Effects of pH on nitrogen cycling in agricultural soils

A thesis submitted to the University of Wales

by

Sarah Jane Kemmitt

In candidature for the degree of

Philosophiae Doctor

School of Agricultural and Forest Sciences

University of Wales

Bangor

January 2002

Summary.

This thesis reports a series of experiments performed to investigate the effects of soil pH on mineralisation processes in agricultural soils. The experiments utilised a short term soil incubation assay which measured the mineralisation of a mixture of 15 uniformly ^{14}C labelled amino acids to $^{14}CO_2$. Amino acids were chosen to represent the labile pool of dissolved organic matter and their mineralisation provides a means of comparing the activity of the soil microbial population performing mineralisation of nitrogenous substrates.

An examination of mineralisation in five profiles of acidic soils (chapter 4) showed that the rate of amino acid mineralisation decreased with depth and was correlated significantly and linearly with total C and total N within each profile. The profiles examined contained high levels of nitrate suggesting the presence of significant populations of acid tolerant nitrifying bacteria. A strong positive correlation between basal respiration rate and the rate of amino acid mineralisation was demonstrated within most profiles.

A study investigating the impact of varying intensities of sheep grazing from three upland regions of the UK on soil mineralisation processes was undertaken (chapter 5). This showed that the soil microbial biomass was maximal at low to intermediate levels of grazing across the three regions and declined as the legacy of grazing was reduced through the long term removal of sheep. High intensity of grazing tended to reduce the phenotypic evenness (a component of diversity) of the microbial community. Net mineralisation rates were highest in mid-successional and lightly grazed treatments in all regions and were generally lowest at the extremes of grazing influence. However, the rate of amino acid mineralisation was generally lowest in the short-term ungrazed and lightly grazed treatments and were fastest at the highest grazing pressure in all regions, supporting the model that heavily grazed grassland favours fast nutrient cycles dominated by labile substrates. Multiple regression of data from all sites showed that the impact of grazing on the activity of the SMB that actively mineralises nitrogenous substrates appears to function primarily through its effect on soil pH.

A field study was conducted to investigate the feasibility of decreasing nitrate leaching in cereal and grass plots by acidification of soil (chapter 6). The results from porous ceramic cup extracted soil water showed that nitrate concentrations in drainage water were greater in cereal plots than in grass plots. Soil acidification lowered nitrate concentrations in drainage water, substantially over winter and spring in grass plots and in cereal plots the effect was minimal during winter but became more substantial in spring. Data indicates that soil acidification decreased nitrification rates, causing the ammonium pool to accumulate. Soil acidification also lowered levels of dissolved organic nitrogen in soil water, usually to a greater degree in grass than in cereal plots. It was concluded that it may be possible to use careful soil pH management as a tool to control nitrate leaching without compromising the quality of drainage water, this may be more effective on grassland than on arable crops.

Long term experimental plots from Rothamsted Experimental Station, Woburn Experimental Farm and the Scottish Agricultural College Craibstone Estate were sampled to investigate the effect of soil pH on a range of microbially mediated soil mineralisation characteristics and processes (chapter 7). The results showed that soil pH did not significantly affect indigenous mineral nitrogen levels at the time of sampling and had little consistent effect on levels of soluble organic nitrogen or carbon across soil types. Soil pH also did not show any great influence on net ammonification, net nitrification or net total mineralisation in a 30 d aerobic

incubation. Soil pH was positively correlated with soil microbial biomass carbon and nitrogen and soil basal respiration in each soil type. The proportions of organic C and N that were biomass C and N were positively correlated to soil pH, indicating an increase in availability of the C and N present in the soil with rising pH. Glucose-C and urea-C mineralisation rates were fastest at intermediate points of the pH range studied in each soil type. Arginine-C mineralisation was positively and linearly related to soil pH.

Results of chapters 5, 6 and 7 showed that soil pH had a significant impact on the rate of amino acid mineralisation. Acidity increased the proportion of added amino acid-C used in respiration and decreased the proportion used in biomass, implying an acidity-induced stress on the microbial population. Soil microbial biomass C or N, basal respiration rates, total soil C and N and dissolved organic C and N were shown not to adequately predict the rate of amino acid mineralisation over the range of soil types studied. Suggestions for further investigations into the soil pH effects on characteristics of organic substrates and how they may determine carbon and nitrogen cycling rates are made.

Acknowledgements.

I would like to acknowledge the guidance and assistance of my supervisors, Dr. Davey Jones and Dr. David Wright, whose help, advice and support were invaluable.

The help of the following is acknowledged:

Mr. Ian Kelso for devising the initial concepts of the field project.

Ms. Annie Britain of the Centre for Environment and Hydrology, CEH, Bangor, for assistance with nitrate and ammonium analysis in early experimental work.

Mr. Steve Hughes of the Centre for Environment and Hydrology, CEH, Bangor, for assistance with analysis of dissolved organic carbon.

Llinos Hughes, Ted Lloyd-Jones and John Thomas at Henfaes, the University Research Farm for assistance with management of the field site.

Dr. Andrew Owen and the late Hilton Trow for general technical support.

Dr. K.W.T. Goulding for permitting sampling of soils at the Rothamsted Experimental Station.

Dr. A. Edwards for providing soil samples from the pH plots of the Scottish Agricultural College, Aberdeen.

Dr. Richard Bardgett for providing opportunities for collaborative research.

I would like to thank my husband, Mark Kemmitt and son, Marley, who we had during the course of this research. Their support and motivation spurred me on. Gratitude also goes to my mother and father who provided support and advice.

I also acknowledge the help and support provided by my colleagues at the School of Agricultural and Forest Sciences and my friends and family.

Finally, I would like to acknowledge the Sir William Roberts Memorial Studentship, which funded this research.

Table of contents

Table of Figures

Table of Tables

SIGNED DECLARATION FORM

NOT TO BE INCLUDED IN THE DIGITISED THESIS

Abbreviations Used

CEC	Cation Exchange Capacity
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DON	Dissolved Organic Nitrogen
NPC	Ninhydrin Positive Compound
NSA	Nitrate Sensitive Area
NVZ	Nitrate Vulnerable Zone
R	Rothamsted soil
S	SAC soil
SAC	Scottish Agricultural College
SIR	Substrate Induced Respiration
SMB	Soil Microbial Biomass
SMB-C	Soil Microbial Biomass Carbon
SMB-N	Soil Microbial Biomass Nitrogen
SMC	Soil Microbial Community
SOC	Soluble Organic Carbon
SOM	Soluble Organic Matter
SON	Soluble Organic Nitrogen
TDN	Total Dissolved Nitrogen
TSN	Total Soluble Nitrogen
W	Woburn soil

Chapter 1 Introduction

1.1 General introduction

Over recent decades, there has been increased interest in sustainable agriculture due to problems associated with conventional agricultural systems, which have grown in magnitude since the Second World War (Kristensen et al., 1995). Loss of nitrogen (N) from the soil-plant system via leaching of nitrate (NO_3^-) and other routes has been a matter of increasing concern (Addiscott et al., 1991). This loss partially stems from the use of increased quantities of N fertilizer. There is a tendency for farmers to apply excess so that crop growth and yield are not limited by insufficient N availability and because of the relative cheapness of the fertiliser (Addiscott et al., 1991).

This N loss not only represents a loss of a vital resource, which is replaced with environmentally costly industrial N-fixation in the form of artificial fertilisers, but also each avenue of N loss has other associated disadvantages. NO_3^- leaching can result in eutrophication of water bodies with detrimental ecological consequences and has possible implications for human health. NO_3^- levels in water have been controlled by the European Union since 1980 (in drinking water) and 1991 (in water bodies). Denitrification can result in gaseous emissions of N_2O, a greenhouse gas, and ammonia volatilisation can result in acid deposition. The manufacture, distribution and application of N fertilisers are energy consuming and so contribute to industrial CO_2 emissions. These concerns are given serious consideration by the UK Government; NO_3^- losses to freshwater and ammonia, methane and nitrous oxide emissions are included in a proposed list of indicators for development of sustainable agriculture in the UK (MAFF, 1998d).

NO_3^- in soil can originate either from additions of NO_3^- fertiliser, or from the nitrification of ammonium (NH_4^+). The NH_4^+ may be from a fertiliser source, or be a resultant product of the mineralisation of organic nitrogenous compounds in the soil. The mineralisation process is performed by the soil biota, and the organic substrates may be from new additions of animal or plant matter or from the indigenous soil organic matter (which will have originally come from plant material) (Haynes, 1986).

The major increase in N use by agriculture during the last few decades has inevitably been associated with large rises in N losses as NO_3^- in drainage water and in gaseous emissions (Davies, 2000). Although a statistical relationship can be established, which links increases in N fertiliser usage with increased NO_3^- in surface and groundwaters, the relationship is not simple (Addiscott et al., 1991; Goulding and

Poulton, 1992). Although increased use of artificial fertilisers has contributed to some of these problems, if good practice is followed, very little fertiliser N is leached (Goulding et al., 2000). Organic compounds indigenous to the soil and from inputs of crop residues or external sources have not been 'innocent' players in these issues. Sources of organic N (e.g. from manures, wastes, slurry, crop residues and green manures) are generally of sustainable origin and confer other benefits to soil fertility by maintaining soil organic matter which improves the soil structure, water and nutrient holding capacity and microbial populations. However, these sources can also give rise to NO_3^- leaching if NO_3^- released from mineralisation and nitrification is greater than crop demand or if applied when drainage losses are high.

Application of inorganic fertiliser is a much more controllable activity than managing the nutrients from organic sources, which show resistance to being 'steered' into desirable pools of nutrients. Thus, low input and organic agricultural systems are not necessarily less 'leaky' than their 'conventional' counterparts.

Fertiliser use has increased the N return to the soil in crop residues, and a decrease in fertiliser use will therefore only reduce NO_3^- leaching in the long term (Goulding and Poulton, 1992). There is evidence that a major source of organic N that has ended up being leached as NO_3^- has been the ploughing in of permanent grassland which occurred to a great extent during the second world war and up to the 1970s (Addiscott et al., 1991). The incorporation of N into the carbon compounds of organic matter provides relative stability, but this is only temporary due to the difficulty of conserving organic matter, especially in arable soils (Davies, 2000).

The key to minimising loss of excess NO_3^- N in the soil is to apply N in a way that reflects crop N requirements (in *amount* and *timing,* Addiscott et al., 1991) and balances the N that becomes available from the soil during the life of the crop. However, we are still unable to adequately predict fertilizer requirement as affected by mineralisation of soil organic matter due to considerable variability between fields (Shepherd et al., 1996). The relationship between soil organic matter and N supply is not strong enough to use predictively, due to the non-uniform nature of the organic matter and the wide range of soil and environmental conditions (Shepherd et al., 1996). In the UK, the vulnerable period for NO_3^- leaching is late autumn to spring, when the majority of drainage occurs (Addiscott et al., 1991; Goulding, 2000) and plant uptake is lowest.

The requirement for increased efficiency and reduced environmental impact of agricultural systems provides a strong motive for obtaining information on

mineralisation (Jarvis et al., 1996). The processes of mineralisation and nitrification are crucial, whether the available N originates from the native soil organic matter, an organic amendment or a chemical fertilizer. This is however a formidable task due to the complexity of the N cycle in soil and the heterogeneity of the soil resource.

1.2 The need for research

Much of the research geared towards understanding the N cycle, and how management options affect it, has failed to examine one of the most important pools of N in the system, dissolved organic N (DON) (Murphy et al., 2000). The importance of DON as a major source of N for the mineralisation and subsequent nitrification and denitrification processes has emerged in recent research. Mobile DON possibly contributes to the 'unaccounted for' fraction in farm N budgets (Jarvis, 2000).

The role of soil pH in the N cycle is unclear. While much literature reports that the mineralisation process is insensitive to soil pH (Haynes, 1986), it is commonly accepted that acid conditions slow organic matter decomposition rates (Jenkinson, 1981). Nitrification is known to be pH sensitive, yet nitrification is detected in very acid soils and the role of heterotrophic or chemical nitrification may be significant at low pHs. Liming maintains soil pH at levels suitable for desired crops or pasture and releases organic compounds into the soluble pool, which are then open to mineralisation and subsequent transformations. Much of the research on soil pH and microbial processes has been carried out on forest soils (e.g. Anderson and Domsch, 1993), which can behave very differently from cultivated pasture or arable soils.

In the light of greater understanding of the importance of DON in soil-plant-environment interactions, and the continuing need to improve our understanding of N dynamics so that the sustainability of agricultural systems may be improved, there is a need for experimental work to re-evaluate the impact of soil pH on the dynamics of nitrogen cycling in arable and pasture soils.

1.3 Plan of the thesis and strategy of study

The remainder of this thesis is divided into seven chapters, which are summarised in Table 1-1. These start with a review of the literature in Chapter 2, establishing the current understanding of N cycling in soil, focussing on the mineralisation, immobilisation and nitrification processes and how they are influenced by soil pH. This is presented within the context of the drive to improve the management of N within agricultural systems relevant to the UK, and how the sustainability of such systems may be increased. Relevant legislation is briefly described.

Methods that were common to each avenue of research pursued are described in Chapter 3 (where a method has been used in only one chapter, it is described therein). The experimental work is described in the subsequent four chapters.

Acid soils (surface pH<5.5) are an important agricultural resource (Malhi et al., 1988; von Uexküll and Mutert, 1995; Curtin and Ukrainetz, 1997) and in the UK, most soils would naturally be acidic if liming were not carried out (ALPC, 1995). A preliminary exploration of DON cycling in acid soil profiles was therefore carried out (Chapter 4).

N cycling in uplands is of importance because of their distinctive, sensitive ecology and our reliance on upland regions as sources of pure water for the water supply industry (Cuttle et al., 1996). Therefore, an examination of DON cycling and N mineralisation in upland regions of the UK across a range of grazing pressures was carried out (Chapter 5). This was part of a wider collaborative study that has been reported by Bardgett et al., (2001), which is included in appendix 1.

That soil pH appeared to have significant influence on N mineralisation warranted further investigation, with the exclusion of confounding influences of varying site and soil types. A field study was therefore conducted in order to examine the effect of soil pH on DON and mineral N dynamics on arable and grass land, with a focus on NO_3^- leaching (Chapter 6). Soil acidification was carried out to examine the effect of pH, rather than liming as is prevalent in the literature.

Long-term pH plots from Rothamsted and Woburn experimental stations, and the Scottish Agricultural College were utilised in a laboratory based exploration of soil pH and its influence on microbially mediated processes (Chapter 7). Methods used in this chapter are included in appendixes 2 and 3, and a correlation matrix of some of the data is included in appendix 4.

Chapter 8 includes a general discussion of elucidated themes from the results of the experimental work, conclusions are drawn and avenues for further research are identified. This includes consideration of the use of soil pH control as a strategy for management of N in agro-ecosystems, in the light of the findings of the experimental work presented herein.

Two posters that were presented at the British Society of Soil Science annual conference at Edinburgh, UK in 1999 are included in appendixes 5 and 6.

1.4 Aims and objectives

The overall objective of this work was to examine relationships between fluxes of soluble nitrogenous compounds and soil pH. Specifically this research aimed to:

1. Examine the factors regulating amino acid mineralisation in acid soils and to establish the nature of any relationship between soil C and N, microbial activity and amino acid turnover (Chapter 4).
2. Examine the spatial distribution of amino acid mineralisation activity throughout acid soil profiles (Chapter 4).
3. Examine how successional transitions and grazing intensity in uplands influence the biomass, activity, and structure of soil microbial communities in relation to N dynamics, especially mineralisation of soluble organic N (SON) (Chapter 5).
4. Observe the effect of artificially decreasing soil pH on N dynamics, specifically total N, NO_3^-, NH_4^+, and DON pools in the soil and drainage water under field conditions (Chapter 6).
5. Study the effect of soil pH reduction on the cycling of low molecular weight DON (Chapter 6).
6. Examine the impact of pH on various aspects of microbially mediated carbon and N (especially DON) cycling, avoiding confounding influences of site and soil specific factors (Chapter 7). This chapter explored relationships between measurements of soil microbial biomass C and N and microbial activity and how these parameters were linked with various soil characteristics with a focus on soil pH.

Table 1-1 Chapter headings and summary of contents

Chapter	Title	Summary
Chapter one	Introduction	General introduction, aims and plan of thesis.
Chapter two	Literature review	The N cycle in soil, focussing on soil pH and the context of furthering the sustainability of agricultural systems relevant to the UK.
Chapter three	Methods	Methods used in each of the four experimental chapters.
Chapter four	Regulation of amino acid biodegradation in acid soils	The mineralisation of amino acids in five acid soil profiles.
Chapter five[1]	Changes in amino acid mineralisation over three successional grazing gradients in UK.	Amino acid mineralisation activity in soils taken from gradients of six levels of grazing influence from three upland regions of the UK, and its relation to various soil and microbial parameters.
Chapter six	Field study of the relationship between soil pH and N dynamics	The impact of soil acidification on N cycling in the field with emphasis on NO_3^- leaching.
Chapter seven[2]	The impact of soil pH on microbially mediated carbon and N dynamics.	The effect of soil pH on carbon and N cycling. Three soils subject to a long-standing and wide induced gradient in pH were studied.
Chapter eight	General discussion Conclusions and future work	Themes from the results of the four experimental chapters. Recommendations for further avenues of research in the light of the findings of this thesis.

[1] Also see appendix 1.

[2] Also see appendixes 2, 3, and 4.

Chapter 2 Literature review

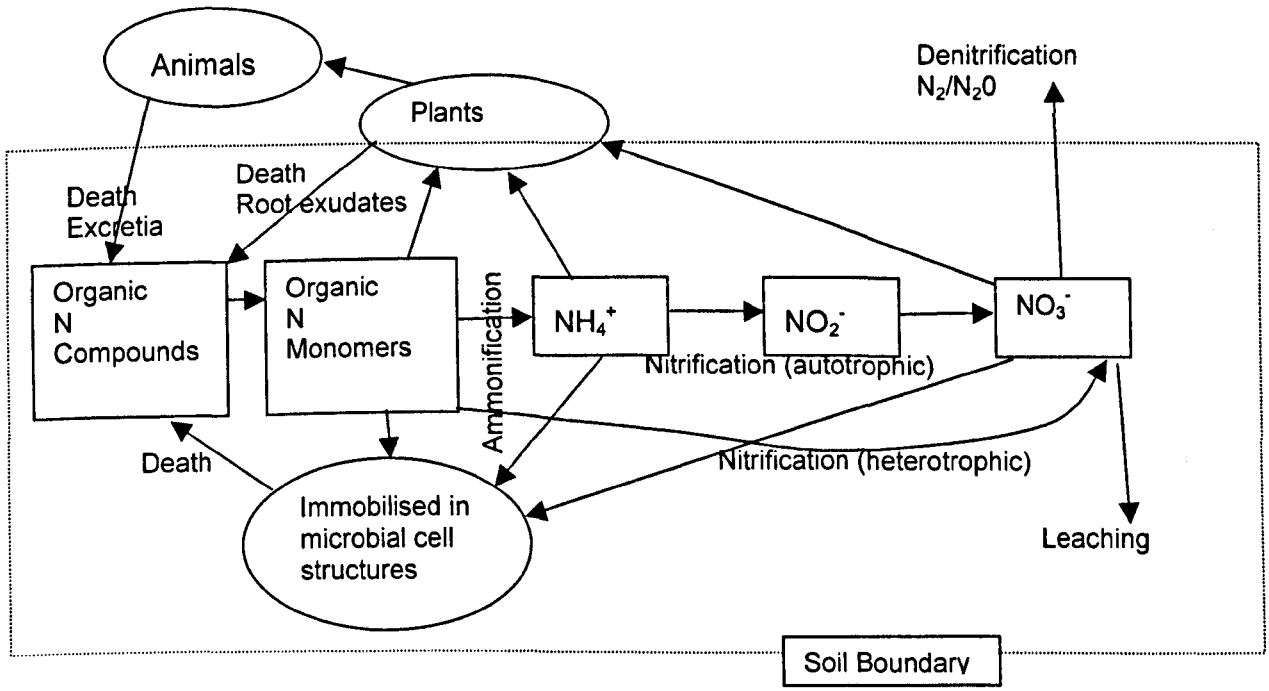
2.1 *Introduction*

This literature review is arranged in three main sections. Section 2.2 covers the aspects of the N cycle in soil that are relevant to the work in this thesis. Section 2.3 reports on factors that influence the processes of ammonification and nitrification, with emphasis on soil pH. NO_3^- leaching is considered in section 2.4, with descriptions of the problems it causes, management options for avoidance of it and the legislative framework that controls it.

2.2 *The nitrogen cycle in soil*

The N cycle in soil is summarised in Figure 2-1, which, although not exhaustive, shows the major pools and fluxes of importance to the work in this thesis.

Figure 2-1: The nitrogen cycle in soil.



Inputs to the system include: biological fixation of atmospheric N_2; atmospheric deposition or anthropogenic addition of NH_3, NH_4^+, NO_3^- and organically bound N. Losses from the system include: leaching, erosion by water or wind (e.g. runoff, sheet erosion), cropping, denitrification and volatilisation.

2.2.1 Pools within the nitrogen cycle

2.2.1.1 Plants

Nitrogen (N) is used in plants as a component of chlorophyll, amino acids, vitamins, enzymes, hormones and it is important to carbohydrate utilisation, root development and activity and uptake of other nutrients (Stevenson, 1986).

Uptake, assimilation and use of mineral N by plants is reviewed by Haynes (1986, chapter 6) and Wild and Jones (1988). Plants vary in their preference for NH_4^+ or NO_3^- (Koppisch et al., 1993), but if a plant takes up NO_3^-, it must be converted to NH_4^+ with the use of NO_3^- and nitrite (NO_2^-) reductase enzymes (Haynes, 1986). Under acid conditions, many plant species show better growth under NO_3^- than NH_4^+ supply, possibly because a predominance of NH_4^+ uptake further acidifies the rhizosphere (Malhi et al., 1988).

Ammonia is taken up via a membrane protein (Ninnemann et al., 1994) and rapidly metabolised into glutamic and aspartic acids, which are then converted to other amino acids as necessary (Stevenson, 1986).

It had long been assumed that plants are only capable of taking up N in inorganic forms (Marschner, 1995). However, work since the 1950s has demonstrated the ability of a range of plants to actively take up organic N (Fischer et al., 1995), irrespective of their mycorrhizal associations (Chapin et al., 1993; Jones and Darrah, 1993, 1994; Kielland, 1994, 1997; Näsholm et al.,1998; Dhont et al., unpublished). This process is especially important in the N budgets of arctic plants because in tundra soils, concentrations of available organic N are often an order of magnitude higher than inorganic N (Atkin, 1996), and this ability may overcome N limitations imposed by the slow rate of organic matter decomposition. However, amino-N uptake has not been demonstrated in the field and the competitive relationships between roots and microbes are not well understood (Jones, 1999).

These observations indicate that the plant available N pool had previously been underestimated and the ability to absorb organic N provides another basis for niche differentiation of what was previously understood to be a single resource (Kielland, 1994).

Leguminous plants have a unique role in the nitrogen cycle and the atmospheric N_2 fixed can be a major input to the plant-soil system, but a detailed consideration of them is beyond the scope of this review.

2.2.1.2 Soil nitrogen

The total N content may range from <0.1% of dry soil by weight in desert to >2% in organic soils (Haynes, 1986). Table 2-1 below gives a breakdown of the composition of soil N.

Table 2-1: The chemical constituents of nitrogen in the soil: (source Paul and Clark, 1989)

Climatic zone	Total N %	%				
		Acid insoluble	NH_3	Amino acids	Amino sugars	Hydrolysed unknown N
Arctic	0.02-0.16	13.9	32	33.1	4.5	16.5
Cool temperate	0.02-1.06	13.5	28	35.9	5.3	17.8
Sub tropical	0.03-0.30	15.8	18	41.7	7.4	17.1
Tropical	0.02-0.16	11.1	24	40.7	6.7	17.6

2.2.1.2.1 Soil organic nitrogen

2.2.1.2.1.1 Non-living organic nitrogen

The organic N pool is always much larger than the mineral N pool. For example an average taken across a range of soil types showed a mineral N content of 76 kg N ha^{-1} compared with 7 t ha^{-1} of organic N (Jarvis et al., 1996). Accumulation of soil N closely follows that of soil organic matter (Haynes, 1986).

Soil organic matter may be divided into several pools stabilised against mineralisation to varying degrees by molecular recalcitrance, physical separation from the soil microbial biomass and or direct association with inorganic ions and clay surfaces. No component has been found to be mineralisable at a rate directly proportional to the overall N-mineralisation rate (Tate, 1995).

In natural ecosystems, the N content of soil is at an equilibrium, which may shift after disturbance. The 0.02-1.0 % of the soil that is organic N is predominantly proteins, nucleic acids (RNA, DNA) (Paul and Clark, 1989), amino acids and amino sugars. Amino acids and sugars constitute 20% of the soil C and 30-40% of soil N (Smith et al., 1993) and are the major source of inorganic N released from soil organic matter by the mineralisation process. Amino sugars are a major constituent of plants and of bacteria and fungal cell walls, and in the soil are bound mostly to humic compounds; 2-8% of humic acid N is amino-sugars (Haynes, 1986). Amino sugars do not contribute as much as amino acids to N-mineralisation (Paul and Clark, 1989).

Amino acids consist of an amino group (NH_2) and a carboxyl group (COOH), attached to the same C atom, along with an R group, which is the distinguishing factor for an amino acid. Amino acids link by 'dehydration synthesis', the joining of the amino group of one amino acid with the carboxyl group of another, forming a

peptide bond and releasing H_2O (Tortora et al., 1995), which occurs on the ribosome during protein synthesis (Mc Kane and Kandel, 1996). Amino acids (except glycine) have stereoisomers D and L; in proteins amino acids are always in their L-isomers. D –amino acids sometimes occur in bacterial cell walls and antibiotics.

Monreal and McGill (1985) found the free amino acid content in cultivated and virgin soil samples ranged between 0.32 and 4.72 μg g^{-1} soil, and the order of abundance was found to be: alanine, phenylalanine, threonine, valine, aspartic-glutamic acid complex, basic amino acids, S-containing amino acids (methionine and cystine), and the isoleucine-leucine pair. Low concentrations of amino acids in this list indicate either high turnover rates due to microbial utilization for energy/biosynthesis and/or strong association with soil organic/inorganic colloids. Virgin soils contained more but not always a wider range of free amino acids than cultivated soils.

Amino acids may be free or bound to humic polymers and clay minerals on external or internal surfaces. Half of the total N in humic acids is amino acid-N bound by peptide bonds, quinone rings and phenolic rings (Haynes, 1986). Amino acid utilisation by the soil microbial community (SMC) depends on rate of supply, rather than solution concentration, and so is related to desorption (Monreal and McGill. 1985).

Amino acids may also enter the soil from root exudation and cell lysis. Free amino acids and proteins in plant and microbial residues are rapidly degraded. Fates of root exudates include sorption (Jones et al., 1994) and fixation to the solid phase (likely to be important for charged solutes (Paul and Clark, 1989)), capture by the microbial biomass, recapture by the root, complexation with metal cations and movement away from the root by diffusion/mass flow (Jones, 1999).

Humus gains stability and resistance to biodegradation from the formation of complexes of organic compounds and inorganic colloids and from its molecular structure. The organic content of soil tends to be higher in clay soil (varying with the absorption capacity of the type of clay) and progressively lower in loamy and sandy textured soils (Haynes, 1986). Haynes (1986) describes the polyphenol theory of humic substance formation in detail.

2.2.1.2.1.1.1 Dissolved or soluble organic nitrogen

Dissolved organic nitrogen (DON) refers to organic N dissolved in the soil solution, whereas soluble organic nitrogen (SON) is the organic N that is solubilised in a water or weak salt extract of soil. The pool of SON is of the same order of magnitude as that of mineral N and of equal size in many cases; typical levels are 20 – 30 kg SON-

N ha^{-1} in a wide range of arable agricultural soils from England (Murphy et al., 2000). The pool size tends to be more constant that that of mineral N (Murphy et al., 2000), but it has the most rapid turnover of the organic pools present in a given ecosystem (Zsolnay, 2001). DON is thought to represent a major pool of N used in the mineralisation process, although only a fraction of the SON pool will be mineralised as some is recalcitrant (Murphy et al., 2000). Smith (1987) found that SON in soil extracts was not exceptionally susceptible to mineralisation and that leaching out of SON did not substantially lower subsequent N mineralisation. However, according to Zsolnay (2001), no part of dissolved organic matter (DOM) can be considered truly non-degradable.

Murphy et al. (2000) review the developments in understanding of DON cycling in forest soils. In the Broadbalk wheat experiment, DON in drainage water is 2-10% of the soil SON pool, and 10% of N leached from drains is organic. High organic matter content in soils may mean that the cation exchange sites are occupied by organic matter, resulting in low sorption of DOM, and greater leaching of DON. Leaching of DON may be of importance by taking with it other nutrients, toxic metals and pesticides (Goulding, 2000).

DOM influences soil development via the downward mobilization and precipitation of iron and aluminium and acts as food for many organisms in soil water (Lofts et al., 2001). Removal of DOM from the soil solution occurs by immobilization on solids in the subsoil and microbial degradation (Lofts et al., 2001). DOM, being mostly anionic (Zsolnay, 2001) sorbs strongly to minerals present in subsurface soils, particularly metal oxides and clays. The physical and chemical processes involved in sorption favour the retention of organic fractions having greater molecular mass or hydrophobicity (Lofts et al., 2001). Dissolved organic matter (DOM) from arable soils has a higher proportion of hydrophobic compounds compared with extracts from grassland/forest soils, and it has been found that total SON and the proportion of amino compounds is usually higher under grassland/forest than arable use (Lofts et al., 2001). Although the quantity of DOM is small, compared to the soil organic matter pool and to C-fluxes in the terrestrial or global carbon and N cycles, it is an important bottleneck with respect to the transformation and transport of highly decomposable organic compounds to a biologically stable soluble organic matter (SOM) fraction (Zsolnay, 2001).

Although sources of SOM are mainly from plant, animal or microbial inputs, Mopper and Zika (1987) found concentrations of dissolved free amino acids in marine rains

averaged 6.5 μM (range 1.1-15.2 μM or 0.015 - 0.21 ppm N), which was a similar magnitude as inorganic N.

2.2.1.2.1.2 Living organic nitrogen

The soil invertebrate fauna make an important contribution to mineralisation by:

1. redistributing organic materials over a range of spatial scales
2. enhancing the rate of change through chemical change during metabolism
3. affecting microbial populations by creating or removing appropriate conditions for their activities (Jarvis et al., 1996).

However, Geissen and Brümmer (1999) found the macro and mesofauna to have a negligible effect on the decomposition of beech forest litter.

Soil flora generally preferentially uses NH_4^+ over NO_3^-, and a select group is able to fix N_2 (Haynes, 1986; McKane and Kandel, 1996). The C:N ratio of microorganisms is variable; in fungi it is 15:1 to 4.5:1, in bacteria it is 3:1 to 5:1 (Paul and Clark, 1989). The soil microbial biomass size (the sum of all soil microbes, SMB) is dependent on the initially available C and N pools (Smith et al., 1993).

Microbial cells are 5-10 % N dry matter, most of which is storage protein, the remainder being in cell walls as polymers of amino sugars and amino acids. The microbial biomass in soil can account for 0.5-15.3% usually c. 3% of soil N (Jarvis et al., 1996), and there is an annual flux of around 1/3rd of the biomass N. This microbial N is very easily mineralised; 5 times faster than the mobile soil organic-N (Haynes, 1986). The flux of N through the SMB is large compared to its size and the size of the mineral N and SON pools at any given time (Murphy et al., 2000). Once N is immobilised in the SMB, a significant proportion of it is no more available to plants than the native soil N, as it is incorporated into the humic and fulvic acid fractions (He et al., 1988).

The microbial population may be split into an autochthonous biomass that decomposes the resistant, complex, humified organic matter at a modest rate and a zymogenous biomass that reacts rapidly to inputs of readily metabolised substrates, decomposes the fresh and soluble organic matter and then returns to dormancy (Ladd and Foster, 1988; Garnier et al., 2001; Reynolds and Pepper, 2000).

Measurements of soil microbial biomass can reveal changes due to soil management before such changes are detectable in total organic C or N content (Glendining et al., 1996).

2.2.1.2.2 Soil mineral nitrogen

Forms of mineral N in soil include NH_4^+, NO_3^- and NO_2^-. Sources of mineral N include atmospheric and anthropogenic inputs and release from the mineralisation of organic N. Mineral N availability determines the productivity and nature of the above ground community, and the N-mineralisation rate is a key indicator of the potential biomass production that can be supported by indigenous mineral N production (Tate, 1995).

2.2.2 Fluxes within the nitrogen cycle

2.2.2.1 Organic nitrogen breakdown

Organic N polymers are broken down to amino acids, purines, pyrimidines, amino sugars (Stevenson, 1986) by enzymes. Proteolytic enzymes (proteases and peptidases) hydrolyse the peptide links, and they are classified by which peptide linkages between amino acids they break (see Paul and Clark, 1989). Microbial enzymes e.g. pronase and subtilisin carry out terminal amino acid chain removal. Fibrous proteins with many cross-links e.g. keratin are resistant to microbial attack because of disulfide bonds between cysteine molecules, but are not resistant to break down by actinomycetes e.g. *Streptomyces* and fungi e.g. *Penicillium* (Paul and Clark, 1989). Nucleic acids (DNA, RNA) are cyclic N compounds connected to phosphate groups by ester linkages, and do not accumulate in organic matter as they are very easily broken down (Paul and Clark, 1989).

The soil fauna and the soil structure affect organic N breakdown. Hassink et al. (1993) found a close positive correlation in grassland soils between the bacterial biomass and soil volume of pores with 0.2-1.2 μ m and between the biomass of nematodes and the soil volume of pores of diameter 30-90 μm. Fungi and protozoa biomass showed no relationship with a specific pore-size class. The amount of N mineralised per bacterium was much higher in soils with a high grazing pressure of bacterivorous nematodes and flagellates than in soils with a low grazing pressure of these groups, although no such relationship was found with amoebae.

Organic N, once broken down into low molecular weight (LMW) compounds, may be taken up by the SMB directly, or mineralised extracellularly and the N absorbed as NH_4^+ (Hadas et al., 1992).

2.2.2.2 Uptake and use of amino acids by soil microbes

There is no single pattern by which the biosynthetic pathways of all twenty amino acids is regulated. Even in prokaryotes the pattern of regulation of a single amino

acid biosynthetic pathway in one bacterium may be different from that in another bacterium, even though the pathway itself may be identical in the two organisms (Umbarger, 1981). That only c. 0.1% of the soil microorganisms have been cultured (Tiedje et al., 2001), highlights the fact that we know so little about their metabolic roles. The soil food web is an extremely complex system, and microbes differ in their preferences and ability to utilise organic or mineral N (Lekkerkerk et al., 1990).

Catabolism of amino acids serves two kinds of function: firstly as a source of energy and carbon by cells that cannot attack carbohydrates or lipids or when the usual energy sources are not in sufficient supply, and secondly to supply N when an adequate supply of N is not available (Umbarger, 1981). Amino acids may also be used in a biosynthetic role in the synthesis of polyamines. While most microorganisms using preformed organic compounds as carbon sources use sugars preferentially, some must rely on other compounds such as amino acids as sources of carbon. Even organisms growing best on a sugar and ammonia as single carbon and N sources will often use certain amino acids as carbon sources.

When used in catabolism, amino acids supply an excess amount of N. An ample supply of carbon in a readily utilised form usually means that not all the N in an amino acid that may be supplied can be used (Umbarger, 1981).

Umbarger (1981) provides a summary of enzyme inducers and whether their formation is conditioned by C or N control for various amino acids. In some cases the amino acid catabolic pathway may have the physiological role either of yielding energy or carbon with N as a by-product or of yielding N with the carbon and energy as a by product, and regulation is accordingly adapted to the physiological role. Some amino acid catabolic enzymes are apparently not subject to either a carbon or a N control.

Amino acids are taken up by bacteria by the use of transporters in the cytoplasmic membrane, using a respiration driven membrane potential, requiring a C source, such as malate (Krulwich et al., 1977), and/or a ΔpH gradient. Amino acid uptake may be via a single homogeneous system (Glover et al., 1975) or, as has commonly been shown, a mixed system with at least two kinetically distinguishable components, usually a high-affinity and a low-affinity one (Gupta and Howard, 1971; Kay and Gronlund, 1971; Tang and Howard, 1973; Hoban and Lyric, 1977). These transport systems have the following characteristics: they vary in the specificity of recognition of amino acids (Anraku, 1980); they require energy and are subject to temperature and pH optima (characteristics of active transport systems); and they

may or may not be inhibited by the presence of certain amino acids (D'ambrosio et al., 1973).

Mixed systems for metabolite transport often include 1) a general non-specific transport system with a low Km value that makes an organism a good 'scavenger', efficient at concentrative uptake, removing traces of amino acids from the external media (Tang and Howard, 1973), and 2) a low-affinity phase, well suited to the transport of large amounts of substrate at high extracellular concentration, where Vmax is most significant (Glover et al., 1975). Transport systems with narrow specificity may be active in the presence of nutrients, but under conditions of starvation, an active transport system with very broad specificity may be used (Tang and Howard, 1973).

Absorbed amino acids enter an internal intermediary pool (in fungi, Tang and Howard, 1973) in the water compartment of the liquid cytoplasm, from where they are taken for protein synthesis on the ribosome or adsorbed into cell components or separate liquid compartments such as submicroscopic vesicles of the endoplasmic reticulum or vacuoles (Zalokar, 1961). There is therefore a lag found in the incorporation of amino acids into proteins. The time necessary to make a molecule of protein (a polypeptide chain) is in the order of magnitude of a few seconds or less (in bacteria, yeast and *Neurospora*; Zalokar, 1961). In bacteria exogenous amino acids are used for protein synthesis in preference to the endogenously formed amino acids and they often suppress the production of the latter (Zalokar, 1961).

Some microbes can use amino acids as a sole C source (Miller and Rodwell, 1971; Tang and Howard, 1973; Krulwich et al., 1977), and some bacteria may be able to take up amino acids as peptides, yielding more energy than the uptake of the equivalent free amino acids (Leach and Snell, 1960).

Mycorrhizal fungi may both enhance mineralisation, by exuding extracellular acid protease enzymes and carry out ammonification (Leake and Read, 1990a and b).

Degens and Harris (1997) examined the substrate induced respiration (SIR) responses to a wide range of substrates, and found that the amino acids asparagine, lysine, serine, phenylalanine, tyrosine, cysteine, arginine, glutamic acid, leucine and histidine were among those that gave the most varied responses across soil types, and the responses were not closely linked to the total soil microbial biomass (SMB). This indicates that differences in the SIR responses of the SMB may exist because of different soil type, or within the same soil type due to differences in the composition of mineralisable organic matter in soil. However, it remains to be proven conclusively

that catabolic diversity characterised by the SIR technique is directly related to the functional diversity of the microbial community (Hopkins et al., 1994).

2.2.2.3 Nitrogen mineralisation

Around 1-2% of organic N is mineralised per year (Smith et al., 1993), and the rate of N mineralisation is closely related to that of organic C (Omay et al., 1997). Examples of mineralisation rates include: cultivated land 11-300 kg N ha^{-1} yr^{-1}, forest 25-200 kg N ha^{-1} yr^{-1}, and grassland 95-380 kg N ha^{-1} yr^{-1} (Smith et al., 1993). The mineralisation rate is affected by many factors including the composition of the decomposing substrates, soil temperature, moisture content and pH (Vinten and Smith, 1993). Tate (1995) describes the modelling of N mineralisation dynamics.

Ammonification (organic N $\rightarrow$ NH_3 or NH_4^+) is carried out by heterotrophic microorganisms, including a wide range of bacteria, actinomycetes, fungi and algae (Stevenson, 1986), in aerobic or anaerobic conditions. Extracellularly, enzymes e.g. proteases, lysozymes, nucleases and ureases, initiate degradation of N polymers found outside the cell such as proteins, cell walls, nucleic acids and urea (Maier, 2000). Monomers (e.g. amino acids) may then be taken up by the cell and degraded further, or digested extracellularly to release NH_4^+ directly to the environment (e.g. urea plus H_2O plus urease gives ammonia and CO_2 (Maier, 2000)).

N surplus to cellular requirements is expelled as NH_4^+, and the C:N ratio of substrates degraded by the soil microbial biomass that divides a state of net N immobilisation (in biomass) or net mineralisation (exudation of NH_4^+) is around 20:1 (Maier, 2000). It is only possible to measure gross rates of mineralisation using isotope methods, which have shown that the gross rates are very different from net rates and much faster than previously thought (Goulding, 2000).

The change in the pool of inorganic N in the soil is calculated by the balance:

$$\Delta N_{inorganic} = N_{\text{min eralized}} - (N_{\substack{usedby \\ microbes}} + N_{\substack{usedby \\ plants}} + N_{volatilized} + N_{leached} + N_{denitrified}) \text{ (Tate,1995)}$$

The NH_4^+ may be:

1. taken up by a plant
2. trapped in particulates (only becoming available for bacterial or chemical oxidation when bioturbation by macrofauna exposes it to O_2 (Jørgensen, 1989).

3. used in microbial growth, where it is generally preferred to NO_3^-, as it is in the correct reduction state for incorporation into amino acids (Paul and Clark, 1989). NH_4^+ is assimilated (immobilised) by cells into amino acids to form protein, cell wall components (e.g. N-acetylmuramic acid), and purines and pyrimidines to form nucleic acids. This may be via 2 pathways:1) reversible incorporation of ammonia from glutamate at high NH_4^+ concentrations. 2) At low NH_4^+ concentrations, NH_4^+ is added to glutamate to form glutamine and a second step transfers an NH_4^+ molecule from glutamine to α-ketoglutrate resulting in the formation of two gutamate molecules; this uses ATP and two enzymes, glutamine synthase and glutamate synthase (Maier, 2000). Most bacteria can make all the 20 amino acids necessary for protein biosynthesis using NH_4^+ (Anraku, 1980) by performing deamination and transamination (Paul and Clark, 1989).

4. held on exchange complexes of clay minerals or organic colloids, where it may be replaced by cations in soil solution (Stevenson, 1986).

5. enter the interlayer portion of clay (as it is approximately the same size as a K^+ ion), which can then get fixed if the interlayer space collapses. This is described in detail by Stevenson (1986).

6. react with soil organic matter to form quinone-NH_2 complexes, which are very stable. NH_3 reacts with lignin, a reaction associated with oxidation and is favoured by an alkaline reaction. At pH>7 fixation by organic matter is greater than fixation by clays (Stevenson, 1986). The reaction is as follows: Phenol + $O_2 \rightarrow$ quinone + $NH_3 \rightarrow$ polymer.

7. used as an energy source by special groups of autotrophs in the nitrification process to form NO_2^- and then NO_3^- (see section 2.2.2.4 below).

2.2.2.4 Nitrification

Nitrification involves the oxidation of NH_4^+, in a two-stage process, first to NO_2^- and then to NO_3^-. As nitrification is carried out by aerobic microbes, NH_4^+ tends to accumulate in anaerobic conditions (Stevenson, 1986). The rate of NO_2^- oxidation is greater than the rate of conversion of NH_4^+ to NO_2^- in most soils, hence the concentration of NO_2^- is rarely over 1 ppm.

It was work by Winogradsky (1891 in Brock and Schlegel, 1989) which first showed that there were two processes carried out by two separate groups of organisms involved in nitrification, and he demonstrated that they used CO_2 as their sole carbon

substrate and demonstrated a link between carbon assimilation and nitrification. Assimilation requires energy and the nitrification process is the only means of producing energy for these organisms (Brock and Schlegel, 1989).

The *Nitrosomonas* genus oxidises NH_4^+ to NO_2^- and *Nitrobacter* oxidises NO_2^- to NO_3^- (Maier, 2000). Nitrifying bacteria, of the family *Nitrobacteraceae* (Bock et al., 1989), with one exception, are obligate lithotrophs, although they can assimilate organic compounds to a limited extent (Bock et al., 1989). These gram-negative chemoautotrophic bacteria are obligate aerobes, but are not obligate autotrophs, i.e. they do not have to use inorganic C for cell synthesis, but most of it is from HCO_3^- in soil water (Stevenson, 1986), which is assimilated via the Calvin Cycle (Bock et al., 1989).

In aerobic conditions NH_4^+ concentration is low as it is quickly oxidised by nitrifiers. Under anaerobic conditions, NH_3 is produced by ammonification or dissimilatory NO_3^- reduction. The ammonia diffuses to the anaerobic interface where nitrifiers are thought to live, where, if the NH_3 concentration is too high to be oxidised at the interface, it diffuses to the aerobic phase and is oxidised under fully aerobic conditions (Keunen and Bos, 1989). Although nitrification peaks at the aerobic/anaerobic interface in soil, many nitrifying autotrophs have a high affinity constant (Ks) for oxygen (it is 10-20 μM, whereas many other aerobes have a Ks of 1-2 μ M, Keunen and Bos, 1989). It is a strategy of some microbes at the anaerobic/aerobic interface to be able to denitrify NO_3^- or NO_2^- using endogenous or exogenous organic compounds as electron donors.

The optimal moisture content for nitrification is 60% of field capacity. *Nitrosomonas sp.* are more susceptible to dry conditions than *Nitrobacter,* but sufficient bacteria survive short periods of desiccation for increased nitrification to occur during the flush of decomposition which follows the rewetting of an air-dry soil (White, 1997).

Nitrification is affected by the concentration of substrates: NH_4^+, O_2, and CO_2 as HCO_3^- in the soil solution. Oxygen may be limited by depletion by heterotrophs in the presence of easily decomposable organic matter, excess moisture and high temperatures (reduced solubility).

Nitrification is predominantly an aerobic autotrophic process, but some methylotrophs can use the methane mono-oxygenase enzyme to oxidise NH_4^+ and a few heterotrophic fungi and bacteria can also perform this oxidation (Maier, 2000). Heterotrophic microbes that oxidise NH_4^+ include some fungi, bacteria and actinomycetes (Paul and Clark, 1989), but they gain no energy from nitrification, so it

is unclear why they carry out the reaction (Maier, 2000). Heterotrophic nitrifiers cannot grow autotrophically and the co-oxidation process is not coupled to growth (Bock et al., 1989). Heterotrophic nitrification is usually an order of magnitude lower than autotrophic, but may be more important in some environments (Maier, 2000). Haynes (1986) reviews autotrophic, heterotrophic, methylotrophic and chemical nitrification in detail.

Nitrification proceeds under a range of conditions (Bock et al., 1989) as there are specialised nitrifiers that occupy specialised ecological niches (with varying conditions of salt concentration, temperature, substrate availability and concentration). In natural ecosystems several different species of nitrifiers coexist (Bock et al., 1989). Most species have an optimum substrate concentration of up to 10 mM, and NO_3^- and NO_2^- become inhibitory at concentrations of 300 mg l^{-1} for *Nitrosomonas* and 4,000 mg l^{-1} for *Nitrobacter* (Bock et al., 1989).

NO_2^- may be subject to chemo denitrification in aerobic conditions, a process favoured by acidic conditions, and it should be noted that at the surface of clay particles the pH may be lower than it is in bulk soil (Stevenson, 1986), e.g. ferric oxyhydroxides (Jørgensen, 1989). The reaction can be summarised by one of the following:

- NO_2^- plus humic compound → nitroso and oximino compounds → organic N complexes → NO_2^- → $N_2 + N_2O$ (Stevenson, 1986)
- With ammonia: $NH_3 + HNO_2 \rightarrow NH_4NO_2 \rightarrow 2H_2O + N_2$
- With amino acids: $RNH_2 + HNO_2 \rightarrow ROH + H_2O + N_2$

However, NO_2^- tends to convert to NO_3^- and NO more than it would react with NH_4^+ and amino acids (Stevenson, 1986).

2.2.2.4.1 Micro-organisms involved in nitrification

The physiology and morphology of ammonia and NO_2^- oxidisers are described in detail by Stevenson (1986), Bock et al. (1989) and Hooper (1989); most, but not all are motile. Table 2-2 below summarises the nitrifying bacteria, and the processes they perform are described in sections 2.2.2.4.2 and 2.2.2.4.3 below.

Table 2-2 The organisms that perform nitrification (Paul and Clark, 1989).

PROCESS	GENUS	SPECIES	HABITAT
$NH_3 \rightarrow NO_2^-$	*Nitrosomonas*	*europaea*	Soil, water, sewage
	Nitrospira	*briensis*	Soil
	Notrosococcus	*nitrosus*	Marine
		mobilis	Soil
	Nitrosovibrio	*tenuis*	Soil
$NO_2^- \rightarrow NO_3^-$	*Nitrobacter*	*winogradskyi**	Soil
		*agilis**	Soil, water
	Nitrospira	*gracilis*	Marine
	Nitrococcus	*mobilis*	Marine
	**N.winogradskyi* has at least 2 serotypes, one of which was referred to as *N.agilis*		

2.2.2.4.2 Stage one – NH_4^+ conversion to NO_2^-

This stage produces energy, which is used to fix CO_2 and provide energy for growth. The reaction is inefficient, requiring 34 moles of NH_4^+ to fix 1 mole of CO_2 and 1 mole of N transformed gives a free energy change of 65 kcal (Paul and Clark, 1989). This stage is catalysed by NH_4^+ mono-oxygenase.

Molecules are taken over the cell membrane by carrier molecules; in eucaryotic cells the electron transport chain is on the inner membrane of mitochondria, in prokaryotic cells it is in the plasma membrane (Tortora et al. 1995). Each molecule in the chain is reduced as it picks up and oxidised as it gives up electrons. The reader is referred to Stevenson (1986), Bock et al. (1989) and Hooper (1989) for the detail of biochemical reactions involved in this stage. Ammonia oxidation in cells is sensitive to inhibition by copper binding agents and activity is destroyed by light of 400-430 nm (Hooper, 1989).

2.2.2.4.3 Stage two – NO_3^- conversion to NO_2^-

This second step is even less efficient than the first, requiring 100 moles of NO_2^- to fix 1 mole of CO_2 (Maier, 2000). 1 mole converted gives a free energy change of 18.2 kcal (Paul and Clark, 1989; 17.8 kcal in Stevenson, 1986). The reader is referred to Stevenson (1986), Bock et al. (1989), Hooper (1989) for details of the biochemical reactions. 2.8 times more CO_2 is fixed per NH_3 oxidised to NO_2^- by *Nitrosomonas* than per NO_2^- oxidised to NO_3^- in *Nitrobacter.*

2.2.2.4.4 Fate of NO_3^-

The generated NO_3^- may then be:

1. denitrified by microbes resulting in gaseous losses of N_2O and N_2 (see section 2.2.2.4.4.1 below).

2. taken up by microbes (assimilatory reduction) whereby it is used in amino acid production and immobilized.
3. taken up by a plant.
4. in the absence of O_2, used by microbes as an electron acceptor and reduced to NH_4^+ (dissimilatory reduction). E.g. in respiration by sulphate reducing bacteria or in reoxidising NADH e.g. clostridia (Jørgensen, 1989).
5. leached (see section 2.4)
6. eroded in run off
7. accumulated in soil.

2.2.2.4.4.1 Denitrification

There are 33 genera of bacteria that can perform denitrification (Stevenson, 1986). The process is favoured by anoxic conditions (Bock et al., 1989; Tate, 1995) as it occurs during anaerobic respiration when NO_3^- acts as a terminal electron acceptor, although there are bacteria that can denitrify in the presence of oxygen (Jørgensen, 1989).

The reduction sequence is as follows (Stevenson, 1986):

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

+5 +3 +2 -1 Reduction state.

Step 1 (NO_3^- to N_2O) requires dissimilatory NO_3^- reductase. Most, but not all denitrifiers can carry out the second step, (N_2O to N_2). N_2O is a gas and may be lost before it is further reduced (Stevenson, 1986). Anaerobic respiration gives less energy than aerobic so a larger amount of substrate must be oxidised per unit C assimilated. In addition, less N is assimilated into microbial tissue so microbes may compete less with plants for available N in wet environments (Stevenson, 1986).

N_2O is a greenhouse gas. It is stable in the troposphere and can diffuse into the stratosphere where it reacts with O_3 following photodissociation (Maier, 2000).

Optimal conditions for denitrification are as follows (from Stevenson, 1986):

- Poor drainage – denitrification is negligible at 2/3 water holding capacity, but is appreciable in flooded soil.
- Temperature over 25°C; denitrification decreases as temperature decreases and practically stops at 2°C.
- pH around 7, there are few denitrifying bacteria at low pH.

- The presence of inorganic N in the form of NO_3^- and a supply of readily decomposable organic matter (hence denitrification is higher in the top soil than sub soil).

Losses of applied fertiliser N due to denitrification may range from 2.5 to over 50% (Vinten and Smith, 1993). Subsoil denitrification is a mechanism whereby the quantity of leached NO_3^- in aquifers may be reduced. Chen et al. (1995) examined the destination of labelled NO_3^- in anaerobic soils, and found recovery on day 6 of 1.0 - 5.8% in organic form, 1.2 - 1.5% as NH_4^+, 0 - 0.8% as NO_2^- and NO_3^-, and 93 - 96% as N_2. Denitrification may be high (over 0.2 kg N ha^{-1} d^{-1}) when soil NO_3^- contents exceed 5 mg kg^{-1}, and so tends to peak in spring and autumn in the UK, and denitrification is increased by high soil carbon contents (Webster & Goulding, 1989). The process of denitrification consumes acidity (Sumner et al., 1991).

2.3 Factors influencing nitrogen cycling processes

This section describes how certain factors affect the mineralisation and nitrification processes, namely temperature, depth, tillage, additions of N, N fixation, the rhizosphere, soil water, aeration and seasonality, with emphasis given to section 2.3.8 on soil pH. A useful review of N release from soil organic matter, and pulses of N released from crop residues (including ploughed grass and cover crops) from manures and from pasture soils, and the effects of cultivation is provided by Shepherd et al. (1996).

2.3.1 Temperature

Temperature affects the metabolic functioning of soil microbes and the access of microbes to substrate pools (MacDonald et al., 1995). It also influences the proportion of C or N substrate used in biomass, with more remaining in biomass at higher temperatures (Nicolardot et al., 1994).

Decomposition of plant residues is temperature sensitive, but even at temperatures as low as 1°C mineralisation is not negligible (Van Schöll et al., 1997); salt content in the soil moisture may lower freezing temperature. The temperature-net mineralisation relationship at optimal soil water content is an Arhenius function with a Q_{10} of approximately 2 (Kladivko and Keeney, 1987 in Jarvis et al., 1996). Nitrification has an optimum temperature of 30-35°C and the process is slow below 5°C and over 40°C (Paul and Clark, 1989).

2.3.2 Depth

Microbial biomass C and N and gross N mineralisation are greatest at the surface of the soil and decline with depth (Murphy et al., 1998) because of decreasing availability of plant residues and chemical degradability of N compounds. In eastern Australian clay soils, subsoil mineralisation made up only a partial contribution to the accumulated NO_3^- pool in subsoils. The major source was leaching of N mineralised in the surface layers of soil under crops and pasture (Weier and MacRae, 1993).

2.3.3 Tillage

Cultivation is an oxidative process, encouraging mineralisation of organic N and nitrification of NH_4^+-N. It may take 50-100 years to reach steady state conditions after ploughing out of mature grassland (Haynes, 1986). Incorporation of residues increases the rate of decomposition compared to no-till (Omay et al., 1997).

For this reason, time of ploughing and cultivation is an important management tool that can influence N dynamics, such as NO_3^- leaching (Djurhuus and Olsen, 1997). Ploughing out of leys should be postponed until spring, especially on coarse sands and sandy loams. This need not have adverse effects on yields of spring cereals (Djurhuus and Olsen, 1997). However, many farmers will plough in the autumn as winter cereals are higher yielding than spring cereals. Autumn re-seeding of pastures also tends to be more successful than spring re-seeding.

No-till soils have a higher amount of organic N and biomass N than under plough tillage, which is likely to have a substantial effect on nutrient cycling in cropping systems. It has been observed that the efficiency of use of N fertilisers by crop plants is lower under no-till than plough-tillage. Tillage method has a marked effect on both the amount and distribution of soil N in the pools of total N, biomass N and C and active N (Simard et al., 1994; McCarty et al., 1995), which in turn will have an impact on the fate of fertiliser N.

2.3.4 Nitrogen additions

2.3.4.1 Inorganic

Artificial fertiliser-N is typically applied to arable crops and grassland at rates up to 400 kg N ha^{-1} in the UK, usually in the form of NH_4^+, NO_3^-, urea or other NH_4^+ or NO_3^- salts. Stevenson (1986) describes the fate of fertiliser N and how it moves through various pools over time. An increase in fertiliser N causes an increase in the uptake of native humus N, especially if the active fraction has been maintained at a high level by making decomposable organic matter continually available. There are two

theories to explain this: increased mineralisation-immobilization turnover "MIT" or a 'priming action of the fertiliser' (Stevenson, 1986). The priming action may work by two mechanisms (Jenkinson, 1981): a) a direct action whereby there is activation of dormant organisms and enhanced attack on humified organic matter through a general increase in enzyme activity, and b) an indirect action caused by the alteration of the microbial environment affecting the decomposition of the indigenous organic matter (e.g. pH change, oxygen may become deficient for a time and microbial nutrients such as N may become limiting). Long term fertiliser treatments have increased the specific mineralisation rate of the biomass (N mineralised per unit of biomass); an extra 30 kg N ha^{-1} is mineralised and taken up by the crop on a plot given 144 kg N ha^{-1} for 135 years compared to a plot given no mineral fertiliser (Glendining et al., 1996).

Fertiliser additions can have far-reaching environmental consequences of disruptions to the N cycle. For example, the capacity of soil to absorb methane (CH_4) from the atmosphere via microbial oxidation can be reduced by the long-term application of mineral N fertiliser, whereas organic fertiliser addition was found not to have the same effect (Hütsch et al., 1993).

Nitrification of fertiliser- NH_4^+-N can be rapid, over a period of weeks, although the rate of NO_3^- production can be decreased by higher application rates (Watson et al., 1995).

Inorganic fertilisers may temporarily decrease the microbial biomass (Thomsen, 1993). However, the continued long-term use of N fertiliser has been shown to slow the decline of or increase the soil organic N, largely by increasing the return of N in residues (Glendining et al., 1996; Jarvis et al., 1996), resulting in more soil organic N and therefore more mineralisable N (Shen et al., 1989; Thomsen, 1993). This increase in organic N return comes from both the amount of crop residue and its N concentration, and the latter also influences its rate of mineralisation (Jenkinson, 1984). Inorganic fertiliser increased the SMB in the Broadbalk wheat experiment compared to the plot that had never received inorganic N but there was little difference in the microbial biomass N content of soils that had 48, 96, 144 or 192 kg N ha^{-1} for many years (Shen et al., 1989). Inorganic nitrogen addition did not affect the rate of amino acid mineralisation or the carbon use efficiency of amino acids by the SMB (Jones and Shannon, 1999).

Greater mineralisation may have increased N loss via leaching and gaseous emissions, but fertiliser N may increase absorption of atmospheric N as the larger crops have a greater surface area (Glendining et al., 1996).

The alternative to inorganic fertiliser inputs, biological N fixation, may use lower inputs of energy (in terms of production, distribution and application of fertilisers), but it may not improve on leaching losses. At equal levels of animal production, leaching losses from pastures are likely to be similar for grass/clover and fertilised grass systems. However, unfertilised grass/clover swards, carrying a reduced stocking level than is possible with fertilised grass, may result in substantially less leaching loss and may be the more economically viable system due to reduced input costs (Cuttle et al., 1998).

Net total atmospheric inputs are in the region of 40-45 kg N ha^{-1} y^{-1}, much of which comes from vehicles and industry; around 30% of this N is leached (from land receiving 200 kg ha^{-1} of fertiliser N) because it is deposited when the crop cannot use it (Goulding and Poulton, 1992).

2.3.4.2 Organic – plant

The N-content of plant material is 0.1 – 6 % depending on the plant species, the part and age. The N concentration of plant tissue tends to decrease with age, and in most mature plants N is < 1 % (Haynes, 1986). Crop residue N after harvest may range from 20 to 145 kg ha^{-1} (Sylvester-Bradley, 1993), and may be over 200 kg ha^{-1} for vegetable crops (Rahn et al., 1992). Root exudates of amino and other organic acids from root tips (via passive diffusion) provide a labile substrate for rhizosphere microbes (Jones and Darrah, 1994).

Crop residues contain three classes of components: 1) cell wall and structural materials (cellulose) and cementing/encrusting materials (carbohydrates predominating in young tissue, lignin in older tissues); 2) reserve substrates (starch, fats, proteins); 3) cell contents (proteins, sugars, unassimilated NO_3^-, NO_2^-, NH_4^+) (Jarvis et al., 1996). The N in leaves and stems is approximately 60% enzyme/membrane protein and 40% water-soluble amino acids. Over 90% of the N in seeds is in the form of storage protein (Haynes, 1986). Plant matter is generally 10-30% protein by weight and contains free amino acids in the region of 10 mM (Jones and Darrah, 1994). After digestion of proteins by a range of proteases concentrations of amino acids in the soil solution may be in the region of 10-200 µM (Monreal and McGill, 1985). Sugars, polysaccharides, proteins, amino acids,

aliphatic acids may lose 80-90% of their C in 3-6 months, whereas lignins, fats and waxes take much longer (Haynes, 1986).

The decomposition of organic detritus is especially important in terms of N availability to crops, in particular how it affects immobilisation. When the substrate C:N ratio is around 20 to 25:1 there is no net immobilization/mobilization (Maier, 2000). Below this there is net mineralisation (an increase in mineral N) and over this there is net immobilisation (a decrease in mineral N). By causing the temporary net immobilisation of N as the carbon is decomposed, straw may be used to ameliorate NO_3^- leaching. Recous et al. (1995) found immobilisation to be 39 mg N g^{-1} added C after 40 days. If the N content of the residue is high, as in legumes, there may be immediate net mineralisation (Haynes, 1986). As organic matter is broken down, the C:N drops as C is lost as CO_2, so the relative proportion of N increases.

Immobilization predominantly uses N from the NH_4^+ pool, but when NH_4^+ is not available, NO_3^- is assimilated by the SMB in the presence of readily accessible C (Jarvis et al., 1996). At a microsite scale there may be direct immobilization of small organic compounds such as amino acids (Drury et al., 1991). N may become immobilised in 3 days (glucose substrate) or over 2 months (a mature crop residue substrate) (Stevenson, 1986). The C:N ratio is only a guide as to whether there will be net immobilization or mineralisation, as not all the C and N is readily available to microbes, and the chemical composition, competitive activities and community structure of the SMB also vary between different systems and therefore influence the efficiency of C use and N demand (Ladd and Foster, 1988).

Recently added organic N from plant residue is seven times more mineralisable than native soil organic N (Shen et al., 1989), and biomass-N increases rapidly (over several weeks) in response to plant residue additions (Thomsen, 1993).

Grass/clover leys increase soil organic matter, and fertiliser-N application can be decreased for two years subsequent to ploughing out, due to increased N mineralisation (MAFF, 2000). NO_3^- leaching may be present in the second and third year after ploughing out a grass/clover ley, irrespective of timing of ploughing out (Djurhuus and Olsen, 1997). It is essential that cropping systems should make use of the mineralised N as it becomes available in order to reduce leaching. The mineralisable pool of N can be increased over the long term by frequent additions of organic matter, especially from systems that produce large amounts of crop residue and are fertilised (Omay et al., 1997). Although organic amendments are an important source of generally readily mineralised N for a following crop they are also

a major source of N lost from the system, so optimal timing and quantity of application is important for the efficient use of the resource (Jarvis et al., 1996).

Approximately 20% of original plant addition is retained in the soil, even though the initial decomposition rates vary, because of the similarity in microbial production and stabilisation of soil organic matter (Paul and Clark, 1989). The decomposition rate constant K describing the decomposition rate of plant material is nearly always independent of the quantity added, if the C added does not exceed 1.5% of the soil dry weight (Paul and Clark, 1989).

The presence of organic matter is not inhibitory to nitrification directly, but the supply of NH_4^+ and O_2 to nitrifiers may be restricted when used in the decomposition process (Paul and Clark, 1989); root exudates may inhibit nitrification in this way (Stevenson, 1986).

2.3.4.3 Organic - animal origin

Animal manures are very variable in composition and depend on species, diet, dilution by water, storage, method of housing, type of bedding and composting. Cattle utilise only 15-20% of the N in their food, and 50-80% is excreted in urine as urea (which is rapidly hydrolysed) and faeces (where the mineral elements are bound to resistant organic fractions so the N is released more slowly than from fresh plant material).

Slurry behaves like a slow release fertiliser, although denitrification losses can be high (Estavillo et al., 1997). Several authors have reported that the application of fertiliser can stimulate, decrease or have no effect on the mineralisation of native organic N in soil (Haynes, 1986). In manures the critical C:N ratio (dividing mineralisation/immobilisation) is 15:1 (Jarvis et al., 1996). A mineralisation peak may only occur during the first hours after slurry application and a few days later the mineralisation rate is similar to that in non fertilized soils (Estavillo et al., 1997). Slurry may have to be applied for years to produce an increase in total soil organic C and N which leads to a permanent increase in soil biomass (Estavillo et al., 1997). The SMB of an organic manure amended soil was the least heterogeneous and the most metabolically active of various farming systems compared by Wander et al. (1995).

Lovell and Jarvis (1996) found urine increased soil respiration rates. High levels of NH_4^+ in urine treated soils were nitrified over a period of 42 days and there was a short lived (<40 days) but substantial emission of nitric and nitrous oxides. Land at

Rothamsted regularly manured with farm yard manure loses four times the amount of N by leaching and by denitrification as that lost from land receiving approximately the same amount of N as inorganic fertiliser (Goulding and Poulton, 1992)

2.3.5 Biological nitrogen fixation (BNF)

Fixation of atmospheric N to ammonia provides a significant input of N to the soil-plant N cycle, and is of key importance in organic and low input agricultural systems during leys. Bacteria that fix atmospheric N may be symbiotic (live within a plant) or non-symbiotic (free living). Symbiotic fixers in legumes are of the genus *Rhizobium* and in non-legumes are of the genus *Frankia* (Stevenson, 1986). Non-symbiotic fixers include some photosynthetic bacteria e.g. *Rhodospirillum*, some aerobic bacteria e.g. *Azotobacter*, some blue green algae of family *Nostocaceae*, some anaerobic bacteria of genus *Clostridium*. Stevenson (1986) provides a description of the biochemistry involved in BNF. Symbiotic N_2 fixation is affected by light, temperature, water relations, soil pH and mineral N in soil. High amounts of available N in soil tends to depress nodule formation because of a low carbohydrate : N ratio in the plant resulting in an inadequate supply of carbohydrate to the root (Stevenson, 1986).

Although N derived from BNF does not incur the external environmental costs of inorganic fertiliser-N, it is not free of environmental considerations. In extensively managed pasture systems, utilising BNF from legumes, soil acidification may be accelerated (von Uexküll and Mutert, 1995). Also, Cuttle et al. (1996) found that there was a direct correspondence between the NO_3^- loss and the proportion of clover in a pasture sward.

2.3.6 Rhizosphere

Rhizosphere processes play a key role in nutrient cycling in terrestrial ecosystems (Toal et al., 2000). Because of the large number of bacteria in bulk soil and their diversity, substrate is consumed until it becomes limiting. Thus these free living bacteria exist in a state of limited starvation and become dwarf bacteria of diameter less than 0.3 µm. The rhizosphere is the one zone in soil where substrate is not limiting as plant photosynthates supply a constant source of available carbon (Reynolds and Pepper, 2000). Between 10-40% of the increase in root dry matter is exuded from the zone immediately behind the root tip which provides hotspots for microbial activity (Jarvis et al., 1996). Populations of bacteria and protozoa are higher with increasing proximity to roots (Badalucco et al., 1996).

The work of Meharg and Killham (1995) on perennial ryegrass suggests that the presence of microbial metabolites stimulates root exudation, but the amount is dependent on the species of microbes present. Under axenic conditions, root exudates of maize constituted 65% sugars, 33% organic acids and 2% amino acids (Kraffczyk et al., 1984). The main amino acid, glutamic acid, was nearly doubled by the presence of microorganisms, whereas other amino acids remained unchanged, and exudates were increased by low K supply. Activities of protease and deaminase enzymes are stimulated in the rhizosphere, enhancing decomposition of root exudates and native soil organic matter (Badalucco et al., 1996). At higher levels of available N, (i.e. where N competition between plants and microbes is reduced) there is an increase in root-induced immobilisation of N (Bremer and Kuikman, 1997).

Carbon lost from roots is readily available and used preferentially, and this results in a stimulatory effect on the net mineralisation of indigenous soil organic N (Griffiths and Robinson, 1992). The mechanism for this is either a) through competition for inorganic N between plants and microbes, or b) the release of C from roots induces microbes to mineralise soil organic N to support their own growth, which subsequently becomes available when the bacteria are grazed by nematodes or protozoa (Griffiths and Robinson, 1992). Either way, Griffiths and Robinson (1992) conclude that microbes do not use plant-derived C to mineralise soil organic N to any great extent, and that root induced N mineralisation merely allows for efficient recycling of N lost through roots.

The influx of amino acids to roots (by active transport) may regulate rhizosphere SMB populations (Jones and Darrah, 1993, 1994), although Owen and Jones (2001) demonstrated that plant roots are poor competitors with the SMB for organic N, capturing only 6% of added free amino acids. Microbes have the competitive advantages of complete coverage of the soil volume, occupying niches where they can filter out resources as they pass towards the root, and some have the ability to move towards hotspots of substrates (Owen and Jones, 2001).

2.3.7 Soil water, aeration and seasonality

Soil water has a number of effects on N processes (Jarvis et al., 1996):

- deficiency/stress limits biological activities
- excess reduces aerobicity and alters the activities of different microbial populations

- it controls solute diffusion and mass distribution of the products of microbial activity
- cycles of wetting and drying increase the availability of substrates. Upon drying, organic N compounds become more soluble and water-stable aggregates are broken down revealing new surfaces to microbes (Stevenson, 1986), although Franzluebbers et al. (1994) suggest that drying and wetting of soil can reduce N mineralisation by increasing the resistance of the organic N to microbial decomposition

Soil moisture is intrinsically linked to soil texture, which influences mineralisation by (Jarvis et al., 1996):

- influencing moisture/aeration status. Microbial metabolism involving aerobic oxidation of organic C occurs in the range of soil water potentials encountered for growth of higher plants; -33 to –1500 kPa, with –40 to –60 kPa optimal for soils of < 40% clay (Voroney et al., 1991). Nitrification proceeds readily at -100 to -1000 kPa water stress, soil water content and aeration are important as nitrifiers are obligate aerobes (Paul and Clark, 1989; Strong et al., 1997).
- affecting the physical distribution of organic materials and hence their potential for degradation
- conferring protection of organic substrates through association with clay particles.

The factors of rainfall, aeration, water status and temperature combine to give a seasonal variation in mineral N accumulation. In temperate regions nitrification is greatest in spring and autumn and slowest in summer and winter (Paul and Clark, 1989). Mineral N levels are low in winter due to leaching; they rise in spring by 4-6 fold as rising temperatures increase mineralisation. Levels are lower in summer as plants take up the available mineral N and then rise in the autumn as plant growth slows and residues decay (Stevenson, 1986).

Temporal fluctuations in microbial C and N concentrations were found to be small and were related to variability in total C and N concentrations in a study by Ross et al. (1995); potential rates of C and N mineralisation were more susceptible to seasonal effects than were the microbial biomass pools. Seasonal changes in biomass C and N may be related to increased availability of root exudates as root growth and turnover increase in spring. Lovell et al. (1995) found the spring biomass N increase came before that of C, increasing the C:N of the biomass.

2.3.8 Soil pH

Soil pH is one of the most important factors influencing decomposition (Haynes, 1986), the dynamics of many aspects of soil biochemistry and the botanical composition of the soil-plant system (Johnston, 1994). It is widely accepted that organic matter decomposition generally proceeds more readily in neutral than in acid soils. Although soil pH has a profound effect on soil organic matter preservation and decomposition, its precise mode of influence has yet to be fully established (Van Bergen et al., 1998).

It is well known that pH influences the availability of many nutrients in soil (Sumner et al, 1991). Concentrations in the soil solution of elements present as anions generally increase with increasing pH (partly explainable by decreasing anion exchange capacity) and concentrations of cationic elements decrease with rising pH (Tyler and Olsson, 2001). The concentration decreases in several cationic elements that form stable soluble complexes with organic matter are reversed above pH 7, causing U or irregular shaped curves of variation with pH (Tyler and Olsson, 2001), although the minimum soil pH examined in the study by Tyler and Olsson (2001) was only 5.2.

Availability of nutrients important to plant growth may increase (e.g. Ca, Mg, Na, available N) or decrease (e.g. Mn) with increasing soil pH (Meharg and Killham, 1990). Below pH 4.5, Al becomes much more soluble, as does Mn (due to oxidation of divalent manganese) (Helyar and Anderson, 1974). Both metals are toxic to plant growth at high concentrations (Meharg and Killham, 1990). Aluminium and manganese toxicity in plants and microbes are described in detail by Foy (1984). Rhizobia survival, multiplication, root infection and nodule initiation are impaired by acidity through the combined effect of proton and aluminium toxicities and other factors (Foy, 1984). Zn and to a lesser degree, Cu induced phytotoxicity causes a reduction in rhizobial N fixation, even at soil pH 6, and effects are exacerbated in acidic soils (Chaudri et al., 2000).

The pH of rhizosphere soil may be higher or lower than the bulk soil (Marschner et al., 1986), although rhizodeposition supplying low molecular weight C substrates to the soil microbial community (SMC) usually results in elevated levels of acidity surrounding the root (Toal et al., 2000). Meharg and Killham (1990) demonstrated the importance of pH in rhizosphere carbon flow by growing *Lolium perenne* in soils of different pHs and exposing them to a pulse of $^{14}CO_2$. Seven days later, the % of ^{14}C lost from the roots and soil varied from 12.3 to 30.6 with changes in soil pH from 4.3 to 6; the greater loss of C from roots with increasing pH may be due to changes in the SMB species composition or activity. A possible explanation is increased NO_3^-

availability at higher pH (NO_3^- availability rose by 400% from pH 4.3 to 5.7) resulting in more bicarbonate being released from roots, enhancing microbial activity.

In acid topsoils where microbial populations are high, the microbes may provide a considerable sink for organic acids, which may markedly compromise root mediated mechanisms for coping with stress, such as Al toxicity or P deficiency (Jones et al., 1996).

2.3.8.1 Liming

It is a traditional practice to apply lime to agricultural land to maintain a suitable pH for crop plants and increase the rate of cycling of N. Liming practices and materials are reviewed by Barber (1984). Ground limestone, chalk, marl and basic slag are used and the active constituents are primarily $CaCO_3$ with some burnt or quick lime (CaO) and hydrated lime ($Ca(OH)_2$) (White, 1997). The effectiveness of a liming material is dependent on its neutralising value (NV, the effectiveness of a liming material compared to pure calcium oxide CaO), its fineness of grinding and the hardness of the parent rock. Ground limestone or ground chalk generally has a NV of 50-55 (MAFF, 2000). The lime requirement depends on the pH buffering capacity of the soil, and is expressed as the quantity of ground limestone or chalk required to raise the soil pH to a desired value as t ha^{-1} to a depth of 15 cm (White, 1997).

White (1997) describes the hydrolysis of lime on dissolving:

$$CaCO_3 + 2H_2O \leftrightarrow Ca(OH)_2 + H_2CO_3$$

$$CaCO_3 + CO_2 + H_2O \leftrightarrow Ca(HCO_3)_2$$

And gives the final pH to be predicted by: $pH = K - 1/2\log(P_{CO_2}) - 1/2\log(Ca)$

Where Pco_2 is the partial pressure of CO_2, (Ca) is the activity of Ca^{2+} ions in solution, and K = 4.8 if the carbonate has the solubility of pure calcite. For carbonates which are more soluble than pure calcite $K \approx 5.2$.

MAFF (2000) gives liming recommendations to maintain pHs (1:2.5 dry soil:water suspension shaken 15 min) at the targets described in Table 2-3 below.

Table 2-3 Target soil pHs for farming systems (MAFF, 2000)

	Optimum soil pH	
	mineral soils	peaty soils
Continuous arable cropping	6.5[a]	5.8
Grass with occasional barley crop	6.2	5.5
Grass with occasional wheat or oat crop	6.0	5.3
Continuous grass or grass/clover sward	6.0	5.3

[a] in arable rotations containing acid sensitive crops such as sugar beet, maintaining a pH of 6.5-7.0 may be justified.

Lime loss is determined by soil type, crops grown, through drainage, atmospheric and fertiliser inputs of nutrients and pollutants and the pH of the soil itself (Goulding et al., 1989). MAFF's advisory service uses the buffer method of Woodruff (1948, in Goulding et al., 1989) to determine the amount of lime needed to adjust the soil pH to 6 or 6.5 and to maintain that pH for 4-5 years. Helyar and Anderson (1974) describe the effect of liming on the solubility and sorption of some plant nutrients.

2.3.8.2 Soil acidification

The chemistry of soil acidity and buffering mechanisms are explained in detail by Thomas and Hargrove (1984) and Rowell (1988). Soil acidification is defined as a decrease in acid neutralising capacity and is accomplished by removal of cationic components from a mineral soil or to a lesser extent by addition of acidic components (van Breemen et al., 1984). An acidifying process produces a proton or consumes a hydroxyl ion (Helyar, 1976). Soil alkalisation is an increase in pH and may involve both the weathering or formation of soil minerals and adsorption or desorption reactions (van Breemen et al., 1984). An alkaline process consumes a proton or produces a hydroxyl ion (Helyar, 1976). Proton consuming and producing processes are summarised in Table 2-4.

In near neutral soils rich in weatherable minerals, soil acidification is mainly associated with H_2CO_3 deprotonation as the principal internal H^+ source (van Breemen et al., 1984). In acidic soils cation assimilation and organic acid deprotonation are the principle internal H^+ sources. Atmospheric deposition of anthropogenically-derived acidic substances (mainly H^+, SO_2, and NH_4^+) may be 1-5 kmol $ha^{-1}y^{-1}$, which may exceed internally generated protons in soils with low to intermediate rates of soil acidification. As a result, potentially toxic inorganic Al is released into the soil solution and H^+ and Al base-neutralising capacity is transported to drainage waters.

Table 2-4 Proton producing and consuming processes in soil (van Breemen et al., 1984)

Proton producing processes	Proton-consuming processes	notes
atmospheric input	drainage	
assimilation of cations	mineralisation of cations	important cations include Ca, Mg, Na, K, NH_4^+
mineralisation of anions	assimilation of anions	$H_2PO_4^-$, SO_4^{2-}, NO_3^-
dissociation of acids	protonation of anions	[a]
oxidations	reductions	reduction oxidation couples include: NH_4^+/NO_3^-, N_2/NO_3^- H_2S/SO_4^{2-}, SO_2/SO_4^{2-}, Fe^{2+}/Fe_2O_3. Organic matter (CH_2O) is usually the electron donor in reduction reactions, organic matter is oxidised during aerobic respiration.
reverse weathering of cations	weathering of metal oxide components	important cations include Ca, Mg, Na, K, Al^{3+}
weathering of anionic components	reverse weathering of anions	$H_2PO_4^-$, SO_4^{2-}

[a]carbonic, organic and nitric acids are continually available to the system through CO_2 and dinitrogen fixation. The protons of these acids are not leached and do not accumulate in soils because they react with the soil minerals, releasing cations into solution; permanent acidification of the ecosystem occurs only when the anion of these acids is lost from the ecosystem as the anion (Helyar, 1976).

Blake et al. (1999) highlight the increasing importance of acid deposition in determining the ratio of external : internal proton inputs. At Rothamsted in 1883 and 1904, 50-60% of the acid inputs were as acid deposition, whereas in 1964 and 1991 this figure was 80-90%. Deposition of acidity because of sulphur and N emissions during the last 110 years is the major cause of soil acidification in Geesecroft Wilderness Rothamsted forest site (Svedrup et al., 1995).

Increasing acidity decreases the base saturation and exchangeable cations, with base cations moving down the soil profile (Blake et al., 1999). Clay minerals are irreversibly weathered and Mn and Al are progressively mobilised (van Breemen et al., 1984), with Al occupying an increasing proportion of the exchange complexes in surface soil (Blake et al., 1999). A detailed description of the silicon-aluminium, manganese and iron buffer systems and associated movement of cations over the range of soil pHs is provided by Blake et al. (1999). Van Breemen (1991) details buffering, acid neutralising capacity and soil acidification.

Although some of the results of soil acidification related to pH dependent charge may be reversible through careful liming, part of the reduction in exchangeable cations represents an irreversible degradation of soil quality as increasing amounts of Al-hydroxy interlayer material blocks interlayer surfaces and reduces expansibility (Svedrup et al., 1995; Blake et al., 1999).

Where natural recovery is possible, it will take a long time, in the order of centuries, especially if acidification occurs to a point where the ion exchange reservoir is nearly empty of nutrient cations. In such cases, liming needs to take into account the temporary effect of increased concentrations of soluble Al^{3+}, Mn^{2+} and other potentially toxic metals displaced from exchange surfaces by Ca^{2+} (Svedrup et al., 1995; Blake et al., 1999).

N cycling in a closed ecosystem is neutral because both processes of N oxidation (acid) and N reduction (alkaline) occur within the plant or microorganism metabolising the NO_3^- (Helyar, 1976); NO_3^- reduction is coupled at least indirectly to the synthesis of organic anions or the solution of carbon dioxide. For this reason, the rate of acidifying processes is slow under natural conditions, but generally accelerates under agricultural practices and soil acidification is recognised as a major problem internationally (de Klein et al., 1997).

At the root-soil boundary, mineral N is actively taken up with the excretion of H^+, OH^- or HCO_3^- ions to maintain electro neutrality (Burle et al., 1997). When NH_4^+ is the dominant form of N absorbed, the plant absorbs more cations than anions resulting in a net efflux of H^+ ions, with a consequent reduction in the pH of the rhizosphere. Conversely, when NO_3^- is the dominant form of N taken up the pH in the rhizosphere is increased (Haynes, 1986). The maximum absorption of NH_4^+ by plants is at pH 7-8 and of NO_3^- at pH 4-5 (Haynes, 1996).

Soil acidity changes due to fluxes in the N cycle are associated with (Helyar, 1976):

1. gains or losses of N from the system
2. the form or quantity of N gains being different from the form and or quantity of N losses
3. the accumulation of N in various forms in the system
4. the removal from the system of residual acid or alkaline by-products of reactions involving N (Dolling et al., 1994; Burle et al., 1997; de Klein et al., 1997)

Nitrification generates acidity as in the following equation (Haynes, 1986):

$$NH_4^+ + 2O_2 + H_2O \rightarrow 2H_3O^+ + NO_3^-$$

The H_3O^+ displaces exchangeable cations (e.g. Ca and Mg (Burle et al., 1997)), which move down as counter ions with NO_3^- resulting in a reduced pH and base saturation in surface soil (Haynes, 1986). In fertile non-saline soil, even under irrigation, leaching of NO_3^- is generally the dominant factor determining the quantity of exchangeable bases leached (Haynes, 1986). Hence avoiding excess NO_3^- accumulation is important to prevent acidification generated by nitrification (Paul and Clark, 1989) and acidification from losing NO_3^- via leaching (Haynes, 1986; Burle et al., 1997; Evans et al., 1998). NO_3^- leaching and nitrification causes one mole of acid to be generated for every mole of NO_3^- leached or created through nitrification (Ridley et al., 2001). However, according to Helyar (1976), cation losses with NO_3^- from the system has little or no effect on the acidity of the system.

NH_4^+ leaching losses are generally small because of the restriction caused by cation adsorption, although NH_4^+ loss that does occur will be accompanied by counter ions. Assuming CO_2 and non- NO_3^--N are the inputs, if these counter ions are Cl^- and SO_4^{2-} there will be little or no effect on the acidity of the system, but if they are NO_3^- or HCO_3^- their loss has a net acid effect on the system (Helyar, 1976).

N fertilization can cause soil acidification (McAndrew and Malhi, 1992; Clay et al., 1993; Dolling et al., 1994). A fertiliser induced decrease in pH may cause an increase in soil organic C and N (Janzen, 1987; McAndrew and Malhi, 1992), a reduction in microbial biomass C and N (McAndrew and Malhi, 1992) and a reduction in mineralisable C and N and a build up in NH_4^+ (Mason, 1992; McAndrew and Malhi, 1992). However, use of the sustainable alternative, legumes, also increases soil acidification (Burle et al., 1997). The type of fertiliser is important (Logan and Thomas, 1999; Mason, 1992; McAndrew and Malhi, 1992).

Soil organic matter accumulation can also be a cause of acidification (Dolling et al., 1994; de Klein et al., 1997), although organic and inorganic N additions resulted in minimal stratification in soil pH in a pot experiment by Evans et al. (1998), in which no leaching occurred. pH buffering capacity is closely related to organic matter content, so maintaining high organic matter content will help both to slow the rate of pH decline, and increase the amount of lime needed to reverse a pH decline.

Soil acidification and N deposition have caused a decline in the diversity of plant species on the Park Grass Experiment at Rothamsted (Blake et al., 1999).

2.3.8.3 Acid soils

Acid soils (pH <5.5 in surface zones) occupy approximately 30% or 3950 m ha of the world's ice free land area, around 4.5 % (179 m ha) of which is used for arable crops (von Uexküll and Mutert, 1995). von Uexküll and Mutert, (1995) provide a detailed description of the global distribution and land uses of acid soils.

Poor crop growth in acid soils is usually due to a combination of toxicity of protons, aluminium (Al^{3+}), manganese (Mn^{2+}) and iron (in reduced soil conditions only), and deficiencies of accessible phosphorous (PO_4^{3-}), calcium (Ca^{2+}), magnesium (Mg^{2+}), molybdenum (Mo) and potassium (Foy, 1984; Wilkinson, 1994; von Uexküll and Mutert, 1995). Protons, aluminium and manganese toxicities in plants function through disruption of enzymes, hormones and oxygen donor ligands (Alexander, 1980; Wilkinson, 1994). At extremely low pH (pH 4.2), the hydrogen ion concentration may hinder or even reverse cation uptake by plant roots (Wilkinson, 1994). Al^{3+} is the dominant species resulting in the expression of toxicity in plants, but other monomeric Al species i.e. $AlOH^{2+}$, $Al(OH)_2^{+}$ are just as detrimental to root growth in a number of plant species (Sumner et al., 1991). Associated physical properties may also hinder fertility, e.g. low water holding capacity and susceptibility to crusting, erosion and compaction. Thus, acid soil stress presents a composite ecological problem to each plant and the importance of each factor is a product of the total plant-soil ecosystem (Wilkinson, 1994). Problems of nutrient status and toxicity in acid soils are detailed by Foy (1984), Rowell (1988) and Sumner et al. (1991).

Recalcitrance of organic N may be related to complexation with aluminium. Gonzalez-Prieto and Carballas (1991) found that the proportion of total organic N which was recalcitrant increased with decreasing organic N levels, and proposed an indirect mechanism to explain the relationship they found between extractable Al and N and a negative correlation of organic N with the net N mineralisation rate. As organo-Al compounds are highly resistant to biological attack, N mineralisation is blocked and organic C and N accumulate in high-Al soils, creating a lack of available N. This may cause a selective pressure for N-fixing legumes, which predominate in that soil. Once the capacity of the extractable Al to sequester organic matter has been saturated, the fresh N-rich organic matter provided by the N-fixing vegetation constitutes a largely rhizospheric reserve of labile N, which is readily extractable and almost independent of the more recalcitrant N.

2.3.8.4 Soil pH and mineralisation

Mineralisation of native soil organic N is not very sensitive to pH because it is carried out by a range of microflora (Alexander, 1980; Haynes, 1986; Sumner et al. 1991) that are rather insensitive to acid soil toxicities (Curtin et. al., 1998). If one organism is 'knocked out', another can easily fill the niche and take over the function (Alexander, 1980). Mineralisation is more tolerant than nitrification at low pH so NH_4^+ is generally the dominant form of N in acidic soil whereas NO_3^- predominates in non-acidic soil (Haynes, 1986). However, anaerobic mineralisation of N is sensitive to pH, with an optimum around neutral. Ryegrass decomposition rates were found to be similar in soils of pH 6.9 and 4.8 but slower in a very acid soil of pH 3.7, although by the end of 5 years the difference had almost disappeared; the slowing of decomposition by acidity was largely confined to the early stages (Jenkinson, 1981). The extent of suppression of mineralisation due to acidity varies with the soil and nature of organic materials (Alexander, 1980).

Nyborg and Hoyt (1978) found no relation between the amounts of mineral N released per unit of organic N in 120 days incubation and soil pH, base saturation, soluble Fe, Al or Mn in 40 soils. Lyngstad (1992) found that mineralisation of organic N in non-limed samples was highly correlated with the total N concentration, but not significantly related to the original pH of the soils. Curtin et al. (1998) found no relationship between the rate constant, *K*, or potentially mineralisable N with soil pH. They concluded that reduction of *K* because of pH is likely to be confined to soils that support toxic soluble Al or Mn. Geissen and Brümmer (1999) found that a higher soil pH achieved by liming 2 years previously did not increase beech forest litter decomposition rates. Dancer et al. (1973) also found ammonification to be insensitive to soil pH in the range 4.7 to 6.6.

However, Weier and Gilliam (1986) found cumulative net mineralisation to be significantly influenced by acidity in four of six histosol soils examined. Purnomo et al. (2000) found heterotrophic activity, as measured by CO_2 evolution, to be strongly correlated with organic C and soil pH. The % of organic C released as CO_2 was highly correlated with pH, implying that acidity limits heterotrophic activity. They also found net N mineralisation was correlated with CO_2 evolution and pH, and concluded there was strong circumstantial evidence to suggest that pH influences the availability of organic substrates to the microbial biomass. Tlustos and Blackmer (1992) observed a strongly pH dependent rate of inorganic N release from various fractions of ureaforms (slow release N fertilisers). Soil pH was found to have a significant effect on the preservation of higher plant derived biomolecules, with

preservation at low pH due to retarded activities of micro organisms by van Bergen et al. (1998). Acidity, by virtue of governing the kinds, numbers and activities of microorganisms, regulates the rate of organic matter mineralisation and therefore reduces the number of simple organic molecules available for further decomposition to render N and other elements soluble (Sumner et al., 1991).

Although mineralisation is often regarded as being relatively insensitive to acidity (Curtin et al., 1998), decomposition is known to be inhibited under strongly acidic conditions (Jenkinson, 1981) and there are many reports of liming causing agronomically significant increases in N mineralisation. Liming of soils (from pH 4.0 - 5.6 to pH 6.7) in the study by Nyborg and Hoyt (1978) approximately doubled the amount of N mineralised during incubation, and in the field, lime increased uptake of soil N by 15-42 kg ha^{-1} in the first year and 7-10 kg ha^{-1} in the 3rd year. The conclusion is generally that soil acidity does not restrict mineralisation of organic N, and lime has a temporary effect in increasing mineralisation of N (Nyborg and Hoyt, 1978; Clay et al., 1993). Curtin et al. (1998) found that addition of $Ca(OH)_2$ stimulated the mineralisation of C and N (*K* increased). Initially the rate increased 2-3 times but declined rapidly after 7-10 days, and over 100 days the amended soils produced $1/3^{rd}$ to $2/3^{rds}$ more CO_2 than untreated counterparts, with comparable increased rates for N mineralisation. The percent of total N extracted by (unbuffered) hot KCl showed no significant pH dependence, whereas a phosphate-borate buffer (pH 11.2) N extraction did. Wheeler et al. (1997) observed an increase in grass N uptake for 3 years following liming due to an increase in N mineralisation. In mowing trials 5 000 kg ha^{-1} lime application increased grass N uptake by an average of 16 kg ha^{-1}, and with an application of 10 000 kg ha^{-1} by an extra 33 kg N ha^{-1}.

The effect of $Ca(OH)_2$ is attributable to release of labile organic matter when pH is increased. The sorption and solubility of organic matter in soil is influenced by pH (Lofts et al., 2001; Tyler and Olsson, 2001). Sorption was found to be maximal at pH 4-5 decreasing with pH>5 by Shen (1999). Reduced bonding between organic constituents and clays at higher pH, results in release (solubilisation) of organic matter (Curtin et al., 1998). Sorption of organic matter was also found to be related to the ionic strength of the soil solution and the ion species present in soil solution (and whether they bind to mineral sites or complex with DOM). Thus liming will change the sorption capacity of soil and the resulting composition of the DOM in solution (Shen, 1999). Clay et al. (1993) found that as a result, liming may reduce the fertiliser-N requirement by 15 kg N ha^{-1} y^{-1} in the year of liming.

