	1	*L. festivus*	0	0
3B	3	1	1	*L. rubellus*	0	0
3B	3	1	1	*L. terrestris*	24.33	10.30
3B	3	1	1	*Lumbricus* sp.	16.22	0.65
3B	3	1	1	*O. cyaneum*	64.88	123.03
2C	1	0	1	*A. chlorotica*	149.12	14.07
2C	1	0	1	*D. octaedra*	18.64	0.19
2C	1	0	1	*D. rubidus*	447.36	19.39
2C	1	0	1	*E. fetida*	0	0
2C	1	0	1	*L. castaneus*	0	0
2C	1	0	1	*L. festivus*	0	0
2C	1	0	1	*L. rubellus*	18.64	5.87
2C	1	0	1	*L. terrestris*	0	0
2C	1	0	1	*Lumbricus* sp.	0	0
2C	1	0	1	*O. cyaneum*	102.52	30.38
2C	2	0	1	*A. chlorotica*	715.33	58.34
2C	2	0	1	*D. octaedra*	0	0
2C	2	0	1	*D. rubidus*	102.19	3.07
2C	2	0	1	*E. fetida*	0	0
2C	2	0	1	*L. castaneus*	0	0
2C	2	0	1	*L. festivus*	9.29	0.84
2C	2	0	1	*L. rubellus*	0	0
2C	2	0	1	*L. terrestris*	0	0
2C	2	0	1	*Lumbricus* sp.	0	0
2C	2	0	1	*O. cyaneum*	130.06	167.50
2C	4	0	1	*A. chlorotica*	0	0
2C	4	0	1	*D. octaedra*	0	0
2C	4	0	1	*D. rubidus*	547.3	19.28

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
2C	4	0	1	*E. fetida*	0	0
2C	4	0	1	*L. castaneus*	0	0
2C	4	0	1	*L. rubellus*	8.42	2.11
2C	4	0	1	*L. terrestris*	16.84	42.35
2C	4	0	1	*Lumbricus* sp.	42.1	3.54
2C	4	0	1	*O. cyaneum*	75.78	60.88
2C	5	0	1	*A. chlorotica*	567.87	38.93
2C	5	0	1	*D. octaedra*	0	0
2C	5	0	1	*D. rubidus*	131.68	13.83
2C	5	0	1	*E. fetida*	0	0
2C	5	0	1	*L. castaneus*	0	0
2C	5	0	1	*L. festivus*	0	0
2C	5	0	1	*L. rubellus*	0	0
2C	5	0	1	*L. terrestris*	0	0
2C	5	0	1	*Lumbricus* sp.	8.23	1.32
2C	5	0	1	*O. cyaneum*	139.91	35.47
2C	6	0	1	*A. chlorotica*	309.96	24.19
2C	6	0	1	*D. octaedra*	0	0
2C	6	0	1	*D. rubidus*	103.32	7.66
2C	6	0	1	*E. fetida*	0	0
2C	6	0	1	*L. castaneus*	0	0
2C	6	0	1	*L. festivus*	0	0
2C	6	0	1	*L. rubellus*	0	0
2C	6	0	1	*L. terrestris*	8.61	40.38
2C	6	0	1	*Lumbricus* sp.	17.22	1.46
2C	6	0	1	*O. cyaneum*	60.27	121.75
2C	1	1	1	*A. chlorotica*	0	0
2C	1	1	1	*D. octaedra*	17.22	0.34
2C	1	1	1	*D. rubidus*	120.54	6.37
2C	1	1	1	*E. fetida*	0	0
2C	1	1	1	*L. castaneus*	0	0
2C	1	1	1	*L. festivus*	0	0
2C	1	1	1	*L. rubellus*	25.83	3.01
2C	1	1	1	*L. terrestris*	0	0
2C	1	1	1	*Lumbricus* sp.	0	0
2C	2	1	1	*O. cyaneum*	17.22	12.57
2C	2	1	1	*A. chlorotica*	751.98	81.81
2C	2	1	1	*D. octaedra*	0	0
2C	2	1	1	*D. rubidus*	54.36	5.16
2C	2	1	1	*E. fetida*	0	0
2C	2	1	1	*L. castaneus*	0	0
2C	2	1	1	*L. festivus*	0	0
2C	2	1	1	*L. rubellus*	9.06	2.17
2C	2	1	1	*L. terrestris*	0	0
2C	2	1	1	*Lumbricus* sp.	72.48	4.35

