Soil-Solution Partitioning of Metals

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ABSTRACT

Soil- solution partitioning of metals determines the behaviour and toxicity of metals. Lead, copper, zinc and nickel are common pollutants, and due to historic metal deposition from the atmosphere, high levels of these metals have accumulated in upland organic soils in the UK. Atmospheric deposition of sulphur and nitrogen, and climate change, can affect soil solution pH and dissolved organic carbon (DOC) concentrations, and both pH and DOC are known to affect soil-solution partitioning of metals. In this thesis, metal concentrations were determined in archived soil and soil solution samples from a regional survey of upland sites in northern England with contrasting soils, and two experiments were undertaken to assess the effect of temperature and nitrogen deposition composition on metal concentrations in soil solution. In each case, a common objective was to assess whether variation in metal concentrations in soil solution could be explained by changes in soil solution pH and DOC concentration.

Lead concentrations in soil solution were modified by heating, but not the composition of nitrogen deposition, and lead showed a strong affinity for organic matter in soils and soil solution. Zinc concentrations were affected by both heating and nitrogen deposition, with the strongest effect being through changes in pH. However, in the case of both zinc and nickel, there were also associations with DOC concentrations, indicating that the organic phase becomes more significant for partitioning of metals between soil and soil solution in organic-rich soils. For copper, there was little effect of heating or nitrogen deposition, and the strongest association was with nitrate, rather than pH or DOC, in soil solution. Future research should be focused on more comprehensive studies dealing with the relationship between DOC, pH, climate, nitrogen deposition and metal in the field, with supporting laboratory experiments.

Key words: metals, soil solution partitioning, nitrogen deposition, temperature, pH, DOC.

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Afia zia

DECLARATION BY CANDIDATE

I hereby declare that this thesis is my own work and effort and that it has not been submitted anywhere for any award. Where other sources of information have been used, they have been acknowledged.

Signature: ……………………………………….

Date: …………………………………………….

Dedication

two men behind my success

“***My Great Father***

&

My lovely Husband”

Chapter 1 – Introduction

The availability and accumulation of trace elements and contaminants in soil is of concern because of plant uptake and bioaccumulation, effecting ground water and e human health (Abrahams, 2002). Soil is the medium through which these contaminants move from the land surface to groundwater. During their movement through the soil, contaminants undergo transformations. Understanding the processes controlling the behaviour of contaminants within the soil is important to Predict soil contamination, and reestablishment of contaminated soils.

This thesis deals with some of the processes and factors which can affect the availability of metal contaminants in the soil environment. In particular, the thesis focuses on the soil liquid phase, and its interaction with the solid phase. Individual chapters provide a more comprehensive literature review; the present introduction summarizes the general concepts and theoretical background underlying the project, and introduces the key aims and objectives, and the structure of the thesis.

1.1 Trace metals in the soil system

Amongst trace elements, cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) are common pollutants in urban and industrial soils (Dudka *et al.,* 1996). These metals are toxic and risk to human health from contamination of groundwater and their accumulation in food crops (Martinez & Motto, 2000). Their environmental concentrations have increased due to human activities, such as waste disposal or utilization (Wolt, 1994) and anthropogenic atmospheric emissions. The contribution of human activities to metal concentrations in soils has increased over the last few centuries (Nriagu, 1990)

The work described in this thesis focuses primarily on organic-rich soils from the uplands of England. These soils have accumulated metals from atmospheric deposition, both long-distance and localised, over a long period of time, and in some cases also from the weathering of soil mineral matter and local mining activity. The UK has had a particularly long history of atmospheric metal pollution. It has been a globally significant producer of metal ores over the last two millennia (Raistrick & Jennings, 1965) and from the mid-18th century (the start of the industrial revolution) metal emissions from mining and smelting have been bolstered by other industrial emissions including metal refining, energy production, manufacturing processes, waste incineration and vehicular combustion (Nriagu, 1990).

Many studies have shown that atmospheric deposition in the UK e.g. for lead, increased markedly at industrial revolution after about 1850, followed by a decrease after decline of heavy industry, awareness of environmental protection practices and the decreasing use of unleaded petrol. The increase in concentration of lead over the past 1000 years is revealed in dated peat cores (Fig 1.1; Steinnes et al, 1997).

Emissions of metals to the atmosphere in the UK have decreased by a factor of 10 or more since 1980 (RoTAP, 2012). However, critical limits for effects of metals may still be exceeded in some parts of upland Britain which have received a high historical load of metal deposition from the atmosphere (Smith et al., 2005; RoTAP, 2012). Furthermore, leaching of this accumulated burden of metal may pose a threat to upland freshwaters (Tipping *et al,* 2010). Dynamic modelling of metal behaviour in upland UK catchments suggests that the soil metal burden may remain for periods of decades to centuries, and may be affected by changes in acidity and DOC release, as well as by metal deposition (Tipping *et al*., 2006; RoTAP, 2012).

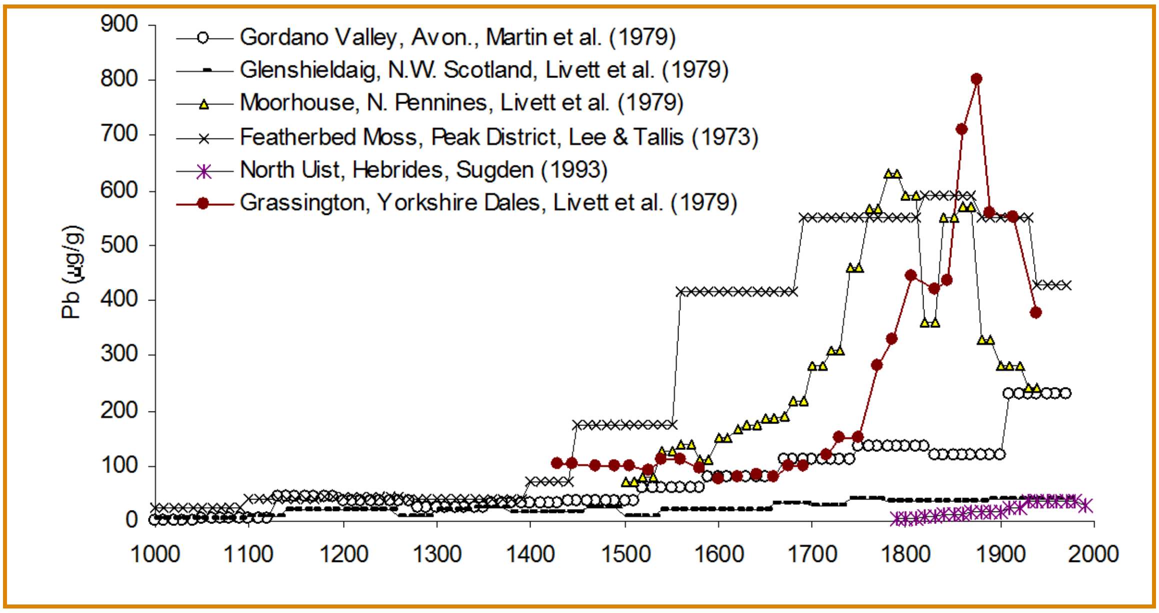


Fig. 1.1: The change in lead concentration in peat cores from the UK over a period of 1000 years. Source: Steinnes et al (1997).

1.2 Soil pore water and the concept of bioavailability

Sposito (1989) gave the definition of phytoavailability and bioavailability as:- “free ionic species is the most available form of element as it can be easily absorbed by the plants”. Thus metal concentrations in the pore water may provide more useful information on its bioavailability and toxicity than total soil concentration (Hani, 1996; Knight *et al.,* 1998; Cances *et al.,* 2003; Percival, 2003; Prokop *et al.,* 2003; Shan *et al.,* 2003), although, soil pore water has not often been utilized as a means of evaluating bioavailability. This has perhaps been due to analytical and technical difficulties related to sampling of the soil pore water.

1.3 Soil pore water definition

The concentrations of contaminants such as metals in the pore water are useful for assessing potential toxicity .Pore water is the water occupying the spaces between soil particles. As defined by the U.S. Geological Survey (2006) pore water is:- "Water occurring in the small openings, spaces, between the root zone and the water table.

1.4 Relationship between soil and pore water metal concentrations

1.4.1. Introduction

The concentration of metals in soils has been examined by many studies and the effects of various factors on metal adsorption and solubility have been described. These include pH (Green *et al.,* 2003), (Davranche & Bollinger, 2001; Davranche *et al.,* 2003; Qafoku *et al.,* 2003), the amount of metals (Garcia-Miragaya, 1984; Basta & Tabatabai, 1992; Sauvé *et al.,* 2000), cation exchange capacity (Ziper *et al.,* 1988), organic matter content (Kashem & Singh, 2001), and soil mineralogy (Warren & Haack, 2001).

The results from these studies demonstrate that total soil metal content is not a very useful tool to define potential risks. (Tack *et al.,* 1995; Sauvé *et al.,* 1998). However, methods of reliably predicting concentrations of metals, and their speciation, in soil pore water and the effects of different environmental factors, remain uncertain. The work in this thesis considered specifically the following factors which affect metal concentrations in soil pore water:- soil metal concentration, soil and soil solution pH, soil organic matter, dissolved organic carbon, nitrogen and temperature. The following sections briefly summarise current knowledge of their effects on metal pore water concentrations; more detailed discussion can be found, where appropriate, in the individual thesis chapters.

1.4.2 Metal concentration

The total soil metal concentration includes all fractions of a metal, from the readily available to the highly unavailable. pH, organic matter, and clay content are important factors to determine the quantity of total metal which is in the soil solution. Consequently, total metal concentration provides a quantity of maximum metal in the soil, (Wolt, 1994). In addition, researchers have found that, while total metal concentration may correlate with bioavailable soil pools of metal, it is inadequate by itself to reflect bioavailability (Lexmond, 1980; Sauve *et al.*, 1996; McBride *et al.*, 1997; Sauve *et al.*, 1997; Peijnenburg *et al.*, 2000).

1.4.3 pH

The equilibrium between solubility and adsorption is well dependent to solution pH (Olomu *et al.*, 1973; Kalbasi *et al.*, 1978; Cavallaro and McBride, 1984; Sauve *et al.*, 1997). Many studies have found soil pH is responsible for metal bioavailability (e.g. Turner, 1994; McBride *et al.*, 1997). For example Mn and Zn bioavailability are strongly affected by soil pH (Fergus, 1954; McGrath *et al.*, 1988; Turner, 1994). As soil pH decreases, Mn and Zn compete with the extra H^+ and Al^{3+} and thus solubility of Mn and Zn increases in the soil solution, (Kalbasi *et al.*, 1978; McBride, 1982; Bar-Tal *et al.*, 1988; Msaky & Calvet, 1990; Sauve *et al.*, 1997).

However not all metals show such a clear effect of pH, For example. although some studies show that soil solution pH affects Cu (Payne and Pickering, 1975; Msaky & Calvet, 1990; Reddy *et al.*, 1995), (Jeffery & Uren, 1983; McGrath *et al.*, 1988; Sauve *et al.*, 1997). because Cu shows high affinity for organic matter (Norvell, 1991).

Over the decades from 1900 up to 1990, high rates of deposition of sulphate and nitrate lead to an acidification of UK upland soils and freshwaters. Since 1980, when emissions of sulphur dioxide in the UK began to rapidly decline, there has been increasing evidence of a recovery in acidity in both soils and waters (RoTAP, 2012).

However, it is unclear what the implications of these changes in soil pH have been for metal concentrations in soil pore water and in upland streams.

1.4.4 Organic matter

Metals complexes with organic matter, The COOH group in both solid and dissolved organic matter form complexes with metals (Stevenson, 1976; Baker & Senft, 1995). These complexes facilitate the transport of metals (Huang *et al.*, 1998). DOC plays an important role in various soil processes. These include the complexion of metals, and formation of hydrophobic organic compounds, substrate for microorganisms to facilitate dentrification (Zsolnay, 1996). There is clear evidence from the UK, and other European countries, that DOC levels in upland streams have increased since 1990. The causes of this change are uncertain but there is increasing evidence that is linked to the reversal of acidification (Evans *et al*., 2006; Monteith *et al*., 2007). Given the high affinity of some metals for DOC, this rise in DOC levels could in principle lead to an increase in metal leaching from upland soils.

1.4.5 Nitrogen

Nitrogen is the largest component of the Earth's atmosphere (Baur & Wlotka, 1969; Stevenson, 1982). The reactive N cycle is one of the most important nutrient cycles, and has been modified greatly by human activities during the last few decades (Jenkinson, 2001; Vitousek *et al.*, 2002). Nitrogen deposition primarily occurs through wet deposition of NH_4^+ and NO_3^- and dry deposition of NH_3, NO_2 and particulate N. The deposition of nitrogen (N) to the ground has increased since the industrial revolution (Fowler *et al.,* 2004). Nitrogen deposition is potentially acidifying, and also can affect DOC behaviour in soils, and hence could also affect metal partitioning between the soil matrix and porewater.