However, the relationship of organic matter solubility to soil pH may not be so simple. Fixation of fulvic and humic acids to clays decreases as pH is increased (Varadachari et al., 1994). This was attributable to greater dissociation of acidic groups of humic and fulvic acids, thereby increasing clay-humus repulsion and reducing the action of the acids on multilayer clays. Sorption reactions of the (non-nitrogenous) organic acids oxalate, citrate, malate and acetate were shown not to be pH dependent (Jones and Brassington, 1998).

Curtin et al. (1998) found that dissolved organic matter was well correlated with the rate of C and N mineralisation. This means that crops may respond to lime even when soil acidity is not directly restricting growth, albeit a temporary effect (Curtin et al., 1998). Liming liberated an additional 1-2% of soil N for crop uptake during a 3 year period in Nyborg and Hoyt's study (1978). On average about 2% of the total soil C is potentially mineralisable, and this percentage is positively correlated to soil pH and negatively to the soil C pool, the soil N pool, and total microbial activity (Riffaldi et al., 1996). Smolander et al. (1994) also found evidence to suggest that lime increases the C substrates suitable for microbes. Their study demonstrated a rise in soil microbial biomass carbon (SMB-C) and soil microbial biomass nitrogen (SMB-N) due to liming. Liming of a range of forest soils increased soluble organic matter and decreased organically-complexed Al and increased organically complexed Ca in a study by Erich and Trusty (1997).

The effect of low pH on the chemical structure and reactions of humic substances are detailed by Schnitzer (1980). He demonstrates that pH has a marked effect on the three dimensional arrangement of fulvic acid particles, which aggregate at low pH, attracted by H bonds, van der Waals interactions and homolytic reactions between free radicals. These forces become weaker at higher pH and there is an increase in ionisation of carboxylic acid and phenolic hydroxyl groups; as the particles separate and repel each other, the molecular arrangement becomes smaller and better oriented. Recalcitrance is also increased at lower pHs due to increased inter-layer adsorption of fulvic acids in expanding minerals (Schnitzer, 1980).

In addition to making the SOM more susceptible to microbial attack, lime may directly affect the microbial population/activity (Lyngstad 1992). Dähne et al. (1995) found that liming increased the number and activity of earthworms, which markedly alters the humus structure, increasing bacterial and protozoal mineralisation rates. They also found that unknown hydrolytic processes caused an increase in dissolved organic N compounds, which in turn triggered increased aminopeptidase activity in the fungal mycorrhizae and raised the host amino acid metabolism.

The effect of lime in increasing crop yield and N offtake may also be due to the effect of Ca on plant roots improving NH_4^+ and or NO_3^- uptake, enabling plants to compete better with the SMB for mineral N (Stevens and Laughlin, 1996). Curtin et al. (1998) do not attribute the effect of liming to increased availability of Ca in their experiment, as Ca was the predominant exchangeable and soluble cation in the unlimed soils, and Ca was not biologically limiting. Results of Bailey (1995) suggest that liming has both effects, each associated with a different phase in the soil Mineralisation Immobilization Turnover (MIT) cycle. During phases of net N mineralisation, liming stimulates biomass activity by raising soil pH and increases the amount of organic N mineralised. In contrast, during phases of net N immobilisation, liming improves the ability of plants to absorb N by increasing Ca availability in the rhizosphere, and thus helps them to compete more effectively with the biomass for mineral N.

On the other hand, Dähne et al. (1995) found that the converse of liming, acid irrigation had no effect on the pH of the humus or mineral soil under *Picea abies* (the soils were in the Al buffer range of pH 3) and the various N forms and their concentrations remained almost unaffected.

2.3.8.5 Soil pH and nitrification

Nitrification is more affected by pH than mineralisation (Dancer et al., 1973; Strayer et al. 1981; Sumner et al., 1991), with an optimum of pH 6.6 - 8.0. The process decreases markedly below pH 6 and is negligible at pH <4.5 (Paul and Clark, 1989). Haynes (1986) gives an optimum pH of 6-7, but notes that some autotrophs may have optima of pH 4 - 5. Bock et al. (1989) give an optimum of pH 7.5 – 8. Although nitrification is generally autotrophic, heterotrophic and chemical nitrification may be of importance in acid soils (Haynes, 1986, see section 2.2.2.4.1), especially acid forest soils (Paul and Clark, 1989).

The growth of *Nitrosomonas* (optimal pH >7.6) is more sensitive to low pH than *Nitrobacter* (optimal pH 6.6 - 7.6) (White, 1997). At low pH, nitrifying organisms in field soils produce less NO_3^- per unit time and hence grow more slowly. However, measurements of the short-term nitrification rate at non-limiting NH_4^+ concentrations suggest that soil nitrifiers adapt to the prevailing pH such that the optimum pH for nitrification is close to the soil pH, although the pH optimum for nitrifiers in any soil is unlikely to exceed 6.6 (Bramley and White 1991, in White, 1997). The generation time of nitrifying bacteria is 34 hours at a pH of 7.6, rising to 104 hours at pH 6.2 (Bock et al., 1989).

The effect of acidity may be an expression of aluminium toxicity (Schmidt, 1982, Haynes, 1986), although in Nyborg and Hoyt's research (1978) nitrification did decrease with pH, but it was not statistically related to base saturation or soluble Fe, Al or Mn; nitrification occurred even when exchangeable Al and Mn were naturally high. Strong et al. (1997) note the importance of pH buffering capacity because although acid sensitive, nitrification is an acidifying process. Stimulation of nitrification by liming lasts for very much longer than the effect of lime on mineralisation.

Despite these observations, nitrification proceeds in soil with lower pH than the limit of pH 3.9-4.5 observed in pure culture, and nitrifying bacteria have been isolated from acid soils (as low as pH 4.1) which are adapted to such soils (Schmidt, 1982, Haynes 1986, Walker and Wickramasinghe 1978 in Bock et al. 1989). Persson and Wirén (1995) found that almost no nitrification could be detected at $pH_{(H2O)}$ values lower than 4.0 in acid Norway spruce forest soil, but NO_3^- was formed in humus layers with pH values of 4.0-4.5, although nitrification was never complete. By contrast, nitrification was almost complete at a depth of 10-50cm where the $pH_{(H2O)}$ was 4.1 - 4.5. Ellis et al. (1998) observed nitrification in aerobic conditions even at pH_{CaCl2} less than or equal to 3.9.

It is possible that there are microsites in the soil where the bacteria perform oxidation at a higher pH than the bulk soil (Strong et al 1997), that acid tolerant strains of nitrifiers exist (Haynes, 1986; Paul and Clark, 1989; DeBoer et al., 1990; Clay et al., 1993), or that heterotrophic nitrification can play an important role (Paul and Clark 1989).

However, using small contiguous soil samples of one soil, employing natural heterogeneity in order to avoid confounding factors of samples from different soil types and sites, Strong et al. (1997) calculated a multiple regression equation predicting NO_3^- concentration following incubation. pH was the only non-significant predictor variable out of NH_4^+, gravimetric water content, bulk density (BD), pH buffering capacity (PHBC), and pH. PHBC had the largest influence on NO_3^- levels, followed by BD (which are both related to organic matter content and clay status). Strong et al. (1997) propose three reasons why they unexpectedly found pH to be a non-significant predictor variable:

1. The microbial population may have adapted to the prevailing pH.

2. Mineralisation was unaffected by pH and even though nitrification was slowed at low soil pH values it was able to keep up with the rate of ammonification, although they note that N mineralisation was stimulated by alkali addition.

3. Actual pH at nitrification microsites was not reflected in the bulk pH measurement.

The pH in the rhizosphere may be up to 2 units higher or lower than in the bulk soil (Marschner et al., 1986). Soil pH may also be higher near clay colloids and in microsites where the local release of ammonia is concentrated during the decomposition of a nitrogenous compound (Alexander, 1980).

The fact that liming acid soils usually stimulates nitrification (Clay et al., 1993; Dähne et al., 1995), (often to a greater extent than ammonification (Haynes, 1986)), indicates the presence of acid sensitive nitrifiers. Nyborg and Hoyt (1978) showed that liming stimulated nitrification permanently. However, no soil exhibited a significant net mineralisation increase from initial pH (<4.5) to limed pH (4.8-5.2) in the study by Weier and Gilliam (1986), and only one soil showed an increase on liming to c. pH 7. Nitrification of mineralised organic matter was complete at all pH values, although high acidities inhibited nitrification of added $(NH_4)_2SO_4$. Persson and Wirén (1995) found that nitrification was sometimes stimulated and sometimes inhibited by the addition of $CaCO_3$ where the nitrification was less easy to characterise as auto/hetero-trophic. In acid soils, the pH is often more favourable for nitrification at depth (Persson and Wirén, 1995). Ridley et al. (2001) found leaching losses of NO_3^- from limed pastures to be consistently greater than from unlimed pastures (by c. 8 kg N ha^{-1} y^{-1}).

The adaptability of nitrifier populations to their environment was highlighted by Bramley and White (1989) and Darrah et al., (1986), who, using a short term nitrification assay, demonstrated that the optimal pH for an indigenous population is never far from the prevailing pH. Ste-Marie and Paré (1999) showed that nitrification was affected by both 'natural' and 'amended' soil pH (both increased and decreased), under a range of forest species. Strayer et al. (1981) examined the effects of acid irrigation on forest soils. They showed that acid rain resulted in inhibition of potential nitrification and N mineralisation was stimulated or unaffected. If no NH_4^+ was added, native NH_4^+ was nitrified by heterotrophic bacteria and unaffected by acid rain, but if NH_4^+ was added, this was nitrified by autotrophic bacteria and was sensitive to acid rain. Their results indicate that acid irrigation causes a shift in the nitrifying organisms (autotrophic to heterotrophic) in some soils.

2.3.8.6 Changes in pH and the soil community

The pH effect on the abundance of most soil animals is not usually very pronounced although different species have different pH optima (Haynes, 1986). According to Jenkinson (1981), it is likely that acidity slows decomposition by restricting the activities of the soil population to a relatively small number of species; e.g. few species of earthworm can tolerate pHs below about 4, and as a result acid grasslands often have a mat of dead litter on the soil surface. A concentration of over 500 μM aluminium may decrease the number of bacteria, and decrease fungi at higher concentrations, but it is possible for some microbial populations to become acclimatised to reduced pH (Alexander, 1980).

Characteristically, the soil microbial community shifts from bacteria to actinomycetes to fungi as the pH drops, although the acid tolerance of individual species varies widely (Haynes, 1986). The relative proportion of fungi increases as pH drops because of lack of competition from other heterotrophs (Alexander, 1980).

Anderson and Domsch (1993) showed that the qCO_2 (respiration:biomass ratio, metabolic quotient, or specific respiration rate, a stress indicator) correlates negatively with natural forest soil pH over a range of pH 3-7, which they attribute to acidity-induced stress. They also demonstrated that the microbial C:organic C ratio increased as pH increased in forest soils, indicating that the C was more available to the SMB at higher pHs.

$qCO2$ is an indication of stress, (Anderson and Domsch, 1985) as it indicates the maintenance carbon requirements of actively-metabolising microbial populations under in situ conditions. However, the value of qCO_2 has been questioned. For example, Dr. John Scullion (pers. Comm.) regards qCO_2 as a useful measure but not one which can be interpreted easily without a range of other microbial indices, because 1) as with any ratio of two determinations you can get the same result with widely differing individual values, and 2) many effects may relate to changes in population composition (e.g. bacterial:fungal ratios and selection for tolerance) and if these changes are accompanied by other responses, interpretation of the result is complicated.

Lime has been shown to increase both the fumigation-extraction and substrate induced respiration derived microbial biomass C, microbial biomass N and its proportion of total soil N and microbial respiration rates in acid forest soils (Smolander et al., 1994). Hojito et al. (1987) showed lime application increased

bacterial numbers. Lime slightly increased the qCO2, indicating that lime increased the C sources suitable for microbes (Smolander et al., 1994).

Anderson (1998) demonstrated adaptation of the SMB population to pH by the application of acid to a limed soil, resulting in a very low SMB-C:Organic C ratio and a high qCO_2 as the maintenance energy demand was increased. However, application of acid to an already acidic environment did not affect the qCO_2 or SMB-C:organic C ratio. Blagodatskaya and Anderson (1998) showed that the pH greatly influenced the fungal:bacterial ratio, which was in turn related to the qCO_2; with increasing fungal presence the qCO_2 declined. The addition of lime has been shown to affect the abundance of mycorrhizal fungi species and their organic N targeted enzyme activity via pH and solubility of organic N effects (Dähne et al. 1995), although acid irrigation did not affect the enzymes measured or soluble protein.

Acid stress (reduction of pH of beech forest soil of pH 6.4 by 1-3 pH units by addition of H_2SO_4) caused a loss of biomass-C (32-87%), an increase in qCO_2 by 1.8 to 7 times, and a strong reduction in bacterial respiration (this did not recover in extreme acid stress) in an experiment by Blagodatskaya and Anderson (1999). Over time there was some recovery of pH, microbial biomass and a concomitant decrease in the qCO_2, indicating adaptation to acidic conditions by the surviving and newly formed biomass over 80-200 days, by which time the percent microbial C of total soil carbon C was close to those for sites of a comparable natural soil pH. After 80 days the total respiratory activity at pH 5 was 18% bacterial, 82% fungal, which agreed with the observed fungal:bacterial ratio. However, the loss of biomass in some soils never recovered. Blagodatskaya and Anderson (1998) found that the qCO_2 was related to the fungal:bacterial ratio, because fungi release less CO_2 per unit cell mass than bacteria, but they point out that fungal:bacterial biomass ratios are not equivalent to activity ratios, and the conversion factor is not known. They concluded that pH is the major independent driving variable controlling composition of microbial community and maintenance demand. At pH 3, 0.5% of total organic C is microbial C, whereas this figure is 2.4% at pH 7 (Blagodatskaya and Anderson, 1999), this may be due to the direct impact of the H^+ ion on the microbial cell.

Wardle (1998) found soil pH to be negatively related to the temporal variability of biomass C in arable and grassland agroecosystems, indicating that alleviating stress on the microbial community has a stabilising effect, reducing its turnover, and this is likely to have important consequences for soil nutrient dynamics and ultimately plant growth and ecosystem productivity (Wardle, 1998).

The literature therefore indicates that soil pH is a significant controlling parameter for microbial biomass build up and the fungal:bacterial ratio as found on natural site studies. pH is the major independent variable controlling soil microflora from soils of different sites and of different types.

2.4 NO_3^- leaching

NO_3^- is an anion and is therefore freely mobile in soil solution, making it vulnerable to leaching. This is especially the case in temperate soils with predominantly 2:1 clay minerals. NO_3^- can be electrostatically held on positively charged sites on soil particles, (e.g. iron and aluminium oxides and hydroxides, 1:1 clay minerals (e.g. kaolinites and allophane)), but soils that have a significant anion exchange capacity are mostly tropical and volcanic soils. The adsorption of NO_3^- in this way decreases as the pH lowers (Haynes, 1986).

In the UK, arable agriculture makes a large contribution to N leaching because of the large land area covered (4.5 m ha, 30% of agricultural land), and horticulture has a tendency to leach large amounts of N but the area covered is small (under 0.2 m ha) (Goulding, 2000).

NO_3^- loss may be via run off (Heathwaite et al., 1993) or leaching whereby it moves down the soil profile in a front when there is downward movement of water. In the UK, NO_3^- leaching is greatest in the winter when there are high levels of mineral N in the soil and there is a downward movement of water due to high rainfall (Stevenson, 1986). The solubility of NO_3^- means that its transport is intimately linked with the hydrological pathways controlling nutrient transport from the land to the stream (e.g. the water holding capacity of the soil, infiltration and percolation rates, precipitation (Stevenson, 1986)), and further controlled by: soil structure and type, rainfall, fertiliser supply, plant cover and root activity (Heathwaite et al., 1993).

NO_3^- may move with soil water in vertical or lateral flow. Soil is not homogeneous and water movement velocity is not equal at all points as the velocity at the centre of pores is greater than at the edge and velocity in channels is greater than in interconnected pores (Paul and Clark, 1989). Flow may be (Armstrong and Burt, 1993):

1. saturated
2. unsaturated - slower than saturated flow, soil water solutions are often near equilibrium

3. by-pass flow - preferential movement of water along specific pathways such as structural fissures and cracks, while soil peds are unsaturated. This water tends to be low in solutes, although drainage from cracked dry soil that has received fertiliser just before heavy rain can contain a lot of nutrients.

Reviews of NO_3^- transport processes are provided by Paul and Clark, (1989) and Armstrong and Burt (1993).

In British conditions, arable soils frequently contain substantial amounts of mineral N in the rooting zone following crop harvest, as a result of mineralisation of crop residues and the presence of unused fertiliser (Vinten and Smith, 1993). Conditions remain favourable for mineralisation into the late summer and early autumn (DoE, 1986). Even if crops are sown in the autumn, the soil mineral N supply will usually exceed crop uptake, because of relatively slow uptake during this stage of growth (DoE, 1986). It is the mineral N generated in the soil in autumn which contributes most to the loss of N by leaching during the winter. Some of this mineral N will be derived from the fertiliser N applied to the previous crop (mainly via organic debris from that crop) but a large part will be derived from the mineralisation of older organic matter (DoE, 1986).

Whilst the concentration of mineralisable N decreases exponentially with depth, 20-30% of the total N released is below 60cm, so the sub soil may contribute substantially to the amount of N mineralised and this has implications for leaching loss (Jarvis, 1996).

When optimal N fertiliser is applied, NO_3^- leaching loss is in the region of 30 kg ha^{-1} y^{-1} (Goulding et al., 2000). Intensive arable systems may leach in the order of 70 kg N ha^{-1} y^{-1} and in areas receiving excessive applications of N fertiliser the losses can exceed 150 kg N ha^{-1} y^{-1} (DoE, 1986). Gill et al. (1995) found that under long term grassland, during November to February, 21 - 38 % of total net annual mineralisation occurred, and an average of 85 kg N ha^{-1} was released. There is an annual loss of 22 kg ha^{-1} of NO_3^--N from agricultural land in NE Scotland (Sinclair et al., 1992).

There has been research into the use of chemicals to conserve fertiliser-N by inhibiting nitrification and therefore leaching and denitrification (Stevenson, 1986), but they are not well suited to use in UK conditions (Addiscott et al., 1991).

2.4.1 Problems resulting from NO_3^- leaching

2.4.1.1 Eutrophication

Eutrophication is an increase in the nutrient status of water courses or bodies. In most freshwater systems, the limiting nutrient is phosphorus and additional NO_3^- has little effect. Where NO_3^- is the limiting nutrient, additional NO_3^- produces rapid increases in plant growth. The reader is referred to the Department of the Environment publication 'Nitrate in Water' (1986) and the Environment Agency report 'Aquatic Eutrophication in England and Wales' (1998) for detailed examinations of the fresh and marine water ecology implications of NO_3^- pollution, the latter containing a proposed management strategy. Briefly, eutrophication can cause the following ecological problems:

1. Changed NO_3^- concentrations can cause qualitative changes in algal communities, e.g. a switch from diatoms to blue-green algae (cyanobacteria), which can have consequences for water treatment due to deterioration in water taste and odour
2. Toxins may be released on bloom decay. In May 1990 in the UK, the NRA detected blue-green algal blooms in 90% of their water regions at 30 locations; at least 70% of these blooms were toxic to humans (Heathwaite et al., 1993).
3. Algal blooms can reduce the depth of light penetration and grow directly on leaves, causing declines in rooted aquatic plants.
4. Excess NO_3^- (over 13 mg NO_3^- l^{-1}) can cause increased shoot biomass, which is not matched by roots, resulting in unstable, more easily eroded reed beds (DoE, 1986).
5. Stimulated aquatic plant growth can cause impeded navigation and drainage (DoE, 1986).
6. Anoxic conditions can be created by the utilisation of available oxygen (DoE, 1986).

2.4.1.2 Human health implications

The health implications of high NO_3^- in drinking water include:

- Infant/animal methaemoglobinemia or 'blue baby syndrome' (Addiscott et al., 1991). NO_3^- in stomachs is reduced to NO_2^- and joins to haemoglobin irreversibly, forming methaemoglobin, preventing it from being able to

transport oxygen. This generally affects infants under 6 months and young animals (Herman and Maier, 2000), because their stomachs are not sufficiently acidic enough to prevent the growth of denitrifying bacteria that convert the NO_3^- to NO_2^-. This can result in brain damage or death. The last recorded death from infant methaemoglobinemia in the UK was in 1950, and the last reported and confirmed case in 1972 (DoE, 1986).

- Denitrification in the stomachs of adults results in NO_2^-, which forms nitrosamines, which are highly carcinogenic. However, there is no proven link between NO_3^- consumption from water and stomach cancer (Addiscott et al., 1991; Herman and Maier, 2000), and the link is not borne out by epidemiological evidence (DoE, 1986). Stomach cancer is highest in western and northern upland areas of the UK, whereas high NO_3^- concentrations occur in southern and eastern arable regions. However, as cancer takes a number of years to develop and NO_3^- concentrations have increased significantly only in the past 20 years, such geographic correlations may be misleading (Heathwaite et al., 1993).

2.4.1.2.1 NO_3^- contamination of water

Extensive leaching of NO_3^- from soils into surface and ground waters has resulted in substantial areas of lowland England with water sources that approach or exceed the limit set by the European Union (Davies, 2000). Agriculture is the main source of NO_3^- in drinking water (MAFF, 1993, 1995). Groundwater sources constitute 30% of public water supplies in Britain; 50% of which are from chalk aquifers and 30% from triassic sandstones (Heathwaite et al., 1993). Groundwater sources may provide 10% to 70% of drinking water regionally (DoE, 1986). Both of the principal aquifers (the Cretaceous Chalk in Southern and Eastern England and the Triassic Sandstone of the North Midlands and West Midlands) are affected by rising groundwater NO_3^- concentrations (DoE, 1986). The worst affected area is the Jurassic Limestone of central Lincolnshire, where some boreholes have had to be taken out of supply, used intermittently or only used when blended with low NO_3^- water. This aquifer is affected by fast fissure flow with marked seasonal fluctuations, which are more typical of surface water quality variations (DoE, 1986).

Numbers of sources exceeding the statutory limit of 50 mg NO_3^- l^{-1} were: 60 in 1960, 90 in 1980, (DoE 1986), 125 in1983-4 (these supplied 1.8 million people), 154 in 1989, and 192 in 1990 (MAFF, 1993). The level of exceedance is increasing in a number of areas in England, notably Norfolk, Cambridgeshire, Lincolnshire , Hereford

and Worcestershire, the Severn-Trent region, Southern, Thames and Yorkshire regions. The reader is referred to the publication 'Nitrate in water' (DoE, 1986) for maps of the UK depicting principle aquifers and their vulnerability to NO_3^- pollution, and relationships between farming systems and rainfall in the UK.

In the west of the UK, excess winter rainfall is often over 400 mm and because of dilution, large amounts of NO_3^- can be leached (e.g. 50 kg N ha^{-1} y^{-1}) without exceeding the EU limit. While in the east, long term excess winter rainfall is as little as 150mm and therefore leaching of as little as 15 kg N ha^{-1} y^{-1} can breach the EU concentration limit (Davies, 2000). Because of the regional nature of NO_3^- contamination of water, the UK government is targeting efforts to control the problem in the designation of Nitrate Vulnerable Zones (MAFF, 1995). This impact of drainage volume on concentration means that even where no N fertiliser is applied, when rain follows a dry summer and autumn, NO_3^- concentrations can be very high (Goulding et al., 2000).

Surface waters provide about 70% of the public water supplies in Britain, in much of Scotland and Wales, where the NO_3^- concentrations are generally very low, surface waters provide over 90% of the supplies. NO_3^- concentrations are higher in central and southern England, comprising the Severn-Trent, Anglian, Thames, Southern and Wessex Water Authorities (DoE, 1986).

2.4.1.2.1.1 Amelioration of NO_3^- contamination of water

Solutions for high NO_3^- concentrations include: water substitution from low -N sources, blending of high and low NO_3^- water and NO_3^- removal through chemical/biological treatment or storage (Heathwaite et al., 1993). These can be costly (MAFF, 1993). Land management options may be more cost-effective (Heathwaite et al., 1993), but there can be a time lag of many years before changes in land use affect NO_3^- concentrations in groundwater supplies (MAFF, 1993).

2.4.2 Agricultural issues

Policy post WWII aimed to increase domestic food production to improve food security, and this goal was achieved as a result of improved crop varieties, better control of pest and diseases, more effective use of crop nutrients and improved agricultural systems with a tendency to larger units, greater use of mechanisation and less fallowing (DoE, 1986). This period saw an increase in fertiliser N used from 60,000 tons y^{-1} in the late 30's to just below 200,000 tons y^{-1} by the mid 40's to 1,580,000 tons y^{-1} in 1985 (DoE, 1986).

The Agricultural Development and Advisory Service (ADAS) of MAFF publish codes of good agricultural practice for the protection of air, soil, and water (MAFF, 1998a, b, and c respectively). These are voluntary codes, aimed at encouraging farmers to minimise the loss of nutrients such as N from their land by measures such as making allowances for any residual N left in the soil after cropping and reducing subsequent N fertiliser applications (Heathwaite et al., 1993). The 'Water Code' (MAFF, 1998c) gives guidance on storage of slurry, treatment of dirty water, use of manures and sludges, inorganic N fertiliser (also in MAFF 2000), crop cover, crop residues, autumn cultivations, grassland management, ploughing up grass and irrigation. Although these codes are voluntary, it is an offence to knowingly discharge pollutants into receiving waters.

Unfortunately, restrictions on fertiliser use needed to reduce leaching to acceptable levels often puts application rates below the economic optimum, which can have serious consequences for the margins of farmers (Davies, 2000). The benefit of this may take several years to become apparent as the soil organic N reserves decline (Davies, 2000). The lag period between changes in management and effect on drinking water can be great: some aquifers retard leaching to the extent that rain falling in the 1940s is only now being extracted as drinking water (Addiscott et al., 1991). Even where no fertiliser N is applied, there is always some leaching loss (e.g. 10 kg ha^{-1} y^{-1} under winter wheat on Broadbalk, (Goulding, 2000)).

2.4.2.1 Set Aside

Set Aside was introduced in 1988 either as 1 year rotational or 3-5 year permanent. Bare fallow was not permitted because it promotes the greatest amount of mineral N accumulation from soil organic N and plant residue mineralisation (Froment and Grylls, 1992). Set aside can reduce NO_3^- leaching, where a dense green cover is established. A high content of ryegrass is effective (more so than natural regeneration), whereas a clover-rich sward may increase NO_3^- leaching (Froment and Grylls, 1992; Sinclair et al., 1992). The effect of management of natural regeneration on set aside land on the mineral N in the soil was examined by Farr et al. (1992).

The crop preceding establishment of set aside influences soil mineral N levels and so NO_3^- leaching via the amount and N content of residues available for mineralisation (Harris et al., 1992). Allowing cover to regenerate in crop stubble results in lower net N mineralisation than if the stubble was cultivated into the soil (Harris et al., 1992).

The ploughing out of set aside can cause high levels of NO_3^- leaching (Goulding, 2000).

2.4.2.2 Autumn cover

Maintaining green cover over autumn and winter (a 'catch crop') is one of the main strategies for reducing winter NO_3^- leaching. The increase in autumn sown cereals over the 1980's helped to reduce NO_3^- leaching (DoE, 1986).

Hewitt et al. (1992) showed that in the autumn, N assimilation was best in forage rape, intermediate in rye grass and lowest in wheat, whereas over winter, ryegrass had the greatest uptake with forage rape and wheat being equal but less and winter stubbles the smallest. Early sowing of winter wheat and barley is recommended (DoE, 1986). Other catch crops include those used for animal fodder crops and green manures, which 'store' the N, and make it available for subsequent plant uptake, depending on the time of incorporation. However, there may be a period of N immobilisation following incorporation, restricting the supply of N to the subsequent crop (Omay et al., 1997). Maintaining a cover crop in organic farming systems may enhance the size and heterogeneity of the microbial biomass (Wander et al., 1995).

However, as cover crops increase soil organic N, they increase the pool of mineralisable and subsequently nitrifiable N, and thus the potential for NO_3^- leaching (Goulding and Poulton, 1992).

2.4.2.3 Other management options

Important management options for avoidance of NO_3^- leaching include: avoiding autumn spreading of manure or sludge, judicious timing of inorganic fertilisers in spring-early summer, more accurate predictions of crop N requirements, direct drilling, immobilisation of NO_3^- by straw incorporation, careful irrigation and drainage practices (DoE, 1986). Ploughing up of permanent or temporary grassland leads to mineralisation of soil organic matter and increased NO_3^- leaching, (Vinten and Smith, 1993) which should be avoided.

Because of the role of mineralisation of organic N in providing NO_3^- at the time when it is vulnerable to leaching, 'organic' or 'low input' agricultural systems are by no means immune from becoming culprits. A poorly managed organic farm can potentially leach much more NO_3^- than a prudently managed conventional farm (Addiscott et al., 1991). Kristensen et al. (1995) conclude that there is considerable potential for ecological farming systems to offer a viable approach to limiting NO_3^- leaching, within an approach which also meets the requirements of broader goals of

sustainability. A range of studies on ecological farming systems and crop rotations are presented in 'Nitrogen leaching in Ecological Agriculture' (Eds. Kristensen et al., 1995). Phillips et al. (1997) argue that organic systems include: 'the rational use of organic manure, the use of appropriate cultivation techniques, the avoidance of soluble fertilisers, the prohibition of agrochemical pesticides and, most importantly, the employment of balanced rotations which inherently require leaching losses to be minimised.' They identify the greatest risk of NO_3^- leaching associated with organic rotations as the establishment of the ley and the transition from the ley to arable phases of the rotation.

Heathwaite et al. (1993) summarise the following prevention strategies:

1) Accurate determination of crop N requirements

2) Application of N fertilisers in late spring when warmer weather will help maximise crop uptake of the supplied N

3) Split applications of fertiliser

4) Encouraging the use of slow-release N fertilisers

5) Use of late summer/autumn cover crops to reduce the area of bare ground

6) A provision of buffer zones between arable land and the drainage network, where conditions conducive to denitrification are encouraged.

Goulding (2000) adds to these precautions: careful use of crop variety (new varieties of wheat are much more efficient at utilising N (Goulding, 1998)), even spreading of fertiliser, careful irrigation and minimisation of pests and disease.

Integrated management decisions need to be made at the catchment scale, involving an understanding of the hydrological pathways and soil processes involving N (Heathwaite et al., 1993).

2.4.3 Legislation and Policy

2.4.3.1 European Community Directives

The EC Directive on drinking water 80/788 set an EC maximum admissible concentration for NO_3^- in drinking water of 50 mg l^{-1} or 11.3 mg NO_3^--N l^{-1} (Heathwaite et al., 1993). The European Community Nitrate Directive (91/676) was adopted by Member States in December 1991, and required restrictions on agriculture in the catchment areas of surface freshwaters and groundwaters intended for drinking water which and natural freshwater lakes, other natural freshwater bodies, estuaries,

coastal waters and seas either already or are at risk of exceeding 50 mg l^{-1}. This directive focussed on organic and inorganic fertilisers (Heathwaite et al., 1993).

2.4.3.2 Nitrate Sensitive Areas (NSAs)

Section 94 and Schedule 12 of the Water Resources Act 1991 cover the designation of Nitrate Sensitive Areas (NSAs) where the Government considers it appropriate to control the amount of NO_3^- entering water from agricultural land (MAFF, 1998b). NSAs started with a pilot scheme in 1990 when 10 NSAs and 9 Nitrate Advisory Areas were set up (MAFF, 1993) and in 1994, 32 NSAs were designated (Dampney et al., 2000). The NSA scheme was closed to new applications in July 1998 (MAFF, 1998c). The scheme included compensation measures for the loss of agricultural production, and has voluntary agricultural restrictions (Heathwaite et al., 1993). NSAs resulted in a significant reduction in N use and N losses (Goulding, 2000).

2.4.3.3 Nitrate Vulnerable Zones (NVZs)

Under the EC Nitrate Directive (91/676), member states were required to establish a code of practice which operates on a voluntary basis as a means for providing all waters with a general level of protection against NO_3^- pollution. In addition, in designated Nitrate Vulnerable Zones (NVZs), farmers are required to comply with mandatory measures consistent with good agricultural practice (MAFF, 1998b). NVZs, many of which overlie aquifers containing major sources of drinking water, were designated in England and Wales in 1996 (Davies, 2000), and compulsory practice was enforced in December 1998 (Goulding, 2000). The 68 NVZs cover 600,000 ha of England and Wales (Dampney et al., 2000; Goulding, 2000).

2.5 Summary

In summary, the literature demonstrates that there is still cause for concern over the perturbations caused to the N cycle by mankind's agricultural and land management activities. Room for improvement remains. We should continue to aim to improve the management of nitrogen resources in order to minimise the direct and indirect pollution problems resulting from movement of nitrogen along undesirable pathways.

Although soil pH is recognised as an important influencing factor on nitrogen dynamics, its influence is somewhat unclear. The conundrum remains that it is known to be a major factor affecting organic matter decomposition, yet mineralisation is generally said to be a pH insensitive process. The effect of soil pH on nitrification is in one sense clear-cut – it is an acid sensitive process, yet NO_3^- is detected in acid

soils. There are many unclear issues and questions to resolve. This is especially true in the light of recent advances in our understanding of DON cycling.

Soil pH is a controllable aspect of agricultural management, yet it has not been given attention as a means of intentionally controlling nitrogen pools and fluxes. The work in this thesis attempts to develop a greater understanding of how soil pH influences certain aspects of nitrogen cycling, and evaluate whether there is potential for using soil pH as a management tool in temperate agricultural systems.

Chapter 3 Methods

This chapter describes analytical methods that are common to the four experimental studies included in this thesis. Where an analytical method was used in only one chapter, the method is described in that chapter.

3.1 pH

Soil pH was determined with the use of a glass electrode, (Gelplas General Purpose Combination Electrode, Cat. No. 309/1050/03, BDH). Soil pH was determined in a salt solution and soil suspension (Thomas and Hargrove, 1984; Rowell, 1988; Thomas, 1996), using either:

- 1:10 w/v of soil : 1M KCl solution (Chapter 4, 6)
- 1:1 w/v of soil : 0.01M $CaCl_2$ solution (Chapter 7).

The salt solution was added to the soil and mixed thoroughly, then allowed to settle for 10 minutes and the electrode was placed in the supernatant.

In soil water extracts from porous cup samplers (Chapter 6), the electrode was placed directly in the soil water.

3.2 Extractions used for NO_3^-, NH_4^+ and SON determination

Soils were extracted in a 1M KCl solution at a soil : solution ratio of 1:10 w/v, by shaking for 1h on a reciprocal shaker (180 strokes min^{-1}) and centrifuging at 5,000 g for 10 min to obtain a supernatant.

3.3 NO_3^-

NO_3^- was determined on an EnviroFlow 3500, Perstorp Flow Injection Autoanalyser, with the exception of analysis in Chapter 4, which was performed on a Skalar segmented flow auto-analyser (Skalar Co., Netherlands), using the same method.

The principle of the method is based on the reduction of NO_3^- to NO_2^- by contact with a copper-cadmium complex. NO_2^- is determined by diazotizing with sulphanilamide and coupling with α-naphthyl-ethylemediamine dihydrochloride to form a highly coloured pink azo dye.

The machine uses a peristaltic pump to circulate reagents. A carrier solution (deionised water) has the sample injected into the flow, to which the buffer (Table 3-1) is then mixed. Pure N_2 gas is then added to segment the flow before it enters an open tubular cadmium reactor (OTCR). The OTCR is maintained in an active state

by washing with hydrochloric acid (very briefly), washing with deionised water and flushing with copper sulphate solution (2%) followed by a final rinse with deionised water. This activation procedure is only carried out when the reduction efficiency of the OTCR drops below acceptable levels (c. 85% reduction). The segmenting bubbles increase the circulation of reagents, increasing the contact with the lining of the OTCR, where reduction of NO_3^- to NO_2^- occurs in contact with the cadmium-copper couple. The colour reagent (Table 3-1) is then added to the flow, which is then de-bubbled prior to entering the spectrophotometer, which measures the density of the pink colour at a wavelength of 540nm.

Table 3-1 Reagents for NO_3^- analysis on the Perstorp EnviroFlow 3500.

Reagent	Ingredients	Weight	Notes
Ammonium Chloride-EDTA Buffer.	Ammonium chloride (NH_4Cl, FW 53.50)	85 g	Dissolve ammonium chloride and disodium EDTA in 900 ml deionised water in a 1 L beaker. Adjust pH to 8.5 with conc. NH_4OH, dilute to 1 L with deionised water. Add Brij and mix.
	EDTA disodium salt dihydrate ($C_{10}H_{14}N_2Na_2O_8.2H_2O$, FW 372.24)	0.1 g	
	Ammonium Hydroxide, conc. (NH_4OH, FW 35.05)	adjust to pH 8.5	
	Brij	2 ml	
Colour Reagent	Phosphoric acid (conc.) H_3PO_4, FW 98.00)	50 ml	Add phosphoric acid to 400 ml deionised water with care, add sulphanilamide and N-1-NED, mix, make upto 500 ml with deionised water.
	Sulfanilamide $C_6H_8N_2O_2S$, FW 172.21)	20 g	
	N-1-naphthylethylenediamine dihydrochloride ($C_{12}H_{14}N_2.2HCl$, FW 259.18)	1 g	
NO_2^- Standard Stock solution (1,000 mg L^{-1})	Potassium nitrite (KNO_2, FW 85.11)	6.072 g	Make up to 1 L with deionised water. Dry potassium nitrite prior to use.
NO_3^- Standard Stock solution (1,000 mg L^{-1})	Potassium nitrate (KNO_3, FW 101.11)	7.218 g	Make up to 1 L with deionised water. Dry potassium nitrate prior to use.

3.4 NH_4^+

This method is known as the Berthelot or indophenol blue reaction (Keeney and Nelson, 1982). Sodium nitroprusside acts as a catalyst, and the blue colour is formed by mixing NH_4^+, phenol and hypochlorite. Sodium salicylate is used as a less toxic alternative to phenol. The method is described by Mulvaney (1996). EDTA chelates di- and trivalent cations, which form insoluble hydroxides at the high pH (11-13) required for the analysis. Unfortunately, the reaction is not entirely specific for NH_4^+, as the colour is also obtained from a variety of organic compounds having a free amino group, including amino acids, amines and amides (Mulvaney, 1996).

Table 3-2 Reagents used in the indophenol blue method of NH_4^+ determination.

Reagent.	Ingredient	Weight	Notes
Sodium-salicylate-nitroprusside reagent	Sodium -salicylate Sodium - - nitroprusside	7.813 g 0.125 g	Add sodium salicylate to 80 ml water, mix, add sodium nitroprusside, make upto 100 ml with deionised water.
Sodium - hypochlorite reagent	Sodium hydroxide, (NaOH) K_2HPO_4 Sodium - hypochlorite	2.96 g 9.96 g 10 ml	Add sodium hydroxide to 80 ml deionised water, mix, add K_2HPO_4, add sodium hypochlorite and adjust to pH 13.0 with sodium hydroxide. Make upto 100 ml with deionised water.
EDTA reagent	Na_2EDTA	6 g	Dissolve EDTA in 100 ml deionised water.

125 µL of sample or standard is added to a cuvette, and 50 µL of EDTA reagent is added and mixed. 200 µ L of Sodium -salicylate-nitroprusside reagent is added and mixed followed by 0.775 ml deionised water, and mixed. 100 µ L of hypochlorite reagent is added and mixed, then the colour is left to develop at room temperature for 1 h. Absorbance is read on a spectrophotometer at 667 nm.

3.5 Total dissolved nitrogen (TDN)

This method (Williams et al., 1995) oxidises total dissolved N (TDN) to NO_3^-, which is determined as in section **Error! Reference source not found.** above. Dissolved or soluble organic nitrogen is then determined by the simple calculation: DON (or SON) =TDN – (NO_3^-+ NH_4^+).

The oxidation procedure is to add 1 ml of soil extract/water or standard to a glass autoclave tube with a PTFE lined lid, and add 1.5 ml of persulfate reagent. Autoclave at 125°C for 30 minutes.

Table 3-3 Reagents used in oxidation of dissolved N.

Reagent	Ingredients	Weight	Notes
Potassium persulfate	Potassium persulfate ($K_2O_8S_2$, FW 270.33, Fluka Co.) Sodium hydroxide (NaOH)	2.7 g 0.6 g	Dissolve potassium persulfate in 150 ml deionised water, dissolve sodium hydroxide and make up to 200 ml with deionised water.
Standards (10 ppm N)	KNO_3, NH_4Cl, urea	-	-

3.6 Soil moisture content

Moisture content was calculated by weight difference before and after drying for 24 h at 80 °C, and expressed as a percentage of dry weight. This temperature was used

so that samples could subsequently be used in the CHN analyser; any higher temperature could have caused some slight losses of volatile organic matter.

3.7 Total C and N

Total C and N were measured on dried soils in a LECO CHN 2000. A weighed sample is combusted in a furnace under a flow of oxygen, which converts all C to CO_2 and N to NO_x. The gas is then passed through an infrared cell to determine the CO_2 content. The gas is passed through a catalyst heater (copper) where NO_x gasses are reduced to N_2, and then CO_2 and H_2O are scrubbed out and the gas goes on to a thermal conductivity cell to determine N_2. Results are given as percentages, and the C:N ratio of the soil is calculated by simple division.

3.8 Amino Acid Mineralisation Assay

This assay was developed by Dr Davey Jones to assess microbial amino acid mineralisation rates in soil. Amino acids are used as the organic N substrate because, upon complete hydrolysis, they constitute 20% of the soil C and 30-40% of soil N (Smith et al., 1993) and are the major source of inorganic N from soil organic matter used in the mineralisation process. Only a small fraction of the amino N in soil is in a directly accessible low molecular weight form (MW<200) (Jones, 1999). The assay can be used to compare mineralisation rates between different soils.

A mixture of 15 uniformly labelled ^{14}C-amino acids (ICN Pharmaceuticals, Irvine, CA), each present at a concentration of 333 μM, giving a final concentration of 5 mM, (25 kBq ml^{-1}) was used as the substrate. This simulates the level of amino acid release into the soil after root cell lysis (Jones, 1999). The amino acids in the L-isomeric form were as detailed in Table 3-4.

50μL of the amino acid mixture was mixed with 0.5g of soil sample (which had been sieved through 2mm mesh) in a 15 ml polypropylene tube. Jones and Shannon (1999) found that sieving and storage of soil at 4 or 18 °C for up to 40 d had little impact on the amino acid mineralisation rate. Evolved CO_2 was trapped using NaOH; initial experimental work (as in chapter 4) used an air flow system, as in Figure 3-1 below. This was modified to use an integral trap, of a small (1.5 ml) Eppendorf phial supported within the incubation tube (Figure 3-2), making it easier to run more samples at once. The two systems were compared to ensure that the integral trap performed as well as the air flow system in terms of the amount of CO_2 captured (experiment 1, section 3.8.1 below).

Table 3-4 Amino Acids used in the mineralisation assay.

Amino Acid.	Number of C atoms per molecule	Weight of C in the molecule	Number of N atoms per molecule	Weight of N in the molecule	Molecular weight	C:N ratio of molecule
Alanine	3	36	1	14	89.09	2.57
Arginine	6	72	4	56	174.2	1.29
Aspartic acid	4	48	1	14	133.1	3.43
Glutamic acid	5	60	1	14	147.13	4.29
Glycine	2	24	1	14	75.07	1.71
Histidine	6	72	3	42	155.16	1.71
Isoleucine	6	72	1	14	131.18	5.14
Leucine	6	72	1	14	131.18	5.14
Lysine	6	72	2	28	146.19	2.57
Phenylalanine	9	108	1	14	165.19	7.71
Proline	5	60	1	14	115.13	4.29
Serine	3	36	1	14	105.09	2.57
Threonine	4	48	1	14	119.12	3.43
Tyrosine	9	108	1	14	181.19	7.71
Valine	5	60	1	14	117.15	4.29

Samples were incubated at 25 °C for 48 hours and liquid in the NaOH trap changed periodically, usually at various times upto 48 h following amino acid addition. The 1ml samples of NaOH were added to 4ml Wallac HiSafe scintillation fluid and counted in a scintillation counter. In this way ^{14}C-CO_2 evolution measures amino acid use in respiration. At the end of the 48hr incubation period the soil was extracted using 5ml of 0.5 M K_2SO_4, shaken for 20 min at 180 rpm, spun down in a microcentrifuge at 15,000 rpm and a 1ml aliquot taken, added to 4ml Wallac HiSafe scintilation fluid and counted on a Wallac 1409 Liquid Scintillation Counter (EG&G Ltd, Milton Keynes, UK). Centrifugation of up to 20,000g does not affect the integrity of microbial cells (Monreal and McGill, 1985) so amino acid-^{14}C used structurally within cells would remain there. The results are multiplied to account for dilution factors and the amount of ^{14}C immobilised in the microbial biomass calculated by:

$$^{14}C \text{ immobilised in biomass} = {}^{14}C \text{ count in added amino acid mixture} - \text{total } {}^{14}C \text{ count in } CO_2 \text{ evolved} - \text{total } {}^{14}C \text{ count in soil solution}$$

The assay is carried out on soils at field capacity, so that soils are compared in a way that is meaningful to field conditions. As most soils show a levelling off in the proportion of carbon in the added amino acid mixture that is respired at approximately 30% added C, it was decided that the rate of amino acid-C mineralisation should be indicated by the reciprocal of the time taken for 15% of added amino acid-C to be respired, hereafter referred to as $1/t_{15}$ (h^{-1}). The sensitivity

of the assay to differences in microbial mineralising activity is demonstrated in experiment 2 (section 3.8.2) below.

3.8.1 Experiment 1 – comparison of two contrasting methods of capturing $^{14}CO_2$ evolved from soil

Three soils were used in the experiment (all dystric cambisols), taking two repeats of the top soil and the sub soil from each, giving 12 samples in total. The amino acid incubation was performed twice on these samples; once using the air flow method of CO_2 capture (Figure 3-1), and once using the integral trap method of CO_2 capture (Figure 3-2). Traps were changed at 1, 4.5, 7, 18 and 24 h following addition of labelled amino acids. Counts performed on the NaOH traps (Decays per Minute, DPM) were corrected for background ^{14}C levels and dilution factors (air flow method only) and made cumulative, so that, for example, the 24 h count included all the $^{14}CO_2$ released between 0-24 h. Voroney et al., (1991) note that the closed systems are generally simpler, but require regular opening to the atmosphere to ensure oxygen does not become limiting. This is unlikely to be a problem with the timings of the regular openings to change NaOH traps in this experiment. Results are shown in Figure 3-3 below, with cumulative data for each time of sampling shown.

The air flow method of CO_2 capture tended to give slightly higher results than the integral trap method (Figure 3-3). The top soil samples for cambisol 2 gave consistently lower values in the air flow method and much larger standard errors. This may indicate a different microbial population. It was concluded that the integral trapping method was sufficiently efficient to be used.

Figure 3-1 Air flow system of CO_2 capture.

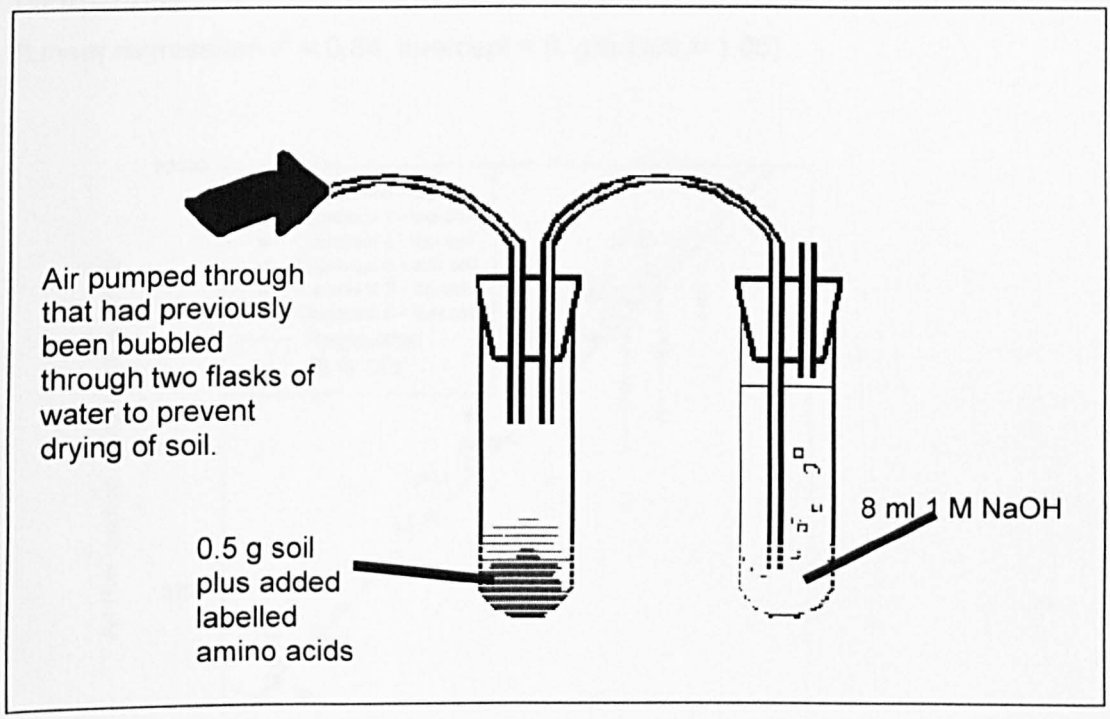


Figure 3-2 Integral CO_2 trap.

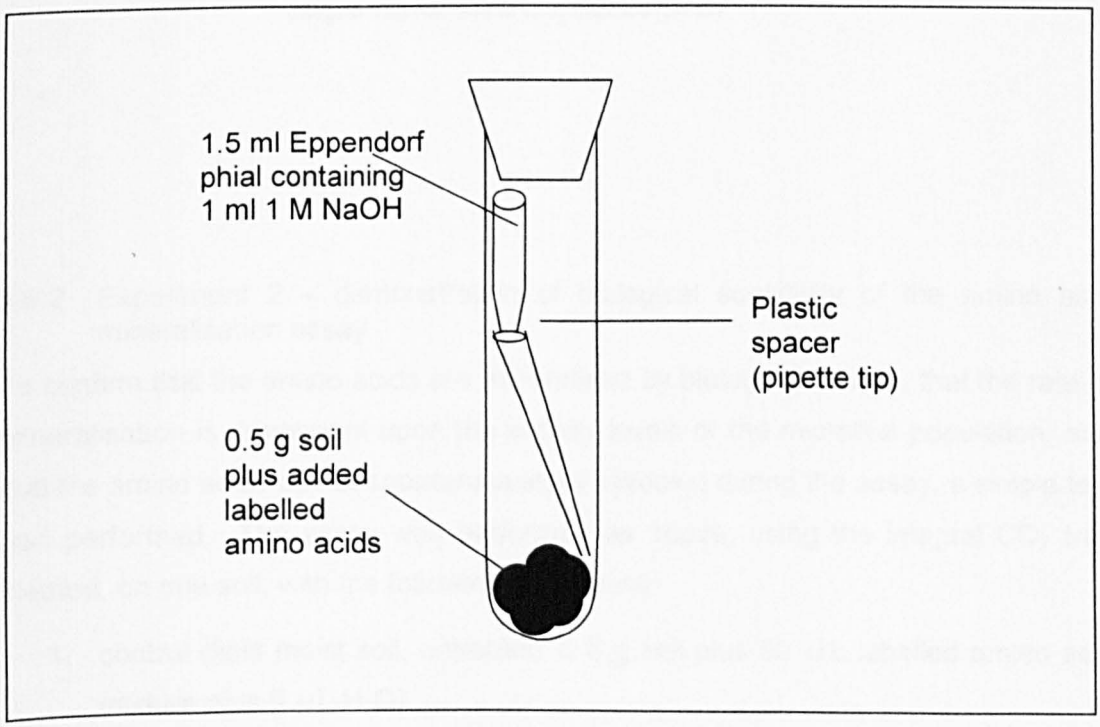
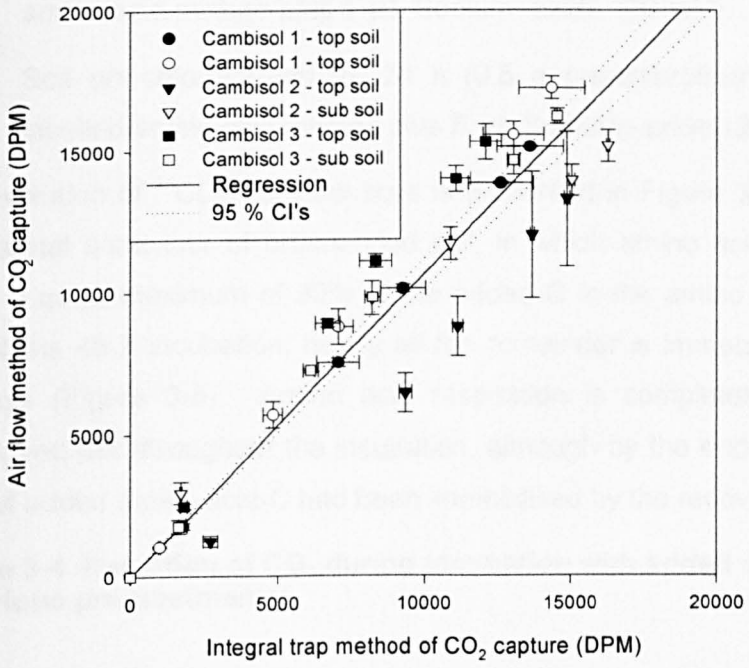


Figure 3-3 Comparison of methods for the capture of ^{14}C-CO_2 from soil during incubations

[Linear regression: $r^2 = 0.84$, intercept = 0, gradient = 1.06]



3.8.2 Experiment 2 – demonstration of biological sensitivity of the amino acid mineralisation assay

To confirm that the amino acids are mineralised by biological activity, that the rate of mineralisation is dependent upon the activity levels of the microbial population, and that the amino acids do not spontaneously breakdown during the assay, a simple test was performed. The assay was performed as above, using the integral CO_2 trap method, on one soil, with the following treatments:

1. control (field moist soil, untreated; 0.5 g soil plus 50 µL labelled amino acid mixture plus 5 µL H_2O).
2. Soil incubated prior to analysis for 1 week at 35°C with chopped fresh grass, at the ratio 4:1 w/w soil:grass. (0.5 g pre-incubated soil plus 50 µL labelled amino acid mixture plus 5 µL H_2O).

3. Soil autoclaved 2 h prior to assay (0.5 g autoclaved soil plus 50 µL labelled amino acid mixture plus 5 µL H_2O).

4. Mercury chloride inhibition of microbial activity (0.5 g soil plus 50 µL labelled amino acid mixture plus 5 µL $HgCl_2$ 120 mM).

5. Sodium azide inhibition of microbial activity (0.5 g soil plus 50 µL labelled amino acid mixture plus 5 µL Sodium -azide 120 mM).

6. Soil pre-chloroformed for 24 h (0.5 g pre-chloroformed soil plus 50 µL labelled amino acid mixture plus 5 µL Sodium -azide 120 mM).

The evolution of $^{14}CO_2$ from the soils is presented in Figure 3-4, and demonstrates the normal behaviour of unamended soil, in which amino acid-C respiration levels reach a quasi-maximum of 30% of the added C in the amino acid mixture. By the end of the 48 h incubation, nearly all the remainder is immobilised in the microbial biomass (Figure 3-5). Amino acid respiration is completely inhibited in freshly autoclaved soil throughout the incubation, although by the end of the 48 h, just over 20% of added amino acid-C had been immobilised by the recovering biomass.

Figure 3-4 Evolution of CO_2 during incubation with added amino acids in soils of various pre-treatments

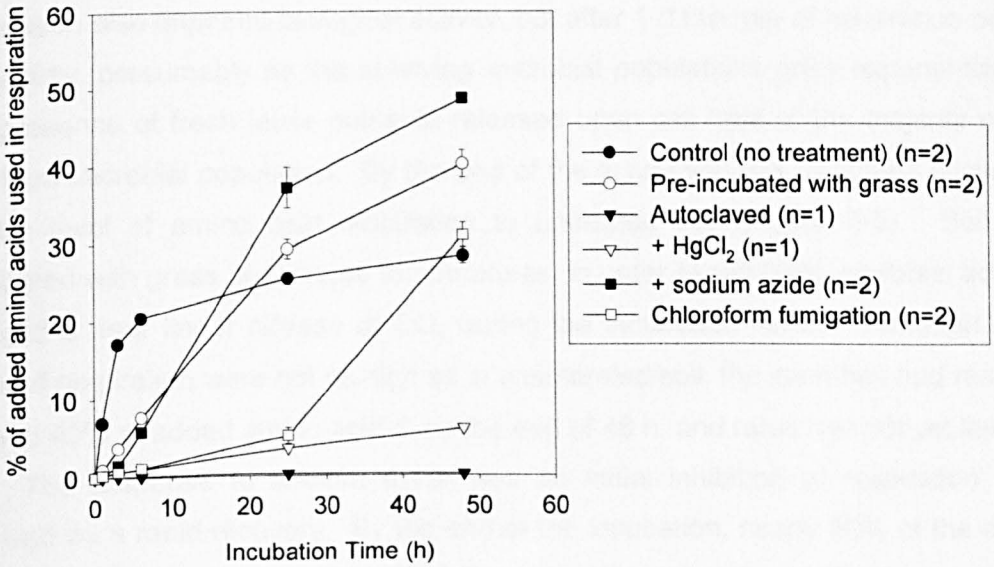
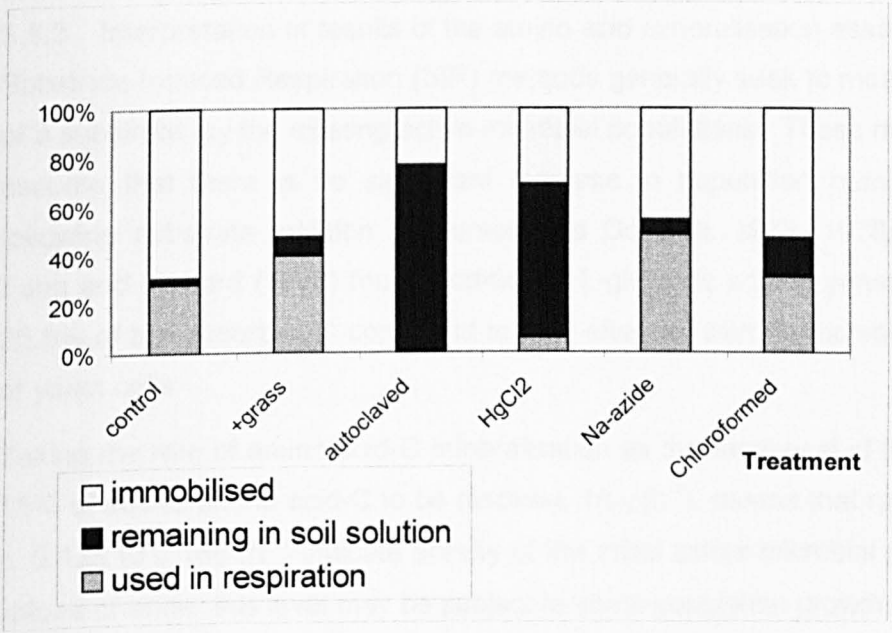


Figure 3-5 Fate of amino acid-^{14}C after 48 h incubation in soils with various pre-treatments



Mercuric chloride severely impaired microbial amino acid respiration. However, the rate of $^{14}CO_2$ production did increase during the incubation period (Figure 3-4), and by the end of the 48 h around 30% of amino acid-C had been taken up by the biomass, although very little was used in respiration (Figure 3-5). Chloroform fumigation also impaired biological activity, but after 1 d the rate of respiration picked up rapidly, presumably as the surviving microbial populations grew exponentially in the presence of fresh labile nutrients released upon cell lysis of the majority of the previous microbial population. By the end of the incubation this treatment showed a similar level of amino acid respiration to untreated soil (Figure 3-5). Soil pre-incubated with grass at elevated temperatures, in order to stimulate microbial activity, showed a near linear release of CO_2 during the incubation period. Although initial rates of respiration were not as high as in unamended soil, the microbes had respired around 40% of added amino acid-C by the end of 48 h, and rates had not yet levelled off. The response to sodium azide was an initial inhibition of respiration rates followed by a rapid recovery. By the end of the incubation, nearly 50% of the amino acid-C had been respired and most of the remainder immobilised. This indicates that the treatment killed some of the microbial biomass, initially inhibiting amino acid respiration rates a little, but the remaining population quickly recovered, feeding off the dead biomass, thereby achieving a stimulated level of metabolic activity.

These data demonstrate the sensitivity of the amino acid mineralisation assay to the level and activity of the soil microbial population and are consistent with the findings of Jones and Shannon (1999).

3.8.3 Interpretation of results of the amino acid mineralisation assay

Substrate Induced Respiration (SIR) methods generally seek to measure metabolism of a substrate, by the existing active microbial populations. These methods generally assume that there is no significant increase in population numbers from 0-6 h following substrate addition (Anderson and Domsch, 1973, 1978). For example, Tang and Howard (1973) found addition of L-glutamic acid to yeast cells resulted in 25.5% of the absorbed C converted to CO_2 after 8h, with no increase in the number of yeast cells.

Taking the rate of amino acid-C mineralisation as the reciprocal of the time taken for 15% of added amino acid-C to be respired, $1/t_{15}$ (h^{-1}), means that rate values of over c. 0.125 to 0.166 (h^{-1}) indicate activity of the initial active microbial population. Rate values of under this level may be subject to some population growth.

Chapter 4 Regulation of amino acid biodegradation in acid soils

4.1 Introduction

To make more effective use of our nitrogen resources and avoid pollution of the atmosphere and freshwaters, we need to gain a greater understanding of the factors that regulate the turnover of soil organic N, the formation of dissolved organic N and its subsequent turnover to inorganic N. The major input of organic matter into most agricultural systems occurs as a result of root turnover, root exudation and residue incorporation. Of this, approximately 5 to 25% can be expected to be in the form of organic N with most present in a labile form as proteins and free amino acids (Marschner, 1995). A critical step in the soil N cycle is therefore the mineralisation of plant proteins to produce free amino acids and their subsequent turnover to CO_2 and NH_4^+ (Stevenson, 1982; Jones et al., 2001). Protein hydrolysis in plant material can occur during apoptosis and after cell death as a result of protease action in the absence of soil microorganisms. It is likely, however, that most protein turnover in soil is mediated by soil microorganisms. The subsequent production and flux of free amino acids in the soil solution can be expected to be dependent on many soil physical, chemical and biological factors which will ultimately determine the quantity and timing of inorganic N becoming available for plant use or nitrified and put at risk of being leached. Amino acids typically constitute around 20% of the soil C and 30-40% of soil N although most of this is present in a polymeric state with free amino acid concentrations ranging from 10 to 100 μM (ca. 0.05% of total amino acid in soil; Monreal and McGill, 1985; Smith et al., 1993). The inclusion of free amino acids and other dissolved organic nitrogen (DON) solutes in N models and as a factor in the calculation of fertiliser recommendations may improve nitrogen resource use, once their fate and behaviour in the environment are more fully understood (Murphy et al., 2000). This is particularly relevant to low input organic systems where amino acids may constitute an important direct source of N for plants (Putnam and Schmidt, 1959; Schobert et al., 1988; Jones and Darrah, 1994).

Currently, no direct link between SON turnover and gross N mineralisation rates has been established (Murphy et al., 2000). Murphy et al. (2000) found that gross mineralisation rates vary less than net N mineralisation across arable soils, possibly a reflection that the turnover of the free amino pool may be a better indicator of gross mineralisation than the net product of mineralisation, because of the confounding issue of immobilisation.

Generally, the mineralisation of soil organic N to NH_4^+ is thought to be relatively insensitive to pH because it is carried out by a range of microflora in which a high level of functional redundancy occurs for common substrates (Haynes, 1986). In contrast, the subsequent conversion of NH_4^+ to NO_3^- appears to be highly pH dependent with nitrification often stimulated by lime application (Haynes, 1986). However, in an effort to achieve more sustainable agronomic systems, a slowing of the N cycle by maintaining a sub-optimal pH for nitrification might lead to less N leaching and better N use efficiency. To date, most studies on the interactions of pH and N mineralisation have focused on the impact of liming (Edmeades et al., 1981; Lyngstad, 1992; Anderson, 1998). The effect of acidity on the rate of microbial processes may be multifacetted with enhanced acidity elevating the levels of general microbial toxins (e.g. Al^{3+}) and inducing shifts in community structure (Schmidt, 1982; Bardgett et al., 2001). Anderson and Domsch (1993) showed that the microbial respiration:biomass ratio, qCO_2, correlated negatively with natural forest soil pH over a range of pH 3-7; this they attributed to acidity-induced stress. However, Nyborg and Hoyt (1978) found no significant relationship between N mineralisation and indices of acidity (soil pH, base saturation, soluble Al, Fe, or soluble Mn), whereas increases in mineralisation due to liming correlated with total soil N, C-to-N ratio and pH.

By tracing the mineralisation of the C in amino acids, one can infer something of the N mineralisation process. Barak et al. (1990) and Barraclough (1997) demonstrated the direct uptake of amino acids into the soil microbial biomass (SMB) as the predominant pathway, as opposed to 'mineralisation-immobilisation-turnover' (MIT) whereby extracellular deamination is followed by NH_4^+ assimilation (Hadas et al., 1992). After entering the soil microbial biomass, amino acids can be immobilised by incorporation into structural cell components such as proteins, nucleic acids etc. In addition, they may also be used for energy production leading to the production of NH_4^+, which may be used internally in amino-compound manufacture or, if the C:N of the microbes is exceeded, exuded from the cell, becoming available to plants or other microbes (Jones, 1999). In this way, measuring the rate of $^{14}CO_2$ evolution gives an indication of the rate of mineralisation of organic N.

The DON pool and its transformations have been largely overlooked in mineralisation research, which has usually concentrated on changes in the size of the soil mineral N pool (Murphy et al., 2000). The aim of this study was to determine the factors regulating amino acid mineralisation in acid soils and to establish the nature of any relationship between C and N, microbial activity and amino acid turnover.

4.2 Materials and methods

4.2.1 Soil sites and sampling regime

Five acid soils were collected from unfertilised unimproved grazing land or natural woodland within North Wales, UK during October 1997. The soil profiles were sampled at sequential depths down to the C horizon using a spade and trowel. Samples were sieved to pass 4 mm and stored in gas-permeable plastic bags at 4°C for a maximum of two weeks prior to analysis. The main characteristics of the soils are provided in Table 4-1. Soil pH was determined using a glass electrode in the supernatant of 1:10 w/v soil:1 M KCl. NO_3^- and NH_4^+ were determined by extraction for 1 h with 1 M KCl (1:10 w/v soil:KCl) with NO_3^- and NH_4^+ determination using a segmented flow auto-analyser (Skalar Co., Netherlands) using the Cu-Cd reduction (Keeney and Nelson, 1982) and modified Berthelot reaction (Searle, 1984) methods respectively. Total C and N were measured on a CHN2000 analyser (LECO corporation, St.Joseph, MI). Basal soil respiration was determined at 18°C using a using a CIRAS-IRGA soil respirometer (PP systems Ltd., Hitchin, UK).

4.2.2 Amino acid mineralisation

Amino acid mineralisation was determined as described in section 3.8, using the air flow system of CO_2 capture. The amount of ^{14}C present in the microbial biomass ($^{14}C_{biomass}$) after 48 h was determined as follows:

$$^{14}C_{biomass} = {}^{14}C_{total} - ({}^{14}CO_2 + {}^{14}C_{K_2SO_4}) \qquad (1)$$

where $^{14}C_{total}$ is defined as the total amount of ^{14}C-amino acid added at the start of the experiment and $^{14}C_{K_2SO_4}$ is the amount of amino acid-^{14}C remaining in the extracted soil solution at the end of the incubation.

4.2.3 Statistical analysis

The time taken for 15% of amino acid-C to be evolved as $^{14}CO_2$ (t_{15}) in hours was estimated by linear interpolation of $^{14}CO_2$ mineralisation curves using the computer package SigmaPlot 4.01 (SPSS Inc., Chicago, ILL). The reciprocal of t_{15} is used as an indicator of the rate of amino acid-C mineralisation. Correlation and regression analyses were performed using SigmaPlot 4.01.

Table 4-1 Sampling sites of the five acid soil profiles studied.

	1	2	3	4	5
Soil type	Leptic podzol	Dystric cambisol	Humic ranker	Dystric cambisol	Orthic podzol
Location	4°01'W 53°14'N	4°01'W 53°14'N	4°15'W 53°12'N	4°15'W 53°12'N	4°06'W 53°10'N
Underlying geology	Slate, shale and grit drift, acid and basic igneous rocks	Colluvium of shales	Mona complex schist	Glacial boulderclay on schist	Acid-igneous drift
Soil series	Denbigh	Cymmer	n/a	Gaerwen	Bodafon
Drainage	Free	Free-excessive	Free	Free	Free-excessive
Rainfall (mm y^{-1})	1130	1120	950	950	1250
Altitude (m)	220	150	40	50	350
Dominant vegetation	*Festuca ovina, Ulex europea*	*Agrostis capillaris*	*Quercus robur*	*Holcus lanatus*	*Calluna vulgaris, Vaccinium myrtillus*
Grazing	Sheep	Sheep	None	Beef cattle	Sheep
Land use	Unfenced upland grazing	Enclosed grassland	Woodland	Enclosed lowland grassland	Unfenced upland grazing

4.3 Results and discussion

4.3.1 Soil characteristics

The pH of the five soil profiles used in this study is shown in Figure 4-1A, which indicates that all the profiles can be classified as acidic. The Orthic podzol possessed the greatest pH range of one pH unit from the soil surface to the subsoil (C horizon). The Humic ranker had a large accumulation of organic matter accompanied by little mineral soil development and exhibited an exceptionally low pH. Following an initial decrease in pH with depth in profiles 1, 2 and 3, all profiles show an increase in pH with greater depth, probably because the lower layers are less heavily leached of cations and greater buffering by soil minerals occurs. A similar pattern of pH throughout the profile of acidic soils was observed by Persson and Wirén (1995) with a pH minimum in the H layer or in the upper E horizon and then rising with increasing depth. As expected, the levels of both soil total C and N declined rapidly with soil depth (Figure 4-1B&C). All soils showed similar levels of total C and N and similar decreases in these with increasing depth, with the exception of the Humic ranker, profile three, which had higher total C and N than the other soils at each depth. The C-to-N ratio of the organic matter in the individual soil profiles typically ranged from 14 to 20.

The distribution of inorganic N down the profile of the five soils surveyed is shown in Figure 4-2. Mineral N decreased with depth and NO_3^- was generally the dominant form in the upper horizons and NH_4^+ generally increased in dominance with greater depth. NO_3^- was the predominant form of mineral N in all profiles except the Orthic podzol possibly due to the inhibition of nitrification by high acidity in this soil. However, NO_3^- dominated in profile three which was the most acidic. The presence of high concentrations of NO_3^- indicates the occurrence of nitrification, which is conventionally thought not be a rapid process in acid soils. It is unlikely that the origin of this NO_3^- was atmospheric deposition. However, this data is supported by a number of studies that have detected significant amounts of nitrification in acid soils (Stams et al., 1990; Deboer et al., 1991).

Figure 4-1 Characteristics of the five soil profiles as a function of soil depth

Panel A shows the relationships of soil depth with pH (determined in 1:10 w/v 1 M KCl), panel B shows the relationships of soil depth with total soil C and the lower panel (C) the relationships of soil depth with total N. All data points represent means (n=2). The legend is the same for all panels.

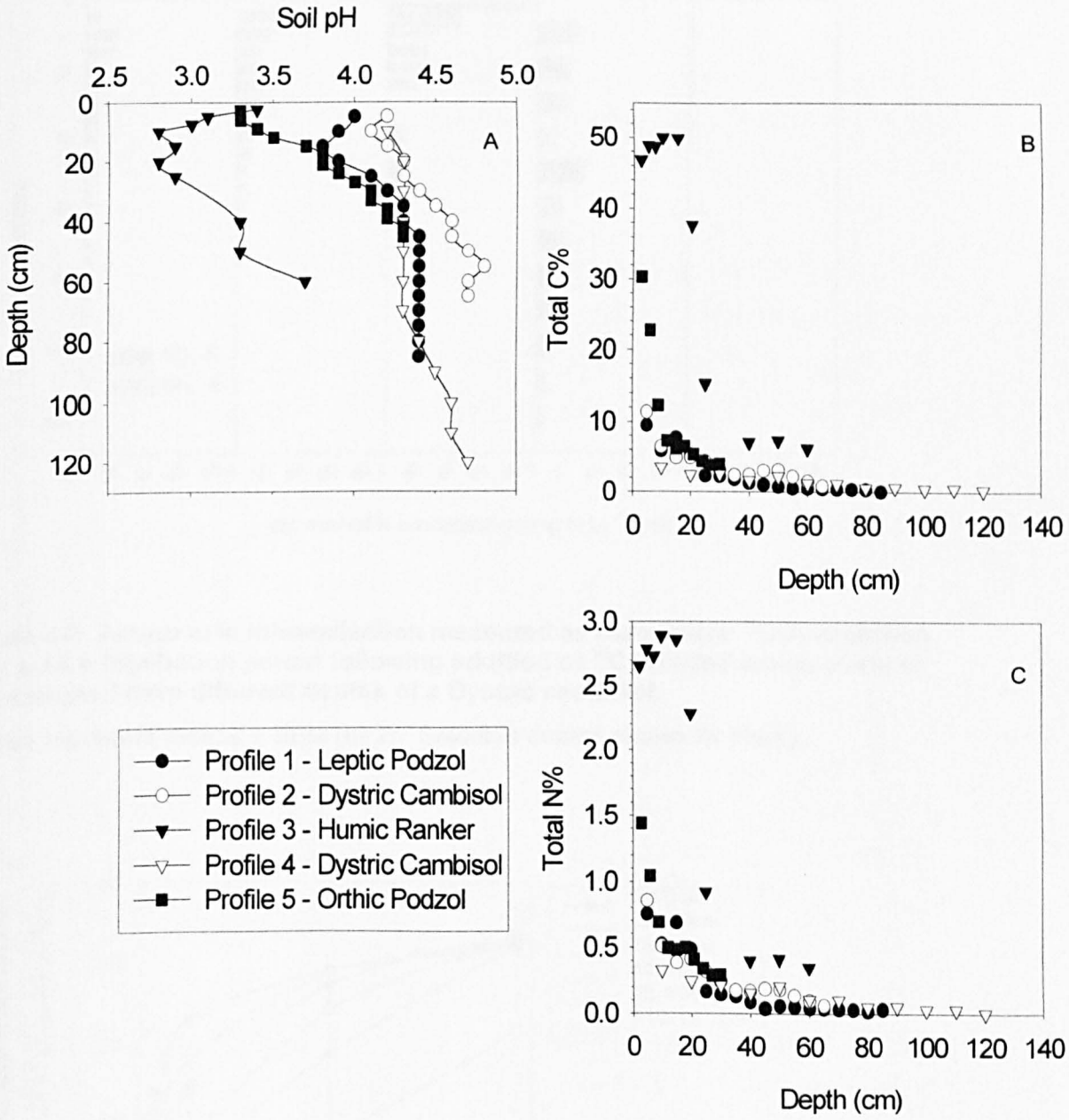


Figure 4-2 Distribution of inorganic N in the five soil profiles as a function of soil depth.

All data points represent means (n=2). The legend is the same for all panels.

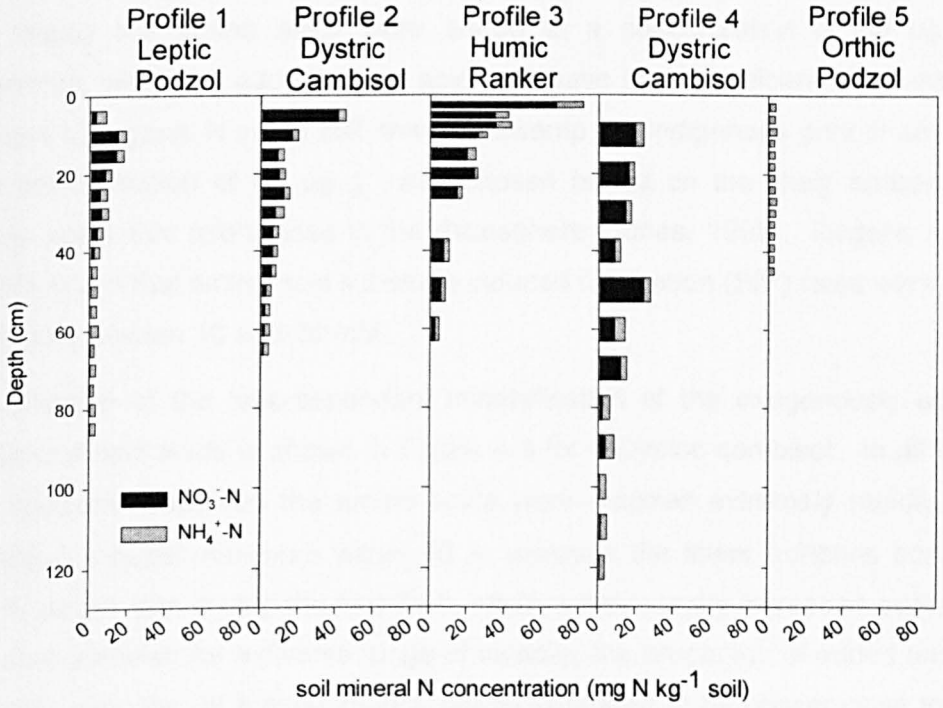
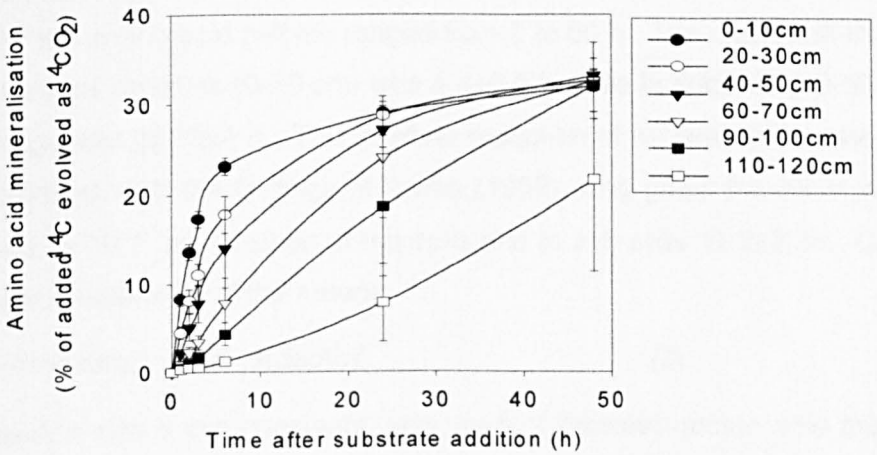


Figure 4-3 Amino acid mineralisation measured as cumulative $^{14}CO_2$ evolution over a 48 h incubation period following addition of ^{14}C-labelled amino acids to soil sampled from different depths of a Dystric cambisol.

Values represent means ± SEM (n=2). Selected depths shown for clarity.



4.3.2 Amino acid mineralisation

Previously reported concentrations of free amino acids in soil typically range from 0.002 to 4.72 μg g^{-1} (Monreal and McGill, 1985; Putnam and Schmidt, 1959), while in this assay the amino acids were added at a concentration of 66 μg g^{-1} soil. Therefore, while the added amino acids will have an insignificant effect on the total amount of organic N in the soil, they will swamp the indigenous pool of amino acids. The concentration of 66 μg g^{-1} was chosen based on the likely concentration of amino acids that might arise in the rhizosphere (Jones, 1999). Degens and Harris (1997) found that amino acid substrate induced respiration (SIR) rates were generally maximal between 10 and 20 mM.

An example of the time-dependent mineralisation of the exogenously added ^{14}C-labelled amino acids is shown in Figure 4-3 for a Dystric cambisol. In all the upper soil horizons examined the amino acids were respired extremely rapidly, the rate reaching a quasi-maximum within 48 h, whereas the lower horizons possessed a much slower rate during the first 24 h, which subsequently increased over time. As previously shown for a diverse range of topsoils, the proportion of added amino acids respired over the 48 h experimental period appeared to be conservative for all soils and depths at around 30% (Jones, 1999; Jones and Hodge, 1999; Jones and Shannon, 1999; Vinolas et al., 2001). To estimate the half-life of the exogenously added amino acids the time taken for 15% of the ^{14}C-labelled amino acid-C to be recovered as $^{14}CO_2$ has been calculated. As soil samples from a depth of more than 30 cm in the Orthic podzols did not respire 15% of the added amino acid-C within the 48 h period, these samples were omitted from further analyses (n=5) in order to avoid error associated with extrapolating data beyond time monitored. Across all soils the amino acid half life ranged from 2 to 50 h. The mean half-life of amino acids in surface horizons (0-10 cm) was 4.4±0.9 h while in subsoils (40-50 cm) it was five-fold greater at 20±4 h. This gradual reduction of mineralisation rate with depth was consistent with the findings of Jones (1999), who gives the mean half-life of amino acids at 18°C as 1.7±0.6h in top soils and in sub soils 12.2±3.3h. Generally, a non-linear relationship of the nature:

$$\text{mineralisation rate} = a(\text{depth})^b \qquad (2)$$

where a and b are constants, was evident between amino acid mineralisation rate and soil depth (r^2=0.89 to 0.97; Figure 4-4). The relationship between mineralisation activity and depth is likely to be a reflection of a higher microbial biomass and activity

in the upper organic horizons, compared to subsoils with lower organic matter and therefore reduced microbial biomass activity. Indeed, the rate of mineralisation correlates well with total soil C and total N within each profile (Figure 4-5), which are in turn strongly related to soil depth (Figure 4-1). The rate of amino acid mineralisation correlates significantly ($P<0.0001$) with total soil C and total N (linear regression r^2 values of 0.85 to 0.98 for C; and 0.82 to 0.98 for N). The linear regression analysis for profile 3 ignored the three outlying points with the fastest mineralisation rates (Figure 4-5). These points corresponded to the surface 7.5 cm of the profile. In support of these results, Tessier et al. (1998) also found a strong correlation between soil microbial biomass and total soil C throughout the profile in a Canadian arable Humic Gleysol. Further, Person and Wirén's (1995) work on acid forest soils also demonstrated mineralisation rates to be distinctly higher in the litter and humus horizons in comparison to the mineral subsoil. When the mineralisation rates of the Person and Wirén's (1995) study were expressed on a per g organic matter basis they also decreased with depth, in agreement with the present study.

Figure 4-4 Relationship between the rate of amino acid-C mineralisation in soil and depth in five acid soils.

Values represent means (n=2). Symbols represent experimental data points whilst lines represent fits of these points to equation 2.

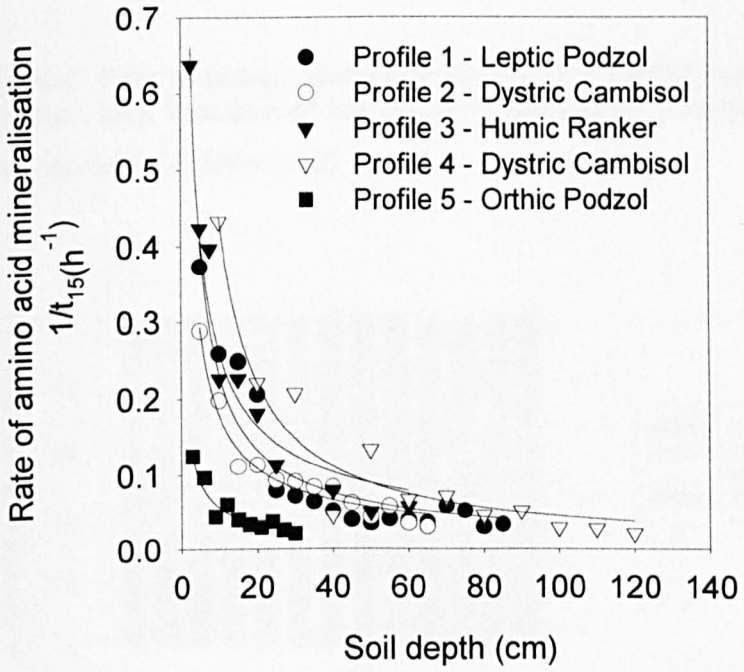


Figure 4-5 Relationship between amino acid half-life in soil and (A) total soil N and (B) total soil C in five acid soils.

Values represent means (n=2). Symbols represent experimental data points whilst lines represent fits of these points to a linear regression equation. Linear regression for profile 3 excludes three boxed outlying points in each panel.

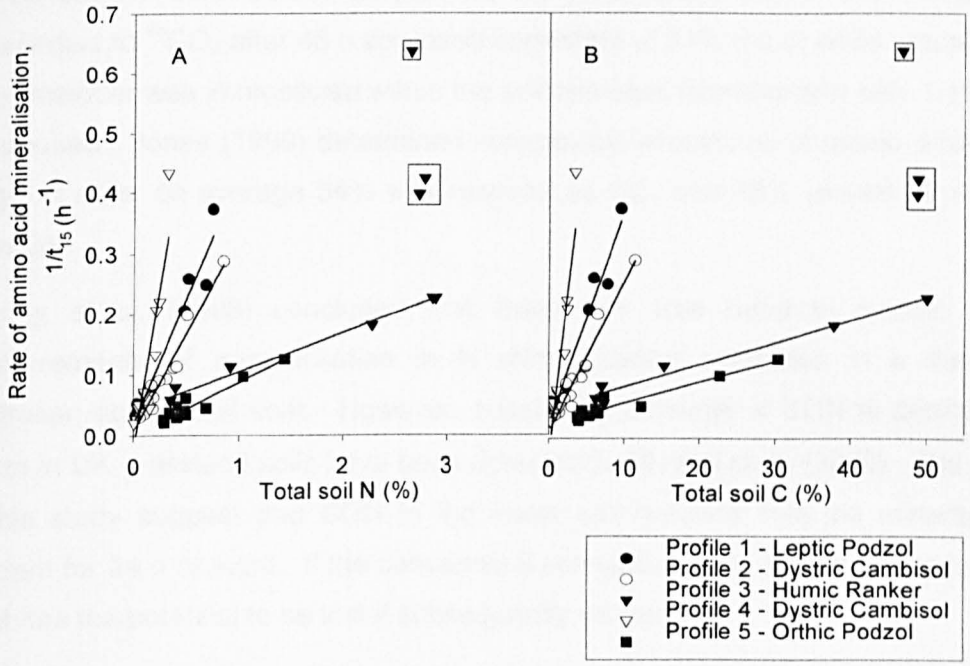
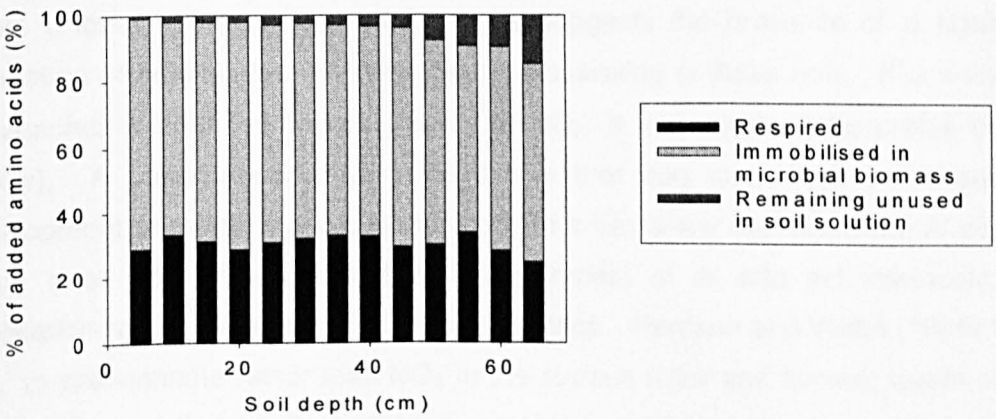


Figure 4-6 Fate of the exogenously added ^{14}C-labelled amino acids after 48 h incubation as a function of soil depth in the Dystric cambisol, profile 2

Values represent means (n=2)



The amount of amino acids remaining unmineralised in the soil after 48 h increased with depth in all the soils studied (e.g. profile 2 represented in Figure 4-6). From Figure 4-3, however, it appears that the rate of amino acid uptake increases with time indicating either an upregulation of amino acid transporters and metabolism enzymes within the microbial community, or less likely, an increase in microbial biomass. In the surface horizons across all profiles, the mean proportion of the amino acids mineralised to $^{14}CO_2$ after 48 h appeared consistent at 31% (±2.0) while virtually all of the remainder was immobilized within the soil microbial biomass with only 1.1% ± 0.1 left unused. Jones (1999) determined comparable allocations of amino acid-C in a range of soils; on average 34% was respired as CO_2 and 66% utilised for new cell biomass.

Murphy et al. (1998) concluded that there was little need to include subsoil measurements of mineralisation in N mineralisation estimates in a variety of Australian agricultural soils. However, substantial amounts of SON at depths up to 90 cm in UK grassland soils have been detected by Bhogal et al. (2000). The results of this study suggest that SON in the lower soil horizons may be mineralised if resident for 24 h or more. If the converted N remains as NH_4^+ it may not be leached, but it has the potential to be lost if subsequently nitrified.

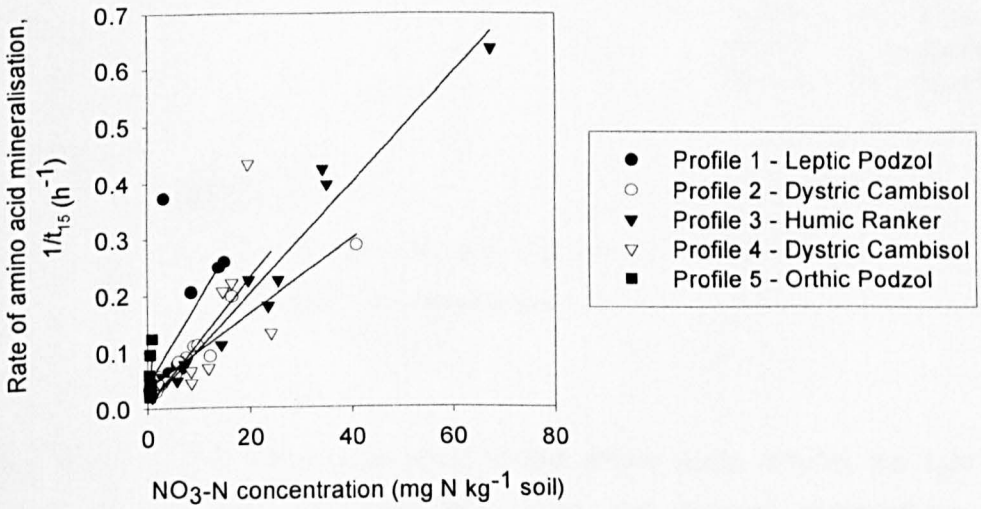
The dominance of NO_3^- as the form of mineral N in four of the soils was unexpected (Figure 4-2) due to the levels of acidity and the widespread reports that nitrification is generally inhibited at low pH. The rate of amino acid mineralisation was found to correlate positively and linearly with soil NO_3^- concentration ($[NO_3^-]$) in each of the soil profiles tested (Figure 4-7, linear regressions significant, $p<0.05$, in all but profile 5). This directly implies that when mineralisation occurs, it is providing a substrate for nitrification to take place, despite the high levels of acidity. There is more NO_3^- present in the upper soil horizons despite the pH generally being lower in those layers (Figure 4-1 and Figure 4-2). This suggests the presence of a significant population of acid-tolerant nitrifying bacteria operating in these soils. It is, however, unexpected to find the highest levels of NO_3^- in the most acidic profile (Humic ranker). A possible explanation for this is that due to its lack of mineral soil development and high organic matter content it has a low exchangeable Al content, which does not inhibit nitrification. The impact of Al and pH interactions on nitrification rates is worthy of further investigation. Persson and Wirén (1995) found NH_4^+ to predominate rather than NO_3^- in the surface (litter and humus) layers of acid forest soils and that levels increased at greater depths. In their organic horizons, nitrification was not found to achieve completion below a pH (H_2O) of 4.7, while in the

mineral subsoil, the ratio of nitrification to net N mineralisation was often 100% down to a pH value of 4.0. They observed no nitrification in subsoils at a pH lower than 3.95 (H_2O) or 2.9 (KCl). In the current study, the most acidic sample, pH 2.8 (KCl) contained over 20 μg NO_3-N g^{-1} soil, although it is possible that in this sample, the NO_3^- could have originated from higher soil layers. Another potential explanation is that the nitrifying bacteria in this acid soil do not rely on microsites with high pH (as do acid sensitive autotrophic nitrifiers) or availability of organic C (as do heterotrophic nitrifiers as suggested by Deboer et al. (1991)). In this situation, the cells probably protect themselves from the toxicity of nitrous acid by aggregating (Deboer et al., 1991).