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
2C	2	1	1	*O. cyaneum*	9.06	28.18
2C	3	1	1	*A. chlorotica*	0	0
2C	3	1	1	*D. rubidus*	48	3.28
2C	3	1	1	*E. fetida*	0	0
2C	3	1	1	*L. castaneus*	0	0
2C	3	1	1	*L. festivus*	0	0
2C	3	1	1	*L. rubellus*	0	0
2C	3	1	1	*L. terrestris*	0	0
2C	3	1	1	*Lumbricus* sp.	16	0.88
2C	3	1	1	*O. cyaneum*	40	69.12
2B	1	0	0	*A. chlorotica*	499.29	49.34
2B	1	0	0	*D. octaedra*	19.58	0.88
2B	1	0	0	*D. rubidus*	215.38	10.48
2B	1	0	0	*E. fetida*	0	0
2B	1	0	0	*L. castaneus*	0	0
2B	1	0	0	*L. festivus*	0	0
2B	1	0	0	*L. rubellus*	0	0
2B	1	0	0	*L. terrestris*	0	0
2B	1	0	0	*Lumbricus* sp.	39.16	2.45
2B	1	0	0	*O. cyaneum*	25.79	27.51
2B	2	0	0	*A. chlorotica*	0	0
2B	2	0	0	*D. octaedra*	18.58	1.02
2B	2	0	0	*D. rubidus*	195.09	13.01
2B	2	0	0	*E. fetida*	0	0
2B	2	0	0	*L. castaneus*	0	0
2B	2	0	0	*L. festivus*	0	0
2B	2	0	0	*L. rubellus*	9.29	2.51
2B	2	0	0	*L. terrestris*	0	0
2B	2	0	0	*Lumbricus* sp.	18.58	1.86
2B	2	0	0	*O. cyaneum*	9.29	13.19
2B	4	0	0	*A. chlorotica*	67.68	12.61
2B	4	0	0	*D. octaedra*	0	0
2B	4	0	0	*D. rubidus*	42.3	14.72
2B	4	0	0	*E. fetida*	0	0
2B	4	0	0	*L. castaneus*	0	0
2B	4	0	0	*L. festivus*	0	0
2B	4	0	0	*L. rubellus*	25.38	7.19
2B	4	0	0	*L. terrestris*	0	0
2B	4	0	0	*Lumbricus* sp.	8.46	0.59
2B	4	0	0	*O. cyaneum*	126.9	94.92
2B	5	0	0	*A. chlorotica*	215.54	31.00
2B	5	0	0	*D. octaedra*	49.74	1.99
2B	5	0	0	*D. rubidus*	8.29	0.41
2B	5	0	0	*E. fetida*	0	0
2B	5	0	0	*L. castaneus*	0	0

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
2B	5	0	0	*L. festivus*	0	0
2B	5	0	0	*L. rubellus*	0	0
2B	5	0	0	*Lumbricus* sp.	0	0
2B	5	0	0	*O. cyaneum*	132.64	163.23
2B	6	0	0	*A. chlorotica*	174.9	20.91
2B	6	0	0	*D. octaedra*	23.85	1.03
2B	6	0	0	*D. rubidus*	0	0
2B	6	0	0	*E. fetida*	0	0
2B	6	0	0	*L. castaneus*	0	0
2B	6	0	0	*L. festivus*	0	0
2B	6	0	0	*L. rubellus*	0	0
2B	6	0	0	*L. terrestris*	0	0
2B	6	0	0	*Lumbricus* sp.	0	0
2B	6	0	0	*O. cyaneum*	151.05	54.70
2B	1	1	0	*A. chlorotica*	0	0
2B	1	1	0	*D. octaedra*	0	0
2B	1	1	0	*D. rubidus*	0	0
2B	1	1	0	*E. fetida*	0	0
2B	1	1	0	*L. castaneus*	0	0
2B	1	1	0	*L. festivus*	0	0
2B	1	1	0	*L. rubellus*	0	0
2B	1	1	0	*L. terrestris*	0	0
2B	1	1	0	*Lumbricus* sp.	8.45	1.01
2B	2	1	0	*O. cyaneum*	0	0
2B	2	1	0	*A. chlorotica*	8.88	1.60
2B	2	1	0	*D. octaedra*	0	0
2B	2	1	0	*D. rubidus*	8.88	0.62
2B	2	1	0	*E. fetida*	0	0
2B	2	1	0	*L. castaneus*	0	0
2B	2	1	0	*L. festivus*	0	0
2B	2	1	0	*L. rubellus*	133.2	12.96
2B	2	1	0	*L. terrestris*	8.88	15.90
2B	2	1	0	*Lumbricus* sp.	0	0
2B	2	1	0	*O. cyaneum*	0	0
2B	3	1	0	*A. chlorotica*	0	0
2B	3	1	0	*D. octaedra*	0	0
2B	3	1	0	*D. rubidus*	16.46	1.40
2B	3	1	0	*E. fetida*	0	0
2B	3	1	0	*L. castaneus*	0	0
2B	3	1	0	*L. festivus*	0	0
2B	3	1	0	*L. rubellus*	0	0
2B	3	1	0	*L. terrestris*	0	0
2B	3	1	0	*Lumbricus* sp.	0	0
2B	3	1	0	*O. cyaneum*	0	0
1F	1	0	0	*A. chlorotica*	9.26	1.85