Furthermore, DOC is involved in nutrient cycling, especially for N, P and S, in metal and organic pollutant transportation, and as an energy source for microorganisms (Tipping & Hurley, 1992; Hagedorn *et al.*, 2000; Kaiser *et al.,* 2001; McDowell, 2003). Many studies have been conducted to address the interactions between N additions and DOC production; however, contrasting findings have been reported. For example, McDowell *et al.* (1998) studied N addition and its impact on DOC and DON production, but found no significant effects. In contrast, Pregitzer *et al.* (2004) showed consistent increases in DOC and DON production in a long-term field-based N addition experiment. The detailed consequences of DOC production and its interactions with N dynamics with the release of metals are discussed in Chapters 4. which describes a long term experiment to test the effect of the ratio of NH_4^+/NO_3^- deposition on soil solution pH and DOC and on metal partitioning between soil and pore water.

1.4.6 Temperature

Temperature is an important limiting factor in the field. DOC concentrations in soil solutions are often elevated during summer when microbial activity is high (Guggenberger & Kaiser 2003).

The increased production of DOC at higher temperatures means that soil solution concentrations of some metals could increase at higher temperatures. However, there is a scarcity of studies on the effect of temperature as a factor governing metal concentrations in soil solution. Hence one objective of the thesis, as described in Chapter 3, was to assess how temperature alters the release of DOC from organic soils and hence the release of metals with a high affinity for organic matter. (Andersson *et al.* (2000)

1.5 Aims and objectives

Environmental risk assessment of metals depends on predicting the fate of metals based on soil–solution partitioning. For large-scale risk assessment, models need to be appropriate so that the data requirements for application do not constrain their use. For example, models currently applied to assess critical loads in the UK, and more widely across Europe, as described in Chapter 2, are based on the assumption that the soil solution concentrations of potentially toxic metals can be predicted as a function of soil solution pH and DOC. However, these assumptions have not been widely tested, and it is not clear if these simple models can explain the effects of major environmental drivers such as temperature and N deposition.

Therefore, the main aim of this study was to use a combination of field survey data and experiments to assess whether the soil solution concentrations of metals, and specifically of Ni, Cu, Zn, Cd and Pb, can be explained by variation in soil solution pH and DOC concentration, in addition to the total soil metal concentration. The specific objectives were:-

- To assess if the soil solution metal concentrations measured in a wide range of upland soil types can be explained as a function pH, DOC and organic content, in addition to HNO_3 extractable soil metal concentrations.
- To assess experimentally if changes in temperature affect the concentrations of metals in soil solution, and if these changes can be explained by changes in DOC and pH
- To assess experimentally if different chemical forms of N deposition affect the concentrations of metals in soil solution, and to assess if these changes are directly associated with changes in soil solution nitrate and ammonium concentrations, or indirectly through changes in DOC or pH.
- To assess experimentally if any effect of different chemical forms of N deposition on metal concentrations in soil solution is associated with acidification, by determining the effect of adding lime.

1.6 Structure of the thesis

To address the issues identified above, the thesis is organized as follows.

Chapter 2 describes a study using archived soil and soil solution samples from a regional survey of upland sites in northern England with contrasting soil and vegetation types. The samples had already been characterised for a range of chemical parameters that are relevant to the solid-solution partitioning of heavy metals, including soil solution DOC and pH. The main aim of the study was to assess whether simple models based on soil metal concentrations, and soil solution pH and DOC, can explain spatial variation in the metal concentrations that were determined in soil solution.

Chapter 3 describes an experiment in which samples from two of the field survey sites with high levels of metal contamination were subjected to different temperatures to investigate how this influenced metal concentrations in soil solution. Cores taken from the two sites were divided between unheated and heated glasshouses at York, and the change in soil solution and leachate chemistry was determined over time. The main aim of the study was to assess whether soil-solution partitioning of metals is perturbed by changes in temperature, and whether this is associated with changes in soil solution pH, DOC concentration, and concentrations of major anions and cations.

Chapter 4 describes an experiment to compare the effects of reduced and oxidized nitrogen deposition on metal concentrations in soil solution. This study made use of a long-term experiment in which soil/vegetation cores from the Isle of Skye had been subjected to different rates of N deposition, and different ratios of oxidized and reduced N, to determine the ecological effects over a period of three years. Towards the end of the study, soil solution and soil samples were taken for determination of metal concentrations. The aims of the study were to determine if soil-solution partitioning of metals is perturbed by changes in N deposition, and whether this is associated with changes in soil solution pH, DOC concentration, and concentrations of major anions and cations.

Chapter 5 integrates the results from Chapter 2-4, summarises the findings of thesis and discusses whether the different studies are consistent with a simple chemical model of soil solution chemistry controlling metal mobility and bioavailability in organic soils.

Chapter 2 – Simple predictive models of soil solution metal concentrations: A regional survey of upland sites

2.1. Introduction

The application of critical loads methodology requires the understanding of biogeochemistry and bioavailability for the protection of upland sites from the toxic effects of metal deposition, (Pačes, 1998). In the study of Tipping *et al.* (2003), soil solutions extracted from soil samples from 98 upland UK sites were analysed for total dissolved metal concentrations, and free metal ion concentrations were modelled. Comparison with critical limits showed that 14 of the sites exceeded the critical limit for cadmium, and 63 for lead. Therefore, in view of the very long residence periods of trace metals in most soils, it is important to assess the long term legacy from metal deposition in UK upland soils.

Environmental risk assessment of metals depends on modelling the mobility of metals based on soil−liquid partitioning coefficients. In the specific context of critical loads as a risk assessment method, for example as applied in the UK (Hall *et al*. 2007), it is assumed that a steady state is eventually reached at which the deposition to the soil from the atmosphere (inputs) are balanced by the outputs – primarily loss of metal in leaching, and uptake and removal of metal in harvested vegetation. Since the rate of loss of metal in leaching, in particular, increases with metal concentrations in soils, the metal concentration will increase with increasing deposition until leaching and other losses balance the increased deposition. At the critical annual deposition load, the metal concentration in soil, or soil solution at steady state, is equal to the concentration above which adverse ecological or environmental impacts may occur.

In this critical loads approach, relationships between total soil metal concentrations and those in soil solution (so-called transfer functions) are important because (a) they determine the proportion of the total soil metal pool which may be leached annually and (b) as discussed in Chapter 1, the effects of metals are better described by the metal concentrations in soil solution than the total soil metal pool. Hence relationships to predict soil solution concentrations, which cannot be readily measured, from available data on total soil metal concentrations play a central role in calculations of critical loads of metals, and hence in assessments of the need for national and international control of metal emissions to the atmosphere (RoTAP, 2012).

Tipping *et al.* (2003) derived relationships for UK upland soils between the metal concentration in soil solution and the total soil concentration, using loss on ignition (LOI), soil solution DOC and soil solution pH. These relationships were derived for cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn), and explained between 39% (for copper) and 81% (for lead) of the variation in soil solution concentration. These findings indicated that these transfer functions could be used for predicting soil solution concentrations from other soil properties and databases of total soil concentrations.

These predictive relationships were of the form

$$\log[M_{SS}] = a \cdot \log[LOI] + b \cdot pH + c \cdot \log[M_{HNO3}] + d \cdot \log[DOC] + e,$$

where M_{ss} is the metal concentration in soil solution, M_{HNO3} is the total soil metal concentration, and *a, b, c, d* and *e* are constants.

However, Tipping *et al.* (2003) did not collect soil solution samples in the field. Instead, soil samples were collected in the field, and then brought back to the laboratory, where they were placed in sealed plastic boxes at laboratory room temperature, after storage at 4°C. Rhizon samplers were then inserted into the box to extract soil solution. This is a highly artificial set-up, and it is far from certain that the metal concentrations in soil solution found using this method are representative of real concentrations in the field.

Therefore, there is a need to determine if similar simple predictive equations could be used to predict concentrations of metals in soil solutions sampled under field conditions. The aim of the work described in this chapter was to further contribute to understanding of the factors influencing soil solution concentrations of Cu, Zn, Cd and Pb under field conditions in different soils with a wide range of pH, DOC and organic content, in order to better predict the soil solution concentration of Cu, Zn, Cd and Pb as a function of pH, DOC and organic content. The work focussed on the feasibility of predicting concentrations of dissolved metals in porewater from data on (i) total metal concentrations (HNO_3 extracts) and (ii) soil and soil solution properties such as organic matter content, pH, and DOC. The prospective value of such tactic is the derivation of valuable estimates of metal bioavailability based on empirical relationships which relate concentrations of total dissolved metal to key soil and soil solution variables.

There is a strong body of evidence that soil solution pH and DOC strongly influence metal concentrations and speciation in pore-water, and that these effects vary between different metals. DOC strongly complexes heavy metals, influencing metal exchange processes between the soil and soil solution and facilitating metal leaching and metal transport through the soil profile towards streams and ground water (Jones 1998; Tipping & Hurley 1992). DOC makes complexes with metals and thus mobilize them in soil solution, while organic matter immobilizes,e.g. Cu is forms stronger complexes with organic ligands (Florence, 1982) however Zn in soil solution (Milne *et al.*, 2003). Hence DOC exerts significant influence for metals in soils (Antoniadis & Alloway, 2002; Tipping, 2002).

Generally pH is considered to be the most important ,it effects the availability and plant uptake of many elements. Metal uptake by plants may be increased due to decreasing pH (Brown *et al.*, 1994), and it is clear that both the complexing capacity of organic acids and low pH are major factors related to mobilization of metals in soil and their accumulation in plants (McGrath *et al*., 1997). According to Bhogal *et al.* (1993), the bioavailability of Cu and Zn is mainly controlled by pH and organic carbon content, while Ni depends in soil with soil organic compounds (Kuo *et al.*, 1985), soil water pH and DOC solution concentrations.

Furthermore, changes in soil solution pH may modify dissolved organic matter (DOM). At High pH, DOM molecules have expanded structure (Rice *et al.*, 2000), because charges attempt to position themselves far as possible. Thus this structure with the presence of negative charge, make them become hydrophilic, therefore it is stated that the changes in pH actually helps to change the adsorption and desorption mechanisms of DOC. (Jardine *et al*., 1989 and Gu *et al*., 1994). water-soluble material are formed when degradation of organic matter occur due to enhanced activity of microbes in the soil dependent on high pH (Higashida & Takao, 1986),

This study made use of a database collected by van den Berg *et al.*, (2012) to determine DOC concentrations in soil solution collected *in situ* using Rhizon samplers from a range of sites in upland Britain, and to identify factors which could be used to predict DOC concentrations. This analysis was extended in the work described in this chapter to factors predicting metal concentrations in soil solution, by analysis of stored soil and soil solution samples for metal concentrations. Although van den Berg *et al.* (2012) used data from a range of sites which used different extraction and measurement methods, the analysis described in this chapter was constrained to those sites within the study of van den Berg *et al.* (2012) that were sampled by the University of York. This ensured a consistent high quality dataset. Furthermore, although van den Berg *et al.* (2012) sampled soil solution every 3 weeks through a year, the analysis described in this chapter was constrained to annual mean concentrations, as it is these concentrations that are used when applying transfer functions in mapping critical loads.

Although Tipping *et al.* (2003) sampled sites with a range of soil types, they present no comparison of soil or soil solution concentrations of metals in different soil types. Since, in environmental risk assessment, maps of total metal concentrations are often associated with information on soil classification, an additional analysis was undertaken to assess differences between total metal concentration and soil solution metal concentration on major groups of upland soil. In addition, this study extends the analysis of Tipping *et al.* (2003) from lead, copper, zinc and cadmium, to include nickel, since critical load assessment in the UK has now been extended to include this metal (RoTAP, 2012). The use of a constant temperature regime for soil solution extraction by Tipping et al. (2003) also raises interesting questions about the effect of variation in temperature on the release of metals into soil solution – this was examined further in the experiment that is described in Chapter 3.

The specific objectives of this study were therefore threefold:-

1. To characterise the solid-solution partitioning of heavy metals from a regional survey of sites with contrasting soil types for which the soil solution DOC and pH have already been determined.

2. To identify relationships between soil solution metal concentrations, total soil concentrations, and pH and DOC in soil solution, and specifically to assess whether decreasing pH and increasing DOC are associated with higher soil solution metal concentrations.

3. To compare the predictive relationships for metal concentrations of soil solution extracted in the field derived in this study with those derived by Tipping *et al.* (2003) using soil solution extracted in the laboratory.