Inspection of the data revealed no obvious relationship between amino acid half-life and soil C:N, the proportion of N that is mineral (NO_3^- or NH_4^+) or initial NH_4^+ levels. This is in general agreement with Persson and Wirén (1995) who found that in litter layers, sites with a high N mineralisation rate had a low C:N ratio and vice versa, but overall C:N of the soil was not well correlated with mineralisation rates in acid forest soils.

Figure 4-7 Relationship between the rate of amino acid-C mineralisation in soil and intrinsic soil NO_3^- concentration in five acid soils.

Values represent means (n=2). Symbols represent experimental data points whilst the lines represent fits to linear regressions.



With the exception of one of the Dystric cambisols, there was a strong positive correlation between basal respiration rate in unamended soils versus the rate of

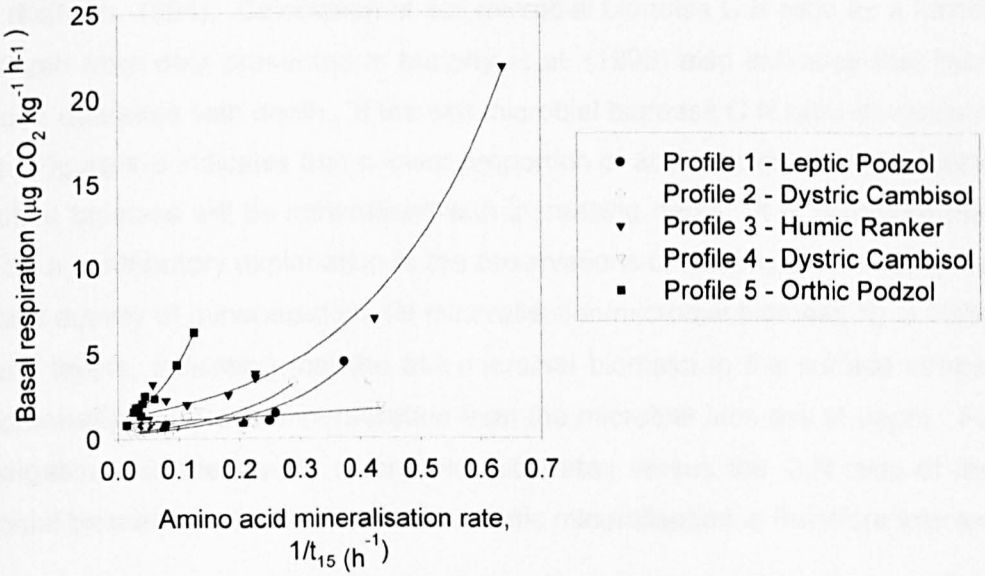
amino acid mineralisation (Figure 4-8). This relationship could be reasonably well described by the exponential equation

$$\text{Rate of amino acid-C mineralisation} = a \times e^{-b(\text{basal soil respiration})} \quad (3)$$

where a and b are constants, with r^2 values of 0.90, 0.88, 0.98, 0.16 and 0.96 for soil profiles 1 to 5 respectively ($p<0.0001$ in all but the Dystric cambisol, profile 4). Although basal respiration is not a direct indicator of soil microbial biomass, it can be used as an indicator of general soil catabolic activity. This relationship suggests that overall, the mineralisation of this range of amino acids is linked to general metabolic activity, reflecting the widespread ability of the soil microbial community to mineralise low molecular weight organic compounds quickly. The reason why one of the Dystric cambisols should not conform to this relationship is not clear.

Figure 4-8 Relationship between amino acid-C mineralisation rate and soil basal respiration in five acid soils.

Values represent means (n=2). Symbols represent experimental data points whilst the lines represent fits to exponential regression equations.



The rapid use of the exogenously added amino acids reflects the fact that their transport into the microbial cells does not require extracellular enzymes (Barraclough, 1997) and that most amino acids are weakly sorbed to the soil's solid phase (Jones, 1999). Putnam and Schmidt (1959) showed that basic amino acids, whilst complexed with clay particles in a bentonite suspension and a soil matrix, were no less susceptible to microbial attack than was the free amino acid. Utilisation of

bound amino acids may be related to the relative affinity for the clay and microbial permeases, the location on the clay, and the yield of energy necessary for their transport from the clay into the cell (Dashman and Stotzky, 1986). Barraclough (1997) verified the direct route for amino acid assimilation, and developed the following relationship

$$P=(1-S.E/B) \qquad (4)$$

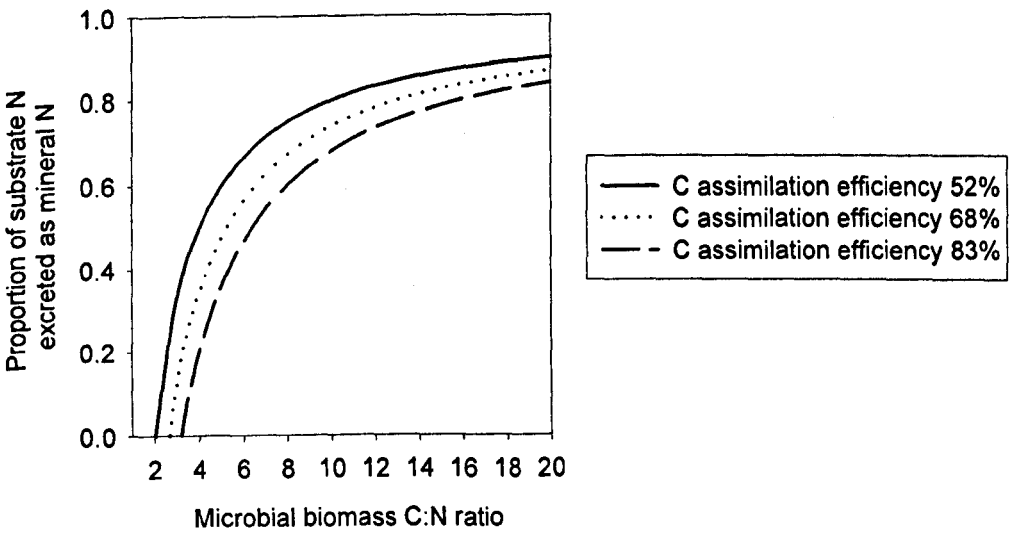
where P is the proportion of N in the substrate that is mineralised, S is the C:N of the substrate, B is the C:N of the assimilating organism and E is the assimilation efficiency of the organism (ratio of assimilated C to C in the substrate). In this study E was calculated to be on average 68% for the surface soil horizons with a range of 52 to 83% throughout the whole soil profile and S to be 3.86 (average C:N of the 15 added amino acids). Based upon the data and equation 4, Figure 4-9 shows the theoretical proportion of the amino acid-N which is taken up by the soil microbial biomass that will be excreted as NH_4^+, with varying C:N ratio of the soil microbial biomass, over the range of C assimilation efficiency observed in these experiments. Although soil microbial biomass C-to-N ratios were not calculated here it is generally thought that the C:N ratio of bacteria ranges from 4:1 to 5:1 and fungi from 10:1 to 15:1 (Killham, 1994). Calculation of soil microbial biomass C:N ratio as a function of soil depth from data presented in Murphy et al. (1998) also indicates that microbial C:N can decrease with depth. If the soil microbial biomass C:N ratio decreases with depth, Figure 4-9 indicates that a lower proportion of amino acids taken up by the soil microbial biomass will be mineralised with increasing depth. It is proposed that this may be a contributory explanation to the observations of Murphy et al. (1998) that the 'specific activity of mineralisation' (N mineralisation/microbial biomass N) is highest in surface layers, indicating that the soil microbial biomass in the surface contributed proportionally more to N mineralisation than the microbial biomass at depth. Further investigation into the quality of organic substrates versus the C:N ratio of the soil microbial biomass as a determinant of specific mineralisation is therefore warranted.

The rate of amino acid mineralisation is significantly and negatively correlated with pH within each profile (mineralisation rate decreases as pH increases, data not presented), an unexpected result. However, this is probably an artefact of a co-correlation of pH with depth. No significant correlation ($p>0.05$) was found between pH and rate of amino acid mineralisation in samples of the same depth across different profiles.

The relationships between amino acid mineralisation rate with depth, organic C and N and basal metabolic rate only hold within each profile, not between all profiles. While similar relationships exist between these factors in most profiles, it is not clear why the coefficients of the models differ between profiles, or some profiles do not fit the models. This suggests that there are important factor(s) controlling gross mineralisation other than organic matter content of the soil, which were not determined here. These may include microclimate aspects such as temperature and rainfall, physical soil aspects such as texture or differences in microbial community structure including microbial C:N ratio.

Figure 4-9 Theoretical calculation of the impact of microbial biomass C-to-N ratio on the amount of amino acid N excreted into the soil as a function of different amino acid assimilation efficiencies.

See results and discussion for further details of the calculations.



The extremely rapid use of amino acids by the soil microbial biomass demonstrated here, alongside the typically low free amino acid pool in the soil solution (1 to 100 μ M; Monreal and McGill, 1985), indicates that the continual breakdown of proteins by protease enzymes must occur, rapidly replenishing the quickly consumed free amino acid pool (Jones, 1999). It seems probable that mineralisation of organic N, whether from an input or from native organic matter, will be limited by the activity of extracellular protease enzymes and availability of digestible substrates than by the mineralisation of amino acids into NH_4^+ and CO_2. Characterisation and quantification of dissolved organic nitrogen by identifying pools of varying digestibility in soil or

functional groups will yield a better understanding of the fluxes and pathways of N compounds within the plant-soil-microbe system.

Table 4-2 Main conclusions of experimental work of Chapter 4

- The rate of amino acid mineralisation decreased non-linearly with depth.
- In the surface horizons across all profiles, the mean proportion of the amino acids mineralised to $^{14}CO_2$ after 48 h appeared consistent at 31% (±2.0) while virtually all of the remainder was immobilized within the soil microbial biomass with only 1.1% ± 0.1 left unused.
- The relationship between amino acid mineralisation activity and depth is likely to be a reflection of a higher microbial biomass and activity in the upper organic horizons, compared to subsoils with lower organic matter and therefore reduced microbial biomass activity. The rate of mineralisation correlated significantly and linearly with total soil C and total N within each profile.
- The acid soil profiles examined contained large quantities of NO_3^-, suggesting occurrence of nitrification.
    - There was more NO_3^- present in the upper soil horizons despite the pH generally being lower in those layers. This suggests the presence of a significant population of acid-tolerant nitrifying bacteria operating in these soils.
- In most horizons there was a strong positive correlation between basal respiration rate in unamended soils versus the rate of amino acid mineralisation. This relationship suggests that overall, the mineralisation of this range of amino acids is linked to general metabolic activity, reflecting the widespread ability of the soil microbial community to mineralise low molecular weight organic compounds quickly.
- It seems probable that mineralisation of organic N, whether from an input or from native organic matter, will be limited by the activity of extracellular protease enzymes and availability of digestible substrates than by the mineralisation of amino acids into NH_4^+ and CO_2.

Chapter 5 Changes in amino acid mineralisation over three successional grazing gradients in UK

5.1 Introduction

Natural, semi-natural or managed ecosystems are dynamic and as they change, so do the functions they perform. In recent years there has been increased focus on the roles of soil organisms in regulating ecosystem processes such as nutrient cycling and organic matter decomposition and there is increasing study of how decomposer organisms respond to changes in land management (Bardgett et al., 2001, included in appendix 1).

A change in one component of an ecosystem can be expected to feed back and influence all other parts, a point illustrated by Brussaard et al. (1996):

> "Nutrient mineralisation/immobilisation in soil and biomass production/decomposition are important processes in ecosystems... ...changes in these processes, following major changes in land use are associated with changes in ecosystem structure, which are co-determined by biological interactions below-ground and the rates of colonisation of both plants and soil organisms."

Thus community structure and ecosystem function are inextricably linked.

Sheep (*Ovis aries*) grazing is an economically important agricultural practice in the uplands of the North-West region of the UK, and it has major ecological consequences. Long-term variations in the frequency (in some cases, dating back 2000-3000 y) and intensity of grazing by sheep has led to the development of successional transitions, from ancient, unmanaged oak (*Quercus petraea*) woodland, which is thought to represent the climax community (Pearsall, 1950), through to intensively grazed grasslands where more than 90% of the annual aboveground productivity (AAP) is consumed (Miles, 1985).

Few studies have examined such successional transitions and this chapter seeks to extend current understanding of the effects of grazing on biological properties of soils by comparing three vegetation gradients, at different locations, for which the primary varying factor is grazing intensity. Rough upland grazing is not generally thought to contribute to NO_3^- leaching because it is not ploughed, it is marginally fertilised and rainfall is high enough to dilute what little NO_3^- is leached. However, there is some concern regarding increased NO_3^- in water from these sources (Addiscott et al., 1991). N cycling in uplands is of importance because of their distinctive, sensitive ecology and our reliance on upland regions as sources of pure water for the water supply industry (Cuttle et al., 1996).

5.1.1 Herbivory, the plant community and the soil microbial community

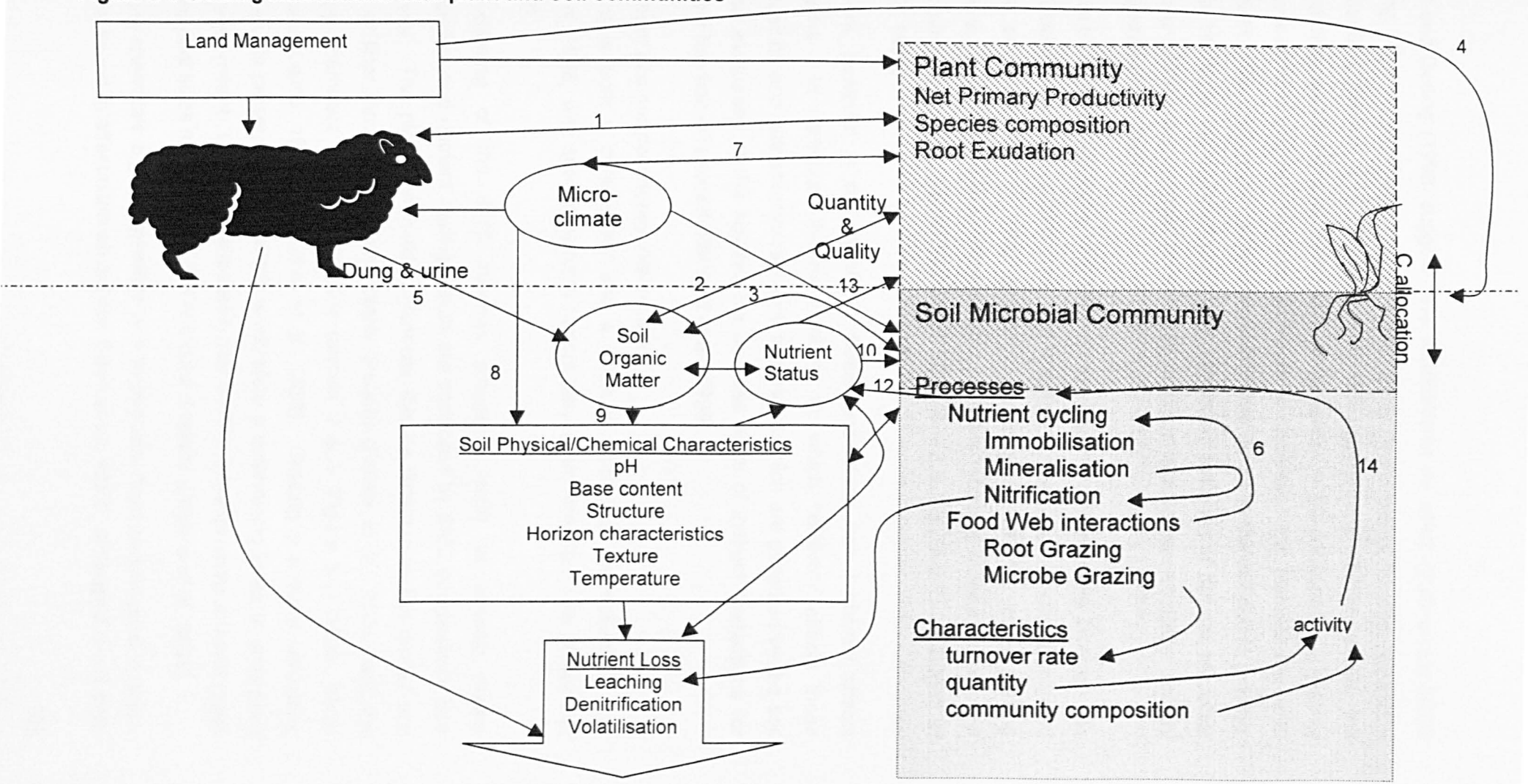
Herbivores affect the plant community via the physical effects of selective removal of plant material and trampling, thus acting as a continual disturbance to succession (arrow 1, Figure 5-1), preventing the establishment of woody mid-late successional plant species. The precise impact of grazing on the plant community will depend on the species, breed and density of livestock or wild herbivore involved.

This interruption to the progress of succession is likely to have large impacts on nitrogen dynamics. The conversion of woodland to grassland results in a decrease in soil N levels, taking 50-100 years for steady state conditions to be reached (Haynes, 1986), and if secondary succession is permitted to resume, the organic matter and N content of the soil increase again. As all inputs of detritus to the soil are derived from living plant biomass (directly or indirectly), levels of soil organic matter and nitrogen follow trends similar to those of total ecosystem biomass during succession (Haynes, 1986).

Grazing has indirect effects on ecosystem productivity and the soil food-web by influencing the quality and quantity of organic matter input from animal excreta and plant litter (arrows 2 & 3, Figure 5-1), the plant C allocation (arrow 4, Figure 5-1) and root exudation (Bardgett et al., 1998). This may affect the net primary productivity (NPP), the below ground biomass and eventually the composition of the plant community. Grazing may accelerate nutrient cycling in grasslands by increasing the soil carbon supply, which, in turn, increases the size and activity of the soil microbial biomass (Tracey and Frank, 1998; Bardgett et al., 1998). This could further stimulate net nutrient mineralisation and increase soil nutrient availability (Mawdsley and Bardgett, 1997; Bardgett et al., 1998), thus accounting for the greater shoot nutrient content and productivity that is often reported in grazed grasslands (Holland and Detling, 1990). Holland and Detling (1990) found grazing by prairie dogs resulted in an increase in plant available N because grazing decreased C allocation below-ground, which limited C availability to decomposers, causing decreased N immobilisation, increased net N mineralisation, and thus an increase in plant-available N.

Herbivory can induce non-linear or biphasic (both positive and negative) growth and development in plants, with the result that at low levels of herbivory overall community responses show increases in production potential, whereas extreme herbivory causes extreme reduction in productivity (Dyer et al., 1993). The transition between these two extremes defines a point of optimal herbivory with respect to C and N processes and production potential (Dyer et al., 1993).

Figure 5-1 Grazing interactions with plant and soil communities



Holland and Detling (1990) suggest other mechanisms via which grazing may affect N cycling: (1) dung deposition may contribute to net N mineralisation and decreased immobilisation (arrows 5 & 3, Figure 5-1); (2) increased predation on the soil microbial biomass (SMB) (e.g. by parasitic nematodes); or (3) enhanced root grazing with above ground grazing may also contribute to decreased root biomass (arrow 6, Figure 5-1). The change in nature of organic matter inputs to the soil due to grazing, can also influence the composition and/or enzymatic activities of the soil microbial community (SMC), e.g. urease activity may increase with enhanced grazer activity (McNaughton et al., 1997).

A general trend in the influence of grazing pressure on the soil microbial biomass has been elucidated: heavily grazed grassland appears to favour fast cycles dominated by labile substrates and a SMC dominated by bacteria, while a lower grazing pressure supports slow cycles characterised by more resistant substrates with soil fungi dominating decomposition pathways (arrows 1,2,3, Figure 5-1) (Bardgett et al., 1993, 1996).

More work, however, is still needed to better understand how herbivory affects ecosystems. In particular, the mechanisms by which herbivory affects those decomposition and nutrient mineralisation processes which are governed by the soil biota and evaluation of the significance of these sorts of indirect interactions for ecosystem function is required (Bardgett et al., 1998).

5.1.2 Plant and soil community interactions

The plant is both a determinant and a result of ecological interactions in soil (Brussard, 1998) with species-specific factors playing an important role (Wardle et al., 1999).

The functioning of the SMC includes processes such as organic matter decomposition and nutrient cycling, which are controlled by SMC composition, size and activity. The plant community influences this by determining the quality and quantity of litter inputs (above and below ground) (Felske et al., 2000), and the secondary chemicals that litter contains (arrows 2 & 3, Figure 5-1) (Miles, 1985; Barford and Lajtha, 1992; Van Cleve et al., 1993). Grazing is a factor controlling succession, its presence and intensity is therefore a determining factor in what plant species are present. Ecosystems creating litter additions that promote a broad range of organic pool sizes may enhance soil microbial diversity (Degens et al., 2000).

Secondary chemicals e.g. polyphenols and terpenoids, from certain plant species, have been found to affect nitrification (Van Cleve et al., 1993), although it is not clear

whether this is direct inhibition or substrate limitation due to immobilisation caused by the high C:N ratio of these compounds (Barford and Lajtha, 1992). A feedback system may exist whereby increased nutrient availability across the successional gradient affects N mineralisation by stimulating decomposition of phenolic compounds, which form recalcitrant phenol-protein complexes in the organic layer of nutrient poor acid soils (Kristensen and Henriksen, 1998) (arrows 2, 3, 12, 11, Figure 5-1). N mineralisation was closely related to recalcitrance of the detrital materials typified by widening lignin:N ratios of the litter layer across the sequence of vegetation types in the work by Van Cleve et al. (1993); a very large change in net N mineralisation occurred among successional stages for relatively small changes in the lignin:N ratio.

Secondary chemicals and products of decomposition also directly alter the soil pH (Kristensen and Henriksen, 1998) and may accelerate eluviation (Miles, 1985) (arrow 9, Figure 5-1). There is also variation between species in the extent to which precipitation is chemically modified and reaches the ground as stemflow. Therefore changes in plant species will change labile soil properties such as topsoil pH, organic matter and exchangeable base content, and under long lived species more profound changes will occur such as structure, horizon thickness, colour and boundary sharpness (Miles, 1985).

Plant community composition will also affect the microclimate (arrow 7, Figure 5-1) (Kristensen and Henriksen, 1998) and hence associated physical soil properties, for example soil temperature (arrow 8, Figure 5-1), which may decline with advance in succession (Van Cleve et al., 1993).

The nutrient supply from soil may determine the species richness and diversity of the vegetation. Direct interactions between roots and soil organisms also affect plant nutrition, colonisation and productivity (Brussard et al., 1996). The relationship between soil nitrogen availability and plant community structure (arrow 13, Figure 5-1) was investigated in old-fields in the shortgrass steppe of Colorado by Paschke et al. (2000). The addition of N generally resulted in increased abundance of annual forbs and grasses relative to perennials at all of the previously cultivated sites. Conversely, experimental reduction of N availability (via sucrose additions) generally increased the relative abundance of perennials. The degree to which N additions increased N availability at the various sites supported the idea that late-seral plant communities are less effective at N capture relative to earlier-seral communities. Contrastingly, while Huberty et al. (1998) found that nitrogen addition significantly increased above ground plant biomass, it did not alter the displacement or

persistence of different life-history plant groups, or affect mean species density or the Shannon index of diversity. The work of Foster and Gross (1998) suggested that the declines in species richness associated with fertilisation could largely be due to the suppressive effects of increased litter and living biomass, due to their capacities to attenuate light to very low levels.

5.1.3 Soil food web interactions

The soil food web and trophic structure, which is influenced by the distribution of pore spaces as determined by soil texture (Hassink et al., 1993), has important impacts on mineralisation (arrows 14 & 6, Figure 5-1). Grazing of bacteria by bacterivorous nematodes and flagellates may considerably increase N mineralisation (Hassink et al., 1993); the direct contribution of bacterial grazers to N mineralisation may be significant as their C:N ratio is relatively high compared with the C:N ratio of their food (not the case with bacteria). Faunal grazing on bacteria may keep the microbial population in a metabolically more active state, and therefore result in an increased turnover of bacterial biomass and increased mineralisation of both C and N.

Soil macrofauna mix and inoculate litter and graze on bacteria and fungi that grow on dead organic substrates. In addition, they carry hot spots of microbial activity in their guts and create them in their excrements (Smith and Steenkamp, 1992). Soil micro and meso fauna do not contribute greatly to total soil respiration but under nutrient limited circumstances they accelerate the turnover of organic matter/microbial biomass/nutrients, which has been reported to lead to contributions of 30-80% to total net nitrogen mineralisation (Smith and Steenkamp,1992; de Ruiter et al., 1994; Griffiths, 1994; Brussard et al., 1996; Yeates and Wardle, 1996).

The difficulty of determining the functional diversity of the SMB is confounded by the problem of determining the functionally active portion of the SMB (Klein et al., 1998; Degens et al., 2000). Klein et al. (1998) suggest that the increasing role of bacterial functional biomass in later successional soils is indicative of major changes in root derived materials as succession proceeds.

5.1.4 Objectives

The objective of this study was to examine how successional transitions and grazing intensity influence the biomass, activity, and structure of soil microbial communities in relation to nitrogen dynamics. Nitrogen mineralisation, especially mineralisation of dissolved organic nitrogen (DON) was examined. Consistency of any trends over the three locations was evaluated.

5.2 Methods

5.2.1 Site and sampling

Samples were collected during June 1998 from three successional grazing gradients in the North West region of the UK, representative of the biogeographic zones of western UK. These were: A - Dentdale, Yorkshire Dales National Park, B – Patterdale, Lake District National Park and C - Cwm Dyli and Abergwyngregyn, Snowdonia National Park, and are detailed in Table 5-1 (taken from Bardgett et al. 2001). Sampling was performed by Dr Richard Bardgett, with assistance in Snowdonia from Dr Davey Jones and the author.

This work was a large collaborative study, coordinated by Dr. Richard Bardgett of Lancaster University, and published by Bardgett et al. (2001); this publication contained total C and N analysis performed by the current author. The current chapter reports work carried out by the author on amino acid mineralisation activity and supporting work by Dr. Andrew Owen (University of Wales, Bangor) on net mineralisation and exchangeable aluminium and calcium on the same samples, not presented in Bardgett et al. (2001).

At each location six vegetation types were selected, representing a successional transition based on the history and intensity of sheep grazing (hereafter referred to as a gradient of grazing influence). These are considered as treatments:

1) unmanaged, ancient oak (*Quercus petraea*) woodland, which acted as an ungrazed control

2) long-term (20 y plus) unmanaged and ungrazed grassland with woodland regeneration

3) short-term (5-to-10 y) unmanaged and ungrazed grassland

4) lightly grazed *Nardus-Galium* grassland where some 50% AAP is consumed

5) moderately grazed *Festuca-Agrostis* grassland where some 60-80% AAP is consumed

6) intensively sheep grazed *Festuca-Agrostis* pasture where more than 90% AAP is consumed (Miles, 1985).

All soils were formed on glacial till, but the underlying geology differed at each location. Underlying rocks in Yorkshire are Silurian slates and shales, whereas in the Lake District and Snowdonia they are volcanic rocks of Ordovician age. All parent

material was base poor, and the topography and altitude of treatments at each location were very similar.

The sampling protocol involved bulking ten 3.5 cm diameter soil cores (10 cm depth) taken in close proximity. This was repeated in three randomly located replicate plots (10 x 10 m) at each site, giving 3 samples from each site. Some of the treatments were represented by single exclosures making pseudo-replication unavoidable. Samples were sieved past 6 mm mesh and refrigerated at 4 °C until analysed.

5.2.2 Soil analysis

Total C and N were measured on a LECO CHN 2000 (LECO corporation, St.Joseph, MI). The water content, organic matter content and pH of soils were determined by standard methods (Allen, 1989). Exchangeable NH_4^+ and NO_3^- were determined in 1:5 soil:1 M KCl extracts by the methods of Downes (1978; hydrazine, N-1-napthylethylenediamine) and Keeney and Nelson (1982; indophenol blue). Net nitrogen mineralisation was determined using a laboratory aerobic incubation method (Hart et al. 1994). Exchangeable Al and Ca were determined by ICP analysis of 1 M KCl soil extracts (1:5 w/v soil:solution).

Amino acid mineralisation analysis was carried out as described in Chapter 3.

The biomass and structure of the soil microbial community structure was assessed by analysing the ester-linked phospholipid fatty acids (PLFA) composition (Bardgett et al., 2001).

5.2.3 Statistical analysis

Analysis of total C, total N and the amino acid mineralisation assay were performed twice on each sample and the results meaned to give one result for each sample. Therefore, n=3 for each of the six sites at each of the three locations.

Regression analysis was performed using SigmaPlot for Windows Version 4.01. Analysis of Variance (ANOVA) and Stepwise multiple regression were performed using Minitab Version 13. Comparisons between treatments following ANOVA were made using Fishers Comparisons with a family error rate p=0.05 using Minitab Version 13.

Table 5-1 Site characteristics along the successional transitions in the Yorkshire Dales, Lake District and Snowdonia National Parks. (Source: Bardgett et al., 2001).

The three areas were grazed by different pure-bred sheep breeds, as follows: Yorkshire Dales, Swaledale and Kendal Rough Fell ewes; Lake District, Herdwick and Swaledale ewes; and, Snowdonia, Welsh Mountain ewes. Dominant vegetation is given as legend beneath the table.

		Gradient of grazing influence					
National Park	Character	Permanently ungrazed (1)	Long-term ungrazed (2)	Short-term ungrazed (3)	Lightly grazed (4)	Moderately grazed (5)	Heavily grazed (6)
Yorkshire Dales	Location	54°18'N,2°31'W	54°18'N,2°,32'W	54°18'N,2°,32'W	54°18'N,2°,32'W	54°18'N,2°,33'W	54°18'N,2°,33'W
	Altitude	250-300m	250-300m	250-300m	250-300m	250-300m	250-300m
	Grazing	None	Ungrazed 25 y	Ungrazed 12 y	1-2 ewe ha^{-1} y^{-1}	4-8 ewe ha^{-1} y^{-1}	8 - 16 ewe ha^{-1} y^{-1}
	Vegetation	*Quercus-Betula*[1]	*Calluna-Deschampsia*[2]	*Nardus-Galium*[3]	*Nardus-Galium*[4]	*Festuca-Agrostis*[5]	*Festuca-Agrostis*[6]
	Soil	Brown podzolic earth	Humus stagnopodzol	Humus stagnopodzol	Ferric stagnopodzol	Humic brown podzol	Brown podzolic earth
	Bulk density	0.40 (0.06)	0.66 (0.03)	0.59 (0.03)	0.57 (0.04)	0.52 (0.03)	0.62(0.00)
Lake District	Location	54°35'N,3°54'W	54°35'N,3°54'W	54°35'N,3°54'W	54°35'N,3°54'W	54°30'N,3°54'W	54°30'N,3°54'W
	Altitude	200m	250m	300m	300m	250-300m	250-300m
	Grazing	None	Ungrazed 40 y	Ungrazed 20 y	1-2 ewe ha^{-1} y^{-1}	4-8 ewe ha^{-1} y^{-1}	8 - 16 ewe ha^{-1} y^{-1}
	Vegetation	*Quercus-Betula*[1]	*Calluna-Deschampsia*[2]	*Nardus-Galium*[3]	*Nardus-Galium*[4]	*Festuca-Agrostis*[5]	*Festuca-Agrostis*[6]
	Soil	Brown podzolic earth	Humus stagnopodzol	Humus stagnopodzol	Ferric stagnopodzol	Humic brown podzol	Brown podzolic earth
	Bulk density	0.54 (0.02)	0.55 (0.08)	0.43 (0.04)	0.51 (0.04)	0.43 (0.01)	0.54 (0.01)
Snowdonia	Location	53°02'N,4°01'W	53°04'N,4°03"W	53°04'N,4°03"W	53°04'N,4°03"W	53°02'N,4°01"W	53°02'N,4°01"W
	Altitude	300m	430m	430m	430m	300m	250m
	Grazing	None	Ungrazed 41 y	Ungrazed 4 y	1-2 ewe ha^{-1} y^{-1}	4-8 ewe ha^{-1} y^{-1}	8 - 16 ewe ha^{-1} y^{-1}
	Vegetation	*Quercus-Betula*[1]	*Calluna-Vaccinium*[7]	*Nardus-Calluna*[8]	*Nardus-Galium*[4]	*Festuca-Agrostis*[5]	*Festuca-Agrostis*[6]
	Soil	Brown podzolic earth	Humus stagnopodzol	Humus stagnopodzol	Ferric stagnopodzol	Humic brown podzol	Brown podzolic earth
	Bulk density	0.38 (0.07)	0.33 (0.03)	0.43 (0.02)	0.43 (0.01)	0.60 (0.01)	0.71 (0.02)

[1] *Quercus petraea, Betula* spp. woodland with an understory dominated by *Deschampsia flexuosa, Vaccinium myrtillus, Pteridium aquilinum*, [2] *Calluna vulgaris* and *D. flexuosa*, with lesser amounts of *V. myrtillus, Molinia caerulea*, and *Nardus stricta*. Regeneration of *Q. petraea, Betula* spp. and *Sorbus aucuparia;* [3] *N. stricta, Galium saxatile*, with regeneration *C. vulgaris* and *V. myrtillus*; [4] *N. stricta* and *G. saxatile*,, and *G. saxatile*; [6] *Festuca ovina* and *Agrostis capillaris*; [7] *C. vulgaris* and *V. myrtillus*, with regeneration of *S. aucuparia* and *Betula spp.*; [8] *N. stricta* grassland with regeneration of *C. vulgaris*.

5.3 Results[3]

5.3.1 Soil conditions

Data on various intrinsic soil properties are provided in Table 5-2 below. At the Lake District and Snowdonia experimental sites, the amount of soil C differed significantly along the successional transitions, being highest in the lightly grazed (treatment 4) and short-term ungrazed grasslands (treatment 3), and lowest in the heavily grazed grassland (treatment 6). In Snowdonia, the total amount of N in the soil varied significantly along the gradient of grazing influence, being highest in the lightly grazed grassland (treatment 4); in the Lake District, total N was highest in the lightly grazed (treatment 4) and short-term ungrazed grasslands (treatment 3). The soil C-to-N ratio was affected significantly by grazing influence at all locations; soil C-to-N ratios were highest in soils of the long-term ungrazed grasslands (treatment 2) at all locations, and lowest in more heavily grazed grasslands (treatments 5 and 6) in Snowdonia. The amount of mineral-N in soil differed significantly with grazing influence in the Lake District only, and was significantly higher in the completely ungrazed control (treatment 1) than in other treatments. Soil pH was highest in the heavily grazed grassland (treatment 6) at all locations and showed a general trend of increasing acidity with reduced grazing pressure from the heavily grazed to the lightly grazed grassland. This relationship is demonstrated in Figure 5-2 with quadratic regression lines (not shown) with r^2 values of 0.75 ($p<0.0001$), 0.56 ($p=0.002$) and 0.95 ($p<0.0001$) in the Yorkshire Dales, Lake District and Snowdonia respectively. This trend is very similar in the Yorkshire Dales and Lake District, but is more pronounced in Snowdonia.

The relationship between exchangeable Al and Ca is shown in Figure 5-3a, with a significant ($p<0.0001$) negative correlation (r^2 of 0.55). Where Ca is at low concentrations, Al is present in high concentrations, and where Ca is plentiful, there is very little Al. This relationship is mediated through soil pH (Figure 5-3b). Calcium and Aluminium are both significantly ($p<0.0001$) correlated with pH, (polynomial regressions, $r^2=0.59$ in both cases).

Organic matter content varies with pH (Figure 5-4); a weak negative relationship ($r^2=0.31$) across all sites, although The Yorkshire Dales and Lake District show little

---

[3] Refer to Bardgett et al. (2001) in appendix 1 for results sections on abundance and activity of soil biota and microbial community structure.

correlation between these two factors, Snowdonia shows a much stronger relationship (r^2=0.66, individual lines not shown).

Table 5-2 Changes in soil conditions in relation to grazing influence in upland ecosystems. (Source: Bardgett et al., 2001).

[Each value is a mean that is derived from three random quadrats within each treatment. All samples were taken in June 1998. Site 1 = ungrazed control, 2 = long-term ungrazed, 3 = short-term ungrazed, 4 = lightly grazed, 5 = moderately grazed, and 6 = heavily grazed.]

	Gradient of grazing influence								
Measure	1	2	3	4	5	6	LSD	F value	P value
Total C (kg m^{-2})									
Yorkshire Dales	5.5	4.3	4.6	5.1	5.2	4.7	1.0	2.03	NS
Lake District	4.7	5.4	5.8	5.8	5.1	4.5	0.9	3.37	0.039
Snowdonia	5.9	6.3	6.1	9.3	4.2	3.5	1.3	23.23	0.0001
Total N (kg N m^{-2})									
Yorkshire Dales	0.38	0.30	0.38	0.40	0.41	0.37	0.05	4.55	0.015
Lake District	0.38	0.35	0.46	0.47	0.41	0.36	0.07	4.62	0.014
Snowdonia	0.40	0.39	0.41	0.59	0.38	0.34	0.09	9.08	0.0009
C-to-N ratio									
Yorkshire Dales	14.5	14.3	12.1	12.8	12.7	12.7	1.4	5.28	0.009
Lake District	12.4	15.4	12.6	12.3	12.4	12.5	1.3	8.74	0.001
Snowdonia	14.8	16.2	14.9	15.8	11.1	10.3	0.8	92.49	0.0001
Soil pH									
Yorkshire Dales	4.6	4.6	4.5	4.7	5.2	5.5	0.4	8.84	0.001
Lake District	4.7	5.0	4.6	4.8	5.2	5.7	0.6	4.10	0.021
Snowdonia	4.0	4.2	4.1	4.3	5.0	6.2	0.2	104.90	0.0001
Mineral N (g m^2)									
Yorkshire Dales	3.5	3.8	3.9	2.9	2.4	3.9	1.2	2.48	NS
Lake District	4.8	3.1	2.9	2.6	2.0	3.4	1.6	3.49	0.035
Snowdonia	2.6	2.0	2.5	1.8	2.4	3.0	1.2	1.16	NS

Figure 5-2 Impact of grazing intensity on soil pH within three regions of the UK.

[Treatment 1 = ungrazed control, 2 = long-term ungrazed, 3 = short-term ungrazed, 4 = lightly grazed, 5 = moderately grazed, and 6 = heavily grazed. For further details see Table 5-1 and Table 5-2. Symbols represent experimental data points.]

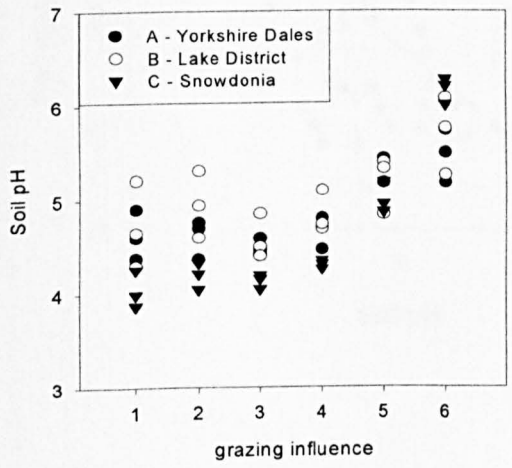


Figure 5-3 Relationships between a) exchangeable calcium and aluminium and b) soil pH and exchangeable calcium and aluminium.

[data points represent all samples over all three regions]

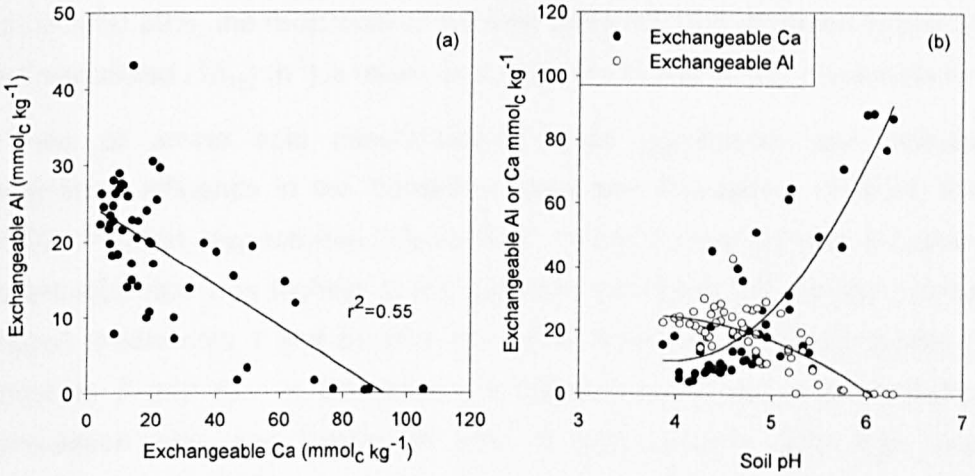
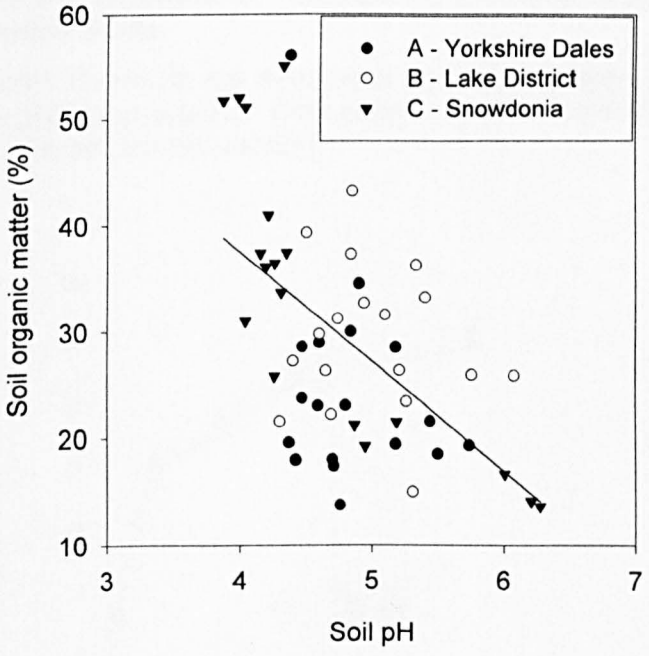


Figure 5-4 Relationship between soil pH and soil organic matter content.



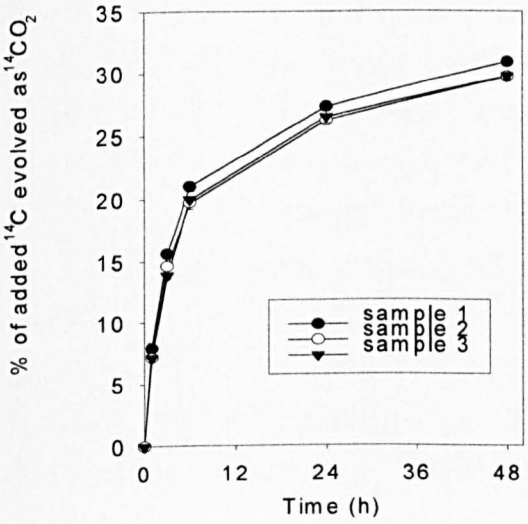
5.3.2 Amino acid mineralisation

The evolution of $^{14}CO_2$ over a 48 hr period following incorporation of ^{14}C-amino acids is shown in Figure 5-5, using the three replicates of treatment 4 from Snowdonia (lightly grazed) as an example. This demonstrates the extremely rapid mineralisation of the added amino acids. As the proportion of added ^{14}C respired generally levelled out at around 30%, the reciprocal of the time taken for 15% of added amino acid-^{14}C to be mineralised ($1/t_{15}$) (h^{-1}) is taken as a measure of amino acid mineralisation rate.

The rate of amino acid mineralisation varied significantly with successional stage/grazing influence in the Yorkshire Dales and Snowdonia ($F=4.31$, $P=0.018$; $F=15.92$, $P<0.001$ respectively) (Figure 5-6). In the Yorkshire Dales the amino acid mineralisation rate was highest at the extremes of heavily grazed and permanently ungrazed (treatments 1 and 6), and slowest at short term ungrazed/lightly grazed, (treatments 3 and 4). In Snowdonia, a different trend was evident; amino acid mineralisation rate was similar at zero to light grazing, and then increased significantly at moderate and heavy grazing. The Lake District did not show any significant trend, but had the lowest rate of amino acid mineralisation at the short-term ungrazed treatment. The rate of amino acid mineralisation was fastest at the highest grazing pressure in all regions.

Figure 5-5 Evolution of $^{14}CO_2$ during a 48 h incubation following addition of ^{14}C-amino acids.

[Samples shown for the three replicate samples taken from Snowdonia, Treatment 4, lightly grazed grassland. Data points are the means of two repetitions, standard error bars included but not visible.]



Amino acid mineralisation rate was strongly negatively related to exchangeable Al in the Yorkshire Dales and Snowdonia (r^2=0.63 and 0.75 respectively, P<0.0001) (Figure 5-7). This relationship was much weaker in the Lake District (r^2=0.21). Amino acid mineralisation rate was weakly positively correlated with exchangeable Ca in the Yorkshire Dales and Lake District, (r^2= 0.15 and 0.34 respectively) and more strongly in Snowdonia (r^2=0.67) (graphs not shown). Soil pH influenced the mineralisation of amino acids significantly (Figure 5-8) in all regions, with linear regression lines showing the relationships with r^2 values of 0.44 (P=0.0027), 0.47 (P=0.0017) and 0.82 (P<0.0001) in the Yorkshire Dales, Lake District and Snowdonia respectively.

The relationship of amino acid mineralisation with basal soil respiration rate, an indicator of general microbial activity, is positive in the Yorkshire Dales (r^2=0.49, P=0.04) and negative in the Lake District and Snowdonia (r^2=0.31, P=0.21 and r^2=0.65, P=0.003 respectively) (Figure 5-9a). Across all sites, there is a (non significant) positive correlation for treatments 1-4 (r^2 = 0.58, 0.27, 0.40, 0.68 respectively) and then it becomes negative for treatments 5 (r^2=0.63) and 6 (r^2=0.61) (Figure 5-9b).

There was a weak negative correlation between amino acid mineralisation rate and total PLFA (an indicator of SMB) (Figure 5-10a, r^2 =0.29 for all samples; individual r^2s on linear regressions of 0.38, 0.21, 0.42 for The Yorkshire Dales, Lake District and Snowdonia respectively, lines not shown). Examination of the data by treatment (Figure 5-10b) showed that total PLFA and amino acid mineralisation rate were not strongly related except in moderately and heavily grazed sites over all regions with negative correlations of r^2=0.58 and 0.68 for treatments 5 and 6 respectively.

This relationship of PLFAs with amino acid mineralisation was similar when looking only at bacterial PLFAs (Figure 5-10c) with again weak negative correlations of r^2=0.49, 0.13, 0.41 in The Yorkshire Dales, Lake District and Snowdonia respectively. Again, there was no relationship evident within exclosure treatments, but there were negative correlations in lightly, medium and heavily grazed treatments with r^2 values of 0.37, 0.60, 0.80 respectively (Figure 5-10d). Fungal PLFAs were also weakly negatively correlated with amino acid mineralisation (Figure 5-10e) with r^2 values of 0.32, 0.20, 0.15 in The Yorkshire Dales, Lake District and Snowdonia respectively. Inspection of the data revealed no strong relationship between the fungal to bacterial PLFA ratio and amino acid mineralisation within regions or treatments.

Figure 5-6 Rate of amino acid mineralisation at sites of varying grazing intensity of three regions.

[Site 1 = ungrazed control, 2 = long-term ungrazed, 3 = short-term ungrazed, 4 = lightly grazed, 5 = moderately grazed, and 6 = heavily grazed. Bars show means of three replicates from each site, bars show standard errors of means. Within each region, values with the same letter are not significantly different.]

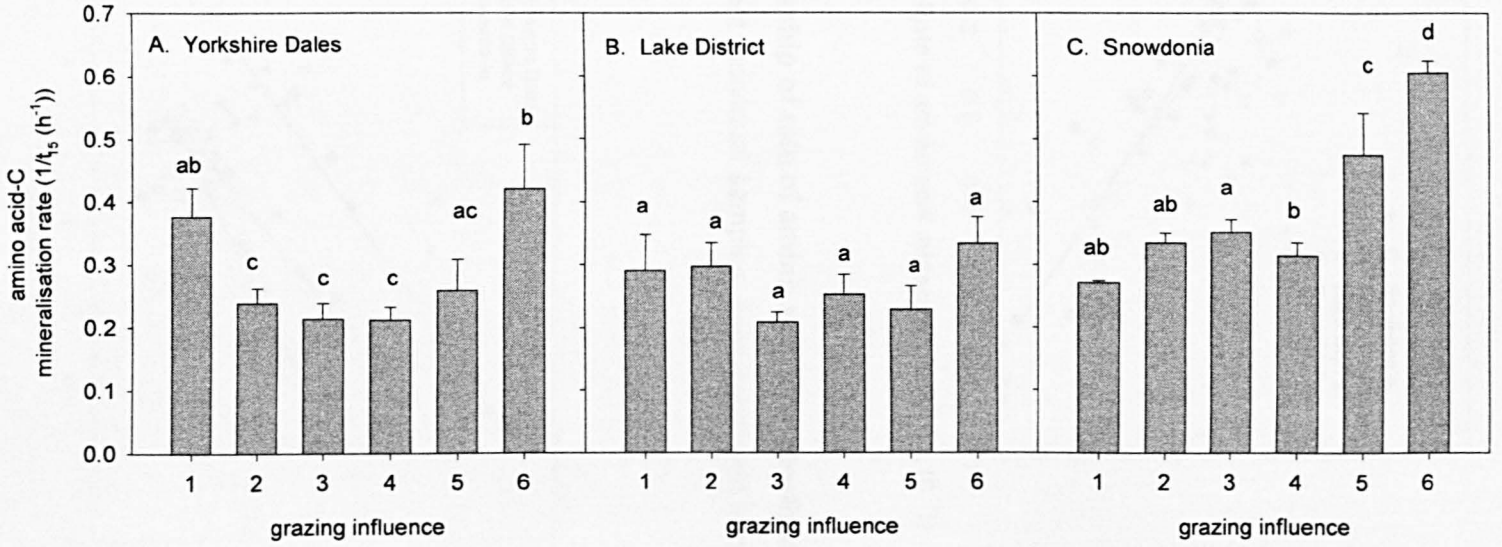


Figure 5-7 Relationship of rate of amino acid-C mineralisation with exchangeable aluminium.

[Data points represent individual samples, lines represent linear regressions at three regions]

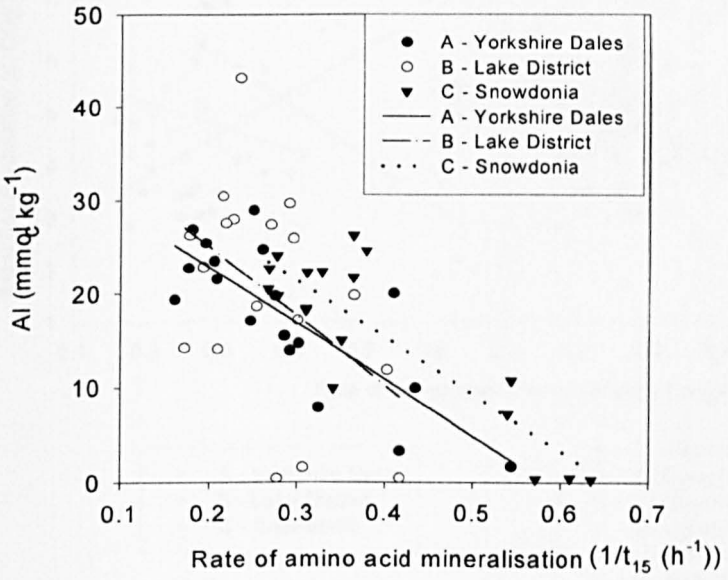


Figure 5-8 Relationship of rate of amino acid-C mineralisation with soil pH.

[Data points represent individual samples, lines represent linear regressions of data from each region]

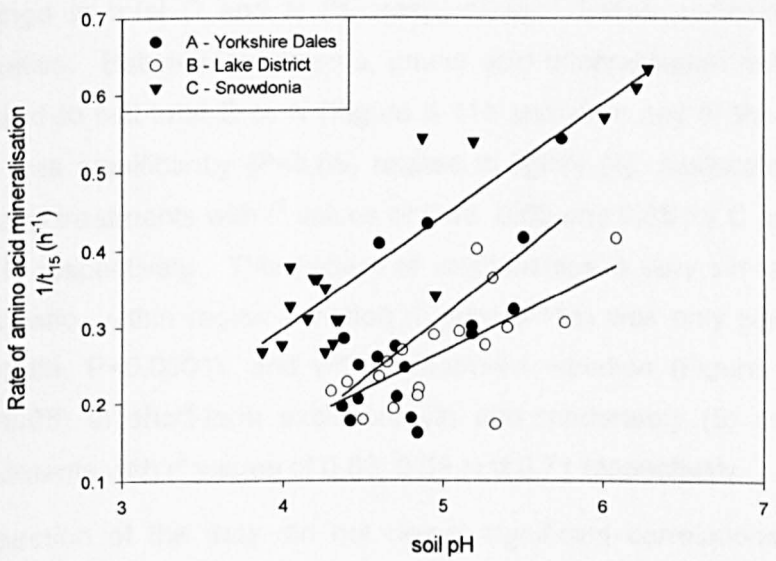
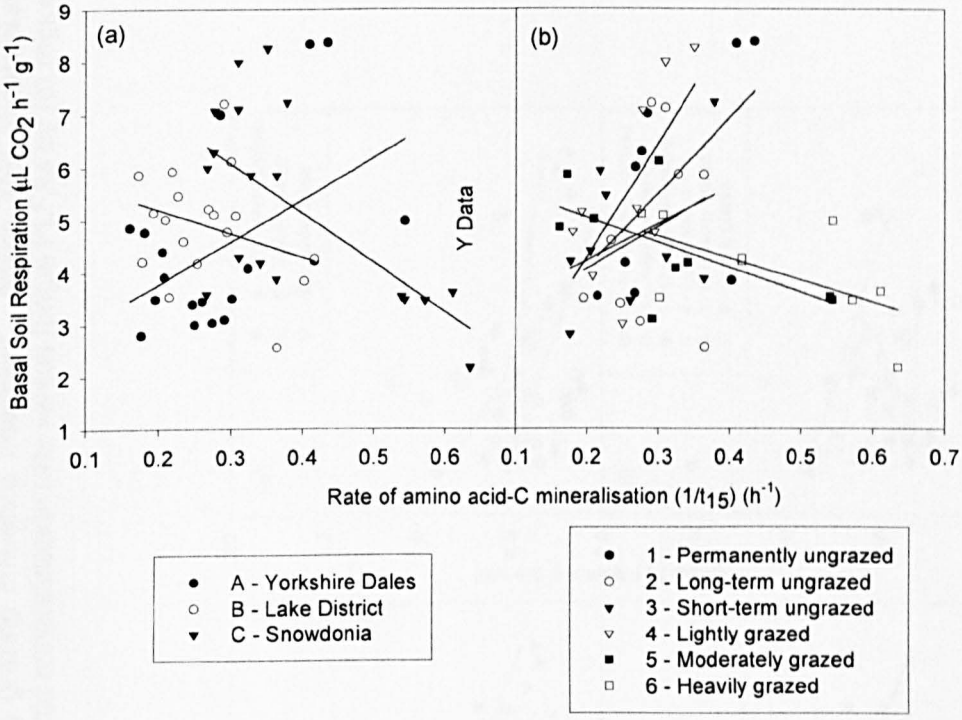


Figure 5-9 Rate of amino acid-C mineralisation versus basal soil respiration by a) region and b) treatment.



Across all the samples, the rate of amino acid mineralisation was not significantly correlated with total soil C, N or C:N. Within-region C and N were not significantly related to amino acid mineralisation (Figure 5-11a and c), except in Snowdonia where 57% and 52% of variability in amino acid mineralisation rate was explained by change in total C and N (%) respectively. These correlations were surprisingly negative. Between treatments, amino acid mineralisation rate was not significantly related to soil total C or N (Figure 5-11b and d) in any of the exclosure treatments, but was significantly ($P<0.05$) related in lightly (4), moderately (5) and heavily (6) grazed treatments with r^2 values of 0.68, 0.60 and 0.65 for C and 0.78, 0.50 and 0.55 for N respectively. This pattern of relationships is very similar when looking at the C:N ratio; within region variation (Figure 5-11e) was only significant in Snowdonia, ($r^2=0.69$, $P<0.0001$), and within treatment variation (Figure 5-11f) was significant ($P<0.05$) in short-term exclosure (3) and moderately (5) and heavily (6) grazed treatments with r^2 values of 0.80, 0.59 and 0.71 respectively.

Inspection of the data did not reveal significant correlations between amino acid mineralisation rate and soil NH_4^+ or NO_3^- content (data not presented).

Figure 5-10 Relationship between amino acid-C mineralisation rate with phospholipid fatty acids (PLFAs)

[Amino acid mineralisation rate versus total PLFAs by (a) region, (b) grazing influence treatment. Amino acid mineralisation rate versus bacterial PLFAs by (c) region, (d) grazing influence treatment. Amino acid mineralisation rate versus fungal PLFAs by (e) region and (f) grazing influence treatment.]

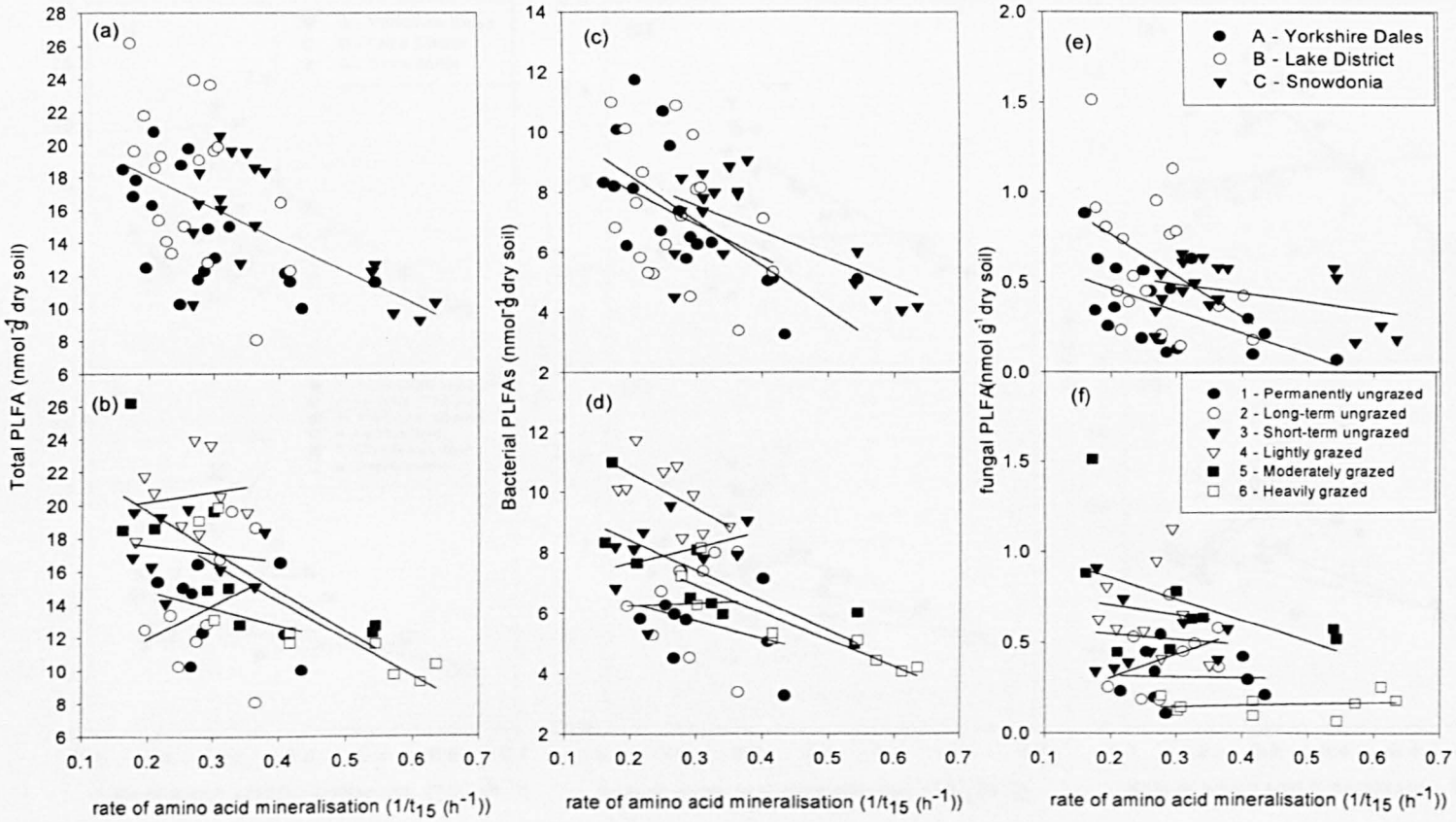
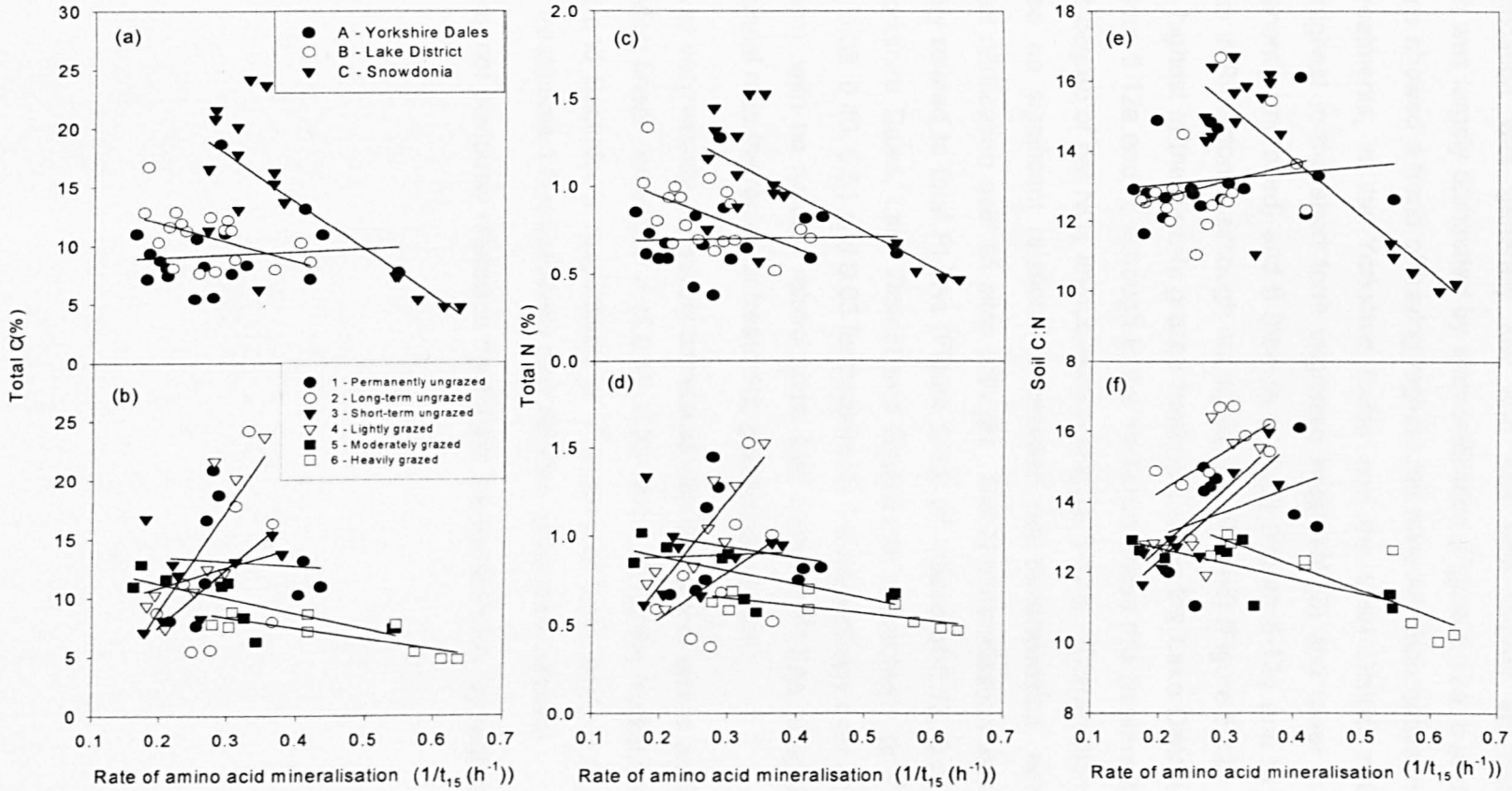


Figure 5-11 Relationship between rate of amino acid mineralisation and total C, total N and soil C:N ratio.

[Amino acid-C mineralisation rate versus total C by (a) region, (b) grazing influence treatment. Amino acid-C mineralisation rate versus total N by (c) region, (d) grazing influence treatment. Amino acid –C mineralisation rate versus soil C:N ratio by (e) region and (f) grazing influence treatment.]



5.3.3 Net nitrogen mineralisation

Figure 5-12 shows net ammonification, net nitrification and net N mineralisation (net ammonification plus net nitrification) for each treatment in each of the three study regions. Net nitrification was generally low and had little impact on net mineralisation, which was largely dominated by ammonification (Figure 5-12a, b and c). The three regions showed a trend of having highest net mineralisation between the two extreme treatments; in the Yorkshire Dales and the Lake District net mineralisation was highest in the short term ungrazed treatment (3) and lower in treatments 1 (permanently ungrazed) and 6 (heavily grazed) (Figure 5-12g and h). This trend was similar in Snowdonia, although not significant (P=0.05) (Figure 5-12i). Net nitrification was highest in the heavily grazed treatment (6) in the Lake District and Snowdonia (Figure 5-12e and f), although in the Yorkshire Dales this treatment showed the greatest degree of net NO_3^- immobilisation (Figure 5-12d). Examination of the data revealed no significant relationship between net mineralisation, net ammonification or net nitrification over all sites with pH. Net N mineralisation was very weakly positively related to total PLFAs (Figure 5-13) (r^2 values of 0.12, 0.04 and 0.31 for the Yorkshire Dales, Lake District and Snowdonia respectively or r^2 values of 0.29, 0.04, 0.08, 0.40, 0.51 and 0.03 for treatments 1-6 respectively over all sites, lines not shown), with no notable relationships with bacterial PLFAs, fungal PLFAs or fungal:bacterial ratio (by region or treatment, graphs not shown).

Net mineralisation was very weakly negatively correlated with the rate of amino acid mineralisation, providing linear correlation r^2 of 0.10, 0.23 and 0.02 for the Yorkshire Dales, Lake District and Snowdonia respectively or r^2 values of 0.18, 0.01, 0.43, 0.02, 0.23, 0.006 for treatments 1-6 respectively over all sites (graphs not shown).

The soil C:N ratio was not obviously related to the fungal :bacterial PLFA, by region or treatment.

Figure 5-12 Impact of grazing influence on mineralisation activity.

[Grazing influence versus net ammonification in (a) Yorkshire Dales, (b) Lake District, (c) Snowdonia. Grazing influence versus net nitrification in (d) Yorkshire Dales, (e) Lake District, (f) Snowdonia. Grazing influence versus net mineralisation (nitrification plus ammonification) in (g) Yorkshire Dales, (h) Lake District, (i) Snowdonia. All bars show the means of three replicates for each treatment at each location, bars show standard errors, within each chart values with the same letter are not significantly different (p=0.05)]

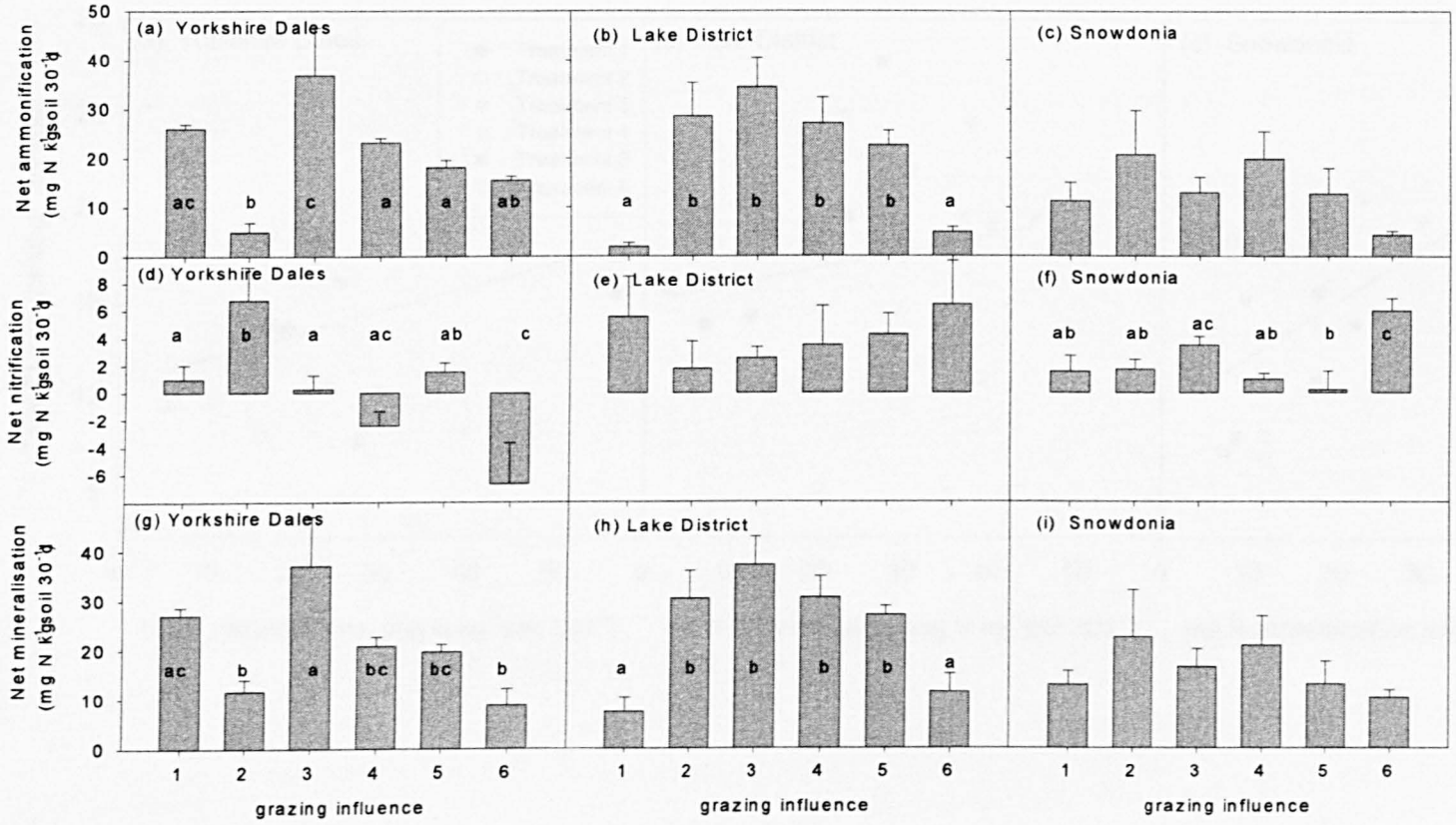
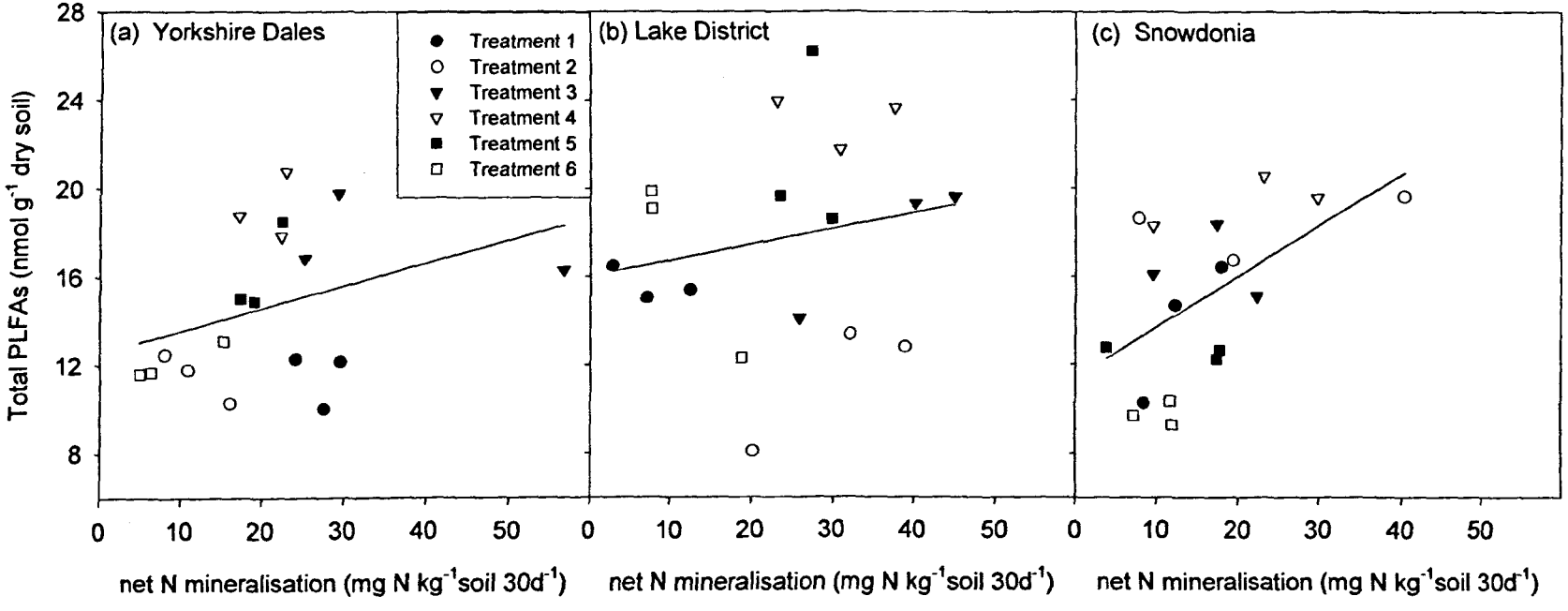


Figure 5-13 Net nitrogen mineralisation versus total phospholipid fatty acids (PLFAs)

[Individual data points represent net nitrogen mineralisation versus total PLFAs by treatment in (a) the Yorkshire Dales, (b) the Lake District and (c) Snowdonia. Lines represent linear regressions on all data points for all treatments at each region.]



5.4 Discussion[4]

This study has shown that both chemical and biological properties of upland soils vary significantly along successional gradients that are related to the intensity and history of grazing. Notable features revealed by the study include:

1) Microbial biomass was generally at its greatest in the soils that were at the intermediate level of influence from grazing, and declined as the legacy of grazing was reduced through the long-term removal of sheep.

2) Several individual 'signature' fatty acids varied along the successions, but the most obvious effect on microbial community structure was that, at two locations, the evenness of PLFA - an index of the degree of dominance of different groups of microorganisms - declined with increasing intensity of grazing.

3) The proportion of fungi relative to bacteria varied significantly along the successions, being at its lowest in the treatments that were most intensively grazed; in general, however, this measure showed few consistent responses to grazing influence.

4) Some of these trends were related to alterations in the chemical composition of the soil along the successional transitions, most notably, soil pH.

5.4.1 Soil pH

The trend of decreased soil pH with lower grazing is probably related to vegetation cover with associated litter inputs, base cation recycling versus leaching and is probably also influenced by parent material. Although the vegetation type did not change between treatment 6 and treatment 5 in each region, the soil type changed from a brown podzolic earth to a humic brown podzol; the development of podzolisation brought an associated drop in pH. *Agrostis-Festuca* grasslands are characteristically associated with brown profiles with mull humus lacking an Ea horizon, properties maintained by high grazing pressures, which promote rapid cycling of nutrients (Miles 1985). Floate (1972, in Miles 1985) showed the reversal of the trend demonstrated here by experimentally increasing grazing levels on heather; the bents, fescues and associated herbs became more abundant and the soil properties changed from those of heather soils towards those characteristics of bent-fescue grasslands. In particular the depth of surface organic matter decreased and

---

[4] Refer to Bardgett et al. (2001) in appendix 1 for discussion sections on patterns of microbial biomass and activity, patterns of microbial community evenness and composition, and fungal to bacterial ratios.

base saturation increased while the mean pH rose from 3.6 to 4.3, and depodzolisation occurred at some sites. Contrastingly, Kirkwood (1964, in Miles, 1985) showed light grazing of bent fescue swards permitted a surface mat of dead plant material to accumulate, which caused decreases of up to 90% in exchangeable calcium and up to 1.1 units in pH.

Treatments 3 and 4 saw a change to a *Nardus-Gallium* vegetation cover with regeneration of *Calluna vulgaris* in Snowdonia. The *Calluna, Deschampsia, Vaccinium*, vegetation types of treatment 2 (long term ungrazed) brought low pHs; *Calluna vulgaris*, *Vaccinium myrtillus* and *Deschampsia flexuosa* are recognised as strong acidifiers of soils, acting even within a decade, and they also promote podzolisation (Miles, 1985). Miles (1985) has reported an increase in pH from 4 to 5 as *Betula sp.* colonised heather moorland with an associated change in the humus from mor to mull or mull like moder humus, due to increases in exchangeable calcium content and rates of organic matter decomposition. However, the transition from long-term ungrazed exclosure, treatment 2, to permanently ungrazed woodland, treatment 1, did not show an increase in pH in any of the regions studied.

Grazing may increase the soil pH relative to ungrazed sited via addition of urea, and the lower plant biomass may provide fewer organic acids and less complexes on which cations can bind. The accumulation of biomass over a successional gradient contributes to acidification of the soil by (i) the action of the canopy as an effective collector of acid deposition (gases, aerosols and particulates), increasing the acid load to the site by approx 50% when the canopy closes in the mature stand (Svedrup et al., 1995) and (ii) net uptake of base cations for incorporation in biomass contributes to the removal of base cations from the soil (van Breemen, 1991; Ulrich, 1991; Svedrup et al., 1995). Blake et al. (1999) suggest that the latter process contributes much less to soil acidification than the former.

5.4.2 Amino acid mineralisation and net nitrogen mineralisation

The 'grazing optimisation hypothesis' (that light grazing causes a peak in soil microbial biomass due to compensatory responses related to enhanced circulation of nutrients) is now evaluated in the light of data on process rates, namely net nitrogen mineralisation and amino acid mineralisation.

Net nitrogen mineralisation rates show a supporting trend, with rates generally highest between the extreme treatments (highest in short term ungrazed, treatment 3, in the Yorkshire Dales and the Lake District), and net nitrogen mineralisation rates are weakly positively correlated with microbial biomass, as measured by total PLFAs.

This concurs with results of Tracey and Frank (1998) who found that net N mineralisation rates were highest in grazed grassland successional sequences in the Yellowstone National Park. However, our findings are contrary to the results of van Wijnen et al.(1999) who found net mineralisation was low where grazing was present, and it increased with successional development; they found evidence to imply that herbivores reduced the mineralisation rate by preventing litter accumulation in a salt marsh sequence of exclosures. Kristensen and Henriksen (1998) examined succession from *Calluna* heathland and found a very low in situ net mineralisation rate, which increased with succession. Our results showed that transition from Calluna vegetation (treatment 2) to Quercus-Betula woodland brought an increase in net mineralisation in the Yorkshire Dales, but a decrease in the Lake District and Snowdonia. No consistent trends were observed in net nitrification rates, as were by Barford and Lajtha (1992), who found nitrification to generally increase with successional age.

However, amino acid mineralisation rates show the opposite trend, whereby they were slowest between treatment extremes and surprisingly were negatively correlated with total, bacterial and fungal PLFAs. One might expect a higher SMB to mineralise amino acids faster. Amino acid mineralisation rates did not show any consistent relationships with soil respiration rates. The marked increase in the relative abundance of the fatty acid a15:0 in the more heavily grazed grasslands, indicating an increase in the dominance of Gram-positive bacteria (O'Leary and Wilkinson, 1988), appears to be related mostly to the increase in pH of soil which occurred as a result of increasing grazing pressure. Amino acid mineralisation rate follows a similar trend to the relative abundance of the fatty acid a15:0 (Figure 5-14). Examining correlations of individual data points for amino acid-C mineralisation rate with the relative abundance of the fatty acid a15:0 gives linear regressions of r^2=0.31, P=0.0173; r^2=0.11, P=0.1851, and r^2=0.81, P<0.0001 for the Yorkshire Dales, the Lake District, and Snowdonia respectively.

Amino acid mineralisation rate is negatively related to basal respiration in the Lake District and Snowdonia and in all regions in treatments 5 and 6, indicating that amino acid mineralisation is not reflective of general SMB metabolic activity. The association of amino acid mineralisation with the fatty acid a15:0, which is also positively correlated to pH, indicates that there may be some relationship of rapid amino acid respiration with a specific microbial group within the community. It is of note that the abundance of the fatty acid a15:0 shows different patterns to other fatty acids also associated with gram positive bacteria.

Figure 5-14 Trends in rate of amino acid-C mineralisation and relative abundance of the fatty acid a15:0 over range of grazing influence in the three study regions.

[Relative abundance of fatty acid a15:0 represented by open bars. Calculated as proportion of selected PLFAs. Rate of amino acid-C mineralisation represented by grey bars with standard errors. n=3 for all bars. Site 1 = ungrazed control, 2 = long-term ungrazed, 3 = short-term ungrazed, 4 = lightly grazed, 5 = moderately grazed, and 6 = heavily grazed]

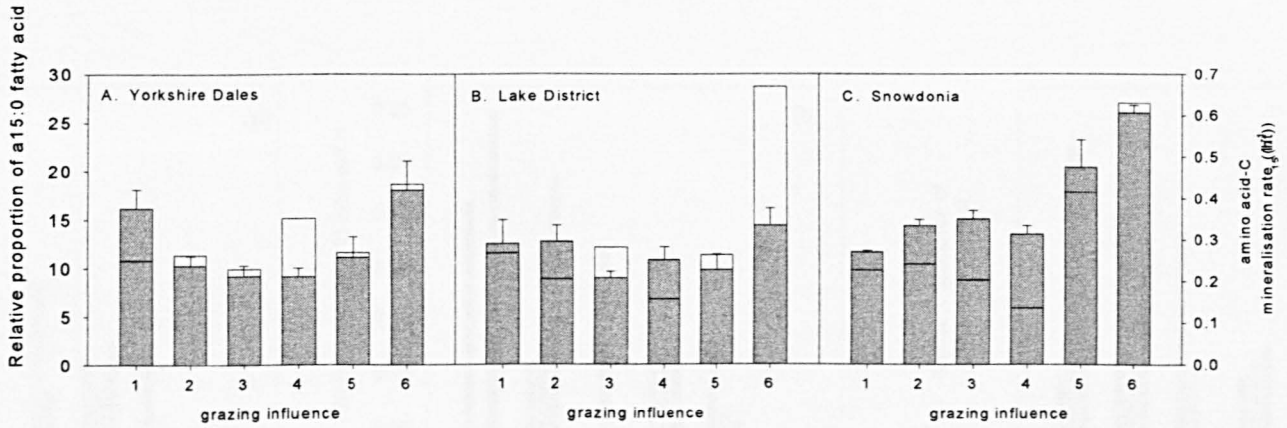
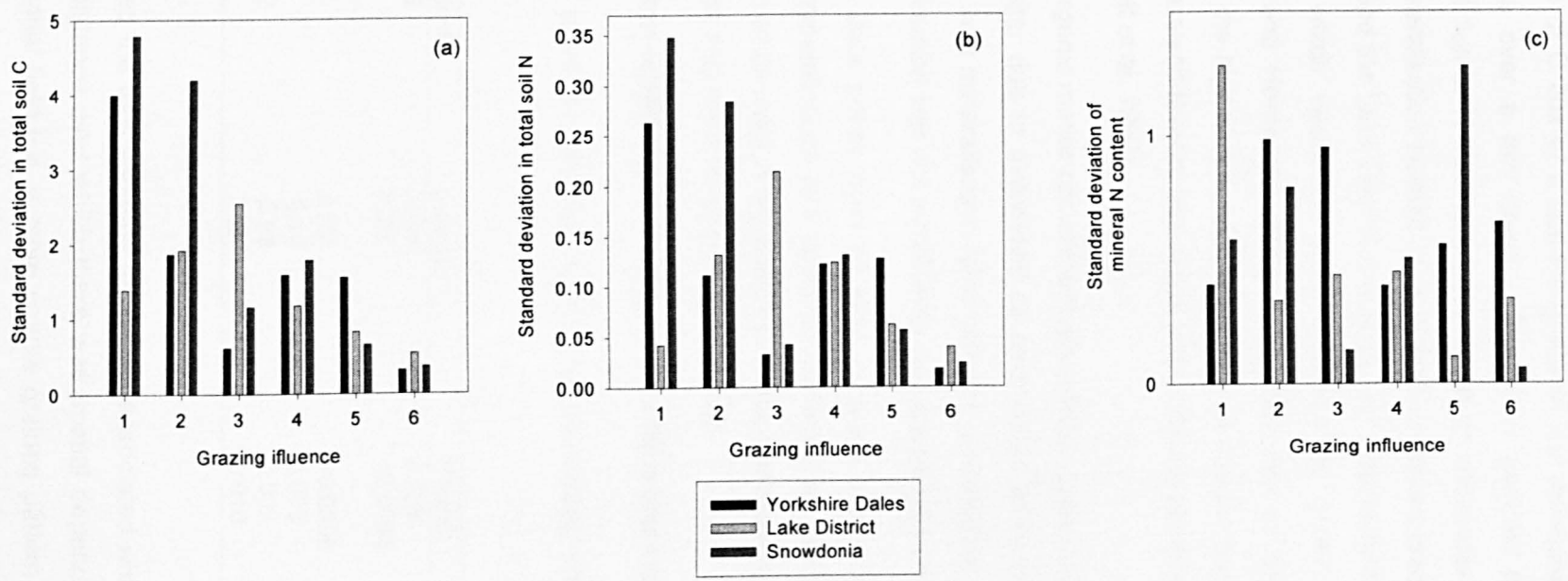


Figure 5-15 Impact of grazing influence on variability in soil total C, total N and mineral N.

[Grazing influence versus (a) standard deviation in total soil C, (b) standard deviation in total soil N, and (c) standard deviation in total mineral N.]



The lack of correlation between amino acid mineralisation rate and net nitrogen mineralisation is likely to be a reflection that the amino acid mineralisation method used measures a gross rate of C flux of a specific group of low molecular weight dissolved organic substrates over a few hours. This is in contrast to net N mineralisation which is a net flux of N from a diverse range of substrates over 30 days. While net nitrogen mineralisation peaked in short term ungrazed (treatment 3) plots in the Yorkshire Dales and the Lake District, this does not necessarily reflect the speed of nutrient cycling, which would be better reflected by gross rates of transformations (Kristensen and Henriksen, 1998). That the rate of amino acid mineralisation was fastest at the highest grazing pressure in all regions supports the model that heavily grazed grassland favours fast cycles dominated by labile substrates (Bardgett et al. 1996, Bardgett et al. 1993).

The negative correlation of organic matter content with pH is likely to be related to an accumulation of organic matter due to decreased decomposition at lower pH; the positive correlation of amino acid mineralisation rates with pH is consistent with this, although net nitrogen mineralisation was not significantly associated with pH.

Multiple regression over all data points from all sites revealed that most of the variance in the amino acid mineralisation rate could be explained by exchangeable aluminium, the ratio of amino acids used in respiration to those used structurally, soil pH, total PLFA, net nitrification and initial mineral N (Table 5-3).

Table 5-3 Multiple regression equation predicting amino acid mineralisation rate.

[Stepwise regression listing predictor variables in order of decreasing importance. ($n=54$, $r^2=0.814$, $P<0.0005$)]

Soil Property	Coefficient	t-value	P value
Exchangeable Al	-0.0036	-3.04	0.004
Amino acids respired:biomass	0.88	8.09	<0.0005
pH^2	0.0094	4.76	<0.0005
Total PLFA	-0.006	-3.14	0.003
Net nitrification	-0.0052	-2.93	0.005
Initial mineral N	0.0011	2.68	0.010

Gross et al. (1995) examined the total variation for factors associated with nitrogen availability and found the distance over which there was spatial dependence was greater in the mid-successional field (i.e. a more coarse grained pattern of spatial heterogeneity in soil nitrogen) and lower in a newly abandoned field and forest.

These differences may be a reflection of changes in the species composition or size of individual plants in these communities, but they also propose that spatial variability in N availability can influence occurrence of individual plants and therefore succession. Figure 5-15 shows the standard deviations of the total C, total N and mineral N in the samples over the range of grazing influence. For the Yorkshire Dales and Snowdonia, it can be seen that there was a general trend for variation in total C and N to increase as succession progressed, whereas variation in total C and N in the Lake District peaked in treatment 3 (short term ungrazed) (concurring with Gross et al., 1995). Variation in the mineral N content of the soil showed no discernable trends over the gradient of grazing influence (Figure 5-15c).

This work shows that grazing intensity appears to be a strong driving force influencing soil characteristics and processes. Treatments 2 and 3 (long and short term ungrazed) in each region were sampled from experimental exclosures. However, it should be noted that the land use of the other treatments (woodland and lightly, moderately and heavily grazed upland grasslands, treatments 1, 4, 5 and 6 respectively) is also a reflection of local climate, topography and soil conditions, which to some extent determine the type of vegetation and its stock carrying capacity. Caution must therefore be exercised in attributing differences in soil processes purely to the effects of intensity of grazing, as the intensity of grazing itself is a culmination of many interrelated facets of each site.

5.5 Conclusions

The limitation of this study is that it was a snapshot of a single sample date, and further work is needed to establish whether these trends are consistent over seasons and to determine the significance of these changes in relation to soil-level ecosystem processes of decomposition and nutrient cycling. This study detected consistent 'broad-scale' trends in soil microbial communities of upland grasslands along successional gradients that are related to grazing intensity, these are summarised in Table 5-4.

Table 5-4 Main conclusions of experimental work of Chapter 5

- Grazing intensity affected soil C and N levels: soil C and N were generally highest in the lightly grazed and short-term ungrazed grasslands, and lowest in the heavily grazed grassland.

- Soil pH was highest in the heavily grazed grassland at all locations and showed a general trend of increasing acidity with reduced grazing pressure from the heavily grazed to the lightly grazed grassland.

- Organic matter levels were weakly linearly negatively related to soil pH, indicating that at lower pH, mineralisation of organic matter is retarded, resulting in accumulation.

- The soil microbial biomass was maximal at low-to-intermediate levels of grazing influence across three regions and declined as the legacy of grazing was reduced through the long-term removal of sheep.

- The proportion of fungi relative to bacteria was lowest in the treatments that were most intensively grazed; however, this measure showed few consistent responses to grazing influence.

- The phenotypic evenness (a component of diversity) of the microbial community declined as the intensity of grazing increased across three regions. The PLFA evenness index was negatively related to soil pH and positively related to total soil C and C-to-N ratio.

- Net mineralisation rates were highest in mid-successional and lightly grazed treatments in all regions and were generally lowest at the extremes of grazing influence.

- Rates of mineralisation of dissolved organic nitrogen in the form of amino acids were generally lowest in the short-term ungrazed/lightly grazed treatments and were fastest at the highest grazing pressure in all regions. This supports the model that heavily grazed grassland favours fast cycles dominated by labile substrates (Bardgett et al. 1996, Bardgett et al. 1993).
    - Multiple regression over all data points from all sites revealed that most of the variance in the amino acid mineralisation rate could be explained by exchangeable aluminium, the ratio of amino acids used in respiration to those used structurally, soil pH, total PLFA, net nitrification and initial mineral N.
    - The impact of grazing intensity on the activity of the SMB that actively mineralises nitrogenous substrates appears therefore to function primarily through its effect on soil pH.