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
1F	1	0	0	*D. octaedra*	0	0
1F	1	0	0	*D. rubidus*	444.48	44.63
1F	1	0	0	*L. castaneus*	0	0
1F	1	0	0	*L. festivus*	0	0
1F	1	0	0	*L. rubellus*	0	0
1F	1	0	0	*L. terrestris*	0	0
1F	1	0	0	*Lumbricus* sp.	0	0
1F	1	0	0	*O. cyaneum*	0	0
1F	2	0	0	*A. chlorotica*	0	0
1F	2	0	0	*D. octaedra*	18.12	0.82
1F	2	0	0	*D. rubidus*	27.18	2.45
1F	2	0	0	*E. fetida*	0	0
1F	2	0	0	*L. castaneus*	9.06	1.90
1F	2	0	0	*L. festivus*	0	0
1F	2	0	0	*L. rubellus*	27.18	5.80
1F	2	0	0	*L. terrestris*	0	0
1F	2	0	0	*Lumbricus* sp.	0	0
1F	2	0	0	*O. cyaneum*	0	0
1F	4	0	0	*A. chlorotica*	8.34	1.50
1F	4	0	0	*D. octaedra*	16.68	1.50
1F	4	0	0	*D. rubidus*	291.9	23.35
1F	4	0	0	*E. fetida*	0	0
1F	4	0	0	*L. castaneus*	0	0
1F	4	0	0	*L. festivus*	0	0
1F	4	0	0	*L. rubellus*	0	0
1F	4	0	0	*L. terrestris*	0	0
1F	4	0	0	*Lumbricus* sp.	0	0
1F	4	0	0	*O. cyaneum*	16.68	72.89
1F	5	0	0	*A. chlorotica*	32.4	6.80
1F	5	0	0	*D. octaedra*	32.4	1.54
1F	5	0	0	*D. rubidus*	510.3	32.81
1F	5	0	0	*E. fetida*	0	0
1F	5	0	0	*L. castaneus*	0	0
1F	5	0	0	*L. festivus*	0	0
1F	5	0	0	*L. rubellus*	0	0
1F	5	0	0	*L. terrestris*	0	0
1F	5	0	0	*Lumbricus* sp.	0	0
1F	5	0	0	*O. cyaneum*	40.5	86.27
1F	6	0	0	*A. chlorotica*	0	0
1F	6	0	0	*D. octaedra*	8.34	0.42
1F	6	0	0	*D. rubidus*	100.08	5.92
1F	6	0	0	*E. fetida*	0	0
1F	6	0	0	*L. castaneus*	0	0
1F	6	0	0	*L. festivus*	0	0
1F	6	0	0	*L. rubellus*	8.34	0.92

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
1F	6	0	0	*L. terrestris*	33.36	33.69
1F	6	0	0	*Lumbricus* sp.	0	0
1F	1	1	0	*A. chlorotica*	0	0
1F	1	1	0	*D. octaedra*	0	0
1F	1	1	0	*D. rubidus*	17.8	1.34
1F	1	1	0	*E. fetida*	0	0
1F	1	1	0	*L. castaneus*	0	0
1F	1	1	0	*L. festivus*	0	0
1F	1	1	0	*L. rubellus*	0	0
1F	1	1	0	*L. terrestris*	0	0
1F	1	1	0	*Lumbricus* sp.	8.9	4.18
1F	2	1	0	*O. cyaneum*	0	0
1F	2	1	0	*A. chlorotica*	0	0
1F	2	1	0	*D. octaedra*	0	0
1F	2	1	0	*D. rubidus*	8.98	7.90
1F	2	1	0	*E. fetida*	0	0
1F	2	1	0	*L. castaneus*	0	0
1F	2	1	0	*L. festivus*	0	0
1F	2	1	0	*L. rubellus*	0	0
1F	2	1	0	*L. terrestris*	0	0
1F	2	1	0	*Lumbricus* sp.	0	0
1F	2	1	0	*O. cyaneum*	0	0
1F	3	1	0	*A. chlorotica*	0	0
1F	3	1	0	*D. octaedra*	0	0
1F	3	1	0	*D. rubidus*	8.09	1.05
1F	3	1	0	*E. fetida*	0	0
1F	3	1	0	*L. castaneus*	0	0
1F	3	1	0	*L. festivus*	0	0
1F	3	1	0	*L. rubellus*	0	0
1F	3	1	0	*L. terrestris*	0	0
1F	3	1	0	*Lumbricus* sp.	8.09	0.08
1F	3	1	0	*O. cyaneum*	0	0
1B	1	0	1	*A. chlorotica*	224.71	26.18
1B	1	0	1	*D. octaedra*	9.77	0.49
1B	1	0	1	*D. rubidus*	9.77	2.34
1B	1	0	1	*E. fetida*	0	0
1B	1	0	1	*L. castaneus*	0	0
1B	1	0	1	*L. festivus*	0	0
1B	1	0	1	*L. rubellus*	19.54	8.60
1B	1	0	1	*L. terrestris*	0	0
1B	1	0	1	*Lumbricus* sp.	19.54	2.54
1B	1	0	1	*O. cyaneum*	48.85	12.51
1B	2	0	1	*A. chlorotica*	1290.64	121.19
1B	2	0	1	*D. octaedra*	0	0
1B	2	0	1	*D. rubidus*	47.45	3.04