2.2 Materials and methods

2.2.1 Sites and data sources

This study partly used the data already collected and analysed by University of York for the regional survey done in 2005-6. The sites that were included in this survey are listed in Table 2.1. The 25 sites included four habitats (grasslands, woodlands, moorlands and heathlands), and five different soil classes (brown soils, lithomorphic soils, organic soils, podzolic soils and ground and surface water gley soils). Sites were assigned to a broad soil class according to the world reference base (WRB) as listed in the European soil atlas (Jones et al. 2005).

2.2.2 Soil pore water sampling

Soil pore water was collected using Rhizon samplers (Eijkelkamp, Giesbeek Netherlands). As described by van den Berg *et al.* (2012), samplers were installed horizontally in the middle of the A horizon in mineral soils, or at 7.5cm. depth relative to the top of the O horizon in organic soils. Five sampling locations were established at each site, and samples were taken every three weeks. Samples from each location were pooled to produce one sample per site for chemical analysis. At the start of the sampling period, in May 2005, five soil samples per site were taken to a depth of 10cm., using an auger, with all samples were pooled prior to analysis and extraction.

The extraction methods for soils, and the analytical methods for soils and soil solution, for non-metal determinants were as described by van den Berg et al. (2012); since similar analytical methods were employed in the experiments described in Chapter 3 and 4, more detail can be found there. Details relevant to the determination of the metal concentrations that formed the focus of the analysis in this chapter are described below. However, before this section, the use of rhizon samplers to extract soil solution is described in more detail, as this is central to the work described in this chapter, and in Chapters 3 and 4 of the thesis.

Table 2.1: Summary of sample sites that provided data used in the analysis in this chapter

Site	**Soil class**	**Grid reference**	**Vegetation type**	**County**
Wardlow calcareous	brown soil	SK177739	Calcareous grassland	Derbyshire
Longstone	lithomorphic soil	SK198731	Calcareous grassland	Derbyshire
Sherwood	podzolic soil	SK162908	Coniferous woodland	Derbyshire
Whernside	lithomorphic soil	SD722764	Calcareous grassland	N.Yorkshire
Hawthorntwaithe	podzolic soil	SD563517	Heath	Lancashire
Castle Howard	brown soil	SE734678	Deciduous woodland	N.Yorkshire
Norwood	brown soil	SE387416	Deciduous woodland	W.Yorkshire
Strensall dry	podzolic soil	SE653600	Heath	N.Yorkshire
Strensall wet	podzolic soil	SE653600	Heath	N.Yorkshire
Trough of bowland	organic soils	SD625531	Neutral to acid grassland	Lancashire
White Hill	organic soils	SE002126	Bog	W.Yorkshire
Holme Moss	organic soils	SE090042	Bog	Derbyshire
Ing bog	podzolic soil	SD747716	Bog	N.Yorkshire
Bishop Wilton	lithomorphic soil	SE821557	Calcareous grassland	N.Yorkshire
Ing grass	surface water gley	SD773775	Neutral to acid grassland	N.Yorkshire
Wardlow neutral	brown soil	SK177739	Neutral to acid grassland	Derbyshire
Rays	organic soils	SD747716	Coniferous woodland	N.Yorkshire
Etherow heath	organic soils	SK125999	Heath	Derbyshire
Derwent Ings	ground water gley	SE702430	Acid grassland	S. Yorkshire
Ryburn up	podzolic soil	SE023188	Coniferous woodland	W.Yorkshire
Etherow grass	surface water gley	SK125999	Neutral to acid grassland	Derbyshire
Coniston	brown soil	SD998658	Calcareous grassland	N.Yorkshire
Over silton up	podzolic soil	SE451933	Coniferous woodland	N.Yorkshire
Ellerswood	podzolic soil	SE854846	Deciduous woodland	N.Yorkshire
Featherbed Moss	organic soils	SK090921	Bog	Derbyshire

2.2.3. Rhizon samplers

*“*Standard Rhizon samplers (Fig. 2.1) consist of a length of porous, chemically-inert hydrophilic polymer plastic. This is capped with nylon at one end, and attached to a 5 or 10 cm length of polyethylene tubing, with a connector at the other end.” (*Di Bonito, 2005)*

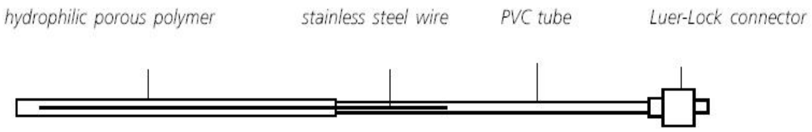


Figure 2.1 Sketch of a Rhizon sampler (from “Rhizon soil moisture sampler: operating instructions”. www.eijkelkamp.nl

Rhizon works under capillary action, when the capillary pressure becomes lower in the soil after suction, water flows in the sampler until equilibrium state is developed between sampler and surrounding soil. (Schubert, 1982).

2.2.3.1 Pore water extraction

For the soil pore water sampling, the procedure described by Knight *et al*. (1998) and Tye *et al*. (2003) was followed. One sampler was inserted into each of the soil containers. Pore water was extracted by linking a syringe to each sampler and applying suction. Soil pore water was sampled with Rhizon samplers (Rhizon Soil moisture sampler, Eijkelkamp, Agrisearch Equipment, Netherlands). The Rhizon samplers were placed horizontally in the field, just under the vegetation, after piercing the soil with a wooden stick with the same diameter. The Rhizon samplers were coupled to a syringe, which was pulled out to create a vacuum. The syringes were covered in aluminium foil to exclude light.

Samples were taken every three weeks using five replicates per location between May 2005 and April 2006. After collection, the volume obtained per sampler was noted and all individual samples were pooled to produce one sample for each sampling period per site. Immediately after pooling, the pH was measured, samples were filtered, and then samples were stored at 4^{o}C until further analysis. 0.4ml 0.1M citric acid was added to a 10 ml sample, to prevent metals from settling. The samples were frozen until further analyses.

2.2.4. Acid digest of soil samples

The soil samples were transported in a cooling box to the lab and stored at 4^{o}C until analysis. Soils were stored in airtight plastic bags, from which as much air as possible was removed. Soils were homogenized by hand, well mixed to avoid internal variation between sub samples, and roots and shoots were removed. Approximately 10g duplicate sub-samples of each field moist soil were weighed into pre-weighed, dried foil dishes, oven-dried overnight at 105^{o}C, and cooled in desiccators, The oven-dried soil residues obtained were individually finely ground with a Retsch ball mill for 3 minutes at 25 Hz before being used for acid digest extracts through microwave digestion.

Each 200 mg dried soil samples had 4 ml nitric acid (65%) and 1 ml hydrogen peroxide (30%) added. The digestion was then carried out using a Milstone microwave (type MLS 1200 mega, Milstone Inc., Sorisole, Italy). Soil samples were heated in microwave digestion system, for 1 min at 250 W, 2 min at 0 W, 5min at 250 W, 5 min at 400 W, and finally 5 min. at 500 W. After cooling for 30 minutes at 4^{o}C, the vessel contents were flushed with deionised water while filtering them with Whatmann filter paper, then diluted to 100 ml. with deionized water, and then the samples were stored at 4^{o}C until further analysis.

2.2.5 Metal analysis

Concentrations of metals were determined by ICP-MS and ICP-OES. Zn, was measured using an ICP Spectrometer (IRIS Intrepid II, Thermo Electron Corporation, Franklin, MA). Other metals were determined using ICP Spectrometer (X series; Thermo Fisher Scientific, Waltham, MA, U.S.A).

Blanks are used to correct for extraction buffer quality and reference solutions for quality checks on the concentrations. Where the blank values were significantly above zero, they were subtracted from the measured values. The drift and QC control was done by the ICP-computer programme which was set at a level of 2.5%. If the ICP found that the standards and reference solutions diverted by more than 2.5% from, it gave a "fail" message and reanalysed the samples. The instrumental drift was also corrected. The standards used were multiple element stock standards of a specific concentration (2, 10 and 50 ppb for Ni, Cu, and Pb; 50 ppb and 500 ppb for Zn).

2.2.6. Statistical analysis

The data were tested for normality using the Kolmogorov-Smirnov test and log transformed to run the multiple regression analysis. The effect of soil type on pH, DOC, C/N ratio and LOI was analysed in a one-way analysis of variance, as were soil type effects on metal concentrations in HNO_3 extracts and in pore water. Pearson correlation coefficients were used to assess the significance of relationships between HNO_3 extract and pore water metal concentrations with pH, LOI, DOC and C/N ratio. Multiple regression models were used to explore potential combinations of predictor variables to assess (a) the effects of soil pH, C/N ratio and LOI on soil metal concentrations and (b) the effects of soil metal concentrations, LOI, pore water pH and DOC on pore water metal concentrations, based on the model of Tipping *et al.* (2003). All statistical analyses were performed using SPSS 19.

2.3. Results

2.3.1 Overview of data

Table 2.2 shows the soil and soil solution chemistry at each site, while Table 2.3 shows the metal concentrations at each site. For the 25 sites, the mean soil solution pH ranged between 3.16 and 7.48; Bishop Wilton, a calcareous grassland site on lithomorphic soil had the highest pH, and the lowest pH was recorded at Over Silton up, on a podzol. The DOC concentration ranged between 6-81.5 mg l^{-1}; most sites on surface water gley soils or organic soils showed DOC concentrations higher than 20 mg l^{-1}. Likewise, the LOI ranged widely, from 7.6 to 94%, with the lowest value at Castle Howard, on a brown earth, and highest at Etherow Heath on an organic soil. The C/N ratio ranged between 10.55-32.75 with the lowest value on a lithomorphic soil and the highest value on an organic soil.

Table 2.2: Soil chemistry parameters (LOI, C:N and pH$_{(NaCl)}$) and rhizon samples (pH and DOC) of all selected sites.

Site	pH(Soil solution)	pH(NaCl)	DOC(mg l^{-1})	LOI (%)	C/N
Wardlow calcareous	6.99	6.68	13.7	32.6	14.9
Longstone	5.34	4.26	20.5	21.7	14.1
Sherwood	3.61	2.84	21.9	25.9	19.5
Whernside	6.64	4.85	7.8	28.8	11.7
Hawthorntwaithe	4.07	2.85	25.3	91.8	20.1
Castle howard	4.36	3.74	47.4	7.6	17.7
Norwood	6.97	6.69	12.8	16.4	11.1
Strensall dry	3.97	3.33	37.1	9.5	25.9
Strensall wet	3.69	3.33	48.0	30.3	28.9
Bowland	4.02	2.73	19.6	69.8	22.8
White hill	3.87	2.92	81.5	90.7	28.9
Holme	4.01	2.97	63.1	76.6	25.3
Ing bog	3.86	2.99	61.5	93.4	25.5
Bishop wilton	7.48	6.64	12.7	27.1	10.5
Ing grass	5.86	4.72	6.0	23.5	11.6
Wardlow neutral	5.42	4.24	23.2	21.7	14.9
Rays	3.94	2.95	50.2	82.4	22.2
Etherow heath	3.96	2.64	31.5	94.1	32.7
Derwent ings	6.43	5.42	24.1	22.5	12.3
Ryburn up	5.45	3.66	47.5	17.6	17.6
Etherow grass	4.58	3.71	27.9	52.3	16.0
Coniston	7.2	5.61	15.7	35.1	13.1
Over silton up	3.16	2.92	31.3	17.2	20.0
Ellerswood	3.88	3.15	43.5	14.9	13.7
Featherbed moss	3.97	3.01	32.5	92.8	30.5

Table 2.3 shows the metal concentrations in nitric acid extracts and in soil solution. Cd concentrations were very low in the dataset, with many values below the detection limit. Therefore no further analysis was carried out for Cd. The HNO_3 extract Ni concentration ranged between 0.01-0.19 µmol g^{-1}, while the soil solution Ni concentration ranged between 0.02-0.24 µmol l^{-1}.

Table 2.3: Summary of mean metal concentrations in HNO_3 digests (µmol g^{-1}) and Rhizon samples (µmol l^{-1}).