Chapter 6 Field study of the relationship between soil pH and nitrogen dynamics

6.1 Introduction

6.1.1 General introduction

Farmland at present comprises a large part of the UK catchments supplying water to boreholes and aquifers which subsequently become a major source of potable water; legislation has set an upper limit of 11.3 mg NO_3^--N l^{-1} in this water supply (Webster et al., 1993). Certain situations culminate in the risk of high levels of NO_3^- being leached. These include aspects of soil texture and structure, parent material, precipitation pattern and agricultural management history. Nitrate Sensitive Areas have been designated by the European Union in areas where these factors combine to produce a significant NO_3^- leaching risk, with payments to farmers to implement management strategies aimed at reducing NO_3^- leaching. Ridley et al. (2001) highlight that our inability to control the supply of mineral N in relation to demand from plants presents a major challenge for land managers to minimize the degradation of land and water.

Nitrification when accompanied by NO_3^- leaching can cause acidification of the soil, whereby one mole of NO_3^- leached or formed corresponds to a mole of acid generated (Ridley et al., 2001), although paradoxically nitrification is sensitive to low pH (e.g. Schmidt, 1982; Paul and Clark, 1989; Persson and Wirén, 1995). It is therefore expected that a reduction in lime application to soil, and allowing natural acidification, or even intentionally lowering the soil pH, might lead to a drop in NO_3^- leaching. This would have negative consequences for arable yields or carrying capacity of grazing land, but it may be an effective management tool for which compensatory payments could be made under the NSA scheme.

Studies on the impact of soil pH on nitrogen dynamics and NO_3^- leaching to date have largely focussed on the effects of liming (e.g. Nyborg and Hoyt, 1978; Weier and Gilliam, 1986; Bailey, 1995; Persson and Wirén, 1995; Stevens and Laughlin, 1996; Wheeler et al., 1997; Curtin et al., 1998) or heterogeneity within one or more soils (Nyborg and Hoyt, 1978; Strong et al., 1997). Ridley et al. (2001) found losses of NO_3^- from limed pastures to be consistently greater than from unlimed pastures (by c. 8 kg N ha^{-1} y^{-1}). While some of these studies have measured soluble organic matter (e.g. Nyborg and Hoyt, 1978; Curtin et al., 1998) they have not measured dissolved organic nitrogen (DON).

Although liming has been shown to increase the solubility of organic matter (Curtin et al., 1998), there is no experimental evidence to suggest that a reduction in pH will decrease organic matter solubility, giving a lower pool of N for ammonification and consequently leading to both a lower pool of NH_4^+ for nitrification and to the direct inhibitory effect of pH on nitrifying bacteria. To the author's knowledge, this is the first study to examine the effect of a soil pH reduction on NO_3^- leaching or DON dynamics in the field.

6.1.2 Objectives of the study

This field study sought to examine the effect of a soil pH reduction on nitrogen dynamics, specifically total N, NO_3^-, NH_4^+, and DON pools in the soil and drainage water under field conditions.

The effect of soil pH reduction on the cycling of low molecular weight DON was also studied in the laboratory.

6.1.3 Porous ceramic cup samplers

Ceramic cups were originally used in 1904 as artificial roots to study soil water availability to plants and its composition (Grover and Lamborn, 1970). Since then, porous cups have been a popular method of sampling water from the vadose zone (Creasey and Dreiss, 1988) for a plethora of reasons (Litaor, 1988) including many studies on soil NO_3^- (e.g. Webster et al., 1993; Poss et al., 1995; Ridley et al., 2001). Soil solution is defined as the soil interstitial water, its solutes and dissolved gasses (Litaor, 1988). The size of soil pores from which the soil water is drawn by a ceramic cup is defined by the tension applied (Armstrong and Burt, 1993). The largest pore to remain full of water has diameter, r whereby:

$r = 2\gamma / \rho g s$, where:

γ is the applied suction,
ρ is the density of water,
g is the acceleration due to gravity
s is the water surface tension.

A tension of -80 kPa imposes a limit to the minimum size of pore sampled, and as the constituents of soil water are affected by the pore size in which it resides, there is inevitably a systematic bias of results (Armstrong and Burt, 1993).

Magesan et al. (1994) highlight the difficulties of predicting NO_3^- movement in a field soil due to the spatial and temporal variability in water flow and NO_3^- concentrations.

However, they do note that porous ceramic cup samplers provide a cheap and simple means of measuring the constituents of soil water. Goulding (2000) proposes that porous cups are the best practicable method for freely draining structureless soils. A thorough review comparing various methods of soil solution sampling was published by Litaor (1988). Some advantages and disadvantages of four common methods of obtaining soil solutions are summarised in Table 6-1 below, and a brief discussion of some disadvantages and a brief review of some recent comparative studies follows.

Table 6-1 Some advantages and disadvantages of four soil solution sampling methods

Method	Advantage	Disadvantage	Reference
Porous ceramic cup samplers.		Do not provide a measure of drainage volume, so must be calculated indirectly, usually using a meteorological model.	Williams and Lord, 1997. Webster et al., 1993.
	Cheap, fairly easy to use and can be installed with very little disturbance to the soil profile.		Magesan et al., 1994.
		Widely regarded as being a flawed technique in soils subject to cracking as water can bypass the cups.	Webster et al., 1993.
		Doubts as to the extent and in what conditions do cups preferentially sample field drainage with bias from preferential sampling from macropores.	Webster et al., 1993. Poss et al., 1995 Armstrong and Burt, 1993. Williams and Lord, 1997.
	Can be left in place, so changes over time are not confounded with changes due to sampling from differing locations.		Poss et al., (1995)

Method	Advantage	Disadvantage	Reference
Porous ceramic cup samplers, continued...		The maximum pressure that can be applied to the cup is approximately -80 kPa to –40 kPa, so a limited range of soil pore sizes are sampled.	Williams and Lord, 1997. Poss et al., 1995.
		Adsorption of anions and possible leaching of cations from ceramic material.	Litaor, 1988.
Lysimeters	Provides a measure of drainage volume.		Webster et al., 1993
		Lacks the subsoil matric potential of field soils.	Webster et al., 1993. Poss et al., 1995.
		Preferential flow along the walls.	Poss et al., 1995.
Soil laboratory extraction e.g. in 1M KCl.		Does not provide a measure of drainage volume.	Webster et al., 1993
		Added variability due to sampling from differing locations.	Poss et al., 1995. Lord and Shepherd, 1993
		Samples whole range of pore sizes, so may not be representative of drainage water.	Poss et al., 1995. Lord and Shepherd, 1993
		Imprecise as many factors other than leaching affect soil NO_3^- concentration.	Goulding and Poulton, 1992
Tensionic porous cups. (Same advantages as porous ceramic cups above).	Does not selectively sample from macro pores.		Poss et al., 1995.
	Extends possible sampling period as water held at a greater tension can be sampled.		Poss et al., 1995.
	Allows simultaneous measurement of soil-water potential.		Poss et al., 1995.
		Optimal sample extraction time is a fortnightly interval, equilibrium time is 6-10 days.	Poss et al., 1995.

Opinions differ as to whether the samples from ceramic cup samplers are biased towards flux-averaged concentrations or the volume averaged concentrations and whether they measure mobile or immobile water (Magesan et al., 1994). Solute concentrations measured by extracting soil represent volume-averaged concentrations in contrast to flux-averaged concentrations (the solute flux divided by

the water flux), which can only be measured unambiguously by sampling the soil drainage water. The distinction can be particularly important in structured soils in which only part of the soil water transports solute because of preferential flow in macropores (Magesan et al., 1994). If the degree of equilibration between fissure and matrix water is substantial, then the accuracy of porous cup measurements is likely to be satisfactory (Williams and Lord, 1997). Tensionic porous cups were designed to overcome the pore-size issue, by placing deionised water in the sampler, and allowing passive diffusion to equilibrate it with the outside soil solution.

Poss et al., (1995) found that porous cups gave similar results to those obtained from soil extractions (1M KCl), and concluded that porous cups are a reliable and accurate method for monitoring NO_3^- leaching on soil that is fairly homogeneous and contains a moderate amount of clay. Ceramic cup and lysimeter results were in good agreement in the 2nd and 3rd years of a study by Webster et al. (1993). Soil core extracts from 130 cm depth gave lower loss estimates than the other methods and significantly different peak concentrations, although total over winter N leached was not significantly different.

Magesan et al. (1994) compared suction cup and soil extraction with drainage water from a sub-surface drained soil and found that porous cups provided the better estimate of leaching loss for non reactive solutes, but for NO_3^-, a biologically reactive solute, there was no clear pattern in the differences between the estimated and measured leaching losses. The flux-averaged concentration in the drainage water was about midway between those measured in the suction cup samples and in the soil extracts; porous cups underestimating and soil extraction overestimating NO_3^- loss.

NO_3^- concentrations were measured by Ridley et al. (2001) in an Australian duplex soil in suction-cup extracted soil water and directly collected subsurface flow. Discrepancies between the two may have been due to spatial and temporal variation of point measurements of NO_3^- concentrations under grazed pastures or that the suction cups were not sampling a true flux-averaged concentration but were sampling a fraction of immobile soil solution. They found the mean concentration in the suction cups to be consistently smoother than was the change in subsurface flow concentrations, although they state that fluctuations could have been due to microbial effects on the NO_3^- in subsurface flow samples that remained in collection bottles for up to 2 days.

6.2 Materials and Methods

6.2.1 Field site characteristics

The field used for the experiment was the 'Bont' field at the University of Wales, Bangor's research farm, Henfaes, in Abergwyngregyn, approximately 6 miles East of Bangor, details specified in Table 6-2 below, and location shown in Figure 6-1.

Table 6-2 Site details.

Site Characteristic	Description
Grid reference	SH 657 729 (Outdoor Leisure Map 17) 53°15'N4°00'W
Location	Henfaes (University Research Farm), Abergwyngregyn.
Parent Material	River Alluvium/boulder clay (Ordovician)
District Topography	Alluvial plain (level)
Site	North West facing slope 2°
Altitude	25m
Drainage	Adequate drainage
Vegetation and management history.	Resown 1987 with long ley mixture of perennial ryegrass (*Lolium perenne L.*), Timothy (*Phleum pratense*) and white clover (*Trifolium repens*). Permanent pasture for grazing and silage.
Land Owner	University of Wales, Bangor.

6.2.2 Soil characteristics

The soil is a low base status Brown Earth of the Denbigh series, freely draining, with a parent material of glacial drift containing shales of acidic igneous rocks with hard grits and slates. A detailed profile description of a Denbigh profile from Abergwyngregyn can be found in Ball (1963) in which it is stated that the cation exchange capacity (CEC) falls from 260 meq kg^{-1} at the soil surface to 90 meq kg^{-1} at 40 cm, the percentage base saturation is low, being below 50 throughout (Ball, 1963). Ball (1963) describes the soils of the Denbigh series as:

> "...the basis of the general farming of a large part of Wales, and, though not inherently well supplied with lime and nutrients, their physical properties make them easily worked and their finer texture makes them resistant to the effects of drought...the structure is easily destroyed....Although it is possible to grow oats in soil with pH values from 5.2 to 5.7, in which range most Denbigh soils fall, it is necessary to lime regularly to maintain the optimum pH value of 6.0 to 6.5 for most other crops and normally there are good responses to applications of nitrogenous, phosphatic or potassic fertilizers."

Figure 6-1 Location of experimental site.

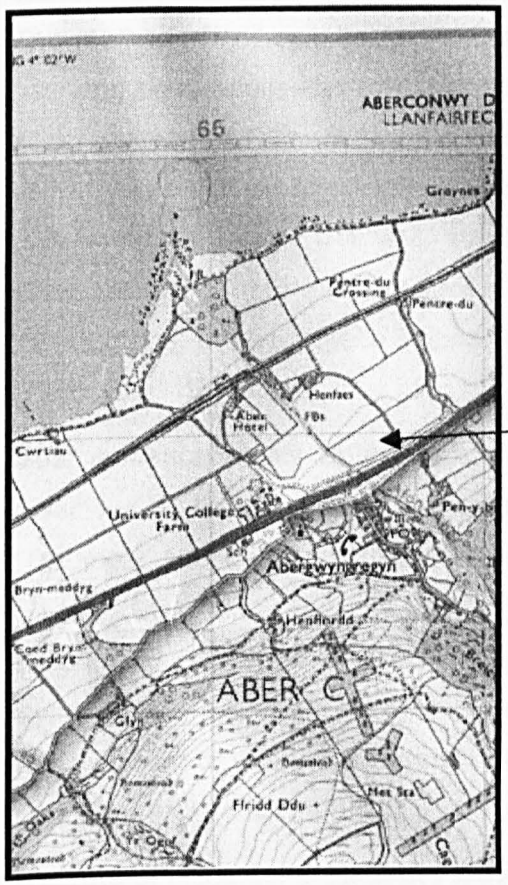
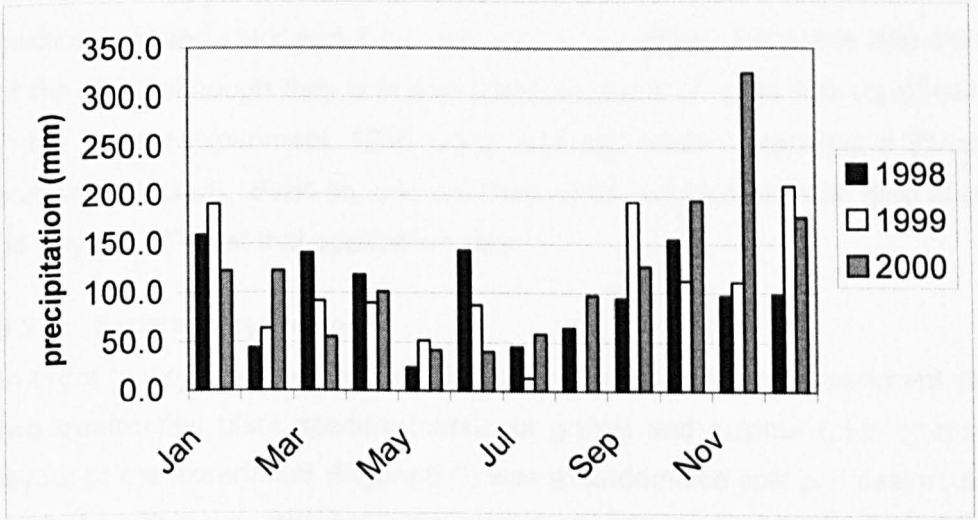


Figure 6-2 Annual rainfall distribution.

(Annual totals: 1998 – 1185 mm, 1999 – 1216 mm, 2000 – 1466 mm)



6.2.3 Soil acidification

The method of soil acidification chosen was application of elemental sulphur, which has long been applied as a fertiliser and as a means of acidifying alkaline soils (Nor and Tabatabai, 1977). Sulphur is oxidised chemically and by microorganisms in the soil: most, if not all *Thiobacilli spp.* (e.g. *Thiobacillus thiooxidans, T. thioparus* and *T.denitrificans*; Li and Caldwell, 1966; Trudinger and Loughlin, 1981); all strains of *Chromatiaceae* and *Chlorobiacea* (Trudinger and Loughlin, 1981); several heterotrophs, photosynthetic sulphur bacteria and colourless, filamentous S bacteria (Tabatabai, 1994).

Most evidence points to a requirement for a close contact between the sulphur particle and the bacterium, rather than excretion of sulphur solubilising agents (Trudinger and Loughlin, 1981). Oxidation of elemental sulphur proceeds through $S_2O_3^{2-}$, $S_4O_6^{2-}$ and SO_3^{2-} to SO_4^{2-} (Nor and Tabatabai, 1977; Tabatabai, 1994) although thiosulphate and polythionates are not essential intermediates in the oxidation of elemental sulphur (Trudinger and Loughlin, 1981). A thorough investigation of the biochemical oxidation of elemental sulphur is provided by Trudinger and Loughlin (1981).

The rate of oxidation is generally lower in acid soils than in alkaline soils. After a 70 day incubation, Nor and Tabatabai (1977) found that 69% of sulphur was oxidised in alkaline soils, and 52% in acid soils. The formation of SO_4^{2-} increases the H^+ concentration in the soil, lowering the pH (Li and Caldwell, 1966; Nor and Tabatabai, 1977). Li and Caldwell (1966) found that the amount of S applied did not affect the fraction oxidised. Nor and Tabatabai (1977) noted that there was little change in pH of the soils, although they only examined additions of up to 200 $\mu g\ g^{-1}$soil, whereas in the current experiment, 1280 $\mu g\ g^{-1}$ soil was added (assuming a 35 cm depth of soil was affected). Even so, one soil they examined showed a pH drop of one unit (in 56 days at 30°C) at that application rate.

6.2.4 Experimental design

In order to examine the effect of pH on agricultural soils, the experiment consisted of two treatments: plant species (cereal or grass) and sulphur (plus or minus). The layout of the experiment (Figure 6-3) was a randomised split plot design, arranged in 4 blocks. This was decided upon as the ploughing and drilling of a full 100 m strip was more practical than cultivating smaller plots. The treatments within the blocks were randomised: the location of the 4 ploughed 100 m strips and the side of main

plot to which sulphur was applied were determined randomly, by coin tossing. This gave four plots of each treatment combination, namely 4 x Cereal plus sulphur (C+); 4 x Cereal with no sulphur (C-); 4 x Grass with sulphur (G+); 4 x Grass with no sulphur (G-) and each plot was 50 m x 6 m = 300 m^2.

6.2.5 Site management

6.2.5.1 Sulphur application

Commencement of the experiment was delayed by difficulties regarding procurement of the Sulphur. It was hoped that the experiment could have started before the beginning of the leaching period in autumn 1998.

Elemental sulphur ('Flowers of Sulphur', S_2) (Univar Plc, Basingstoke) was applied at a rate of 4.167 tons ha^{-1} (125 kg S_2 300 m^{-2} plot or 0.42 kg m^{-2}) during 13-17/11/98. This was based on the following calculation (Ian Kelso, pers. Comm.):

The soil had a CEC[5] of 0.15 mol kg^{-1} and an average $pH_{(1M\ KCl)}$ of 5.2 (n=80). Assuming the soil was near base saturation and the actual CEC is 0.12 mol kg^{-1} accounting for stones. We aimed to lower it to 30% base saturation, 0.04 mol kg^{-1} (pH 4.5), requiring a loss of 0.08 mol of charge kg^{-1} soil. In each 1 m^2 area, taking into account a soil depth of 0.35 m with a bulk density of approximately 1 g cm^{-3}, this is 350 kg soil, requiring a drop in charge of 28 mol. As 1 M charge of sulphur is 16 g, 448 g sulphur is required per m^2. Treating 2,400 m^2 therefore required 1,075,200 g sulphur, or approximately 1 ton.

Application was carried out by hand, by marking out a 10 x 6 m section of a plot and using a jug to distribute a 25 kg sack of elemental sulphur as evenly as possible within that area. Protective boots, gloves, overalls and a mask were worn.

6.2.5.2 Ceramic porous cup soil water samplers

Porous cups (Soil moisture Equipment Corporation, P.O.Box 30025, Santa Barbera, CA 93105, USA) of 5 cm O.D., represented in Figure 6-4, were used to sample soil water. Prior to use, these were buried in a pit in the field with the ceramic part in contact with the soil, to allow equilibration of the cation exchange capacity of the ceramic with the soil, for over 1 month. No pre-treatment of the porous ceramic cup with acid was deemed necessary as Ca, Na, K, P or trace elements were not being

---

[5] CEC determined by Dr. Andy Owen to be 15.125 $mmol_c100g^{-1}$ on a randomly chosen soil sample Plot 15 sample 3 pre-treatment (pH 5.55).

Figure 6-3 The design of the experimental plots on Bont field, Henfaes.

A55 road to the South.

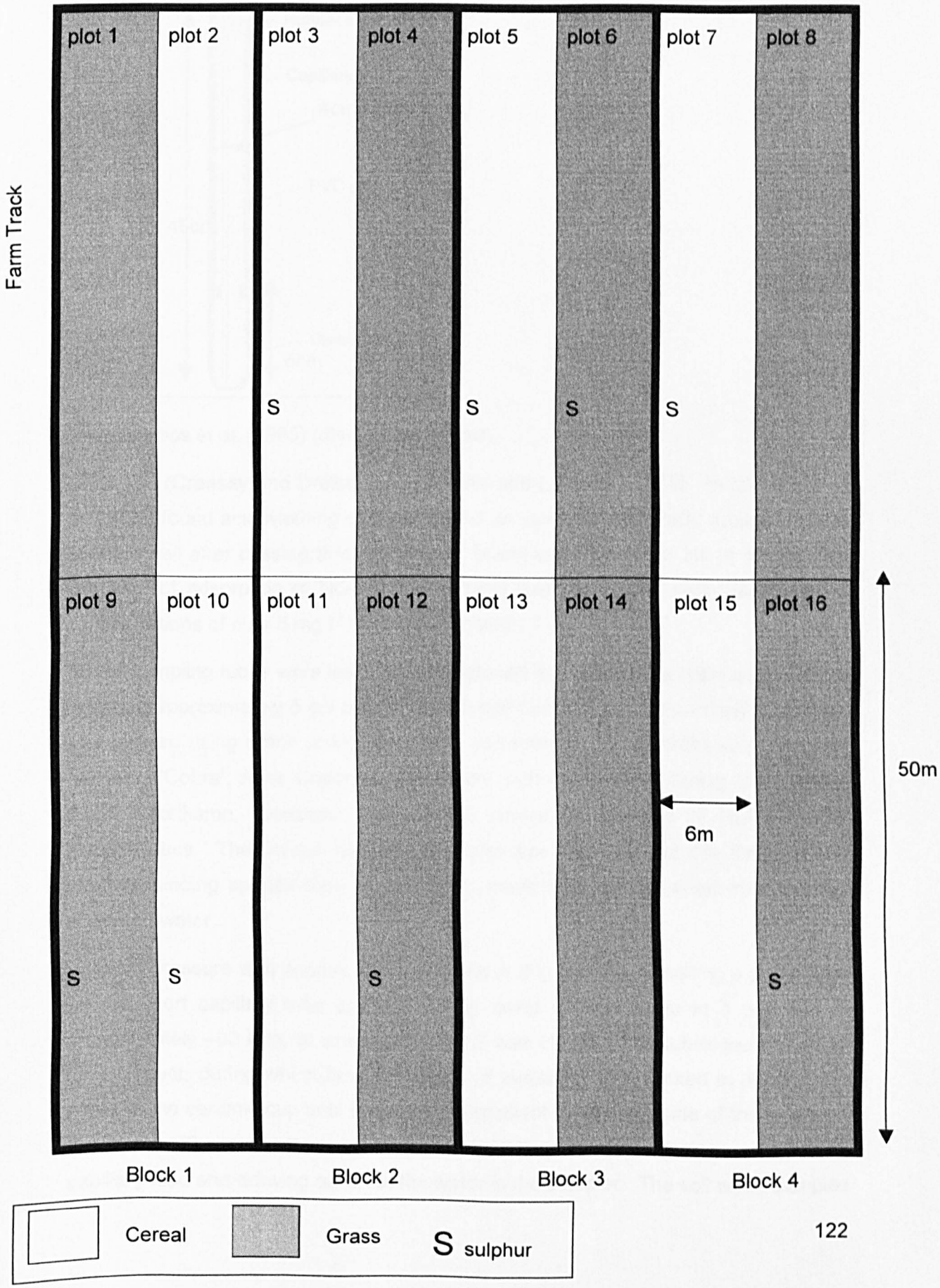
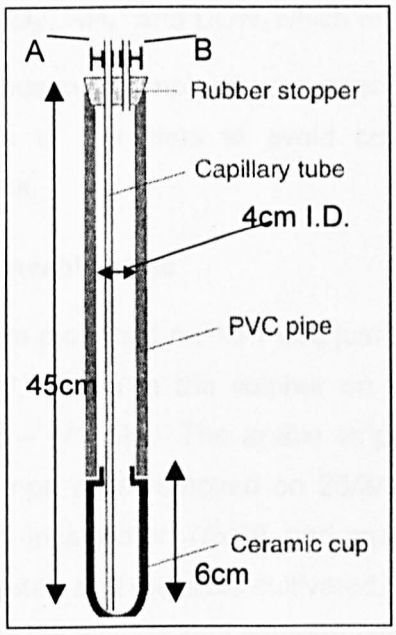


Figure 6-4 Porous Ceramic Cup Water Sampler in Cross Section.



Source: Poss et al. (1995) (dimensions added).

examined (Creasey and Dreiss, 1988; Grover and Lamborn, 1970). In fact, Poss et al. (1995) found acid washing of cups caused an increase in the NO_3^- retained by the ceramic wall after passing through 'Coinda' brand samplers (from 2% to 11%). The low level of adsorption of NO_3^- to the ceramic was considered negligible for NO_3^- concentrations of over 5 mg l^{-1} (Poss et al., 1995).

These sampling tubes were inserted in the ground at a depth of approximately 40 cm (allowing approximately 5 cm of tube to protrude from the ground surface). The hole was created using a soil coring apparatus, comprising of a petrol-driven percussion hammer ('Cobra', Atlas Copco Ltd., Sweden) with an attached coring tube (Model 08.18 Eijkelkamp, Giesbeek, Netherlands) containing a series of HDPE plastic sample tubes. The porous cup sampling tube was then inserted into the hole and any surrounding spaces filled in with slurry made from soil obtained from the soil corer and water.

Vacuum pressure was applied by closing clip A (Figure 6-4), attaching a pump tube on the short capillary tube and evacuating using a hand pump to a pressure of approximately –80 kPa, at which point clip B was closed. The tubes were then left for 24 hours, during which time soil water (if available) was sucked in through the pores of the ceramic cup until the pressure gradient in- and out-side of the tube was equalised. The soil water was then extracted by attaching a syringe on the long capillary tube and drawing out all of the water in the sampler. The soil water samples

were then immediately taken to the laboratory and refrigerated (<4°C) until analysed for pH, NO_3^-, NH_4^+ and DON, which were usually completed within 48 h.

Five porous cup samplers were inserted in each plot, avoiding a 1 m border around the edge of the plots to avoid cross-seepage of soil water between different treatments.

6.2.5.3 Arable plots

Plots were ploughed on 10/11/98 just before the sulphur was applied, and were then cultivated, mixing in the sulphur on 26/11/98. Porous cups were inserted during 30/11/98 – 4/12/98. The arable strips were left fallow and the porous cups in the arable strips were removed on 26/3/99 to allow cultivation and sowing with spring barley, re-inserted on 7/5/99, and again removed on 8/9/99 so that the barley could be harvested and the plots cultivated. It was intended that the crop be sampled and tested for dry weights and nitrogen content of above ground biomass and seed yield. However, shortly before sampling of the crop in early September 1999, the crop was destroyed, trampled and eaten by birds to such an extent that meaningful sampling was not possible. The porous cups were re-inserted following cultivation on 26/10/99 and were removed on 31/3/00, so that the strips could be cultivated and sown with barley; during that sampling period the arable strips were left fallow. Management practices and dates are summarised in Table 6-3 below. No fertiliser was applied in either year.

6.2.5.4 Grass plots

Sulphur was applied to the surface of the soil and vegetation. Porous cups were inserted in the soil during 30/11-4/12/98 and soil solution sampling commenced on 22/1/99. These samplers remained in the soil until the end of the experiment. The grass was mown using a wide hand driven mower or a tractor, with cutting around the porous cup samplers done with a strimmer. Plots were mown prior to commencement of the experiment and cutting was not necessary over the winter of 1998/9. Monthly cutting of the grass commenced on 20/5/99; grass was raked off by hand and removed from the plots. Cutting continued until October 1999 and was not necessary over the winter or spring until the end of the experiment. No fertiliser was applied in either year. Management practices and dates are summarised in Table 6-3 below.

Table 6-3 Management of field experiment on Bont, Henfaes

Management	Date
Plots marked out	3/11/98
Arable strips ploughed	4/11/98
Sulphur applied	13/11/98 plots 16,12,10.9 16/11/98 plots 7,6 17/11/98 plots 6,3
Arable strips cultivated.	26/11/98
Arable & grass PCs put in	30/11/98 &1, 3, 4/12/98
Porous cup sampling starts	6/1/99
Arable porous cups taken out	26/3/99
Arable strips cultivated	6/4/99
Arable strips sown with barley	4/5/99
Porous cups put in	7/5/99, 'soiled in' 10/5/99
Grass mown & raked off	20-25/5/99 & then monthly until October.
Arable porous cups taken out	8/9/99
Barley 'harvested'	15/9/99
Arable strips cultivated	5/10/99
Arable porous cups re-inserted	26/10/99
Arable porous cups removed	24/3/00
Arable strips cultivated	3/00
Arable strips sown with barley	4/00

6.2.6 Sampling regimes

6.2.6.1 Soil samples

1) Soil was sampled at the start of the experiment in order to ascertain initial conditions, this was carried out on 5.11.98, after ploughing but prior to application of Sulphur. 5 soil samples (0-15 cm) were taken per plot, using a soil corer. Samples were sieved (4 mm) and analysed for: total C, total N, soil moisture and amino acid mineralisation. Soil extracts (1 g soil:10 ml 1 M KCl) were analysed for pH, NH_4^+, and NO_3^-.

2) 6 months after Sulphur application (17.5.99), soil was sampled from each plot, taking profile depths 0-5, 5-10, 10-15, 15-20 cm in order to determine the extent and depth of the influence of the Sulphur. These were sieved (4 mm), extracted by shaking 5 g of soil in 50 ml 1 M KCl and analysed for pH, NO_3, NH_4, and DON.

3) At the end of the experiment (9.10.00) soil was sampled in order to compare soil conditions with those at the start of the experiment. The sampling and analysis was performed as in paragraph 1 above, with the addition of DON analysis.

6.2.6.2 Porous cup extracted soil water

Arable strips

The first porous cup samples were taken on 8.1.99, but as only around half of the samplers worked, data from these samples is not presented. Sampling was performed fortnightly from 22.1.99 to 16.3.99, during which time the plots were vegetation free. Sampling was carried out fortnightly from 7.5.99 – 8.9.99 but no sample was obtained due to insufficient soil water. Sampling recommenced after agricultural operations on 29.10.99 and took place fortnightly until 31.3.00. Sampling every 2 weeks (about every 25mm of drainage) has proved adequate (Lord and Shepherd, 1993).

Grass strips

The first samples were taken on 8.1.99, but as only around half of the samplers worked, data from these samples is not presented. Sampling was performed fortnightly from 22.1.99 to 16.3.99 after which no sample was obtained due to lack of soil water. Sampling commenced again from 29.10.99, when it was possible to start sampling the arable plots, and continued until 19.4.00 at which point there was no sample available due to lack of soil water.

6.2.7 Soil and soil solution analysis

Soil and soil solution samples were analysed for pH, NO_3^-, NH_4^+, DON, amino acid mineralisation and total C and N as described in chapter 3.

$CaCO_3$ content was determined according to the method and apparatus described by Bascomb (1974). Briefly, 10 g of soil was placed in the bottle of a calcimeter and drops of 3M HCl were carefully added until gas evolution ceased. The % $CaCO_3$ is then calculated by (Bascomb, 1974):

$$\%CaCO_3 = \frac{vol.CO_2(ml)}{sampledrymass(g)} \times \frac{barometricpressure(mmHg)}{temperature(^\circ C + 273)} \times k$$

where: $k = \frac{273 \times 100}{760 \times 224} = 0.1604$

Soil texture was determined by sequential sieving (to sand fractions >2000 µm, 630-2000 µm, 200-630 µm, 63-200 µm) and sedimentation of silt (2-63 µm equivalent spherical diameter (e.s.d.)) and clay (<2 µm e.s.d.) measured on an X-ray SediGraph 5000ET (Micromeritics Inc.).

Exchangeable cations were determined by a modified variation of the method of Rowell (1994), briefly 5 g of air-dried soil sample was placed in a tall 1l beaker, 25 ml water and then successive 5 ml portions of 100 vol. hydrogen peroxide were added until effervescence ceased. Drops of pentan-1-ol were used to destroy the froth. Soil was then heated vigorously to destroy excess peroxide. The soil sample was then placed in 100 ml centrifuge tubes with 40 ml of 1 M ammonium acetate, stoppered and shaken thoroughly for several minutes, and then centrifuged for 5 min. at 2 000 rpm. Supernatant was decanted into a volumetric flask. This washing was performed 3 further times, and the bulked supernatants then made up to 200 ml with 1 M ammonium acetate. The soil sample was washed 3 times with 40 ml portions of 95% ethanol, rejecting the washings and avoiding loss of soil. The adsorbed NH_4^+ was displaced from the soil by washing with 3 successive 30 ml portions of 1 M KCl (acidified to pH 2.5), collecting supernatants from each sample and made up to 100ml. NH_4^+ content was measured by the method described in Chapter 3. Cation concentrations in the NH_4^+ acetate were determined on an Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES, Jobin Yvon 38).

6.2.8 Statistical analysis

Statistical analysis was performed using Minitab version 13. Analysis of variance (ANOVA) was performed according to the split plot design. Individual sample data (n = 5 per plot in most samplings) were meaned and used as single data points for each plot. Data were checked for normality (Anderson Darling test) and equal variances (Bartlett's or Levene's tests depending on if parametric or not), and where these conditions were not met, the data was transformed so that ANOVA could be used. Various transformations of the data were attempted (e.g. ln(x), ln(x+1), $\sqrt{(x)}$, $\sqrt{(x+0.5)}$, e^x, arcsin(x), $(x)^2$). If no suitable transformation could be found to normalise the data, but the variances were equal according to Levene's test (P=0.05), the generalised linear model ANOVA was used.

To determine differences between treatments, LSD values were calculated by the following equation: (Clewer and Scarisbrick, 2001).

$$LSD = SED \times t_{(5\%,\ error\ df)}$$

Where:

$$SED = \sqrt{((2 \times Error\ MS)/n)}$$

Statistical significance was calculated at the 95% (P=0.05) level, unless otherwise stated.

6.3 Results

6.3.1 Soil samples

6.3.1.1 Descriptive soil characteristics

Soil characteristics on Bont, determined on samples taken from under grass before the experiment commenced, (0-10 cm depth) are reported in Table 6-4 below.

6.3.1.2 Soil samples from experimental plots, pre-sulphur treatment

Table 6-5 below shows the soil characteristics on Bont plots by species treatment. At the time of sampling the cereal plots had recently been ploughed. Figure 6-5 shows the soil characteristics by all treatments, although the sulphur had not yet been applied.

Prior to treatment with sulphur, mineral N varied between the plots. The grass plots that were to be treated with sulphur had significantly higher NO_3^- concentrations than all the other treatments (Figure 6-5i).

Table 6-6 provides an example of an ANOVA table, and shows the ANOVA results for NH_4^+ data at the start of the experiment. The NH_4^+ content was significantly higher in the grass plots than in the cereal plots, which had recently been ploughed, with the only significant difference between treatments being between G- and C+ plots (Figure 6-5ii). There were no significant differences between treatments or future treatments in soil pH (Figure 6-5viii), moisture (Figure 6-5iii), rate of amino acid mineralisation (Figure 6-5iv), total C (Figure 6-5v), total N (Figure 6-5vi) or the C:N ratio (Figure 6-5vii).

Table 6-4 Field soil characteristics prior to experiment

(b.l.d. indicates below limit of detection, n=3 on all tests).

Characteristic	value	unit
$CaCO_3$	0.51	%
LOI	9.1	%
Stone	4.50	%
Sand	47.73	%
Silt	28.97	%
Clay	23.30	%
Texture	Clay loam	UK classification
Exchangeable Ca	55.33	mg kg^{-1}
Exchangeable Na	7.33	mg kg^{-1}
Exchangeable Mg	3.91	mg kg^{-1}
Exchangeable K	12.09	mg kg^{-1}
Exchangeable P	0.12	mg kg^{-1}
Exchangeable Fe	1.11	mg kg^{-1}
Exchangeable Al	0.15	mg kg^{-1}
Exchangeable Mn	0.53	mg kg^{-1}
Exchangeable Zn	b.l.d.	mg kg^{-1}
Exchangeable Pb	b.l.d.	mg kg^{-1}
Exchangeable Cd	b.l.d.	mg kg^{-1}
Cation Exchange Capacity	20.05	$mmol_c$ $100g^{-1}$
Base saturation	20.69	%
Bulk density[6]	1.1	g cm^{-3}

Table 6-5 Initial soil sample characteristics on plots (5.11.98)

	Cereal (n=8)		Grass (n=8)	
	mean	St.err.	mean	St.err.
$pH_{(1:10\ 1MKCl)}$	5.29	0.07	5.18	0.06
NO_3^-N (mg N kg^{-1} soil)	134.43a	12.19	214.0b	38.1
NH_4^+-N (mg N kg^{-1} soil)	4.5a	1.0	9.4b	1.86
C:N	11.7	0.4	11.8	0.4
Total C %	3.5	0.2	3.3	0.2
Total N %	0.30	0.01	0.28	0.01
Amino acid mineralisation rate, $1/t_{15}(h^{-1})$	0.37	0.02	0.36	0.03
Moisture Content %	34.5	0.5	34.9	0.4

[6] As accurate measurement of bulk density in such stony soil is somewhat complicated, the bulk density is assumed to be 1.2 g cm^{-3} on average or 1.1 g cm^{-3} in the top 20cm, 1.3 g cm^{-3} below 20cm (Ian Kelso pers. comm.).

Table 6-6 ANOVA results for NH_4^+ concentrations in soil samples at the start of the experiment.

[g/c indicates grass or cereal treatment, s/ns indicates sulphur or no sulphur to be applied, * indicates interaction.]

Source	DF	SS	MS	F	P
block	3	6.78	2.26	0.30	0.823
g/c	1	95.75	95.75	12.88	0.037
block*g/c	3	22.30	7.43	0.24	0.867
s/ns	1	25.70	25.70	0.82	0.400
g/c*s/ns	1	7.21	7.21	0.23	0.649
Error	6	188.09	31.35		
Total	15	345.84			

6.3.1.3 Soil sampled from different depths

Six months after sulphur application (17.5.99) there was still some elemental sulphur visible at the surface of the soil of both the grass and cereal treated plots, that had not been oxidised or thoroughly mixed in. It was therefore expected that the pH of the soil would drop further with time. Pair-wise comparisons on this six month data were made using Tukey tests. The results of soil pH, NH_4^+, NO_3^- and DON are shown in Figure 6-6i-iv. Results of the ANOVA for soil pH (Table 6-7) showed that there were significant sulphur treatment, depth and interaction effects, with sulphur treated plots having a significantly lower pH than non-sulphur treated plots and pH increasing with depth. Pairwise comparisons of individual treatments at each depth showed that the G+ plots had a significantly lower pH in the 0-5 cm layer than in all other treatments and than in the G+ 10-20 cm depth samples. (Figure 6-6i).

ANOVA results showed that NH_4^+ concentrations varied significantly with depth (surface 0-5 cm NH_4^+ concentrations were higher than all other depths) and there was a significant grass/cereal x depth interaction, meaning the NH_4^+ levels were affected by depth differently in cereal and grass plots. NH_4^+ was lower in the surface 0-5 cm of cereal plots than the grass plots, but not significantly so (Figure 6-6ii). In the C- plots the NH_4^+ content of the soil at 15-20 cm was significantly greater than at 0-5 cm, whereas in the C+ plots the NH_4^+ content of the soil at 15-20 cm was significantly lower than at 0-10 cm. In grass plots, NH_4^+ levels were highest at 0-5 cm and lower in other horizons.

NO_3^- concentrations showed significant species, sulphur and depth effects and species x depth and species x sulphur interactions. NO_3^- levels were much higher in

cereal than grass plots, and in sulphur treated than non-sulphur treated plots. NO_3^- levels in the 15-20 cm depth samples were higher than those at depths 0-15 cm. The species x depth interaction indicates that NO_3^- concentrations varied with depth differently in the grass and cereal plots: this is demonstrated in Figure 6-6iii which shows NO_3^- levels in the soil increased with depth in the cereal plots, whereas in the G+ plots NO_3^- levels were lower at 5-10 and 10-15 cm than in the surface 0-5 cm layer. There were no significant treatment or depth effects on DON concentrations at this time (Figure 6-6iv).

Table 6-7 ANOVA results for soil pH from samples obtained from different depths.

[g/c indicates grass or cereal treatment, s/ns indicates sulphur or no sulphur treatment, depth indicates depth of sample, * indicates interaction.]

Source	DF	SS	MS	F	P
Block	3	0.14672	0.04891	0.25	0.861
g/c	1	0.11989	0.11989	0.60	0.494
Block*g/c	3	0.59758	0.19919	2.77	0.053
s/ns	1	2.97994	2.97994	41.44	0.000
depth	3	4.26340	1.42113	19.76	0.000
s/ns*depth	3	0.33914	0.11305	1.57	0.210
g/c*depth	3	1.17244	0.39081	5.44	0.003
g/c*s/ns	1	0.00069	0.00069	0.01	0.922
g/c*s/ns*depth	3	0.70502	0.23501	3.27	0.030
Error	42	3.02008	0.07191		
Total	63	13.34490			

Figure 6-5 Soil properties of Bont plots prior to sulphur application.

(n=4 plots per treatment, each plot value being the mean of 5 samples. $P < 0.05$, $pH_{(1:10\ 1\ M\ KCl)}$).

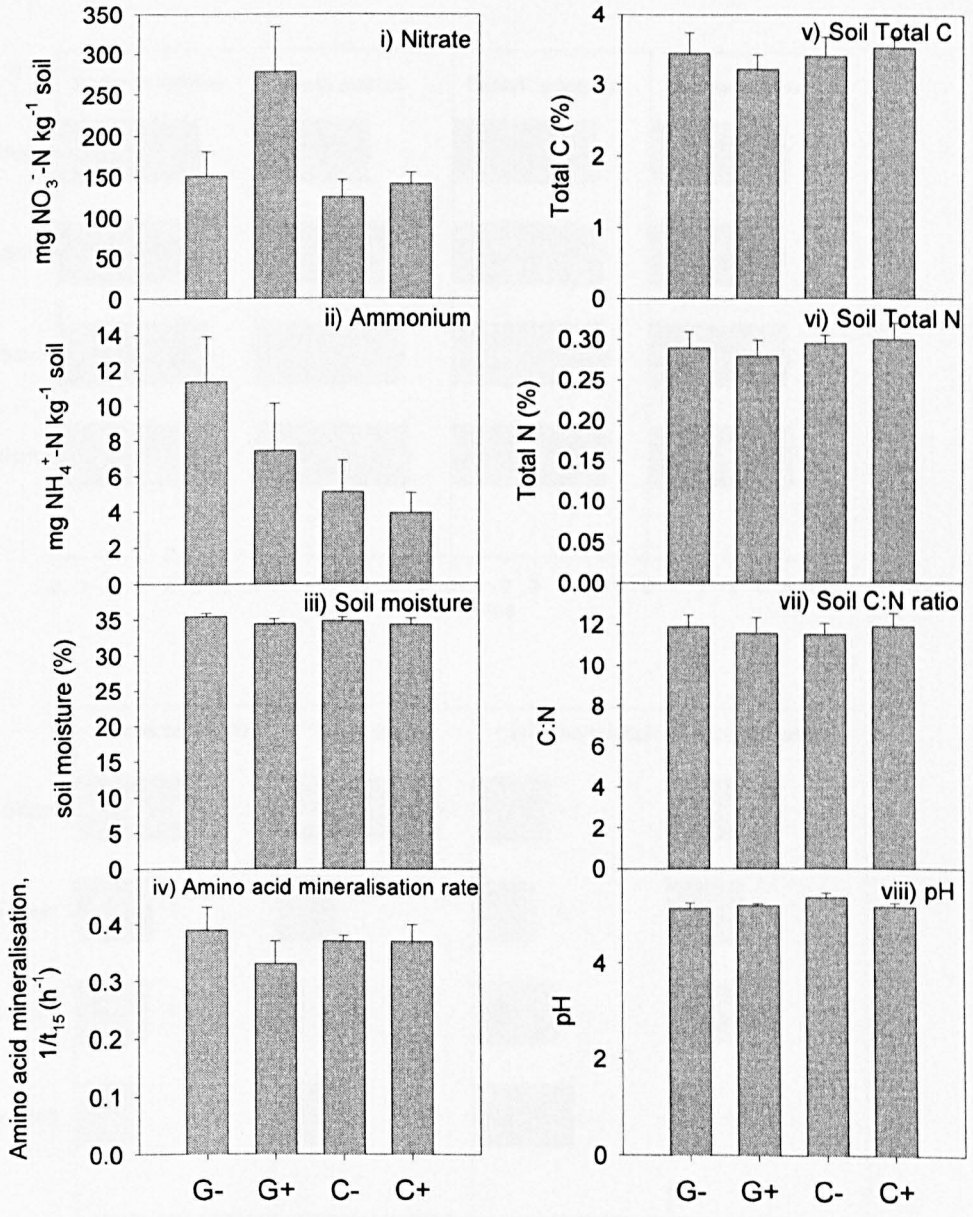


Figure 6-6 pH, NH_4^+, NO_3^- and DON in soil samples 6 months post sulphur application.

(n=4 plots per treatment, one sample per plot).

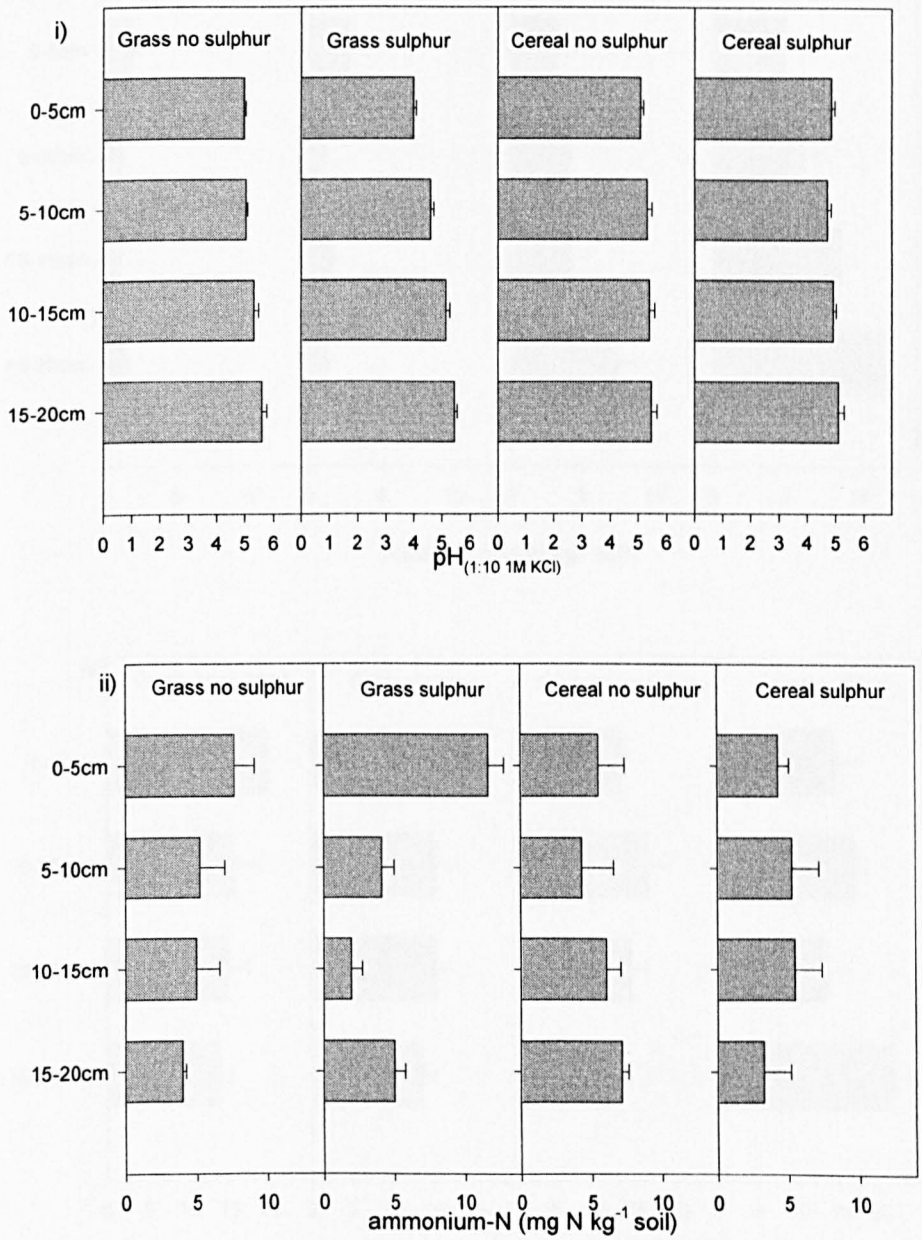
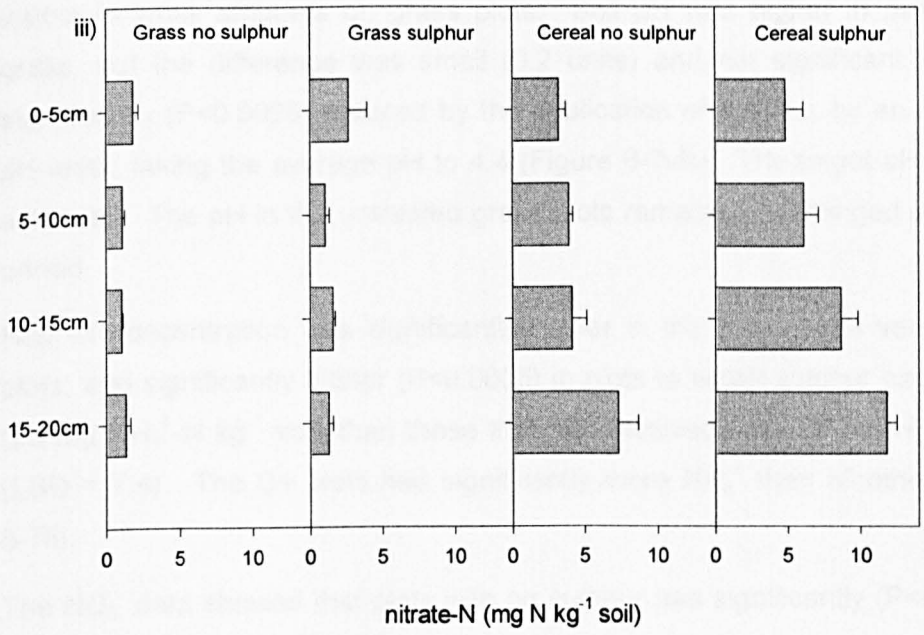
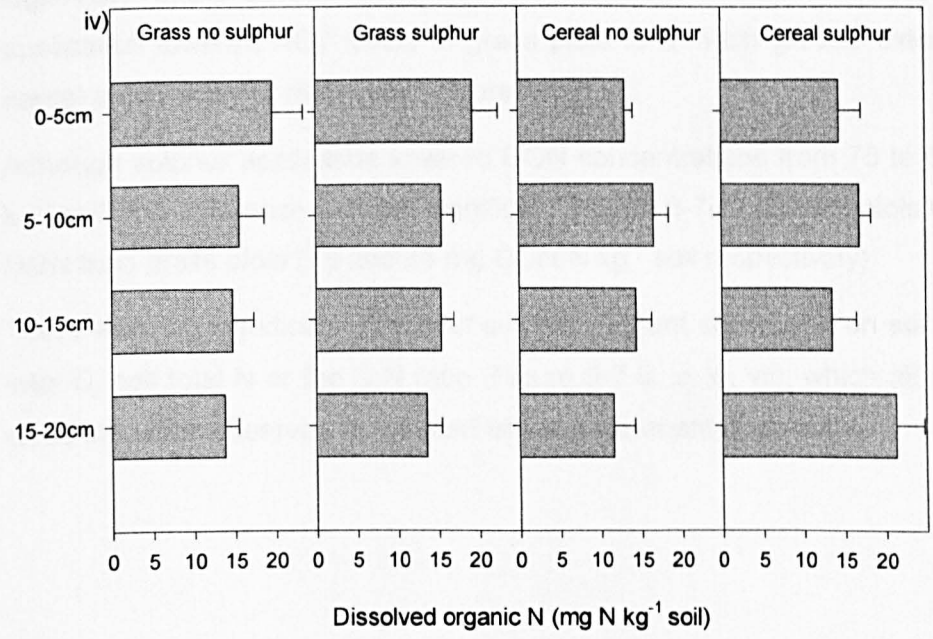


Figure 6 continued...





6.3.1.4 Soil sampled at the end of the experiment (9.10.00)

By the end of the experiment, nearly 2 years after the sulphur was applied, there were some dramatic changes to the soil (Figure 6-7). Elemental sulphur was still visible in small amounts on grass plots. Soil pH was higher in cereal plots than grass, but the difference was small (0.2 units) and not significant. Soil pH was significantly ($P<0.0005$) reduced by the application of sulphur, by an average of 0.8 pH units, taking the average pH to 4.4 (Figure 6-7viii). The target pH was therefore achieved. The pH in the untreated grass plots remained unchanged over the 2 year period.

NH_4^+-N concentration was significantly higher in the grass plots versus the cereal plots, and significantly higher ($P<0.0005$) in plots to which sulphur had been applied (25 mg NH_4^+-N kg^{-1} soil) than those that had received none (6 mg NH_4^+-N kg^{-1} soil) (LSD = 7.4). The G+ plots had significantly more NH_4^+ than all other plots (Figure 6-7ii).

The NO_3^- data showed that plots with no sulphur had significantly ($P<0.0005$) higher NO_3^- levels than those with sulphur applied. Plant cover type did not have a significant effect at this time, and the apparent interaction effect (the sulphur application lowered NO_3^- levels in grass plots to a much greater extent than in the cereal plots) was not significant (Figure 6-7i).

Although sulphur application lowered DON concentrations from 75 to 64 mg DON-N kg^{-1} soil, this difference was not significant (Figure 6-7ix). Cereal plots were higher in DON than grass plots (73 and 66 mg DON-N kg^{-1} soil respectively).

There were no significant effects of sulphur or plant cover type on soil moisture, soil total C, soil total N or the C:N ratio (Figure 6-7 iii, v, vi, vii), which all remained very close to values observed at the start of the experiment (Figure 6-5).

Figure 6-7 Soil properties of Bont soil 2 years post sulphur application (9.10.00)

[n=4 plots per treatment, each plot value being the mean of 5 samples. $pH_{(1:10\ 1M\ KCl)}$]

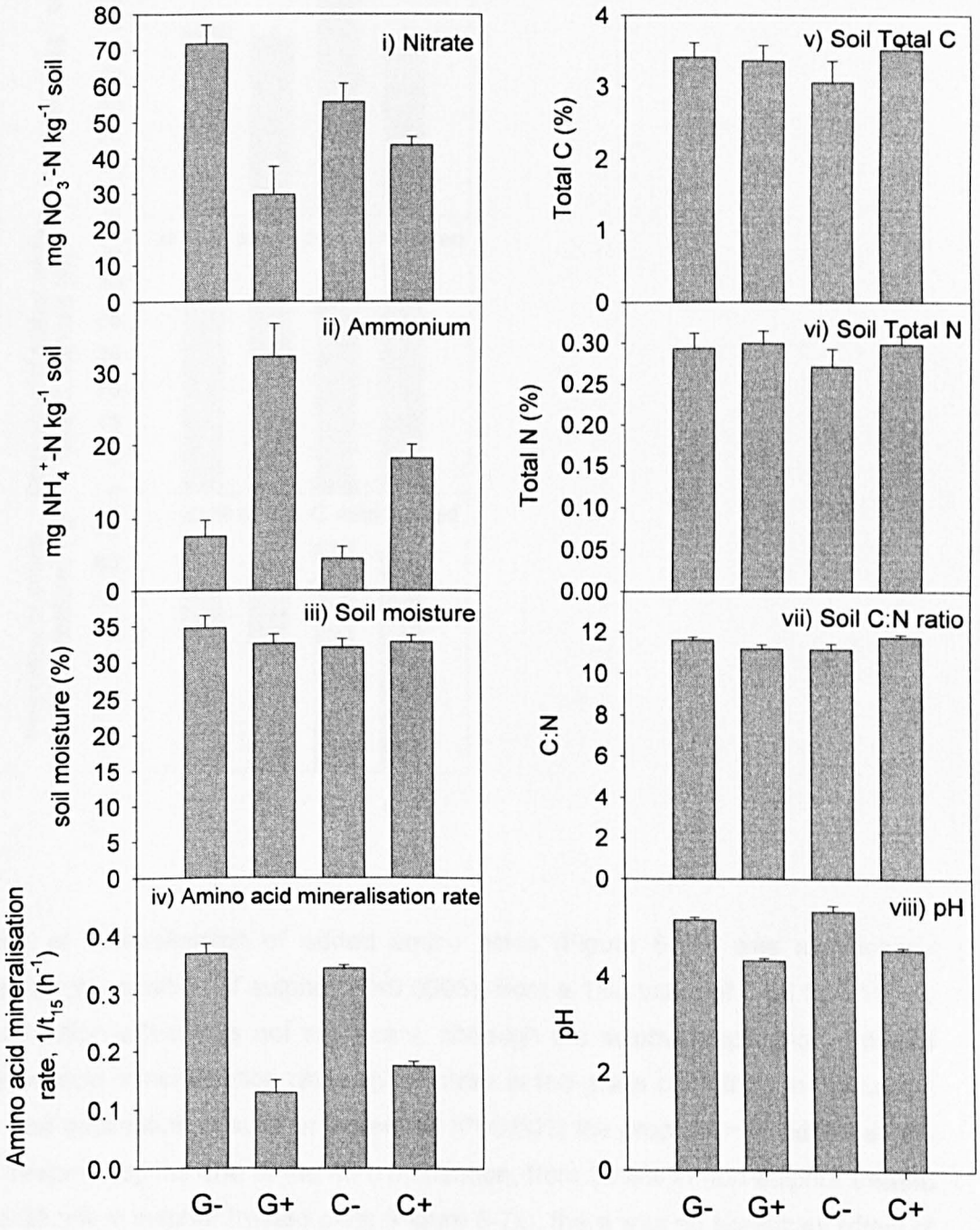
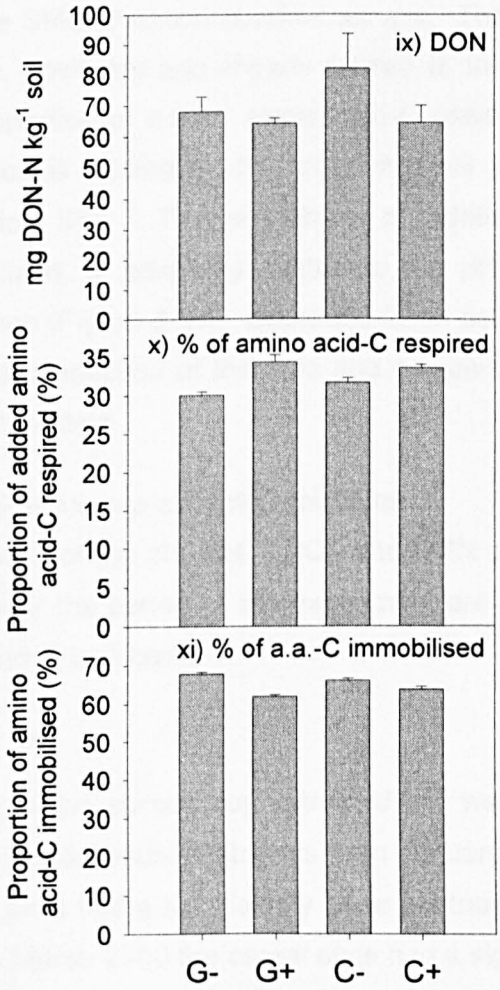


Figure 6.7 continued...



The rate of mineralisation of added amino acids (Figure 6-7iv) was significantly reduced by the addition of sulphur ($P<0.0005$), from a $1/t_{15}$ value of 0.36 to 0.15 h^{-1}. The interaction effect was not significant, although the sulphur application reduced the amino acid mineralisation rate slightly more in the grass plots than in the cereal plots. The application of sulphur increased ($P=0.001$) the proportion of added amino acid-C respired by the end of the 48 h incubation, from 30.8% in non-sulphur treated plots to 33.9% in sulphur treated plots (Figure 6-7x); there was no significant effect of vegetation type or interaction. Sulphur addition caused a corresponding drop in the proportion of added amino acid-C used in cell biomass, from 66.9% in non-sulphur treated plots to 62.7% in sulphur treated plots. There was a significant interaction effect as sulphur addition caused a much greater decrease in the proportion of amino

acid-C immobilised in the grass plots (6%) than in the cereal plots (2.4%) (LSD = 1.9), but there was no vegetation type effect.

Figure 6-8 shows the relationships between pH and the utilisation of the amino acid-C by the SMB of each individual sample. The rate of amino acid mineralisation was strongly, positively and linearly related to the pH of the soil sample (Figure 6-8a). The proportion of added amino acid-C used in respiration by the end of the 48 h incubation is related to the pH of the soil sample (Figure 6-8b) with a quadratic regression line. The proportion of added amino acid-C used structurally or 'immobilised' is positively related to the pH of the soil sample, with a quadratic regression (Figure 6-8c). Quadratic fits in panels b and c of Figure 6-8 were chosen on visual inspection of the data and comparison of correlation coefficients between different models.

6.3.2 Porous cup extracted soil water

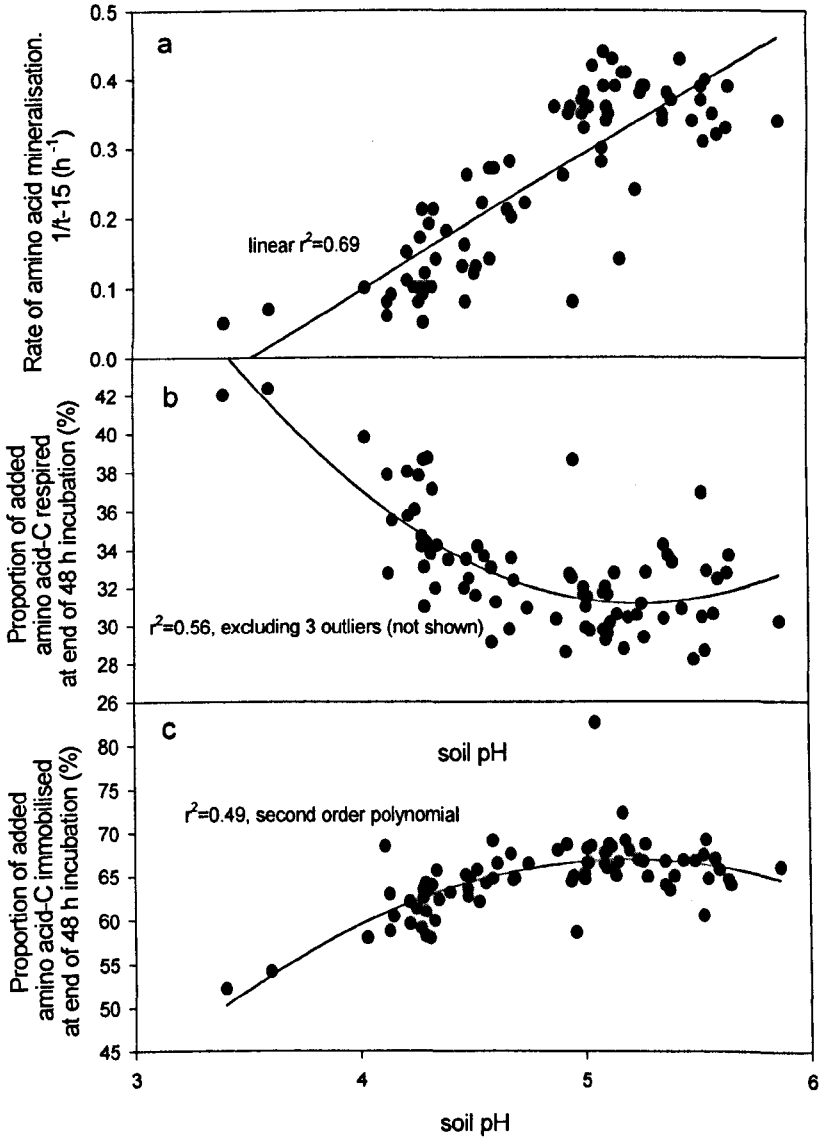
The results of the pH, NH_4^+, NO_3^- and DON content of the porous cup extracted soil water over the period of the experiment are shown in Figure 6-9, and each is now described in turn below.

6.3.2.1 pH

The pH of the porous cup extracted soil water (Figure 6-9i) showed no significant differences between treatments from January to March 1999 with the exception that the G+ plots had a significantly lower pH than the G- plots on 2.2.99. From October 1999 to March 2000 the cereal plots had a significantly lower pH than the grass plots (with the exception of one date, 14.12.99) and the sulphur treated plots had a significantly lower pH than the plots not treated with sulphur. The trends from October 1999 are identified as: although the pH was fairly consistently lower in C- plots than in G- plots, the C- and G- plots were never significantly different; pH in the G+ plots was always (and nearly always significantly) lower than in the G- and C- plots; and in the C+ plots pH was always significantly lower than in all other treatments. There was a significant interaction effect only on two of the sample dates from October 1999 to March 2000 (12.1.00 and 9.2.00), although sulphur always decreased pH to a much greater degree in cereal than in grass plots. It can be seen from figure Figure 6-9i that the pH of the soil water in the C- and G- plots usually fell within the range 6.0 - 6.6, and between 5.55 - 5.85 in the G+ plots and between 4.2 - 5.1 in the C+ plots.

Figure 6-8 Effect of soil pH on amino acid mineralisation on final Bont soil samples.

[Relationships between the $pH_{(1:10\ 1MKCl)}$ of each individual soil sample (n=80) and: a) the rate of amino acid mineralisation, the reciprocal of the time taken for 15% of added amino acid-C to be evolved as CO_2, $1/t_{15}$ (h^{-1}); b) the proportion of added amino acid-C that was respired after 48 h and c) the proportion of added amino acid-C that was immobilised after 48 h.]



6.3.2.2 NH_4^+

Figure 6-9ii shows the NH_4^+-N concentration of porous cup extracted soil water. NH_4^+ levels were always low, with values less than 2 mg NH_4^+-N l^{-1}. From January 1999 to March 2000 there were no significant differences between sulphur or species treatment, or the interaction of each, with the exception of 2.3.99 when the soil water NH_4^+ concentration in grass plots was significantly higher than in cereal plots.

During the period October 1999 to March 2000, the G- plots consistently had more NH_4^+ in the soil water than the C- plots, although the difference was never significant. From October 1999 to April 2000, the sulphur treated plots always had more NH_4^+ in the soil water than the untreated plots; this was significant ($P<0.05$) on 6 of the 12 sampling dates during this period. The NH_4^+ concentration in the soil water was always higher in the C+ plots than the G+ plots (with the exception of 21.3.00), although this difference was rarely significant. Sulphur treatment caused NH_4^+ levels to always be higher in the soil water in the grass plots (although rarely significant), but the effect was much more pronounced in the cereal plots (significant on 6 of the 10 sampling dates). However, the ANOVA showed a significant interaction effect on only one sampling date.

NH_4^+ levels in all treatments were highest during October to December 1999. NH_4^+ concentrations were at similar levels in untreated plots in January to March of 1999 and 2000, but during this period, sulphur treated plots generally had higher NH_4^+ levels in 2000 than in 1999.

6.3.2.3 NO_3^-

During the period January 1999 to March 1999, the effect of treatments on NO_3^- concentration of the porous cup extracted water (Figure 6-9iii) was very consistent. Cereal plots had a significantly higher soil water NO_3^- concentration than grass plots at all sampling dates (between 2 and 4.5 times the concentration) and while sulphur usually resulted in lower NO_3^- concentrations, the effect was small and not significant.

During October 1999 to March 2000 cereal plots had a significantly higher NO_3^- concentration than grass plots at all but one sampling date (14.12.99). Sulphur application lowered the NO_3^- concentration of the soil water significantly at most dates during this period, and there was never a significant interaction effect. The G+ plots showed a gradual build up in soil water nitrate levels from October 1999 to January 2000 (up to 8 mg NO_3^--N l^{-1}), which then fell steadily until April when they

were very low (1 mg NO_3^--N l^{-1}). During this period the trend in G- plot NO_3^- concentrations was very similar, but NO_3^- concentrations were significantly higher (up to 19 mg NO_3^--N l^{-1} in February 2000, and down to 5 mg NO_3^--N l^{-1} in April), and the decrease started around a month later. During December 1999 to February 2000, NO_3^--N concentrations in the G- plots were not significantly different to those in the C- or C+ plots, and were significantly higher than those in the G+ plots from late November. The NO_3^- concentration in the G- plots began to fall in March and returned to being significantly lower than in the cereal plots, but still significantly higher than the G+ plots.

The NO_3^- levels in the cereal plots were fairly stable from November 1999, and always lower in sulphur treated plots. In March 2000 the difference between C+ and C- plots widened, with the C- plots becoming significantly higher in NO_3^- concentration than the C+ plots.

6.3.2.4 DON

During the period January to March 1999, DON-N levels were 16-33% of NO_3^--N levels in cereal plots and 27-63% of those in grass plots. DON in the soil water of grass plots was consistently and significantly lower than in cereal plots during this period. At most dates, sulphur slightly lowered the amount of DON in both cereal and grass plots, but significantly only on 2.3.99 and 16.3.99.

DON levels were exceptionally high at the end of October 1999, from 2-4 times higher than NO_3^--N levels at that time. After this time, DON levels fell quickly and levels were fairly consistent from the end of November until DON levels in cereal plots started to rise during March. On half of the dates between October 1999 and March 2000 grass plots had significantly lower DON levels in the soil water than cereal plots, and sulphur treated plots have a significantly lower DON level than plots not treated with sulphur. During this period there was a significant interaction effect on only one date (25.1.00), where sulphur lowered DON levels much more in grass than in cereal plots.

6.3.3 Weather data

Weather conditions at the field site are presented in Figure 6-10. Average daily temperatures (Figure 6-10a) were very erratic. During January to March 1999 there was a slight trend of increasing temperature. From October 1999 the average daily temperature tended to fall until the end of the year. Temperatures picked up slightly during February 2000, to a warm spell in mid March, after which temperatures fell

again. Weekly total rainfall prior to sampling dates was also very variable, with little discernible pattern of wet or dry weeks evident (Figure 6-10b).

6.4 Discussion

6.4.1 Site management and experimental design

6.4.1.1 Sampling

6.4.1.1.1 Porous cup sampler depth

When sampling drainage water, porous ceramic cup samplers are usually installed at or below 90 cm depth to sample water that has moved beyond the main rooting zone of most crops (Williams and Lord, 1997; Djurhuus and Olsen, 1997). Cereals can extract water down to 120 cm, possibly deeper (Poss et al., 1995). In this experiment, the size of porous cup sampler used only allowed insertion to 40 cm depth. Insertion to any greater depth without substantial soil disturbance was precluded due to the stoniness of the site. However, this means that the porous cup extracted soil water may have higher nitrogen levels than the water leaching from the site, as roots will probably have had opportunity to capture this nitrogen at depths >40 cm.

6.4.1.1.2 Porous cup sampler success rate

Poss et al. (1995) had a suction cup failure rate of 6%, whereas in the current study the failure rate was 2.5%. This reflected the careful sealing of air leaks around joins in the samplers prior to installation in the field.

6.4.1.1.3 Number of samplers

Webster et al. (1993) raised the question of how many cups are needed to overcome problems of spatial heterogeneity and provide a reliable mean. Clewer and Scarisbrick (2001) describe how to determine the sample size in an experiment. In order to approximate the population mean (μ) with a sample mean ($\bar{x}$) that differs from the population mean by less than d with 95% confidence, a sample size of n is required, such that $1.96(\sigma/\sqrt{n}) < d$. Although σ is unknown, we can estimate it from experimental evidence, with the sample standard deviation, s. Thus n must be greater than $((1.96s)/d)^2$.

Figure 6-9 The pH, NH_4^+, NO_3^- and DON content of the soil solution over time.

[G = grass, C = cereal, + = plus sulphur, - = no sulphur, bars represent treatment means with standard errors (n=4 plots per treatment, each plot value is mean of five samples), $pH_{(1:10\ 1MKCl)}$]

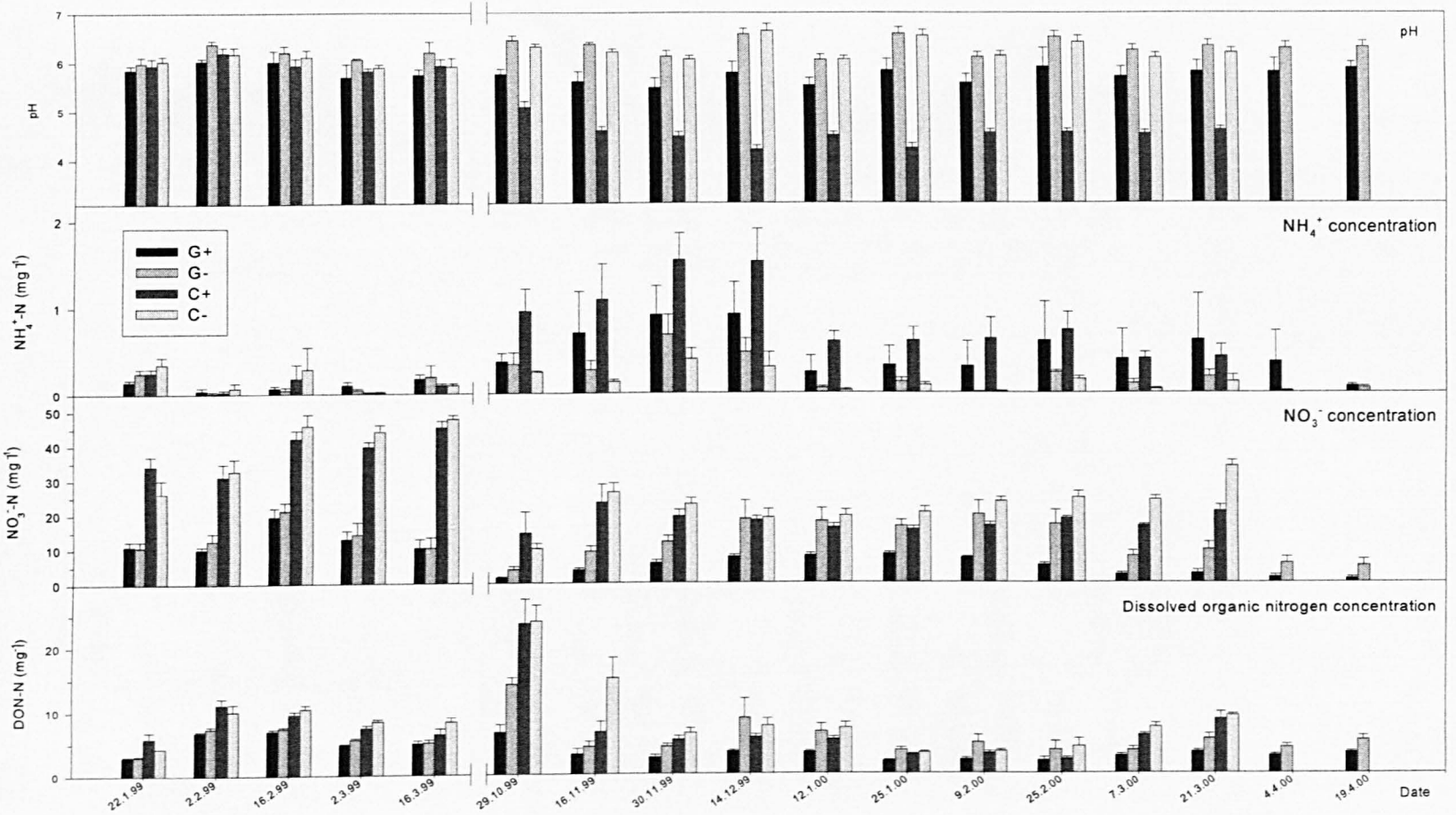
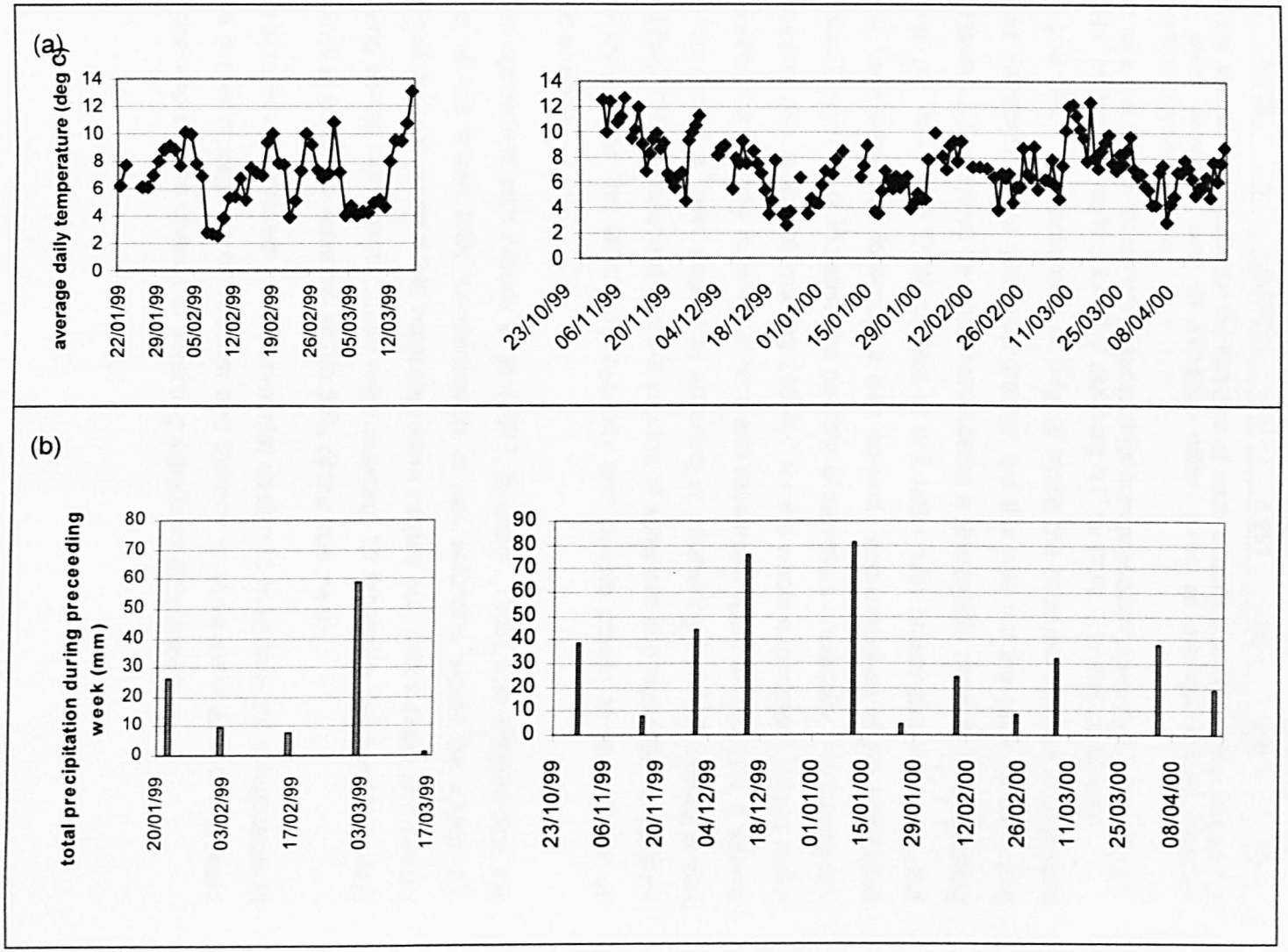


Figure 6-10 Weather conditions at the field site: a) average daily temperature and b) total weekly rainfall during sampling periods.

[(a) Average daily temperature = (min. temp + max. temp) / 2, (b) only weeks ending on sampling dates shown. Data for week ending 21.3.00 not visible, value 0.2mm]



The results of the porous cup samplers are presented in Table 6-8 below, with the corresponding sample sizes required to give reasonable accuracy at the 95% confidence level.

Table 6-8 Sample size, (n), required to give reasonable approximation of sample mean to population mean for porous cup extracted water.

	pH		NH_4^+		NO_3^-		DON	
treatment	s[a]	n[b]	s	n	s	n	s	n
G+	0.623	37	0.974	15	4.736	22	1.614	3
G-	0.313	9	0.259	1	10.288	102	3.424	11
C+	0.352	12	0.754	9	8.433	68	2.954	8
C-	0.228	5	0.212	1	8.257	65	3.477	12

[a]Taking the standard deviation for the samples of each treatment (n=20) of the porous cup sampler water, averaged over all sampling dates, gives an average sample standard deviation for each treatment, s.

[b]Sample size required, n, for sample mean to differ from population mean by 0.2 units for pH, 0.5 mg N l^{-1} for NH_4^+, 2 mg N l^{-1} for NO_3^- and 2 mg N l^{-1} for DON, with 95% confidence.

For pH and NH_4^+, the application of sulphur made the required number of samplers larger, as variability in the data was greater, but this was not the case for NO_3^- and DON. Having 20 samplers per treatment seems a reasonable number for estimating the actual pH, NH_4^+ and DON content of soil water with reasonable accuracy, but with NO_3^-, one either has to accept a less accurate approximation of the population mean than 2 mg N l^{-1}, or increase the number of samplers drastically. As having 60-100 samplers per treatment, making 240-400 for the whole experiment, would make the experiment too costly in terms of time and resources spent on analysis, it seems sensible to accept a lower degree of accuracy in estimating true NO_3^- levels in soil water. Litaor (1988) observed that the amount of samplers and replicates necessary will 'quickly exceed the laboratory capacity and people power of most if not all scientific projects'.

This is in agreement with Alberts et al. (1977, in Litaor, 1988) who showed that the estimate of the mean NO_3^- concentration in soil solutions would be within 5% ($P<0.05$) of the true mean if 246 replicates were carried out, with sample size rapidly decreasing as the significance levels were lowered; 10 replicates per sampler would be required to obtain an estimate within 30% of the true mean.

It would also have improved the experimental design to have had more replicates to increase the error degrees of freedom and thereby improve estimates of treatment means and improve the chance of detecting significant differences.

6.4.1.2 Vegetation management

The results from the grass plots, where the grass was cut and raked off, may not be an accurate reflection of nitrogen dynamics under a grazed regime. Although arable land is more prone to leaching than cut grassland, the situation is quite different if the grass is grazed in situ, because when nitrogen is returned in faeces and urine in grazed systems the leaching loss is generally much higher (Addiscott et al., 1991; Vinten and Smith, 1993). This is because the nitrogen removed in live weight gain or milk is much smaller than that removed in cut grass, and very high concentrations of NO_3^- can develop under urine patches, which exceeds the uptake capacity of the grass.

Cuttle et al. (1998) found leaching from grass/clover plots to be positively correlated with the numbers of lamb grazing days in the latter part of the grazing season. That relationship, along with the high spatial variability associated with measurements, indicated that N derived from excreta was the main source of leached NO_3^-. However in Cuttle et al. (1996), the quantity of NO_3^- leached was found to be independent of stocking rate.

Ruminants excrete over 75% of the N they ingest, returned in localised patches of dung and urine (Cuttle et al., 1998). N in urine patches is readily hydrolysed in soil to mineral forms which are susceptible to loss by leaching and other pathways, whereas dung is thought to be less important as a source of leached NO_3^-. Loss from 'camping' areas is very high (Cuttle et al., 1996). Ryden et al. (1984) showed, on a loam overlying chalk in the UK, that NO_3^- leaching below a grass sward grazed by cattle was 5.6 times greater than that from a cut sward and exceeded NO_3^- losses normally observed on arable land.

Cutting and raking off the majority of the plant biomass production on the grass plots will have given different results to if the site been grazed, as the dynamics of organic N breakdown will have been quite drastically altered. However, grazing of the plots was not possible due to logistical constraints. Grass was raked off, as, if it had been left on after a monthly cut, there would have been occasional large amounts of organic matter on the surface breaking down, which would have suppressed pasture growth. A much more regular cutting regime (possibly weekly), with grass left on the plots, may have provided a more accurate simulation of grazing, as in that case cut vegetation would not have been in large enough quantities to suppress pasture production, and breakdown of the organic matter would have been more constant, as

it is in grazed systems. However, this does not invalidate the effects of soil acidification on N dynamics that were observed, and leaves avenues for future work.

Good agricultural practice was not followed in management of the cereal plots, e.g. timing of cultivations, lack of winter cover crop. This was intentional, as the experiment intended to expose differences between sulphur treatments, without them being ameliorated by 'best practice'. It should also be pointed out that no fertiliser was applied, so that differences could be attributed to the internal N cycle, rather than compounded by the dynamics of added organic or inorganic N. The impact of soil pH on fertilised (organically and inorganically) systems is an avenue worthy of research.

6.4.2 Discussion of results

6.4.2.1 Soil pre-treatment

The NH_4^+ levels in the soil of the cereal plots may have been initially lower than in grass plots because they had just been ploughed, the turf, having been inverted, would be being mineralised quickly. This may have caused some immobilisation of NH_4^+, as the C:N of the sward is likely to be higher than that of the microbes mineralising it. Immobilisation may also have accounted for the lower NO_3^- levels in cereal than grass plots. However, the difference between the G- and G+ plot NO_3^- levels is a surprise as, at this stage no sulphur had been applied and there was no soil disturbance. The high average NO_3^- concentrations in the G+ plots were caused by a few samples of extremely high NO_3^- concentrations, which may have come from old urine patches or camping areas, as the site had been grazed by sheep prior to being fenced off for the experiment.

6.4.2.2 Soil sampled 6 months post-treatment from different depths

Six months after treatment, sulphur application resulted in a small but significant decrease in soil pH. The only notable effect was a significant lowering of soil pH in the G+ plots in the surface 0-5 cm, presumably because of lack of mixing, the sulphur was very concentrated and had the greatest effect. This explains the minimal effect of sulphur treatment on porous cup extracted soil water in the period monitored up to March 1999 (Figure 6-9). Where sulphur had impacted on the pH of the surface 0-5 cm of the grass plots, it caused a (non-significant) increase in soil NH_4^+ concentrations over the untreated grass plots. This may have been due to a pH induced inhibition of nitrification resulting in a build up in the NH_4^+ pool. However,

NO_3^- levels in G+ samples were higher than G- at this depth, indicating that this is probably not the case. In the C- plots, NH_4^+ levels were significantly higher at 15-20cm depth than 0-5cm depth, whereas in C+ plots, NH_4^+ levels were lower at 15-20cm depth than 0-5cm; this may have been due to an inhibition of mineralisation by the sulphur, the effect being at 15-20 cm because of incorporation of sulphur by cultivation. However, this is the opposite effect to that which was observed in surface layers of grass plots as described above.

Soil in the cereal plots contained more NO_3^- than grass plots, perhaps due to enhancement of oxidative processes by cultivation, and lack of uptake by vegetation. The higher NO_3^- levels in C+ than C- plots, suggests that sulphur addition is actually stimulating nitrification, which may be possible as the sulphur had not yet lowered the soil pH in C plots. There were no significant treatment effects on NO_3^- or DON concentrations.

Overall, the effects of sulphur on soil properties six months after application were smaller than those observed at the end of the experiment.

6.4.2.3 Soil at the end of the experiment

Sulphur application successfully reduced soil pH and target soil pHs were reached two years after sulphur application. That sulphur treated plots had significantly more NH_4^+ in the soil than non-treated plots, indicates that either mineralisation rate was stimulated, or the more likely explanation is that nitrification was lessened, thus allowing the NH_4^+ pool of mineral N to accumulate. The latter explanation is supported by the NO_3^- data, which shows that NO_3^- is lower in sulphur treated plots, suggesting that nitrification was impeded by the pH reduction in sulphur treated plots.

The acidic conditions created by sulphur addition caused interesting changes in the ability of the SMB to utilise low molecular weight (LMW) DON in the form of a mixture of amino acids. The acidity greatly reduced the rate of mineralisation of amino acid-C (Figure 6-7iv). The possible explanations for this include:

1) a reduced population of amino acid metabolising soil microbes;
2) reduced general metabolic activity of the SMB;
3) a stress resulting in reduced functioning of amino acid cell membrane transporters;
4) a diversion of amino acids from catabolic pathways to structural use.

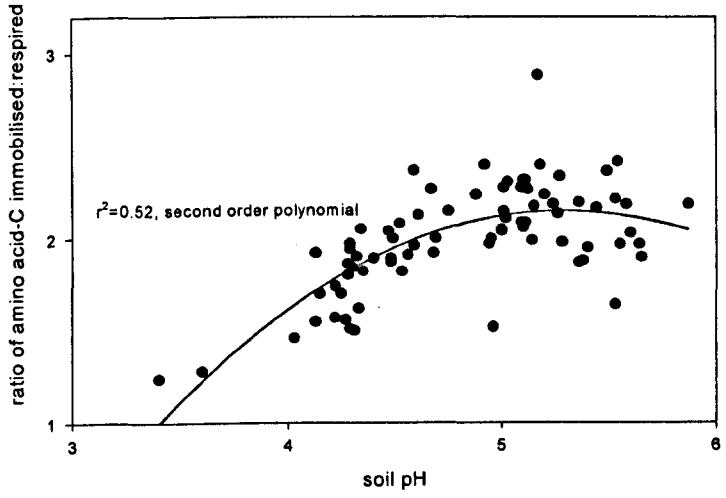
The data obtained at the end of the 48 h incubation on the proportions of amino acid-C respired (Figure 6-7x) or used in biomass (Figure 6-7xi) indicate that the acidity required the microbes to use more amino acid-C in catabolism and less in biomass. This does not support point 4 above.

The mineralisation rate in this experiment, measured as $1/t_{15}$ (h^{-1}) shows that 15% of amino acid-C is usually respired within 2 to 7 h (Figure 6-8a). At this point, there has been insufficient time for microbes to reproduce to any great extent, so this measure is an indication of both initial SMB biomass and activity. By the time the incubation is finished at 48 h there has been sufficient time for the SMB to have reproduced and numbers increase greatly. It is therefore impossible with the available data to discount the possibilities of points 1 and 2 above. However, that the SMB were able to use less of the amino acid-C for biomass and had to use more in catabolism in acidic conditions, indicates that the acidity induced some form of stress, lending some support to point 3 above.

Soil sampled two years after sulphur application, at the end of the experiment, provides samples with a range of pHs. Data in Figure 6-8 demonstrates a strong relationship between soil pH and the rate of mineralisation of amino acid-C, the rate slowing down with increasing acidity. If one accepts that microbes are experiencing the least stress, or optimal conditions, when the ratio of amino acid-C immobilised : respired is maximum, then differentiation of the second order polynomial regression equation in Figure 6-11 shows the optimal $pH_{(1:10\ 1M\ KCl)}$ to be 5.30. This may seem somewhat low, although the pH determination was in a salt solution, which may give values approximately 0.5 (Rowell, 1994) to 1 (Thomas, 1996) unit lower than those obtained in water. No values have been found in the current literature against which to compare this finding. A wider range of soil pHs would be of benefit in defining this relationship and fitting a model.

Figure 6-11 The pH of soil samples taken at the end of the experiment versus the ratio of amino acid-C immobilised : respired.

[excluding 2 outliers, not shown, $pH_{(1:10\ 1M\ KCl)}$.]



6.4.2.4 Porous cup extracted soil water

Porous ceramic cups require some independent measure of drainage volume in order to calculate total N leached. This is usually performed using a model with climatic data inputs and estimates of evapotranspiration. However, estimating the flux of water through soil is notoriously difficult (Addiscott et al., 1991) and errors in calculating water flux could influence the results considerably. The estimate of NO_3^- loss can be greatly affected by the calculated date of start of drainage, when soil moisture deficit is zero (Lord and Shepherd, 1993; Webster et al., 1993). As the volume of water leached will be the same on the plots of this experiment, the main factor of interest here was how the concentration of nitrogen solutes varied between treatments. It was therefore not deemed necessary to estimate the volume of drainage.

6.4.2.4.1 pH

The sulphur treated plots had a significantly lower pH than those that were untreated, throughout the period October 1999 to April 2000. G- and C- plots had similar pHs but sulphur application had a much greater effect on the pH of the soil solution in C+ plots than G+ plots. Presumably, this was due to a higher degree of sulphur oxidation, as a result of mixing from cultivation. Also, the soil water in the grass plots

may percolate through larger pore spaces, such as worm burrows or fissures between soil peds, whereas the disturbance on the cereal plots caused by cultivation may result in the soil water percolating through smaller pore spaces, thus 'picking up' more of the free H^+ ions in the soil generated through addition of sulphur.