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
1B	2	0	1	*E. fetida*	0	0
1B	2	0	1	*L. castaneus*	0	0
1B	2	0	1	*L. rubellus*	37.96	10.15
1B	2	0	1	*L. terrestris*	0	0
1B	2	0	1	*Lumbricus* sp.	0	0
1B	2	0	1	*O. cyaneum*	0	0
1B	4	0	1	*A. chlorotica*	115.08	11.67
1B	4	0	1	*D. octaedra*	0	0
1B	4	0	1	*D. rubidus*	789.12	49.90
1B	4	0	1	*E. fetida*	0	0
1B	4	0	1	*L. castaneus*	0	0
1B	4	0	1	*L. festivus*	0	0
1B	4	0	1	*L. rubellus*	16.44	2.71
1B	4	0	1	*L. terrestris*	0	0
1B	4	0	1	*Lumbricus* sp.	24.66	1.81
1B	4	0	1	*O. cyaneum*	0	0
1B	5	0	1	*A. chlorotica*	0	0
1B	5	0	1	*D. octaedra*	0	0
1B	5	0	1	*D. rubidus*	0	0
1B	5	0	1	*E. fetida*	0	0
1B	5	0	1	*L. castaneus*	0	0
1B	5	0	1	*L. festivus*	0	0
1B	5	0	1	*L. rubellus*	0	0
1B	5	0	1	*L. terrestris*	0	0
1B	5	0	1	*Lumbricus* sp.	0	0
1B	5	0	1	*O. cyaneum*	0	0
1B	6	0	1	*A. chlorotica*	413.1	52.49
1B	6	0	1	*D. octaedra*	8.1	0.08
1B	6	0	1	*D. rubidus*	16.2	0.82
1B	6	0	1	*E. fetida*	0	0
1B	6	0	1	*L. castaneus*	0	0
1B	6	0	1	*L. festivus*	0	0
1B	6	0	1	*L. rubellus*	24.3	7.53
1B	6	0	1	*L. terrestris*	0	0
1B	6	0	1	*Lumbricus* sp.	0	0
1B	6	0	1	*O. cyaneum*	16.2	37.58
1B	1	1	1	*A. chlorotica*	213.5	34.67
1B	1	1	1	*D. octaedra*	0	0
1B	1	1	1	*D. rubidus*	51.24	3.59
1B	1	1	1	*E. fetida*	0	0
1B	1	1	1	*L. castaneus*	0	0
1B	1	1	1	*L. festivus*	0	0
1B	1	1	1	*L. rubellus*	0	0
1B	1	1	1	*L. terrestris*	0	0
1B	1	1	1	*Lumbricus* sp.	8.54	0.60

plot	treatment	dist/und	lime/unl	Species	abundance/ m^2	biomass g/m^2
1B	2	1	1	*O. cyaneum*	17.08	45.60
1B	2	1	1	*A. chlorotica*	572.18	41.88
1B	2	1	1	*D. rubidus*	8.54	1.03
1B	2	1	1	*E. fetida*	0	0
1B	2	1	1	*L. castaneus*	0	0
1B	2	1	1	*L. festivus*	0	0
1B	2	1	1	*L. rubellus*	0	0
1B	2	1	1	*L. terrestris*	0	0
1B	2	1	1	*Lumbricus* sp.	0	0
1B	2	1	1	*O. cyaneum*	0	0
1B	3	1	1	*A. chlorotica*	208.78	0.00
1B	3	1	1	*D. octaedra*	0	0
1B	3	1	1	*D. rubidus*	0	0
1B	3	1	1	*E. fetida*	0	0
1B	3	1	1	*L. castaneus*	0	0
1B	3	1	1	*L. festivus*	0	0
1B	3	1	1	*L. rubellus*	8.03	0.00
1B	3	1	1	*L. terrestris*	8.03	0.00
1B	3	1	1	*Lumbricus* sp.	0	0
1B	3	1	1	*O. cyaneum*	32.12	52.52

Appendix H Botanical composition of the mesocosm boxes

Table H.1 Botanical survey of mesocosm boxes using the Braun-Blanquet cover scale, October 2001

plot	lime	earthworm treatment	*Mycena sp.*	*Atrichum undulatum*	*Pseudoscleropodium purum*	*Pteridium aquilinium*	*Agrostis capillaris*	*Cynosurus cristatus*	*Festuca ovina*	*Holcus lanatus*	*Luzula multiflora*	*Achillea millefollium*	*Cardamine pratensis*	*Cerastium fontanum*	*Cirsium palustre*	*Cirsium vulgare*	*Galium saxatile*	*Potentilla erecta*	*Potentilla reptans*	*Ranunculus repens*	*Rumex acetosa*	*Trifolium repens*	*Vaccinium myrtillus*
1B	L	1,0	0	0	3	0	5	0	4	0	3	0	0	0	0	0	0	0	0	0	5	0
1B	L	2,0	0	0	3	0	5	0	5	0	4	0	0	0	0	0	0	3	0	0	0	0	0
1B	L	3,1	0	0	3	0	5	0	3	0	4	0	0	0	0	2	0	3	0	3	0	4	0
1B	L	4,0	0	0	3	0	6	0	4	0	4	0	0	0	0	0	0	2	0	0	0	0	0
1B	L	5,0	0	0	0	0	6	0	5	0	3	0	0	0	0	0	0	0	0	0	0	5	0
1B	L	6,0	0	0	0	0	5	0	5	0	3	0	0	0	0	0	0	0	0	0	0	5	0
1B	L	1,1	0	0	3	0	5	0	4	0	3	0	0	0	0	2	0	0	0	2	0	0	0
1B	L	2,1	0	0	4	1	5	0	4	0	4	0	0	0	0	0	0	0	0	0	0	0	0
1F	UnL	1,0	0	0	5	0	3	0	0	3	0	0	0	0	0	0	6	0	0	0	0	3	0
1F	UnL	2,0	0	0	5	0	5	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1F	UnL	3,1	0	0	0	0	6	0	5	4	0	0	0	0	0	0	0	0	0	0	0	0	0
1F	UnL	4,0	0	0	0	0	6	0	5	0	3	0	0	0	0	0	0	0	0	0	0	2	0
1F	UnL	5,0	0	2	6	0	4	0	2	0	2	0	0	0	0	0	2	0	0	0	0	0	0
1F	UnL	6,0	0	0	0	0	3	0	6	0	3	0	0	0	0	0	0	0	0	0	0	0	0
1F	UnL	1,1	0	0	0	0	5	0	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0
1F	UnL	2,1	0	0	0	0	6	0	5	0	0	0	0	0	0	0	0	3	0	0	0	0	0
2B	UnL	1,0	0	0	4	0	5	0	4	0	0	0	0	0	0	0	0	5	4	0	0	0	0
2B	UnL	2,0	1	0	4	0	4	0	6	0	2	0	0	0	0	2	0	0	0	0	0	0	0
2B	UnL	3,1	0	0	0	0	5	0	4	0	5	0	0	2	0	2	5	0	3	0	0	0	0
2B	UnL	4,0	0	0	0	0	5	0	4	0	4	0	0	0	0	2	0	3	0	0	0	0	0
2B	UnL	5,0	0	0	4	0	4	0	5	0	3	0	0	0	0	2	0	0	0	0	0	0	0
2B	UnL	6,0	0	0	0	0	4	0	5	0	4	0	0	0	0	0	0	3	0	0	0	2	0
2B	UnL	1,1	0	0	5	0	4	0	2	0	0	0	0	0	0	2	0	3	0	0	0	0	0
2B	UnL	2,1	0	0	5	0	4	0	2	0	0	0	0	2	0	0	0	4	2	0	0	0	0
2C	L	1,0	0	0	3	0	5	0	5	0	0	0	0	0	0	0	0	0	0	0	0	3	0
2C	L	2,0	0	0	3	0	5	0	5	0	0	0	0	0	0	0	0	0	0	0	0	2	0
2C	L	3,1	0	0	3	0	4	0	5	0	0	0	0	0	0	4	0	3	0	0	3	3	0
2C	L	4,0	0	0	4	0	4	0	5	2	0	0	0	0	0	0	0	0	0	0	0	2	0
2C	L	5,0	0	0	5	0	5	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0