SITE	Ni_HNO_3	Ni_Rhiz	Cu_HNO_3	Cu_Rhiz	Zn_HNO_3	Zn_Rhiz	Pb_HNO_3	Pb_Rhiz
Wardlow Calcareous	0.15	0.03	0.12	0.65	1.56	6.62	1.56	0.01
Longstone	0.08	0.04	0.1	1.36	0.81	2.09	1.88	0.6
Sherwood	0.02	0.17	0.06	1.32	0.06	12.5	0.15	0.26
Whernside	0.1	0.03	0.12	1.41	0.51	2.56	1.56	0.28
Hwthorntwaithe	0.02	0.02	0.1	1.27	0.1	1.62	0.2	0.05
Castle howard	0.03	0.13	0.02	1.02	0.09	8.59	0.03	0.03
Norwood	0.09	0.04	0.06	1.42	0.37	7.8	0.06	0.01
Strensall dry	0.01	0.02	0.02	1.68	0.14	1.62	0.02	0.07
Strensall wet	0.01	0.09	0.05	1.28	0.06	2.27	0.05	0.06
Through of bowland	0.02	0.02	0.08	1.26	0.09	1.26	0.23	0.04
White hill	0.04	0.11	0.28	1.25	0.09	2.69	0.47	0.01
Holme	0.03	0.06	0.2	1.46	0.07	5.56	0.37	0.11
Ing bog	0.02	0.03	0.08	1.08	0.06	2.2	0.24	0.16
Bishop wilton	0.12	0.03	0.06	0.98	0.55	2.1	0.1	0.01
Ing grass	0.08	0.02	0.07	1.03	0.29	1.42	0.08	0.01
Wardlow neutral	0.12	0.04	0.08	1.13	0.63	1.68	0.24	0.03
Rays	0.03	0.04	0.09	2.33	0.07	3.89	0.47	0.1
Etherow heath	0.03	0.02	0.21	0.9	0.12	0.58	0.85	0.19
Derwent ings	0.08	0.15	0.06	0.92	0.27	1.13	0.05	0.01
Ryburn up	0.05	0.24	0.09	1.59	0.13	14.5	0.12	0.04
Etherow grass	0.03	0.03	0.14	1.15	0.13	1.4	0.29	0.07
Coniston	0.19	0.05	0.09	0.73	1.82	2.7	0.33	0
Over silton up	0.02	0.09	0.03	0.96	0.05	13.9	0.08	0.17
Ellerswood	0.02	0.05	0.02	1.04	0.07	3.41	0.05	0.07
Featherbed Moss	0.04	0.05	0.36	2.06	0.14	1.24	0.66	0.08

The highest brown soil concentration was found at Coniston, while the highest podzol soil solution concentration was at Ryburn up. Cu concentrations in HNO_3 extracts ranged between 0.02-0.36 µmol g^{-1}, with those in Rhizon samples ranging between 0.65-2.33 µmol l^{-1}. The highest organic soil concentration was at Featherbed

Moss, while the highest organic soil solution concentration was at Rays. Hence the highest concentrations in bulk soil and in soil solution were not found at the same site. A similar pattern was found for zinc, for which the highest concentration in HNO_3 extracts (1.82 µmol g^{-1}) was, as for Ni, at Consiton, on a brown soil, while the highest concentration in soil solution (14.5 µmol l^{-1}) was, as also for Ni, from Ryburn up, on a podzol. However, in the case of Pb, the highest concentrations in both soil extracts and pore water were found at the Longstone site.

2.3.2 Effect of soil type on metal concentrations

All the 25 areas were divided according to their soil categories, as shown in Table 2.4. Lithomorphic and surface water gley soils were put together under same category.

Table 2.4: Summary of division of sites by soil type

Brown soil	**Podzolic**	**Organic soil**	**Lithomorphic and soil+surface water gley soil**
Wardlow calcareous Castle howard Norwood	Sherwood Hawthorntwaithe Strensall dry Strensall wet Ing bog Ryburn up Over silton up Ellerswood Llyan lagi Hafren	Through of bowland White hill Holme Rays Etherow heath Featherbed moss Whim	Longstone Whernside Bishop wilton Ing grass Etherow grass

Table 2.5 summarises the soil characteristics for the different soil types, In terms of the metal concentrations, the pattern for the four soil types differed in some cases between the HNO_3 extract and the Rhizon extract. For example, the highest Ni concentrations in HNO_3 extracts were 0.09 and 0.08 µmol g^{-1} on brown and lithomorphic soils respectively, but the highest soil solution mean Ni concentration was 0.09 µmol l^{-1} on podzolic soils. However, the highest mean Zn concentration in both the acid digest (0.67 µmol g^{-1}) and in soil solution (10.94 µmol l^{-1}) was on brown soils. The highest pH values were found on brown and lithomorphic soils, while the highest DOC concentrations and LOI values were, as expected, on organic soils.

Table 2.5: The mean value and range of the soil and soil solution parameters for the four different soil types.

Parameter	**Extract**	**Brown**	**Podzol**	**Organic**	**Lithomorphic**
		average	Average	average	average
Ni	HNO_3 (µmol g^{-1})	0.09	0.01	0.03	0.08
	Rhizon (µmol l^{-1})	0.06	0.09	0.05	0.02
Cu	HNO_3 (µmol g^{-1})	0.07	0.05	0.2	0.1
	Rhizon (µmol l^{-1})	1.03	1.38	1.38	1.18
Zn	HNO_3 (µmol g^{-1})	0.67	0.08	0.09	0.47
	Rhizon (µmol l^{-1})	10.94	6	2.59	3.89
Pb	HNO_3 (µmol g^{-1})	0.55	0.11	0.5	0.59
	Rhizon (µmol l^{-1})	0.02	0.11	0.13	0.13
pH	Rhizon	6.11	3.95	3.95	5.97
DOC (mg l^{-1})	Rhizon	24.6	39.5	46.4	15
LOI (%)	soil	18.87	37.5	84.4	31.81

Table 2.6 shows the results of the analysis of variance of the effects of soil type on soil characteristics and metal concentrations in HNO_3 extracts and in soil solution from Rhizon samples, while Figure 2.2 shows the differences in mean metal concentrations in these two sets of samples between soil types. Soil solution pH and DOC, and soil LOI and C/N ratio all showed significant effects of soil types at $P<0.001$, as might be expected from a survey design selecting sites on a wide range of soil types. However, the effects of soil type on metal concentrations were less clear-cut.

Table 2.6: Anova results to test the significance of differences between soil type for (a) soil and soil solution parameters and (b) metal concentrations

a		Soil type(df=3)	
	Parameter	F	P
	pH	22.8	0.000
	DOC	8.6	0.000
	CN	28.8	0.000
	LOI	17.4	0.000

b			
Extract	Metal	F	P
HNO_3	Ni	8.4	0.001
	Cu	6.5	0.004
	Zn	4.5	0.016
	Pb	1.2	0.353
Rhizon	Ni	1.3	0.316
	Cu	0.9	0.442
	Zn	3.5	0.036
	Pb	0.6	0.633

Pb was the only metal that showed no significant effect of soil type on concentrations in either HNO_3 extracts or pore water. The data in Figure 2.2 suggest a lower HNO_3 extract Pb concentration in podzols, but the very high variation and the limited number of sites meant that firm conclusions could not be drawn. In the case of nickel, there was a significant effect of soil type (F=8.4; P <0.001) for the HNO_3 extracts but not for pore water concentrations. HNO_3 extract concentrations were 3-4 times higher on brown and lithomorphic soils than on organic soils and podzols, but these differences were not reflected in the pore water concentrations. For Cu, as for Ni, there was a significant effect of soil type (F=6.5; P <0.005) for the HNO_3 extracts but not for pore water concentrations, although in both cases the highest concentrations were found on organic soils. Zinc was the only metal for which significant effects of soil type were found both for HNO_3 extracts and for pore water concentrations, with higher concentrations being found on brown and lithomorphic soils.

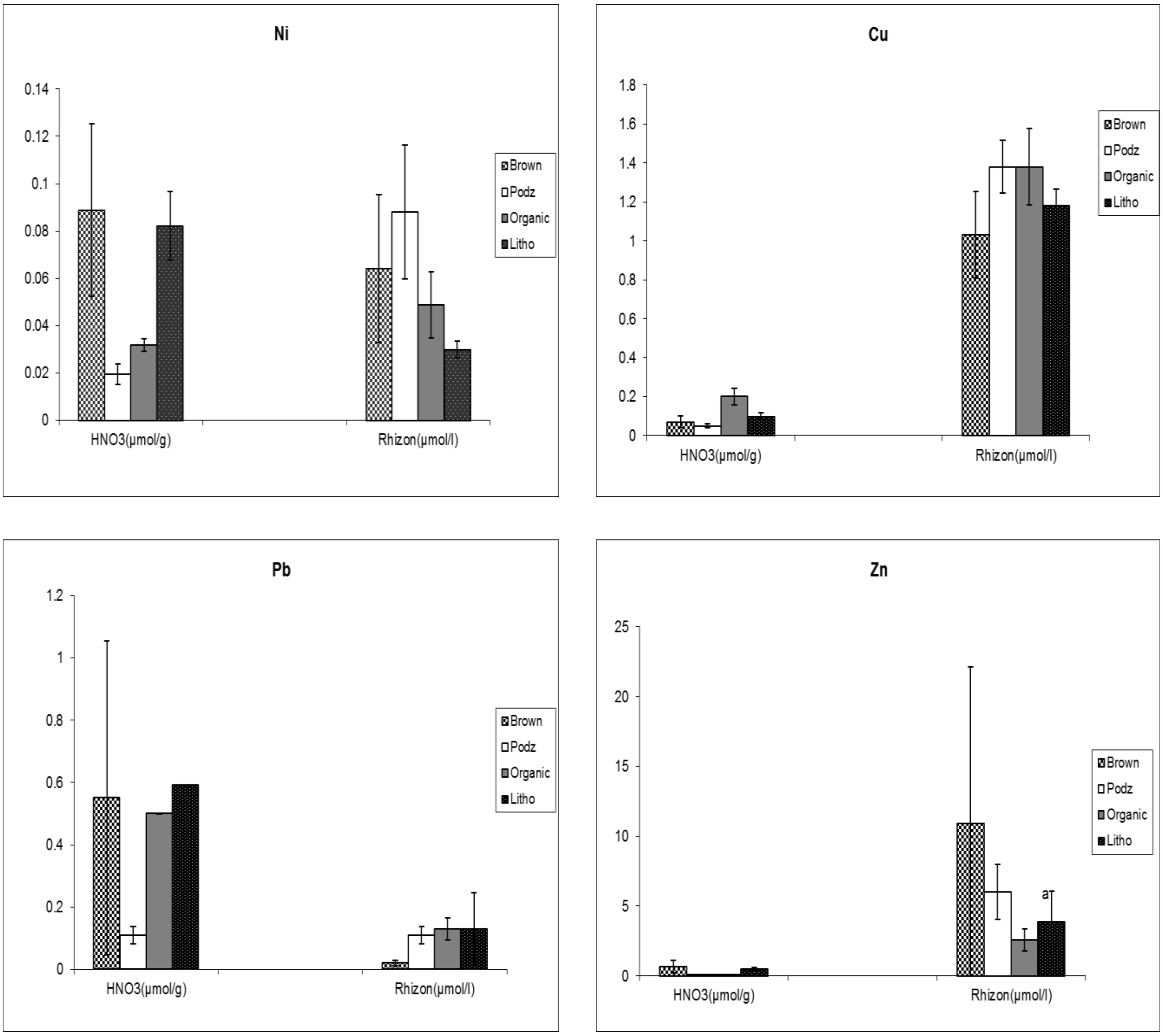


Fig. 2.2: Mean metal concentrations in HNO_3 extracts (µmol g^{-1}) and pore water (µmol l^{-1}) in the four soil categories. Error bars indicate standard errors of means (±1.s.e)

2.3.3. Correlation of metal concentrations with soil and soil solution parameters

Table 2.7 shows the correlation coefficients between the metal concentrations in HNO_3 extracts and in soil solution, and the soil and soil solution parameters that were determined. The soil solution concentrations were not significantly correlated with either DOC or pH in soil solution, and also showed no significant correlation with any of the soil variables. There was also no significant correlation between the soil solution and HNO_3 extract concentrations of the metals, except in the case of Pb, for which there was a significant positive correlation (Fig 2.3f). In contrast, the HNO_3 extract concentrations did show significant relationships with soil and soil solution variables (Table 2.7). In the case of Ni and Zn, there was a positive relationship between HNO_3 extract concentrations and pH, and a negative relationship with soil solution DOC and soil C/N ratio. These relationships are shown in Figure 2.3d. In contrast, Pb concentrations in HNO_3 extracts were not significantly correlated with any of the variables, whereas Cu concentrations were positively correlated with soil organic content and C/N ratio (Table 2.7; Fig 2.3e)

*Table 2.7: Correlation matrix (n=25) between the various soil and soil solution properties and metal concentrations in soil solution and HNO_3 extracts. Significance is indicated as:- * $P<0.05$; ** $P<0.01$.*

Metal		pH(ss)	pH(NaCl)	DOC	LOI	C/N	Soil solution metal
Nickel	soil solution	-0.082	-0.114	0.315	-0.309	-0.018	-
	HNO_3	.891**	0.831**	-.566**	-0.291	-0.786**	-.153
Copper	soil solution	-0.365	-0.385	0.237	0.103	0.217	-
	HNO_3	-0.137	0.219	0.274	.698**	.519**	-0.008
Zinc	soil solution	0.128	-0.155	0.025	-0.381	-0.248	-
	HNO_3	.721**	0.691*	-.487*	-0.252	-.469*	-0.024
Lead	soil solution	-0.354	-0.219	0.246	0.146	0.242	-
	HNO_3	0.124	0.191	-0.1	0.202	0.078	.636**