The ratio of water to soil in a suspension has the effect of increasing pH as the ratio increases, but the buffering capacity of soil maintains quite a stable pH over a wide range of dilutions (Thomas, 1996). The slight variation in pH within non-sulphur treated grass and cereal plots may be due to variation in the dilution effect of varying soil water contents and varying salt concentrations (Thomas, 1996). However, the level of precipitation during the preceding week to sampling date did not show any link with variation in the pH of non-sulphur treated grass or cereal plots (Figure 6-9i and Figure 6-10b).

6.4.2.4.2 NH_4^+

In untreated plots, grass plots generally showed higher levels of NH_4^+ in soil solutions than cereal plots, (although not significantly), especially during November-December 1999. This may be caused either by increased mineralisation of organic nitrogen or by decreased nitrification causing the NH_4^+ pool to accumulate. Sulphur increased NH_4^+ levels from October 1999 onwards, to a much greater degree in cereal plots than grass. This meant that sulphur reversed the trend evident in untreated plots; NH_4^+ levels were greater in G- than C- plots and greater in C+ than G+ plots. This may be due to the pH effect of sulphur being much greater in cereal plots than in grass, having a greater impact on nitrification and so causing the NH_4^+ pool to accumulate more. Figure 6-12a shows NH_4^+-N concentrations of all individual samples at all dates. This demonstrates that NH_4^+ levels were generally low, presumably as the NH_4^+ pool was kept at low levels due to nitrification. As pH levels fell below c. 4.5 there was an increase in the incidence of higher NH_4^+ levels, presumably as the pool was allowed to accumulate due to inhibited nitrification. NH_4^+ levels were highest during October to December 1999 (Figure 6-9ii), although treatment averages were still under 2 mg N l^{-1}, and low during January to March 1999 and January to March 2000. The high levels in autumn 1999 may have originated from mineralisation of crop residues (cereal plots) and senescing sward (grass plots). Comparison with temperature data (Figure 6-10a) did not indicate that high NH_4^+ levels at this time were due to a stimulation of mineralisation due to temperature, as the temperature was decreasing from October to December, whereas ammonium levels were increasing.

It should be noted that changes in the concentration of NH_4^+ levels in soil water is only indicative of changes in the magnitude of NH_4^+ levels in the bulk soil. NH_4^+ levels in the bulk soil will be much higher than levels in the soil water due to adsorption of NH_4^+ on cation exchange sites on soil particles (e.g. compare NH_4^+ levels in soil, Figure 6-7ii, and porous cup water, Figure 6-9ii).

6.4.2.4.3 NO_3^-

Over the sampling periods that yielded soil water samples, the 'cereal' plots consisted of bare soil, which may account for the trend that on nearly every sampling date the cereal plots contained significantly more NO_3^- in the soil water than the grass plots. This may be due to both the fact that the grass will have been taking up some NO_3^-, even though it was not growing very much, and that the cereal strips were ploughed and cultivated, which stimulates oxidative processes. NO_3^- levels during January to March 1999 were very high in cereal plots, with concentrations up to 48 mg NO_3^--N l^{-1} in individual samples. This exceeds the EU limit on drinking water of 11 mg NO_3^--N l^{-1} greatly, and it is notable that this was in the absence of fertiliser-N (organic or inorganic). While it is likely that NO_3^- in drainage water leaving a field will be diluted or denitrified between drain and stream or soil and aquifer, it is useful to take the EU limit as an exceedance target for drainage waters (Goulding et al., 2000).

The fall in NO_3^- levels in grass plots that occurred from early February in G+ plots and from late February in G- plots was probably due to commencement of grass growth as solar energy and temperature became sufficient. In March the NO_3^- levels in the C- plots began to increase, as temperatures increased and there was no vegetation to take up generated NO_3^-.

In both grass and cereal plots, differences between sulphur and non-sulphur treated plots became more marked over time, as the differences in pH became more established. From November 1999, sulphur always had the effect of reducing NO_3^- concentrations in soil water; this effect was substantial over winter and spring in grass plots, and in cereal plots the effect was minimal during winter, but became more substantial in spring.

Figure 6-12b demonstrates the effect of pH on NO_3^- concentration of soil water. It shows that in all soil water samples from all treatments over all dates there is a cut off point at c. pH 5. Below this, NO_3^--N levels are almost always under 40 mg l^{-1}, whereas above this, NO_3^--N levels can approach 100 mg l^{-1}.

6.4.2.4.4 DON

DON in soil water of grass plots was significantly lower than in cereal plots from January to March 1999. Possible explanations of this include:

1) Uptake of DON by grass sward, although this is unlikely because the sward was not growing at this stage.

2) Higher DON formation in cereal plots than in grass plots. This may be generated from the breakdown of the inverted sward when ploughed in during November 1998. There may have also been higher microbial degradation of soil organic N, which may have been stimulated by the dead organic matter from the sward.

3) Faster mineralisation of DON in grass plots resulting in a lower DON pool. This possibility is not supported by the rate of amino acid mineralisation measured in soil at the end of the experiment, where amino acid mineralisation rate was not found to be significantly different between untreated grass and cereal plots (Figure 6-7iv).

From October 1999, soil acidification by sulphur addition did generally reduce DON levels in soil water (often significantly), usually to a slightly greater degree in grass than in cereal plots. Although Figure 6-12c shows that DON content of soil water is not strongly determined by pH, it does have a certain controlling effect. Above c. pH 5 high DON levels (above 20 mg N l^{-1}) are more frequent, and extremely high levels are possible (>60 mg N l^{-1}), whereas below pH 5, DON levels above 20 mg N l^{-1} are unusual.

It should be noted that as DON contains molecules that adsorb and absorb strongly, ceramic suction cups may not be a suitable method for measuring DON (Murphy et al., 2000).

6.4.3 General discussion

These results show that NO_3^- levels leached from this soil were often over the statutory limit for drinking water of 11.3 mg NO_3^--N l^{-1} in all treatments except G+. An acidic soil (here $pH_{(1:10\ 1M\ KCl)}$ 4.3) may successfully lower NO_3^- leachate concentrations to below 11 mg NO_3^--N l^{-1} from grassland, which at a higher pH (here $pH_{(1:10\ 1M\ KCl)}$ 5.1) would be up to twice this level for significant periods over the winter. Although increased acidity lessened NO_3^- leaching on arable land, substantially in the spring of 2000, it did not bring NO_3^- leaching levels below the statutory limit. The ameliorative effect of soil acidity on NO_3^- leaching may be beneficial on bare arable

land in the spring, although a longer period of monitoring than was possible here would be needed to confirm this.

Crop growth was greatly compromised in the acidified plots, with grass growth becoming patchy, with bare patches of ground, and poor cereal growth with more weeds. This could have been a direct consequence of the acidity and resultant raised soluble aluminium levels. There is also the possibility of toxic effects of intermediate products of sulphur oxidation such as $S_2O_3^{2-}$ (Tabatabai, 1994). Although these intermediate products rarely persist for long, as oxidation proceeds to sulphate rapidly, the application rates of sulphur were so high that there may have been the possibility of toxic effects.

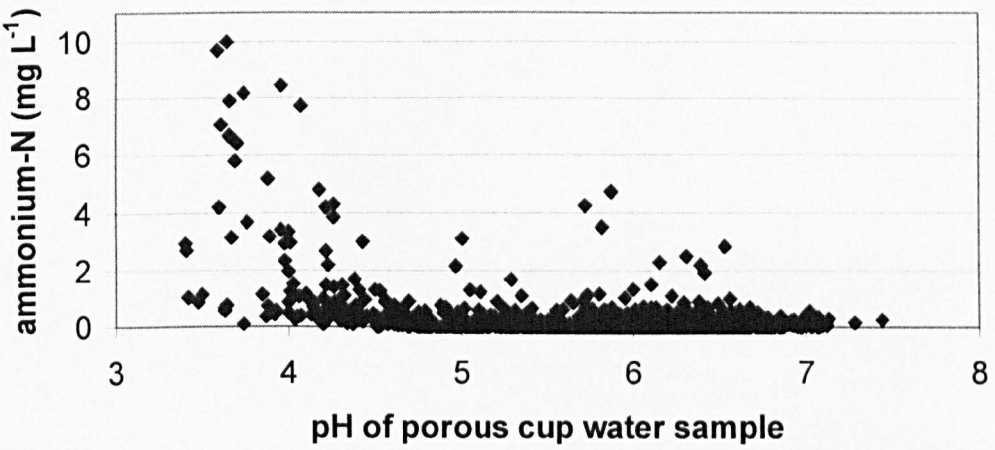
It must be stressed that this lowering of NO_3^- loss through reduced soil pH will have a serious impact on crop yield or pasture carrying capacity. Ridley et al. (2001) point out that decisions on liming pastures should consider the trade-off between degrading the soil resource through acidification of individual fields (compromising the future ability to grow certain species) and the off-site impacts of degrading water resources.

Although it was known that nitrification rates are reduced at low pH, it was not known at what pH nitrification rates become insufficient to keep pace with the availability of NH_4^+ produced by the mineralisation process. These results indicate that the acidity levels induced by sulphur treatment created a bottleneck at the nitrification stage, causing the NH_4^+ pool to increase. Further work on a pH range in different soils would be beneficial to elucidate the pH at which nitrification can no longer keep pace with the availability of NH_4^+ supply, and the pH necessary to lower NO_3^- levels to acceptable levels in a range of agricultural systems.

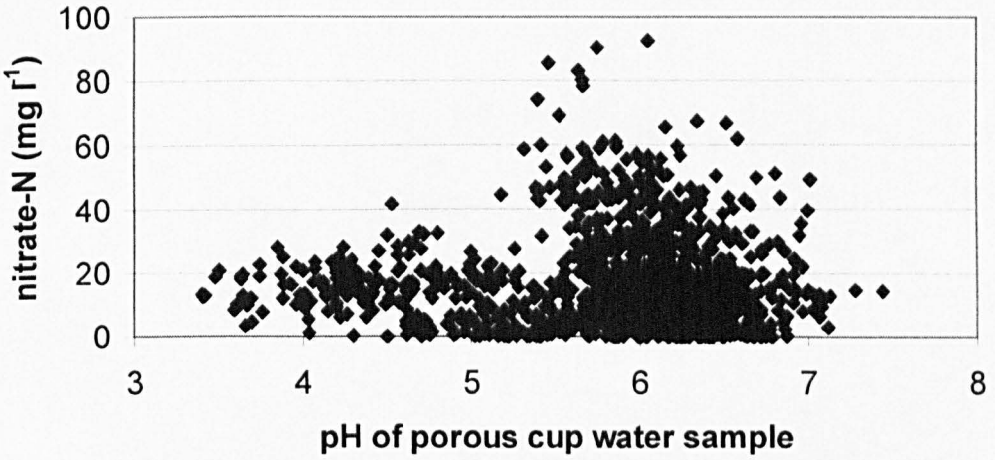
If further research corroborates the findings of this work, that by reducing soil pH (by acidification or allowing natural acidification and precluding lime application) NO_3^- leaching can be ameliorated, there will be a need for more research into crop species and varieties that are tolerant of soil acidity. In areas that are especially prone to NO_3^- contamination of surface or ground waters, this may be a useful management tool. Future research should also include an examination of the impacts of heavy metals entering water bodies or courses. If, as Figure 6-12 suggests, soil water pHs would only need to be prevented from rising above pH 5 to gain NO_3^- leaching diminution, this may mean that careful soil pH management could ameliorate NO_3^- leaching without resulting in serious heavy metal contamination of water resources.

Figure 6-12 pH of porous cup water samples versus a) NH_4^+-N, b) NO_3^--N and c) dissolved organic N over all samples at all dates.

(a)



(b)



(c)

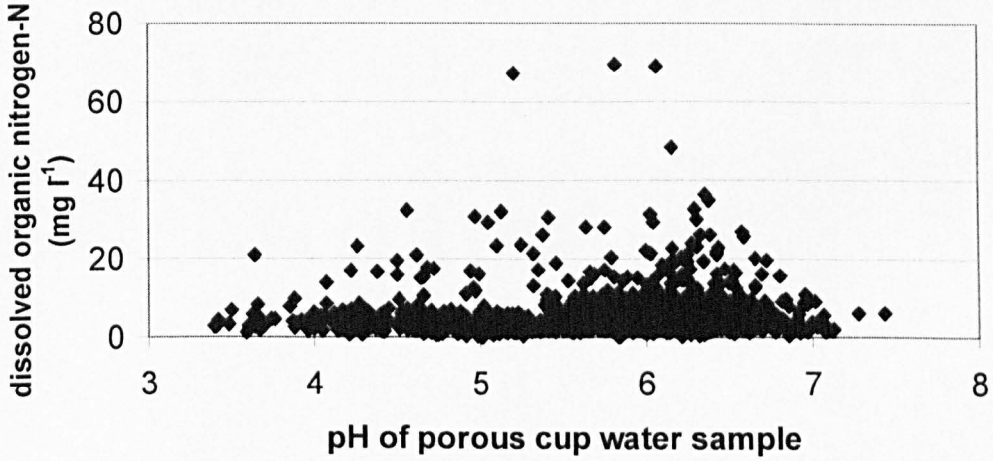


Table 6-9 Main conclusions of experimental work of Chapter 6

- Sulphur addition took around 6 months to lower the soil pH significantly and impact on the nitrogen dynamics. It decreased the pH of cultivated cereal plots to a much greater degree than it did on grass plots.

- NH_4^+ levels in soil solution were always low, with values less than 2 mg NH_4^+-N l^{-1}.
    - NH_4^+-N concentrations were significantly higher in the soil of the grass plots versus the cereal plots, and the lower soil pH resulted in significantly higher NH_4^+ levels.

- NO_3^--N concentration of the porous cup extracted water was significantly higher in cereal than grass plots. The lower pH always had the effect of lowering NO_3^- concentrations in soil water; this effect was substantial over winter and spring in grass plots, and in cereal plots the effect was minimal during winter, but became more substantial in spring.

- Data on NO_3^--N and NH_4^+-N concentrations suggest that sulphur treatment induced pH decrease resulted in lowering of nitrification rates, allowing the NH_4^+ pool to accumulate.

- DON in the soil water of grass plots was consistently significantly lower than in cereal plots.
    - The lower soil pH significantly lowered DON levels in soil water, usually to a slightly greater degree in grass than in cereal plots.

- In soil samples taken 2 years after sulphur application, the rate of amino acid-C mineralisation was significantly, positively and linearly related to the pH of the soil sample.
    - Acidity increased the proportion of added amino acid-C used in respiration and decreased the proportion of added amino acid-C immobilised: these proportions were related to the pH of the soil sample, and can be described by quadratic regression lines.
    - Optimal conditions for amino acid use by the soil microbial biomass, when the ratio of amino acid-C immobilised : respired was maximum, was calculated to be $pH_{(1:10\ 1M\ KCl)}$ 5.30.

- It may be possible to use careful soil pH management as a tool to control NO_3^- leaching, without compromising the quality of drainage water. This may be more effective on grassland than on arable crops.

Chapter 7 The impact of soil pH on microbially mediated carbon and nitrogen dynamics

7.1 Introduction

The process of mineralisation is often regarded as being insensitive to soil pH, (Haynes, 1986; Curtin et al., 1998) and nitrification is usually considered to be highly pH sensitive (Strayer et al., 1981; Paul and Clark, 1989). However, it is known that low soil pH has the potential to retard organic matter decomposition (Chapters 5 and 6 of this thesis) and that nitrification can occur at low pHs (Schmidt, 1982, Haynes 1986, Chapters 4 and 6 of this thesis). Despite this confusion, soil pH is recognised as a dominant factor governing microbial turnover of organic matter Adams and Adams, 1983). In comparison, differences in microbial community structure, water content and concentrations of toxic ions such as Al or Mn may be of secondary importance, although Adams and Adams (1983) conclude that they contribute to increased variability in the proportion of the organic matter being degraded in soils of a similar pH.

Where soils in an area develop with different pHs (as those examined in chapter 5), the discrepancies in pHs are a result of inconsistency in physical, biological and chemical processes. Much of this variation will be due to spatial differences in the major factors of soil formation; climate, parent material, topography, vegetation and soil organisms, and the time over which the soil has been forming (Jenny, 1961). These factors integrate to form differing soil textures, mineralogy, organic matter contents, hydrological characteristics and soil type, which affect the macro- and microbial- populations present in the soil. It is therefore difficult to elucidate the effect of pH on soils of different natural pHs, due to confounding influences of many interacting factors. Most experiments in which pH is altered by liming (e.g. Nyborg and Hoyt, 1978; Smolander et al., 1994; Wheeler et al., 1997; Curtin et al., 1998) or acid addition (e.g. Strayer et al., 1981; Dähne et al., 1995) are relatively short term experiments, and so the soil microbial biomass (SMB) may not have had time to adjust to the radically altered conditions. Kaiser et al. (1992) found relationships at a low level of significance between pH and various soil microbial properties, although most soils examined were from a small range around neutrality. A narrow pH range in limed plots studied by Adams and Adams (1983) may also have accounted for the lack of significant relationship of soil pH with basal respiration rate. Lime application also has the consequence of solubilising organic matter so microbial responses will be due to that flush, rather than simply a different pH.

The aim of these experiments was to examine relationships between soil pH and the rate of nitrogen and carbon cycling, especially dissolved organic nitrogen (DON), using soils from long term experiments, in order to clarify contradictory evidence and fill gaps in current knowledge.

7.1.1 Measurement of SMB carbon and nitrogen content

The fumigation-extraction (FE) method (Brookes et al., 1985) was used to determine SMB C and N. This has advantages over the fumigation-incubation (FI) method (Jenkinson and Powlson 1976; Paul and Clark, 1989), which is not suitable for acid soils (Vance et al., 1987; Jenkinson and Wilson 1988), and is subject to controversy over what control method to use (Brookes et al., 1985). The principle of the FE method involves lysing microbial cells during fumigation with chloroform ($CHCl_3$) and measuring the 'flush' of C and N released from the cytoplasm, by subsequently extracting the soil and measuring dissolved organic C (DOC) and N while deducting the DOC and N found in non-fumigated soil (control). The N measurement can be either via the detection of ninhydrin–positive compounds (NPC), which includes amino acids, NH_4^+, peptides and proteins (Amato and Ladd, 1988 and 1994; Joergensen and Brookes, 1990) or detection of total dissolved N (TDN) (Joergensen and Brookes, 1990) by persulfate oxidation (Cabrera and Beare, 1993). Both methods were used here in order to compare outcomes. The flush of C or N is divided by a constant (K_{EC} and K_{EN}) to estimate the microbial C and N, correcting for the extractability of the compounds in question.

Originally KCl was used as a soil extractant in the FE method (Amato and Ladd 1988), but was modified by Joergensen and Brookes (1990), who used K_2SO_4 as an extractant. This requires a modification of the ninhydrin assay used by Amato and Ladd (1988), but means that SMB C and N can be measured on the same extracts (chloride from KCl interferes with the UV persulphate oxidation method used to measure DOC).

7.1.1.1 Arginine mineralisation

Arginine ammonification has been used by a number of researchers to indicate microbial activity potentials in soils (Alef and Kleiner, 1986, 1987; Alef et al., 1988; Lin and Brookes 1999b). Ammonification starts immediately after the addition of arginine and is typically linear for 1 to 6 h (Alef and Kleiner, 1986). The physiological status and number of microbes have been shown to remain stable during the assay, indicating that 'resting' bacterial cells do not ammonify arginine.

Arginine is a basic amino acid with three amino groups and is catabolised by most, if not all, heterotrophic soil bacteria (Alef and Kleiner, 1986). It can be used as a primary C and N source via at least four major biochemical assimilation pathways (Lin and Brookes, 1999b):

1. the arginine-urease or arginase-urea amidolyase pathway

2. the arginine transmidinase pathway

3. the arginine deaminase pathway

4. the arginine decarboxylase pathway.

With the exception of pathway no. 2, NH_4^+ is an end product which is commonly excreted and which can be quickly nitrified in soil.

Lin and Brookes (1999b) added 0.3 mg arginine g^{-1} soil, and found that the NO_3^-, NH_4^+ and total inorganic N produced after 2 h were closely related to soil ATP content, biomass C (FE method) and CO_2 evolution (SIR). Alef et al. (1988) also found highly significant positive correlations between arginine ammonification and biomass C, heat output, soil ATP content, soil protease activity and soil organic matter content. Lin and Brookes (1999b) found the method to be little, if at all improved by including NO_3^- measurement rather than NH_4^+ alone because relatively little NO_3^- was produced compared to background NO_3^-. They concluded that arginine mineralisation appears to be a fast and rapid method for estimating soil microbial biomass.

7.1.2 Soils

The three soils examined in this chapter are described in Table 7-1 below. The plots at the experimental field sites provided an excellent resource on which to examine the effect of long-term differences in soil pH on various microbial processes without the confounding influence of varying soil, site and climate conditions. Although soil pH will not have been completely static since the establishment of the plots 40 years ago, the microbial communities have had a long time to adjust to the prevailing conditions. The plots were expected to have different pHs and provide a wide, relatively stable range of pH. However, the exact pHs were measured and results were related to the measured pHs.

The plots are subsequently referred to as RO, RL, RM, RH and WO, WL, WM, WH for zero, low, medium and high pH treatments in the Rothamsted and Woburn soils respectively, and S1-7 for the SAC soil treatments, as specified in Table 7-1.

Table 7-1 Experimental Plots Examined.

Site	Rothamsted Experimental Station, Harpenden, Herts., UK.	Woburn Experimental Farm, Husborne Crawley, Bedford, UK.	Scottish Agricultural College (SAC), Craibstone Estate, Bucksburn, Aberdeen, UK.
Referred to as	Rothamsted (R)	Woburn (W)	SAC (S)
Field plots	Sawyers field section I	Stackyard field section C	Woodlands Beds, K, L, M and N.
Soil type and cited characteristics	Silty clay loam (coarse sand 14%, fine sand, 40%, silt 23%, clay 21%; Bolton, 1977) 23% clay (K.Goulding, 2001 pers comm.)	sandy loam (coarse sand 50%, fine sand, 27%, silt 7%, clay 11%; Bolton, 1977) 10% clay (K.Goulding, 2001 pers comm.)	Sandy loam, free draining. Organic C, 6.5%; total N, 0.32%; CEC 134 meq kg^{-1}. (Meharg and Killham,1990; Mackay and Watson, 1990).
Soil series	Batcombe series derived from drift and loess over clay with flints (Bolton, 1977).	Cottenham series developed on drift parent material over Lower Greensand (Bolton, 1977).	Countesswells series (Meharg and Killham, 1990) and association (Mackay and Watson, 1990).
Vegetation	Louisa Red Fescue *Festuca rubra* sown at 50 kg ha^{-1} on 3.10.97.	Italian ryegrass, *Lolium multiflorum* 'Atalja' sown at 30 kg ha^{-1} on 9.9.96.	Samples taken from 2nd year grass from 8 year rotation[2].
Treatments	Amount of lime applied 1962-87 (t ha^{-1})[1] 0 none L 15.0 M 24.5 H 52.5	Amount of lime applied 1962-78 (t ha^{-1})[1] 0 none L 9.0 M 25.5 H 45.5	Established 1961 by addition of ferric sulphate[3] 1 pH 4.5 2 pH 5 3 pH 5.5 4 pH 6 5 pH 6.5 6 pH 7 7 pH 7.5
Design	2 randomised blocks of 4x4 plots split into 2, making 32 plots, 6.0m by 16.1 m (0.0097 ha) each.	2 randomised blocks of 4 x 4 plots split into 2, making 32 plots, 6.0 m by 16.1 m (0.0097 ha) each	Each year of the 8 course rotation is represented in each pH treatment, making 56 plots.

[1]initially 4 rates of lime (0, 5, 10 and 20 t $CaCO_3$ ha^{-1} at Rothamsted and 0, 4.6, 10.9, 17.3 t $CaCO_3$ ha^{-1} at Woburn) were applied in 1962 and 1963. Further smaller amounts were applied in 1978, 1982 and 1983. Local soft cretaceous limestone (chalk) was used (100%< 6mm, 60%<0.35mm, 25% < 0.15mm) in the original experiment (Bolton, 1977). Both of the Rothamsted and Woburn experimental plots were split to include P and S application at fertiliser rates. All areas of plots sampled in this study had not received any S or P.

[2] 8 year rotation of: winter wheat, potatoes, barley, turnips, spring oats (undersown), 1st yr grass (hay) (plus NPK at 70:30:50 kg ha^{-1}), 2nd yr grass (pasture, topped, not grazed, no fertiliser), 3rd yr grass (pasture, topped, not grazed, no fertiliser).

[3]Plots are sampled regularly and calcium carbonate added if required to maintain the stated pH, very occasionally ferric sulphate has been added to the very low pH plots to maintain the stated values.

7.1.3 Long term liming experiments at Rothamsted and Woburn

The pH plots at Rothamsted and Woburn were used by Bolton (1977) to construct a mechanistic model to predict lime losses. The pH treatments had the intended effect of increasing the soil pH, but after the first year, soil pH decreased in the 5 t ha^{-1} treatment on both sites. However, the 20 t ha^{-1} application rate caused the soil pH to increase for 6 years at Rothamsted and 3 years at Woburn. Exchangeable Ca decreased linearly in all plots of both experiments from the first year, the rate depending on the average pH. Bolton predicted that Ca loss was a function of leaching rate, anion (NO_3^-, SO_4^{2-}, Cl^-) inputs in fertilisers and from the atmosphere, temperature, CO_2 partial pressure and soil pH.

The model of Goulding et al. (1989) takes lime loss to be a function of soil type, crop grown, through drainage, soil pH and the amount and form of fertiliser N applied. The impacts of atmospheric deposition, and SO_4^{2-} and Cl^- in fertilisers were assumed to be negligible compared to the above factors on fertilised agricultural soils.

Liming resulted in a linear increase in pH in the soils. Regression equations provided by Goulding et al. (1989) for 12 years after lime application are:

Rothamsted: $\text{Change in pH} = 0.109\ (\text{lime added as t ha}^{-1}\ CaCO_3) - 0.690$ $(r=0.99, p<0.001)$

Woburn: $\text{Change in pH} = 0.110\ (\text{lime added as t ha}^{-1}\ CaCO_3) - 1.160$ $(r=0.98, p<0.001)$

The gradients of the above regression lines are the same, but the constant is different. Goulding et al. (1989) suggest that the slope of the line will be affected by the site management (e.g. crop grown and fertiliser applied), and the intercept might reflect site factors (rainfall and soil type).

7.1.4 Objectives

The experiments reported in this chapter sought to examine the impact of pH on various aspects of microbially mediated carbon and nitrogen (especially DON) cycling, avoiding confounding influences of site and soil specific factors. This was achieved by thoroughly examining one soil type in one location in which the pH had been altered over a long period of time. Lime had not recently been added, avoiding the influence of a recently solubilised pool of organic C or N. Three very different soil types were examined in this way, providing the opportunity of identifying the consistency of the effects of pH in different soil types.

Specifically, relationships between measurements of soil microbial biomass C and N and microbial activity (amino acid, arginine, glucose, urea, and net indigenous

mineralisation and basal respiration rates) were examined. Analysis of how these parameters were linked with soil characteristics (soil pH, aluminium, calcium, total C and N, soil C:N, NH_4^+, NO_3^-, dissolved organic C and N, soil moisture) was also performed with a focus on soil pH.

7.2 Methods

7.2.1 Sampling techniques

The soils were sampled as described in Table 7-2 below.

Table 7-2 Sampling Regime.

Soil	Rothamsted	Woburn	SAC.
Sampling date	25.3.99	25.3.99	11.6.99
Sampling regime	6 cores (5cm diameter, 0-10cm depth) were taken in each plot. 2 plots were sampled for each of the 4 treatments.	6 cores (5cm diameter, 0-10cm depth) were taken in each plot. 2 plots were sampled for each of the 4 treatments.	6 cores (3cm diameter, 0-7.5cm depth) were taken in each plot. 1 plot was sampled for each of the 7 treatments.
Sampling performed by	The author, Dr. D. Jones with assistance from Dr. K. Goulding	The author, Dr. D. Jones with assistance from Dr. K. Goulding	Dr. T. Edwards, (samples sent by post)

7.2.2 Chemical analyses

The following soil characteristics were analysed using methods as described in chapter 3: pH (1:1 0.01M $CaCl_2$), total C, total N, C:N, moisture content, amino acid mineralisation, NH_4^+, NO_3^-, DON. In addition, the following were measured:

7.2.2.1 Glucose and urea mineralisation

Urea and glucose substrate induced respiration were determined by incubation with uniformly labelled ^{14}C urea and glucose substrates (ICN Pharmaceuticals Ltd.) in tubes containing sodium hydroxide CO_2 traps (1 ml). 0.5 g of field moist soil was weighed into a plastic tube and 50 µ l of substrate was added; substrate concentrations were 100 mM for glucose and 50 mM for urea. Sealed tubes were incubated for 3 h, and the NaOH traps measured for $^{14}CO_2$ by adding 1 ml samples of NaOH to 4ml Wallac Optiphase 3 HiSafe scintillation fluid and counting in a scintillation counter (Wallac 1409 Liquid Scintillation Counter, EG&G Ltd, Milton Keynes, UK).

7.2.2.2 Basal respiration

Basal respiration was measured on a CIRAS-SC version 2.4 soil respirometer (PP Systems, Hitchin, Herts., UK). 15 g of moist soil was weighed into a tube, left to settle for 2 h and then monitored at 20°C until CO_2 output was stable (96 minutes). Measurements were recorded with the units pmol CO_2 s^{-1} and then divided by the dry weight of soil to give pmol CO_2 $g^{-1}s^{-1}$.

7.2.2.3 DOC, SMB C and N

Soil microbial biomass C and N were determined using the chloroform-fumigation-extraction method as described by Horwath and Paul (1994).

Field moist samples (10 g dry soil weight) were fumigated for 5 days in ethanol free chloroform with anti-bumping granules in a desiccator with wet towels to prevent desiccation of soils, as described by Horwath and Paul (1994). The desiccator was evacuated until the chloroform boiled vigorously, the air was let back in, and this was repeated three more times. On the fourth time chloroform was boiled for 2 min. Samples were left for 5 days in the dark at 25°C, after which the desiccator was evacuated in a fume cupboard for 3 minutes, 8 times letting air in after each evacuation. Duplicate samples (5 g dry soil weight) were weighed and left unfumigated in a desiccator next to the fumigating samples for the 5 d incubation period. Fumigated and unfumigated samples were then extracted (1:5 0.5 M K_2SO_4, shaken on a reciprocal shaker 180 strokes min^{-1} for 1 h, filtered through Whatman no. 1) and the filtrate was frozen until analysed. DOC was measured on a Skalar autoanalyser using the UV persulfate digestion method (appendix 2). NPCs were measured according to Joergensen and Brookes (1990) and TDN was measured using the persulflate digestion method (chapter 3).

Microbial biomass C was calculated using the formula:

$$Biomass\ C = \frac{(C_f - C_{uf})}{K_{EC}}$$

where C_f is the soluble organic C (SOC) in the fumigated soil, C_{uf} is the SOC in the unfumigated soil, and K_{EC} is the proportion of the microbial C that is extracted from the soil (Horwath and Paul, 1994). K_{EC} will depend on the physical and chemical properties of the soil, but in this study was assumed to be 0.45 (Joergensen and Brookes, 1990).

Microbial biomass N was calculated using the formula:

$$Biomass\ N = \frac{(N_f - N_{uf})}{K_{EN}}$$

where N_f is N in the fumigated soil, N_{uf} is N in the unfumigated soil, and K_{EN} is the proportion of the microbial N that is extracted from the soil (Horwath and Paul, 1994). K_{EN} was assumed to be 0.45 (Joergensen and Brookes, 1990) for TDN in K_2SO_4 extracts, and 0.154 (reciprocal of 6.5, Sparling and Zhu, 1993) for NPC.

DOC content was obtained from the results of the unfumigated samples.

7.2.2.4 Net mineralisation and change in DON

Net nitrification, ammonification and change in DON were determined using an aerobic incubation method, as described by Hart et al. (1994).

Baseline conditions were obtained from field-moist samples (sieved past 4mm mesh) which were extracted (5 g soil:25 ml 0.5 M K_2SO_4) and measured for NO_3^-, NH_4^+ and SON (Chapter 3). Duplicate samples (10 g) were incubated for 30 days at 24°C in thin gas permeable plastic bags, and then extracted in 50 ml 0.5 M K_2SO_4 and the extracts measured for NO_3^-, NH_4^+ and SON.

Net nitrification, ammonification, mineralisation and change in SON were calculated by subtracting the initial from the final levels of NO_3^-, NH_4^+, total mineral nitrogen (NH_4^+ plus NO_3^-) and SON respectively.

7.2.2.5 Aluminium

Exchangeable aluminium was determined using the pyrocatechol violet colorimetric method (Appendix 3, Kerven et al., 1989) on K_2SO_4 extracts (5g soil : 25 ml 0.5 M K_2SO_4).

7.2.2.6 Calcium

Exchangeable calcium was determined from K_2SO_4 extracts (5g soil : 25 ml 0.5 M K_2SO_4) which were measured on an atomic adsorption spectrometer.

7.2.2.7 Arginine mineralisation

A trial experiment was performed using one sample from the pH extremes of each of the three soil types (6 samples) plus 2 blanks. NH_4^+ and NO_3^- were measured in sample extracts (1 g:5 ml 0.5 M K_2SO_4) at the start of the experiment, and paired samples (1g) were incubated for 24h at 18°C with 100 µl of 25 mM ^{14}C-arginine (Amersham Co., L-arginine hydrochloride, $C_6H_{14}N_4O_2.HCl$, FW 210.7, labelled at

0.0453 μCi ml^{-1}). This amounted to an addition of 0.52 mg arginine g^{-1}soil (25 mM arginine stock solution was made up as follows: 0.078g arginine plus 13.6μl labelled stock solution, made up to 15ml with distilled water). NaOH traps were changed at 1, 3, 6, 24 h intervals and the $^{14}CO_2$ counted (as in section 7.2.2.1). After 24 h incubation, soil was extracted using 5 ml 0.5 M K_2SO_4, shaken for 30 min and then centrifuged at 14,000 g for 10 min. A 1 ml aliquot was taken and measured for ^{14}C content. NH_4^+ and NO_3^- were measured on the remaining extractant. Balances were calculated for C and N.

As the results showed that this concentration of arginine gave detectable levels of NH_4^+, NO_3^- and CO_2 evolution upon mineralisation, and that there was a difference between treatments (see results section 7.3.1.7), a more thorough experiment was designed, using a longer incubation time in the light of the results obtained in this trial.

The full experiment consisted of a soil incubation with arginine addition as described above. Three soil core samples for three treatments of the Rothamsted and Woburn soils were selected randomly, and three (pseudoreplicates, see section 7.2.3) of the SAC samples were taken from plots 1, 5 and 7, making 27 samples in all.

Control samples of each soil (1g) were monitored at the beginning and end of the experiment for NH_4^+ and NO_3^-. Times of sampling were 1, 3, 6, 24.5, 48, 192 (8 days) and 404 (nearly 17 days) h.

Table 7-3 Sampling regime in arginine mineralisation experiment.

Time (h)	Sampling activity
0	27 Controls extracted Arginine added to all tubes.
1	27 samples destructively sampled, NaOH traps taken out and soils extracted
3	27 samples destructively sampled, NaOH traps taken out and soils extracted
6	27 samples destructively sampled, NaOH traps taken out and soils extracted
24.5	27 samples destructively sampled, NaOH traps taken out and soils extracted
48	27 samples destructively sampled, NaOH traps taken out and soils extracted Changed NaOH traps in 192 and 404 h samples, to prevent saturation.
192	27 samples destructively sampled, NaOH traps taken out and soils extracted Changed NaOH traps in 404h and end control samples
404	27 samples and 27 control samples destructively sampled, NaOH traps taken out and soils extracted

The extraction was performed by adding 5 ml 0.5 M K_2SO_4 to the 1 g soil in the tube, shaking on ice for 20 min and centrifuging at 14,000 g for 10 min. Supernatants were frozen and subsequently analysed for NH_4^+, NO_3^- and ^{14}C.

7.2.2.8 PLFA analysis

Analysis of the phospholipid fatty acid (PLFA) fraction of microbial cell membranes allows some characterisation of the microbial populations, as groups of microbes contain certain signature patterns of membrane lipids (Zelles, 1999). Frostegård et al. (1993) showed that addition of lime and wood ash to coniferous forest soils caused changes in the PLFA pattern, indicating that the increased pH caused a shift in the bacterial community to more Gram-negative and fewer Gram-positive bacteria, while the amount of fungi were unaffected (although the species composition changed). There was also an indication of an increase in actinomycetes in limed soils.

PLFA analysis was performed on the samples studied in this chapter; extraction of the PLFAs (as in Bardgett et al., 1996) was performed during the week starting 21/6/99, and the extracted material dried and frozen, to await analysis by GC-MS by a third party. However, unfortunately the results had not been returned in time to include them in this thesis.

7.2.2.9 Protease activity

Protease activity determines the rate of release of amino acids which subsequently become available for ammonification. Alef et al. (1988) found a good correlation between protease activity and arginine ammonification, reflecting the close relationship between the hydrolysis of proteins and the ammonification of amino acids in soils.

Several attempts on measuring protease were attempted unsuccessfully. These included:

1. ^{14}C labelled protein mineralisation (using cultured microbial protein, plant labelled protein and purchased labelled protein). During characterisation of molecular size, using centrifugal sieving through molecular filters of 100 and 3 kDa, radioactivity went 'missing', count balances did not calculate, and so was eventually abandoned.
2. Protease activity measurement (measuring breakdown of Sodium-Casein followed by addition of TCA and Folin-Ciocalteau's reagent; Alef and Nannipieri, 1995) with a paired control for each sample was attempted, but was not successful, as spectrophotometer readings were not stable, and did not make sense compared against controls, standards and blanks.

3. MUF substrate. L-leucine-4-methyl-7-coumarinylamide hydrochloride (leucine-MUF, $C_{16}H_{20}N_2O.HCl$, MW 324.81) was used as a substrate in soil incubations for measurement of leucine aminopeptidase activity. The principle of the assay (Freeman et al., 1995) is that once the leucine is cleaved off the substrate molecule by the enzyme, the remaining molecule fluoresces, and levels of fluorescence can be measured on a fluorimeter. However, during preliminary work in determining the optimal substrate concentration to use, problems were encountered as raw leucine-MUF substrate was found to fluoresce, and so differences post incubation did not yield meaningful results.

More work would be required to overcome measurement of protease activity at indigenous soil pH (rather than at any buffered pH). The use of controls for each sample is important to overcome experimental artefacts caused by differences in solubility of organic matter within a soil over a pH range.

7.2.3 Statistical analysis

For the Rothamsted and Woburn experimental plots, the 6 cores per plot were kept separate, and each assay was performed on a sub-sample of each core. This gave 6 replicates for each plot, which were meaned to give a plot average. Statistical analysis was then performed on data, giving n=2 for each of the 4 treatments on each soil type. Unfortunately, a confusion arose in obtaining the soil samples from the SAC. 6 cores were requested from each plot, but these were sent bulked rather than separate. This meant that in order to avoid pseudoreplication, only one (or in some cases two which were then meaned) replication was performed on each sample. Because only one plot was sampled for each treatment, n=1, which meant that statistical analysis was not meaningful. Therefore tables do not include standard errors or ANalysis Of VAriance (ANOVA) results for these samples.

ANOVA was performed using Minitab (version 13), and least significant difference (LSD) values were calculated according to Clewer and Scarisbrick (2001):

$$LSD = SED \times t_{(5\%,\ error\ df)}$$

Where:

$$SED = \sqrt{((2 \times Error\ MS)/n)}$$

Statistical significance was calculated at the 95% (P=0.05) level, unless otherwise stated.

Functional relationships were examined using individual data points for each sample taken (n=48 for Rothamsted and Woburn soils, n=7 for SAC soil), and correlation analysis was performed using SigmaPlot for Windows Version 4.01. Straight lines or quadratic curves were fitted to the data based on visual inspection of data plots and comparison of correlation coefficients.

7.3 Results

7.3.1 Soil characteristics and their relationship to soil pH

7.3.1.1 Soil pH, total C, total N, soil C:N and soil moisture

The soil characteristics are summarised in Table 7-4, with significant differences shown where applicable. Average treatment $pHs_{(CaCl2)}$ ranged from 3.45 to 6.19 for the Rothamsted soil, 3.67 to 6.10 for the Woburn soil, and from 4.29 to 6.54 for the SAC soil. All the different pH treatments resulted in significantly different pHs in the Rothamsted and Woburn soils, with the exception of RM and RH treatments which were not significantly different. The seven SAC plots increased sequentially by 0.3 – 0.5 pH units.

Total C and N did not show significant differences between the different pH treatments (Table 7-4), although in the SAC soil, the two plots of the highest pH possessed the highest total C and N levels. Individual data points plotting total C and N against pH are presented in Figure 7-1i and ii. Only the SAC soil shows strong positive correlations of total C and N with pH, with r^2 values of 0.6 in both cases. Total soil C and N are strongly correlated in each soil (appendix 4). Soil C:N ratios also showed little trend with lime application rate (Figure 7-1iii), although in the Rothamsted soil the RH treatment was significantly higher than the others (Table 7-4).

Soil moisture in the three soil types ranked in the same order as the organic matter content among the soil types, in descending order: SAC, Rothamsted, Woburn. In Rothamsted and Woburn soils the soil moisture was greatest in L and M pH treatments, although there were no significant differences in soil moisture between pH treatments (Table 7-4). However, the SAC soil showed a significant (r^2=0.94, p=0.0003) trend of increasing soil moisture with increasing pH treatment

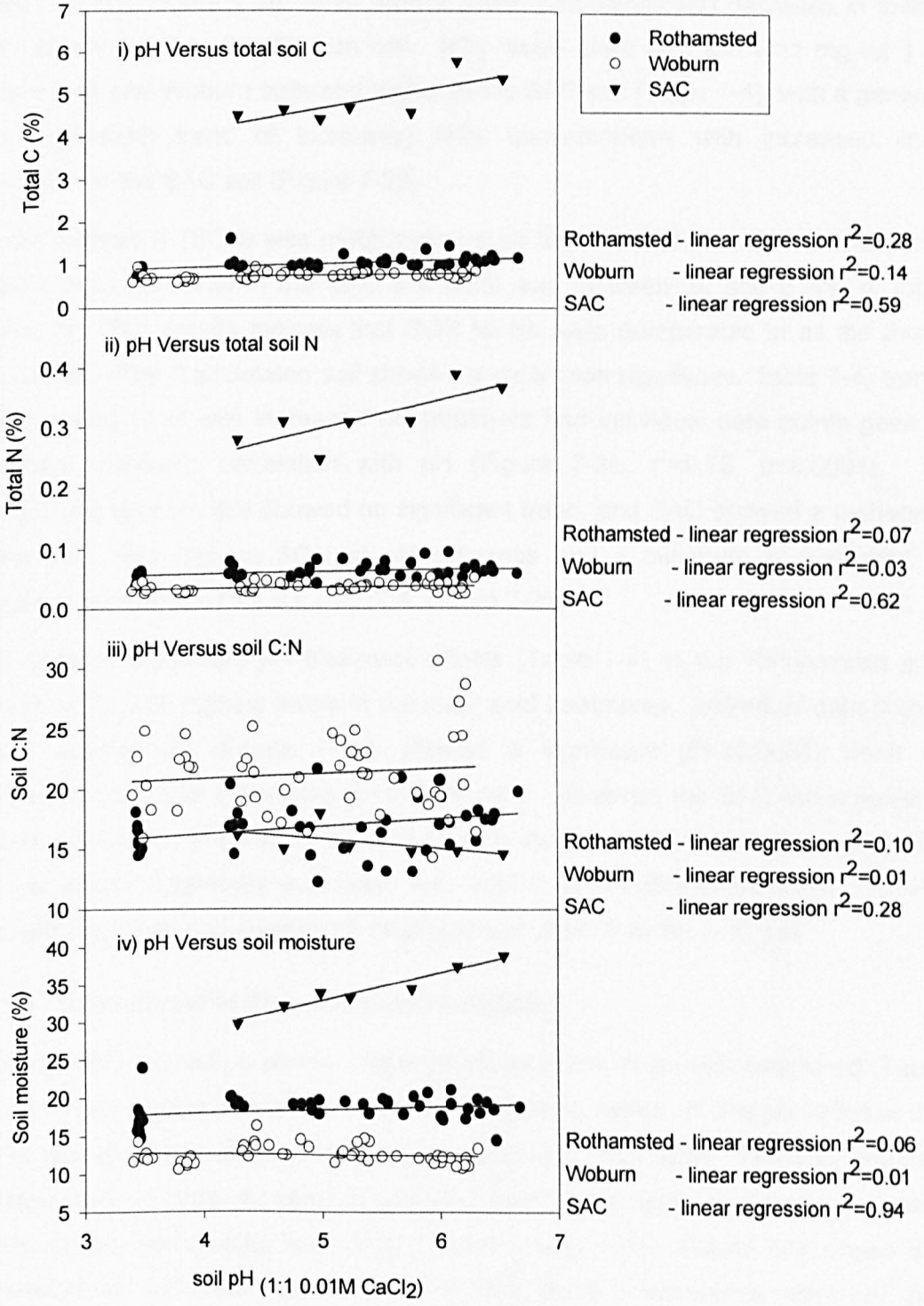
Table 7-4 Soil Characteristics[7].

Soil & Treatment	$pH_{(CaCl2)}$	Soil moisture %	Total C %	Total N %	C:N	NH_4^+ mg kg^{-1}	NO_3^- mg kg^{-1}	SON mg kg^{-1}	SOC mg kg^{-1}	Exch. Al mmol kg^{-1}	Al transformed $1/\sqrt{(x+1)}$	Ca mg kg^{-1}	Ca transformed $\sqrt{(x)}$
R0	3.45c (0.01)	17.01 (1.48)	0.85 (0.08)	0.05 (0.01)	15.80b (0.40)	0.41 (0.41)	0.28 (0.03)	16.22 (0.45)	126.58b (7.16)	31.57 (0.52)	0.151b	1.30 (0.46)	1.12b
RL	4.58b (0.31)	19.25 (0.28)	1.04 (0.06)	0.06 (0.00)	17.01ab (0.31)	0.98 (0.35)	0.35 (0.02)	11.20 (2.50)	92.65ab (13.11)	3.05 (2.59)	0.446b	28.01 (0.64)	5.29a
RM	5.72a (0.00)	19.53 (0.31)	1.12 (0.05)	0.07 (0.01)	15.47b (0.63)	0.17 (0.03)	0.27 (0.01)	8.81 (2.05)	64.89a (10.25)	0.00 (0.00)	0.954a	29.50 (5.16)	5.41a
RH	6.19a (0.23)	17.86 (0.13)	1.07 (0.07)	0.06 (0.01)	19.19a (1.06)	0.37 (0.05)	0.24 (0.01)	7.47 (0.14)	58.52a (0.53)	0.00 (0.00)	1.00a	24.65 (0.61)	4.96a
P	0.002	n.s.d.	n.s.d.	n.s.d.	0.054	n.s.d.	n.s.d.	n.s.d.	0.02	c.r.	0.003	c.r..	0.001
LSD	0.76	n.a.	n.a.	n.a.	2.63	n.a.	n.a.	n.a.	35.57	n.a.	0.30	n.a.	1.03
W0	3.67d (0.18)	11.96 (0.53)	0.68 (0.03)	0.03 (0.00)	21.11 (0.83)	0.77 (0.54)	0.40 (0.06)	11.68 (0.59)	81.96b (5.51)	16.78 (3.83)	0.20c	6.71 (4.54)	2.42b
WL	4.59c (0.20)	13.60 (0.29)	0.75 (0.02)	0.04 (0.00)	20.26 (1.27)	0.58 (0.38)	0.42 (0.06)	13.92 (0.47)	60.95a (5.35)	2.50 (0.89)	0.40b	24.37 (0.54)	4.94a
WM	5.37b (0.13)	12.86 (0.28)	0.77 (0.01)	0.04 (0.00)	21.87 (0.98)	0.44 (0.16)	0.44 (0.00)	11.86 (0.30)	49.01a (5.40)	0.01 (0.01)	0.91a	25.35 (0.44)	5.04a
WH	6.10a (0.06)	11.81 (0.48)	0.76 (0.02)	0.04 (0.01)	21.79 (4.13)	0.12 (0.04)	0.32 (0.01)	12.99 (1.07)	54.36a (2.37)	0.02 (0.00)	0.88a	27.31 (2.15)	5.22a
P	0.001	n.s.d.	n.s.d.	n.s.d.	n.s.d.	n.s.d.	n.s.d.	n.s.d.	0.03	c.r.	<0.0005	c.r.	0.039
LSD	0.60	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	19.00	n.a.	0.12	n.a.	1.89
S1	4.29	29.85	4.51	0.28	16.16	0.51	3.09	22.11	87.36	12.67		12.14	
S 2	4.68	32.30	4.64	0.31	15.02	15.09	4.31	14.04	113.25	6.64		27.31	
S 3	4.99	34.00	4.42	0.25	17.97	15.50	11.57	13.49	102.90	2.53		24.28	
S 4	5.24	33.40	4.68	0.31	15.19	23.51	7.12	7.32	100.31	2.04		25.04	
S 5	5.76	34.54	4.57	0.31	14.79	16.74	7.26	8.49	115.84	0.02		25.04	
S 6	6.15	37.53	5.78	0.39	14.86	9.96	8.33	11.41	118.43	0.01		27.31	
S 7	6.54	38.91	5.37	0.37	14.63	17.66	7.80	17.02	136.55	0.02		25.80	

[7] For Table 7-4 to Table 7-7, The treatment symbols are: R=Rothamsted soil, W=Woburn soil, S= SAC soil. 0=zero, L=low, M=medium, H=high lime application rate, 1-7 are pH treatments on SAC soil as specified in Table 7-1. Values are means (n=2 Rothamsted and Woburn, n=1 SAC), followed by standard error of mean in parentheses. n.s.d. is no significant difference at P=5%; LSD is least significant difference; n.a. is not applicable; m.d. is missing data; c.r. is contravenes requirements of ANOVA; different letters denote significant differences ($p<0.05$) within a soil type.

Figure 7-1 Relationships between soil pH and total C and N, soil C:N and soil moisture.

[Data points represent individual samples.]



7.3.1.2 Mineral and soluble organic N and C

NH_4^+ levels were very low (<1 mg kg^{-1}) in the Rothamsted and Woburn soils, and quite high (up to 25 mg kg^{-1}) in the SAC soil, with no trend evident with pH treatment (Table 7-4 and Figure 7-2i), other than a slight (non significant) decrease in levels with increasing pH in the Woburn soil. NO_3^- levels were also low (< 1 mg kg^{-1}) in Rothamsted and Woburn soils and higher in the SAC soil (Table 7-4), with a general (non significant) trend of increasing NO_3^- concentrations with increased lime application in the SAC soil (Figure 7-2ii).

Soluble organic N (SON) was much higher than total mineral N in Rothamsted and Woburn soils, whereas in the SAC soil SON was between 24 and 614% of total mineral N. The results indicate that SON levels were comparable in all the three soils tested. The Rothamsted soil showed a clear (non significant, Table 7-4) trend of decreasing SON with increased pH treatment and individual data points gave a significant quadratic correlation with pH (Figure 7-2iii, r^2=0.79, p<0.0001). In contrast, the Woburn soil showed no significant trend, and SAC showed a u-shaped relationship, with highest SON at pH extremes and a minimum at treatment 4 (quadratic regression, r^2=0.93, p=0.004, Figure 7-2iii).

SOC showed significant pH treatment effects (Table 7-4) in the Rothamsted and Woburn soils, with highest levels in the most acid treatments. Individual data points plotted against pH (Figure 7-2iv) showed a significant (P=<0.0001) trend of decreasing SOC with increasing pH in both soils. However, the SAC soil showed a positive correlation (Figure 7-2iv, p=0.05), with increasing SOC with increasing pH. SOC is weakly negatively correlated with total C in the Rothamsted and Woburn soils, although the (non-significant) relationship is positive in the SAC soil.

7.3.1.3 Exchangeable Aluminium and Calcium

Exchangeable aluminium showed clear trends in all the three soils examined (Table 7-4), with very high levels in the lowest pH treatments, falling off sharply to below the limit of detection at medium and high lime application treatments. The data required transformation in order to perform ANOVA, which gave highly significant treatment effects in the Rothamsted and Woburn soils (Table 7-4). Figure 7-3i shows the untransformed individual data points in near perfect correlation with soil pH. Aluminium levels were strongly correlated to total C (negatively) and SOC levels (positively) in Rothamsted and Woburn soils (appendix 4).

Exchangeable calcium showed the opposite trend to aluminium (strong negative correlation with Al in all soils, appendix 4), unsurprisingly with low Ca levels at zero lime applications, to high levels at high lime applications. Transformed data showed a significant treatment effect (Table 7-4) with the lowest pH treatment having a significantly lower exchangeable calcium level than low, medium and high lime applications (which did not differ significantly from each other) in Rothamsted and Woburn soils. Figure 7-3ii shows highly significant ($P<0.0001$) quadratic regressions for Rothamsted and Woburn soils when individual data points are plotted against pH, although this relationship is not significant in the SAC soil ($P=0.13$). Calcium levels were strongly correlated with total C (positively), DOC levels (negatively) in Rothamsted and Woburn soils (appendix 4).

7.3.1.4 Soil basal and substrate induced respiration

Table 7-5 summarises data on soil basal and substrate induced respiration. Basal respiration showed a clear trend of increasing with lime application treatment in the Rothamsted soil, although this was not statistically significant (Table 7-5). A similar trend in the Woburn soil was highly significant, and the same trend could be seen in the SAC soil data. Figure 7-4iii shows the correlations of individual data points with soil pH, which were significant in all three soil types ($p<0.0001$ for Rothamsted and Woburn, $p=0.0036$ for SAC soil). Basal respiration was correlated with DOC in each soil (negatively in Rothamsted and Woburn, positively in SAC), and negatively correlated with aluminium levels in Rothamsted and Woburn (appendix 4).

The proportion of added glucose respired in 3 h was significantly lower at the high lime application treatment in Woburn soil (Table 7-5), and the trends in all soils was for a slightly faster glucose mineralisation rate at intermediate pHs (Figure 7-4i). Quadratic regressions (significant in Woburn and SAC soil) gave maximum rates at pH 4.95, 4.45 and 5.06 in Rothamsted, Woburn and SAC soils respectively.

Figure 7-2 Relationships between soil pH and NH_4^+, NO_3^-, SON and SOC.

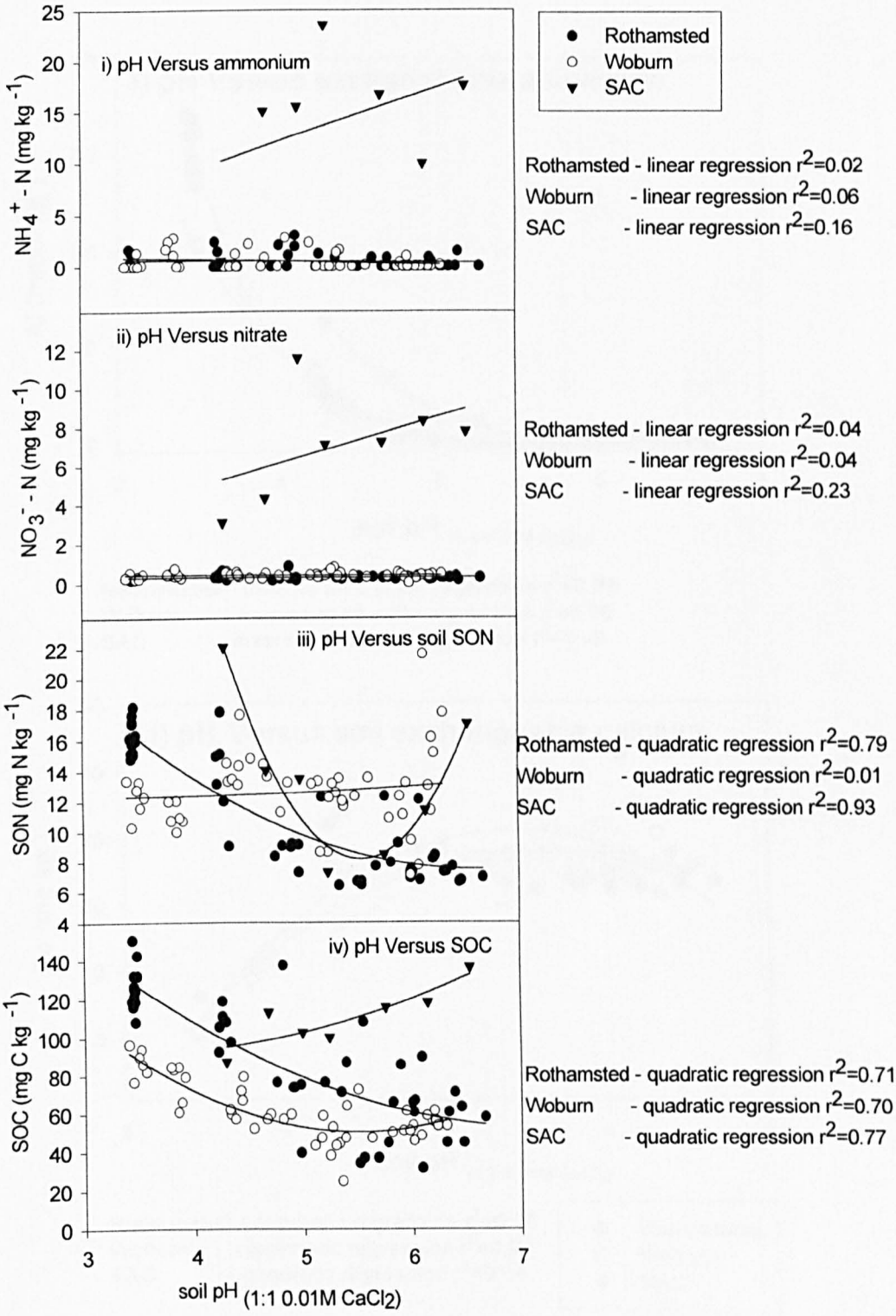
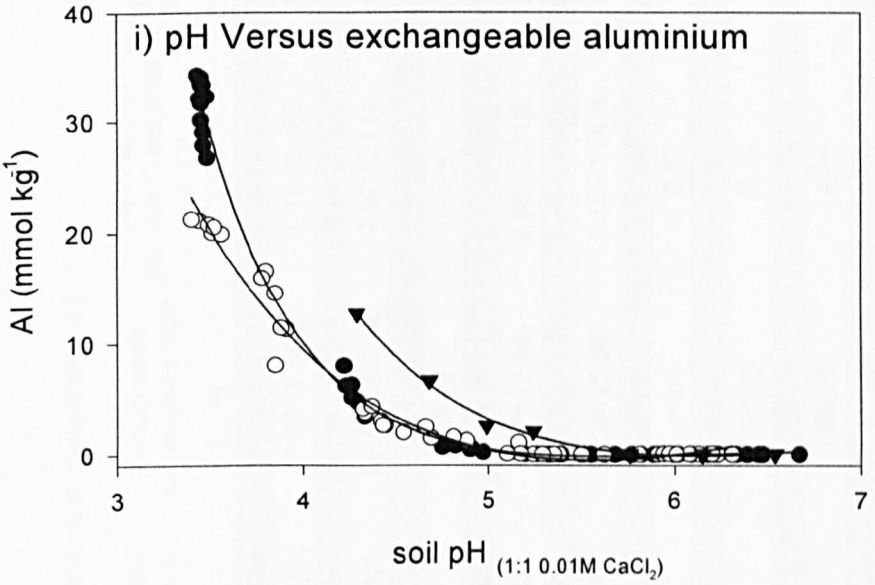
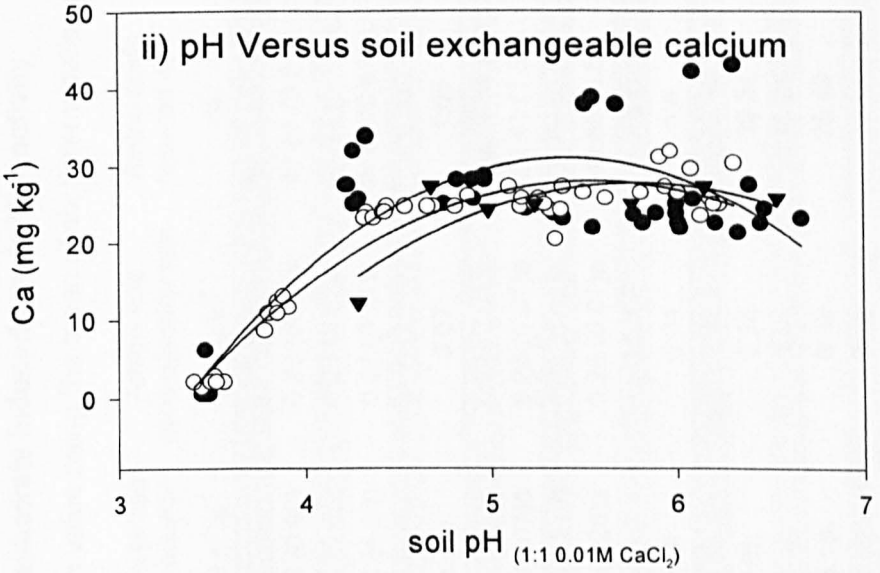


Figure 7-3 Relationships between soil pH and exchangeable aluminium and calcium.



Rothamsted - inverse third order regression r^2=0.99
Woburn - inverse third order regression r^2=0.98
SAC - inverse third order regression r^2=0.99



Rothamsted - quadratic regression r^2=0.76
Woburn - quadratic regression r^2=0.92
SAC - quadratic regression r^2=0.64

● Rothamsted
○ Woburn
▼ SAC

Table 7-5 Soil basal and substrate induced respiration activity

[Data are means (n=2, each value being the mean of six determinations), standard errors in parentheses.]

Soil & treatment	Basal respiration (CO_2 evolution)	Amino acid mineralisation rate	Amino acids respired 48 h	Amino acids unused 48 h	Amino acids immobilised 48 h	Proportion of glucose-C respired in 3 h	Proportion of urea-C respired in 3 h
	p mol s^{-1} g^{-1}	$1/t_{15}$ h^{-1}	%	%	%	%	%
R0	7.76 (0.89)	0.11 (0.01)b	52.26 (0.64)b	7.51 (0.41)c	40.23 (1.05)b	2.59 (0.10)	2.56 (0.68)
RL	10.06 (0.92)	0.23 (0.02)a	47.87 (0.27)c	4.84 (1.31)ac	47.30 (1.58)a	2.75 (0.06)	3.87 (0.79)
RM	14.39 (2.68)	0.28 (0.02)a	46.17 (0.11)a	3.80 (0.83)a	50.03 (0.72)a	2.91 (0.14)	5.07 (0.17)
RH	19.51 (4.29)	0.27 (0.01)a	46.85 (0.47)ac	2.61 (0.24)a	50.54 (0.71)a	2.52 (0.11)	4.48 (0.07)
P	n.s.d.	0.006	0.002	0.05	0.007	n.s.d.	n.s.d.
LSD	n.a.	0.07	1.65	3.19	4.22	n.a.	n.a.
W0	5.43 (0.19)c	0.11 (0.01)c	53.59 (0.97)	7.24 (0.80)	39.17 (1.77)	2.70 (0.02)b	3.23 (0.24)
WL	6.46 (0.07)b	0.23 (0.01)a	50.41 (1.54)	4.80 (0.92)	44.79 (2.46)	2.92 (0.31)b	4.25 (0.50)
WM	6.33 (0.21)b	0.36 (0.02)b	50.64 (0.72)	3.71 (0.08)	45.65 (0.64)	2.66 (0.05)b	4.24 (0.09)
WH	9.81 (0.20)a	0.25 (0.01)a	43.66 (5.50)	4.10 (0.35)	52.24 (5.86)	1.85 (0.04)a	3.16 (0.57)
P	<0.0005	<0.0005	n.s.d.	n.s.d.	n.s.d.	0.033	n.s.d.
LSD	0.70	0.04	n.a.	n.a.	n.a.	0.63	n.a.
S 1	8.82	0.32	32.98	2.56	64.46	3.92	19.96
S 2	9.62	0.34	32.34	2.03	65.63	3.76	16.14
S 3	10.48	0.41	30.85	2.21	66.94	4.66	21.49
S 4	14.15	0.38	29.49	1.75	68.76	4.66	16.68
S 5	17.88	0.40	31.44	2.01	66.55	3.04	18.47
S 6	27.68	0.29	30.47	1.73	67.79	4.00	15.32
S 7	56.82	0.11	29.46	2.17	68.37	1.99	7.52

Urea mineralisation was not significantly affected by treatment in either soil (Table 7-5), although Figure 7-4ii shows again a trend for faster urea mineralisation rates at intermediate pHs; quadratic regressions (all significant) gave maximum rates at pH 5.62, 4.92 and 4.91 in Rothamsted, Woburn and SAC soils respectively. Urea mineralisation rates were significantly correlated with total C and N (appendix 4).

The rate of amino acid mineralisation (the reciprocal of the time taken for 15% of added amino acid-^{14}C to be evolved as $^{14}CO_2$ ($1/t_{15}$ (h^{-1}))) was significantly different between treatments in the Rothamsted and Woburn soils (Table 7-5). The amino acid mineralisation rate was slower in the most acid samples and peaked in the medium lime application treatment in both soils. Data pertaining to the amino acid mineralisation incubation are shown plotted against soil pH in Figure 7-5. All three soils show significant ($P<0.0001$) quadratic correlations of amino acid mineralisation rate with soil pH, with the rate peaking at pH 5.8, 5.5 and 5.2 in Rothamsted, Woburn and SAC soils respectively. The rate of amino acid-C mineralisation was strongly correlated to total C (positively) and DOC levels (negatively) in Rothamsted and Woburn soils (appendix 4)

The proportion of amino acid-C respired within 48 h was significantly higher in the lowest pH treatment in Rothamsted, but there was no significant treatment effect in the Woburn soil, although it and the SAC soil show the same trend. These trends with soil pH are evident in Figure 7-5ii, and are significant in Rothamsted and Woburn soils ($P=<0.0001$ and 0.0011 respectively). More amino acids are left unused after 48 h at low pHs (Figure 7-5iii and Table 7-5), whereas the proportion of amino acids immobilised in microbial biomass increases with increasing lime application (significant treatment effect only in Rothamsted soil, Table 7-5, Figure 7-5iv). More amino acids were immobilised, fewer were left unused and less were respired in the SAC soil than either the Rothamsted or Woburn soils. The partitioning of amino acid-C use was similar in the Rothamsted and Woburn soils.

The ratio of amino acid-C immobilised:respired over the 24 h incubation was highly correlated with DOC (negatively); SMB-C (positively); SMB-N_{TDN} (positively); basal respiration (positively); aluminium (negatively); calcium (positively); and the proportion of total C that is microbial C (positively) in Rothamsted and Woburn soils (appendix 4).

7.3.1.5 Soil microbial biomass C and N

Data on soil microbial biomass (SMB) C and N is summarised in Table 7-6 below. SMB-C was significantly affected by pH treatment in the Rothamsted and Woburn soils, with the lowest pH treatment having significantly lower SMB-C than other treatments in each soil. Individual data points for each sample are shown in Figure 7-4iv with linear regressions ($p<0.0001$ in Rothamsted and Woburn, not significant in SAC) showing increasing SMB-C with increasing soil pH. SMB-C was highly correlated with DOC (negatively), aluminium levels (negatively) and calcium levels (positively) in Rothamsted and Woburn soils (appendix 4).

Two methods of determining SMB-N were used; the flush of ninhydrin positive compounds (NPCs hereafter termed SMB-N_{NPC}) and the flush of total dissolved N (NO_3^-, NH_4^+ and soluble organic) released upon fumigation (hereafter termed SMB-N_{TDN}). Neither method revealed significant treatment effects (Table 7-6), although in Rothamsted and SAC soils, the highest pH treatments gave the highest SMB-N_{NPC} and Rothamsted and Woburn soils showed increasing SMB-N_{TDN} with increasing pH treatment. Individual data points plotted against pH in Figure 7-6i and ii showed a general trend of increasing SMB-N with increasing pH in all soils, in both methods (significant regressions in Rothamsted and Woburn soils for both methods), with the exception of the Woburn SMB-N_{NPC} which started to fall above approximately pH 5.5. The SMB-N_{TDN} was significantly correlated with DOC (negatively), SMB-C (positively), aluminium (negatively), and calcium (positively) levels in Rothamsted and Woburn soils (appendix 4).

SMB C:N ratios gave very dubious results, as one would not expect this value to be greater than 12. There were no trends in SMB C:N with soil pH (Figure 7-6iii and iv) in either methods.

Soil microbial biomass C and N as a percentage of total soil C and N are shown in Figure 7-7, plotted against soil pH. Figure 7-7i shows that in the Rothamsted and Woburn soils, there is a linear trend for the % of total C that is microbial C to increase with pH, both regression lines are significant and have similar gradients. The SAC soil shows little relationship between microbial C as a % of total C and the linear regression is not significant. The proportion of total C that can be attributed to SMB-C is highly correlated with: DOC (negatively); SMB-C (positively); SMB-N_{TDN} (positively); basal respiration (positively); aluminium (negatively) and calcium (positively) levels in Rothamsted and Woburn soils (appendix 4).

Figure 7-4 Relationship of soil pH to glucose and urea substrate induced respiration, basal respiration and SMB-C

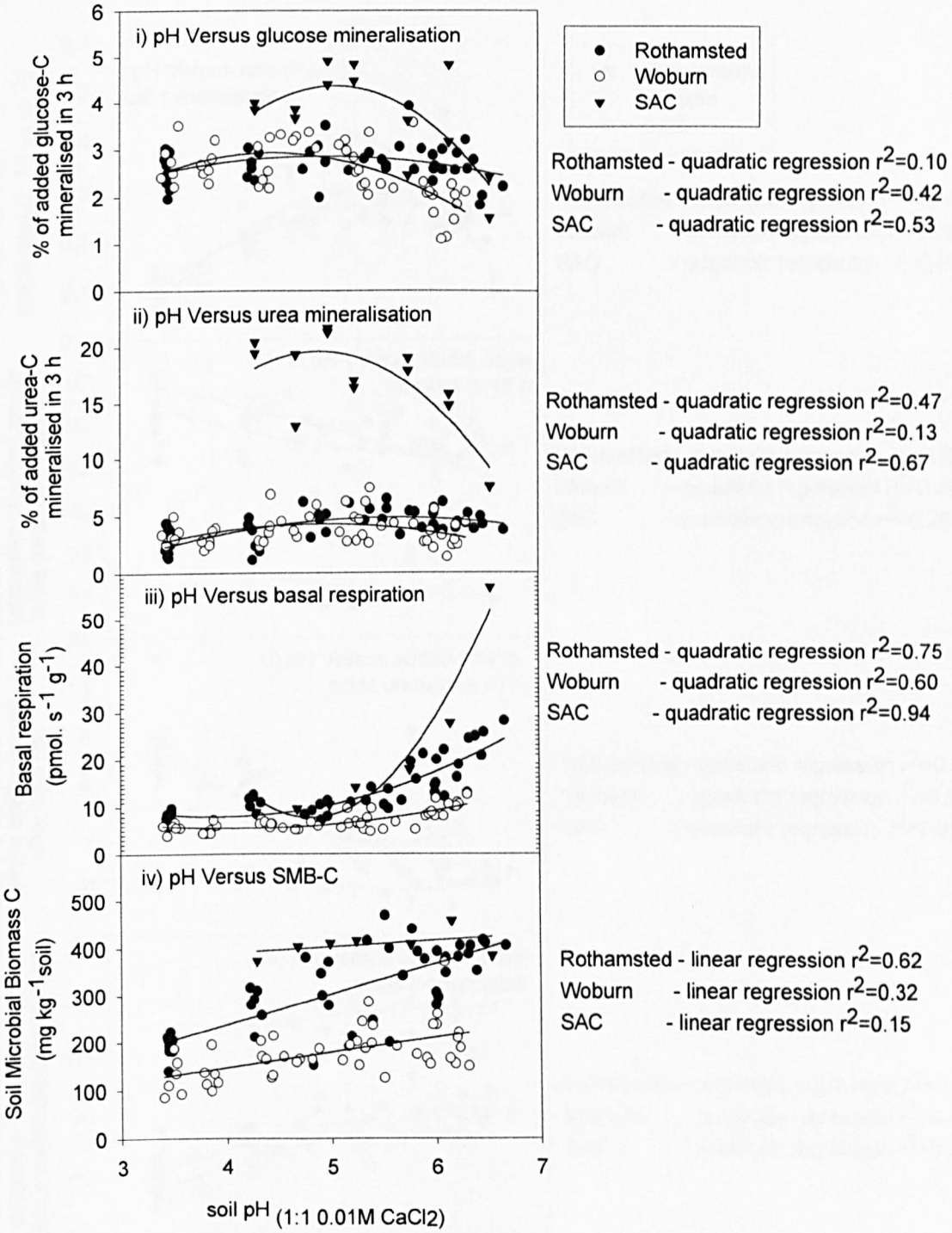


Figure 7-5 Relationships of soil pH to rate of amino acid mineralisation and proportion of amino acids respired, immobilised and left unused after a 48 h incubation.

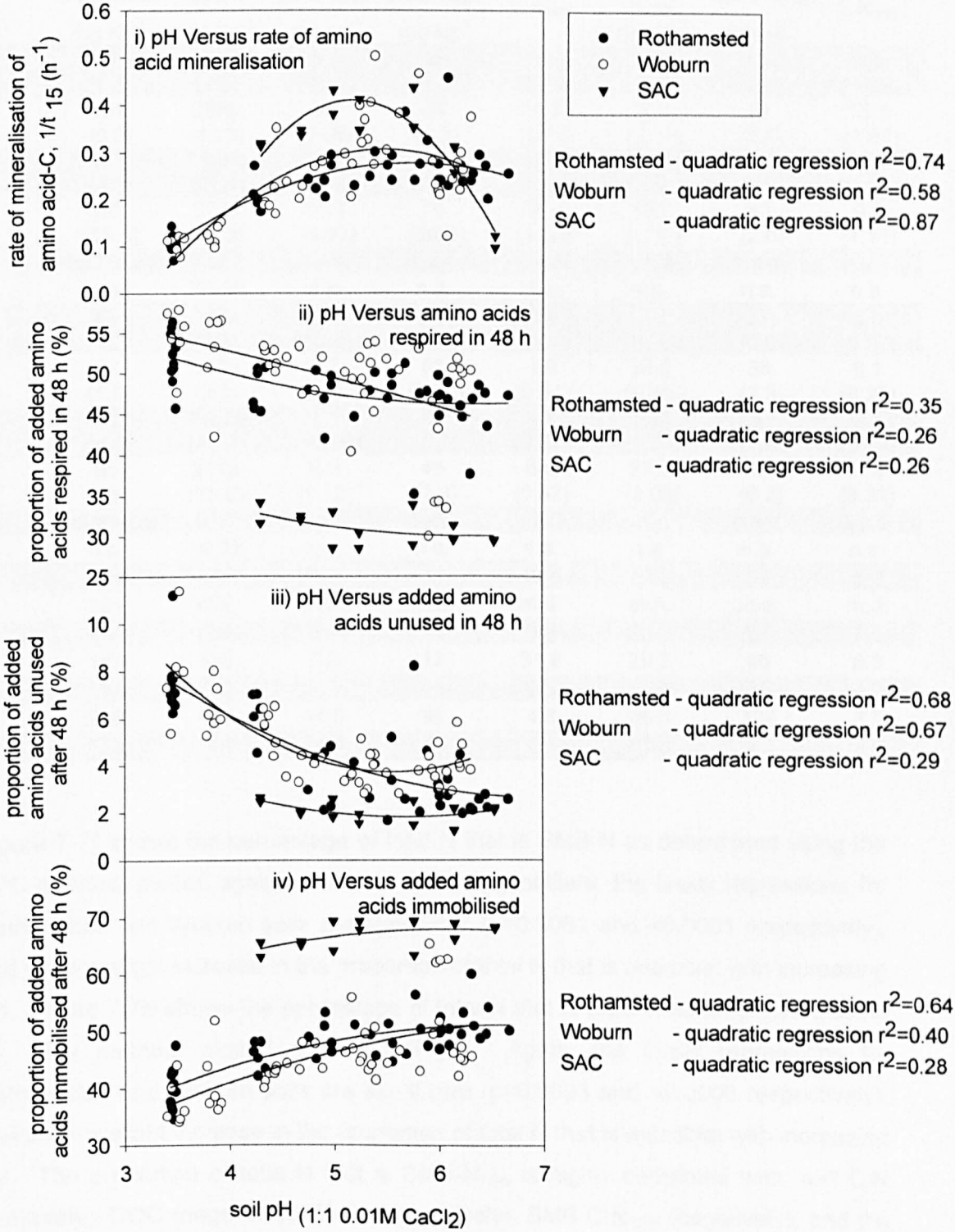


Table 7-6 Soil microbial biomass C, N and C:N determined by the fumigation extraction method, with two methods of microbial N calculation

[Data are means, n=2, each value being the mean of six replicate determinations, standard errors in parentheses]

	DOC flush	SMB C	NPC flush	SMB-N_{NPC}	SMB C:N_{NPC}	TDN flush	SMB-N_{TDN}	SMB C:N_{TDN}
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}		mg kg^{-1}	mg kg^{-1}	
R0	87 (0.8)	193c (1.8)	5.1 (0.20)	33 (1.3)	6.1 (0.33)	11.0 (1.58)	24 (3.5)	8.4 (1.48)
RL	131 (6.0)	292b (13.3)	3.8 (0.66)	25 (4.3)	15.6 (3.05)	10.6 (1.11)	24 (2.5)	13.4 (1.07)
RM	157 (9.6)	349ab (21.4)	7.8 (2.00)	51 (13.0)	8.2 (1.77)	17.0 (3.52)	38 (7.8)	10.7 (1.88)
RH	168 (11.5)	373a (25.5)	11.7 (4.03)	76 (26.2)	9.1 (4.53)	19.5 (1.16)	43 (2.6)	9.0 (1.11)
P	Not tested	0.007	n.s.d.	n.s.d.	n.s.d.	n.s.d.	n.s.d.	n.s.d.
LSD	n.a.	70.39	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
W0	58 (1.0)	130c (2.3)	3.8 (0.34)	25 (2.2)	5.5 (0.51)	12.0 (0.00)	27 (0.0)	5.0 (0.07)
WL	77 (1.0)	171b (2.3)	9.7 (3.25)	63 (21.1)	3.9 (0.51)	16.0 (0.80)	36 (1.8)	5.1 (0.29)
WM	88 (4.8)	196ab (10.6)	7.1 (0.87)	46 (5.7)	4.4 (0.77)	16.4 (1.33)	36 (3.0)	5.5 (0.13)
WH	95 (6.1)	211a (13.5)	6.9 (1.12)	45 (7.3)	5.0 (0.52)	21.6 (3.06)	48 (6.8)	4.4 (0.32)
P	Not tested	0.01	n.s.d.	n.s.d.	n.s.d.	n.s.d.	n.s.d.	n.s.d.
LSD	n.a.	34.33	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
S1	168	372	4.6	30	12.5	32.3	72	5.2
S2	181	403	m.d.	m.d.	m.d.	m.d.	m.d.	m.d.
S3	184	409	6.8	44	9.3	25.7	57	7.2
S4	187	415	2.0	13	31.9	29.7	66	6.3
S5	184	408	7.0	46	9.0	43.9	98	4.2
S6	206	457	14.6	95	4.8	58.5	130	3.5
S7	173	384	14.4	94	4.1	43.6	97	4.0

Figure 7-7ii shows the percentage of total N that is SMB-N as determined using the NPC method, plotted against soil pH. Excluding outliers, the linear regressions for Rothamsted and Woburn soils are significant ($p=0.0061$ and <0.0001 respectively), and show a slight increase in the proportion of total N that is microbial with increasing pH. Figure 7-7iii shows the percentage of total N that is SMB-N as determined using the TDN method, plotted against soil pH. Again, the linear regressions for Rothamsted and Woburn soils are significant ($p=0.0003$ and <0.0006 respectively), and show a slight increase in the proportion of total N that is microbial with increasing pH. The proportion of total N that is SMB-N_{TDN} is highly correlated with: soil C:N (positively); DOC (negatively); SMB-C (positively); SMB C:N_{TDN} (negatively); and the proportion of total C that is microbial C (positively), in Rothamsted and Woburn soils (appendix 4).

Figure 7-6 Relationship between soil pH and Soil Microbial N and C:N determined by two methods.

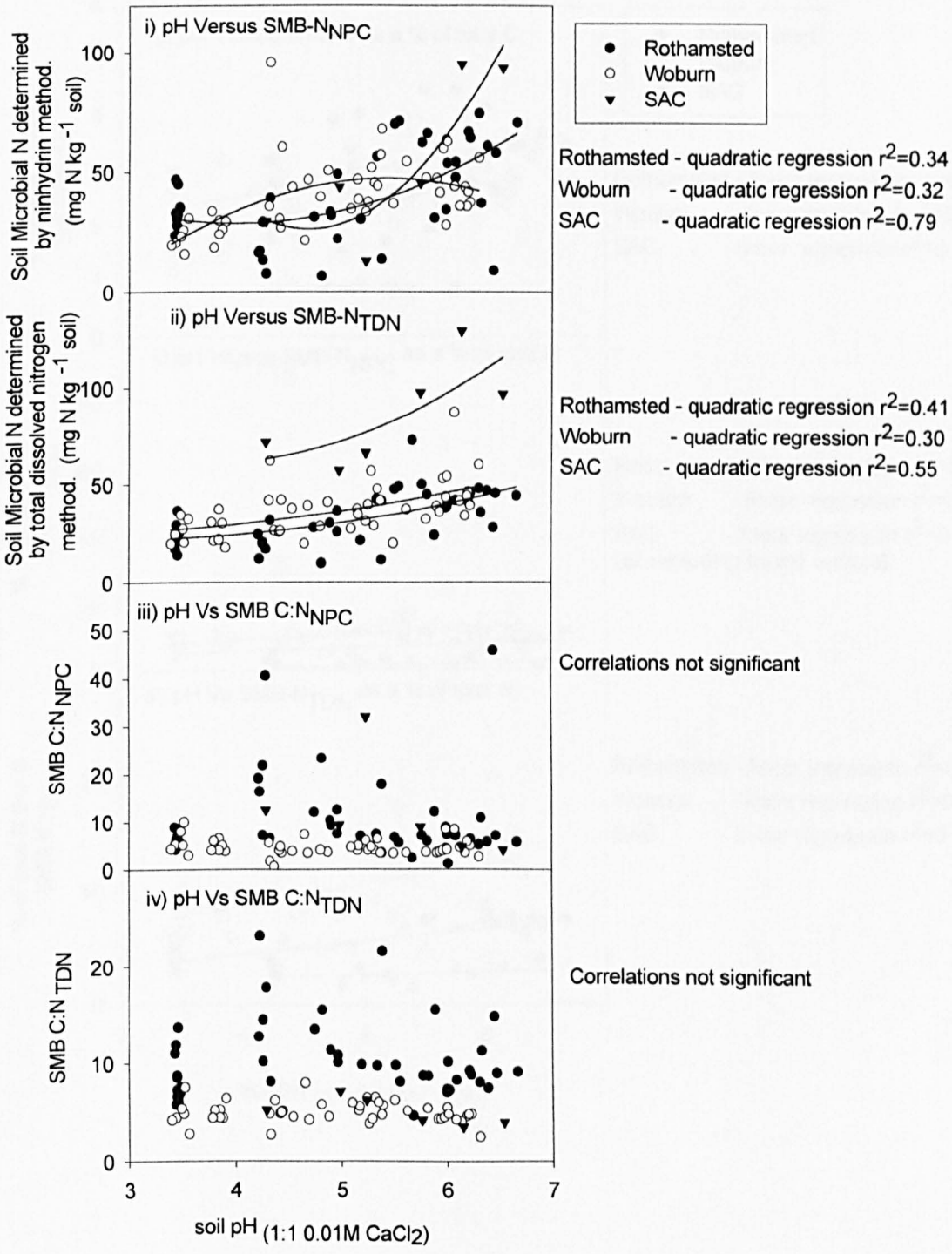
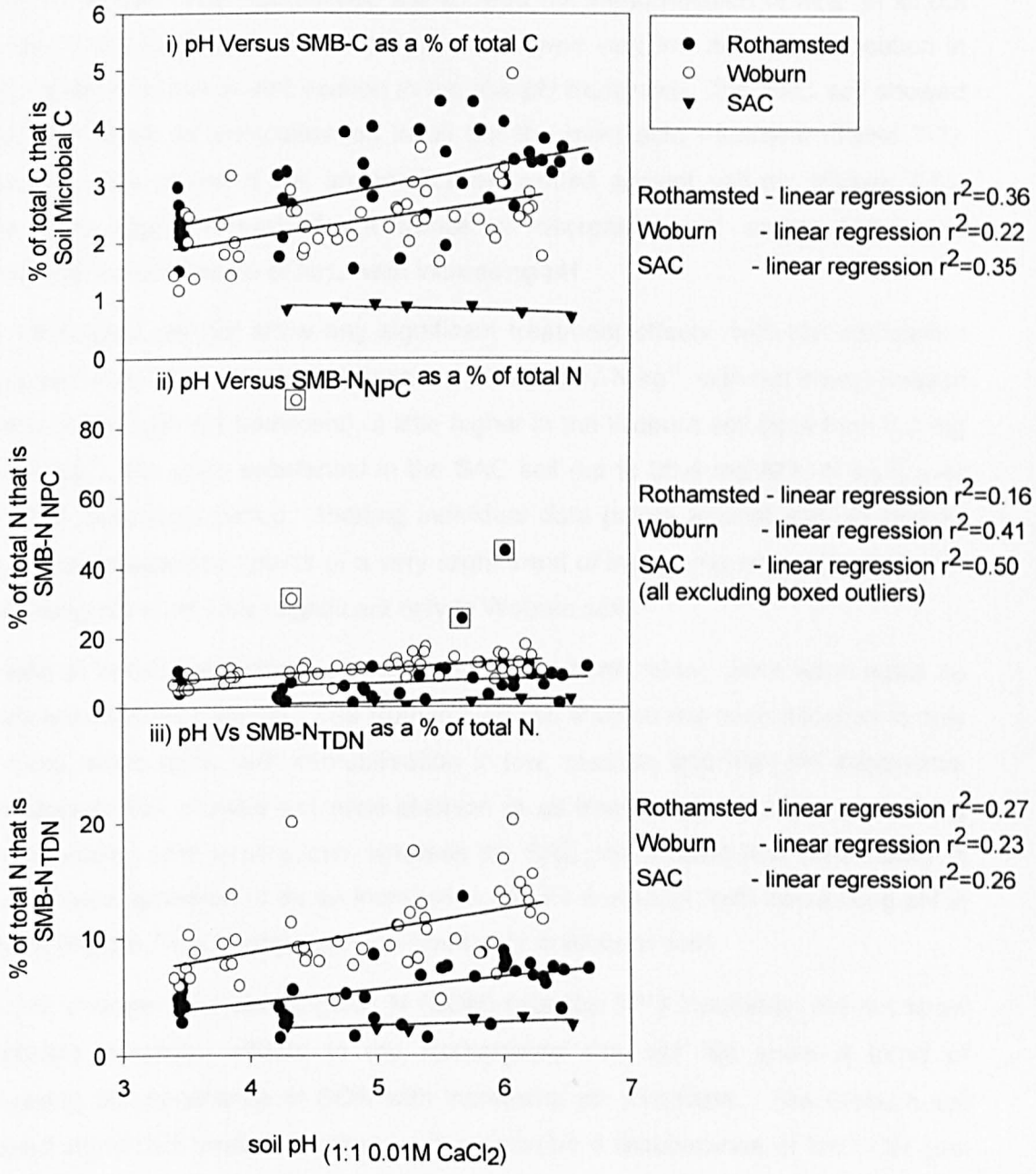


Figure 7-7 The relationship of soil pH to soil microbial biomass C and N as a % of total C and N.



7.3.1.6 Net mineralisation

The data on net mineralisation activity over a 30 d incubation are summarised in Table 7-7 below. The Rothamsted soil showed net immobilisation of NH_4^+ in all but the lowest pH treatment. The Woburn soil showed very low net ammonification in most treatments, but immobilisation in the low pH treatment. The SAC soil showed quite high levels of immobilisation in all but the most acid treatment (Table 7-7). Individual data points of net ammonification plotted against soil pH (Figure 7-8ii) show very slight, non-significant trends of decreasing net ammonification or increasing immobilisation of NH_4^+ with increasing pH.

Net nitrification did not show any significant treatment effects, with net nitrification being low in the Rothamsted soil (less than 2 mg NO_3^--N kg^{-1}, with net immobilisation of NO_3^- in the high pH treatment), a little higher in the Woburn soil (less than 5.1 mg NO_3^--N kg^{-1}), but quite substantial in the SAC soil (up to 26.4 mg NO_3^--N kg^{-1}) over the 30 d incubation period. Plotting individual data points against soil pH (Figure 7-8i) gave unexpected results of a very slight trend of increasing net nitrification with decreasing pH in all soils (significant only in Woburn soil).

Looking at net mineralisation (ammonification plus nitrification), there were again no significant treatment effects. The Rothamsted soil showed net mineralisation in only the most acidic plots, with immobilisation in low, medium and high pH treatments. The Woburn soil showed net mineralisation in all treatments, the level decreasing with increasing lime applications, whereas the SAC soil showed little consistency in trend. There appeared to be an increase in net mineralisation with decreasing pH in all soils (Figure 7-8iii, regression significant only in Woburn soil).

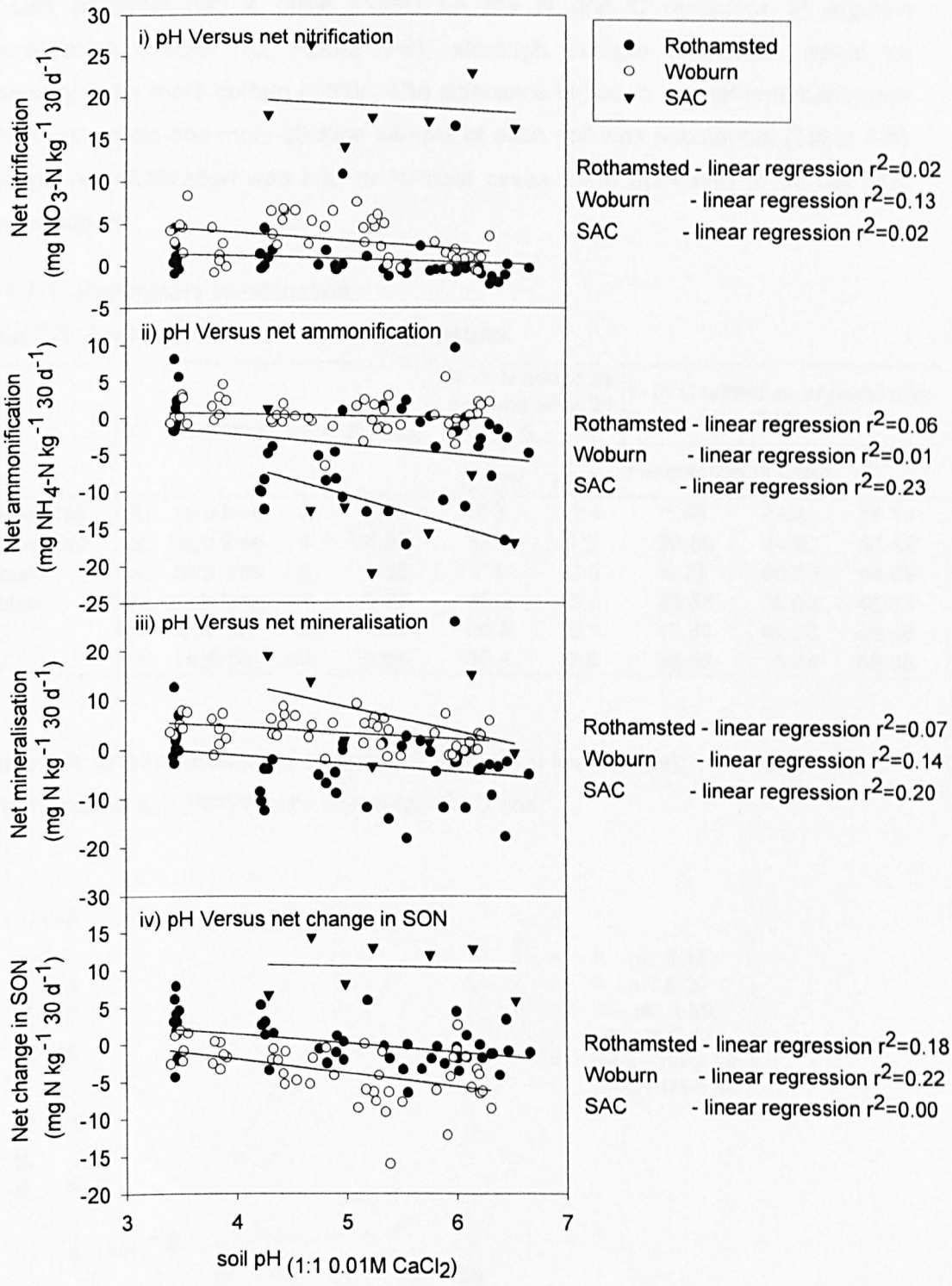
The net change in soluble organic N (SON) over the 30 d incubation did not show significant treatment effects in the Rothamsted soil, but did show a trend of increasing disappearance of SON with increasing pH treatment. The Woburn soil showed significant treatment effects with increasing disappearance of the SON pool over the 30 d incubation with increasing pH treatment (with the exception of the high pH treatment). Again, the SAC soil showed little discernible trend. The individual data points plotted against pH (Figure 7-8iv) showed similar trends to those for net mineralisation, in that the SON pool showed a slight linear trend of diminution with increasing pH, which was significant in the Rothamsted ($p=0.0026$) and Woburn ($p=0.001$) soils.