plot	lime	earthworm treatment	*Mycena sp.*	*Atrichum undulatum*	*Pseudoscleropodium purum*	*Pteridium aquilinium*	*Agrostis capillaris*	*Cynosurus cristatus*	*Festuca ovina*	*Holcus lanatus*	*Luzula multiflora*	*Achillea millefollium*	*Cardamine pratensis*	*Cerastium fontanum*	*Cirsium palustre*	*Cirsium vulgare*	*Galium saxatile*	*Potentilla erecta*	*Potentilla reptans*	*Ranunculus repens*	*Rumex acetosa*	*Trifolium repens*	*Vaccinium myrtillus*
2C	L	1,1	0	0	4	0	5	0	5	0	0	0	0	0	0	4	0	0	0	0	0	0	0
2C	L	2,1	0	0	4	0	5	0	5	3	0	0	0	0	0	0	0	0	0	0	0	3	0
3B	L	1,0	0	0	0	0	6	0	3	0	4	0	0	0	0	0	0	2	0	0	0	0	0
3B	L	2,0	0	0	3	0	5	0	4	0	3	0	0	0	0	0	3	3	0	0	0	0	0
3B	L	3,1	0	0	4	0	5	0	3	0	4	1	0	2	0	0	4	0	2	0	0	0	0
3B	L	4,0	0	0	3	0	5	0	3	0	3	0	0	0	0	0	0	4	0	0	0	0	0
3B	L	5,0	0	0	4	0	4	0	6	0	3	0	0	0	0	0	0	3	0	0	0	0	0
3B	L	6,0	0	0	4	0	6	0	5	0	2	0	0	0	0	0	0	0	0	0	0	0	0
3B	L	1,1	0	0	0	0	5	0	2	0	0	0	0	2	0	0	5	3	0	0	0	0	0
3B	L	2,1	0	0	3	0	5	0	4	0	4	0	0	0	0	0	4	3	0	0	0	0	0
3D	UnL	1,0	0	0	0	0	6	0	3	3	0	0	0	0	0	0	0	0	0	0	0	3	0
3D	UnL	2,0	0	0	4	0	3	0	3	3	2	0	0	0	0	0	0	0	0	0	0	4	0
3D	UnL	3,1	0	0	3	0	5	0	3	0	3	0	0	0	0	2	0	0	0	0	0	2	0
3D	UnL	4,0	0	0	0	0	6	0	3	0	0	0	0	0	0	0	0	0	0	0	0	4	0
3D	UnL	5,0	0	0	0	0	3	0	6	0	0	0	0	0	0	0	0	2	0	0	0	3	0
3D	UnL	6,0	0	0	3	0	0	0	5	0	4	0	0	0	0	0	0	0	0	0	0	4	0
3D	UnL	1,1	0	2	0	0	6	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3D	UnL	2,1	1	0	5	0	5	0	3	0	5	0	0	0	0	0	3	0	0	0	0	0	0
4D	UnL	1,0	0	0	3	0	6	0	5	0	3	0	0	0	0	0	0	0	0	0	0	0	0
4D	UnL	2,0	0	0	0	0	4	2	5	0	3	0	0	0	0	0	0	2	0	0	0	0	0
4D	UnL	3,1	0	0	0	0	5	3	5	0	3	3	0	0	0	0	0	0	3	0	0	0	0
4D	UnL	4,0	0	0	3	0	3	3	5	0	3	0	0	2	0	0	0	2	0	0	0	0	0
4D	UnL	5,0	0	0	4	0	4	0	5	0	5	0	0	0	0	0	4	2	0	0	0	0	0
4D	UnL	6,0	0	0	3	0	4	0	6	3	3	0	0	0	0	0	0	0	0	0	0	0	0
4D	UnL	1,1	0	0	4	0	4	0	5	0	4	0	0	0	0	0	4	3	0	0	0	0	0
4D	UnL	2,1	0	0	5	0	2	0	5	0	4	0	0	2	0	0	2	0	4	0	0	0	0
4F	L	1,0	0	0	3	0	6	0	3	0	0	0	0	0	0	0	0	0	0	0	0	2	0
4F	L	2,0	0	0	3	0	6	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4F	L	3,1	0	0	3	0	6	0	3	0	3	0	0	2	0	3	0	0	0	0	0	0	0
4F	L	4,0	0	0	3	0	6	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4F	L	5,0	0	0	3	0	5	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4F	L	6,0	0	0	5	0	3	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4F	L	1,1	0	0	4	0	6	0	3	0	0	0	0	0	1	0	0	0	0	0	0	0	0
4F	L	2,1	0	0	6	0	4	0	3	0	0	0	0	4	0	2	0	0	0	0	0	0	0