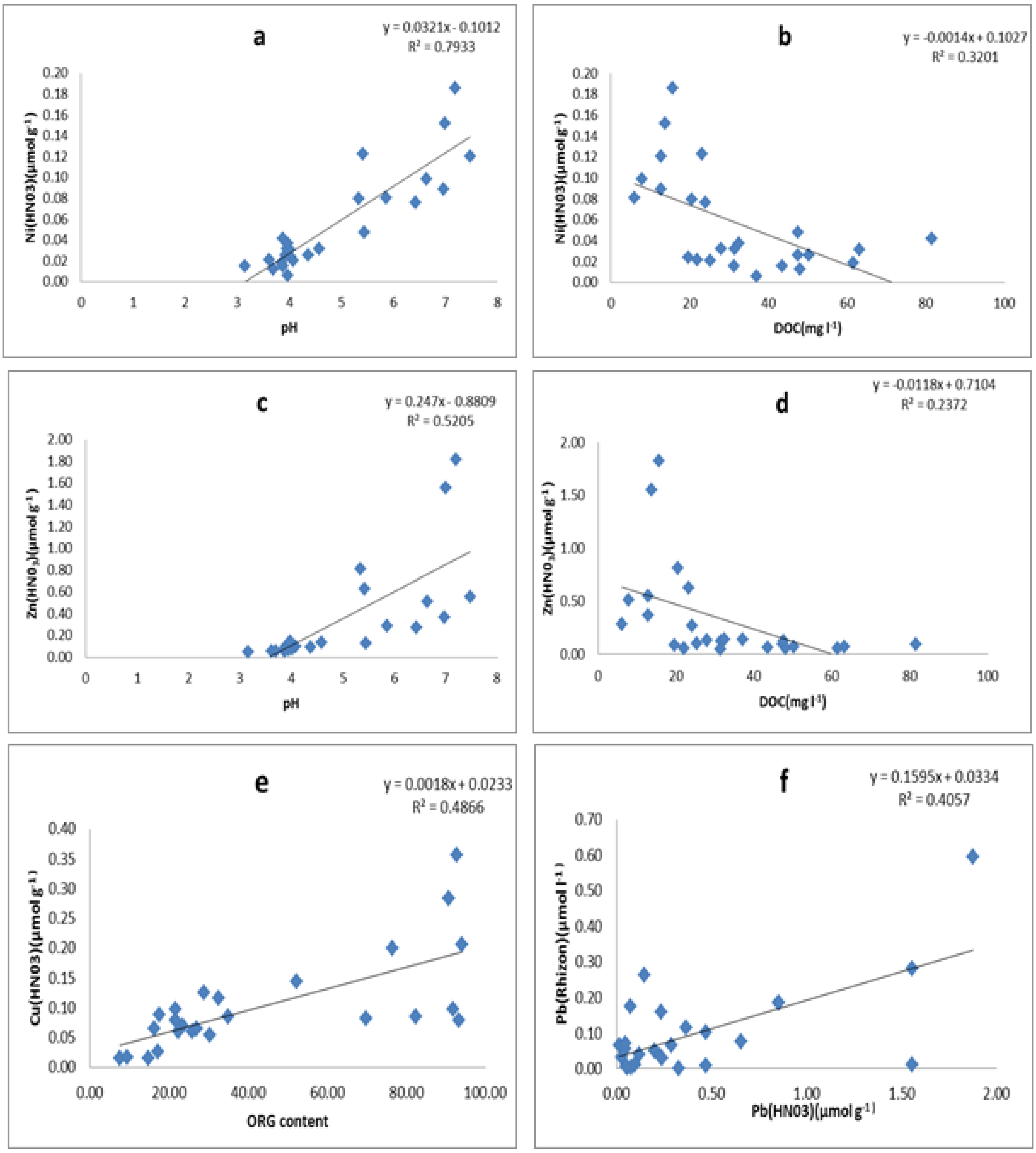


Fig. 2.3: Relationships between Ni (a,b) and Zn (c,d) concentrations in acid digest extracts and pore water pH and DOC, along with relationships between Cu concentrations in acid digest extracts and LOI (e), and Pb pore water concentrations and Pb concentrations in HNO_3 *extracts (f).*

However, interpretation of the relationships shown for metal concentrations in Table 2.7 is constrained by the high correlations between soil variables, as shown in Table 2.8. As C/N ratio highly significantly correlated with pH and LOI, it was excluded from the subsequent multiple regressions analysis. As expected, the two measures of pH were also highly correlated, but these were not included in the same multiple regression equation. Soil solution pH was significantly negatively correlated with pH, reflecting the suggested suppression of DOC production at high soil acidity, and both variables were significantly correlated with LOI. However, since all three are significant variables in the model of Tipping *et al.* (2003), they were included in subsequent multiple regression analysis.

These univariate relationships are shown in Figure 2.4 , which clearly demonstrate the strong and highly significant linear relationships between organic matter and Pb and Cu, and pH and Ni and Zn.

*Table 2.8: The correlation matrix for the measured soil and soil solution properties. Significance is indicated as:- * P<0.05; ** P<0.01; *** P<0.001.*

Variables	*pH(SS)*	*pH(NaCl)*	*DOC*	*LOI*	*C/N*
ph(SS)	—				
pH(NaCl)	0.94***	—			
DOC	-0.62**	-0.60**	—		
LOI	-0.41*	0.49*	0.42*	—	
C/N	-0.75**	-0.74**	0.64**	0.66**	—

2.3.4 Multiple regression analysis

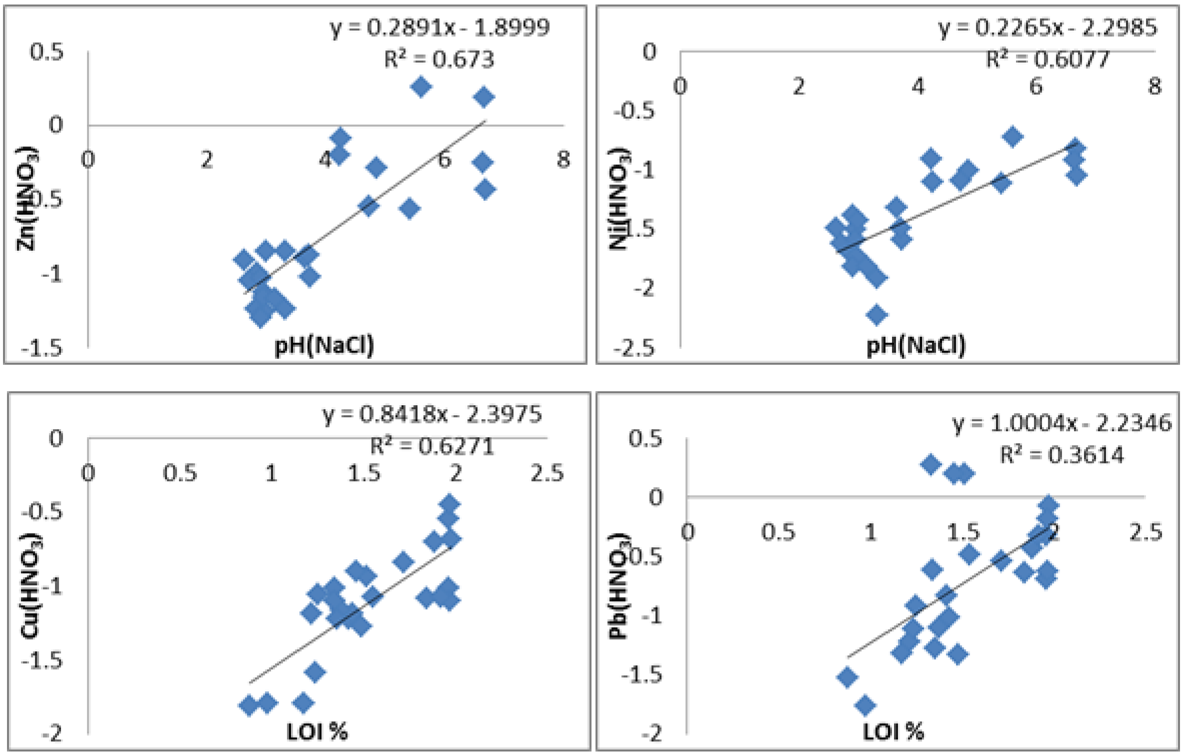


Fig. 2.4: Relationships between metal concentrations in HNO_3 extracts and LOI (for Cu and Pb) and pH (for Zn and Ni). All plotted values are log-transformed.

2.3.4 Multiple regression analysis

All the analyses described above were conducted with untransformed data. Since a key aim of the work reported in this chapter was to make a comparison with the predictive model of Tipping *et al.* (2003), all data except pH (as this is already expressed on a log scale) were log-transformed for multiple regression analysis. Firstly, a best fit model for prediction of HNO_3 extractable metal concentrations from other soil variables was determined, following a step-down procedure, using the formula

$$\log[M_{HNO3}] = a + b \cdot \log[LOI] + c \cdot pH[NaCl]$$

The results are shown in Table 2.9. For each metal, the equations only included one variable, possible reflecting the close correlations between soil variables. For Ni and Zn, a negative relationship with soil pH was derived, while for Cu and Pb, the relationship was a positive one with soil organic content. These univariate relationships are shown in Figure 2.4, which clearly demonstrate the strong and highly significant linear relationships between LOI and Pb and Cu, and pH and Ni and Zn. The lower value of R^2 for Pb than for the other three metals may be explained by three outlier sites with high metal concentrations but average values of LOI; these may possibly reflect local sources of Pb.

*Table 2.9: Multiple regression parameters for models of metal concentrations in HNO_3 extracts. Significance is indicated as:- * P<0.05; ** P<0.01; *** P<0.001*

µmol g^{-1}	constant	pH(NaCl)	log[LOI]%	F	P	R^2
log[Pb(H)]	-2.235	-	1.000***	13.0	<.001	.361
log[Zn(H)]	-1.900***	.289***	-	47.3	<.000	.673
Log[Ni(H)]	-2.298***	.226***	-	35.6	<.000	.608
log[Cu(H)]	-2.397***	-	.842***	38.7	<.000	.627

Table 2.10 shows the result of an analysis of the soil solution data to fit a multiple regression model of the form

$$\log(\text{rhizon}) = a + b \cdot \log[LOI] + c \cdot pH + d \cdot HNO3 + e \cdot \log[DOC]$$

as proposed by Tipping *et al* (2003). A step-down procedure was again used in model fitting.

*Table 2.10: Multiple regression parameters for models of metal concentrations in pore waters. Significance is indicated as:- * P<0.05; ** P<0.01; *** P<0.001*

µmol l^{-1}	constant	DOC(mg l^{-1})	pH(SS)	HNo_3 µmol g^{-1}	log[LOI]%	R^2	F	P
log[Pb]	2.800***	-	-.404***.	858***	-1.030***	.666	13.9	<.000
log[Zn]	1.187*	-	-	-	.477*	.179	5.0	<.003
log[Ni]	-1.353***	0.954***	-	0.393*	-0.513***	.507	7.2	<.002
Cu	.259*	-	-.037*	-	-	.152	4.1	<.05

For all four metals, the model fitted explained a significant proportion of the variation in metal concentrations in porewater. However, whereas the fitted regression equation explained two thirds of the variation in lead concentrations in porewater and half of the variation in nickel concentrations in porewater, it explained less than 20% of the porewater concentrations of Cu and Zn. A key reason for this difference is that the

HNO_3 extractable metal concentration was a significant term in the equations for Pb and Ni, as well as LOI, but it did not enter the equations for Zn and Cu, for which only organic content and porewater pH respectively were significant terms. Hence, for these two metals the regression model was not based on parameters such as DOC, pH and LOI modifying the relationship between HNO_3 metal concentrations and porewater metal concentrations.

Since Tipping *et al.* (2003) provided a version of the model that included coefficients for all variables. This analysis was therefore repeated to allow comparison with the results shown in Table 2.11

*Table 2.11: Multiple regression parameters for models of metal concentrations in pore water, including all variables. Significance is indicated as:- * P<0.05; ** P<0.01; *** P<0.001*

µmol l^{-1}	constant	DOC(mgl^{-1})	pH(SS)	HNO_3 µmol g^{-1}	log[LOI]%	R^2	F	P
log[Pb]	4.015 *	-.169	-.352	.817***	-.940*	0.687	6.6	<.001
log[Zn]	.593	.425	-.051	-.309	-.375	.322	1.4	.260
log[Ni]	-.165	.874*	-.052	.640*	-.611	.540	3.5	.01
log[Cu]	.710	.035	--.001	.066	-0.149	.255	1.0	.441

Adding additional terms improved the R^2 values, but the inclusions of redundant terms in the model decreased the values of F. The improvement in R^2 was small for Pb and Ni, for which the additional terms did not add much to an already good model, but was higher for Zn and Cu. However, for these two metals, neither the overall model nor any of the individual coefficients was significant. Further discussion of this multiple regression model, when compared to that of Tipping et al. (2003), is provided below.