Table 7-7 Net nitrification, ammonification and mineralisation activity and net change in soluble organic nitrogen pool over a 30 d aerobic incubation

[Data are means, n=2, each value being the mean of six replicate determinations, standard errors in parentheses]

	net nitrification mg kg^{-1}	net ammonification mg kg^{-1}	net mineralisation mg kg^{-1}	net change in SON mg kg^{-1}
R0	1.00 (0.26)	1.19 (0.75)	2.18 (1.01)	2.18 (0.79)
RL	1.74 (0.35)	-7.19 (1.09)	-5.45 (1.44)	0.53 (1.25)
RM	0.96 (1.65)	-4.33 (2.20)	-3.37 (0.55)	-0.59 (1.53)
RH	-0.39 (0.49)	-4.25 (1.86)	-4.63 (2.35)	-1.18 (0.32)
P	n.s.d.	n.s.d.	n.s.d.	n.s.d.
LSD	n.a.	n.a.	n.a.	n.a.
W0	2.89 (1.54)	1.34 (0.56)	4.23 (0.98)	-0.77d (0.61)
WL	5.13 (0.11)	-0.87 (1.03)	4.27 (1.14)	-2.58c (0.11)
WM	3.23 (2.04)	0.36 (0.49)	3.60 (2.52)	-7.18b (0.24)
WH	0.95 (0.32)	0.43 (0.14)	1.38 (0.46)	-4.32a (0.06)
P	n.s.d.	n.s.d.	n.s.d.	0.001
LSD	n.a.	n.a.	n.a.	1.31
S1	17.81	1.24	19.05	6.77
S2	26.42	-12.59	13.83	14.44
S3	14.05	-12.12	1.93	8.21
S4	17.52	-20.95	-3.42	13.06
S5	17.05	-15.67	1.38	12.08
S6	22.95	-7.83	15.12	12.85
S7	16.24	-16.91	-0.67	5.97

Figure 7-8 Relationship between soil pH and net mineralisation activity over a 30 d incubation.



7.3.1.7 Arginine mineralisation

The results from the arginine mineralisation trial showed that the pH of the soil samples probably had a great impact on the N and C dynamics of arginine mineralisation (Table 7-8, Figure 7-9), although sample replication would be necessary to be more certain of this. The difference between the net ammonification in the most acidic and most alkaline sample of each soil was substantial (Table 7-8), although net nitrification was low, or in most cases there appeared to be net NO_3^- immobilisation.

7.3.1.7.1 Preliminary investigation

Table 7-8 Arginine mineralisation trial results.

soil	plot	Treatment	core	$pH_{(CaCl2)}$	% of N added as arginine after 24 h.		% of C added as arginine after 24 h.		
					net amfc'n	net nitfc'n	mineralised	unused	Immobl's d
Rothamsted	10b	zero lime	3	3.46	2.3	-1.4	1.68	24.21	74.12
Rothamsted	3a	high lime	4	6.00	43.1	1.2	20.55	24.92	54.52
Woburn	55a	zero lime	5	3.85	7.4	-1.9	4.73	40.50	54.78
Woburn	59b	high lime	4	5.97	40.3	-2.0	20.65	32.62	46.73
SAC	4.5	Low pH	n.a.	4.29	26.5	-0.1	13.87	42.76	43.38
SAC	7.5	High pH	n.a.	6.54	50.4	3.8	23.58	13.74	62.68

Figure 7-9 Mineralisation of arginine-C over 24 h incubation.

R=Rothamsted soil, W=Woburn soil, SAC=SAC soil.

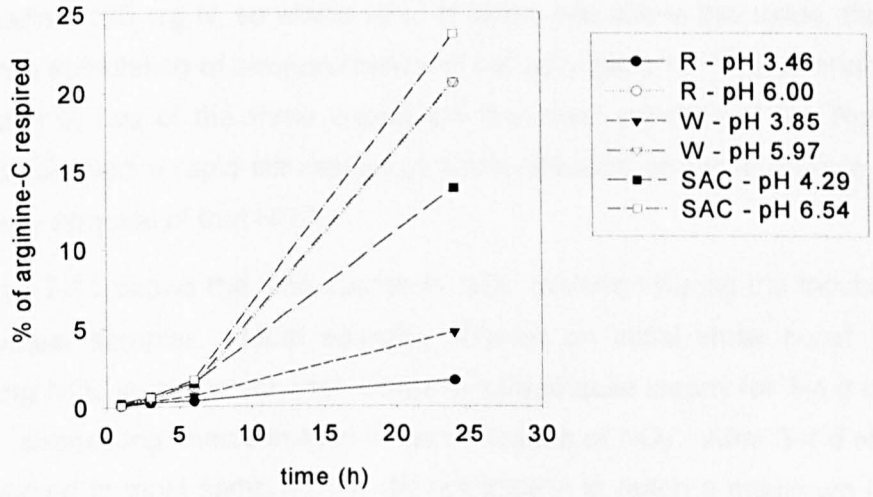


Figure 7-9 shows the time course of $^{14}CO_2$ evolution during the 24 h incubation, and demonstrates that the more alkaline samples had a much faster rate of arginine C mineralisation than the acid samples, but that during this incubation the rates did not start to level off. A longer incubation time than 24 h was required to monitor full progress of the process, as 14-43% of added arginine-C was unused after 24 h (Table 7-8).

Both the Rothamsted soil samples used the same proportion of added arginine in the 24 h incubation (Table 7-8), but the acidic sample immobilised around 75% and mineralised very little of the C, whereas the most alkaline sample immobilised just over 50% and mineralised around 20% of the C. Very little of the added N was mineralised in the acidic sample, whereas in the most alkaline sample, over 40% of the N was mineralised. All three soils showed similar patterns in terms of N and C utilisation, although in the Woburn and SAC soils, the acidic sample of the pair left more substrate unused than the more alkaline sample.

7.3.1.7.2 More thorough investigation

The time-course of NH_4^+ levels in individual samples are shown in Figure 7-10, demonstrating that samples and soils behave quite differently. Some samples reached maximum NH_4^+ production within 24 h, whereas in others this took 8 days, and in one sample levels continued to rise during the whole 17 d incubation. Once the maximum NH_4^+ production was reached, in some samples NH_4^+ concentration decreased (most pronounced in the high lime application treatment in the SAC soil), and in others it remained quite steady. The falling NH_4^+ levels suggest removal from the pool by either nitrification or immobilisation. The arginine added to each sample contained 138 µg N, so where NH_4^+-N levels rise above this value, there must have been a stimulation of ammonification of native organic N. This phenomenon is most notable in two of the three lowest pH treatment samples of the Rothamsted soil, which showed a rapid stimulation of ammonification of native organic N followed by speedy removal of that NH_4^+.

Figure 7-11 shows the time-course of NO_3^- evolution during the incubation period in individual samples. Most samples showed an initial small boost in nitrification, raising NO_3^- levels, which then either remained quite steady for 3-4 d or decreased a little, suggesting immobilisation or denitrification of NO_3^-. After 3-4 d NO_3^- production increased in most samples, but did not appear to reach a maximum in the samples (with the exception of one high pH treatment SAC soil sample) within the

Figure 7-10 NH_4^+ production throughout the arginine incubation.

C_e shows the NH_4^+ levels in paired control samples at the end of the incubation period. (Legend: R=Rothamsted soil, W=Woburn soil, S=SAC soil, 0=zero lime treatment, L=low lime treatment, M= medium lime treatment, H=high lime treatment)

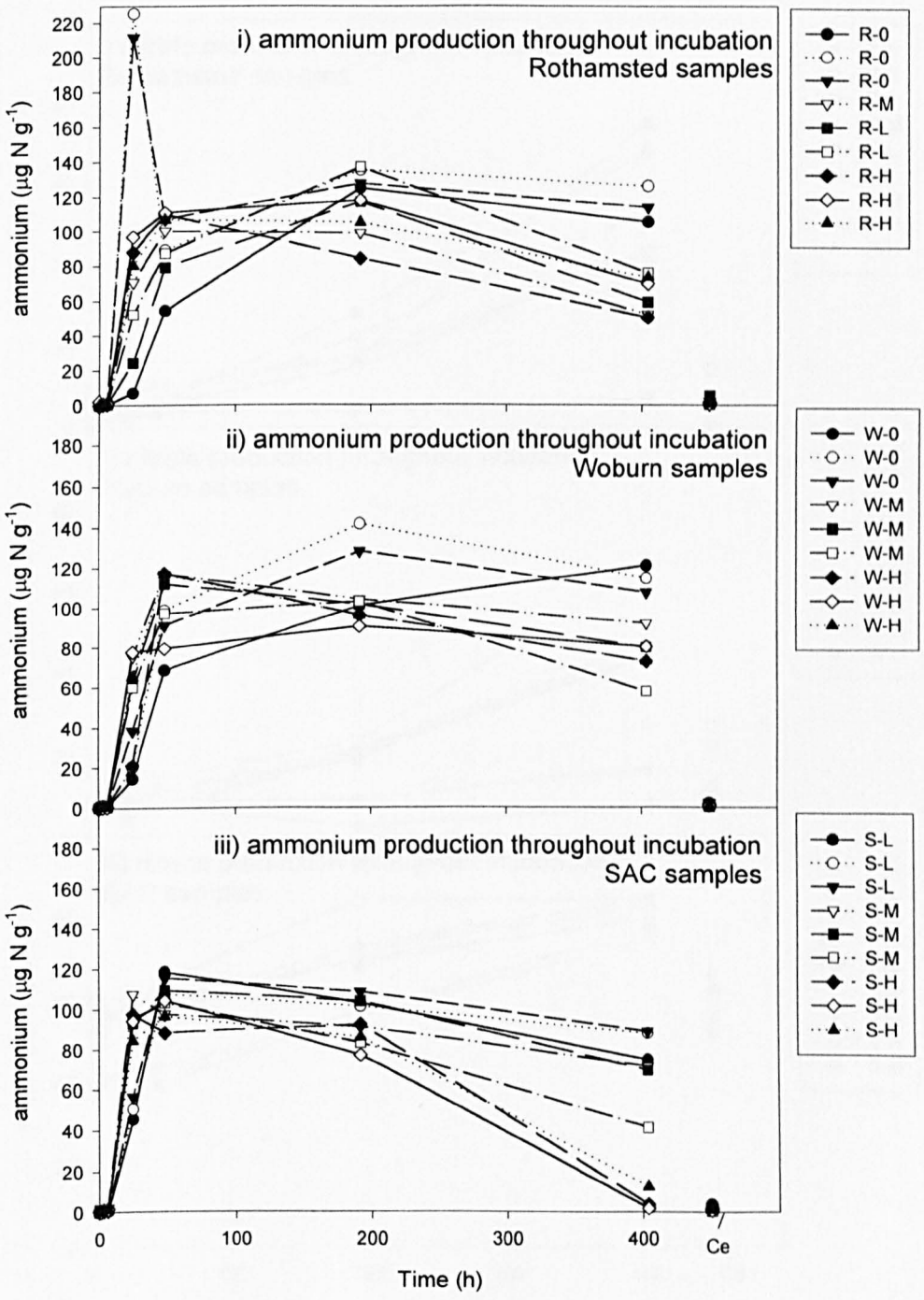
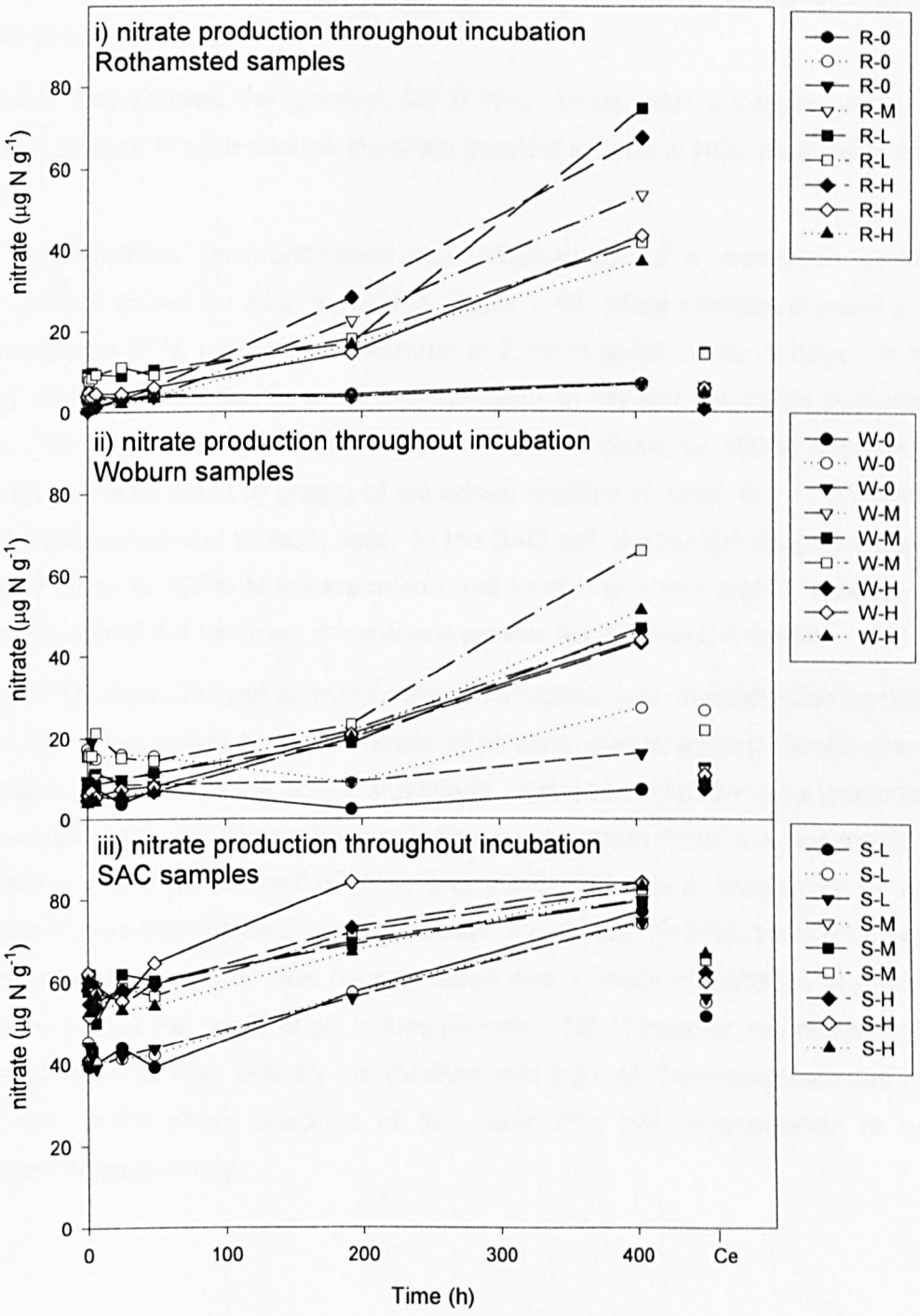


Figure 7-11 NO_3^- production throughout the arginine incubation.

C_e shows the NO_3^- levels in paired control samples at the end of the incubation period. (Legend: R=Rothamsted soil, W=Woburn soil, S=SAC soil, 0=zero lime treatment, L=low lime treatment, M= medium lime treatment, H=high lime treatment)



17 d incubation. The Rothamsted and Woburn soils showed that the lowest pH treatment samples had little or no net nitrification above control samples. In the SAC soil, all samples started the incubation with quite high NO_3^- levels, but the low pH treatment samples started off with the lowest NO_3^- levels. However, by the end of the incubation NO_3^- levels in these acidic samples were similar to samples of much higher pHs.

Samples that showed the greatest fall in NH_4^+ levels after maximum levels were reached (Figure 7-10) tended to show the greatest pick up in NO_3^- production (Figure 7-11).

Net mineralisation (ammonification plus nitrification) as a proportion of added arginine-N is shown for each sample in Figure 7-12. Most samples showed a rapid mineralisation of N, reaching a maximum in 2, or in some cases, 8 days. In some cases, this was preceded by a net immobilisation in the first few hours equivalent of up to 7% of added N. Most samples reached close to 100% mineralisation (reference line included in graph) of the added arginine-N within the 17 d incubation in the Rothamsted and Woburn soils. In the SAC soil, the low pH treatment samples reached close to 100% N mineralisation and levels remained static. However with increasing pH of the samples, there was a greater fall in mineral N levels.

Figure 7-13 shows the net ammonification, nitrification and mineralisation by the end of the 404 h incubation for each sample of all soils, plotted against its pH, giving an indication of the fate of the added arginine-N. Net ammonification as a proportion of added arginine-N fell, almost linearly (although the curve fitted is a quadratic), with increasing pH (r^2=0.66, p<0.0001). Net nitrification as a proportion of added arginine-N rose with increasing pH (quadratic regression, r^2=0.50, p=0.0003) with an 'optimum' at $pH_{(CaCl2)}$ 5.6. Net mineralisation was 77-95% of added N in almost all samples, across the range of pH values (Figure 7-13). However, the regression line is pulled down at high pHs by the medium and high pH treatment samples of the SAC soil, which show averages of 59% and 21% net mineralisation of added substrate N respectively.

Figure 7-12 Net mineralisation as a proportion of added arginine-N throughout the incubation.

Net mineralisation = (NH_4^+-N – initial NH_4^+-N) + (NO_3^--N – initial NO_3^--N). (Legend: R=Rothamsted soil, W=Woburn soil, S=SAC soil, 0=zero lime treatment, L=low lime treatment, M= medium lime treatment, H=high lime treatment).

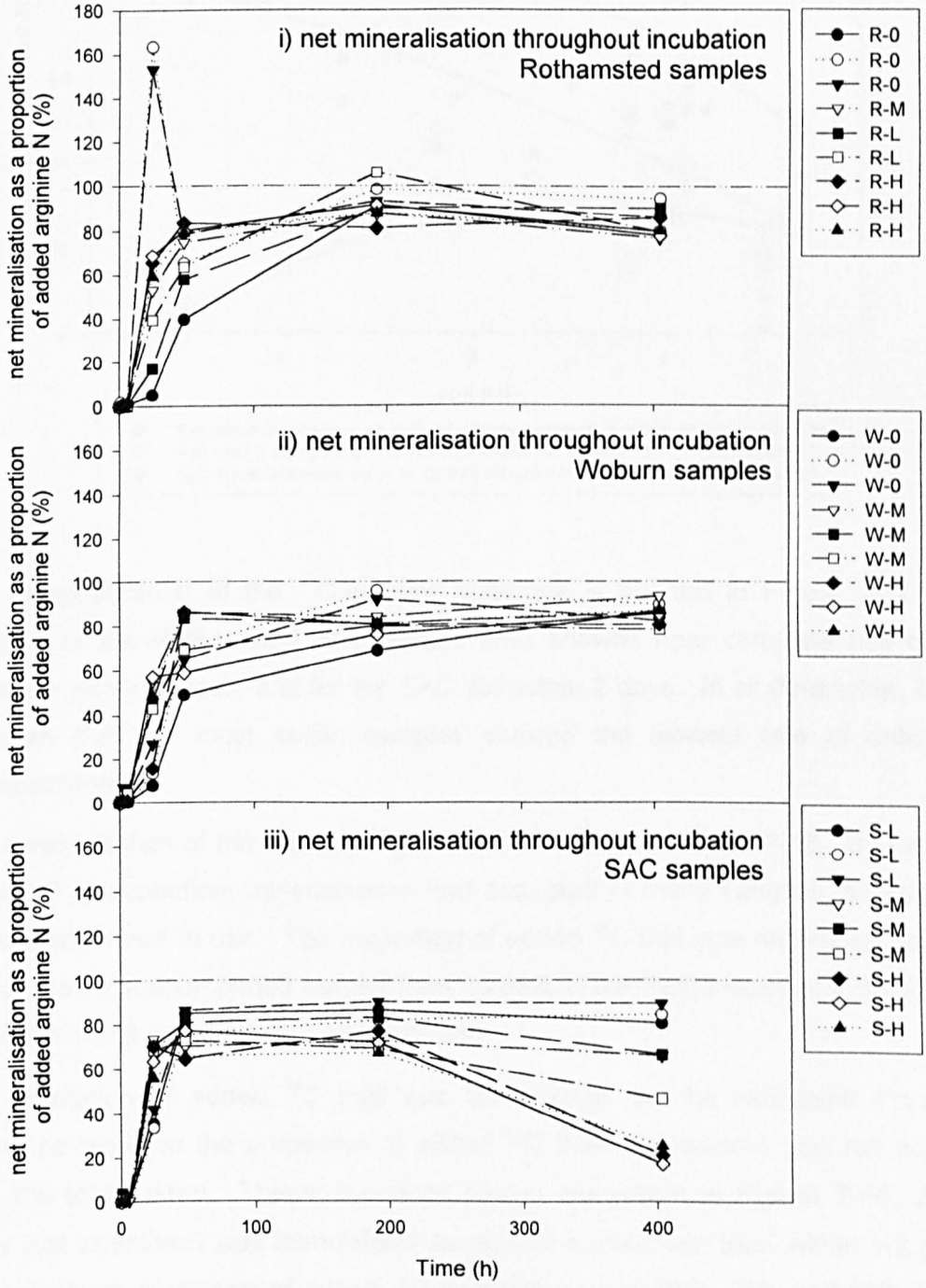
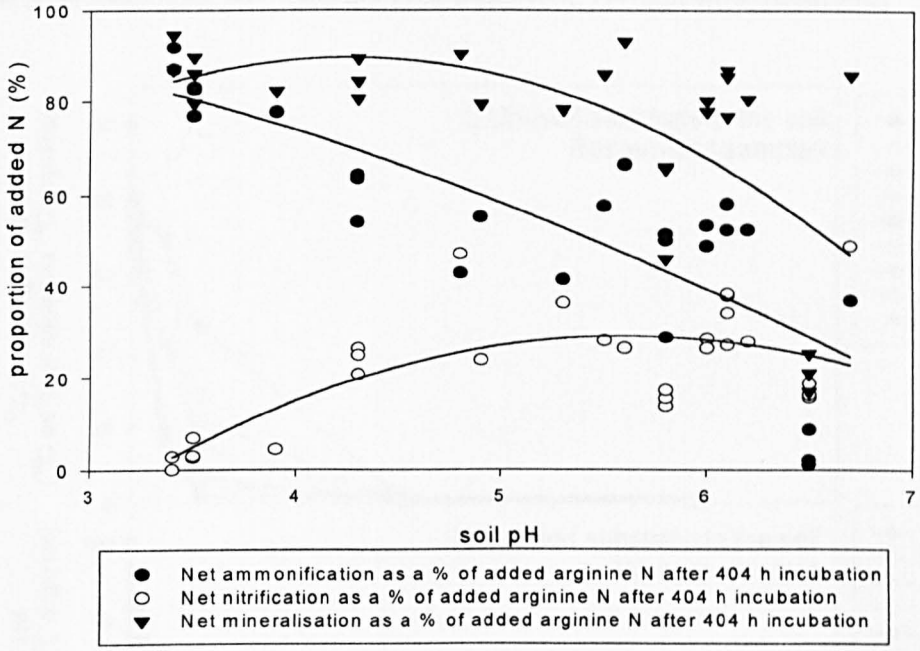


Figure 7-13 Effect of soil pH on the fate of arginine-N at the end of the incubation period across all samples



The disappearance of the ^{14}C-arginine substrate is charted in Figure 7-14. All samples of the Rothamsted and Woburn soils showed near complete use of the substrate within 1 week, and for the SAC soil within 2 days. In all three soils, it can be seen that the most acidic samples showed the slowest rate of substrate disappearance.

The mineralisation of the arginine-^{14}C to $^{14}CO_2$ is shown in Figure 7-15. By the end of the 17 d incubation, mineralisation had saturated in many samples, although in some it continued to rise. The proportion of added ^{14}C that was mineralised by the end of the incubation period ranged from 53-68% in the Rothamsted soil, 53-67% in the Woburn soil and 61-65% in the SAC soil.

The proportion of added ^{14}C that was immobilised can be calculated from the difference between the proportion of added ^{14}C that was respired plus left unused from the total added. These calculated figures are shown in Figure 7-16, which show that arginine-C was immobilised quickly, to a maximum level within 1-2 days. The maximum proportion of added ^{14}C immobilised was 59%, 74% and 65% in the Rothamsted, Woburn and SAC soils respectively. Once the maximum immobilisation

Figure 7-14 Unused ^{14}C-arginine substrate remaining in the soil over the incubation period

^{14}C remaining in the soil, as extracted by 0.5 M K_2SO_4, as a percentage of added ^{14}C. (Legend: R=Rothamsted soil, W=Woburn soil, S=SAC soil, 0=zero lime treatment, L=low lime treatment, M= medium lime treatment, H=high lime treatment).

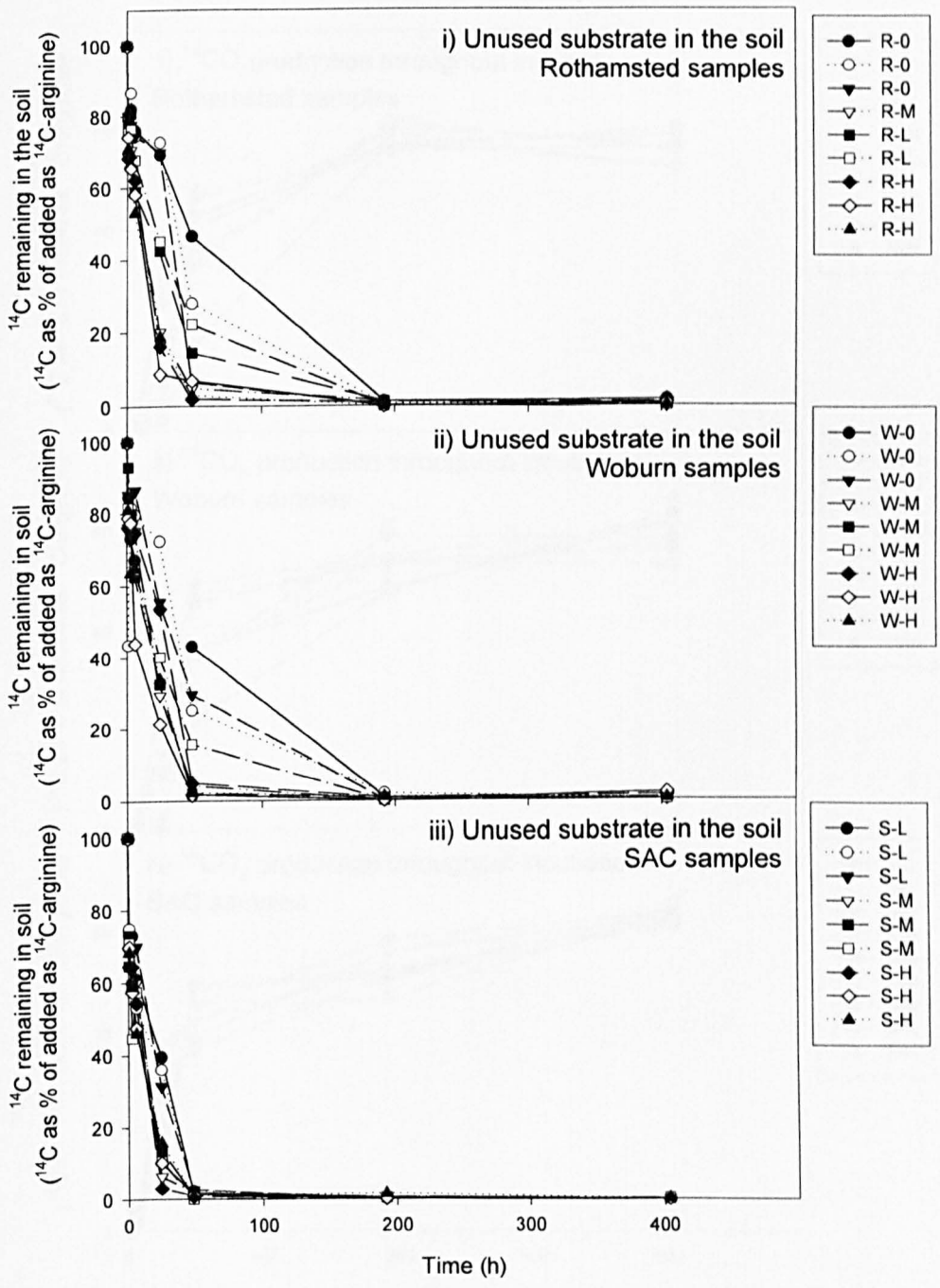


Figure 7-15 Mineralisation of arginine-C over the incubation period.

$^{14}CO_2$ trapped in NaOH, as a percentage of added ^{14}C. (Legend: R=Rothamsted soil, W=Woburn soil, S=SAC soil, 0=zero lime treatment, L=low lime treatment, M= medium lime treatment, H=high lime treatment).

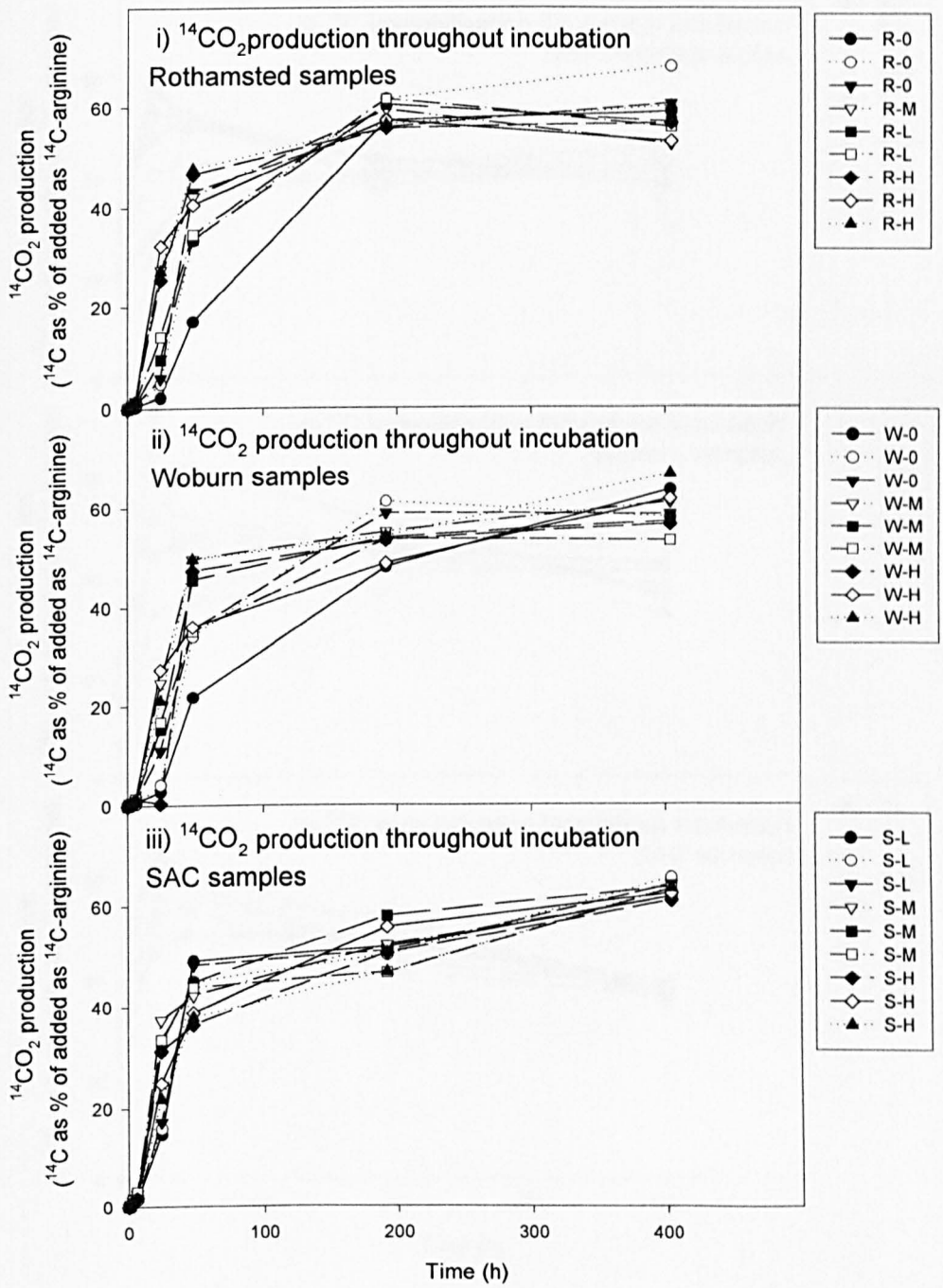
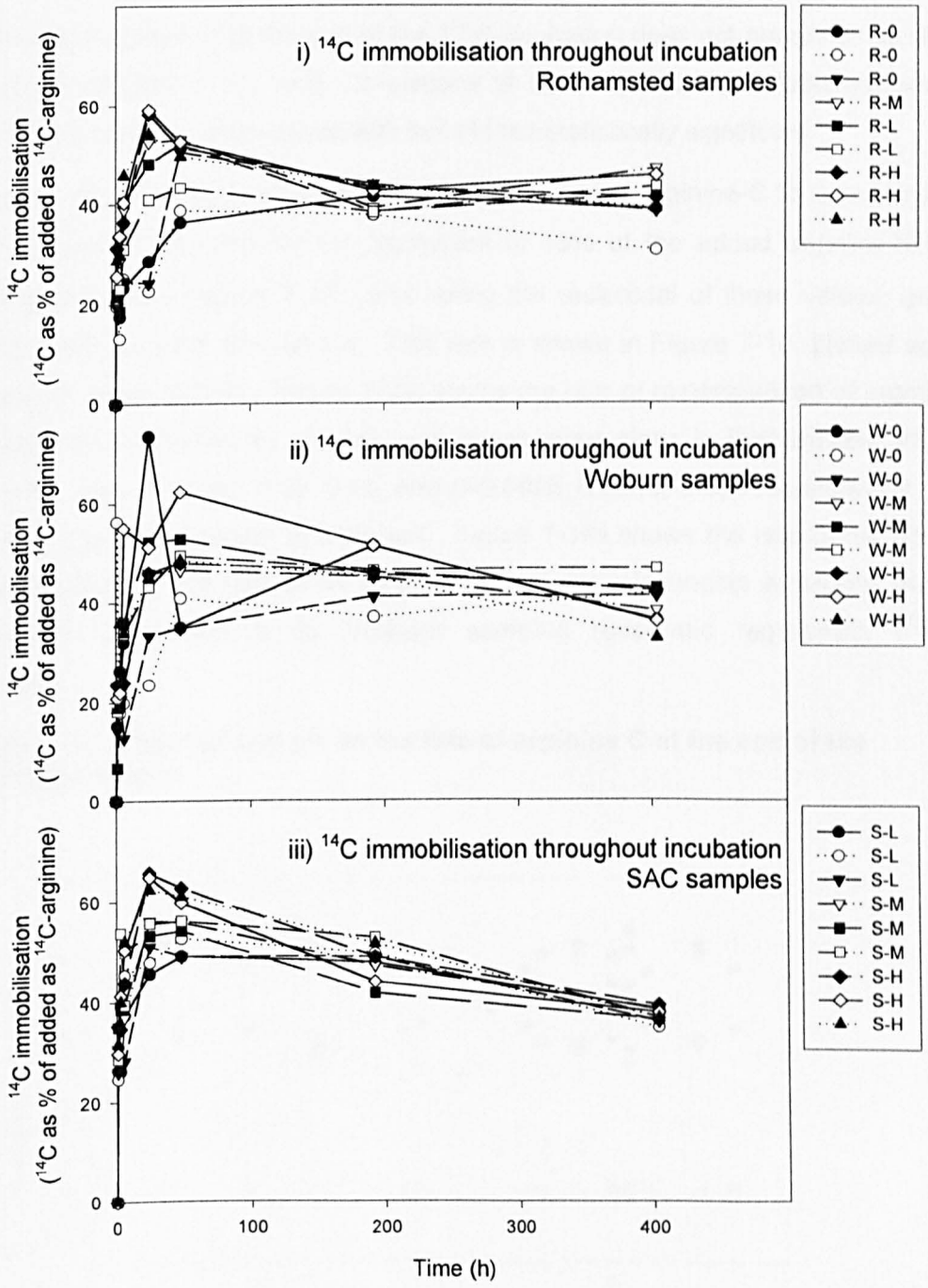


Figure 7-16 Immobilisation of arginine C over the incubation period.

% of added ^{14}C immobilised measured as 100-(% of added ^{14}C respired + % of added ^{14}C unused in soil). (Legend: R=Rothamsted soil, W=Woburn soil, S=SAC soil, 0=zero lime treatment, L=low lime treatment, M= medium lime treatment, H=high lime treatment).



levels were reached, immobilisation of ^{14}C reduced to the 8 day sampling, and then either continued to decrease or rose again slightly to the levels of immobilisation seen at the end of the experiment, on the 17th day. The proportion of added ^{14}C that was immobilised at the end of the experiment ranged from 31-47%, 33-46% and 35-39% for the Rothamsted, Woburn and SAC soils respectively.

The fate of arginine-C at the end of the 17 d incubation does not appear to be related to soil pH (Figure 7-17), with correlations of the proportion of added arginine ^{14}C respired, unused or immobilised with soil pH not statistically significant.

Reading off the time taken (in h) for 15% of the added arginine-C to be mineralised (from Figure 7-15) and for the equivalent of 15% of the added arginine-N to be mineralised (from Figure 7-12), and taking the reciprocal of these values, gives a rate of mineralisation of C and N. This rate is shown in Figure 7-18, plotted against soil pH for each sample. Figure 7-18i shows the rate of mineralisation of arginine-C is significantly affected by soil pH, with linear regressions in Rothamsted, Woburn and SAC soils (r^2= 0.84, 0.85, 0.50, and p=0.0005, 0.0010, 0.034 respectively). Note that the slopes are similar in each soil. Figure 7-18ii shows the rate of net nitrogen mineralisation versus soil pH for each sample. Little relationship is evident; the only significant correlation is for Woburn samples (quadratic regression, r^2=0.75, p=0.015).

Figure 7-17 Effect of soil pH on the fate of arginine C at the end of the incubation period

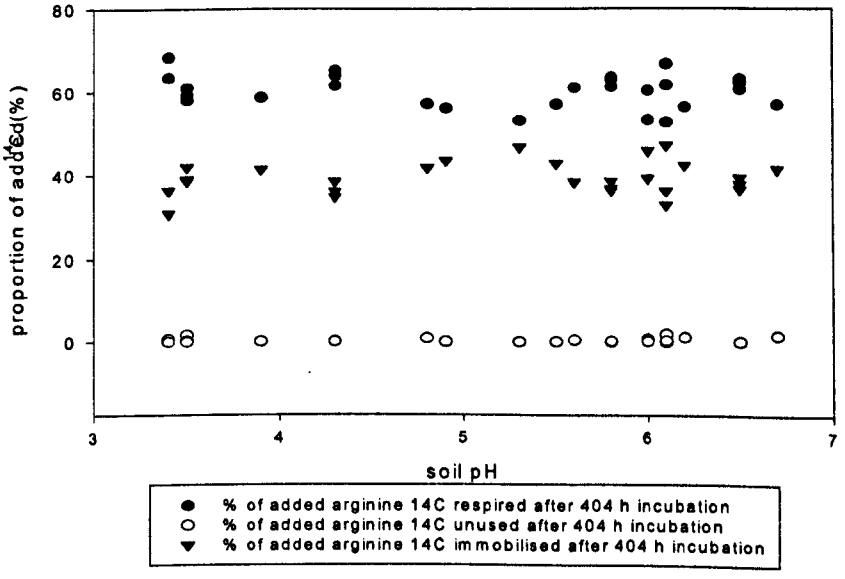
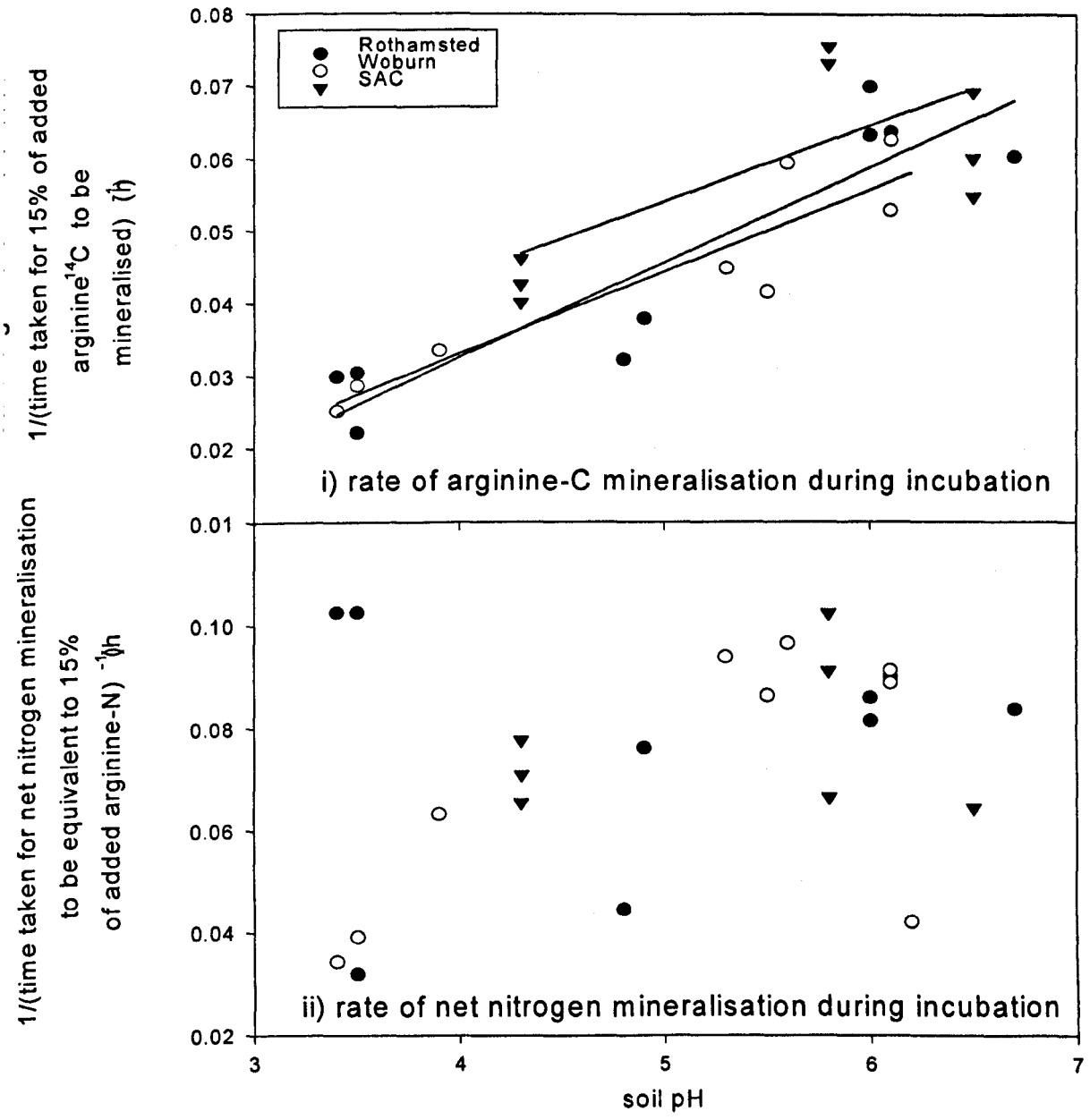


Figure 7-18 Relationships between soil pH and the rate of mineralisation of arginine (i) C and (ii) N



7.4 Discussion

7.4.1 Sample pHs

The samples provided an opportunity to study microbially-mediated processes and soil characteristics within a range of 3.2, 2.9 and 2.25 pH units in the Rothamsted, Woburn and SAC soils respectively. It should be noted that pH values obtained were in a 1:1 (w/v) 0.01 M $CaCl_2$ supernatant, and the salt effect may give values approximately 0.5 units lower than those obtained in a water suspension (Rowell, 1994). It would also be of interest to examine the effect of pH on N cycling processes over a wider pH range, extending the upper pH limit above neutrality.

7.4.2 Total C, N, soil C:N and soil moisture

Total C and N rise with increasing soil pH in the SAC soil, suggesting a higher primary productivity resulting in more organic inputs to the soil. However, if organic matter breakdown were impaired at low pH, one would expect higher organic matter levels at low pH, the reverse of what was observed in this soil. The Rothamsted and Woburn soils possess much lower organic matter (C and N) contents and do not show any significant trend with pH. If the mineralisation of C and N were affected differently by soil pH, one would expect to find a trend in the C:N ratio with soil pH; this is not borne out by the data here. It is likely that biomass production and decomposition are in balance with organic inputs, resulting in no net build up. It is probable that the primary production of the soils decreases with lower pH, although root turnover may increase. Further studies would be required to ascertain these balances of inputs with cycling rates. The importance of organic matter in determining soil moisture, a factor important to microbial activity, can be seen from the data (Figure 7-1).

7.4.3 Mineral and soluble organic N and C

Mineral N levels were extremely low in the Rothamsted and Woburn soils. This is probably due to the date of sampling (25th March), as significant winter leaching will have just occurred, yet mineralisation in the spring will not yet have boosted levels. Nevertheless, this demonstrates that these experimental plots have very low available nutrients. Mineral N levels were much higher in the SAC soil, possibly reflecting a later sampling date, its higher organic matter status, the fact that it had received 70 kg N in fertiliser the previous year (when mown for hay) and that it was down to pasture in the year of sampling. The SAC soil shows a trend of increasing

NO_3^- concentrations with increasing pH, suggesting that nitrification may be inhibited at low pH, but more data points are needed to confirm this. Previous work on sampling of the Craibstone plots (SAC soil) by Meharg and Killham (1990) showed similar results in that available N in the soil solution was pH dependent, with NH_4^+ and NO_3^- concentrations increasing with increasing pH.

SON levels in the Rothamsted and Woburn soils were much higher than mineral N levels, which is surprising as typically both soluble organic and inorganic N levels of the same magnitude, as occurred in the SAC soil (Murphy et al., 2000). SON showed little consistent trend with soil pH among the three soil types tested.

Liming is known to increase the solubility of organic matter in soils, as it is closely and positively correlated with soil solution pH (Nyborg and Hoyt, 1978; Smolander et al., 1994; Riffaldi et al., 1996; Curtin et al., 1998; Tyler and Olsson, 2001). However, the organic matter released by liming that is available for microbial use is quickly mineralised or leached, and so the effect is temporary. The observation that SOC increased with decreasing pH in the Rothamsted and Woburn soils is unsurprising, because catchments of low pH soil generally have high DOC levels in soil and drainage waters, however, this DOC is generally of high molecular weight and is recalcitrant.

The SOC at lower pHs may not be available to the microbes because:

a) metabolic functions are impaired as a direct result of the soil pH,

b) the SOC may be more recalcitrant due to chelation with toxic metals, such as Al causing accumulation (Gonzalez-Prieto and Carballas, 1991 and Gonzalez-Prieto et al., 1992) (exchangeable Al is highly positively correlated with DOC in these soils).

c) The SOC may be more recalcitrant and of high molecular weight, as the inputs of vegetation in the lower pH soils may be of lower quality (e.g. higher lignin content), resulting in SOC higher in phenolics.

Possibility (a) is supported over (b) by work by Sanders (1983), which suggests that at higher soil pHs a greater proportion of trace metals (Cu, Mn, Zn, Ni, Co) were complexed on soluble organic matter and as soil pH falls a greater proportion of the trace metals are present as free ions.

The SAC soil shows the opposite trend of increasing SOC with increasing pH. This may be because of the higher levels of total organic matter (C and N). As a result, the SOC fraction may not be as 'polluted' as toxic elements may have more sites on

which to bind, the concentration of the toxic elements may not be so high on the SOC fraction at low pHs. Also, the rotational vegetation management may have resulted in residue inputs of a higher quality, avoiding the build up of recalcitrant SOC at lower pHs. The SAC soil showed a (non-significant) negative correlation of exchangeable Al with SOC. Further investigation of the make up of the SOC would be required to draw conclusions on these hypotheses.

7.4.4 Soil basal respiration

Soil basal respiration strongly decreased with soil pH (Figure 7-5iii), concurring with Adams and Adams (1983) who showed basal CO_2 production increased with soil pH. That correlations of basal respiration with SOC were negative in the Rothamsted and Woburn soils demonstrates the recalcitrance of the SOC pool that has accumulated in these soils. However, the relationship is positive in the SAC soil. Although there is less SOC available to the SMC at higher pHs in the Rothamsted and Woburn soils, they are producing more CO_2. This implies the SOC at low pHs is of limited use as a substrate to the microbial population in these soils.

7.4.5 Glucose substrate induced respiration

The Substrate Induced Respiration (SIR) method of estimating soil microbial activity is based on stimulating the microbial biomass, most of which is in a dormant state with a low rate of respiration, by adding an easily decomposable C substrate. The glucose-SIR method rapidly increases microbial respiration to a maximum level, which remains constant for over 4h. The magnitude of the microbial SIR response over 0-6 h is characteristic of the initial microbial community in soil before selective growth of the organisms occurs in response to the added substrates (Anderson and Domsch, 1973, 1978). The initial maximum respiration rate is therefore proportional to the size of the original soil microbial biomass. Coody et al. (1986) showed that CO_2 evolution was shown to be a poor indicator of the rapid disappearance of glucose in soils, because of the production of transitory intermediates (e.g. maltose), exuded into the growth medium. The optimal time for CO_2 measurement in glucose SIR is between 0.5 and 2.5 h after substrate addition and it is unnecessary to make any correction for CO_2 dissolved in the soil solution for soils below pH 6.5 (Lin and Brookes, 1999a).

Glucose-C mineralisation rates show an optima at intermediate pHs (5.0, 4.5 and 5.1 in Rothamsted, Woburn and SAC soils respectively). Why rates should decline at higher pHs while the SMB-C remains constant (Figure 7-5iv) is not known; a more

thorough investigation of glucose-C allocation within the microbial cells would need to be made to resolve this. The likely cause is an increase in use in non-catabolic metabolic functions at higher pHs.

7.4.6 Urea substrate induced respiration

Soil ureases are microbial enzymes that can persist outside the cell in the soil because they are highly resistant to environmental degradation (McNaughton et al., 1997). They hydrolyse urea to inorganic compounds, allowing microbial populations to grow to levels leading to significant mineralisation of organic matter, which may take from several hours to a few days (McNaughton et al., 1997). Urease activity increases with the level of above ground grazer activity at a site (McNaughton et al., 1997), and as the samples taken were from long term ungrazed sites, this may account for low levels of urea-C mineralisation (c. 5% in 3 h incubation) in Rothamsted and Woburn soils. The low proportion of added urea mineralised in 3 h may also have been because of the high concentration that was added. However, urease activity was much higher in the SAC soil, which was in the first year of two consecutive years at pasture in the 8 year rotation (Table 7-1). It is not known why urease activity was much higher in this soil as this pasture was topped to simulate grazing, but not actually grazed.

Urea SIR also shows a convex quadratic relationship between urea-C mineralisation rate and soil pH. Again, more data is needed to fully explain this, but the falling off at higher pHs is likely to be related to use of a higher proportion of urea-C in non-catabolic cellular functions.

7.4.7 Amino acid mineralisation

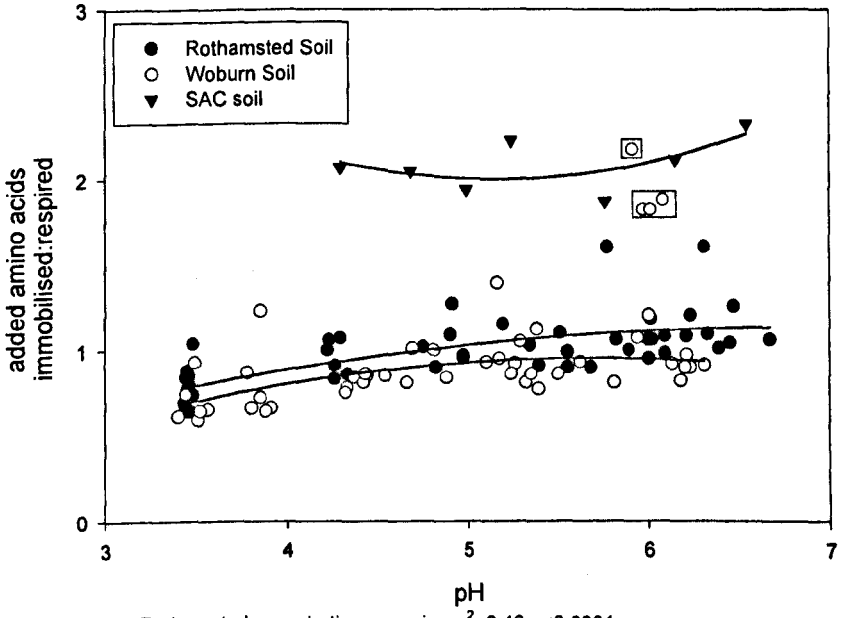
The rate of amino acid-C mineralisation appears to be strongly affected by soil pH (Figure 7-4i), with optima at pH 5.8, 5.5 and 5.2 in Rothamsted, Woburn and SAC soils respectively. This optima can be explained by Figure 7-4 which indicates that the rate of evolution of amino acid-C as CO_2 is limited at low pHs by slow uptake of amino acids from the free soil pool (indicated by Figure 7-4iii), and is limited at higher pHs as a higher proportion of the amino acids taken up are utilised structurally (suggested by Figure 7-4iv), and fewer are used catabolically (Figure 7-4ii). During the incubation period 0-6 h, the assay measures the intrinsic activity of the initial SMB, as there has been insufficient time for significant increases in population levels (Anderson and Domsch, 1978). Amino acid mineralisation rates of over approximately 0.17 h^{-1} (1/time taken for 15% of added amino acid-C to be respired as

CO_2) are an indication of the potential activity of the initial microbial population. Most of the observed rates fall above this level, with only the samples of the lowest and highest pHs mineralising below this rate. The rate of amino acid-C mineralisation was negatively related to SOC levels in Rothamsted and Woburn soils.

The ratio of amino acid-C immobilised to that respired by the end of the 48 h incubation plotted against soil pH is shown in Figure 7-19 below. If one accepts that the microbial population is functioning at optimal conditions when this ratio is at a maximum, the optimal pH in the Rothamsted soil is 6.8 and in the Woburn soil is 5.7. The outlying data points (n=4) for the Woburn soil make the quadratic regression for that soil a slightly concave curve if included, rather than a convex one. The concave curve shown for the SAC soil samples is unexpected, and possibly due to the low number of data points available.

Figure 7-19 Relationship between soil pH and the ratio of amino acids immobilised:respired after 48 h incubation.

[4 boxed Woburn soil data points are excluded from the regression analysis.]



Rothamsted - quadratic regression, r^2=0.46 p<0.0001
Woburn - quadratic regression (excluding 4 outliers), r^2=0.30 p=0.0008
SAC - quadratic regression, r^2=0.37 p=0.3961

7.4.8 SMB C and N

The accuracy of the FE method for determining SMB-C depends on the size of the difference in DOC between fumigated and unfumigated samples. Kaiser et al., (1992) found the quotient of fumigated to non-fumigated extractable C to be on average 3.2 (range 2.01 - 6.55), with smaller values in two peat soils. Similar values were obtained here; the ratios of fumigated:non-fumigated extractable C were on average 3.08 (range 1.42-7.14), 2.45 (1.39-6.17) and 2.68 (2.27-2.92) in the Rothamsted, Woburn and SAC soils respectively.

K_{EC} and K_{EN} values of 0.45 were used in SMB-C and N_{TDN} calculations (Joergensen and Brookes, 1990; Kaiser et al., 1992). K factors for the FE method might be expected to vary, by a greater degree than those for the FI method (Brookes et al., 1985), because of variation in solubilisation of components released during fumigation, and variation in interaction of soluble components with the soil during extraction (Sparling and Zhu, 1993). K factors may therefore be influenced by soil texture (Kaiser et al., 1992) and may vary by around 15% between soil types (Voroney et al., 1991).

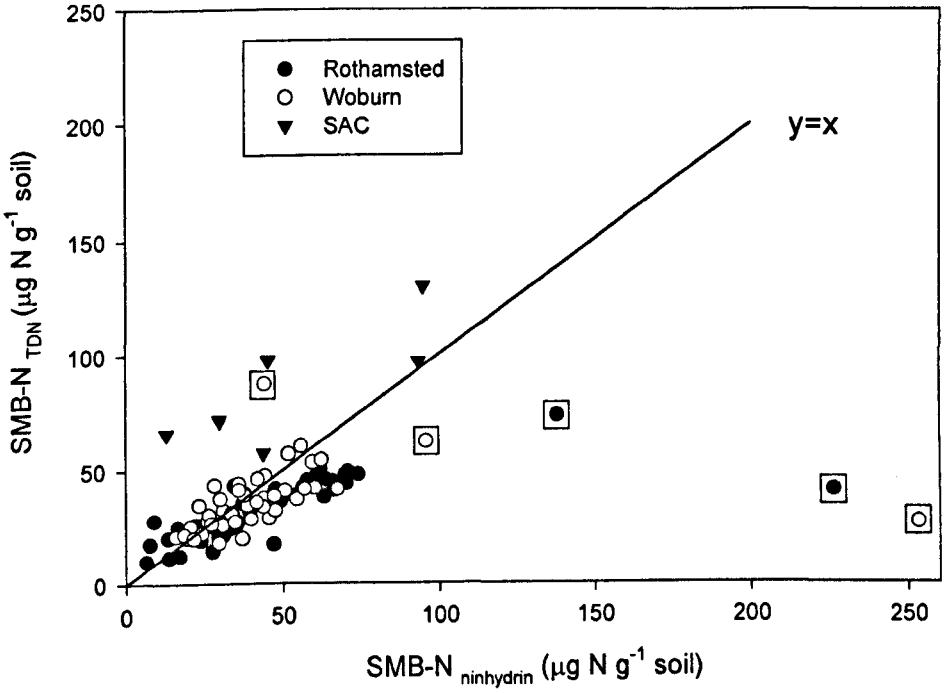
Although FE can be used over the whole range of soil pH (Vance et al., 1987), it is possible that extraction efficiencies vary between soil types and within a soil type in samples of differing pH's. It may therefore have created experimental artefacts to use the same K values for all samples examined here. However, as the FI method is known not to be very accurate in acidic soils, further work on calibrating K values by comparison with microscopic methods would be required to obtain an unbiased baseline.

Microbial C ranged from 85-469 $\mu g\ C\ g^{-1}$, which is comparable to the 50-421 $\mu g\ C\ g^{-1}$ determined by Sparling and Zhu (1993). SMB-C increased linearly with increasing pH in Rothamsted and Woburn soils. The lack of evidence of this trend in the SAC soil may be due to insufficient data points, although Meharg and Killham's (1990) work on these plots also showed that biomass C did not vary greatly with soil pH. They did find that the soil of the lowest pH had a SMB-C 60% lower than that in the other soils, but they used the FI method, which underestimates biomass C at low soil pH.

SMB-C was negatively correlated with DOC, indicating that at high SMB-C levels, the organisms had successfully scavenged all available DOC and/or at low SMB-C levels conditions resulted in the DOC being less bio-available, allowing the pool to accumulate.

Here a biomass N / NPC flush ratio of 6.5 was used to determine biomass N (Sparling and Zhu, 1993). This was higher than values of 5 determined by Joergensen and Brookes (1990) and 5.1 reported by Amato and Ladd (1988). In this study the value of 6.5 gave a better match with the SMB-N_{TDN} values.

Figure 7-20 Relationship between biomass N determined by measurement of ninhydrin positive compounds and total dissolved nitrogen



Boxed data points are excluded from the following linear regressions (which are not shown in Figure 7-20):

Soil	Linear regression equation	r^2	P value
Rothamsted	SMB-N_{TDN}=10.32 + (0.5239 x SMB-N_{NPC})	0.76	<0.0001
Woburn	SMB-N_{TDN}=12.97 + (0.5757 x SMB-N_{NPC})	0.52	<0.0001
SAC	SMB-N_{TDN}= 52.77 + (0.6320 x SMB-N_{NPC})	0.62	0.0605

Figure 7-20 above shows that in general, the two methods of determining SMB-N corroborate reasonably, with linear regression equations (excluding outliers) for Rothamsted and Woburn soils having slopes close to 0.5, and similar intersects.

SMB-N_{TDN} values for the SAC soil are, in general, much higher than for the SMB-N_{NPC} method. This may be related to the SAC soil having high background levels of TDN.

The flush of NPC in all three soils ranged from 1-49 $\mu g\ N\ g^{-1}$, which falls within the range 2-60 $\mu g\ N\ g^{-1}$ found by Joergensen and Brookes (1990). Microbial N ranged from 7-253 $\mu g\ N\ g^{-1}$ for the NPC method and 10-130 $\mu g\ N\ g^{-1}$ for the TDN method. These values are a much greater range than the 7-65 $\mu g\ N\ g^{-1}$ determined by Sparling and Zhu (1993), although they were examining nutrient poor Australian soils.

Both methods of determining SMB-N show a trend of increasing SMB-N with increasing pH in all soils (except Woburn soil, SMB-N_{NPC}). These results generally concur with Adams and Adams (1983) who found that lime application increased SMB C and N.

Despite reasonable data for both SMB-C and SMB-N, calculated SMB C:N ratios gave very dubious results, with many values far higher than is feasible, especially in the SMB-N_{NPC} method. This suggests that, for many samples, either the SMB-C estimate is too high or the SMB-N estimate is too low. The out of range values show no consistent trend with soil pH (Figure 7-6iii and iv). It is therefore unlikely that this is an error due to using the same extraction efficiency values ($K_{EN\&C}$) for all samples, when it might be the case that $K_{EN\&C}$ varies with soil pH. Cited SMB C:N ratios include 4.1 (Joergensen and Brookes, 1990), 6.7 (Amato and Ladd, 1988), 6 (Shen et al., 1984), 4.3 or 12 depending on the method used to calculate microbial C (whether a control was taken into account using the FI method) (Sparling and Zhu, 1993). One would expect the SMB C:N to increase at low pHs due to a community shift from bacterially dominated communities at higher pHs through actinomycetes to fungal domination at low pH (Haynes, 1986). This is because the C:N ratio of micro organisms is not constant; in fungi it is 5:1 to 15:1 and in bacteria it is 3:1 to 5:1 (Paul and Clark, 1989). Adams and Adams (1983) found a SMB C:N of 4.2 below pH 4.5 and SMB C:N of 5.8 in pH > 4.5, in a study of limed plots, but they note that low values may be an artefact of FI method not being well suited to low pH soil.

These problematic microbial C:N results may be of several sources. $CHCl_3$ may cause an increase in solubility of organic matter, making the 'flush' of C or N larger than that attributable to microbial origin. The lysis of cells caused by the chloroform fumigation causes the cell contents (cytoplasm) to spill out into the soil solution, while the cell walls can remain adhered to soil particles. These cell walls may not be

extracted in the procedure, so the difference in C and N contents between the cytoplasmic and wall components may therefore introduce a procedural bias.

Figure 7-7i and iii show that the ratios of biomass C:soil organic C and biomass N:soil organic N increase with increasing pH in the Rothamsted and Woburn soils, in agreement with Anderson and Domsch (1993), indicating an increase in availability of the C and N present and more efficient substrate utilisation (Kaiser et al., 1992). These ratios are indicators of the condition, quality and health of the soil (Doran and Parkin, 1996).

7.4.9 Net mineralisation

These results show very little relationship of net nitrification, net ammonification, net mineralisation, and net change in SON over the 30 d incubation to soil pH (Figure 7-8). Where any trend is evident, it is the reverse of what would be expected, with very slight increases in rates of the above processes with reducing soil pH. It would be of interest to perform measurements using better methods in the field, and especially of interest to measure gross rates, which often vary greatly from net rates (Goulding, 2000). However, this would necessitate the use of ^{15}N isotope labelling techniques, which were not available in this study.

7.4.10 Arginine mineralisation

Previous studies using arginine mineralisation as an indicator of microbial populations and activity have used short incubations, and found that the rate of arginine ammonification remains linear for 6 h, i.e. the SMB mineralises arginine without changing their activity during that period (Alef and Kleiner 1986; Lin and Brookes, 1999b). The results of Lin and Brookes (1999b) showed that ammonification rates ranged from 0.1 to 17.1 $\mu g\ NH_4^+\text{-}N\ g^{-1}\ h^{-1}$, nitrification ranged from 0 - 6.7 $\mu g\ NO_3^-\text{-}N\ g^{-1}\ h^{-1}$ and total inorganic N production ranged from 0.1-23.7 $\mu g\ g^{-1}\ h^{-1}$. Other studies have shown ammonification ranges of 0.51 - 13 $\mu g\ NH_4^+\text{-}N\ g^{-1}\ h^{-1}$ (Alef and Kleiner, 1987, in 34 soils) and 0.03 to 2.71 $\mu g\ NH_4^+\text{-}N\ g^{-1}\ h^{-1}$ (Kaiser et al., 1992); neither study took account of nitrification. In the current experiment, ammonification rates over the first 6 h were negligible, and in several cases negative (i.e. immobilisation occurred). Average ammonification rates over the first 25 h period ranged from 0.3 to 9.0 $\mu g\ NH_4^+\text{-}N\ g^{-1}\ h^{-1}$.

Nitrification was boosted in most samples in the first 6 hours following arginine addition, but after that time net nitrification did not proceed to any great extent for several days (Figure 7-11). Rates then picked up, presumably due to population

increases in nitrifier populations in all samples of the SAC soil, and to a greater extent in the less acid samples of Rothamsted and Woburn soils.

The calculated net mineralisation figures (Figure 7-12) are somewhat deficient, in that the control (i.e. unamended) NH_4^+ and NO_3^- levels were only measured at the start and end of the incubation. Therefore, net mineralisation here only deducts (mineral-N at time t in amended sample – mineral-N at time 0), rather than (mineral-N at time t in amended sample – mineral-N at time t in control), as it should strictly be. This was due to constraints of one person being able to deal with numbers of replicate samples within the time period available. In the SAC soil, the low pH treatment samples reached close to complete N mineralisation and levels remained static. However, with increasing pH of the samples, there was an increasing fall in mineral N levels. This must be either due to immobilisation of the mineral N during a period of microbial growth, or denitrification causing loss from the mineral N pool. Why this should happen in the SAC soil and not in Rothamsted or Woburn is unknown. Higher levels of organic nutrients could have permitted more denitrification in the SAC soil than the other two.

The large peak in net mineralisation at the 1 d reading in two of the acid Rothamsted samples went up to around 160% of added arginine-N, indicating a sudden peak in net mineralisation of indigenous organic N. This suggests that the added arginine somehow made a portion of indigenous organic N accessible to microbes. Why this occurred only in these two samples is not known. The mineral-N released was immobilised or denitrified within 1 d. Gonzalez-Prieto et al. (1992) also found that addition of glycine stimulated microbial oxidation of unhumified organic matter in acid soils.

Figure 7-13 shows that most samples (with the exception of medium and high pH treatments of the SAC soil) reached a high level (77-95 %) of net N mineralisation by the end of the incubation period. There was little trend of net N mineralisation with soil pH. Net ammonification decreased with increasing pH, because at higher pHs more was transformed to NO_3^- via nitrification, which was positively correlated with soil pH.

Figure 7-16 shows that immobilisation reached a maximum, and then dropped, suggesting that some of the ^{14}C that was taken up and held within the cell was respired at a later date. Either the ^{14}C took time to work its way through to catabolic pathways, or this represents turnover of microbial-C by death or predation of microbes, the biomass of which were subsequently mineralised. There appeared to

be no soil pH effect on the partitioning of arginine-C (unused, respired or immobilised) at the end of the incubation time (Figure 7-17). However, the rate of arginine-C mineralisation (1/the time taken for 15% of added arginine-C to be mineralised), was strongly, linearly, and positively related to soil pH (Figure 7-18i). Figure 7-18ii shows little relationship between net N mineralisation and pH. This may be because N mineralisation measurements were net, not gross (the C mineralisation measurement was gross, and a good relationship was evident there). Alternatively, this may be because some samples showed a brief period of net N immobilisation (Figure 7-12), so the time for net mineralisation to reach the equivalent of 15% of added N was very variable. Lin and Brookes (1999b) concluded that arginine mineralisation can not be used as an estimate of microbial biomass in soils which are immobilising inorganic N during the early phase of substrate decomposition (which occurred in their rye grass amended and fumigated soils). Alternatively, it may be that there is no relationship with soil pH, as observed by Alef and Kleiner (1986), Alef et al., (1988) and Lin and Brookes (1999b).

Lin and Brookes (1999b) cite evidence that measurement of NH_4^+-N by the automated indophenol blue procedure was interfered with by presence of amino acids in KCl solution. In the work of Lin and Brookes (1999b) an increased amount of arginine gave lower levels of NH_4^+-N when levels were in fact static, and no NH_4^+-N could be detected at levels of 2000 mg arginine l^{-1} in KCl solution. Because of this, they concluded that best results were obtained using a small amount of arginine and a longer incubation time. If measurement of NH_4^+-N was disrupted by high levels of arginine in the early stages of the incubation, this may account for the lack of correlation discussed above.

In theory, the net N mineralisation from arginine use should be related to the C:N of the SMB, as organic N is taken in by the cell and N in excess of the C:N ratio of the cell is exuded as mineral N (Barraclough, 1997). Examination of this relationship was not possible in all soils due to inaccurate SNB C:N values, although it was attempted on the Woburn soil, as the SMB $C:N_{TDN}$ values all fall within a feasible range in this soil. The model of Barraclough (1997) is:

$P=(1-S.E/B)$

where:

P is the proportion of N in the substrate that is mineralised, S is the C:N of the substrate (here 1.29), B is the C:N of the assimilating organism and E is the assimilation efficiency of the organism (ratio of assimilated C:C in the substrate).

The fit of the model is shown in Table 7-9 below, which shows that the model fits exactly for some samples, and the maximum deviation from the model is 10%. This demonstrates the important controlling influence of substrate quality and the SMB C:N on net mineralisation activity.

Table 7-9 Fitting data to the model of Barraclough (1997) to predict net arginine-N mineralisation for Woburn soil.

Plot	Lime treatment	Core no.	SMB C:N_{TDN}	Proportion of C assimilated[1]	Model prediction of proportion of N mineralised	Actual equivalent proportion of added N net mineralised[1]	Model prediction minus actual value
33b	0	1	4.42	0.4023	0.883	0.869	0.01
33b	0	2	4.86	0.3410	0.909	0.895	0.01
55a	0	2	6.42	0.3742	0.925	0.821	0.10
61a	m	4	5.16	0.4674	0.883	0.931	-0.05
61a	m	6	6.26	0.4451	0.908	0.859	0.05
42a	m	6	4.32	0.4968	0.852	0.781	0.07
48a	h	1	4.6	0.4229	0.881	0.804	0.08
48a	h	6	4.03	0.4639	0.852	0.854	0.00
59b	h	5	4.24	0.6279	0.809	0.867	-0.06

[1] at the end of the incubation period (17 d).

7.4.11 Linking measurements of microbial activity/biomass

The following parameters measured on the samples examined in this chapter give an indication of the size and/or activity of the soil microbial biomass: amino acid mineralisation rate, glucose SIR, urea SIR, SMB-C (FE method), SMB-N (FE method, two extraction measurements), arginine ammonification and basal respiration. As arginine ammonification was only performed on 27 samples across the three soil types, it must be noted that the following correlations involving arginine mineralisation only have n=9 for each soil type, all other correlations have n=48 for Rothamsted and Woburn soils, n=7 for SAC soil. Amino acid-C mineralisation rate was not found to correlate significantly with arginine ammonification, which was consistent with the findings of Jones (1999).

Table 7-10 shows which indicators of microbial biomass or activity were well correlated with each other. Amino acid-C mineralisation rate was well correlated with the rate of urea SIR and basal respiration in all three soils. SMB-C was well correlated with SMB-N_{TDN} and basal respiration and SMB-N_{TDN} was well correlated

with basal respiration in Rothamsted and Woburn soils. It should be noted that this correlation analysis will not necessarily have detected non-linear relationships.

Table 7-11 shows the correlations between indicators of microbial biomass or activity and indigenous substrate availability. Amino acid-C mineralisation rate, urea SIR and SMB-C were positively related to total C in Rothamsted and Woburn soils. With the exception of glucose SIR, most indexes of microbial biomass/activity in most soils showed reasonable and negative correlations with SOC. This indicates that high levels of microbial populations/activity, the available soluble organic C substrates are being mopped up and kept low. Again, it should be noted that this correlation analysis will not necessarily have detected non-linear relationships.

Table 7-10 Linear correlation matrix of microbial biomass/activity indicators.

	Amino acid mineralisation[1]	Glucose SIR[2]	Urea SIR[3]	SMB-C (F-E)	SMB-N_{NPC}(F-E)	SMB-N_{TDN}(F-E)	Basal Respiration	Arginine-C mineralisation[4]
Glucose SIR[2]	R* W n.s. S*							
Urea SIR[3]	R*** W*** S**	R n.s. W** S*						
SMB-C (F-E)	R*** W* S n.s.	R n.s. W* S n.s.❾	R*** W* S n.s.					
SMB-N_{NPC}(F-E)	R* W n.s. S n.s.	R n.s. W n.s. S n.s.	R n.s. W n.s. S n.s.	R* W n.s. S n.s.				
SMB-N_{TDN}(F-E)	R*** W n.s. S n.s.	R n.s. W** S n.s.	R* W n.s. S n.s.	R*** W*** S n.s.	R*** W n.s. S n.s.			
Basal respiration	R*** W* S**	R n.s. W*** S*❾	R** W n.s. S**	R*** W** S n.s.❾	R* W n.s. S n.s.	R** W*** S n.s.		
Arginine-C mineralisation[4]	R** W n.s. S n.s.	R n.s. W* S n.s.	R n.s. W n.s. S n.s.	R* W n.s. S***	R* W* S n.s.	R* W n.s. S**	R* W* S n.s.	
Arginine-N mineralisation[5]	R n.s. W n.s. S n.s.	R n.s. W n.s. S n.s. ❺❼	R n.s. W n.s. S n.s.	R n.s. W n.s. S n.s. ❺❻	R n.s. W* S n.s.	R n.s. W n.s. S n.s.	R n.s. W n.s. S n.s. ❶	R n.s. W** S n.s.

[Legend: R=Rothamsted soil, W=Woburn soil, S=SAC soil, n.s.= not significant (p>0.05), * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001]

[1] rate of amino acid-C mineralisation, 1/(time taken for 15% of added amino acid-C to be respired) (h^{-1}).

[2] proportion of added glucose-C respired in 3 h.

[3] proportion of added urea-C respired in 3 h.

[4] 1/(time taken for 15% of added arginine-C to be respired) (h^{-1})

[5] 1/(time taken for equivalent of 15% of added arginine-N to be net mineralised) (h^{-1})

The following symbols show where significant correlations were found by the following researchers:

❶ Alef and Kleiner, 1986.

❷ Lin and Brookes, 1999a.

❸ Kaiser et al., 1992.

❹ Alef and Kleiner, 1987a.

❺ Lin and Brookes, 1999b (acid soils removed from correlations)

❻ Alef and Kleiner, 1987b.

❼ Alef et al., 1988.

❽ Adams and Adams, 1983.

❾ Anderson and Joergensen, 1997.

Table 7-11 Correlation of soil microbial biomass/activity indicators with soil organic matter levels.

	a.a. minz'n[1]	Glucose SIR[2]	Urea SIR[3]	SMB-C (F-E)	SMB-N_{NPC}(F-E)	SMB-N_{TDN}(F-E)	Basal respiration	Arginine-C minz'n rate[4]	Arginine-N minz'n rate[5]
SOC	R***- W***- S n.s.-	R n.s.- W n.s.+ S*-❾	R***- W n.s.- S*-	R***- W***- S n.s.+ ❾	R***- W n.s.- S*+	R***- W***- S n.s.+	R***- W*- S*+ ❷	R **- W*- S n.s.+	R **- W*- ❶ S n.s.- ❺
SON	R***- W n.s.+ S n.s.-	R n.s.- W n.s.- S n.s.-	R***- W n.s.+ S n.s.-	R***- W n.s.- S n.s.-	R**- W n.s.+ S n.s.+	R***- W n.s.+ S n.s.-	R***- W n.s.+ S n.s.+	R **- W n.s.+ S***-	R **- W n.s.+ S n.s.-
Total C	R***+ W***+ S n.s.-	R n.s.+ W n.s.+ S n.s.- ❾	R***+ W**+ S n.s.-	R***+ W*+ S n.s.+ ❸❾	R n.s.+ W n.s.+ S*+	R n.s.+ W*+ S*+	R***+ W n.s.+ S n.s.+ ❷	R n.s.+ W n.s. S n.s.+	R n.s.+ ❸ W n.s.- ❹ S n.s.- ❺
Total N	R**+ W n.s.+ S n.s.-	R*+ W n.s.+ S n.s.-	R**+ W**+ S*-	R n.s.+ W n.s.+ ❸ S n.s.+ ❽	R n.s.- W n.s.- S n.s.+ ❽	R n.s.- W*+ S*+	R*+ W n.s.+ S n.s.+ ❷	R n.s. - W n.s.+ S n.s.+	R n.s.- ❸ W n.s.+ ❹ S n.s.- ❺

[Legend: R=Rothamsted soil, W=Woburn soil, S=SAC soil, n.s.= not significant (p>0.05), * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001, + = positively correlated, - = negatively correlated.]

[1] rate of amino acid-C mineralisation, 1/(time taken for 15% of added amino acid-C to be respired) (h^{-1}).

[2] proportion of added glucose-C respired in 3 h. [3] proportion of added urea-C respired in 3 h.

[4] 1/(time taken for 15% of added arginine-C to be respired) (h^{-1})

[5] 1/(time taken for equivalent of 15% of added arginine-N to be net mineralised) (h^{-1})

The following symbols show where significant correlations were found by the following researchers:

❶ Alef and Kleiner, 1986.
❷ Lin and Brookes, 1999a.
❸ Kaiser et al., 1992.
❹ Alef and Kleiner, 1987a.
❺ Lin and Brookes, 1999b (acid soils removed from correlations)
❻ Alef and Kleiner, 1987b.
❼ Alef et al., 1988.
❽ Adams and Adams, 1983.
❾ Anderson and Joergensen, 1997.

7.5 Conclusions

Table 7-12 summarises the main findings of the work presented in this chapter.

Table 7-12 Main conclusions of experimental work of Chapter 7

- Long term alteration of soil pH only significantly affected soil total C and N in the SAC soil, where pH and total C and N were positively correlated.
- Long term alteration of soil pH did not significantly effect indigenous mineral nitrogen levels at the time of sampling.
- SON showed little consistent trend with soil pH among soil types.
- The relationships of SOC with soil pH and total C were not consistent across soil types.
- Basal respiration was significantly and positively correlated with soil pH in all three soil types.
- Glucose-C and urea-C mineralisation were fastest at intermediate pHs with maximum glucose rates at pH 5.0, 4.5 and 5.1 and urea rates at pH 5.62, 4.92 and 4.91 in Rothamsted, Woburn and SAC soils respectively.
- The rate of amino acid-C mineralisation was strongly affected by soil pH with optima at pH 5.8, 5.5 and 5.2 in Rothamsted, Woburn and SAC soils respectively. This optima was a result of a limited rate of uptake of amino acids from the free soil pool at low pHs (more amino acids are left unused after 48 h at low pHs), and due to a greater proportion of the amino acids taken up being utilised structurally at higher pHs with fewer used catabolically.
    - The rate of amino acid-C mineralisation was strongly correlated to total C (positively) and negatively related to DOC levels in Rothamsted and Woburn soils.
    - The ratio of amino acid-C immobilised to that respired by the end of the 48 h incubation indicates that the microbial population was functioning at optimal conditions at pH 6.8 in the Rothamsted soil and 5.7 in the Woburn soil. This ratio was highly correlated with DOC (negatively); SMB-C (positively); SMB-N_{TDN} (positively); basal respiration (positively); aluminium (negatively); calcium (positively); and the proportion of total C that is microbial C (positively) in Rothamsted and Woburn soils.
- SMB-C and N showed significant positive linear relationships with soil pH.
- The ratios of biomass C:soil organic C and biomass N:soil organic N, indicators of the condition, quality and health of the soil, increased significantly with increasing pH, in the Rothamsted and Woburn soils. This indicates an increase in availability of the C and N present and more efficient substrate utilisation.
- The results show very little relationship of net nitrification, net ammonification, net mineralisation, and net change in SON over the 30 d incubation to soil pH.

- At the end of a 17 d incubation, net arginine ammonification as a proportion of added arginine-N fell significantly with increasing pH. This was because at higher pHs more was transformed to NO_3^- via nitrification.
- The rate of arginine-C mineralisation was linearly and positively related to soil pH although there appeared to be no soil pH effect on the partitioning of arginine-C (unused, respired or immobilised) at the end of the 17 d incubation.

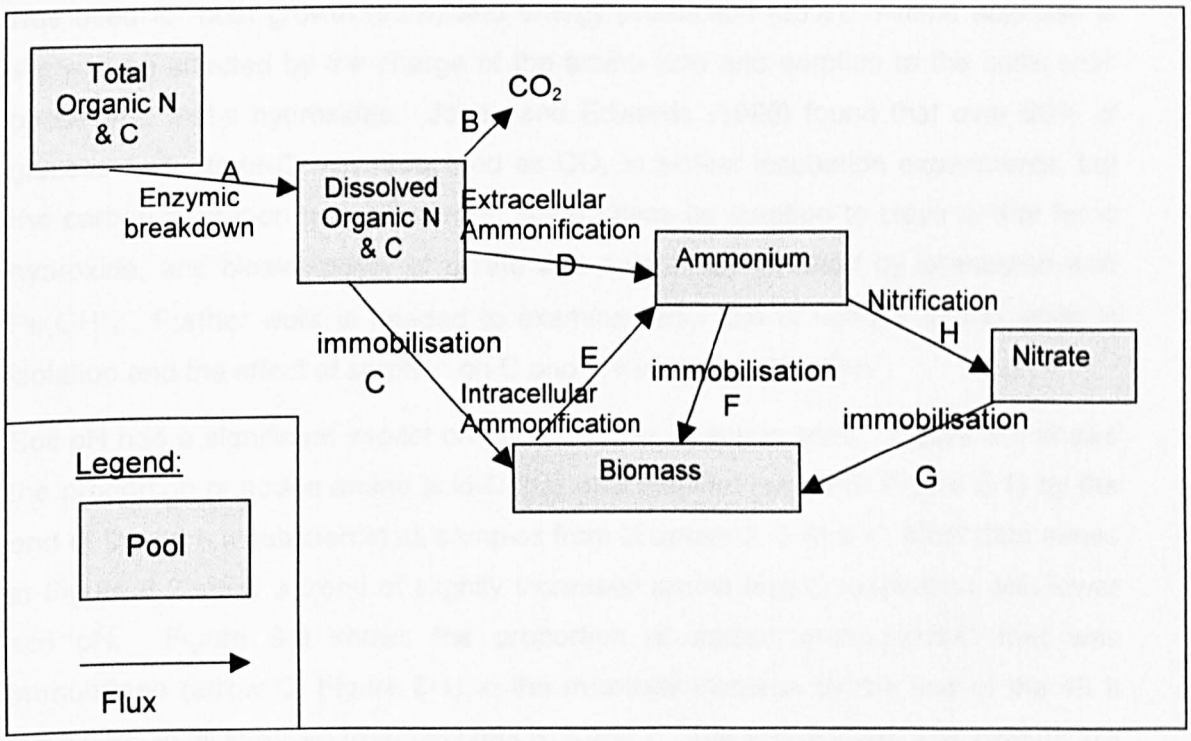
- With the exception of glucose SIR, most indexes of microbial biomass/activity measured generally showed reasonable and negative correlations with SOC levels, indicating that further work is needed to establish controlling mechanisms for SOC turnover.

Chapter 8 General discussion

8.1 Discussion of results

Figure 8-1 below depicts the flows of C and N within the soil pertinent to the work of this thesis. The effect of soil pH on some of these fluxes and pools will be discussed below.

Figure 8-1 Pools and fluxes of soil nitrogen and carbon.



8.1.1 Amino acid mineralisation and immobilisation

The work of this thesis used amino acids to represent the more labile fraction of the dissolved organic C and N. An amino acid mineralisation assay was used, as described in Chapter 3, section 3.8 and Table 3-4, in each of the four experimental chapters. This used a mixture of fifteen amino acids in equal proportion. The proportion of amino acid-C mineralised to CO_2 (arrow B, Figure 8-1) usually reached a plateau within a 48 h incubation period at a level in the region of 30%, with the exception of the Rothamsted and Woburn samples reported in Chapter 7, which showed respiration of around 50% of added amino acid-C. However, this proportion in a similar assay in which only the amino acid arginine was added was approximately 60% in three different soils, and the time taken to level out was longer; between 2 to 8 days (Chapter 7, section 7.3.1.7.2). This may have been due to a

higher concentration of a single amino acid causing a blockage of enzymatic or transport pathways. Very little substrate was left unused in the soil, and the remainder was immobilised in the microbial biomass (arrow C, Figure 8-1).

It is not clear what determines the allocation of amino acid C between respiration and microbial yield. The microbial yield of amino acid-C has been shown to be independent of substrate concentration (Jones and Hodge, 1999; Vinolas et al., 2001). Jones and Hodge (1999) found that lysine and glycine were predominantly used in the production of new cell biomass (80±1% of total taken up) while glutamate was used for both growth (65%) and energy production (35%). Amino acid use is likely to be affected by the charge of the amino acid and sorption to the soil's solid phase and metal hydroxides. Jones and Edwards (1998) found that over 60% of glucose and citrate-C was recovered as CO_2 in similar incubation experiments, but the carbon allocation was affected in some cases by sorption to clays and/or ferric hydroxide, and bioavailability of citrate was completely inhibited by interaction with $Fe(OH)_3$. Further work is needed to examine SMC use of various amino acids in isolation and the effect of sorption on C and N metabolic allocation.

Soil pH had a significant impact on microbial use of amino acids. Figure 8-2 shows the proportion of added amino acid-C that was respired (arrow B, Figure 8-1) by the end of the 48 h incubation in all samples from chapters 5, 6 and 7. Most data series in Figure 8-2 show a trend of slightly increased amino acid-C respiration with lower soil pH. Figure 8-3 shows the proportion of added amino acid-C that was immobilised (arrow C, Figure 8-1) in the microbial biomass by the end of the 48 h incubation in all samples from chapters 5, 6 and 7. The Rothamsted and Woburn soil samples show a much higher allocation of amino acid-C to respiration (Figure 8-2) and a lower allocation of amino acid-C to microbial biomass (Figure 8-3) than the other soils. The trend is also different; the Rothamsted and Woburn soils show a more linear response to soil pH, with immobilisation increasing with rising pH, whereas the other series show immobilisation of 60-80% of added amino acid-C with a quadratic relationship, maximal at around pH 5. This anomalous behaviour of the SMB of these two soil types may be attributable to the much lower organic matter status of these soils compared to the other soils, as highlighted in Table 8-1. The examination of acid soil profiles (chapter 4) showed that the amino acid-C mineralisation rate within profiles was strongly positively related to total C and N. The results suggest that at any given soil pH, the SMB of the Rothamsted and Woburn soils were more stressed than the SMB of the other soils. There are no

other obvious causal factors such as differences in time or method of sampling, sample preparation, method or site factors such as vegetation or land use.

Amino acid-C mineralisation rate was not related to total C or N in the one site studied in chapter 6, although insufficient time had elapsed for total soil C and N to have been altered greatly by treatments. Nearly forty years had elapsed since pH was originally adjusted in the sites examined in Chapter 7, and amino acid mineralisation rate was positively related to total C in this study. This suggests that it takes time for changes in chemical soil characteristics (e.g. pH) to work through subsequent effects on the microbial population, it's activity, available nutrient form and levels, plant composition, litter composition, litter degradation and the eventual make up of the soil organic matter pool.

Table 8-1 Ranges of total C and total N observed in the individual samples examined in experimental chapters 5, 6 and 7

[See individual chapters for more information on sampling regimes, soil types, numbers of samples and treatments examined in each soil]

Chapter	Soil	Total C	Total N
5	Yorkshire Dales	5.5-18.7	0.38-1.28
5	Lake District	7.7-16.8	0.52-1.34
5	Snowdonia	4.8-24.2	0.47-1.53
6	Field trial soil pre sulphur treatment	2.5-4.9	0.21-0.40
6	Field trial soil 2 years post sulphur treatment	2.2-4.8	0.20-0.41
7	Rothamsted pH plots	0.7-1.6	0.04-0.09
7	Woburn pH plots	0.6-10	0.02-0.05
7	SAC pH plots	4.4-5.8	0.25-0.39

The amount of amino acid-C remaining unused in the soil at the end of the 48 h incubation in all samples is shown in Figure 8-4. Generally, above pH 4.5 less than 5% of added amino acid-C is left unused in the soil solution, whereas below this pH level, between 5 and 10 % is generally left unused.

These results demonstrate that microbes at a lower soil pH have to use more of the amino acid-C in metabolic maintenance (respiration) and as a consequence have to use a lower proportion of amino acid-C in the formation of new biomass. This acid stress may be a result of proton or aluminium toxicity.

Amino acid-C mineralisation rate was affected by soil pH, however, relationships did not fit a consistent model. Relationships were fitted, based on visual inspection of the data and comparisons of correlation coefficients between different models, with convex quadratic curves in Chapter 7, and with linear relationships in Chapters 5 and 6. In Chapter 7 arginine-C mineralisation rates were fitted with straight lines in relationship to soil pH. Bringing together all data points from the amino acid-C

mineralisation assays described in chapters 5, 6 and 7 in Figure 8-5, it can be seen that despite a large degree of scatter, there is a linear trend for increased rate of amino acid-C mineralisation with increasing soil pH. Above pH 6 there is a tendency for the mineralisation rate in some series to decrease, others level off and others continue to rise.

The data points for the soil samples from Snowdonia, as reported in chapter 5, indicate that the SMB is well adapted to the soil pH, as at each soil pH, they tended to exhibit among the fastest rates of amino acid-C mineralisation (Figure 8-5). The samples that had been subject to soil acidification in the field (as described in chapter 6) and had their pH lowered to less than 5, showed the slowest rates of amino acid-C mineralisation (Figure 8-5). This may indicate that the SMB had not yet adapted to the acidity, despite a period of 2 years having elapsed since sulphur application. During this period, acidity will probably have continuously been generated from the oxidation of the elemental sulphur applied.

At pH levels above 6, despite 'favourable' conditions, there are a number of samples that exhibited slow rates of amino acid-C mineralisation. These included samples from the Woburn, SAC and the Rothamsted pH plots, and the reasons are not known.