plot	lime	earthworm treatment	*Mycena sp.*	*Atrichum undulatum*	*Pseudoscleropodium purum*	*Pteridium aquilinium*	*Agrostis capillaris*	*Cynosurus cristatus*	*Festuca ovina*	*Holcus lanatus*	*Luzula multiflora*	*Achillea millefollium*	*Cardamine pratensis*	*Cerastium fontanum*	*Cirsium palustre*	*Cirsium vulgare*	*Galium saxatile*	*Potentilla erecta*	*Potentilla reptans*	*Ranunculus repens*	*Rumex acetosa*	*Trifolium repens*	*Vaccinium myrtillus*
5A	UnL	1,0	1	0	5	0	3	0	4	0	0	0	0	0	0	0	4	0	3	0	0	0	0
5A	UnL	2,0	0	0	5	0	2	0	3	0	4	0	0	0	0	0	4	0	0	0	0	0	0
5A	UnL	3,1	1	0	0	0	5	0	4	0	5	2	0	0	0	0	0	0	2	0	0	0	0
5A	UnL	4,0	0	0	4	0	0	0	0	0	5	0	0	0	0	0	0	0	3	0	0	0	1
5A	UnL	5,0	0	0	3	0	5	0	5	0	4	0	0	0	0	0	0	0	0	0	0	0	0
5A	UnL	6,0	0	0	3	0	5	3	3	0	0	0	0	0	0	0	2	4	0	0	0	0	0
5A	UnL	1,1	1	0	4	0	5	0	4	0	3	0	0	0	0	0	4	4	4	0	0	0	0
5A	UnL	2,1	0	0	0	0	4	0	3	0	4	0	0	0	0	0	3	4	3	0	0	0	0
5B	L	1,0	0	0	3	0	5	0	5	0	2	0	0	0	0	0	0	0	0	0	0	0	0
5B	L	2,0	0	0	0	0	6	0	3	0	3	0	0	0	0	0	0	0	0	0	2	0	0
5B	L	3,1	0	0	4	0	4	0	5	0	0	0	0	0	0	0	0	0	0	0	2	0	0
5B	L	4,0	0	0	0	0	5	0	0	4	0	0	0	0	2	0	0	0	0	0	0	0	0
5B	L	5,0	0	0	3	0	5	0	3	0	3	0	2	0	0	0	0	0	0	0	0	0	0
5B	L	6,0	0	0	0	0	5	4	3	2	3	0	0	0	0	0	0	0	0	0	3	0	0
5B	L	1,1	0	0	4	0	6	0	3	0	3	0	0	0	0	2	0	0	0	0	0	0	0
5B	L	2,1	0	0	4	0	5	0	5	0	4	0	4	0	0	0	0	0	0	0	3	0	0

Key to Braun-Blanquet cover scale:

Scale value	% Cover
0	Not present
1	< 1% cover, a single individual
2	1-5 %
3	6-25 %
4	26-50 %
5	51-75 %
6	76-100 %