2.4. Discussion

2.4.1 Direct comparison with Tipping et al. (2003)

One specific objective of this analysis was to test whether the multiple regression model proposed by Tipping *et al.* (2003) could reliably be applied to predict pore water concentrations sampled in the field. Tipping *et al.* (2003) took soil samples in the field and then extracted soil solution under uniform controlled conditions in the laboratory, and focussed on samples collected from five study areas – Lake District, Yorkshire Dales, Peak District, North Wales and Dartmoor. All these are upland sites with high rainfall, but cover a wider range of climatic conditions would be found across the sites in this study.

Table 2.12 provides a comparison of the multiple regression equations derived in this study with those derived by Tipping *et al.* (2003). No comparison was possible for Ni, for which Tipping *et al.* (2003) did not provide data. As noted in the results section, this is not necessarily an optimal model, because of the high degree of correlation between soil variables, but all variables were allowed to enter the model in order to make a direct comparison with the results of Tipping et al. (2003). The intercept values in Table 2.12 were reduced by 3 from the values in Table 2.11 to allow a direct comparison, because of the different units used. No values of Ni are included, as Tipping et al. (2003) did not include Ni.

Overall, the regression equations provided a better fit (higher R^2 values) in both studies for Pb than for Zn and Cu. Although higher R^2 values overall were reported by Tipping *et al.* (2003), their analysis was based on a greater number of sites. In both studies, there were consistent negative relationships with pH, i.e. metal levels increased in pore water with increasing acidity, although these relationships were very weak in both studies in the case of Cu. There were also consistent negative relationships in both studies with LOI, reflecting the effect of metals binding to the organic matrix in the soil.

However, in the case of the other two parameters, the relationships were rather different in the two studies. Both studies showed a positive relationship between Pb concentrations in porewater and in the HNO_3 extracts, but, while Tipping et al. (2003) showed similar strong relationships for Zn and Cu, this was not the case in this study, and indeed a negative coefficient was found for Zn. For DOC, the positive relationships with porewater concentrations reported by Tipping *et al.* (2003) were only found for Zn in this study, with a negative coefficient being found for Pb, and a small coefficient for Cu, despite the reported affinity of these metals for dissolved organic matter.

Table 2.12: Multiple regression parameters for models of metal concentrations in pore water, including all variable from this study and from Tipping et al. (2003).

(a) This study

µmol l^{-1}	constant	DOC(mgl^{-1})	pH(SS)	HNO_3 µmol g^{-1}	LOI %	r2
Pb	1.01	-.169	-0.35	0.82	-0.94	0.69
Zn	-2.41	.425	-0.05	-0.31	-0.37	0.32
Cu	-2.29	.035	--0.001	0.07	-0.15	0.25

(b) Tipping et al (2003)

µmol l^{-1}	constant	DOC(mgl^{-1})	pH(SS)	HNO_3 µmol g^{-1}	LOI %	r2
Pb	-1.23	0.79	-.0.20	0..89	-.0.47	0.81
Zn	-1.11	0.39	-0.18	0.60	-0.54	0.57
Cu	-4.50	0.51	--0.03	0.38	-0.61	0.39

However, any comparison of regression models fitted in different studies, especially those using different sites, and different methods of extracting soil solution, needs to take account of the range of values of the different parameters in the datasets. Table 2.13 presents a summary of the range of the different soil and porewater parameters measured in this study and that of Tipping *et al.* (2003). Note that the values listed for Tipping *et al.* (2003) were estimated from Fig 2 of their paper, and are only rough indications of the range of values. No values for Ni were reported by Tipping *et al.* (2003), and so no comparison is possible.

Table 2.13: Comparison of the range of values of different soil and soil solution parameters in this study and as reported by Tipping et al. (2003)

Parameter	Range of values (this study)	Estimated range of values (Tipping *et al.*, 2003)
pH	3.05-7.56	3.4-8.0
LOI (%)	6.2-95.1	10-98
DOC (mg l^{-1})	5.1-95.8	0-500
HNO_3 Pb (µmol g^{-1})	0.01-1.91	0.05-50
HNO_3 Zn (µmol g^{-1})	0.01-1.56	0.02-50
HNO_3 Cu (µmol g^{-1})	0.01-0.48	0.02-1
Porewater Pb (µmol l^{-1})	0-1.48	0.001-50
Porewater Zn (µmol l^{-1})	0.5-19.0	0.1-10
Porewater Cu (µmol l^{-1})	0.55-2.33	0.03-5

The range of values of pH and LOI is comparable in the two datasets. However, those of DOC are quite different, with the maximum values recorded by Tipping *et al.* (2003) being five times higher than in this study. The range of values reported by Tipping *et al.* (2003) is clearly unrealistic, compared to the reported range; for example a range of 1.6-195 mg l^{-1} was reported by van den Berg *at al.* (2012), who collected DOC samples from 41 UK upland sites. As Tipping *et al.* (2003) themselves admit, this probably reflects disturbance to the soils resulting from the artificial method of extracting soil solution. The greater, but artificial, range in the study of Tipping et al. (2003) means that stronger effects of DOC on soil solution metal concentrations are likely to be found, as is clearly the case from the comparison of regression models in Table 2.12.

A further important difference is the range of metal concentrations in the collected samples. For lead and zinc, in particular, much higher ranges and maximum values were found in the HNO_3 extracts of Tipping *et al.* (2003) than in this study. While sample sites in this study were selected at random from a list of sites for which access and security could be ensured over the year of study, it is likely that Tipping *et* *al.* (2003) specifically targeted areas of high contamination from historic mining activities. These areas however, are not typical of the wider UK countryside. The wider range of metal concentrations is likely to increase the chance of finding strong correlations between metal concentrations in soil and porewater; as shown in Table 2.12, the positive effect of HNO_3 extract metal concentrations was much stronger in the multiple regression model of Tipping *et al.* (2003) than in this study. This leads to the conclusion that the predictive equations proposed by Tipping *et al.* (2003) may not be applicable to most field areas, where the range of DOC and metal concentrations is much lower than in their study.

2.4.2 Controls on pore water metal concentrations

Metal concentrations in the HNO_3 extracts showed a clear effect of soil type, for all metals except Pb. These concentrations were also very well explained by a single soil variable – pH in the case of Ni and Zn, and LOI in the case of Pb and Cu. Ni and Zn are bound fairly weekly and are sensitive more to changes in pH, compared to Cu and Pb, which are held strongly by soil. A similar conclusion for Pb was reached by Bergkvist (2001) in field observations of Swedish forest soils. Thums *et al.* (2008) reported the partitioning of Pb into soil solution increases as pH decreased. This significant relationship implies that in more acidic soils have high quantity of exchangeable metals. Thums *et al.* (2008) also reported the increased partitioning of Zn in alkaline soils is dependent on the expense of other cations.

However, the pore water concentrations showed less variation between soil types, and in no case was there a single correlated variable, except for the positive relationship between Pb concentrations in porewater and in the HNO_3 extracts. For Ni and Pb, for which the multiple regression equations included a significant relationship with metal concentrations in the HNO_3 extracts, there was also a negative relationship with LOI in the model. This suggests that the extent to which metals are partitioned into soil solution is lower on soils with a high LOI and high organic content. This is consistent with the binding of these metals to organic matter in soils. Thums *et al.,* (2008) observed that the absorption of Ni and Cu increased when organic carbon content (estimated by LOI) of soil increased. It is concluded that the organic phase palyed an important role for Ni, Cu and Zn. For Pb, the negative coefficient with pH reflects the greater partitioning of metal into soil solution as acidity increases. This was also reported by Reddy *et al.* (1995) who found that low pH was associated with an increase in Pb solubility in soil waters, and that at low pH the organic Pb complexes may become more soluble and hence become available to plants. However at higher pH, Pb forms insoluble Pb complexes even if the organic content is high; if the organic content is low and pH is higher at 6-8, Pb forms Pb phosphate and Pb oxide precipitates and become unavailable to plants (Chaney & Rayan, 1994; Chaney *et al*., 1997).

For Ni, the positive coefficients with DOC indicate a competition between binding with soil organic matter and DOC – at greater DOC concentrations, more Ni is found in soil solution. Ni was also strongly bound to organic matter in the results observed by Meadows & Watmough (2012) in a soil survey conducted in 415 sites in Canada. However, it is important to note that soil solution DOC and pH were themselves negatively correlated, making it difficult to be sure about causal mechanisms.

In the case of Zn and Cu, no significant multiple regression model could be found, possibly because of the competing effects of pH, DOC and LOI on both soil and soil solution concentrations, and the correlations between the possible causal variables in the dataset. This highlights the limitations of deriving empirical regression models from field survey data. For this reason experimental approaches, where specific factors can be manipulated, may help to better understand the underlying causal mechanisms. Furthermore, predictive equations such as that of Tipping *et al.* (2003) may not be appropriate for prediction of the effects of changing environmental conditions at a particular site, for example the effects of atmospheric nitrogen deposition (Findlay 2005), and elevated CO_2 levels and temperature (Freeman *et al.* 2001). For these reasons, the rest of this thesis, as described in Chapters 3 and 4, focuses on experimental approaches to assessing controls on pore water concentrations of metals, and the effects of temperature and nitrogen deposition in particular.

Chapter 3 – Temperature effects on DOC, pH and elemental release from a metal contaminated soil

3.1 Introduction

In UK for several years, Upland sites have been contaminated by the atmospheric deposition of metals (Shotyk, 2002), they are mainly derived from industry emissions (Smith *et al.*, 2005; Tipping *et al.*, 2006). According to (Damman, 1978; Pakarinen *et al.*, 1983; Gignac & Beckett, 1986; Glooschenko & Arafat, 1988; Glooschenko, 1989), most of the Peatlands has elevated heavy metals deposition as compare to mineral soils. Toxic metals, eg Pb and Cu, are found in the top layers of peats around the UK (Mighall *et al.*, 2002; Tipping *et al.*, 2003; Farmer *et al.*, 2005; Rothwell *et al.*, 2005; Smith *et al.*, 2005). “The blanket peats in the southern Pennines, are situated in the core of the 19th century English Industrial Revolution, between the cities of Manchester and Sheffield. So, very high concentrations many toxic heavy metals, are deposited in the upper peat layer in this region” (Jones, 1985; Markert & Thornton, 1990; Jones & Hao, 1993; Hutchinson, 1995).

For this reason, peat soils are of concern due to presence of deposited metals transported to receiving waters, and have the effects that such pollutants are quite alarming (Lawlor & Tipping, 2003; Tipping *et al.*, 2003b; Tipping *et al.*, 2006; Vinogradoff *et al.*, 2005; Graham *et al.*, 2006). Lucassen *et al*.(2002) and Tipping *et al*.(2003) have confirmed that “heavy metals stored in the upper layer of peatlands can be remobilize at low pH, such conditions in drainage can result in high dissolved heavy metal concentrations in surface waters draining heavy metal-contaminated peatland catchments, with likely unfavourable effects on aquatic entities.”

However according to Salomons & Forstner,(1984) “Range of physical, chemical and biological conditions are effecting the transport of metals”. Increase in concentrations of complexing agents and H^+ ions in solution can favour to the release of metals from soils into solution (Franchi & Davis, 1997). As Catchments surrounded by blanket peat are at high risk due to their carbon-rich nature in the form of DOC.(Worrall *et al.*, 2002, which are major complexing agents that control the behaviour of many metals (Gao *et al.*, 1999). “Thus the role of DOC in mobilising metals has been reported in a variety of circumstances, including snowmelt events in the Alaskan Artic” (Rember & Trefry, 2004), contaminated wetlands in Germany

(Kalbitz & Wennrich, 1998), and in mid-western Finland catchments affected by acid sulphate soils (Astrom & Corin, 2000).

Increases in temperature affect soil biota, in general resulting in increase in microbial and enzymatic activity up to a certain threshold temperature. However decrease in pH and attachment to dissolved organic matter is also dependent on soil temperature and has been linked with the release of trace elements from soils. In a recent study, Richards & Kump (2003) suggested that temperature affects the release of solutes from soils in two ways; first directly, by modifying equilibrium constants of the mineral present, and second, indirectly, by influencing the level of biological activity. Temperature will therefore affect both the volume of water withdrawn from a soil and its chemical composition.