It was expected that the rate of amino acid-C mineralisation would be a function of either a higher number of organisms and/or a greater level of activity or efficiency. Unfortunately, microbial biomass was not determined in all samples. However, the samples examined in chapter 5, which were from a range of upland sites exposed to different grazing pressures, did not show a strong or consistent relationship between amino acid-C mineralisation rate and basal soil respiration rate (an indicator of general catabolic activity) and amino acid-C mineralisation rate was actually weakly negatively related to SMB as measured by total PLFAs. The samples examined in chapter 7, which were from long term experimental plots where three soil types had pH gradients induced, also did not demonstrate the expected relationship consistently. Amino acid-C mineralisation rate was significantly but weakly positively related to basal respiration in Rothamsted and Woburn samples, but strongly negatively related in SAC samples (Table 8-2). Jones (1999) also found a weak correlation between amino acid half life and basal respiration in topsoils (r^2=0.34) and subsoils (r^2=0.15) over a range of soil types. Amino acid-C mineralisation rate was related positively to SMB-C fairly strongly in the Rothamsted soil, weakly but significantly in the Woburn soil and not significantly in the SAC soil (Table 8-2).

Amino acid-C mineralisation rate was weakly but significantly positively related to SMB-N in the Rothamsted soil, not significantly in the Woburn soil and negatively (but not significantly) in the SAC soil (Table 8-2).

Table 8-2 Linear correlation coefficients and P values for rate of amino acid-C mineralisation with microbial biomass C and N and basal respiration in soil samples examined in chapter 7

[amino acid-C mineralisation rate determined as the reciprocal of the time taken for 15% of added amino acid-C to be mineralised to CO_2, using a substrate mixture of 15 uniformly labelled equimolar amino acids. SMB determined by fumigation extraction. NPC indicates SMB-N was determined by measurement of ninhydrin positive compounds, TDN indicates SMB-N was determined by measurement of total dissolved nitrogen. See chapter 7 for more details of methods, sampling and soil treatments.]

		SMB-C	SMB-N_{NPC}	SMB-N_{TDN}	Basal Respiration
Rothamsted	r	0.672	0.308	0.498	0.564
	P	0.000	0.035	0.001	0.000
Woburn	r	0.357	0.171	0.230	0.301
	P	0.014	0.250	0.119	0.040
SAC	r	0.235	-0.727	-0.438	-0.905
	P	0.612	0.102	0.385	0.005

It therefore appears that microbial biomass and basal respiration rates are not reliable predictors of amino acid mineralisation activity across a range of soil types. The data from chapter 5 (the multiple regression summarised in Table 5-3, page 110) and from chapter 7 (section 7.3.1.4) indicate that exchangeable Al, soil pH (which influences the proportion of amino acids respired:immobilised), total C and SOC are important variables. These factors are all facets of the level and quality of the organic substrates that are available to the SMB in situ and further investigation into this relationship is therefore warranted.

The ratio of amino acids immobilised:respired in the samples of chapters 5, 6 and 7 are shown in Figure 8-6. The anomalous behaviour of the Rothamsted and Woburn soil samples is demonstrated, as the ratio is nearer 1, with approximately equal proportions of amino acid-C respired and immobilised. These samples show a linear trend with soil pH, with the ratio increasing slightly with increasing soil pH. The other data series show a more quadratic relationship with ratio levels generally fluctuating around 2, with a maximum around pH 5.

Figure 8-2 The influence of soil pH on the proportion of added amino acid-C respired after 48 h in soil samples of chapters 5, 6 and 7.

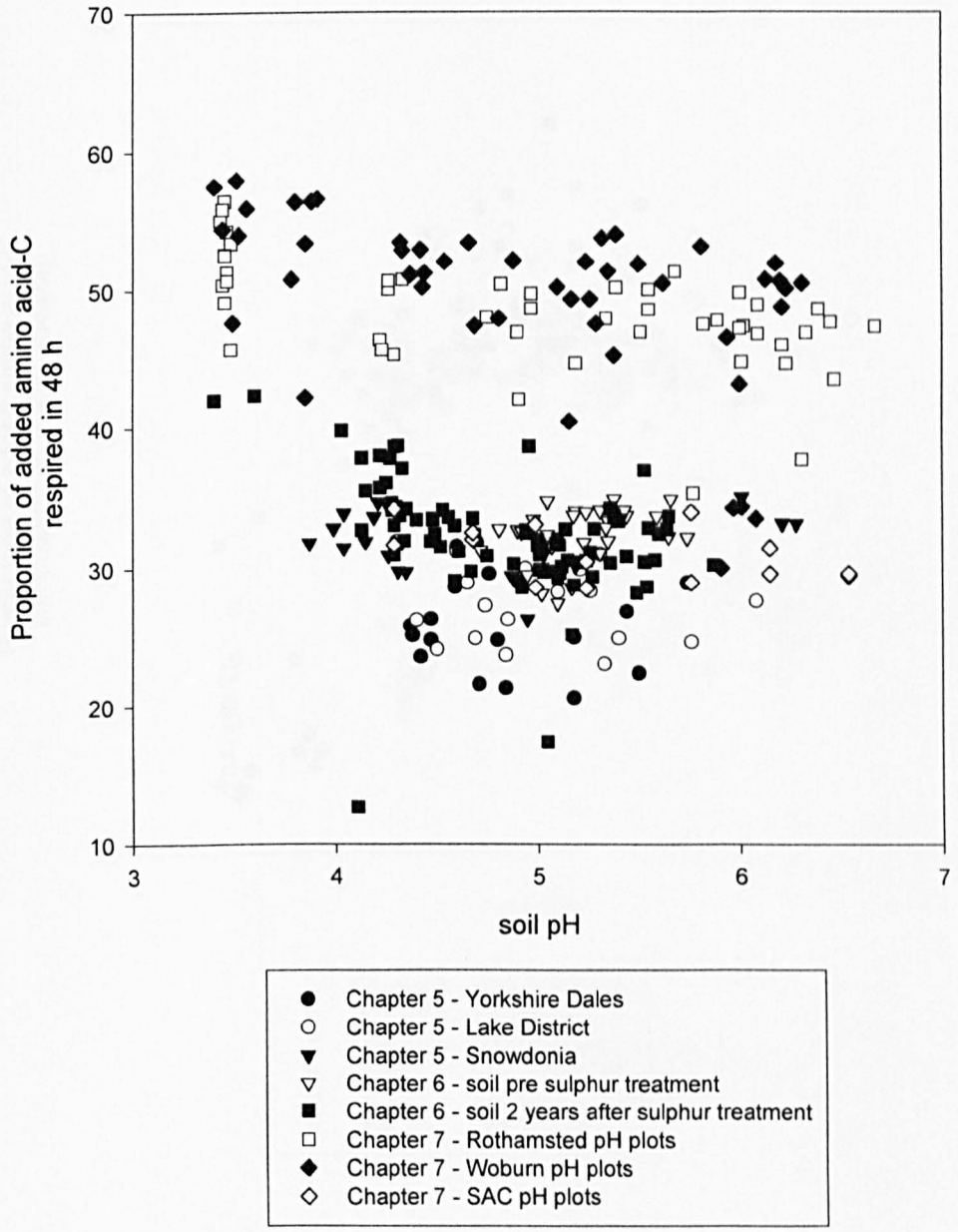


Figure 8-3 The influence of soil pH on the amount as a % of added amino acid-C immobilised after 48 h in soil samples of chapters 5, 6 and 7.

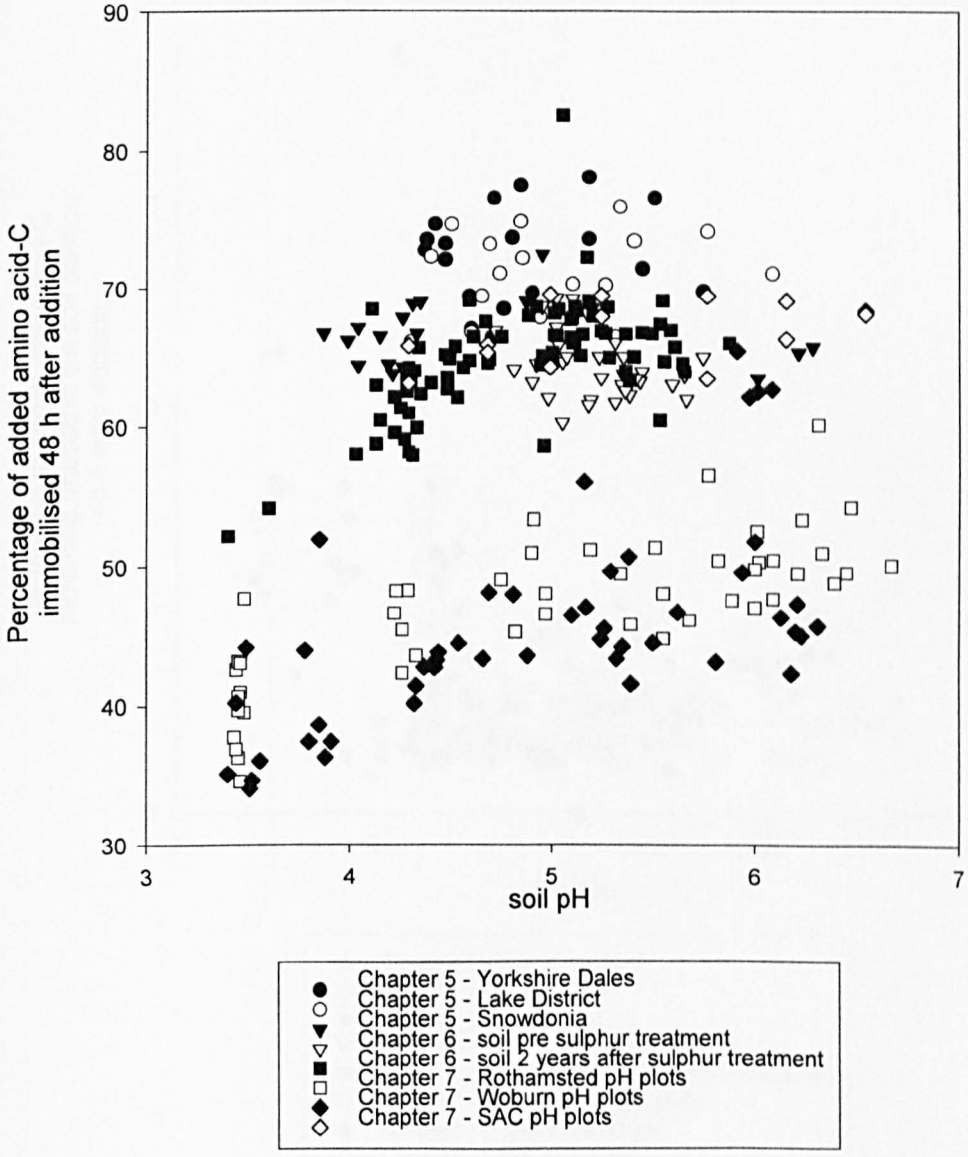


Figure 8-4 The influence of soil pH on the proportion of added amino acid-C left unused after 48 h in soil samples of chapters 5, 6 and 7.

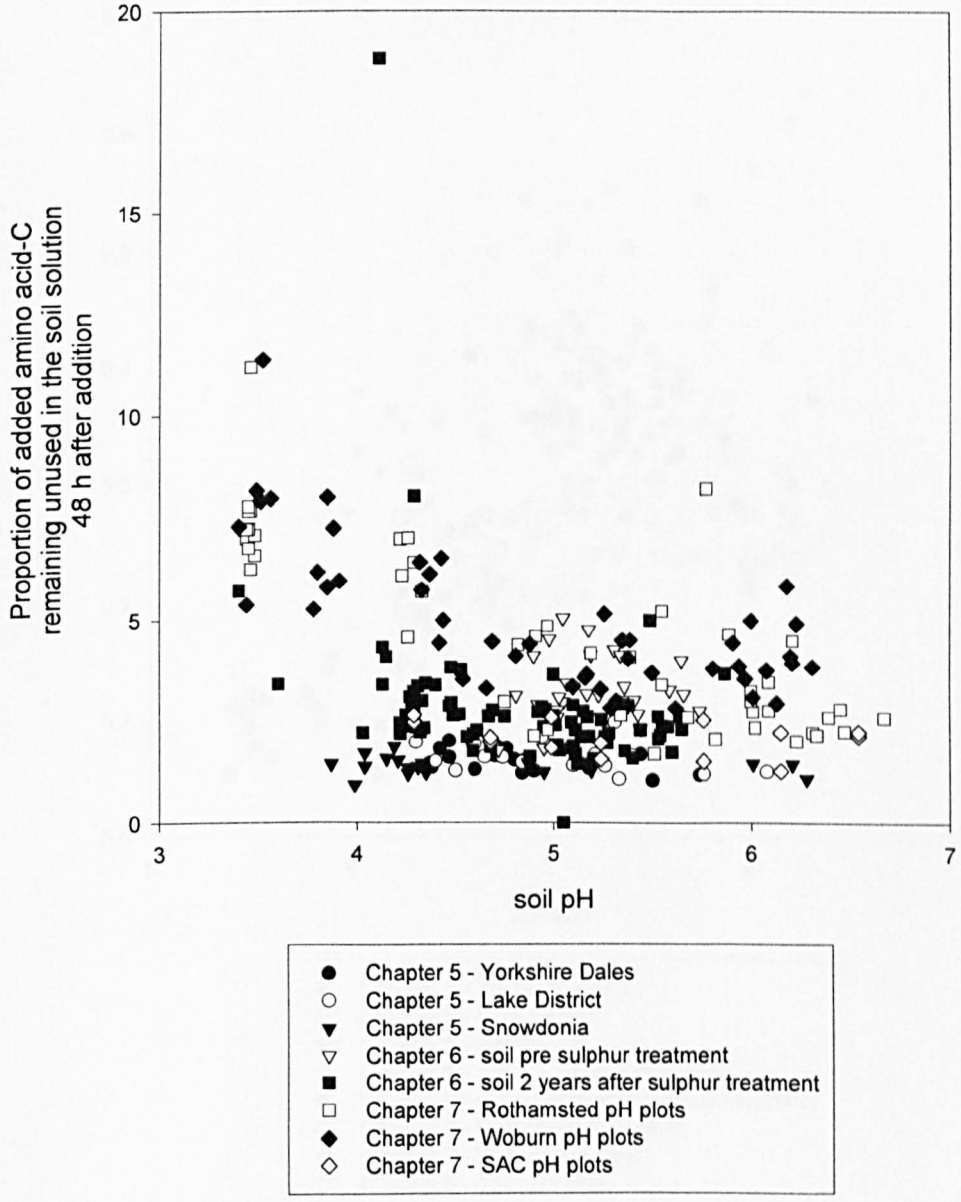


Figure 8-5 The influence of soil pH on the rate of mineralisation of added amino acid-C in soil samples of chapters 5, 6 and 7.

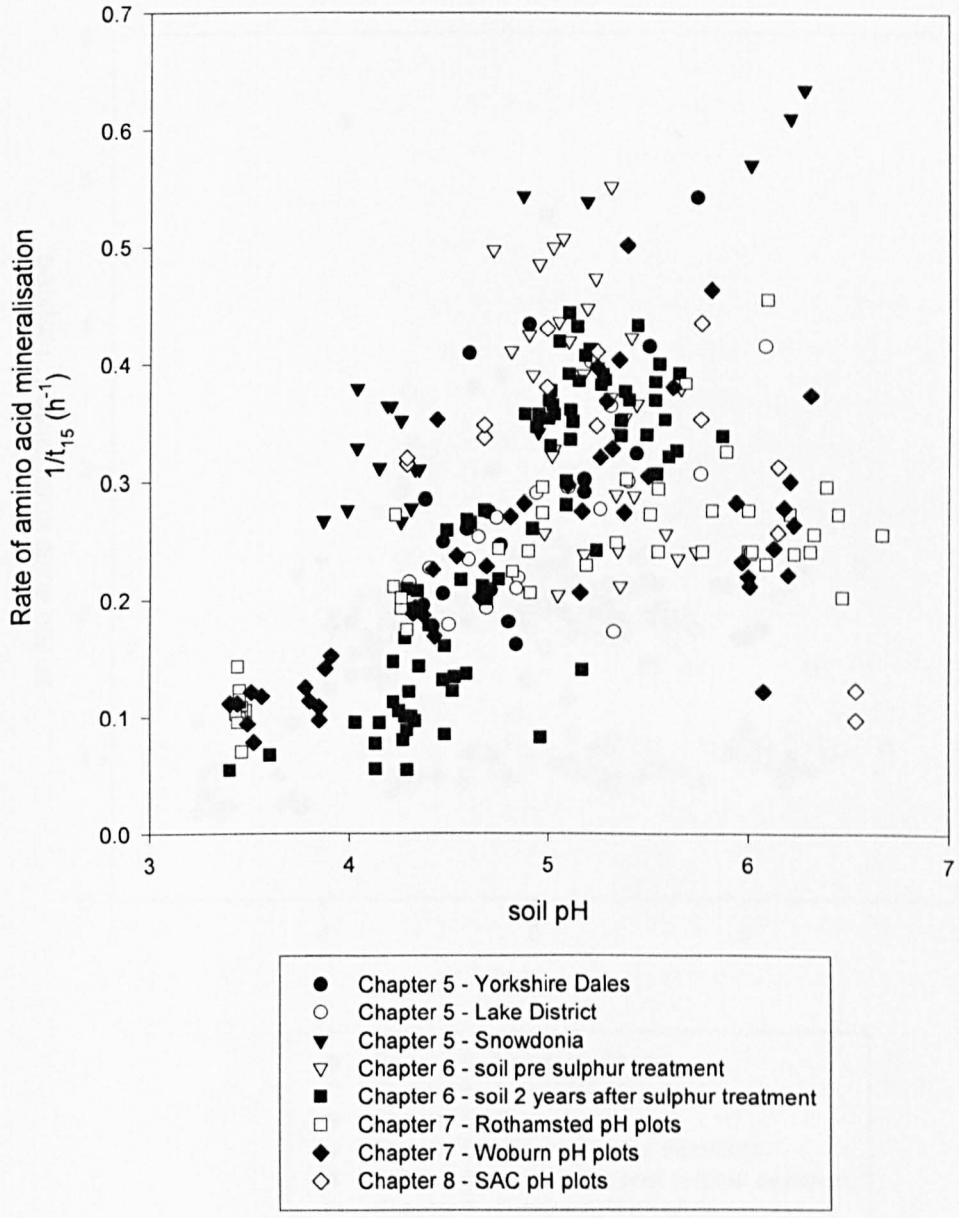
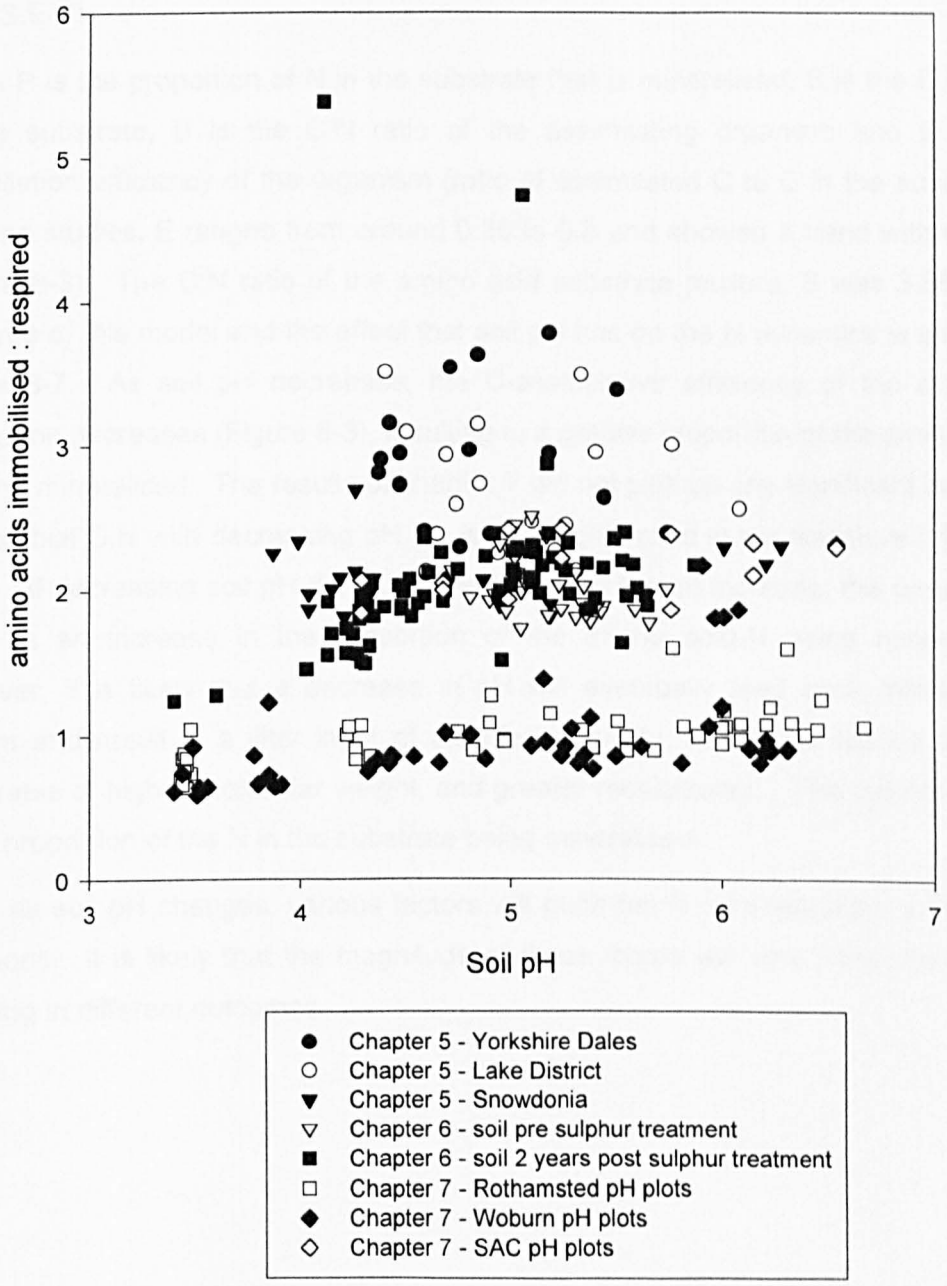


Figure 8-6 The influence of soil pH on the ratio of added amino acid-C immobilised : respired after 48 h in soil samples of chapters 5, 6 and 7.



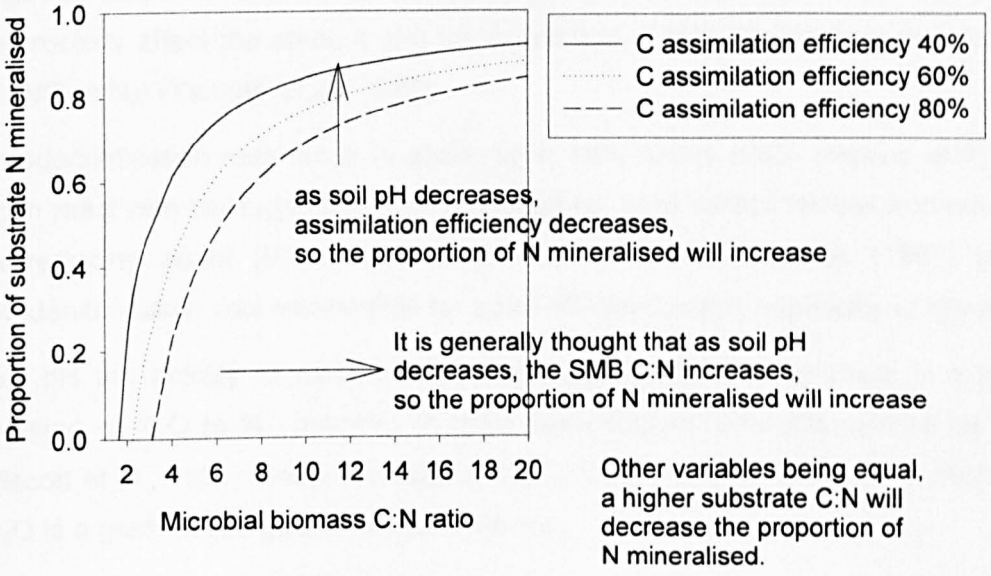
Although it was not possible to use nitrogen isotopes to track the movement of the amino acid-N in this study, the results of the C allocation study can tell us something of the N dynamics, based on the model described by Barraclough (1997) which was considered in Chapter 4 and verified in Chapter 7. Briefly, the model states:

$$P=(1-S.E/B)$$

where P is the proportion of N in the substrate that is mineralised, S is the C:N ratio of the substrate, B is the C:N ratio of the assimilating organism and E is the assimilation efficiency of the organism (ratio of assimilated C to C in the substrate). In these studies, E ranged from around 0.35 to 0.8 and showed a trend with soil pH (Figure 8-3). The C:N ratio of the amino acid substrate mixture, S was 3.86. The outcome of this model and the effect that soil pH has on the N dynamics is shown in Figure 8-7. As soil pH decreases, the C-assimilative efficiency of the microbial population decreases (Figure 8-3), resulting in a greater proportion of the amino acid-N being mineralised. The results of chapter 7 did not pick up any significant increase in microbial C:N with decreasing pH, as is commonly cited in the literature (Haynes, 1986). If decreasing soil pH did cause the microbial C:N to increase, this would also result in an increase in the proportion of the amino acid-N being mineralised. However, it is likely that a decrease in pH will eventually feed back through the system and result in a litter input of an inferior quality, leading to soluble organic substrates of higher molecular weight, and greater recalcitrance. This will result in a lower proportion of the N in the substrate being mineralised.

Thus, as soil pH changes, various factors will push net N mineralisation in different directions. It is likely that the magnitude of these forces will vary from site to site, resulting in different outcomes.

Figure 8-7 Theoretical proportion of substrate-N that is mineralised with varying SMB C:N, and how changes in soil pH influence processes.



8.2 Discussion of relevant issues

Improvement of agricultural systems involves considering management options, while weighing up costs and benefits. In conjunction with considering agricultural output and farming profitability, one aims to 'steer' nutrients into pools where they are most useful or cause minimal negative environmental impacts. Ideally, strategies can be found that 'win' on both economic and environmental grounds, although this is affected by the wider climate of economic, political, social and policy considerations (Addiscott et al., 1991), which extend well beyond the scope of this thesis.

However, an important consideration in the management of nutrients is the interrelatedness of pools and fluxes within cycles, and the recognition that altering one flux to control one pool will inevitably have knock-on effects on other fluxes and pools. While we would seek to reduce all forms of N loss, the interrelatedness of all parts of the N cycle mean that attempts to control NO_3^- leaching have implications for the loss of other forms of N from farms (Davies, 2000). It would therefore be of value to look at all components of a production system and all processes or issues (e.g. leaching, denitrification, volatilisation) to develop nutrient budgets (Jarvis, 1998).

8.2.1 Shifting the N loss

For example, if a lowering of soil pH is used as a means of controlling NO_3^- leaching, as considered in chapter 6, the possibility of altering the denitrification process must be considered. The effect of pH on denitrification is unclear, as some effects attributed to changes in pH in the past have been shown to be due to associated changes in carbon availability (Vinten and Smith, 1993; Stevens et al., 1998). pH and aerobicity affect the amount and the proportion of the different gasses released (N_2O, NO or N_2) (Yamulki et al., 1997).

Chemodenitrification may occur in acidic soils: NO_2^- forms HNO_2 (nitrous acid) and this can react with soil organic matter to release N_2, or in subsoil ferrous iron may act as a reducing agent (Vinten and Smith, 1993). Yamulki et al. (1997) found chemodenitrification was responsible for some NO production, especially at low pH.

At low pH the activity of nitrous oxide reductase is inhibited so there is a lower conversion of N_2O to N_2, resulting in more denitrification products emitted as N_2O (Addiscott et al., 1991; Vinten and Smith, 1993; Maier, 2000). This is less desirable as N_2O is a greenhouse gas, whereas N_2 is not.

However, Ellis et al., (1998) found that CO_2 and N_2O production decreased with decreasing pH under aerobic and anaerobic conditions and that there were significant effects of pH on the number of micro-organisms and on the kinetics of the reduction enzymes involved in denitrification (Ellis et al., 1998). Yamulki et al. (1997) also found that mean fluxes of N_2O from soil to atmosphere decreased with increasing acidity within the pH range 3 to 8. Significant production of N_2O was observed even at pH 3.9, whilst NO fluxes showed little dependence on pH. They also found that increasing the pH of acid soil reduced NO and N_2O fluxes, leading to the conclusion that the microbial community of the soil had adjusted to the low pH and was responsible for the entire production of the N_2O and much of the NO release.

Understanding the effect of pH on gaseous emissions is complex as nitrification and denitrification can occur simultaneously. Where denitrification is the main source of N_2O, emissions tend to decrease with increasing pH (at least in acid soils, below pH 5-6), but where nitrification is the main source, emissions of N_2O tend to increase with increasing pH (at least in the range pH 6-8) (Yamulki et al., 1997).

Liming may increase dissimilatory NO_3^- reduction to NH_4^+ (Stevens et al., 1998), which poses the question as to whether cessation of liming or commencement of acidification would increase dissimilatory reduction.

8.2.2 Shifting the problem

Soil acidification affects botanical diversity, the soil food web and so the higher trophic levels of ecosystems, and affects the water draining from the land. Acid water draining from acidic soils is high in aluminium and often low in bases (ALPC, 1995). Ridley et al. (2001) point out that decisions on liming annual pastures should consider the trade-off between degrading the soil resource through acidification of individual fields (compromising the future ability to grow certain species) and the off-site impacts of degrading water resources.

Soil acidity of the degree that has a great impact on the toxic metal content of drainage water is likely to have such severe impacts on crop growth that it would not be considered in agricultural management. However, the quality of drainage water and the environmental impacts should be considered in any further study of soil pH reduction as a means of ameliorating NO_3^- leaching.

Sustainable agricultural systems, including organic farming, relies greatly on biological nitrogen fixation (BNF) during crop rotations to maintain soil fertility. BNF is known to be sensitive to low pHs, so the sustainability of such systems may be seriously compromised by the attempt to make the system less 'leaky' through soil acidification or cessation of liming.

8.2.3 Agricultural systems

There is however, a theoretical limit to the potential result of *precision farming*, even given perfect knowledge about N fluxes. Farm managers will always want sufficient N to be present to allow maximum potential crop yields, but other conditions that reduce crop yield such as bad weather or pests and disease during the growing season may prevent the use of all the available N by the crop, resulting in excess N potentially being lost (Goulding, 2000; Jarvis, 2000).

Leaching cannot be prevented in arable systems. Certain measures will minimise it, but cultivation and the growing of annual crops inevitably results in a leaky nitrogen cycle (Addiscott et al., 1991; Goulding and Poulton, 1992). Large reductions in leaching loss will only come about through change from intensive arable or horticulture to extensive unfertilised grass (Goulding, 2000)

In many regions, cereal yields are restricted by soil acidity (Simard et al., 1994). Surface applied lime does not generally neutralise acidity in subsoils (Hojito et al., 1987), which may restrict rooting depth and proliferation and increase proneness to drought (Foy and Paterson, 1994). If agricultural soils are to be 'run' at a lower pH, as studied in chapter 6, the use of acid tolerant strains of crops (e.g. 'Atlas 66' wheat) and pasture species must be researched and developed. Acid or aluminium tolerance in wheat may be associated with a high grain protein, ability to use NO_3^--N and a capacity to increase the pH of the rhizosphere (Foy and Paterson, 1994). Aluminium seems to inhibit K uptake in acid sensitive varieties, but not in acid tolerant varieties (Foy and Paterson, 1994). It may be worth returning to research on older varieties of crops, as Foulkes et al. (1994) found that older varieties of wheat were generally better at acquiring soil-N than modern varieties, which therefore needed more fertiliser.

Perennial cropping systems, including agroforestry systems, could also be looked into as less leaky alternatives that may suit lower pH soils.

Ian Kelso (2000, pers. comm.) suggests that an ideal progression for temperate sustainable agriculture is to develop perennial wheat varieties, so that it can be grown in combination with clover, in a permanent sward with cattle grazing between harvest and the onset of flowering, while soil structure considerations allow. This may be facilitated by the (controversial) development of herbicide tolerant varieties of perennial wheat and clover, so that weeds could be controlled without the need for cultivation and spraying out all vegetation. This would confer the advantages of no-tillage systems to soil organic matter and soil fertility.

8.3 Future work

Fertile soil typically harbours a few billion prokaryotes per gram and often an equivalent amount of fungal biomass, and the diversity is exhibited as metabolic, genetic, kinetic, morphological and life history variation (Tiedje et al., 2001). We know very little about these populations as we have only cultured 0.1% of the soil microorganisms. Soil harbours microsites of a tremendous range of physical and chemical conditions. We can only hope to increase our understanding of how differing populations affect function and how processes are controlled (Tiedje et al., 2001). DOM plays a crucial role by transporting a range of substrates into microsites, which can be utilised in a variety of environmental conditions (Zsolnay, 2001). Better understanding of the role of microsites, different pools of potentially

mineralisable N and the diffusional constraints of mineral N could improve our models of soil N dynamics (Jarvis et al., 1996).

Improved understanding of the links between microbial community structure and function may result in useful information, if changes in specific populations or groups can be used as indicators of environmental stress.

Of course, other processes in the plant-soil system are pH sensitive, and can not be ignored in agricultural considerations. These include sulfate reduction to sulfide, phosphorus mineralisation, and lower populations of blue-green algae resulting in lower CO_2 and N_2 fixation. Changes in soil pH may alter prevalent plant diseases.

The work of this thesis points directly towards the following recommended avenues for research:

1) The impact of soil pH on protease enzyme activities should be examined (Arrow A, Figure 8-1). It was unfortunate that none of the numerous attempts made during the course of this research were successful. The results of the amino acid mineralisation assay used in chapters 4, 5, 6 and 7 of this thesis suggest that accessible dissolved organic nitrogen is used extremely rapidly, within one to two days, which keeps pool sizes in the soil low. Although rates of low molecular weight DON mineralisation are affected by soil conditions such as pH, the substrates are used so quickly that it seems unlikely that this process presents any major bottleneck in the transformation of large organic N molecules to those that may be lost from the plant-soil system. Extracellular protease enzyme activity may pose more of a control on the process. Results from Chapter 6 show that soil acidification lowered DON concentrations in soil solution slightly, which indicates that protease enzymes may have a lower activity at reduced soil pH.

Although protease enzymes are usually thought to function optimally at neutral or alkaline pHs, Kamimura and Hayano (2000) have detected proteases with optima in the acidic region in acidic tea soils. More information would help to ascertain to what extent DON formation is a bottleneck in the N cycle (Zsolnay, 2001), and to what extent soil pH contributes to that 'blockage'. The partitioning of organic matter between phases is partially concentration driven, but microbial activity, e.g. via extracellular enzymes, may play an active role in the partitioning (Zsolnay, 2001). The effect of soil pH may have a significant impact on this.

2) The preliminary exploration of the impact of soil acidity on NO_3^- leaching in chapter 6 should be extended to include a comprehensive N budget over a wider range of soil pHs. This could be achieved with liming as well as sulphur treatments, with several application rates to obtain a higher resolution pH gradient. It would be of benefit to study crop yields and N offtake. Monitoring of nitrogen flows should also include:

a) Monitoring of field measurements of in-situ nitrogen mineralisation and nitrification. The resin-core incubation technique described by Bhogal et al. (1999) would be worth trying for net measurement. This also provides a measure of NO_3^- leaching which has been found to be comparable to that obtained by porous ceramic water samplers. Measurement of gross mineralisation and nitrification rates, using ^{15}N isotope techniques, and their comparison with net measurements would be of interest. The short-term dry ^{15}N isotopic dilution nitrification assay described by Willison et al. (1998) appears to have certain benefits over other methods.

b) Monitoring of gaseous emissions of all forms of N gas

c) Collection of drainage water (possibly using porous ceramic cups inserted deeper than they were in the study reported in chapter 6) and monitoring of its nitrogen content. More detailed characterisation of the DON in the drainage water (e.g. using methods as in Gonzalez-Prieto et al., 1992), especially quantifying:

i) the complexation of DON with polyvalent metal ions and examination of how this affects its recalcitrance.

ii) The extent to which DON complexes with Al and keeps biotoxic inorganic al concentrations low (Schnitzer, 1980; Sumner et al., 1991) and the implications of that process for the microbial populations, and subsequent nutrient dynamics.

iii) Clarification of the effects on the N cycle of lower pH and the presence of polyvalent cations making DON less soluble and changing the functionality of the DON (Zsolnay, 2001)

iv) The extent to which these influences are reversible.

d) Primary productivity of the vegetation and inputs to the soil system from above and below ground sources. This would need to be carried out on long-term pH plots as those studied in Chapter 7, so that the soil-plant system will have had time to reach new quasi-equilibria states in response to the pH alteration.

e) The use of ceramic porous cups should be appraised for their suitability as a suitable collection method for DON analysis.

3) Further research into measurement techniques for the quantification of soil microbial biomass C and N is warranted in the light of comments made in section 7.4.8.

4) The characterisation of the differences in the soil microbial community over a pH gradient in the same soil type would be of interest in order to establish links of groups with functional nitrogen transformations. The establishment of links between critical community characteristics (species/groups/population levels) and function may lead to a means of assessing impacts of management changes in the short term and potential for appraising longer term consequences. It is likely that these indicators will be broad based for mineralisation and more specific for nitrification functions.

5) This type of detailed study could be extended to cover different agricultural systems, notably

a) realistically grazed pastures

b) arable with organic and inorganic fertiliser additions

The concept of using soil pH management as a tool for management of soil organic nitrogen requires further investigation of the following avenues of research:

1) Acid tolerant cereal and pasture crops.

a) Acidity related toxicity escape mechanisms (Wilkinson, 1994). Variation between species and cultivars in:

i) metabolic control of ion pumps and pores in uptake and movement of ions.

ii) root mucilage attenuation of aluminium absorption

iii) amelioration of aluminium toxicity by dicarboxylic organic acid ion chelators internal or external to the plant cell

iv) ability to detoxify by accumulation in vacuole or extracellular spaces.

b) Their productive capabilities under a range of soil pHs

c) Their tolerated and preferred nitrogen nutrition

d) How their growth affects the nitrogen dynamics of the plant-soil system.

2) Acid tolerant biological N fixation.

8.4 Concluding remarks.

It has been demonstrated in this thesis that soil pH has a strong influence on the mineralisation of amino acids, which are representative of the labile pool of soluble soil organic matter. However, soil pH does not appear to have a consistent effect on soluble organic carbon and nitrogen levels across soil types. This is likely to be a reflection of the consideration of soluble organic matter as a single pool. The results of this research have failed to determine a single or set of predictor variables that adequately explain differences in the rate of mineralisation of low molecular weight soluble organic substrates across different soil types. Hence further work is required to improve our understanding of soluble organic fractions, their bio-availability and how these are affected by soil pH. The practical consequences of this, given the heterogeneity of the soil resources and other site specific factors, are that without such a shift in our understanding, one has to concur with Bramley and White (1989) in concluding that it may not be possible to produce a definitive model of mineralisation processes which works for all soil types.

Appendix 1 Bardgett et al. (2001). Soil microbial community patterns related to the history and intensity of grazing in sub-montane ecosystems. *Soil Biology and Biochemistry,* 33, 1653-1664.

PERGAMON

Soil Biology & Biochemistry 33 (2001) 1653–1664

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Soil microbial community patterns related to the history and intensity of grazing in sub-montane ecosystems

Richard D. Bardgett[a,*], Angela C. Jones[b], David L. Jones[c], Sarah J. Kemmitt[c], Roger Cook[d], Phil J. Hobbs[e]

[a]*Institute of Environmental and Natural Sciences, University of Lancaster, Lancaster LA1 4YQ, UK*
[b]*School of Biological Sciences, Stopford Building, University of Manchester, Manchester M13 9PT, UK*
[c]*School of Agricultural and Forest Sciences, University of Wales, Bangor, Gwynedd, LL57 2UW, UK*
[d]*Institute of Grassland and Environmental Research, Aberystwyth, Dyfed, SY23 3EB, UK*
[e]*Institute of Grassland and Environmental Research, North Wyke, Okehampton, Devon EX20 2SB, UK*

Received 23 October 2000; received in revised form 5 March 2001; accepted 26 March 2001

Abstract

Long-term variations in the frequency and intensity of sheep (*Ovis aries*) grazing have led to the development of ubiquitous plant successional transitions in sub-montane regions of the UK. In this study, we measured a range of soil microbial properties across these successional transitions in three biogeographic regions of the UK, to establish how gradients of grazing-influence (in terms of the history and intensity of sheep grazing) alter the biomass, activity, and structure of soil microbial communities. We also measured soil physicochemical variables to relate changes in soil microbial community arrangement along these grazing-related successional transitions to key soil properties. Our results from three locations show that microbial communities of soils display some consistent and 'broad-scale' trends along successional transitions that are related to the history and intensity of grazing. We show that microbial biomass of soil is maximal at low-to-intermediate levels of grazing influence and that the phenotypic evenness (a component of diversity) of the microbial community declines as the intensity of grazing increases. We also provide evidence that soil microbial communities of heavily grazed sites are dominated by bacterial-based energy channels of decomposition, whereas in systems that are less intensively grazed, or completely unmanaged, fungi have a proportionally greater role. Further studies are needed to establish the significance of these changes in relation to soil-level ecosystem processes of decomposition and nutrient cycling. The data show that human disturbances can have profound effects on the biomass and structure of the soil communities that regulate soil processes in these ecosystems and that these effects are consistent across sites.

Keywords: Microbial community; Inorganic N; PLFA; Sheep grazing; Grassland

1. Introduction

Human disturbances that are associated with agriculture have emerged as one of the main shaping-forces of ecosystem diversity, structure, and productivity across the globe (Vitousek et al., 1997). To date, much research effort has been directed at understanding how agriculture and land management practices influence the structure and diversity of above-ground communities (e.g. Collins et al., 1998; Lawton et al., 1998). However, as ecologists become more aware of the important roles of soil organisms in regulating ecosystem processes, such as nutrient cycling and organic matter decomposition, there is increasing study of how decomposer organisms respond to disturbance regimes (Groffman and Bohlen, 1999; Wall and Moore, 1999), and especially those that are related to agriculture (Wardle, 1995; Bardgett and Cook, 1998; Swift et al., 1998).

In the wet, cool, sub-montane regions of north-west Britain, the most ecologically and economically important agricultural practice is sheep (*Ovis aries*) grazing (Pearsall, 1950). Long-term variations in the frequency (in some cases, dating back 2000–3000 years) and intensity of grazing by sheep has led to the development of successional transitions on well drained soils, from ancient, unmanaged oak (*Quercus petraea*) woodland, which is thought to represent the climax community (Pearsall, 1950), through to intensively grazed grasslands where more than 90% of the annual aboveground productivity (AAP) is consumed

* Corresponding author. Tel.: +44-1524-594856; fax: +44-1524-843854.
E-mail address: r.bardgett@lancaster.ac.uk (R.D. Bardgett).

0038-0717/01/$ - see front matter
PII: S0038-0717(01)00086-4

(Miles, 1985). These successional transitions relate to both the direct, physical effects of herbivores (especially the selective removal of plant material and trampling) on the structure of plant communities (Nicholson et al., 1970; Grant et al., 1985; Hill et al., 1992), and to the indirect, positive effects of herbivores on ecosystem productivity (Bardgett et al., 1998). These indirect effects of grazing result from more efficient re-circulation of nutrients via the animal excreta pathway (Floate, 1971a,b; Ruess and McNaughton 1987; McNaughton et al., 1997a; Bardgett et al., 1998), and, in some cases, from improvements in plant litter quality and decomposability of grazed plants (Bardgett et al., 1998). Accelerated nutrient cycling in grasslands that are grazed may also be linked to an increase in soil carbon supply, which, in turn, increases the size and activity of the soil microbial biomass (Tracey and Frank, 1998; Bardgett et al., 1998). Grazing-induced enhancements in soil biological activity could further stimulate net nutrient mineralization and increase soil nutrient availability (Mawdsley and Bardgett, 1997; Bardgett et al., 1998), thus accounting for the greater shoot nutrient content and productivity that is often reported in grazed grasslands (Holland and Detling, 1990).

Several studies have examined the effects of grazing animals on soil biotic communities and their activities, and these generally show that the size and activity of the soil microbial biomass, and numbers of microbial-feeding animals, are higher in grazed than ungrazed grasslands (Bardgett et al., 1993, 1997). Grazing has also been reported to increase the activities of soil enzymes that are fundamental to the cycling of N (McNaughton et al., 1997b). Nearly all studies of the effects of grazing on soil biological properties, however, have been site specific and have simply compared grazed *versus* ungrazed grasslands, and have not examined successional transitions that are related to both the history and intensity of grazing. In this study, we extend our understanding of the effects of grazing on biological properties of soils by comparing three vegetation gradients, at different biogeographic locations, for which the primary varying factor is grazing intensity. Our primary objective was to study successional transitions to determine how variations in the history and intensity of grazing alter the biomass, activity, and structure of soil microbial communities. We also aimed to assess whether patterns of soil biotic community arrangement along these grazing-related successional transitions are consistent at different biogeographic locations. A secondary objective was to determine how changes in soil biotic communities along the successions relate to key soil properties, such as the size of soil nutrient pools.

2. Materials and methods

2.1. Study sites and soils

The study of the upland ecosystem of the UK, below the tree-line, provides an ideal opportunity to examine these biotic interactions due to the extremely long history (2000–3000 years) of forest clearance and agricultural activity, in particular sheep grazing, in these areas. We studied successional transitions at three locations, representative of the biogeographic zones of western United Kingdom (Bardgett et al., 1995). These were: (1) Dentdale, in the Yorkshire Dales National Park; (2) Patterdale, in the Lake District National Park; and (3) Cwm Dyli and Abergwngregyn, in the Snowdonia National Park, North Wales. At each location we selected a range of six vegetation types representing a successional transition based on the history and intensity of sheep grazing (from hereafter referred to as a gradient of grazing influence) from unmanaged, ancient oak (*Q. petraea*) woodland (treatment 1), which acted as an ungrazed control, to intensively sheep grazed *Festuca-Agrostis* pasture where more than 90% AAP is consumed (Miles, 1985) (treatment 6). Treatments of intermediate grazing-influence were selected at each location: long-term (20 years plus) unmanaged and ungrazed grassland with woodland regeneration (treatment 2), short-term (5–10 years) unmanaged and ungrazed grassland (treatment 3), lightly grazed *Nardus-Galium* grassland where some 50% AAP is consumed (treatment 4), and moderately grazed *Festuca-Agrostis* grassland where some 60–80% AAP is consumed (treatment 5). All soils were formed on glacial till, but the underlying geology differed at each location. Underlying rocks in Yorkshire are Silurian slates and shales, whereas in the Lake District and Snowdonia they are volcanic rocks of Ordovician age. All parent material was base poor and the topography and altitude of treatments at each location were very similar. Details of the location, altitude, vegetation, soil, and management of the treatments are given in Table 1. The vegetation, soils, and microbial community of each treatment were taken to be in 'equilibrium' with their long-term grazing regime.

2.2. Sampling of soils

Due to the physical nature of the treatments, in particular the presence of single exclosures within areas, pseudo-replication was unavoidable. The problem of pseudo-replication is omnipresent in most studies of grazing effects of mammals in the field (Stohlgren et al. 1999). In order to minimize the sources of error associated with this problem we used multiple, randomly located, sites within each treatment. Soils were sampled in June 1998 by taking 10 3.5 cm diam (10 cm depth) cores from each of three randomly ($n = 3$) located replicate plots (10 × 10 m) of each treatment at each location. These 10 cores were bulked together and vegetation, plant roots, and large stones were removed by sieving (sieve mesh 6 mm^2). Soil properties and microbial community characteristics were then assessed as described below.

2.3. Soil analysis

The water content, organic matter content and pH of soils

Table 1
Site characteristics along the successional transitions in the Yorkshire Dales, Lake District and Snowdonia National Parks. The three areas were grazed by different pure-bred sheep breeds, as follows: Yorkshire Dales, Swaledale and Kendal Rough Fell ewes; Lake District, Herdwick and Swaledale ewes; and, Snowdonia, Welsh Mountain ewes. Dominant vegetation is given as: [1]*Quercus petraea*, *Betula* spp. woodland with an understory dominated by *Deschampsia flexuosa*, *Vaccinium myrtillus*, *Pteridium aquilinum*; [2]*Calluna vulgaris* and *D. flexuosa*, with lesser amounts of *V. myrtillus*, *Molinia caerulea*, and *Nardus stricta*. Regeneration of *Q. petraea*, *Betula* spp. and *Sorbus aucuparia*; [3]*N. stricta*, *Galium saxatile*, with regeneration *C. vulgaris* and *V. myrtillus*; [4]*N. stricta* and *G. saxatile*; [5]*Festuca ovina* and *Agrostis capillaris*; [7]*C. vulgaris* and *V. myrtillus*, with regeneration of *S. aucuparia* and *Betula* spp.; [8]*N. stricta* grassland with regeneration of *C. vulgaris*

National Park	Character	Gradient of grazing influence					
		Permanently ungrazed (1)	Long-term ungrazed (2)	Short-term ungrazed (3)	Lightly grazed (4)	Moderately grazed (5)	Heavily grazed (6)
Yorkshire Dales	Location	54°18′N,2°31′W	54°18′N,2°,32′W	54°18′N,2°,32′W	54°18′N,2°.32′W	54°18′N,2°.33′W	54°18′N,2°.33′W
	Altitude	250–300 m	250–300 m	250–300 m	250–300 m	250–300 m	250–300 m
	Grazing	None	Ungrazed 25 y	Ungrazed 12 y	1–2 ewe ha^{-1} y^{-1}	4–8 ewe ha^{-1} y^{-1}	8–16 ewe ha^{-1} y^{-1}
	Vegetation	*Quercus-Betula*[1]	*Calluna-Deschampsia*[2]	*Nardus-Galium*[3]	*Nardus-Galium*[4]	*Festuca-Agrostis*[5]	*Festuca-Agrostis*[6]
	Soil	Brown podzolic earth	Humus stagnopodzol	Humus stagnopodzol	Ferric stagnopodzol	Humic brown podzol	Brown podzolic earth
	Bulk density	0.40 (0.06)	0.66 (0.03)	0.59 (0.03)	0.57 (0.04)	0.52 (0.03)	0.62(0.00)
Lake District	Location	54°35′N,3°54′W	54°35′N,3°54′W	54°35′N,3°54′W	54°35′N,3°54′W	54°30′N,3°54′W	54°30′N,3°54′W
	Altitude	200 m	250 m	300 m	300 m	250–300 m	250–300 m
	Grazing	None	Ungrazed 40 y	Ungrazed 20 y	1–2 ewe ha^{-1} y^{-1}	4–8 ewe ha^{-1} y^{-1}	8–16 ewe ha^{-1} y^{-1}
	Vegetation	*Quercus-Betula*[1]	*Calluna-Deschampsia*[2]	*Nardus-Galium*[3]	*Nardus-Galium*[4]	*Festuca-Agrostis*[5]	*Festuca-Agrostis*[6]
	Soil	Brown podzolic earth	Humus stagnopodzol	Humus stagnopodzol	Ferric stagnopodzol	Humic brown podzol	Brown podzolic earth
	Bulk density	0.54 (0.02)	0.55 (0.08)	0.43 (0.04)	0.51 (0.04)	0.43 (0.01)	0.54 (0.01)
Snowdonia	Location	53°02′N,4°01′W	53°04′N,4°03″W	53°04′N,4°03″W	53°04′N,4°03″W	53°02′N,4°01″W	53°02′N,4°01″W
	Altitude	300 m	430 m	430 m	430 m	300 m	250 m
	Grazing	None	Ungrazed 41 y	Ungrazed 4 y	1–2 ewe ha^{-1} y^{-1}	4–8 ewe ha^{-1} y^{-1}	8–16 ewe ha^{-1} y^{-1}
	Vegetation	*Quercus-Betula*[1]	*Calluna-Vaccinium*[7]	*Nardus-Calluna*[8]	*Nardus-Galium*[4]	*Festuca-Agrostis*[5]	*Festuca-Agrostis*[6]
	Soil	Brown podzolic earth	Humus stagnopodzol	Humus stagnopodzol	Ferric stagnopodzol	Humic brown podzol	Brown podzolic earth
	Bulk density	0.38 (0.07)	0.33 (0.03)	0.43 (0.02)	0.43 (0.01)	0.60 (0.01)	0.71 (0.02)

were determined by standard methods (Allen, 1989). Soil total C and N contents were determined using a CHN-2000 (Leco Corp., St Joseph, MI, USA). Exchangeable NH_4^+ and NO_3^- was determined in 1:5 soil:1 M KCl extracts by the methods of Downes (1978; hydrazine, N-1-napthylethylenediamine) and Keeney and Nelson (1982; indophenol blue).

2.4. Soil microbial community analysis

The biomass and structure of the soil microbial community structure was assessed by analysing the ester-linked phospholipid fatty acids (PLFA) composition of the soil, since certain groups of microorganisms have different 'signature' fatty acids (Tunlid and White, 1992). Briefly, lipids were extracted from soil, fractionated, and quantified using the procedure described by Bardgett et al. (1996), which is based on the method of Bligh and Dyer (1959) as modified by White et al. (1979). Separated fatty acid methyl-esters were identified by chromatographic retention time and mass spectral comparison using standard qualitative bacterial acid methyl ester mix (Supelco UK, Poole, Dorset, UK) that ranged from C11 to C20. For each sample, the abundance of individual fatty acid methyl-esters was expressed on a dry weight basis per unit area (m^{-2}). Fatty acid nomenclature used was that described by Frostegård et al. (1993). The fatty acids i15:0, a15:0, 15:0, i16:0, 17:0, cy17:0, 18:1ω7, and cy19:0 were taken to represent bacterial PLFA (Federle, 1986; Tunlid et al., 1989; Frostegård et al., 1993). The polyenoic, unsaturated PLFA 18:2ω6 was used as an indicator of fungal biomass (Federle, 1986). It was assumed that the primary source of this eukaryotic PLFA was soil fungi. The ratio of 18:2ω6:bacterial PLFAs was taken to represent the ratio of fungal:bacterial biomass in soil (Frostegård and Bååth, 1996; Bardgett et al., 1996). The Shannon–Weaver evenness index was used on the above bacterial and fungal PLFAs as a measure of the relative distribution, or degree of dominance, of microbial groups. In accordance with Huston (1994), the more 'even' the distribution of PLFA, intuitively the greater is their diversity.

2.5. Nematode enumeration

Nematodes were extracted from soils using Whitehead and Hemming (1965) trays. Briefly, 100 g (fresh weight) soil samples were placed in trays (20 × 35 cm) with 400 ml distilled water and an extraction time of 24 h at 18–20°C. Samples were repeatedly settled and their volume reduced to approximately 10 ml by suction; all nematodes in 20% aliquots were counted at 50 × magnification.

2.6. Statistical analysis

All data were converted to a soil area basis (calculated from bulk density values), checked for normality, and for each biogeographic location analysed by one-way ANOVA to determine the amount of variance that was attributed to the gradient of grazing influence. Fisher's PLSD test was used to test for significant differences between individual means. Since data for individual sites is unavoidably psuedoreplicated, we also performed an ANOVA using site as a replicate to determine the effects of grazing intensity across all sites on soil parameters. All data are given as means (±SE), and F and P values are provided in the text.

3. Results

3.1. Soil conditions

Data on various intrinsic soil properties are provided in Table 2. At the Lake District and Snowdonia experimental sites, amounts of soil C differed significantly along the successional transitions, being highest in the lightly grazed (treatment 4) and short-term ungrazed grasslands (treatment 3), and lowest in the heavily grazed grassland (treatment 6). In Snowdonia, the total amount of N in soil varied significantly along the gradient of grazing influence, being highest in the lightly grazed grassland (treatment 4); in the Lake District, total N was highest in the lightly grazed (treatment 4) and short-term ungrazed grasslands (treatment 3). The soil C-to-N ratio was affected significantly by grazing influence at all locations; soil C-to-N ratios were highest in soils of the long-term ungrazed grasslands (treatment 2) at all locations and lowest in more heavily grazed grasslands (treatments 5 and 6) in Snowdonia. Soil pH was highest in the heavily grazed grassland (treatment 6) at all locations, and showed a general trend of increasing acidity with reduced grazing pressure from the heavily grazed to the lightly grazed grassland. The amount of mineral-N in soil differed significantly with grazing influence in the Lake District only, and was significantly higher in the completely ungrazed control (treatment 1) than in other treatments.

3.2. Abundance and activity of soil biota

Site specific data on the effects of grazing influence on soil microbial activity, total PLFA, and the abundance of nematodes are given in Fig. 1, whereas means that are derived from using location as a unit of replication are given in Table 3. There were no consistent trends in microbial activity, measured as basal respiration, along the gradient of grazing influence at the three locations (Table 3). The only significant effect of grazing occurred in Snowdonia ($F = 3.19$, $P = 0.046$) where basal respiration was found to be higher in the lightly grazed (treatment 4) grassland than in any of the other treatments. Microbial biomass, measured as total PLFA, varied significantly along the gradients and was highest in the lightly grazed (treatment 4) grassland at each location ($F = 10.56$, $P = 0.005$; $F = 6.64$, $P = 0.004$; $F = 6.72$, $P = 0.003$, for Yorkshire Dales, Lake District and Snowdonia,

Table 2
Changes in soil conditions in relation to grazing influence in upland ecosystems. Each value is a mean that is derived from three random quadrats within each treatment. All samples were taken in June 1998. Site 1 = ungrazed control, 2 = long-term ungrazed, 3 = short-term ungrazed, 4 = lightly grazed, 5 = moderately grazed, and 6 = heavily grazed

Measure	Gradient of grazing influence								
	1	2	3	4	5	6	LSD	*F* value	*P* value
Total C (kg m^{-2})									
Yorkshire Dales	5.5	4.3	4.6	5.1	5.2	4.7	1.0	2.03	NS
Lake District	4.7	5.4	5.8	5.8	5.1	4.5	0.9	3.37	0.039
Snowdonia	5.9	6.3	6.1	9.3	4.2	3.5	1.3	23.23	0.0001
Total N (kg N m^{-2})									
Yorkshire Dales	0.38	0.30	0.38	0.40	0.41	0.37	0.05	4.55	0.015
Lake District	0.38	0.35	0.46	0.47	0.41	0.36	0.07	4.62	0.014
Snowdonia	0.40	0.39	0.41	0.59	0.38	0.34	0.09	9.08	0.0009
C-to-N ratio									
Yorkshire Dales	14.5	14.3	12.1	12.8	12.7	12.7	1.4	5.28	0.009
Lake District	12.4	15.4	12.6	12.3	12.4	12.5	1.3	8.74	0.001
Snowdonia	14.8	16.2	14.9	15.8	11.1	10.3	0.8	92.49	0.0001
Soil pH									
Yorkshire Dales	4.6	4.6	4.5	4.7	5.2	5.5	0.4	8.84	0.001
Lake District	4.7	5.0	4.6	4.8	5.2	5.7	0.6	4.10	0.021
Snowdonia	4.0	4.2	4.1	4.3	5.0	6.2	0.2	104.90	0.0001
Mineral N (g m^{-2})									
Yorkshire Dales	3.5	3.8	3.9	2.9	2.4	3.9	1.2	2.48	NS
Lake District	4.8	3.1	2.9	2.6	2.0	3.4	1.6	3.49	0.035
Snowdonia	2.6	2.0	2.5	1.8	2.4	3.0	1.2	1.16	NS

respectively) (Table 3). In both Yorkshire Dales and Snowdonia, total PLFA was lowest in the historically ungrazed treatment (treatment 1), and at all locations declined significantly following the transition from light grazing (treatment 4) to long-term cessation of grazing (treatment 2) (Table 3). Likewise, the transition from light to moderate grazing (treatment 5) reduced significantly total PLFA in the Yorkshire Dales and Lake District. In Snowdonia, the transition from light (treatment 4) to heavy grazing (treatment 6) decreased significantly total PLFA.

The abundance of soil nematodes varied significantly along the gradient of grazing influence in the Lake District ($F = 6.26$, $P = 0.004$) and Snowdonia ($F = 5.23$, $P = 0.009$). In the Lake District, numbers of nematodes were highest in the lightly grazed (treatment 4) and moderately grazed (treatment 5) grasslands, whereas numbers of nematodes in Snowdonia were lowest in the lightly grazed (treatment 4) and the short- and long-term ungrazed grasslands (treatments 2 and 3). There was no effect of grazing influence on nematodes when location was used as a unit of replication (Table 3).

3.3. Microbial community structure

The fungal-to-bacterial PLFA ratio, a measure of the relative proportion of fungi and bacteria within the microbial community, varied significantly along the gradients of grazing influence at all locations ($F = 10.42$, $P = 0.0005$; $F = 4.85$, $P = 0.012$; $F = 5.74$, $P = 0.06$; for Yorkshire Dales, Lake District, and Snowdonia, respectively) and was at its highest in the moderately grazed grassland (treatment 5) in the Yorkshire Dales and Snowdonia (Fig. 2). When location was used as a unit of replication, the ratio was at its lowest in the heavily grazed grassland (treatment 6) and at its highest in the moderately grazed grassland (Table 3).

Table 3
Effects of grazing influence on soil microbial variables calculated using location as a unit of replication (degrees of freedom = 5). Where significant treatment effects are shown, data with the same letter are not significantly different. Site 1 = ungrazed control, 2 = long-term ungrazed, 3 = short-term ungrazed, 4 = lightly grazed, 5 = moderately grazed, and 6 = heavily grazed

Measure	Gradient of grazing influence								
	1	2	3	4	5	6	LSD	*F* value	*P* value
Nematodes (number $m^{-2} \times 0^3$)	3797	3443	3375	4446	5526	3784	1556	2.199	0.0697
Total PLFA (nmol m^{-2})	605^{a}	654^{ab}	833^{c}	1030^{d}	837^{c}	790^{bc}	154	7.726	< 0.0001
Fungal-to-bacterial PLFA ratio	5.4^{ad}	7.4^{ac}	6.9^{a}	6.8^{a}	9.8^{c}	3.1^{d}	2.6	5.811	0.0003
Fungal PLFA (nmol 18:2ω6 m^{-2})	13.9^{a}	19.9^{ab}	24.6^{b}	34.1^{c}	36.2^{c}	10.0^{a}	8.5	12.57	< 0.0001
PLFA evenness	0.90^{ab}	0.91^{a}	0.91^{ab}	0.88^{b}	0.91^{ac}	0.84d	0.03	6.451	0.0001

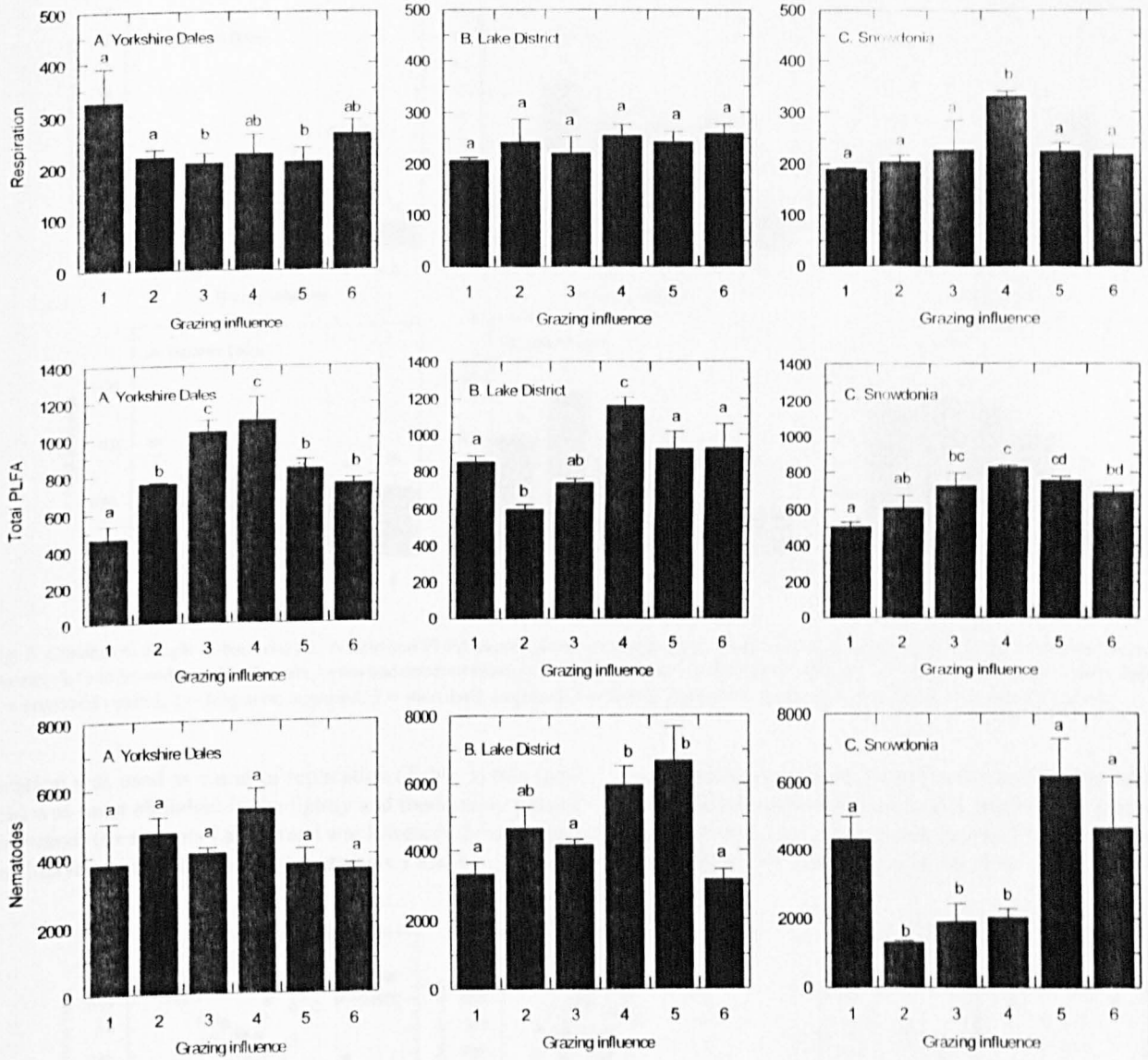


Fig. 1. Changes in soil respiration (ml CO_2-C m^{-2} h^{-1}), total PLFA (μmol m^{-2}) and nematodes (number $m^{-2} \times 10^1$) along grazing-related successional transitions in the Yorkshire Dales (A), Lake District (B) and Snowdonia (C). Data are means and standard errors ($n = 3$) and within each chart values with the same letter are not significantly different. Site 1 = ungrazed control, 2 = long-term ungrazed, 3 = short-term ungrazed, 4 = lightly grazed, 5 = moderately grazed, and 6 = heavily grazed.

The PLFA evenness index (Fig. 2), a measure of the relative distribution of microbial PLFAs, varied significantly along the gradient of grazing influence in the Lake District ($F = 11.72$, $P = 0.0003$) and Snowdonia ($F = 19.15$, $P < 0.0001$). At both locations, there was a trend of decreasing PLFA evenness with increasing grazing influence; PLFA evenness was highest in the ungrazed (treatments 2 and 3) and lightly grazed treatments (treatment 4), and lowest in the moderately and heavily grazed treatment (treatments 5 and 6). This trend was also evident when location was used a replicate; across all sites, PLFA evenness was lowest in the heavily grazed treatment (Table 3). Several individual fatty acids varied significantly in their relative abundance along the gradient of grazing influence (Table 4). However, changes in the PLFA evenness index with grazing influence were related primarily to a marked increase in the relative abundance of the fatty acid a15:0 in the more heavily grazed grasslands; PLFA evenness was significantly ($r = -0.692$ and -0.937 for the Lake District and Snowdonia, respectively; $P < 0.001$ for both) and negatively related to the relative abundance of a15:0 (Fig. 3).

The transition from lightly grazed to short-term ungrazed grassland (treatments 4 to 3) increased significantly the relative abundance of the fatty acids a15:0 and i17:0 in the Lake District and Snowdonia (Table 4). The relative abundance of the fatty acid 18:2ω6 (used as a fungal biomass indicator) was affected significantly by the degree of grazing influence at all locations, and was proportionally at its most abundant in the moderately grazed grassland (treatment 5) in the Yorkshire Dales and Snowdonia, and at its lowest in the heavily grazed grasslands (treatment 6) at all locations (Table 4). When

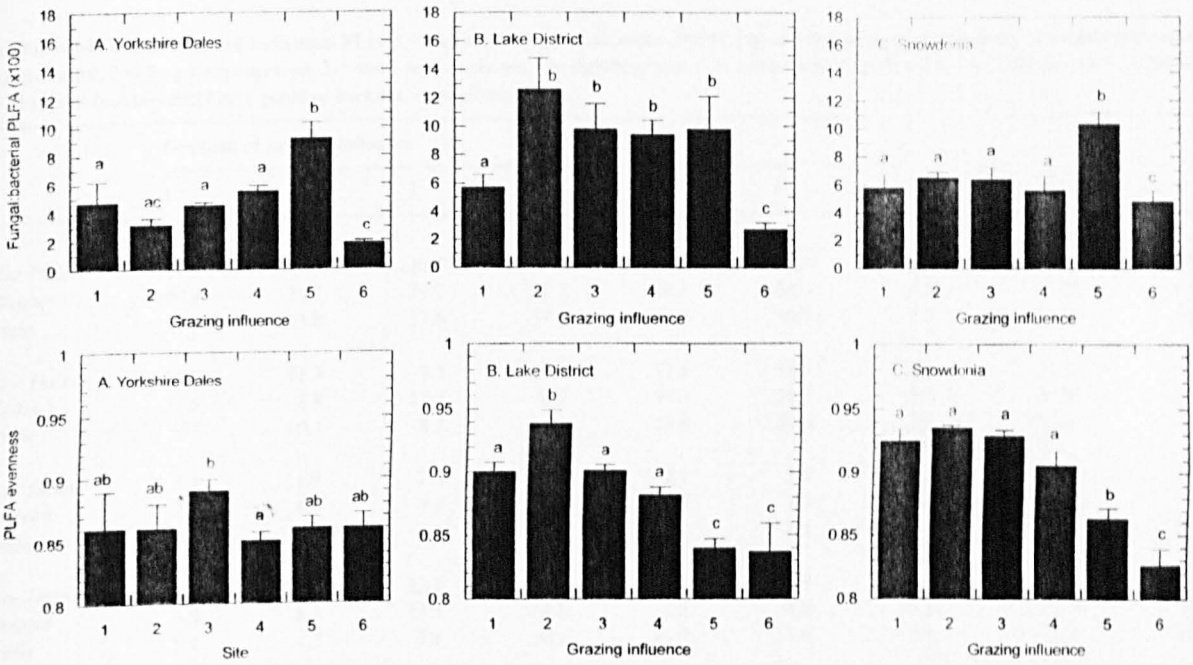


Fig. 2. Changes in fungal-to-bacterial PLFA ratio and PLFA evenness index along grazing-related successional transitions in the Yorkshire Dales (A), Lake District (B) and Snowdonia (C). Data are means and standard errors ($n = 3$) and within each chart values with the same letter are not significantly different. Site 1 = ungrazed control, 2 = long-term ungrazed, 3 = short-term ungrazed, 4 = lightly grazed, 5 = moderately grazed, and 6 = heavily grazed.

location was used as a unit of replication (Table 3) this fatty acid was most abundant in the lightly and moderately grazed grasslands (treatments 4 and 5) and was lowest in the ungrazed and heavily grazed treatments (treatments 1 and 6).

Relationships between soil and microbial variables were constructed using data across the gradients of grazing influence all locations (Figs. 3 and 4). The PLFA evenness index was negatively related to soil pH ($r = -0.48$, $P = 0.0002$),

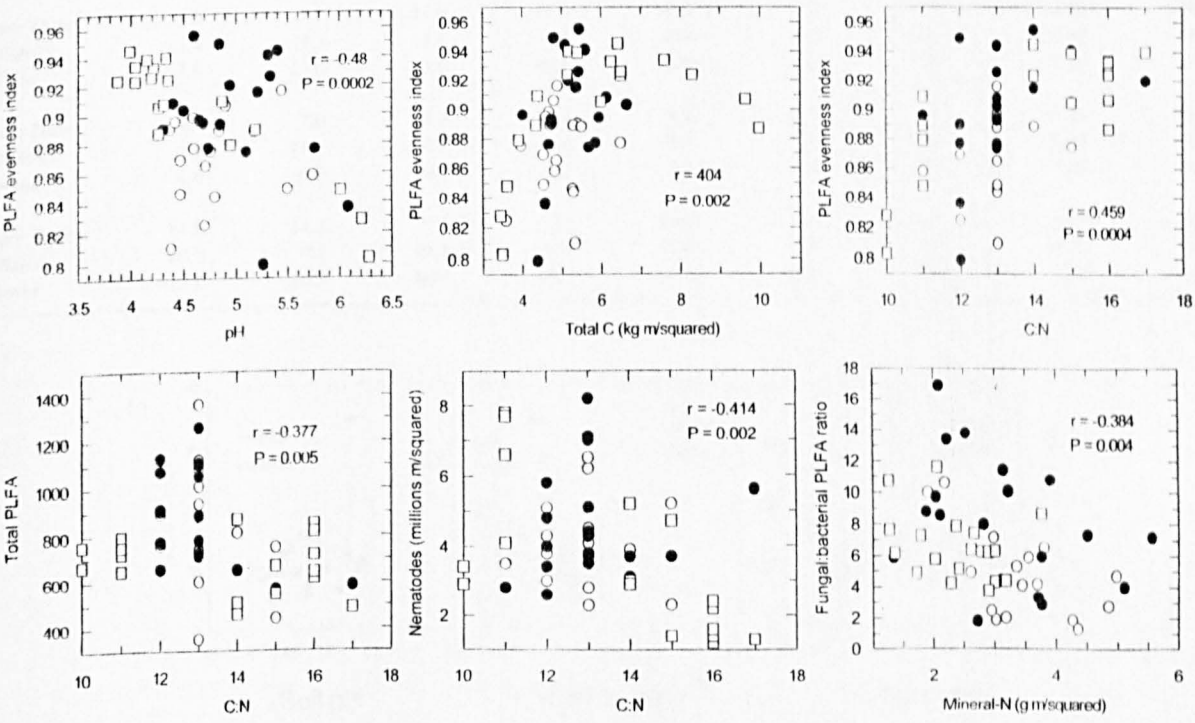


Fig. 3. Relationships between PLFA evenness index and total PLFA with soil pH, total C and C:N ratio across the three biogeographic locations. Symbols represent data for the Yorkshire Dales (○), Lake District (●), and Snowdonia (□).

Table 4
Changes in the relative abundance of individual PLFAs (% of total selected microbial PLFAs) in relation to grazing influence in upland ecosystems. Site 1 = ungrazed control, 2 = long-term ungrazed, 3 = short-term ungrazed, 4 = lightly grazed, 5 = moderately grazed, and 6 = heavily grazed. *, **Employed as indicators of fungal biomass and Gram-positive bacteria, respectively

Measure	Gradient of grazing influence								
	1	2	3	4	5	6	LSD	*F* value	*P* value
i15:0									
Yorkshire Dales	34.9	32.8	30.1	28.7	26.6	32.4	6.9	1.82	NS
Lake District	40.9	36.7	29.7	27.2	36.7	24.6	9.5	4.25	0.019
Snowdonia	39.8	33.8	37.6	37.6	27.6	30.7	5.3	7.30	0.002
a15:0*									
Yorkshire Dales	10.8	11.3	9.9	15.1	11.6	18.6	3.5	8.30	0.001
Lake District	11.5	8.8	12.1	6.7	11.3	28.7	5.4	20.54	0.0001
Snowdonia	9.7	10.4	8.7	5.8	17.8	27.0	2.1	130.61	0.0001
15:0									
Yorkshire Dales	3.4	1.9	2.3	1.8	2.7	2.9	0.7	7.23	0.002
Lake District	2.2	4.3	3.5	2.5	3.9	7.9	2.5	6.37	
Snowdonia	3.5	4.9	4.4	3.5	3.1	2.8	0.7	13.60	0.004
i16:0									
Yorkshire Dales	18.1	23.0	22.8	24.6	22.1	11.8	14.0	1.08	NS
Lake District	6.8	8.3	13.1	24.1	1.2	4.9	13.1	3.59	0.032
Snowdonia	5.1	1.7	1.6	0.7	13.7	1.6	8.5	3.21	0.045
17:0									
Yorkshire Dales	5.2	5.5	6.2	3.8	6.5	7.3	2.9	1.61	NS
Lake District	7.6	6.6	8.2	5.5	9.0	10.4	2.7	3.82	0.027
Snowdonia	6.1	9.6	9.2	13.1	7.0	8.5	1.5	24.35	0.0001
i17:0									
Yorkshire Dales	7.6	6.6	5.9	6.0	5.3	7.0	2.4	1.13	NS
Lake District	8.1	6.0	9.0	5.8	6.6	5.9	2.0	4.44	0.016
Snowdonia	8.0	7.7	7.0	4.7	5.8	8.5	1.8	6.41	0.004
cy17:0									
Yorkshire Dales	2.7	2.5	1.9	1.9	2.4	3.9	1.1	4.60	0.014
Lake District	3.5	4.3	2.3	1.7	2.3	2.6	1.7	3.05	NS
Snowdonia	3.9	2.4	2.2	3.6	2.6	3.7	1.2	1.16	0.027
18:1ω7									
Yorkshire Dales	8.0	7.0	11.0	13.0	10.3	7.7	10.3	0.48	NS
Lake District	3.8	4.3	2.6	9.4	8.9	4.6	5.4	2.60	NS
Snowdonia	3.8	13.0	13.0	10.1	3.9	4.4	5.9	5.54	0.007
18:2ω6**									
Yorkshire Dales	4.5	3.0	4.3	5.2	8.4	1.7	2.2	10.30	0.001
Lake District	5.4	11.1	8.8	8.6	8.8	2.6	4.0	5.39	0.008
Snowdonia	4.6	6.1	6.0	5.2	9.4	4.3	2.1	7.08	0.003
cy19:0									
Yorkshire Dales	11.0	14.3	9.7	3.3	13.0	5.7	7.5	3.07	NS
Lake District	10.2	9.7	10.7	8.6	11.3	7.8	5.8	0.48	NS
Snowdonia	15.8	10.3	10.4	15.7	9.1	8.4	4.4	5.39	0.008

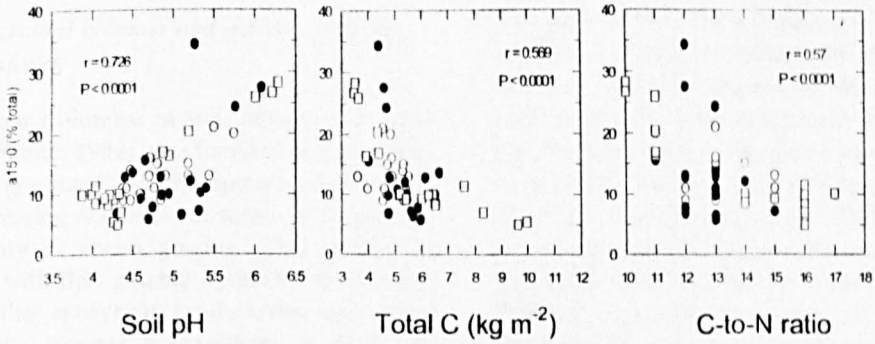


Fig. 4. Relationships between phospholipid a15:0 and soil pH, total C and C-to-N ratio across the three biogeographic locations. Symbols represent data for the Yorkshire Dales (○), Lake District (●), and Snowdonia (□).

and positively related to total soil C ($r = 0.404$, $P = 0.002$) and C-to-N ratio ($r = 0.459$, $P = 0.0004$). Total PLFA was negatively related to soil C-to-N ratio ($r = -0.377$, $P = 0.005$). The ratio of fungal-to-bacterial PLFAs was negatively related to amounts of mineral-N in soil ($r = -0.384$, $P = 0.004$). The relative abundances of the fatty acids i15:0 ($r = -0.371$, $P = 0.006$), 18:1ω7 ($r = -0.298$, $P = 0.029$), and cy19:0 ($r = -0.37$, $P = 0.006$) were negatively related to soil pH, whereas the relative abundance of a15:0 was positively related to soil pH ($r = 0.726$, $P < 0.0001$) (Fig. 4). Multiple regression revealed that most of the variance in the relative abundance of a15:0 could be explained by soil pH, followed by soil C content and C-to-N (Table 5).

Table 5
Multiple regression equations predicting the relative abundance of the fatty acid a15:0 from soil pH, C content and C-to-N ratio. The relationship was constructed using data from all successional stages of all three sites ($n = 54$, $r = 0.760$, $P < 0.0001$)

Soil property	Coefficient	t-value	P value
pH	6.027	4.68	< 0.0001
Total C	−0.925	−1.495	0.141
C-to-N	−0.461	−0.951	0.346

4. Discussion

Our study has shown that both chemical and biological properties of sub-montane soils vary significantly along successional gradients that are related to the intensity and history of grazing. Although several microbial properties of soils did not show any consistent patterns at the different biogeographic regions, there were certain trends that occurred across the locations. First, we found that although microbial activity of soil did not vary along the successional gradients, microbial biomass was generally at its greatest in the soils that were at the intermediate level of influence from grazing, and declined as the legacy of grazing was reduced through the long-term removal of sheep. Second, several individual 'signature' fatty acids varied along the successions, but the most obvious effect on microbial community structure was that, at two locations, the evenness of PLFA—an index of the degree of dominance of different groups of microorganisms—declined with increasing intensity of grazing. Third, the proportion of fungi relative to bacteria varied significantly along the successions, being at its lowest in the treatments that were most intensively grazed; in general, however, this measure showed few consistent responses to grazing influence. Some of these trends were related to alterations in the chemical composition of the soil along the successional transitions. We will now discuss these findings in turn.

4.1. Patterns of microbial biomass and activity, and the abundance of nematodes

The active microbial biomass in soil, measured as total PLFA (Tunlid and White, 1992), was found to be greatest in the lightly grazed grassland, which represented an intermediate level of grazing influence in terms of long-term history and intensity of sheep grazing. This finding is broadly consistent with the 'grazing optimization hypothesis' which states that ecosystem productivity, especially primary productivity, reaches a maximum at moderate levels of herbivory (McNaughton, 1979; Hilbert et al., 1981) due largely to enhanced re-circulation of nutrients within the ecosystem (Loreau, 1995). We do not have data on plant productivity or soil process rates, but the finding that microbial biomass peaked consistently in soils of these lightly grazed grasslands suggests that decomposer-related processes, such as nutrient cycling, might also be optimum at this intermediate level of grazing influence. However, data on soil respiration rates, a measure of microbial activity, does not support this claim, other than in Snowdonia where respiratory activity was also at a maximum in the lightly grazed grassland. Numbers of nematodes in soil, which are generally positively related to microbial biomass in these sub-montane ecosystems (Bardgett et al., 1997), also did not show a peak at this intermediate level of grazing influence, other than at one location. The absence of consistent effects of grazing influence on total numbers of nematodes does not preclude the possibility of responses from different trophic groups of nematodes. The complex nature of trophic relations that occur between different nematode feeding groups (Yeates and Wardle, 1996) and microorganisms, could possibly reduce the likelihood of detecting associations between these components at fixed sampling times (Wardle et al., 1999). Further analysis of nematode-feeding groups, at different times of the year, would be needed to resolve this issue.

Decomposer-related processes of nutrient cycling are regulated by the magnitude of the active soil microbial biomass (Beare et al., 1991). Therefore, the peak in soil microbial biomass in lightly grazed grassland might support the notion that compensatory responses to intermediate levels of herbivory are related to enhanced circulation of nutrients (McNaughton, 1985; Dyer et al., 1993; Loreau, 1995; de Mazancourt et al., 1998; Bardgett et al., 1998). The peak in microbial biomass is likely to be due to the combined, stimulatory effects of increases in the supply of labile C, and other nutrients, through defoliation-induced root exudation (Mawdsley and Bardgett, 1997; Guitian and Bardgett, 2000), changes in root turnover (McNaughton et al., 1998), and the supply of dung and urine (Bardgett et al., 1997; Tracey and Frank, 1998) in these lightly grazed grasslands. The dominance of certain plant species in these grasslands, which are absent from the other extremes of the gradient of grazing influence, might also exert positive effects on the soil microbial biomass through the exudation of rhizodeposits (Bardgett et al., 1999). Effects of changes in plant community structure are also likely to be responsible,

in part, for the lower microbial biomass values that were found in the sites that had been ungrazed for different lengths of time. Here, shifts in vegetation structure towards the dominance of dwarf-shrubs that are intolerant to grazing (e.g. *Calluna vulgaris* and *Vaccinium myrtillis*) will have increased the proportion of litter of low N and high polyphenol content entering the soil. This, in turn, will have increased the amount of recalcitrant organic matter in soil and hence reduced the amount of labile organic matter that is available to support the microbial biomass (Insam and Domsch, 1988). This view is supported by the finding that microbial biomass was positively related to the C-to-N ratio of soil; at all locations the C-to-N ratio of soil was highest in the sites that had been ungrazed for 20 years or more. Our findings, therefore, support the notion that the functional characteristics of dominant plants are important determinants of soil biological properties (Wardle et al., 1999; Bardgett et al., 1999), and hence the functioning of successional ecosystems (Wardle et al., 1997).

4.2. Patterns of microbial community evenness and composition

The evenness of PLFA was lowest in the most heavily grazed sites, and at two locations was highest in those sites that were either lightly grazed or had been ungrazed for different lengths of time. Although the evenness of PLFA cannot be used to measure species or genetic diversity, it can inform about very broad-scale changes in the relative abundance, or dominance (a component of diversity), of certain microbial groups within the decomposer community (Bardgett et al., 1999). Our results, therefore, provide some evidence that high intensities of grazing increase the degree of dominance within the microbial community, and that the microbial community becomes more even as the intensity and legacy of grazing declines. These changes in evenness were related primarily to a marked increase in the relative abundance of the fatty acid a15:0 in the more heavily grazed grasslands, which would indicate an increase in the dominance of Gram-positive bacteria (O'Leary and Wilkinson, 1988). The greater dominance of this microbial group, and consequent reduction in evenness, appears to be related mostly to the increase in pH of soil which occurred as a results of increasing grazing pressure; the PLFA evenness was negatively correlated, whereas the relative abundance of a15:0 was positively correlated to soil pH, and to a lesser extent by changes in soil C and N along the successions. Other individual fatty acids that are synthesized by Gram-negative bacteria (18:1ω7 and cy19) (Wilkinson, 1988) were negatively related to soil pH, but showed no consistent trends along the successional transitions. Although our data suggest that pH is an important shaping force in terms of the evenness of the microbial community, changes in the heterogeneity and availability resources (especially C) along the successional transitions are also likely to be important (Bardgett et al., 1997; Degens et al., 2000). Our findings are broadly consistent with other studies of successional sequences that show that heterotrophic evenness (Schipper et al., 2000) and fungal diversity (Frankland, 1998) generally increase as succession proceeds, due mainly to an increase in the amount and complexity of organic matter. However, we do not report any declines in the evenness of microbial communities in our completely ungrazed sites where low resource quality (high C-to-N ratio) might be expected to lead to the dominance of microorganisms that are strong competitors and the exclusion of others (Bardgett et al., 1999).

4.3. Fungal-to-bacterial ratios

Previous studies show that the microbial community of soil becomes increasingly dominated by fungi as succession proceeds (Wardle et al., 1995; Ohtonen et al., 1999; Bardgett et al., 1999), and that decreasing the intensity, or complete cessation, of grazing leads to a shift from a bacterial-based to a fungal-based energy channel (sensu Moore and Hunt, 1988) in the decomposer food-web (Bardgett et al., 1998). The ratio of fungal-to-bacterial PLFA, a measure of the relative abundance of fungi and bacteria in the microbial community (Frostegård and Bååth, 1996), was consistently at its lowest in the most heavily grazed grasslands. This was largely due to the relatively low abundance of the fungal fatty acid 18:2ω6 (Federle, 1986) in the heavily grazed as compared to the other, less intensively and unmanaged treatments. This finding provides some support for the notion that intensively grazed, or disturbed ecosystems have decomposition channels that are bacterial-based (dominated by Gram-positive bacteria) and that fungi are relatively more important in decomposer food-webs of less disturbed systems. These findings are consistent with other studies of agro-ecosystems which show that the soil fauna of low-input and intensively managed grasslands are dominated by bacterial-feeders and fungal-feeding animals respectively (Siepel, 1996; Yeates et al., 1997), and with studies of alpine grasslands that show that the abandonment of management increases the proportion of fungi relative to bacteria in the microbial community of soil (Zeller et al., 2001). Such changes might be related, in part, to the availability of N in soil. As in other studies (Bardgett and McAlister, 1999; Bardgett et al., 1999), our data reveal that the ratio of fungal-to-bacterial PLFA was negatively correlated with the mineral N concentration of soil.

5. Conclusion

Our data from different biogeographic locations—albeit from a single sample date—show that consistent 'broad-scale' trends in soil microbial communities of sub-montane grasslands are detectable along successional gradients that are related grazing intensity. Across three biogeographic zones, we show that microbial biomass of soil is maximal at low-to-intermediate levels of grazing influence and that

the phenotypic evenness (a component of diversity) of the microbial community declines as the intensity of grazing increases. We also provide evidence from these locations that soil microbial communities of intensively grazed sites are dominated by bacterial-based energy channels of decomposition, whereas in systems that are less intensively grazed, or completely unmanaged, fungi have a proportionally greater role. Further studies are needed to establish whether these trends are temporally robust and to determine the significance of these changes in relation to soil-level ecosystem processes of decomposition and nutrient cycling.

Acknowledgements

We are grateful to the land owners who allowed us to access their land and to the anonymous referees for their constructive comments on the manuscript.

References

Allen, S.E., 1989. Chemical Analysis of Ecological Materials. Blackwell, Oxford.

Bardgett, R.D., Cook, R., 1998. Functional aspects of soil animal diversity in agricultural grasslands. Applied Soil Ecology 10, 263–276.

Bardgett, R.D., McAlister, E., 1999. The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate grasslands. Biology and Fertility of Soils 19, 282–290.

Bardgett, R.D., Frankland, J.C., Whittaker, J.B., 1993. The effects of agricultural practices on the soil biota of some upland grasslands. Agriculture, Ecosystems and Environment 45, 25–45.

Bardgett, R.D., Marsden, J.H., Howard, D.H., 1995. The extent and condition of heather on moorland in the uplands of England and Wales. Biological Conservation 71, 155–161.

Bardgett, R.D., Hobbs, P.J., Frostegård, Å., 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. Biology and Fertility of Soils 22, 261–264.

Bardgett, R.D., Leemans, D.K., Cook, R., Hobbs, P.J., 1997. Seasonality in the soil biota of grazed and ungrazed hill grasslands. Soil Biology and Biochemistry 29, 1285–1294.

Bardgett, R.D., Wardle, D.A., Yeates, G.W., 1998. Linking above-ground and below-ground food webs: how plant responses to foliar herbivory influence soil organisms. Soil Biology and Biochemistry 30, 1867–1867.

Bardgett, R.D., Mawdsley, J.L., Edwards, S., Hobbs, P.J., Rodwell, J.S., Davies, W.J., 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Functional Ecology 13, 650–660.

Beare, M.H., Neely, C.L., Coleman, D.C., Hargrove, W.L., 1991. Characterisation of a substrate-induced respiration method for measuring fungal, bacterial and total microbial biomass on plant residues. Agriculture, Ecosystems and Environment 34, 65–73.

Bligh, E., G, ., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemical Physiology 37, 911–917.

Collins, S.L., Knapp, A.K., Briggs, J.M., Blair, J.M., Steinauer, E.M., 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. Science 280, 745–747.

Degens, B.P., Schipper, L.A., Sparling, G.P., Vojvodic-Vukovic, M., 2000. Decreases in organic matter C reserves in soils can reduce the catabolic diversity of soil microbial communities. Soil Biology and Biochemistry 32, 189–196.

Downes, M.T., 1978. An improved hydrazine reduction method for the automated determination of low nitrate levels in freshwater. Water Research 12, 673–675.

Dyer, M.I., Turner, C.L., Seastedt, T.R., 1993. Herbivory and its consequences. Ecological Applications 3, 10–16.

Federle, T.W., 1986. Microbial distribution in soil—new techniques. In: Megusar, F., Gantar, M. (Eds.), Perspectives in Microbial Ecology. Slovene Society for Microbiology, Ljubljana, pp. 493–498.

Floate, M.J.S., 1970a. Mineralization of nitrogen and phosphorus from organic materials of plant and animal origin and its significance in the nutrient cycle in grazed upland and hill soils. Journal of the British Grassland Society 25, 295–302.

Floate, M.J.S., 1970b. Decomposition of organic materials from hill soils and pastures. II. Comparative studies on the mineralization of carbon, nitrogen and phosphorus from plant materials and sheep faeces. Soil Biology and Biochemistry 2, 173–185.