Appendix I Soil Biodiversity Baseline Data 1998

Block	Main Plot	Wt (mg)	%N	%C	C:N Ratio	Ca (meq/100g)	Na (meq/100g)	K (meq/100g)	Mg (meq/100g)	% Moisture loss	% Loss on Ignition	pH(H_2O)	pH($CaCl_2$)
1	A	5.88	0.49	5.80	11.77	2.01	0.15	0.49	1.16	4.04	13.62	4.75	4.16
1	B	5.09	0.47	6.72	14.29	1.95	0.13	0.68	1.09	4.40	14.94	4.47	3.98
1	C	5.01	0.57	7.65	13.48	2.67	0.14	0.77	1.56	4.51	15.89	4.60	4.03
1	D	5.16	0.62	8.40	13.52	1.40	0.12	0.89	1.08	4.65	16.40	4.50	3.81
1	E	5.75	0.53	7.36	13.79	0.69	0.13	0.85	0.64	4.90	17.31	4.49	3.78
1	F	5.71	0.52	6.71	12.88	0.86	0.12	0.78	0.68	4.12	14.91	4.41	3.78
2	A	5.77	0.57	7.30	12.79	2.10	0.14	0.58	1.16	4.36	15.38	4.74	4.11
2	B	5.89	0.60	8.90	14.87	3.22	0.14	0.58	1.91	5.25	18.84	4.62	3.98
2	C	5.86	0.52	7.17	13.82	3.19	0.14	0.71	1.76	4.82	17.32	4.68	4.00
2	D	5.37	0.56	7.75	13.93	3.60	0.14	0.80	2.05	5.14	18.26	4.55	4.00
2	E	5.37	0.55	7.70	13.91	2.63	0.14	0.65	1.54	4.95	18.07	4.60	3.95
2	F	6.06	0.53	7.12	13.50	2.29	0.12	0.76	1.28	4.38	15.92	4.43	3.92
3	A	5.08	0.62	7.97	12.90	3.00	0.15	0.51	1.75	3.69	17.13	4.76	4.12
3	B	5.88	0.64	9.73	15.21	3.41	0.18	0.63	2.11	6.54	19.66	4.65	4.04
3	C	5.72	0.54	7.58	13.91	2.18	0.13	0.59	1.25	5.20	15.62	4.58	3.95
3	D	5.83	0.61	8.28	13.50	3.37	0.15	0.61	1.85	5.11	18.49	4.76	4.08
3	E	5.54	0.53	7.26	13.65	3.00	0.14	0.72	1.58	4.54	16.95	4.75	4.07
3	F	5.83	0.55	7.71	13.97	2.41	0.14	0.76	1.20	4.53	16.48	4.76	3.95
4	A	6.01	0.56	8.02	14.26	2.96	0.15	0.61	1.59	5.15	17.56	4.82	4.05
4	B	5.70	0.56	7.65	13.59	1.67	0.14	0.65	0.95	4.90	17.96	4.53	3.88
4	C	5.76	0.57	7.20	12.62	1.11	0.13	0.58	0.67	4.46	15.33	4.59	3.91
4	D	5.76	0.51	7.00	13.77	1.49	0.13	0.62	0.75	4.74	15.92	4.47	3.96
4	E	5.83	0.53	6.80	12.94	1.01	0.12	0.62	0.58	4.29	14.60	4.45	3.84
4	F	5.67	0.58	7.73	13.41	1.23	0.12	0.81	0.80	4.61	17.09	4.45	3.64
5	A	5.96	0.58	7.66	13.24	3.32	0.15	0.59	1.66	4.72	15.99	4.75	3.96
5	B	5.95	0.60	8.07	13.45	2.58	0.14	0.67	1.51	4.94	17.07	4.70	3.76
5	C	5.24	0.53	7.48	14.13	3.41	0.16	0.65	2.14	5.05	17.71	4.93	3.94
5	D	5.92	0.57	7.51	13.13	5.07	0.16	0.83	2.89	4.60	16.34	4.95	4.14
5	E	6.01	0.53	7.01	13.23	3.86	0.14	0.71	2.21	4.69	16.42	4.77	3.70
5	F	5.22	0.62	9.07	14.74	3.12	0.17	0.68	1.88	5.19	20.10	4.73	3.53

Appendix J Microcosm experiment data and statistics

J.1 Preliminary L. terrestris *pot size experiment*

1-tailed Mann-Whitney Test. The impact of pot size on soil respiration in limed and unlimed soil, with or without *L. terrestis* present

1-tailed Mann-Whitney test on the effect of pot size on soil respiration in limed soil, no worm

Small pot N = 3 Median = 26.524
Large pot N = 3 Median = 14.901
Point estimate for ETA1-ETA2 is 11.623
91.9 Percent CI for ETA1-ETA2 is (3.641,16.185)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 > ETA2 is significant at 0.0404

1-tailed Mann-Whitney test on the effect of pot size on soil respiration in limed soil, with worm

Small pot N = 2 Median = 46.12
Large pot N = 2 Median = 32.19
Point estimate for ETA1-ETA2 is 13.93
75.5 Percent CI for ETA1-ETA2 is (8.52,19.35)
W = 7.0
Test of ETA1 = ETA2 vs ETA1 > ETA2 is significant at 0.1226

Cannot reject at alpha = 0.05

1-tailed Mann-Whitney test on the effect of pot size on soil respiration unlimed soil, no worm

Small pot N = 3 Median = 13.895
Large pot N = 2 Median = 13.055
Point estimate for ETA1-ETA2 is 1.393
85.1 Percent CI for ETA1-ETA2 is (-4.327,7.116)
W = 10.0
Test of ETA1 = ETA2 vs ETA1 > ETA2 is significant at 0.3864

Cannot reject at alpha = 0.05

1-tailed Mann-Whitney test on the effect of pot size on soil respiration unlimed soil, with worm

Small pot N = 3 Median = 28.90
Large pot N = 3 Median = 14.76
Point estimate for ETA1-ETA2 is 12.43
91.9 Percent CI for ETA1-ETA2 is (0.69,18.48)
W = 15.0
Test of ETA1 = ETA2 vs ETA1 > ETA2 is significant at 0.0404