Olivie-Lauquet *et al.* (2001) also emphasized the soil microbial activity, as it controls the release of metals from soil into water, with the rise in metal concentrations in summer months. A large number of studies have observed an increase in DOC concentration in water with rising temperature (e.g. Kalbitz *et al*., 2000; Evans *et al*., 2005). In addition, seasonal DOC variations are also closely related to temperature in many experiments (e.g. Scott *et al*., 1998; Clark *et al*., 2005; Billett *et al*., 2006), and the DOC model developed by Lumsdon *et al*. (2005) aslo required a temperature-dependent term describing biological activity,

Therefore warming can lead to shifts in both pH and DOC and hence influence metal mobility. Metal behaviour in peat has been studied but uncertainties lies to metal behaviour in these soils, In particular, although there is a potential for temperature to change soil solution chemistry and metal leaching, few experiments have actually studied the effects of warming on metal release.

3.1.1 Aims and objectives

Given this context, the overall aim of this experiment was to investigate the effect of temperature on release of metals from an organic-matter rich, metal contaminated soil. This was achieved by taking cores from the field and subjecting them to different temperature regimes. The specific objectives were as follows:-

- to determine the effect of temperature over time on soil solution concentrations of metals;
- to determine the effect of temperature over time on soil solution pH, and concentrations of DOC and inorganic ions;
- to determine if the changes in metal concentrations in soil solution in response to temperature over time were related to changes in pH and DOC; and
- to determine if soil concentrations at the end of the experiment had been modified by temperature.

Based on the results of the survey data analysis (Chapter 2), the Holme Moss and Featherbed Moss sites were selected for study because they showed elevated concentrations of metals and high concentrations of DOC. Intact cores were sampled from Featherbed Moss and Holme Moss, and divided into pots which were assigned to heated and unheated green houses at York, after an initial acclimation at an ambient temperature. The study specifically concentrated on changes in soil solution and drainage water concentrations of DOC, and pH, NH_4^+ and NO_3^-, as well as the concentrations of metals (Ni, Cu, Cd, Pb and Zn).

3.2 Materials and methods

3.2.1 Sampling sites and soil sampling

Featherbed Moss and Holme Moss are both located at 520 m above sea level in the Southern Pennines of the U.K.; the National Grid References of the sampled sites are SK090921 and SE090042 respectively. On 2nd March 2010, peat samples were collected from the two sites. In order to establish the experiment, six intact cores were taken from each site at a depth of 0-10 cm. This depth was chosen, as Pb concentration is higher in the upper layers of the peat profile (Jones & Hao, 1993) (Rothwell *et al.*, 2007). The intact cores were transferred into rectangular boxes of dimensions 15 cm deep and 20 cm long, and transported to York.

At the same time, in order to determine initial soil characteristics, two soil samples were taken at each site to a depth of 8–10 cm with an augur (diameter *2*.50 cm). Five sub-samples per plot were pooled to create one sample (approx: 200 g fresh weight in total). The soil samples were transported in a cool box to the lab and stored at 4^{o}C until analysis. Soils were stored in airtight plastic bags, from which as much air as possible was removed. Soils were homogenized by hand, well mixed to avoid internal variation between sub samples, and roots and shoots were removed. The samples were thoroughly mixed and sieved (4 mm mesh) prior to analysis.

3.2.2 Experimental design

On arrival in York on 2nd March, the 12 intact cores of soil from both sites were placed in 15*20 cm plastic pots, and were sealed with aluminium foil on the top, to prevent evapo-transpiration and to prevent uncontrolled water addition. Drainage holes were made at 2 cm from the bottom to keep the soil moist, and to collect the drainage water. Soil pore water was sampled by installing Rhizon samplers (Eijkelkamp, Agrisearch Equipment, Netherlands) 5 cm from the bottom of each core. The Rhizon sampler was coupled to a syringe, which was pulled out to create a vacuum. More details of this sampling method are provided in Chapter 2. Sampling was done on a weekly basis for soil pore water. In addition, at the same time, drainage water which had leached down into the tray placed at the bottom of the pots was collected. Sampling was continued for a period of twelve weeks.

The experiment was conducted in 3 replicates, for each site and treatment, with a total of 2 x 3 x 2 = 12 experimental pots. The 6 cores from each site were randomly assigned to one of the two treatments. After the sample was transferred to the pot and compacted, sufficient water was added to bring the soil moisture content to saturation. The cores were all were kept outside in the walled garden of the University of York to establish equilibrium for first two weeks.

After two weeks (on 16th March 2010), the cores were transferred to their pre-assigned temperature treatment. Three cores from each site were placed in a heated glasshouse with a target temperature of 20-30^{o}C. The other three cores were placed in an unheated glass house. Throughout the experiment, the soil moisture content was kept high by adding 200 ml of deionised water to all the cores every day. Air temperature was observed on a daily basis in each glasshouse using max-min thermometers.

After twelve weeks (on 2nd June 2010), each core was divided and cut into three 5cm portions (the lower, middle and upper soil layers) to get soil samples. Soils were homogenized by hand, well mixed to avoid internal variation between sub samples, and roots and shoots were removed before analysis, as described below. These samples were extracted as described below, and stored in refrigerator before further analysis,.

3.2.3 Soil extractions

Acid digests of the pooled soil core samples from each depth were carried out as described in Chapter 2. Water and KCl extractions were also performed, as follows. Pooled core samples were homogenized and approximately 35 g of the homogenized peat soil was weighed, and then shaken with either 100 ml deionized water or 100 ml 0.5 M KCl in a 310 ml glass flasks, sealed with aluminium caps. Shaking was done for 1 h at 100 rpm on a Gallenkamp orbital shaker. Samples were then filtered through 0.45 µm Whatman filters and stored in the refrigerator (4^{o}C) until further analysis.

3.2.4 Analytical methods for soils

Approximately 10 g duplicate sub-samples of each field moist soil were weighed into pre-weighed, dried aluminium dishes, oven dried over night at 105^{o}C, cooled in desiccators, and reweighed to determine the moisture content from the loss of mass, expressed on an oven-dry weight basis. The organic content of the soils sampled was determined as percentage loss on ignition (LOI) after heating the soils for 5 hours at 550 ^{o}C.

The oven-dried soil residues obtained were individually finely ground with a Retsch ball mill for 3 minutes at 25 Hz and used for the measurement of soil C%, N% and C:N mass ratio on an Elementar Vario Macro C and N analyzer calibrated with glutamic acid. The steel grinding balls and containers were carefully cleaned with tissue and a brush between each sample. To minimize any possible effect of carryover of C and N between samples, samples were ground in depth sequence when soils from whole profiles were being studied. At the end of each run, the Vario Macro analyser uses data from glutamic acid check standards, usually run after every 8 samples, to compensate for small amounts of instrument response drift.

The pH of soil was measured in both pore water and extracted soil samples. The soil pH was determined after mixing fresh soil with deionised water (1:2 w:w) and shaking for 1 h at 100 rpm. The pH meter used (MP220 BasicpH/mV/°C Meter, Mettler Toledo International Inc.) was pre-calibrated with commercial standard buffer solutions at pH 4.0, 7.0 and 10.1. The pH 7 buffer was used after every 8-10 samples to confirm instrumental stability.

3.2.5 Analytical methods for soil solution

Weekly soil solution samples were analysed for dissolved organic carbon concentration using a TOC elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Samples were analyzed as soon as possible after collection. Samples to be analysed for DOC were first filtered through 0.45 µm Whatmann filters. From a basic stock solution of 500 mg l^{-1} TIC + 500 mg l^{-1} TOC (using sodium carbonate as the

TIC standard and potassium phthalate as the TOC standard), intermediate stock solutions were produced for calibration. Calibration was done at concentrations of 1, 2. 5, 10, 20 and 50 mg l^{-1} DOC.

The extracts and pore water were stored at 4°C until analysis for ammonium-N and nitrate-N using a standard Auto Analyser (Bran+Luebbe Auto Analyser 3 digital colorimeter) protocol with matrix-matched standards, as soon as possible (generally the next day) after extraction. Ammonium nitrate standards for KCl (for KCl extracts of soils) and H_2O (for pore water and water extracts of soil) at 2 ppm, 1.5 ppm, 1.0 ppm, 0.5 ppm and 0 ppm were made in a 100 ml volumetric flask. The samples were then run through the Auto-Analyser.

3.2.6 Metal analysis

All the pore water and soil samples were than stored in the refrigerator at 4°C until they were analysed .The samples were acidified before storing, with 100 µl of 70% HNO_3 added to every 10 ml of sample to prevent metals from settling. All the samples were analysed using the methods described in Chapter 2.

3.2.7 Statistical analysis

Since the experiment follows a full factorial design with 4 replicates, GLM statistics used to analyse the results. Effects temperature on metal concentration, DOC and pH were tested with General Linear Model (GLM) procedures. Tukey's student range tests were used to identify differences between treatments. Bivariate correlation analysis was used to determine relationships between variables in soil solution and leachate, based both on the mean data determined in each week, and the values for all the individual cores on all measurement dates. An analysis of variance was carried out to assess the effects of temperature on soil concentrations at the end of the experiment. Tukey's student range tests were used to identify differences between treatments. All analyses were carried out in SPSS 19.

3.3 Results

3.3.1 Initial soil properties

Table 3.1 summarizes the properties of the soils taken from the two sample sites, Holme Moss and Featherbed Moss.

Table 3.1: The physical and chemical properties of soil used from Featherbed (F) and Holme Moss (H). 1 and 2 represent separate samples

Parameter	F1	F2	Mean	H1	H2	mean
pH (water)	3.69	3.58	3.64	3.66	3.59	3.63
Moisture content (%)	81.1	84.3	82.7	78.9	74.0	76.4
C (%)	43.1	51.4	47.3	43.5	42.0	42.8
N (%)	1.60	1.81	1.71	1.71	1.65	1.68
C/N ratio	26.9	28.4	27.6	25.5	25.4	25.4
LOI (%)	74.6	73.1	73.8	74.6	77.0	75.8
H_2O-extractable NH_4^+-N (µg $g^{-1)}$	0.86	1.43	1.14	1.00	1.77	1.37
H_2O-extractable NO_3^--N (µg g^{-1})	1.14	0.69	0.91	1.20	0.89	1.03

The pH varied little between the samples, ranging from 3.58 to 3.66. Soils at Featherbed Moss had higher moisture contents than soils at Holme Moss, with mean values of 82.7% and 76.4% respectively. There were high amounts of organic material at both sites, with all values of LOI exceeding 70%. The mean water extractable ammonium was higher at Holme Moss than at Featherbed Moss, with values of 1.37 and 1.14 µg g^{-1} respectively. The extractable nitrate concentration was also slightly higher at Holme Moss. The mean C:N was similar at the two sites.

Table 3.2 shows that the metal concentrations, based on an acid digestion, were similar at Featherbed and Holme Moss. For all five metals, the concentrations at site H1 were lower than those at site H2, and this led to slightly lower mean concentrations at Holme Moss than at Featherbed Moss. The overall ratio of metal concentrations at the two sites was similar, with concentrations ranked in the order Pb>Cu>Zn>Ni>Cd.

Table 3.2: Metal concentration (µg kg^{-1}) of samples from the two sites based on HNO_3 digests. Sites and samples are indicated as in Table 1.

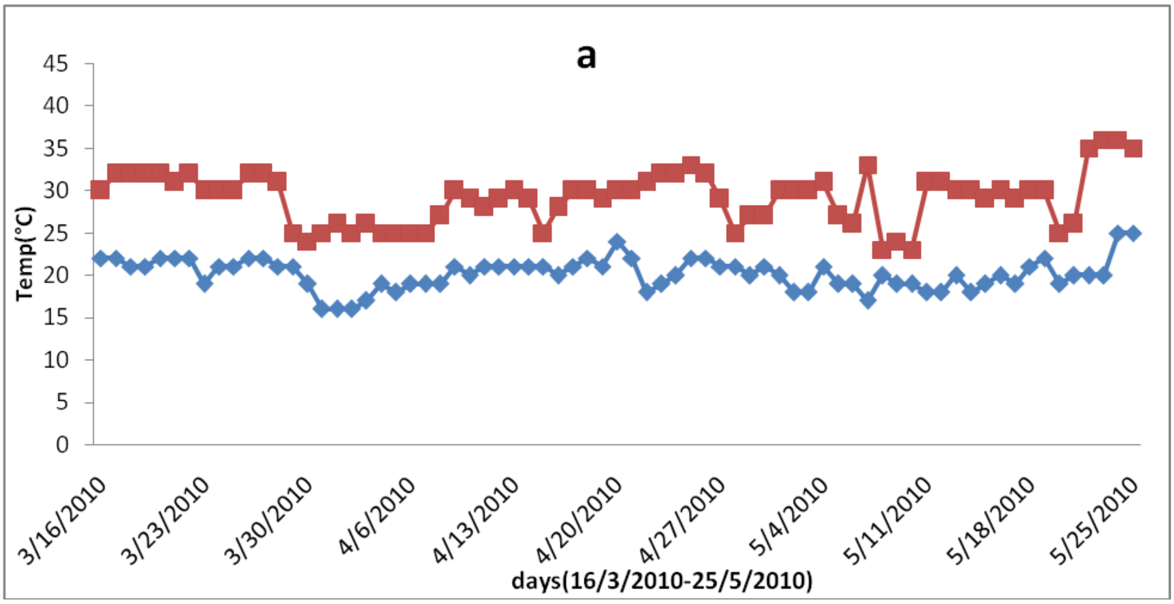
Metal	H1	H2	mean	F1	F2	mean
Ni	25.49	29.76	27.63	24.84	27.33	26.09
Pb	1390	1839	1614.5	1550	1565	1557.5
Cu	177.3	196.2	186.75	166.8	158.1	162.45
Zn	57	87.8	72.4	89.49	107.2	98.35
Cd	0.94	1.93	1.44	1.8	1.79	1.79

3.3.2 Temperature variations during the experiment

The experiment started on 2nd March 2010. From 16th March 2010, the cores were placed in two different glasshouses, one of which was heated to prevent the temperature falling below 20°C at night and below 30°C in the day, and the other of which was unheated. The temperature in both glasshouses fluctuated rapidly because it was dependent on the external weather changes. Table 3.3 summarises the mean weekly air temperatures in each glasshouse, while Figure 3.1 summarises the maximum and minimum temperatures that were recorded in both glasshouses daily.