Frankland, J.C., 1998. Fungal succession—unravelling the unpredictable. Mycological Research 102, 1–15.

Frostegård, Å., Bååth, E., 1996. The use of phospholipid fatty acid to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59–65.

Frostegård, Å., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25, 723–730.

Grant, S.A., Suckling, D.E., Smith, H.K., Torvell, L., Forbes, T.D.A., Hodgson, J., 1985. Comparative studies on the diet selection by sheep and cattle: the hill grassland. Journal of Ecology 73, 987–1004.

Groffman, P.M., Bohlen, P.J., 1999. Soil and sediment biodiversity—Cross-system comparisons and large-scale effects. BioScience 49, 139–148.

Guitian, R., Bardgett, R.D., 2000. Plant and soil microbial responses to defoliation in temperate semi-natural grassland. Plant and Soil 220, 271–277.

Hilbert, D.W., Swift, D.M., Detling, J.K., Dyer, M.I., 1981. Relative growth rates and the grazing optimization hypothesis. Oecologia 51, 14–18.

Hill, M.O., Evans, D.F., Bell, S.A., 1992. Long-term effects of excluding sheep from hill pastures in North Wales. Journal of Applied Ecology 80, 1–13.

Holland, E.A., Detling, J.K., 1990. Plant response to herbivory and belowground nitrogen cycling. Ecology 71, 1040–1049.

Huston, M.A., 1994. Biological Diversity. Cambridge University Press, Cambridge.

Insam, H., Domsch, K.H., 1988. Relationships between soil organic C and microbial biomass on chronosequences of reclamation sites. Microbial Ecology 15, 177–188.

Keeney, D.R., Nelson, D.W., et al., 1982. Nitrogen—inorganic forms. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd ed. Soil Science Society of America, Madison, WI, pp. 643–698.

Lawton, J.H., Bignell, D.E., Bolton, B., Bloemers, G.F., Eggleton, P., Hammond, P.M., Hodda, M., Holt, R.D., Larsen, T.B., Mawdsley, N.A., Stork, N.E., Srivastava, D.S., Watt, A.D., 1998. Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. Nature 391, 72–76.

Loreau, M., 1995. Consumers as maximisers of matter and energy flow in ecosystems. American Naturalist 145, 22–42.

Mawdsley, J.L., Bardgett, R.D., 1997. Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the microbial population of an upland grassland soil. Biology and Fertility of Soils 24, 52–58.

Mazancourt, C., de, ., Loreau, M., Abbadie, L., 1998. Grazing optimization and nutrient cycling: when do herbivores enhance plant production? Ecology 79, 2242–2252.

McNaughton, S.J., 1979. Grazing as an optimization process: grass-ungulate relationships in the Serengeti. American Naturalist 113, 691–703.

McNaughton, S.J., 1985. Ecology of a grazing ecosystem: the Serengeti. Ecological Monographs 55, 329–336.

McNaughton, S.J., Banyikwa, F.F., McNaughton, M.M., 1997a. Promotion of the cycling of diet-enhancing nutrients by African grazers. Science 278, 1798–1800.

McNaughton, S.J., Zuniga, G., McNaughton, M.M., Banyikwa, F.F., 1997b. Ecosystem catalysis: soil urease activity and grazing in the Serengeti ecosystem. Oikos 80, 467–469.

McNaughton, S.J., Banyikwa, F.F., McNaughton, M.M., 1998. Root biomass and productivity in a grazing ecosystem: the Serengeti. Ecology 79, 587–592.

Miles, J., 1985. The pedogenic effects of different species and vegetation types and the implications for succession. Journal of Soil Science 36, 571–584.

Moore, J.C., Hunt, H.W., 1988. Resource compartmentation and the stability of real ecosystems. Nature 333, 261–263.

Nicholson, I.A., Paterson, I.S., Currie, A., 1970. A study of vegetation dynamics: selction by sheep and cattle in Nardus pasture. In: Watson, A. (Ed.). Animal Populations in Relation to their Food Resources. Blackwell Science, Oxford, pp. 129–143.

O'Leary, W.M., Wilkinson, S.G., 1988. Gram-positive bacteria. In: Ratledge, C., Wilkinson, S.G. (Eds.). Microbial Lipids, vol. 1. Academic Press, London, pp. 117–185.

Ohtonen, R., Fritze, H., Pennanen, T., Jumpponen, A., Trappe, J., 1999. Ecosystem properties and microbial community changes in primary succession on a glacier forefront. Oecologia 119, 239–246.

Pearsall, W.H., 1950. Mountains and Moorlands. Collins New Naturalist Series. Bloomsbury Books, London.

Ruess, R.W., McNaughton, S.J., 1987. Grazing and the dynamics and energy regulated microbial processes in the Serengeti grassland. Oikos 49, 101–110.

Schipper, L.A., Degens, B.P., Sparling, G.P., Duncan, L., 2000. Changes in microbial heterotrophic diversity along five plant successional sequences. Soil Biology and Biochemistry, submitted.

Siepel, H., 1996. Biodiversity of soil microarthropods: the filtering of species. Biodiversity Conservation 5, 251–260.

Stohlgren, T.J., Schell, L.D., Heuvel, B.V., 1999. How grazing and soil quality affect native and exotic plant diversity in rocky mountain grasslands. Ecological Applications 9, 45–64.

Swift, M.J., Andren, O., Brussaard, L., Briones, M., Couteaux, M.M., Ekschmitt, K., Kjoller, A., Loiseau, P., Smith, P., 1998. Global change, soil biodiversity, and nitrogen cycling in terrestrial ecosystems: three case studies. Global Change Biology 4, 729–743.

Tracey, B.F., Frank, D.A., 1998. Herbivore influence on soil microbial biomass and nitrogen mineralization in a northern grassland ecosystem: Yellowstone National Park. Oecologia 114, 556–562.

Tunlid, A., White, D.C., 1992. Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. In: Stotzky, G., Bollag, J.M. (Eds.). Soil Biochemistry, vol. 7I. Marcel Dekker, New York, pp. 229–262.

Tunlid, A., Hoitink, H.A.J., Low, C., White, D.C., 1989. Characterization of bacteria that suppress Rhizoctonia damping-off in bark compost media by analysis of fatty acid biomarkers. Applied Environmental Microbiology 55, 1368–1374.

Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M., 1997. Human domination of earth's ecosystems. Science 277, 494–499.

Wall, D.H., Moore, J.C., 1999. Interactions underground—Soil biodiversity, mutualism, and ecosystem processes. BioScience 49, 109–117.

Wardle, D.A., 1995. Impact of disturbance on detritus food-webs in agroecosystems of contrasting tillage and weed management practices. Advances in Ecological Research 26, 105–185.

Wardle, D.A., Yeates, G.W., Watson, R.N., Nicholson, K.S., 1995. Development of the decomposer food-web, trophic relationships, and ecosystem properties during a three-year primary succession in sawdust. Oikos 73, 155–166.

Wardle, D.A., Zackrisson, O., Hörnberg, G., Gallet, C., 1997. The influence of island area on ecosystem properties. Science 277, 1296–1299.

Wardle, D.A., Bonner, K.I., Barker, G.M., Yeates, G.W., Nicholson, K.S., Bardgett, R.D., Watson, R.N., Ghani, A., 1999. Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. Ecological Monographs 69, 535–568.

White, D.C., Davis, W.M., Nickels, J.S., King, J.C., Bobbie, R.J., 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51–62.

Whitehead, A.G., Hemming, J.R., 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology 55, 25–38.

Wilkinson, S.G., 1988. Gram-negative bacteria. In: Ratledge, C., Wilkinson, S.G. (Eds.). Microbial Lipids, vol. 1. Academic Press, London, pp. 299–457.

Yeates, G.W., Wardle, D.A., 1996. Nematodes as predators and prey: relationships to biological control and soil processes. Pedobiologia 40, 43–50.

Yeates, G.W., Bardgett, R.D., Cook, R., Hobbs, P.J., Bowling, P.J., Potter, J.F., 1997. Faunal and microbial diversity in three Welsh grassland soils under conventional and organic management regimes. Journal of Applied Ecology 34, 453–471.

Zeller, V., Bardgett, R.D., Tappeiner, U., 2001. Site and management effects on soil microbial properties of subalpine meadows: A study of land abandonment along a north-south gradient in the European Alps. Soil Biology and Biochemistry in press.

Appendix 2 DOC analysis on the Skalar autoanalyser

Reagent	Chemicals	Formula	Amount	Preparation
Sulphuric acid 0.03 N	Sulphuric acid DI water	H_2SO_4 (96%) H_2O	0.84ml 1000ml	Sulphuric acid is diluted in 900ml DI water, made upto 1 l and mixed.
Digestion reagent	Potassium persulphate Sodium tetraborate DI water, carbonate-free.	$K_2S_2O_8$ $Na_2B_4O_7.10H_2O$ H_2O	12g 34g 1000ml	Potassium persulphate and sodium tetraborate dissolved in 800 ml H_2O, made up to 1000 ml and mixed.
Hydroxylamine solution	Hydroxylammonium chloride Sulphuric acid DI water Triton X-100	$HONH_3Cl$ H_2SO_4 H_2O -	100 g 20 ml 1000 ml 5 ml	Hydroxylammoniumchloride is dissolved in 900ml water, dilute sulphuric acid while swirling and then the Triton X-100. Make up to 1000 ml and mix well. Prepared 12 h before use.
Stock solution Colour reagent.	Sodium carbonate DI water, carbonate free.	Na_2CO_3 H_2O	10.6 g 1000 ml	Sodium carbonate is dissolved in 900 ml water, dilute to 1000 ml and mix well. Store in closed bottle.
Working solution Colour reagent.	Stock solution Phenolphthalein DI water Triton X-100	- $C_2OH_{14}O_4$ (1% in methanol) H_2O	8 ml 0.5 ml 1000 ml 2 ml	Stock solution is dissolved in 800 ml water, the phenolphthalein is added and diluted to 1000 ml. Add Triton X-100 and mix well. Prepared 12 h before use, stir while using in analyser, store in dark bottle.

Principle

The method involves UV persulphate digestion and is applicable within the range 0-10 ppm C. The sample is diluted with acid and purged with oxygen to remove dissolved carbonates. A digestion solution is then added and the sample is led along an ultra violet source. The radicals converted from the persulphate by the U.V. radiation react with the organic material in the sample, which is converted into carbon dioxide and water. By gas dialysis the carbon dioxide is led into a colour reagent, the colour intensity of this solution decreases proportionately to the change in pH caused by the carbon dioxide and is measured at 550 nm. This decrease is in relation to the concentration of the dissolved organic carbon (DOC).

Standards were made from a stock standard solution (1000 ppm C) from potassium hydrogen phthalate $C_8H_5O_4K$ (2.215g) in DI water (1000 ml). The standards were made up in 0.5 M K_2SO_4.

Appendix 3 Aluminium determination using the Pyrocatechol violet colorimetric method

Reagents

Pyrocatechol violet reagent: 0.077 g of pyrocatechol violet was dissolved in 200 ml H_2O (0.0375 %).

Hexamine buffer (15%) reagent: 75 g hexamine is dissolved in 400 ml H_20, then 16 ml of concentrated ammonia solution is added, pH adjusted to 6.2 with 5 M HCl and volume made up to 500 ml with H_2O.

Iron interference reagent: 100 mg 1,10-phenanthroline is added to 80 ml H_2O, 250 mg of ascorbic acid is added and volume made up to 100 ml (prepared daily).

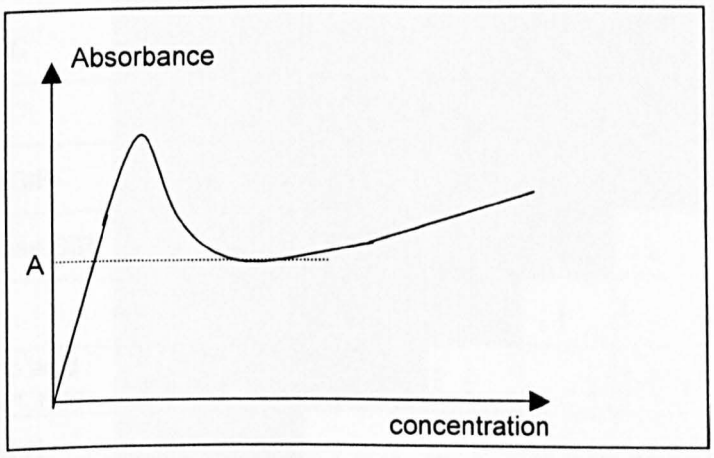
Procedure

0.750 ml of sample or standard is added to a cuvette, then 0.125 ml of the iron interference reagent, mix thoroughly. Then add 0.05 ml of the pyrocatechol reagent and mix. Finally, add 0.25 ml of hexamine buffer and mix. After 20 minutes filter samples through a 0.22 µm membrane and read absorbance at 585 nm.

Standards were made from Aluminium Chloride (FW 241.4) ($AlCl_3.6H_2O$) with concentrations of 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625 and 0 µM Al.

Note: It was found that the standard curve has shape as in figure i below.

Figure i Standard curve in the pyrocatechol violet assay



It was therefore necessary to repeat the assay with different dilutions until a sample gave a reading of absorbance between 0 and A.

Reference: Kerven, G.L. et al. (1989).

Appendix 4 Correlation matrix of soil and microbial parameters of data from chapter 7†

Cell row content: Rothamsted Woburn SAC	Total C	Total N	Soil C:N	Amino acid mins'n. rate	pH	Glucose SIR	Urea SIR	SOC	SMB C	NH_4^+	NO_3^-	SON	Net nitrification	Net ammonific'n	Net mineralis'n	Net change in SON	SMB-N_{NPC}	SMB C:N_{NPC}	SMB-N_{TDN}	SMB C:N_{TDN}	Soil moisture	Basal respiration	Aluminium	Calcium	Amino acids immob:resp	SMB-C % total C	SMB-N_{NPC} % total N
Total N	*** *** **																										
Soil C:N	- - -	*** *** *																									
Amino acid mins'n rate	*** *** -	** - -	- - -																								
pH	*** ** *	- - *	* - -	*** *** -																							
Glucose SIR	- - -	* - -	- - -	* - *	- *** -																						
Urea SIR	*** ** -	** ** *	- * -	*** *** **	*** - -	- ** *																					
SOC	* * -	- - -	** - -	*** *** -	*** *** *	- - *	*** - *																				
SMB-C	*** * -	- - -	* - -	*** * -	*** *** -	- * -	*** * -	*** *** -																			
NH_4^+	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- -																		

† calculated using Minitab V13, only detects linear correlations. [Legend: - = not significant (p>0.05), * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001]

Appendix 4 continued...

	Total C	Total N	Soil C:N	Amino acid mins'n. rate	pH	Glucose SIR	Urea SIR	SOC	SMB C	NH_4^+	NO_3^-	SON	Net nitrification	Net ammonific'n	Net mineralis'n	Net change in SON	SMB-N_{NPC}	SMB C:N_{NPC}	SMB-N_{TDN}	SMB C:N_{TDN}	Soil moisture	Basal respiration	Aluminium	Calcium	Amino acids immob:resp	% total C SMB-C	% total N SMB-N_{NPC}
NO_3^-	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- * -	- - -																	
SON	* - -	- - -	** - -	*** - -	*** - -	- - -	*** - -	*** - -	*** - -	- - *	- - -																
Net nitrification	- - -	- - -	- - -	- - -	- * -	- *** -	- * -	- - -	- - -	- - -	- - -	- - -																
Net ammonif'n	*** - -	*** - -	- - -	** - -	- - -	- - -	* - -	- - -	- - -	- - ***	- - -	- - -	- - -															
Net mineralis'n	*** - -	*** - -	- - -	** - -	- ** -	- ** -	* - -	- - -	- - -	- - *	- - -	- - -	*** *** -	*** *** **														
Net change in SON	- - -	- - -	- - -	* *** -	** *** -	- - -	- - -	** * -	* - -	- - -	- - -	*** - -	- - -	- ** -	- - -													
SMB-N_{NPC}	- - *	- - -	** - -	* - -	** - -	- - -	- - -	*** - *	* - -	- - -	- - -	** - -	- - -	- - -	- - -	** - -												
SMB C:N_{NPC}	- - -	- - -	- - -	- * -	- - -	- - -	- - -	- - -	- * -	- - -	* * -	- * -	- - -	*** - -	*** - -	- - -	*** *** -											
SMB-N_{TDN}	- * *	- * -	*** - -	*** - -	*** *** -	- ** -	* - -	*** *** -	*** *** -	- - -	- - -	*** - -	- - -	* - -	- - -	* - -	*** - -	** - -										
SMB C:N_{TDN}	** - -	* - *	- - *	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	*** - -	*** - -	- - -	** - -	*** ** -	*** *** **									

Appendix 4 continued...

	Total C	Total N	Soil C:N	Amino acid mins'n. rate	pH	Glucose SIR	Urea SIR	SOC	SMB C	NH_4^+	NO_3^-	SON	Net nitrification	Net ammonific'n	Net mineralis'n	Net change in SON	SMB-N_{NPC}	SMB C:N_{NPC}	SMB-N_{TDN}	SMB C:N_{TDN}	Soil moisture	Basal respiration	Aluminium	Calcium	Amino acids immob:resp	% total C SMB-C	% total N SMB-N_{NPC}	% total N SMB-N_{TDN}
Soil moisture	*** *** *	*** ** -	- - -	** * -	- - ***	- ** -	** *** -	- - **	- - -	- - -	- - -	- - -	- *** -	* - -	* * -	- - -	- - *	- - -	- - -	- - -	- - *							
Basal respiration	*** - -	* - -	- - -	*** * **	*** *** *	- *** *	** - **	*** * *	*** ** -	- ** -	- ** -	*** - -	- * -	- - -	- - -	- - -	* - -	- - -	** *** -	- - -	- - *							
Aluminium	*** ** -	* - -	- - -	*** *** -	*** *** *	- - -	*** - -	*** *** -	*** *** -	- - -	- - -	*** - -	- - -	** - -	** - -	** *** -	- - -	- - -	** *** -	- - -	*** - *	*** *** -						
Calcium	*** ** -	- - -	- - -	*** *** -	*** *** -	- - -	*** - -	*** *** -	*** *** -	- - -	- - -	*** - -	- - -	- - -	- - -	* *** -	- * -	- * -	** *** -	- - -	*** - -	* *** -	*** *** *					
Amino acids immob:resp	** - -	* - -	- - -	*** - -	*** *** -	- ** -	*** - *	*** *** -	*** *** -	- - -	- - -	*** - -	- - -	- - -	- - -	* - -	- - -	- - -	** *** -	- - -	* - -	*** ** -	*** ** -	*** ** -				
% total C SMB-C	- - *	- - -	** - -	** - ***	*** *** -	- ** -	* - *	*** *** -	*** *** -	- - -	- * -	*** - -	- - -	- - -	- - -	* - -	* - -	- ** -	*** *** -	- - -	- - -	** ** *	*** *** -	*** ** -	*** *** -			
% total N SMB-N_{NPC}	- - -	** - -	*** - -	- - -	* - -	- - -	- - -	** - -	- - -	- - -	- - -	* - -	- - -	* - -	- - -	* - -	*** *** **	*** *** *	*** - -	** - -	- - -	- - -	- - -	- - -	- - -	- - -		
% total N SMB-N_{TDN}	- - -	*** - -	*** ** -	- - -	** *** -	- *** -	- - -	*** ** -	*** *** -	- - -	- - -	** - -	- - -	*** - -	** - -	- - -	*** - -	** - -	*** *** *	*** ** *	- - -	- *** -	- ** -	- ** -	*** *** -	*** - -		

Appendix 5 "Effect of depth, N regime and Drainage on Mineralisation of Organic Matter"

Poster presented at the British Soil Science Society Meeting

Edinburgh 1999

Effect of depth, N regime and Drainage on Mineralization of Organic Matter

Sarah Gowland[1a], Davey Jones[1] & Roland Bol[2].

[1]School of Agriculture and Forest Sciences, University of Wales, Bangor, Gwynedd, LL57 2UW.

[2]Institute of Grassland and Environmental Research, North Wyke, Okehampton, Devon

[a]afpØaf@bangor.ac.uk.

Introduction.

Previous work at North Wyke indicated that microbial processes and properties in grassland soils are affected by drainage and N fertiliser application. This study used an amino acid mineralization assay (Jones, 1999) to further examine the influence of soil depth, N fertilization regime and drainage type on organic matter mineralization. The soil type was Pelostagnogley (a very poorly drained silty clay loam).

Method.

The following permanent pasture plots at Rowden (IGER North Wyke) were sampled: (1) Conventional N (280kg N ha^{-1}) drained (CND); (2) Conventional N undrained (CNU); (3) Zero N undrained (ZNU); (4) Zero N drained (ZND); (5) Grass/clover sward, drained (G/CD). From each plot 4 samples were taken, and each sample was split into 4 depths: 0-5cm, 5-10cm, 10-15cm and 15-20cm. Samples were analysed for total C and N, moisture content, and amino acid mineralization using ^{14}C labelled amino acids.

Figure 1.

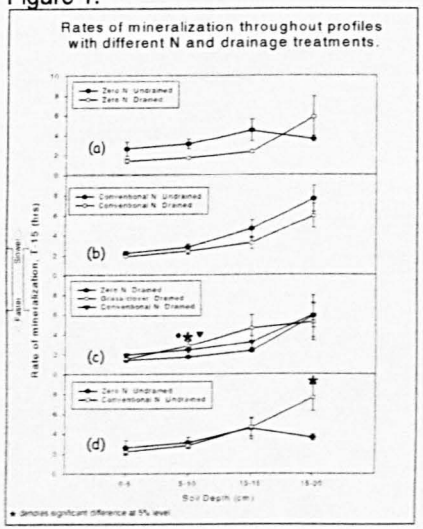
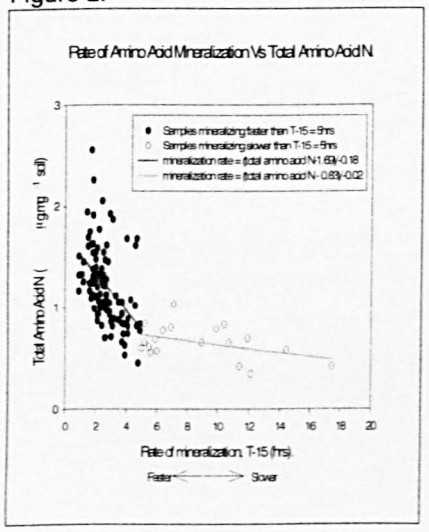


Figure 2.



Conclusions.

The addition of ^{14}C-amino acids followed by the monitoring of evolved $^{14}CO_2$ (Jones, 1999) provides a rapid and easy diagnostic method of comparing mineralization rates of nitrogenous organic matter between treatments.

Results and discussion.

Drainage decreased T-15 mineralization rates (time required for 15% of the added ^{14}C-amino acids to be respired) in Zero-N (Fig. 1a), but not in conventional N treatments (Fig 1b). The G/CD and CND mineralization rates in 0-15 cm depth interval were lower in than in the ZND treatment (Fig 1c). Whereas, in the CNU treatment the mineralization rate was lower than the ZNU at a depth of 15-20cm (Fig 1d). The mineralization rate decreases with depth in all treatments (Fig 1a-d). The mineralization rates were correlated with total C and total N, but moisture content had limited influence (results not shown).

There appears to be a 2-phase relationship between total amino acid N (aa-N) content and mineralization rate, whereby at high aa-N contents, a small drop results in a small decrease in mineralization rate, but at lower organic matter contents a small drop results in a large decrease in mineralization rate. This 'cut-off' point occurs at a T-15 value of ~5hrs, and is likely to be related to the recalcitrance of the o.m. substrate.

Our observed differences in amino acid mineralization rates with increasing depth, drainage status and N fertiliser application generally reflect the variations in soil C and N content measured in other studies on Rowden (McTiernan, *pers. comm.*), with the highest mineralization rates occurring the soils with the highest total C and N contents.

Reference Jones, D.L. (1999). Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. *Soil Biology and Biochemistry*. **31**:613-622.

Appendix 6 "Changes in soil quality following the incorporation of pasture"

Poster presented at the British Soil Science Society Meeting

Edinburgh 1999

Changes in soil quality following the incorporation of pasture.

Sarah Gowland[1a], Davey Jones[1], & Roland Bol[2].
[1]School of Agriculture and Forest Sciences,University of Wales, Bangor, Gwynedd, LL57 2UW
[2]Institute of Grassland and Environmental Research, North Wyke, Okehampton, Devon
[a] afpØaf@bangor.ac.uk

Introduction

The conversion of long term pastures to arable cropping can lead to a reduction in soil organic matter content. An amino acid mineralization assay (Jones, 1999) was employed as a diagnostic tool to determine how such land use changes affect soil organic matter quality in the first 3 years after conversion.

Method

Soils were sampled from a randomised plot experiment; in which grassland had been converted to maize in 1996, '97 and '98, with one set remaining unchanged. Each treatment was replicated three times, and three samples were taken from each plot (0-15cm depth). Two distinct soils were compared: 1. Gleyic Cambisol (Burrows) and 2. Eutric Cambisol (De Bathe). Samples were analysed for moisture content, total C, total N, C:N ratio and mineralization rates of ^{14}C labelled amino acids (Jones, 1999).

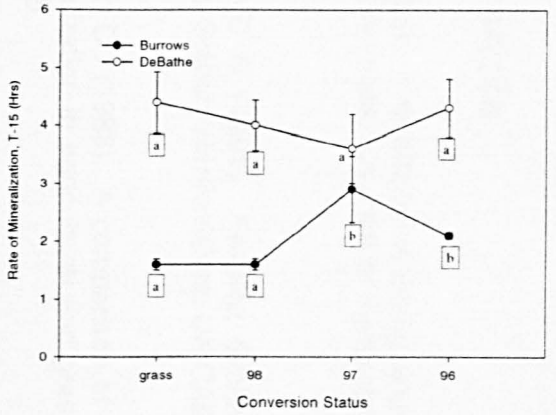
Table 1

Treatment	Burrows Mean ± s.e. (n=9)				DeBathe Mean ± s.e. (n=9)			
	Moisture (%)	Total C (%)	Total N (%)	C:N	Moisture (%)	Total C (%)	Total N (%)	C:N
Grass	51.4±1.26a	4.49±0.17a	0.46±0.02a	9.84±0.11a	21.5±0.65a	1.56±0.03ab	0.13±0.00a	12.04±0.21a
Converted '98	39.0±2.22b	4.29±0.13a	0.44±0.01a	9.69±0.17a	21.1±0.76a	1.68±0.06a	0.14±0.01b	11.76±0.13a
Converted '97	36.2±2.67b	3.43±0.12b	0.36±0.01b	9.46±0.12ab	15.3±1.02b	1.60±0.04ab	0.15±0.01b	10.75±0.47a
Converted '96	34.6±2.22b	3.69±0.08b	0.39±0.01b	9.37±0.08b	19.7±1.63a	1.53±0.03b	0.14±0.00ab	11.08±0.25a

Results and Discussion

After 48hrs incubation the mean percentage of added amino acids (AA) immobilised, respired and unused was 63.5, 34.8 and 1.6 in the Burrows soil and 58.8, 38.7 and 2.5 in the De Bathe soil respectively, with no significant differences between treatments. The T-15 mineralization rate (the time taken for 15% of AA to be respired) in the the Burrows soil was faster than that observed in the De Bathe soil (Fig.1); probably associated with its higher C and N contents and lower C:N ratios (Table 1). The mineralization rates at Burrows were significantly lower in the second and third year after conversion (5% significance level), but no differences were observed for the De Bathe soil. Mineralization rates were (weakly) correlated with total C (r^2 = 0.53), total N (r^2 = 0.38) and C:N (r^2 = 0.38) in the Burrows soil, but not in the De Bathe soil. The lowering of soil moisture content in the Burrows plots following conversion may also have contributed to the observed decrease in its mineralization rates (Table 1).

Figure 1. Rate of amino acid mineralization, T-15, in Plots converted from grass to maize on 2 soil types.



Conclusions

The impact of the conversion of permanent pasture to arable cropping on soil organic matter mineralization, and hence soil quality, was more severe in the Gleyic Cambisol than the Eutric Cambisol. Soil type therefore appears to be an important factor in how soil quality characteristics respond to land use changes and crop rotations.

Reference

Jones, D.L. (1999). Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. *Soil Biology and Biochemistry*. **31**:613-622.

References

Adams, T. McM. and Adams, S. N. (1983). The effects of liming and soil pH on carbon and nitrogen contained in the soil biomass. *Journal of Agricultural Science*, 101, 553-558.

Addiscott T. M., Whitmore A. P., Powlson D. S. (1991). *Farming, fertilizers and the nitrate problem.* Rothamsted Experimental Station, Hertfordshire, UK CABI, 170 pp.

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

Alef, K. and Kleiner, D. (1986). Arginine ammonification a simple method to estimate microbial activity potentials in soils. *Soil Biology and Biochemistry*, 18, 223-235.

Alef, K. and Kleiner, D. (1987). Estimation of anaerobic microbial activities in soil by arginine ammonification and glucose-dependent CO_2 production. *Soil Biology and Biochemistry*, 19, 683-686.

Allen, S. E. (1989). *Chemical Analysis of Ecological Materials.* Blackwell, Oxford.

Alef, K. and Nannipieri, P. (1995). Protease activity. pp. 313-315 In: K. Alef and P. Nannipieri (Eds.) *Methods in Applied Soil Microbiology and Biochemistry.* Academic press.

Alexander, M. (1980). Effects of acidity on microorganisms and microbial processes in soil. pp. 363-374 In: Hutchinson, T. C. and Havas, M. (Eds.) *Effects of acid precipitation on terrestrial ecosystems.* Plenum press.

ALPC (Agricultural Lime Producers Council). (1995). *Agricultural lime and the environment.* 32pp. ALPC, 156 Buckingham Palace Rd, London, SW1 W9TR.

Amato, M. and Ladd, J. N. (1988). Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils. *Soil Biology and Biochemistry*, 20, 107-114.

Amato, M. and Ladd, J. N. (1994). Application of the ninhydrin - reactive N assay for microbial biomass in acid soils. *Soil Biology and Biochemistry*, 26, 1109-1115.

Anderson, J. P. E. and Domsch, K. H. (1973). Quantification of bacterial and fungal contributions to soil respiration. *Archiv für Mikrobiologie,* 93, 113-127.

Anderson, J. P. E. and Domsch, K. H. (1985). Maintenance carbon requirements of actively –metabolizing microbial-populations under in situ conditions. *Soil Biology and Biochemistry,* 17, 197-203.

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

Anderson, T.H. and Domsch, K.H., (1993). The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions such as pH on the microbial biomass of forest soils. *Soil Biology and Biochemistry,* 25, 393-395.

Anderson, T.H. (1998). The influence of acid irrigation and liming on the soil microbial biomass in a Norway spruce (*Picea abies* [L.]K.) stand. *Plant and Soil,* 199, 117-122.

Anderson, T. H. and Joergensen, R. G. (1997). Relationship between SIR and FE estimates of microbial biomass C in deciduous forest soils at different pH. *Soil Biology and Biochemistry,* 29, 1033-1042.

Anraku, Y. (1980). Transport and utilization of amino acids by bacteria. pp. 9-33. In: Payne, J. W. (Ed.) *Microorganisms and Nitrogen Sources.* John Wiley and Sons Ltd.

Armstrong, A.C. and Burt, T.P. (1993). Nitrate losses from agricultural land. Chpt 9, pp. 239-268. In: Burt, T.P., Heathwaite, A.L. and Trudgill, S.T. (Eds.) *Nitrate Processes, Patterns and Management.* Wiley. 444 pp.

Atkin, O. K. (1996). Reassessing the nitrogen relations of Arctic plants: a mini-review. *Plant, Cell and Environment.* 19, 695-704.

Badalucco, L., Kuikman, P. J., Nannipieri, P. (1996). Protease and deaminase activities in wheat rhizosphere and their relation to bacterial and protozoan populations. *Biology and Fertility of Soils,* 23, 99-104.

Bailey, J. S. (1995). Liming and nitrogen efficiency - some effects of increased calcium supply and increased soil pH on nitrogen recovery by perennial ryegrass. *Communications in Soil Science and Plant Analysis,* 26, 1233-1246.

Ball, D. F. (1963). *The soils and land used of the district around Bangor and Beaumaris.* Soil Survey of Great Britain. 182pp.

Barak, P, Molina, J. A. E., Hadas, A., Clapp, C. E. (1990). Mineralization of amino acids and evidence of direct assimilation of organic nitrogen. *Soil Sci. Soc. Am. J.* 54, 769-774.

Barber, S. A. (1984). Liming materials and practices. pp 171-205 In: Adams, F (Ed.) *Soil acidity and liming.* American Society of Agronomy, Madison.

Bardgett, R. D., Frankland, J. C., Whittaker, J. B. (1993). The effects of agricultural practices on the soil biota of some upland grasslands. *Agriculture, Ecosystems and Environment*, 45, 25-45.

Bardgett, R. D., Hobbs, P. J., Frostegård, A. (1996). Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biology and Fertility of Soils,* 22, 261-264.

Bardgett, R. D., Wardle, D. A., Yeates, G. W. (1998). Linking above-ground and below-ground food webs: how plant responses to foliar herbivory influence soil organisms. *Soil Biology and Biochemistry,* 30, 1867-1878.

Bardgett, R. D., Jones, A. C., Jones, D. L., Kemmitt, S.J., Cook, R. and Hobbs, P.J. (2001). Soil microbial community patterns related to the history and intensity of grazing in sub-montane ecosystems. *Soil Biology and Biochemistry,* 33, 1653-1664.

Barford, C. and Lajtha, K. (1992). Nitrification and nitrate reductase activity along a secondary successional gradient. *Plant and Soil,* 145, 1-10.

Barraclough, D. (1997). The direct MIT route for nitrogen immobilization: a ^{15}N mirror image study with leucine and glycine. *Soil Biology and Biochemistry,* 29, 101-108.

Bascomb, C.L. (1974). Physical and Chemical Analyses of <2mm Samples. In: Avery, B.W. and Bascomb, C.L. (Eds.) *Soil Survey Laboratory Methods, Soil Survey Technical Monograph No. 6.* Rothamsted Experimental Station, Harpenden, Herts. 83pp.

Bhogal, A., Hatch, D.J., Shepherd, M.A. and Jarvis, S.C. (1999). Comparison of methodologies for field measurement of net nitrogen mineralisation in arable soils. *Plant and Soil*, 207, 15-28.

Bhogal, A., Murphy, D.V., Fortune, S., Shepherd, M.A., Hatch, D.J., Jarvis, S.C., Gaunt, J.L., and Goulding, K.W.T. (2000). Distribution of nitrogen pools in the soil profile of undisturbed and reseeded grasslands. *Biology and Fertility of Soils,* 30, 356-362.

Blagodatskaya, E.V. and Anderson, T.H. (1998). Interactive effects of pH and substrate quality on the fungal to bacterial ratio and qCO_2 of microbial communities in forest soils. *Soil Biology and Biochemistry,* 30, 1269-1274.

Blagodatskaya, E.V. and Anderson, T.H. (1999). Adaptive responses of soil microbial communities under experimental acid stress in controlled laboratory studies. *Applied Soil Ecology,* 11, 207-216.

Blake, L., Goulding, K. W. T., Mott, C. J. B., Johnston, A.E. (1999). Changes in soil chemistry accompanying acidification over more than 100 years under woodland and grass at Rothamsted Experimental Station, UK. *European Journal of Soil Science,* 50, 401-412.

Bock, E., Koops, H. P. and Harms, H. (1989). Nitrifying bacteria. pp81-96. In: Schlegel H.G. and Bowien B. (Eds.) *Autotrophic Bacteria.* Science Tech., Madison, WI.

Bolton, J. (1977). Changes in soil pH and exchangeable calcium in two liming experiments on contrasting soils over 12 years. *Journal of Agricultural Science, Cambridge,* 89, 81-86.

Bramley, R. G. V. and White, R. E. (1989). The effect of pH, liming, moisture and temperature on the activity of nitrifiers in a soil under pasture. *Australian Journal of Soil Research,* 27, 711-724.

Bremer, E. and Kuikman, P (1997). Influence of competition for nitrogen in soil on net mineralization of nitrogen. *Plant and Soil,* 190, 119-126.

Brock, T.D. and Schlegel, H.G. (1989). Chapter 1 pp1-16. Introduction. In: Schlegel, H.G. and Bowien, B. (Eds.) *Autotrophic Bacteria.* Science tech, Maddison, WI.

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

Brussaard, L., Bakker, J. P. and Olff, H. (1996). Biodiversity of soil biota and plants in abandoned arable fields and grasslands under restoration management. *Biodiversity and conservation,* 5, 211-221.

Brussaard, L. (1998). Soil fauna, guilds, functional groups and ecosystem processes. *Applied Soil Ecology*, 9, 123-135.

Burle, M.L., Mielniczuk, J., Focchi, S. (1997). Effect of cropping systems on soil chemical characteristics, with emphasis on soil acidification. *Plant and Soil*, 190, 309-316.

Cabrera, M. L., Beare, M. H. (1993). Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Sci. Soc.Am. J.* 57, 1007-1012.

Chapin, F.S., Moilunen, L. and Kielland, K. (1993). Preferential use of organic nitrogen for growth by a non-micorrhizal arctic sedge. *Nature*, 261, 150-153.

Chaudri, A.M, Allain, C.M.G., Barbosa-Jefferson, V.L., Nicholson F.A., Chambers, B.J., McGrath, S.P. (2000). A study of the impact of Zn and Cu on two rhizobial species in soil of a long-term field experiment. *Plant and Soil*, 221, 167-179.

Chen, D.L., Chalk, P.M., Freney, J.R. (1995). Distribution of reduced products of ^{15}N-Labelled nitrate in anaerobic soils. *Soil Biology and Biochemistry*, 27, 1539-1545.

Clay, D. E., Clapp, C. E., Dowdy, R., H., Molina, J. A. E. (1993). Mineralization of Nitrogen in Fertilizer-Acidified Lime-Amended Soils. *Biology and Fertility of Soils*. 15, 249-252.

Clewer, A.G., Scarisbrick, D.H. (2001). *Practical Statistics and Experimental Design for Plant and Crop Science*. John Wiley & Sons, Ltd. 332pp.

Coody, P. N., Sommers, L. E. and Nelson, D. W. (1986). Kinetics of glucose uptake by soil microorganisms. *Soil Biology and Biochemistry,* 18, 283-289.

Creasey, C.L., Dreiss, S.J. (1988). Porous cup samplers: cleaning procedures and potential sample bias from trace element contamination. *Soil Science*, 145, 93-101.

Curtin, D., Campbell, C.A., Jalil, A. (1998). Effects of acidity on mineralization: pH-dependence of organic matter mineralization in weakly acidic soils. *Soil Biology and Biochemistry*, 30, 57-64.

Curtin, D. and Ukrainetz, H. (1997). Acidification rate of limed soil in a semiarid environment. *Canadian Journal of Soil Science*. 77, 415-420.

Cuttle, S.P., Hallard, M., Gill, E. K. and Scurlock, R. V. (1996). Nitrate leaching from sheep-grazed upland pastures in Wales. *Journal of Agricultural Science*, 127, 365-375.

Cuttle, S. P., Scurlock, R. V., Davies, B. M. S., (1998). A 6-year comparison of nitrate leaching from grass/clover and N-fertilized grass pastures grazed by sheep. *Journal of Agricultural Science*, 131, 39-50.

Dähne, J., Klingelhöfer, D., Ott, M., Rothe, G. M. (1995). Liming induced stimulation of the amino acid metabolism in mycorrhizal roots of Norway spruce (*Picea abies* [L] Karst.). *Plant and Soil*, 173, 67-77.

D'Ambrosio, S. M., Glover, G. I., Nelson, S. O., Jensen, R. A. (1973). Specificity of the tyrosine-phenylalanine transport system in *Bacillus subtilis*. *Journal of Bacteriology*. 115, 673-681.

Dampney, P. M. R., Lord, E. I., Chambers, B. J. (2000). Development of improved advice for farmers and advisers. *Soil Use and Management*, 16, 162-166. Supplement: Tackling nitrate from agriculture.

Dancer, W. S., Peterson, L. A. and Chesters, G. (1973). Ammonification and nitrification of N as influenced by soil pH and previous N treatments. *Soil Sci. Soc. Amer. Proc.*, 37, 67-69

Darrah, P. R., Nye, P. H. and White, R. E. (1986). Simultaneous nitrification and diffusion in soil. V The effects of pH change, following the addition of ammonium sulphate, on the activity of nitrifiers. *Journal of Soil Science,* 37, 479-484.

Dashman, T., Stotzky, Y. G. (1986). Microbial utilization of amino acids and a peptide bound on homoionic montmorillonite and kaolinite. *Soil Biology and Biochemistry*, 18, 5-14.

Davies, D.B. (2000). The nitrate issue in England and Wales. *Soil Use and Management*, 16, 142-144. (Supplement; Tackling nitrate from agriculture).

DeBoer, W., Gunnewiek Pjak, Troelstra, S. R. (1990). Nitrification in Dutch heathland soils 2. Characteristics of nitrate production. *Plant and Soil*, 127:193-200.

DeBoer, W., Gunnewiek Pjak, Veenhuis, M., Bock, E., Laanbroek, H.J. (1991). Nitrification at low pH by aggregated chemolithotrophic bacteria. *Applied and Environmental Microbiology,* 57, 3600-3604.

Degens, B. P., Harris, J. A. (1997). Development of physiological approach to measuring the catabolic diversity of soil microbial communities. *Soil Biology and Biochemistry,* 29, 1309-1320.

Degens, B. P., Schipper, L. A., Sparling, G. P., Vojvodic-Vukovic, M. (2000). Decreases in organic matter C reserves in soils can reduce the catabolic diversity of soil microbial communities. *Soil Biology and Biochemistry,* 32, 189-196.

De Klein, C. A. M., Monaghan, R. M., Sinclair, A. G. (1997). Soil acidification: a provisional model for New Zealand pastoral systems. *New Zealand Journal of Agricultural Research*, 40, 541-557.

De Ruiter, P. C., Bloem, J., Bouwman, L. A., Didden W. A. M., Hoenderboom, G. H. J., Lebbink, G., Marinissen, J. C.Y., Devos, J. A., Vreekenbuijs, M. J., Zwart, K. B., Brussaard, L. (1994) Simulation of dynamics in nitrogen mineralisation in the belowground foodwebs of two arable farming systems. *Agriculture, Ecosystems and Environment*, 51, 199-208.

Department of the Environment, Central Directorate of Environmental Protection. (1986). *Nitrate in Water, Pollution paper No. 26.* HMSO, London, 104pp.

Dhont, C., Murray, P. J., Cliquet, J. B. (Unpublished) Uptake of amino nitrogen by *Lolium perenne* L.

Djurhuus, J. and Olsen, P. (1997). Nitrate leaching after cut grass/clover leys as affected by time of ploughing. *Soil Use and Management,* 13, 61-67.

Dolling P. J., Porter, W. M., Rowland, I. C. (1994). Acidification rates in the central wheatbelt of Western Australia. 2. on a deep yellow sand. *Australian Journal of Experimental Agriculture*, 34 1165-1172.

Doran, J. W. and Parkin, T. B. (1996). Quantitative indicators of soil quality: a minimum data set. Chapter 2 pp 25-37. In: Doran, J. W. (Ed.) *Methods for assessing soil quality. Special Publication 49.* SSSA.

Downes, M.T. (1978). An improved hydrazine reduction method for the automated determination of low nitrate levels in freshwater. *Water Research,* 12, 673-675.

Drury, C. F., Voroney, R. P., Beauchamp, E. G. (1991). Availability of NH_4^+ to microorganisms and the soil internal nitrogen cycle. *Soil Biology and Biochemistry*, 23, 165-169.

Dyer, M. I., Turner, C. L., Seastedt, T. R. (1993). Herbivory and its consequences. *Ecological Applications*, 3, 10-16.

Edmeades, D. C., Judd, M. and Sarathchandra, S. U. (1981). The effect of lime on nitrogen mineralization as measured by grass growth. *Plant and Soil*, 60, 177-186.

Ellis, S., Howe, M.T., Goulding, K.W.T., Mugglestone, M.A. and Dendooven, L. (1998). Carbon and nitrogen dynamics in a grassland soil with varying pH: effect of pH on the denitrification potential and dynamics of the reduction enzymes. *Soil Biology and Biochemistry*, 30, 359-367.

Environment Agency. (1998). *Aquatic Eutrophication in England and Wales - a proposed management strategy*. Consultative Report. Environmental Issues Series. Environment Agency. 37pp.

Erich, M. S., Trusty, G. M. (1997). Chemical characterization of dissolved organic matter released by limed and unlimed forest soil horizons. *Canadian Journal of Soil Science*, 77, 405-413.

Estavillo, J. M., Rodrigues, M., Lacuesta, M. and Gonzalez-Murua, C. (1997). Effects of cattle slurry and mineral N fertilizer applications on various components of the nitrogen balance of mown grassland. *Plant and Soil*, 188, 49-58.

Evans, C.M., Conyers, M.K. Black, A.S., Poile, G.J. (1998). Effect of ammonium, organic amendments, and plant growth on soil pH stratification. *Australian Journal of soil Research*, 36, 641-653.

Farr, E, Sinclair, A. H., Lee, K. M. and Atkinson, D. (1992). Effect of the cutting management of naturally regenerated Set Aside land on the mineral N content in the soil profile. In: Clarke J. (Ed.) *Set Aside BCPC Mono Nop.50*. BCPC.

Felske, A., Wolterink, A., Van Lis, R., De Vos, W. M., Akkermans, A. D. L. (2000). Response of a Soil Bacterial Community to Grassland Succession as Monitored by 16S rRNA Levels of the predominant Ribotypes. *Applied Environmental Microbiology*, 66, 3998-4003.

Fischer, W. N., Kwart, M., Hummel, S., Frommer, W. B. (1995). Substrate specificity and expression profile of amino acid transporters (AAPs) in *Arabidopsis*. *The Journal of Biological Chemistry*, 270, 16315-16320.

Foster, B. and Gross, K. L. (1998). Species richness in a successional grassland: effects of nitrogen enrichment and plant litter. *Ecology*, 79, 2593-2602.

Foulkes, M.J., Scott, R. K., Sylvester-Bradley, R., Clare, R. W., Evans, E. J., Frost, D. L., Kettlewell, P. S., Ramsbottom, J. E. and White, E. (1994). Suitabilities of UK winter wheat (*Triticum aestivum*, L) varieties to soil and husbandry conditions. *Plant varieties and seeds*, 7, 161-181.

Foy, C. D. (1984). Physiological effects of hydrogen, aluminium and manganese toxicities in acid soil. pp 57-98 In: Adams, F (Ed.) *Soil acidity and liming*. American Society of Agronomy, Madison.

Foy, C. D. and Peterson, C. J. (1994). Acid soil tolerances of wheat lines selected for high grain protein content. *Journal of plant nutrition*, 17, 377-400.

Franzluebbers, K., Weaver, R. W., Juo, A. S. R., Franzluebbers, A. J. (1994). Carbon and nitrogen mineralisation from cowpea plants part decomposing in moist and in repeatedly dried and wetted soil. *Soil Biology and Biochemistry*, 26, 1379-1387.

Freeman, C., Liska, G., Ostle, N. J., Jones, S. E. and Lock, M. A. (1995). The use of fluorogenic substrates for measuring enzyme activity in peatlands. *Plant and Soil*, 175, 147-152.

Froment, M. A. and Grylls, J. P. (1992). Changes in soil mineral N during set aside and the effect of rotational fallows on the yield and nitrogen-optima of subsequent cereal crops. pp23-28 In: Clarke J. (Ed.). *Set Aside. BCPC mono. No. 50.* BCPC.

Frostegård, A., Bååth, E. and Tunlid, A. (1993). Shifts in the structure of soil microbial communities in limed forest as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry*, 25, 723-730.

Garnier, P., Neel, C., Mary, B., Lafolie, F. (2001). Evaluation of a nitrogen transport and transformation model in a bare soil. *European Journal of Soil Science*, 52, 253-268.

Geissen, V., Brümmer, G. W. (1999). Decomposition rates and feeding activities of soil fauna in deciduous forest soils in relation to soil chemical parameters following liming and fertilization. *Biology and Fertility of Soils*, 29, 335-342.

Gill, K., Jarvis, S. C., Hatch, D. J. (1995). Mineralization of nitrogen in long-term pasture soils: effects of management. *Plant and Soil*, 172, 153-162.

Glendining, M. J., Powlson, D. S., Poulton, P. R., Bradbury, N. J., Palazzo, D. and Li, X. (1996). The effects of long term applications of inorganic nitrogen fertilizer on soil nitrogen in the Broadbalk Wheat experiment. *Journal of Agricultural Science,* 127, 347-363.

Glover, G. I., D'Ambrosio, S. M., Jensen, R. A. (1975). Versatile properties of a nonsaturable homogeneous transport system in *Bacillus subtilis*: genetic, kinetic and affinity labelling studies. *Proc. Nat. Acad. Sci.*, 72, 814-818.

Gonzalez-Prieto, S. J. and Carballas, T. (1991). Composition of organic N in temperate humid region soils (NW Spain). *Soil Biology and Biochemistry,* 23, 887-895.

Gonzalez-Prieto, S. J., Carballas, M. and Carballas, T. (1992). Incorporation of the degradation products of ^{14}C, ^{15}N-Glycine in various forms of organic carbon and nitrogen in two acid soils. *Soil Biology and Biochemistry,* 24, 199-208.

Goulding, K. W. T. (1998). Managing nitrogen in arable systems: what more can we learn from the Broadbalk Experiment? Society of Chemical Industry Symposium: Managing nitrogen in crop rotations, 17th March 1998.

Goulding, K.W.T. (2000). Nitrate leaching from arable and horticultural land. *Soil Use and Management*, 16, 145-151. (Supplement; Tackling nitrate from agriculture).

Goulding, K. W. T. (2001). Personal Communication. Soil Science Department, IACR-Rothamsted, Harpenden, Herts. AL5 2JQ, UK.

Goulding, K.W.T., McGrath, S. P. and Johnston, A. E. (1989). Predicting the lime requirement of soils under permanent grassland and arable crops. *Soil Use and Management*, 5, 54-58.

Goulding, K. and Poulton, P. (1992). Unwanted nitrate. *Chemistry in Britain*, 28, 1100-1102.

Goulding, K. W. T., Poulton, P. R., Webster, C. P. and Howe, M. T. (2000). Nitrate leaching from the Broadbalk Wheat Experiment, Rothamsted, UK, as influenced by fertilizer and manure inputs and the weather. *Soil Use and Management*, 16, 244-250.

Griffiths, B. and Robinson, D. (1992). Root-induced nitrogen mineralisation: a nitrogen balance model. *Plant and Soil,* 139, 253-263.

Griffiths, B. S. (1994). Microbial-feeding nematodes and protozoa in soil – their effects on microbial activity and nitrogen mineralisation in decomposition hotspots and the rhizosphere. *Plant and Soil*, 164, 25-33.

Gross, K. L., Pregitzer, K. S. and Burton, A. J. (1995). Spatial variation in nitrogen availability in three successional plant communities. *Journal of Ecology*, 83, 357-367.

Grover, B.L., Lamborn, R.E. (1970). Preparation of porous ceramic cups to be use for extraction of soil water having low solute concentrations. *Soil Science Society of America Proceedings*, 34, 706-708.

Gupta, R. K. and Howard, D. H. (1971). Comparative physiological studies of the yeast and mycelial forms of *Histoplasma capsulatum*. uptake and incorporation of L-Leucine. *Journal of Bacteriology*, 105, 690-700.

Hadas, A., Sofer, M., Molina, A.E., Barak, P., Clapp, C.E., (1992). Assimilation of Nitrogen by Soil Microbial Population: NH_4 versus organic N. *Soil Biology and Biochemistry* 24, 137-143.

Harris, G. L., Rose, S. C., Muscutt, A. D., Mason, D. J. (1992). Changes in hydrology, pesticides and soil - nitrogen levels under set-aside. In: Clarke, J. (Ed.) pp 35-40. *Set Aside. BCPC Mono No.50.* BCPC.

Hart, S.C., Stark, J.M., Davidson, E.A., Firestone, M.K., (1994). Nitrogen mineralization, immobilization and nitrification. In: Weaver, R.W., Angle, J.S., Bottomley, P.S. (Eds.) pp 985-1018 *Methods of Soil Analysis Part 2 – microbiological and biochemical properties.* SSSA Book series, no. 5.

Hassink, J., Bouwman, L. A., Zwart, K. B. and Brussaard, L. (1993). Relationships between habitable pore space, soil biota and mineralization rates in grassland soils. *Soil Biology and Biochemistry*, 25, 47-55.

Haynes, R.J., (1986). *Mineral Nitrogen in the Plant-Soil System.* Academic Press. pp483.

He, X. T., Stevenson, F. J., Mulvaney, R. L. and Kelley, K. R. (1988). Incorporation of newly immobilized ^{15}N into stable organic forms in soil. *Soil Biology and Biochemistry*, 20, 75-81.

Heathwaite, A.L., Burt, T.P. and Trudgill, S.T. (1993). Overview - the nitrate issue. In: Burt, T.P., Heathwaite, A.L. and Trudgill, S.T. (Eds.) pp. 3-22 *Nitrate Processes, Patterns and Management.* Wiley. 444 pp.

Helyar, K.R., (1976). Nitrogen cycling and soil acidification. *The Journal of the Australian Institute of Agricultural Science*, 42, 217-221.

Helyar, K.R., Anderson, A.J. (1974). Effects of Calcium Carbonate on the availability of nutrients in an acid soil. *Soil Science Society of America Proceedings*, 38, 341-346.

Herman, D.C. and Maier, R.M. (2000). Consequences of biogeochemical cycles gone wild. Chapter 15 pp. 347. In: Maier, R.M., Pepper, I.L., Gerba, C.P. *Environmental microbiology*. Academic press.

Hewitt, M. V., Webster, C.P. and Bacon, E.T.G. (1992). The effect of cultivation and plant establishment on nitrogen conservation set-aside land. pp 51-58 In: Clarke, J. (Ed.) *Set Aside*. BCPC Mono No.50. BCPC.

Hoban, D. J. and Lyric, R. M. (1977). Glutamate uptake in *Thiobacillus novellas*. *Canadian Journal of Microbiology*, 23:271-277.

Hojito, M., Higashida, S., Nishimune, A., Takao, K. (1987). Effects of liming on grass growth, soil solution composition and microbial activities. *Soil Science and Plant Nutrition*, 33, 177-185.

Holland, E. A. and Detling, J. K. (1990). Plant response to herbivory and below ground nitrogen cycling. *Ecology*, 7, 1040-1049

Hooper, A. B. (1989). Biochemistry of nitrifying Lithoautotrophic bacteria. pp239-259 In: Schlegel H.G. and Bowien B (Eds.) *Autotrophic Bacteria*. Science Tech, Madison, WI.

Hopkins, D. W., Isabella, B. L., Scott, S. E. (1994). Relationship between microbial biomass and substrate induced respiration in soil amended with D- and L-isomers of amino acids. *Soil Biology and Biochemistry* 26, 1623-1627.

Horwath, W. R. and Paul, E. A. (1994). Microbial Biomass. In: Page, A. L. (Ed.) *Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties*. Soil Science Society of America Book Series No 5. SSSA inc., Madison WI.

Huberty, L. E., Gross, K. L. and Miller, C. J. (1998). Effects of nitrogen addition on successional dynamics and species diversity in Michigan old-fields. *Journal of Ecology*, 86, 794-803.

Hütsch, B. W., Webster, C. P. and Powlson, D. S. (1993). Long-term effects of nitrogen fertilization on methane oxidation in soil of the Broadbalk wheat experiment. *Soil Biology and Biochemistry*, 25, 1307-1315.

Janzen, H. H. (1987). Soil organic matter characteristics after long-term cropping to various spring wheat rotations. *Canadian Journal of Soil Science*, 67, 845-856.

Jarvis, S. C. (1998). Approaches to managing nitrogen: lessons from systems studies. Society of Chemical Industry Symposium: Managing nitrogen in crop rotations, 17.3.98, SCI, London.

Jarvis, S. C. (2000). Progress in studies of nitrate leaching from grassland soils. *Soil Use and Management*, 16, 152-156. (Supplement; Tackling nitrate from agriculture).

Jarvis, S. C., Stockdale, E. A., Shepherd, M. A. and Powlson, D. S. (1996). Nitrogen mineralization in temperate agricultural soils: processes and measurement. *Advances in Agronomy*, 57, 187-235.

Jenkinson, D. S. (1984). The supply of nitrogen from the soil. pp. 79-93. In: *The nitrogen requirement of cereals*. Reference book no. 385. ADAS, MAFF, London.

Jenkinson, D. S. and Powlson, D. S. (1976). The effects of biocidal treatments on metabolism in soil V. a method for measuring soil biomass. *Soil Biology and Biochemistry*, 8, 209-213.

Jenkinson, D. S. (1981). The fate of plant and soil animal residues in soil. Chapter 9 In: Greenland, D. J. and Hayes, M. H. B. (Eds.) *The Chemistry of Soil Processes*. John Wiley and Sons Ltd.

Jenkinson, D. S. and Wilson, J. R. (1988). Determination of microbial biomass carbon and nitrogen in soil. pp 368-386. In: Advances in nitrogen cycling in agricultural ecosystems. Proceedings of the symposium on advances in nitrogen cycling in agricultural ecosystems held in Brisband, Australia, 11-15th May 1987. CABI, Wallingford, Oxford.

Jenny, H. (1961). Derivation of state factor equations of soils and ecosystems. *Soil Science Society of America Proceedings*, 25, 385-388.

Joergensen, R. G. and Brookes, P. C. (1990). Ninhydrin-reactive nitrogen measurements of microbial biomass in 0.5 M K_2SO_4 soil extracts. *Soil Biology and Biochemistry*, 22, 1023-1027.

Johnston, A. E. (1994). The Rothamsted classical experiments. pp9-38 In Leigh, R. A. and Johnston, A. E. (Eds.) *Long-term experiments on agricultural and ecological sciences.* CAB international.

Jones, D. L. (1999). Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. *Soil Biology and Biochemistry,* 31, 613-622.

Jones, D. L. and Darrah, P. R. (1993). Influx and efflux of amino acids from *Zea mays* L. roots and their implications for N-nutrition and the rhizosphere. *Plant and Soil,* 156, 87-90.

Jones, D. L. and Darrah, P. R., (1994). Amino-acid influx at the soil-root interface of *Zea mays* L. and its implications in the rhizosphere. *Plant and Soil* 163, 1-12.

Jones, D. L., Edwards, A.C., Donachie, K., and Darrah, P. R. (1994). Role of proteinaceous amino acids released in root exudates in nutrient acquisition from the rhizosphere. *Plant and Soil* 158, 183-192.

Jones, D. L., Prabowo, A. M., Kochian, L. V. (1996). Kinetics of malate transport and decomposition in acid soils and isolated bacterial population: the effect of microorganisms on root exudation of malate under Al stress. *Plant and Soil,* 182, 239-247.

Jones, D.L. and Brassington, D. S. (1998). Sorption of organic acids in acid soils and its implications in the rhizosphere. *European Journal of Soil Science,* 49, 447-455.

Jones, D. L. and Edwards, A. C. (1998). Influence of sorption on the biological utilization of two simple carbon substrates. *Soil Biology and Biochemistry,* 30, 1895-1902.

Jones, D. L. and Hodge, A. (1999). Biodegradation kinetics and sorption reactions of three differently charged amino acids in soil and their effects on plant organic nitrogen availability. *Soil Biology and Biochemistry,* 31, 1331-1342.

Jones, D. L. and Shannon, D. (1999). Mineralization of amino acids applied to soils: Impact of soil sieving, storage and inorganic nitrogen additions. *Soil Sci. Am. J.*, 63, 1199-1206.

Jones, D. L., Eldhuset, T., de Wit, H. A., Swensen, B. (2001). Aluminium effects on organic acid mineralisation in a Norway spruce forest soil, *Soil Biology and Biochemistry,* 33, 1259-1267.

Jørgensen, B. B. (1989). Biochemistry of chemo autotrophic bacteria. pp117-146. In: Schlegel, H. G. and Bowien, B. (Eds.) *Autotrophic bacteria.* Science Tech, Madison WI.

Kaiser, E. A., Mueller, T., Joergensen, R. G., Insam, H., Heinemeyer, O. (1992). Evaluation of methods to estimate the soil microbial biomass and the relationship with soil texture and organic matter. *Soil Biology and Biochemistry*, 24, 675-683.

Kamimura, Y.;Hayano, K. (2000). Properties of protease extracted from tea-field soil. *Biology and Fertility of Soils*, 30, 351-355.

Kay, W. W. and Gronlund (1971). Transport of aromatic amino acids by *Pseudomonas aeruginosa. Journal of Bacteriology,* 105, 1039-1046.

Kelso, I., (2000). Personal communication. School of agricultural and forest sciences, University of Wales, Bangor, Gwynedd, LL57 2UW.

Keeney, D. R. and Nelson, D. W. (1982). Nitrogen – inorganic forms. pp.643-698. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.). *Methods of Soil Analysis. Part 2. Chemical and Microbiological properties.* 2nd Edition. ASA-SSSA. Madisonn, WI.

Kerven, G. L., Edwards, D. G., Asher, C. J., Hallman, P. S.. Kokot, S. (1989). Aluminium determination in soil solution. II. Short-term Colorimetric procedures for the measurement of inorganic monomeric aluminium in the presence of organic acid ligands. *Australian Journal of Soil Research*, 27, 91-102.

Keunen, J. G. and Bos, P. (1989). Habitats and ecological niches of chemolitho(auto)trophic bacteria. pp 53-80. In: Schlegel, H. G. and Bowien, B. (Eds.) *Autotrophic bacteria.* Science Tech, Madison WI.

Kielland, K. (1994). Amino Acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling. *Ecology*, 75, 2373-2383.

Kielland, K. (1997). Role of free amino acids in the nitrogen economy of arctic cryptogams. *Ecoscience*, 4, 75-79.

Killham, K. (1994) *Soil Ecology.* Cambridge University Press, Cambridge. 242pp.

Klein, D. A., Paschke, M. W., Redente, E. F. (1998) Assessment of fungal-bacterial development in a successional shortgrass steppe by direct integration of chloroform-fumigation extraction (FE) and microscopically derived data. *Soil Biology and Biochemistry,* 30, 573-581.

Koppisch, D., Schulze, E. D., Gebauer, G. (1993). ^{15}N-labelled ammonium and nitrate uptake by the grass *Calamagrostis villosa*. *Isotopenpraxis*, 29, 77-84.

Kraffczyk, I., Trolldenier, G., Beringer, H. (1984). Soluble root exudates of maize: influence of potassium supply and rhizosphere microorganisms. *Soil Biology and Biochemistry,* 16, 315-322.

Kristensen, H. L. and Henriksen, K. (1998). Soil nitrogen transformations along a successional gradient from *Calluna* heathland to *Quercus* forest at intermediate atmospheric nitrogen deposition. *Applied Soil Ecology,* 8, 95-109.

Kristensen, L., Stopes, C., Kolster, P, Granstedt, A. (1995) Nitrogen leaching in ecological agriculture: summary and recommendations, pp 331-340. In: Kristensen, L., Stopes, C., Kolster, P., Granstedt, A. and Hodges, D. (Eds.) *Nitrogen leaching in ecological agriculture*. ABA, UK. 343pp.

Krulwich, T. A., Blanco, R., McBride, P. A. (1977). Amino acid transport in whole cells and membrane vesicles of *Arthrobacter pyridinolis*. *Archives of Biochemistry and Biophysics,* 178, 108-117.

Ladd, J. N. and Foster, R. C. (1988). Role of soil microflora in nitrogen turnover. pp 113-133. In: Wilson, J. R. (Ed.) *Advances in Nitrogen Cycling in Agricultural Ecosystems*. CAB International, Wallingford.

Leach, F. R. and Snell, E. E. (1960). The absorption of glycine and alanine and their peptides by *Lactobacillus casei*. *The Journal Of Biological Chemistry,* 235, 3523-3531.

Leake, J. R. and Read, D. J. (1990a). Proteinase activity in mycorrhizal fungi II The effects of mineral and organic nitrogen sources on induction of extracellular proteinase in *Hymenoscyphus ericae*. *New Phytologist*, 116, 123-128.

Leake, J. R. and Read, D. J. (1990b). Proteinase activity in mycorrhizal fungi III Effects of protein, protein hydrolysate, glucose and ammonium on production of extracellular proteinase by *Hymenoscyphus ericae* (Read) Korf & Kernan. *New Phytologist*, 116, 123-128.

Lekkerkerk, L., Lundkvist, H. and Agren, G. I. (1990). Decomposition of heterogeneous substrates; an experimental investigation of a hypothesis on substrate and microbial properties. *Soil Biology and Biochemistry,* 22, 161-167.

Li, P. and Caldwell, A. C., (1966). The oxidation of elemental Sulphur in soil. *Soil Science Society of America Proceedings*, 30, 370-372.

Lin, Q. and Brookes, P. C. (1999a). An evaluation of the substrate-induced respiration method. *Soil Biology and Biochemistry,* 31, 1969-1983.

Lin, Q. and Brookes, P. C. (1999b). Arginine ammonification as a method to estimate soil microbial biomass and microbial community structure. *Soil Biology and Biochemistry*, 31, 1985-1997.

Litaor, M.I., (1988). Review of soil solution samplers. *Water resources Research*, 24, 727-733.

Lofts, S, Simon, B. M., Tipping, E. and Woof, C. (2001). Modelling the solid-solution partitioning of organic matter in European forest soils. *European Journal of Soil Science*, 52, 215-226.

Logan, K. A. B., Thomas, R. J. (1999). Interactions between N supply and N uptake by perennial ryegrass, ^{15}N recovery and sol pH for four acid Scottish soils. *Grass and Forage Science*, 54, 42-51.

Lord, E. I. and Shepherd, M. A. (1993). Developments in the use of porous ceramic cups for measuring nitrate leaching. *Journal of Soil Science*, 44, 435-449.

Lovell, R. D. and Jarvis, S. C. (1996). Effects of urine on soil microbial biomass: methanogenesis, nitrification and denitrification in grassland soils. *Plant and Soil,* 186, 265-273.

Lovell, R. D., Jarvis, S. C. and Bardgett, R. D. (1995). Soil microbial biomass and activity in long-term grassland: effects of management changes. *Soil Biology and Biochemistry,* 27, 969-975.

Lyngstad, I. (1992). Effect of liming on mineralization of soil nitrogen as measured by plant uptake and nitrogen released during incubation. *Plant and Soil,* 144, 247-253.

MacDonald, N. W., Zak, D. R., Pregitzer, K. S. (1995). Temperature effects on kinetics of microbial respiration and net nitrogen and sulfur mineralisation. *Soil Sci. Soc. Am. J.,* 59, 233-240.

Mackay, J. M. and Watson, C.A . (1990). Organic systems trials in the north of Scotland. pp.251-254 In: *Organic and low input agriculture*, BCPC mono. No. 45. BCPC.

MAFF (1993). Solving the Nitrate Problem – progress in research and development. MAFF, London SE99 7TP. 37pp.

MAFF (1995). Government response to the consultation on the designation of Nitrate Vulnerable Zones in England and Wales. MAFF.

MAFF (1998a). *Code of Good Agricultural Practice for the protection of Air* (MAFF Publications, Admail 6000, London, SW1A 2XX). 80pp.

MAFF (1998b). *Code of Good Agricultural Practice for the Protection of Soil* (MAFF Publications, Admail 6000, London, SW1A 2XX). 66pp.

MAFF (1998c). *Code of Good Agricultural Practice for the protection of Water* (MAFF Publications, Admail 6000, London, SW1A 2XX). 97pp.

MAFF (1998d). Development of a set of indicators for sustainable agriculture in the United Kingdom – a consultation document. 22.6.98. MAFF, London. 54pp.

MAFF (2000) *Fertiliser recommendations for Agricultural and Horticultural crops* (RB209). 7th Edition. The Stationary Office, Norwich, UK. 175pp.

Maier, R. M. (2000) Biogeochemical cycling. Chapter 14 pp. 319-346. In: Maier, R. M., Pepper, I. L., Gerba, C. P (Eds.) *Environmental microbiology.* Academic press.

Magesan, G. N., White, R. E., Scotter, D. R., Bolan, N. S. (1994). Estimating leaching losses from sub-surface drained soil. *Soil Use and Management*, 10, 87-93.

Malhi, S. S., Nyborg, M., Caldwell, C. D., Hoyt, P. B. and Leitch, R. H. (1988). Effect of ammonium and nitrate on growth and yield of barley on acid soils. *Communications In Soil Science And Plant Analysis*, 19, 1049-1063.

Marschner, H., (1995). *Mineral Nutrition of Higher Plants.* Second Edition. Academic Press, London. 889pp.

Marschner, H., Romheld, V., Horst, W. J., Martin, P. (1986). Root-induced changes in the rhizosphere importance for the mineral nutrition of plants. *Zeitschrift-fur-Pflanzenernahrung-und-Bodenkunde*, 149, 441-456.

Mason, M. G., (1992). Effect of nitrogen source and soil type on inorganic nitrogen concentrations and availability in field trials with wheat. *Australian Journal Of Experimental Agriculture*, 32, 175-181

Mawdsley, J. L. and Bardgett, R.D. (1997). Continuous defoliation of perennial rygrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the microbial population of an upland grassland soil. *Biology and Fertility of Soils*, 24, 52-58.

McAndrew, S. W. and Malhi, S. S. (1992). Long-term N fertilization of a solonetzic soil: effects on chemical and biological properties. *Soil Biology and Biochemistry,* 24, 619-623.

McCarty, G. W., Meisinger, J. J. and Jenniskens, F. M. M. (1995). Relationships between total-N, biomass-N and active-N in soil under different tillage and N fertilizer treatments. *Soil Biology and Biochemistry,* 27, 1245-1250.

McKane L. and Kandel J. (1996). *Microbiology - essentials and applications.* 2nd edition. McGraw-Hill inc.

Mc Naughton, S. J., Zuniga, G., McNaughton, M. and Banyikwa, F. F. (1997). Ecosystem catalysis: soil urease activity and grazing in the Serengeti ecosystem. *Oikos,* 80, 467-469.

Meharg, A. A. and Killham, K. (1990). The effect of soil pH on rhizosphere carbon flow of *Lolium perenne. Plant and Soil,* 123, 1-7.

Meharg, A. A. and Killham, K. (1995). Loss of exudates from the roots of perennial ryegrass inoculated with a range of microorganisms. *Plant and Soil,* 170, 345-349.

Miles, J. (1985). The pedogenic effects of different species an vegetation types and the implications of succession. *Journal of Soil Science,* 36, 571-584.

Miller, D. L. and Rodwell, V. W. (1971). Metabolism of basic amino acids in *Pseudomonas putida. The Journal of Biological Chemistry,* 246, 1763-1771.

Monreal, C. M. and McGill, W. B. (1985). Centrifugal extraction and determination of free amino acids in soil solutions by TLC using titrated 1-fluro-2,4-dinitrobenzene. *Soil Biology and Biochemistry,* 17, 533-539.

Mopper, K. and Zika, R. G. (1987). Free amino acids in marine rains: evidence for oxidation and potential role in nitrogen cycling. *Nature,* 325, 15.

Mulvaney, R. L. (1996). Nitrogen – Inorganic Forms. Chpt. 38, pp 1123-1200. In: Sparks, D. L. (Ed.) *Methods of Soil Analysis, Part 3 – Chemical Methods.* SSSA book series No.5. , SSSA, Inc., Madison, Wi., USA.

Murphy, D. V., Macdonald, A. J., Stockdale, E. A., Goulding, K. W. T., Fortune, S., Gaunt, J. L., Poulton, P. R., Wakefield, J. A., Webster, C. P., Wilmer, W. S. (2000). Soluble organic nitrogen in agricultural soils. *Biology and Fertility of Soils,* 30, 374-387.

Murphy, D. V., Sparling, G. P., Fillery, I. R. P. (1998). Stratification of microbial biomass C and N and gross N mineralization with soil depth in two contrasting western Australian agricultural soils. *Australian Journal of Soil Research,* 36, 45-55.

Näsholm, T., Ekbald, A, Nordin, A., Giesler, R., Högberg, P. (1998). Boreal forest plants take up organic nitrogen. *Nature*, 392, 914-916.

Nicolardot, B., Fauvet, G., Cheneby, D. (1994). Carbon and nitrogen cycling through soil microbial biomass at various temperatures. *Soil Biology and Biochemistry,* 26, 253-261.

Ninnemann, O., Jauniaux, J. C., Frommer, W. B. (1994). Identification of a high affinity NH_4^+ transporter from plants. *The EMBO Journal,* 13, 3464-3471.

Nor, Y. M. and Tabatabai, M. A. (1977). Oxidation of elemental Sulphur in soils. *Soil Science Society of America Journal*, 41, 736-741.

Nyborg, M., Hoyt, P. B., (1978). Effects of soil acidity and liming on mineralization of soil nitrogen. *Canadian Journal of Soil Science,* 58, 331-338.

O'Leary, W. M. and Wilkinson, S. G. (1988). Gram-positive bacteria. pp. 117-185. In: Ratledge, C., Wilkinson, S. G. (Eds.). *Microbial lipids, vol. 1.* Academic press.

Omay, A. B., Rice, C. W., Maddux, L. D. and Gordon, W. B. (1997). Changes in soil microbial and chemical properties under long term crop rotation and fertilization. *Soil Sci.Soc.Am.J.,* 61, 1672-1678.

Owen, A. G. and Jones, D. L. (2001). Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. *Soil Biology and Biochemistry*, 33, 651-657.

Paschke, M. W., McLendon, T., Redente, E. F. (2000). Nitrogen availability and old-field succession in a shortgrass steppe. *Ecosystems,* 3, 144-158.

Paul, E. A. and Clark, F. E. (1989). *Soil microbiology and Biochemistry.* Academic Press Inc. 273pp.

Persson, T., Wirén, A. (1995). Nitrogen mineralization and potential nitrification at different depths in acid forest soils. *Plant and Soil,* 169, 55-65.

Pearsall, W. H. (1950). *Mountains and Moorlands.* Collins New Naturalist Series, Bloomsbury Books, London.

Philipps, L., Stopes, C. E. and Woodward, L. (1997). Nitrate leaching losses from organic farming systems. Royal Agricultural Society of England Symposium: Organic Farming – Science into practice. 4.11.97. Stoneleigh Park, Warwickshire.

Poss, R., Noble, A. D., Dunin, F. X., Teyenga, W. (1995). Evaluation of ceramic cup samplers to measure nitrate leaching in the field. *European Journal of Soil Science,* 46, 667-674.

Purnomo, E., Black, A. S., Conyers, M. K., (2000). The distribution of net nitrogen mineralisation within surface soil. 2. Factors influencing the distribution of net N mineralisation. *Australian Journal of Soil Research*, 38, 643-652.

Putnam, H. J., Schmidt, E. L. (1959). Studies on the free amino acid fraction of soils. *Soil Science,* 87, 22-27.

Rahn, C. R., Vaidyanathan, L. V., Pterson, C. D. (1992). Nitrogen residues from brassica crops. *Aspects of Applied Biology*, 30, 263-270.

Recous, S., Robin, D., Darwis D., Mary, B. (1995). Soil inorganic N availability : effect on maize residue decomposition. *Soil Biology and Biochemistry,* 27, 1529-1538.

Reynolds, K. A. and Pepper, I. L. (2000). Microorganisms in the environment. Chapter 2 pp. 7-42. In: Maier, R. M., Pepper, I. L., Gerba, C. P. *Environmental Microbiology.* Academic press.

Ridley, A. M., White, R. E., Helyar, K. R., Morrison, G. R., Heng, L. K. and Fisher, R. (2001). Nitrate leaching loss under annual and perennial pastures with and without lime on a duplex (texture contrast) soil in humid south-eastern Australia. *European Journal of Soil Science,* 52, 237-252.

Riffaldi, R., Saviozzi, A., LeviMinzi, R., (1996). Carbon mineralization kinetics as influenced by soil properties. *Biology and Fertility of Soils*, 22, 293-298.

Ross, D. J., Speir, T. W., Kettles, H. A. and Mackay, A. D. (1995). Soil microbial biomass, C and N mineralization and enzyme activities in a hill pasture: influence of

season and slow-release P and S fertilizer. *Soil Biology and Biochemistry,* 27, 1431-1443.

Rowell, D. L. (1988). Soil acidity and alkalinity. pp 844-898 In: Wild, A. (Ed.) *Russell's soil conditions and plant growth.* 11th Edition. Longman Scientific and Technical.

Rowell, D. L. (1994). *Soil Science, Methods and Applications.* Longman, UK. 350pp.

Ryden, J. C., Ball, P. R., and Garwood, E. A. (1984). Nitrate leaching from grassland. *Nature*, 311, 50-53.

Sanders, J. R. (1983). The effect of pH and organic matter of some soils on the ionic activities and concentrations of trace elements in soil solutions. *Journal of Science Food and Agriculture*, 34, 52-52.

Schmidt, E. L. (1982). Nitrification in Soil. pp253-288 In: Stevenson, F.J. (Ed.) *Nitrogen in Agricultural Soils.* American Society of Agronomy.

Schnitzer, M. (1980). Effect of low pH on the chemical structure and reactions of humic substances. pp. 203-222. In: Hutchinson, T. C. and Havas, M. (Eds.) *Effects of acid precipitation on terrestrial ecosystems.* Plenum press.

Schobert, C., Kockenberger, W., Komor, E., (1988). Uptake of amino acids by plants from the soil: A comparative study with castor bean seedlings grown under natural and axenic soil conditions. *Plant and Soil,* 109, 181-188.

Scullion, John. Personal communication. Soil Science Unit, Institute of Biological Sciences, University of Wales, Penglais, Aberystwyth SY23 3BB

Tel 01970 622304 Fax 01970 622307.

Searle, P. L. (1984). The Berthelot or Indophenol reaction and its use in the analysis chemistry of nitrogen. *The Analyst,* 109, 549-565.

Shen, S. M., Pruden, G. and Jenkinson, D. S. (1984). Mineralization and immobilization of nitrogen in fumigated soil and the measurement of microbial biomass nitrogen. *Soil Biology and Biochemistry*, 16, 437-444.

Shen, S. M., Hart, P. B. S., Powlson, D. S., Jenkinson, D. S. (1989). The nitrogen cycle in the Broadbalk wheat experiment: ^{15}N-labelled fertilizer residues in the soil and in the soil microbial biomass. *Soil Biology and Biochemistry,* 21, 529-533.

Shen, Y. (1999). Sorption of natural dissolved organic matter on soil. *Chemosphere,* 38, 1505-1515.

Shepherd, M. A., Stockdale, E. A., Powlson, D. S. and Jarvis, S. C. (1996). The influence of organic nitrogen mineralization on the management of agricultural systems in the UK. *Soil Use and Management*, 12, 76-85.

Simard, R. R., Angers, D. A., Lapierre, C. (1994). Soil Organic-Matter Quality as Influenced by Tillage, Lime, and Phosphorus. *Biology and Fertility of Soils*, 18, 13-18.

Sinclair, A. H., Still, E. B., Heath, S. B. (1992). Effect of set-aside on soil nitrogen and subsequent cropping. pp29-34 In: Clarke, J. (Ed.). *Set Aside*. BCPC Mono No.50. BCPC.

Smith, J. L., Papendick, R. I., Bezdicek, D. F., Lynch, J. M. (1993). Soil organic matter dynamics and crop residue management. pp. 65-94 In: Blaine Metting Jr., F., (Ed.), *Soil Microbial Ecology*. Marcel Dekker Inc.

Smith, S. J. (1987). Soluble organic nitrogen losses associated with recovery of mineralised nitrogen. Soil Sci. Soc. Am. J., 51, 1191-1194.

Smith, V. R., Steenkamp, M. (1992). Soil macrofauna and nitrogen on a Sub-Antarctic Island. *Oecologia,* 92, 201-206.

Smolander, A., Kurka, A., Kitunen, V. and Mälkönen, E. (1994). Microbial biomass C and N and respiratory activity in soil of repeatedly limed and N- and P-fertilized Norway spruce stands. *Soil Biology and Biochemistry,* 26, 957-962.

Sparling, G. and Zhu, C. (1993). Evaluation and calibration of biochemical methods to measure microbial biomass C and N in soils from Western Australia. *Soil Biology and Biochemistry*, 25, 1793-1801.

Stams, A. J. M., Flameling, E. M., Marnette, E. C .L. (1990). The importance of autotrophic versus heterotrophic oxidation of atmospheric ammonium in forest ecosystems with acid soil. *FEMS Microbiology Ecology,* 74, 337-344.

Ste-Marie, C. and Paré, D. (1999). Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. *Soil Biology and Biochemistry*, 31, 1579-1589.

Stevens, R. J. and Laughlin, R. J. (1996). Effects of lime and nitrogen fertilizer on two sward types over a 10 year period. *Journal of Agricultural Science,* 127, 451-461.

Stevens, R. J., Laughlin, R. J., Malone, J. P. (1998). Soil pH affects the processes reducing nitrate to nitrous oxide and di-nitrogen. *Soil Biology and Biochemistry*, 30, 1119-1126.

Stevenson, F.J. (1982). Organic forms of soil nitrogen. Chapter 3 pp. 67-122 In: *Nitrogen in agricultural soils*. Agronomy Monograph no.22. American Society of Agronomy, Madison, WI.

Stevenson, F.J. (1986). *Cycles of Soil: C,N,P,S, micronutrients*. John Wiley and Sons Inc. 380pp.

Strayer, R. F., Lin, C. J. and Alexander, M. (1981). Effect of simulated acid rain on nitrification and nitrogen mineralization in forest soils. *Journal of Environmental Quality,* 10, 547-551.

Strong, D. T., Sale, P. W. G. and Helyar, K. R. (1997). Use of natural heterogeneity in a small field site to explore the influence of the soil matrix on nitrogen mineralization and nitrification. *Aust.J.Soil.Res.*, 35, 579-590.

Sumner, M. E., Fey, M. V., Noble, A. D. (1991). Nutrient status and toxicity problems in acid soils. pp149-182 In: Ulrich, B., Sumner, M. E. (Eds.) *Soil Acidity*. Springer-Verlag.

Sverdrup, H., Warfvinge, P., Blake, L. and Goulding, K., (1995). Modelling recent and historic soil data from the Rothamsted Experimental Station, UK using SAFE. *Agriculture Ecosystems and Environment*, 53, 161-177.

Sylvester-Bradley, R. (1993). *Nitrate Research*. MAFF Review. MAFF, London.

Tabatabai, M. A. (1994). Sulphur oxidation and reduction in soils. Chpt. 45 pp1067-1078. In: Weaver, R. W., Angle, J. S., Bottomley, P. S. (Eds.) *Methods of Soil Analysis Part 2 – Microbiological and Biochemical Properties, SSSA Book Series 5.* Soil Science Society of America, Inc.

Tang, S. L. and Howard, D. H. (1973). Uptake and utilization of glutamic acid by *Cryptococcus albidus*. *Journal of Bacteriology,* 115, 98-106.

Tate, R.L. (1995). *Soil Microbiology*. Wiley and Sons Inc. New York USA. 398pp.

Tessier, L., Gregorich, E. G., Topp, E. (1998). Spatial variability of soil microbial biomass measured by the fumigation extraction method and K_{ec} as affected by depth and manure application. *Soil Biology and Biochemistry,* 30, 1369-1377.

Thomas, G. W. and Hargrove, W. C. (1984). The chemistry of soil acidity. pp 3-56 In: Adams, F (Ed.) *Soil acidity and liming.* American Society of Agronomy, Madison.

Thomas, G.W. (1996). Soil pH and Soil Acidity. Chpt. 16, pp 475-490. In: Sparks, D. L.(Ed.) *Methods of Soil Analysis, Part 3 – Chemical Methods.* SSSA book series No.5. SSSA, Inc., Madison, WI., USA.

Thomsen, I. K. (1993). Turnover of ^{15}N-straw and NH_4NO_3 in a sandy loam soil: effects of straw disposal and N fertilization. *Soil Biology and Biochemistry,* 25, 1561-1566.

Tiedje, J. M., Cho, J. C., Murray, A., Treves, D., Xia, B., and Zhou, J. (2001). Soil teeming with life: new frontiers for soil science. pp. 393 – 413 In: Rees, R. M., Ball, B.C., Campbell, C.D. and Watson, C.A. (Eds.) *Sustainable Management of Soil Organic Matter.* CABI.

Tlustos, P., Blackmer, A. M. (1992). Release of nitrogen from ureaform fraction as infuenced by soil pH. *Soil Science Society of America Journal,* 56, 1807-1810.

Toal, M. E., Yeomans, C., Killham, K., Meharg, A. A. (2000). A review of rhizosphere carbon flow modelling. *Plant and Soil,* 222, 263-281.

Tortora, G., Funke, B. and Case, C. (1995) *Microbiology - an introduction.* 5th Ed. Benjamin/Cummings. 801pp.

Tracey, B. F., Frank, D. A. (1998). Herbivore influence on soil microbial biomass and nitrogen mineralization in a northern grassland ecosystem: Yellowstone National Park. *Oecologia,* 114, 556-562.

Trudinger, P. A. and Loughlin, R. E. (1981). Metabolism of simple sulphur compounds. Chpt 3 pp165-257 In: Neuberger, A and Van Deenen, L. L. M. (Eds.) *Amino acid metabolism and sulphur metabolism, Volume 19A Comprehensive Biochemistry.* Elsevier.

Tyler, G. and Olsson, T. (2001). Concentrations of 60 elements in the soil solution as related to the soil acidity. *European Journal of Soil Science,* 52, 151-165.

Ulrich, B. (1991). An ecosystem approach to soil acidification. pp28-79 In: Ulrich, B., Sumner, M. E. (Eds.) *Soil Acidity.* Springer-Verlag.

Umbarger, H. E. (1981). Regulation of amino acid metabolism. pp165-257 In: Neuberger, A and Van Deenen, L. L. M. (Eds.) *Amino acid metabolism and sulphur metabolism, Volume 19A Comprehensive Biochemistry.* Elsevier.

Van Bergen, P. F., Nott, C. J., Bull, I. D., Poulton, P. R. and Evershed, R. P. (1998). Organic geochemical studies of soils from the Rothamsted Classical Experiments – IV. Preliminary results from a study of the effect of soil pH on organic matter decay. *Organic Geochemistry*, 29, 1779-1795.

Van Breemen, N., Driscoll, C. T. and Mulder, J. (1984). Acidic deposition and internal proton sources in acidification of soils and waters. *Nature*, 307, 599-604.

Van Breemen, N (1991). Nutrient status and toxicity problems in acid soils. pp149-182 In: Ulrich, B., Sumner, M. E. (Eds.) *Soil Acidity.* Springer-Verlag.

Van Cleve, K., Yarie, J., Erickson, R., Dyrness, C. T. (1993). Nitrogen mineralisation and nitrification in successional ecosystems on the Tanana river floodplain, Interior Alaska. *Canadian J For Res,* 23, 970-978.

Vance, E. D., Brookes, P. C., Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass carbon. *Soil Biology and Biochemistry*, 19, 703-707.

Van Schöll, L., van Dam, A. M. and Leffelaar, P. A. (1997). Mineralisation of nitrogen from an incorporated catch crop at low temperatures: experiment and simulation. *Plant and Soil,* 188, 211-219.

Van Wijnen, H. J., van der Wal, R., Bakker, J. P. (1999). The impact of herbivores on nitrogen mineralization rate: consequences for salt-marsh succession. *Oecologia*, 118, 225-231.

Varadachari, C, Mondal, A. H., Nayak, D. C., Ghosh, K. (1994). Clay-humus complexation: effect of pH and the nature of bonding. *Soil Biology and Biochemistry*, 26, 1145-1149.

Vinten, A. J. A. and Smith, K. A. (1993). Nitrogen Cycling in Agricultural Soils. pp39-74 In: Burt, T. P., Heathwaite, A. L. and Trudgill, S. T. (Eds.) *Nitrate - Processes, Patterns and Management.* Wiley. 444 pp.

Vinolas, L. C., Healey, J. R., Jones, D. L. (2001). Kinetics of soil microbial uptake of free amino acids, *Biology and Fertility of Soils*, 33, 67-74.

Von Uexküll, H. R. and Mutert, E. (1995). Global extent, development and economic impact of acid soils. *Plant and Soil,* 171, 1-15.

Voroney, R. P., Winter, J. P., Gregorich, E. G. (1991). Microbe/plant/soil interactions.p.77-99. In Coleman, D.C. Coleman and Fry, B. (Eds.) *Carbon isotope techniques*. Academic Press, New York.

Wander, M. M., Hedrick, D. S., Kaufman, D., Traina, S. J., Stinner, B. R., Kehremeyer, S. R., White, D. C. (1995). The functional significance of the microbial biomass in organic and conventionally managed soils. *Plant and Soil*, 170, 87-97.

Wardle, D. A. (1998). Controls of temporal variability of the soil microbial biomass: a global-scale synthesis. *Soil Biology and Biochemistry*, 30, 1627-1637.

Wardle, D. A., Bonner, K. I., Barker, G. M., Yeates, G. W., Nicholson, K. S., Bardgett, R. D., Watson, R. N., Ghani, A. (1999). Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity and ecosystem properties. *Ecological Monographs,* 69, 535-568.

Watson, C. J., Kilpatrick, D. J. and Cooper, J. E. (1995). The effect of increasing application rate of granular calcium ammonium nitrate on net nitrification in a laboratory study of grassland soils. *Fertilizer Research,* 40, 155-161.

Webster, C. P., Shepherd, M. A., Goulding, K. W. T. and Lord, E. (1993). Comparisons of methods for measuring the leaching of mineral nitrogen from arable land. *Journal of Soil Science*, 44, 49-62.

Webster, C. P. and Goulding, K. W. T. (1989). Influence of soil carbon content on denitrification from fallow land during autumn. *Journal of the Science of Food and Agriculture,* 49, 131-142.

Weier, K. L., Gilliam, J. W. (1986). Effect of acidity on nitrogen mineralisation and nitrification in Atlantic coastal plain soils. *Soil Sci Soc. Am J.*, 50, 1210-1214.

Weier, K. L. and MacRae, I. C., (1993). Net mineralization, net nitrification and potentially available nitrogen in the subsoil beneath a cultivated crop and a permanent pasture. *Journal of Soil Science*, 44, 451-458.

Wheeler, D. M., Edmeades, D. C. and Morton, J. D. (1997). Effect of lime on yield, N fixation and plant N uptake from the soil by pasture on 3 contrasting trials in New Zealand. *New Zealand Journal of Agricultural Research,* 40, 397-408.

White, R.E. (1997). *Principles and Practice of Soil Sciences*. Third Ed. Blackwell Science. 348pp.

Wild, A. and Jones, L. H. P. (1988). Mineral nutrition of crop plants. pp69-112. In: Wild, A. (Ed.) *Russell's soil conditions and plant growth*. Longman Scientific and Technical.

Wilkinson, R. E. (1994). Acid soil stress and plant growth. pp 125-148 In: Wilkinson, R. E. (Ed.) *Plant-Environment Interactions*. Marcel Dekker, Inc.

Williams, B. L., Shand, C. A., Hill, M., O'Hara, C., Smith, S. and Young, M.E. (1995). A procedure for the simultaneous oxidation of total soluble nitrogen and phosphorus in extracts of fresh and fumigated soils and litters. *Communications in Soil Science and Plant Analysis*, 26, 91-106.

Williams, J. R. and Lord, E. I. (1997). The use of porous ceramic cup water samplers to measure solute leaching on chalk soils. *Soil Use and Management*, 13, 156-162.

Willison, T. W., Baker, J. C., Murphy, D. V. and Goulding, K. W. T. (1998). Comparison of a wet and dry ^{15}N isotopic dilution technique as a short-term nitrification assay. *Soil Biology and Biochemistry*, 30, 661-663.

Yamulki, S., Harrison, R. M., Goulding, K. W. T. and Webster, C. P. (1997). N_2O, NO and NO_2 fluxes from a grassland: effect of soil pH. *Soil Biology and Biochemistry*, 29, 1199-1208.

Yeates, G. W., Wardle, D. A. (1996). Nematodes as predators and prey: relationships to biological control and soil processes. *Pedobiologia*, 40, 43-50.

Zalokar, M. (1961). Kinetics of amino acid uptake and protein synthesis in *Neurospora*. *Biochimica et Biophysica Acta*, 46, 423-432.

Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology and Fertility of Soils*, 29, 111-129.

Zsolnay, Á. (2001). Exploratory ESF Workshop: *The prediction of the Environmental Function of the Dissolved Organic Matter in Ecosystems*. Scientific Report. 15th September 2001.