J.2 Earthworm masses analysis, before and after microcosm experiment

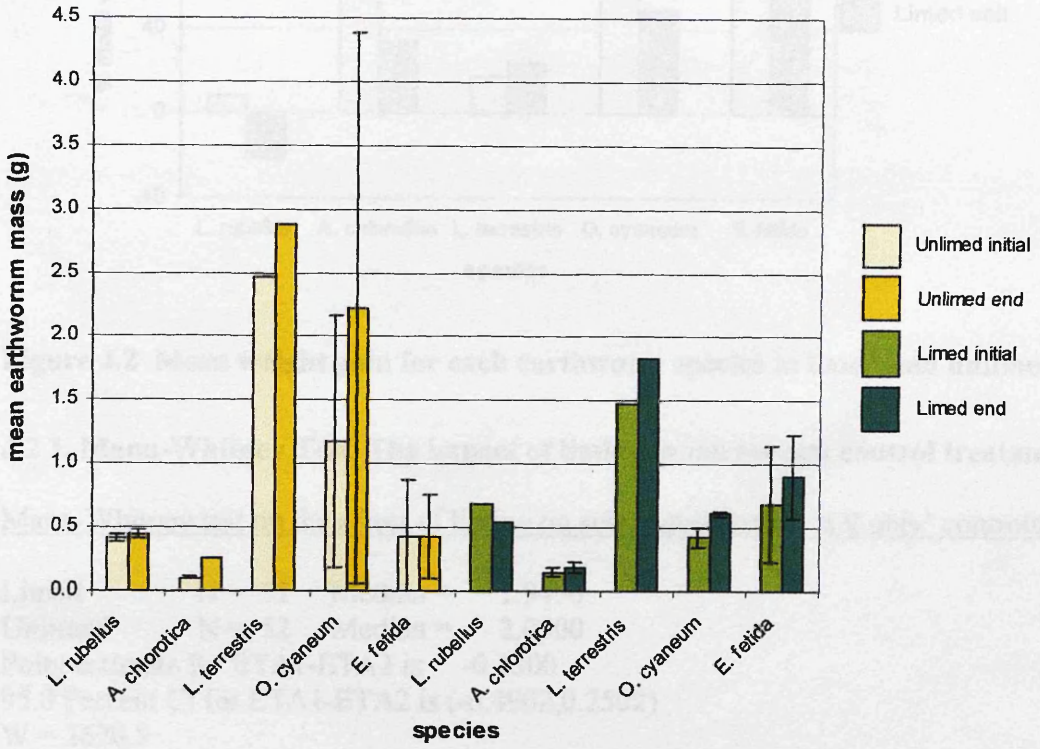


Figure J. 1 Mean earthworm masses at the beginning and end of the microcosm experiment, in limed and unlimed soil.

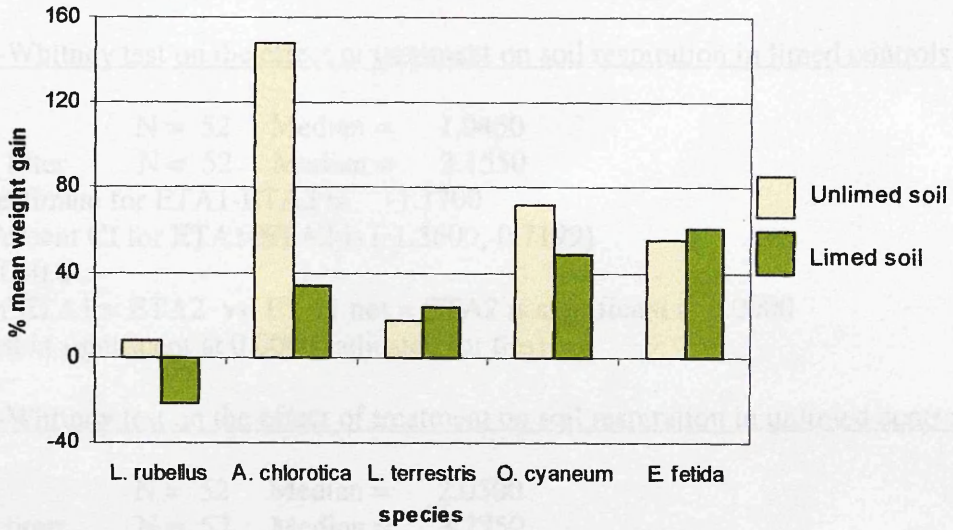


Figure J.2 Mean weight gain for each earthworm species in limed and unlimed soil

J.2 1. Mann-Whitney Test. The impact of liming in microcosm control treatments

Mann-Whitney test on the effect of liming on soil respiration in 'soil only' controls

Limed N = 52 Median = 1.9450
Unlimed N = 52 Median = 2.0500
Point estimate for ETA1-ETA2 is -0.1300
95.0 Percent CI for ETA1-ETA2 is (-0.4902,0.2502)
W = 2620.5
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4786
The test is significant at 0.4785 (adjusted for ties)

Cannot reject at alpha = 0.05

Whitney test on the effect of liming on soil respiration in 'soil + litter' controls

Limed N = 52 Median = 3.1550
Unlimed N = 52 Median = 3.2750
Point estimate for ETA1-ETA2 is -0.1800
95.0 Percent CI for ETA1-ETA2 is (-0.6401,0.3298)
W = 2621.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.4806
The test is significant at 0.4806 (adjusted for ties)

Cannot reject at alpha = 0.05

J.2 2. Mann-Whitney Test. The impact of treatment in microcosm control treatments

Mann-Whitney test on the effect of treatment on soil respiration in limed controls

Soil N = 52 Median = 1.9450
Soil + litter N = 52 Median = 3.1550
Point estimate for ETA1-ETA2 is -1.1700
95.0 Percent CI for ETA1-ETA2 is (-1.5600,-0.7199)
W = 2030.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)

Mann-Whitney test on the effect of treatment on soil respiration in unlimed controls

Soil N = 52 Median = 2.0500
Soil + litter N = 52 Median = 3.2750
Point estimate for ETA1-ETA2 is -1.1800
95.0 Percent CI for ETA1-ETA2 is (-1.6400,-0.6702)
W = 2082.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0000
The test is significant at 0.0000 (adjusted for ties)