Table 3.3: Mean air temperature (°C) in heated and unheated glasshouses, for the period of week 3 to week 12 (16th March -25th May 2010). The mean is the average of the daily maximum and minimum air temperatures.

	Heated	Unheated
Week 3	26.6	16.6
Week 4	25.5	15.4
Week 5	21.2	10.9
Week 6	23.8	13.1
Week 7	24.9	14.4
Week 8	26.2	15.7
Week 9	24.1	13.7
Week 10	22.9	11.8
Week 11	24.4	12.3
Week 12	26.6	20.1



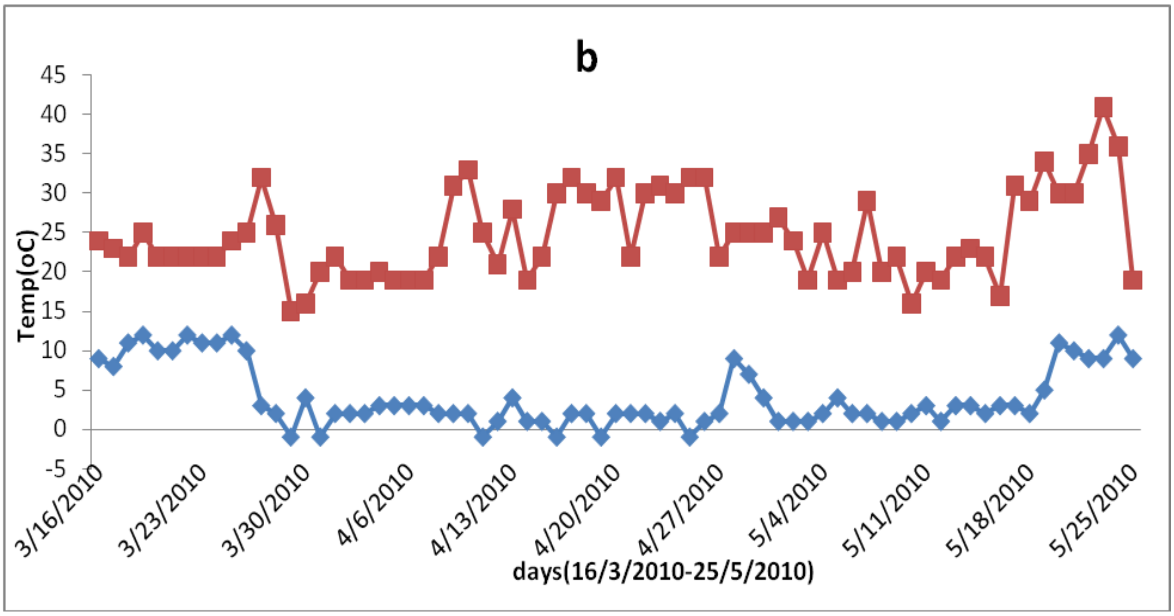


Fig 3.1: Changes in maximum and minimum daily air temperatures in (a) heated and (b) unheated glasshouses over the period from Week 3 to 12.

The temperature changes during the experiment in the unheated glasshouse were more dependent on the changes in external climate. During the two weeks in the second half of March (Weeks 3 and 4), overall mean temperatures in the unheated glasshouse were 16.6^{o}C and 15.4^{o}C, compared to values in the heated glasshouse of 26.6^{o}C and 25.5^{o}C. A maximum temperature of 32.0 °C was recorded in the unheated glasshouse on 23rd March. However, temperatures in the unheated glasshouse fell sharply overnight; in mid-March, the temperatures only fell to about 10°C overnight, but a minimum temperature of -1 °C was recorded at the end of March (Figure 3).

A maximum temperature of 33 °C was recorded in the first three weeks of April (Weeks 5, 6, and 7). Minimum temperatures of between -1 and 2 °C were recorded in the early weeks of the month but the minimum temperature in the unheated glasshouse increased to 7°C briefly in the last week of the month. The low temperatures overnight led to lower mean temperatures in the unheated glasshouse, with mean weekly temperatures between 11°C and 16°C in weeks 5 to 8, compared to 21-27°C in the heated glasshouse.

In May mean weekly temperatures in the unheated glasshouse were low (between 12 and 14°C) in weeks 9-11, but recovered at the end of the month, in a transition to warmer weather, to a mean temperature of 20°C. This compared to an average weekly temperature of 22-27°C during weeks 9-12 in the heated glasshouse.

Overall, the mean, maximum and minimum temperatures showed more variation, as well as lower absolute values, in the unheated glasshouse. In the unheated glasshouse, the mean weekly temperature ranged between 10.9-20.1°C, compared with 21.2-26.6°C in the heated glasshouse. The daily minimum temperature range in the unheated glasshouse was also high, from -1 to 12°C. This reflects changes in weather outside the glasshouse, and especially in the degree of cooling at night. Minimum temperatures were higher at the start and end of the experiment and lower in the middle period. In contrast, the daily minimum temperatures in the heated glasshouse never fell below 16°C and were in the range 16-25°C throughout the experiment. The maximum daily temperature in the unheated glasshouse was similar to that in the heated glasshouse, but the maximum temperature in the heated glasshouse had a more limited range (24°C to 36°C) and never fell below 21°C. In contrast, the range in the unheated glasshouse was much greater (16-42°C), with the highest values on hot days at the end of the experiment.

3.3.3 Changes in soil water chemistry over time

Figure 3.2 shows the changes over the 12 weeks in the mean DOC concentrations for the two sites and glasshouses. Cores from both sites showed a large variation in weekly mean DOC concentrations over the 12 weeks. For Holme Moss, values ranged in the heated glasshouse from 19 to 132 mg l^{-1}, while in the unheated glasshouse they ranged from 21 to 308 mg l^{-1}. For Featherbed Moss, values in the heated glasshouse ranged between 14 and 148 mg l^{-1}, and in the unheated glasshouse between 13 and 207 mg l^{-1}. Both sites and treatments showed an increase in DOC concentrations in the first four weeks, and in weeks 11 and 12. The concentrations were steady, or decreased, between weeks 4 and 10. Only in the last 2-3 weeks was there a clear effect of treatment, with higher DOC concentrations found in the unheated glasshouse in cores from both sites.

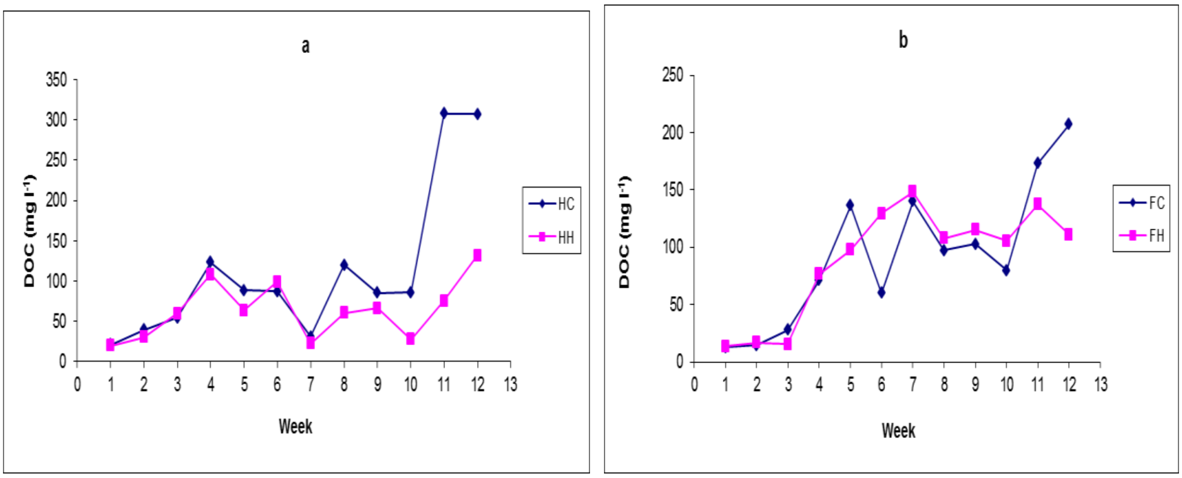


Fig. 3.2: Change in mean weekly DOC concentration (mg l^{-1}) over the experiment in cores taken from (a) Holme Moss and (b) Featherbed Moss. C indicates unheated glasshouse, H indicates heated glasshouse.

The pH of the soil solutions remained relatively unchanged, until week 8 (Fig. 3.3). There was a peak in weeks 9-10 and then a fall in pH until week 12. This coincided with the increase in temperature in week 11 and week 12, and the increase in DOC, at the end of experiment. In Featherbed Moss cores, there was little difference in pH between the two glasshouses. However, in Holme Moss cores, pH in the heated glasshouse increased from week 5, gradually diverging from the pH of unheated cores, and reaching a peak of pH 4.6, compared to pH 3.8, in week 10.

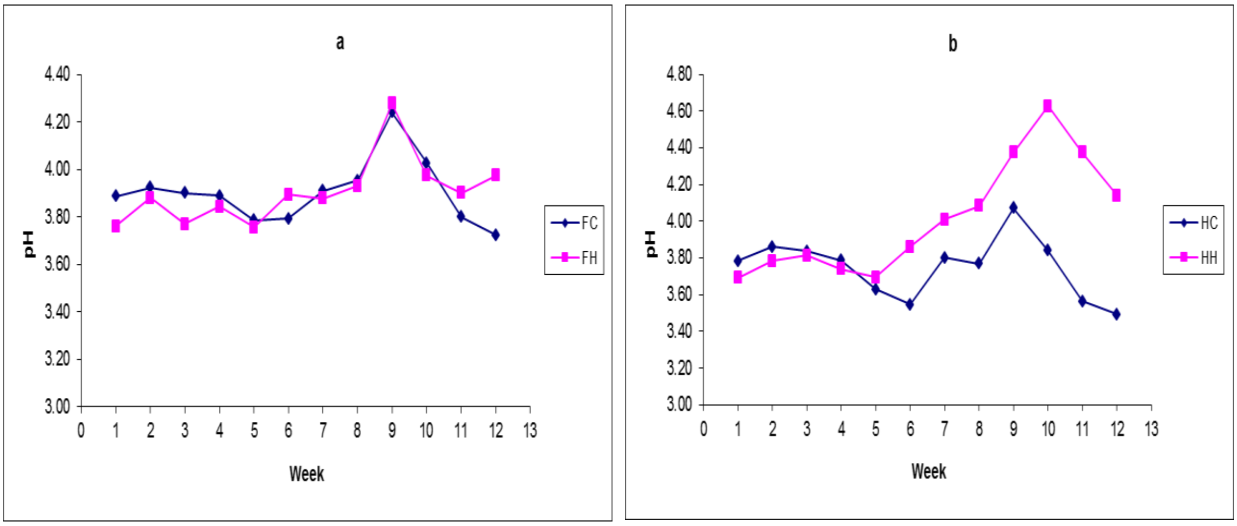


Fig. 3.3: Change in pH over the experiment in cores taken from (a) Holme Moss and (b) Featherbed Moss. C indicates unheated glasshouse, H indicates heated glasshouse.

There was a gradual increase over time in NH_4^+-N concentrations for both sites and treatments (Fig. 3.4). As for pH, the values tended to peak around week 10, and then tended to decline in weeks 11 and 12. There was no obvious difference in values in heated and unheated cores from Holme Moss, but from Week 7, the NH_4^+-N concentrations in the heated cores from Featherbed Moss clearly began to increase faster than in the unheated cores.

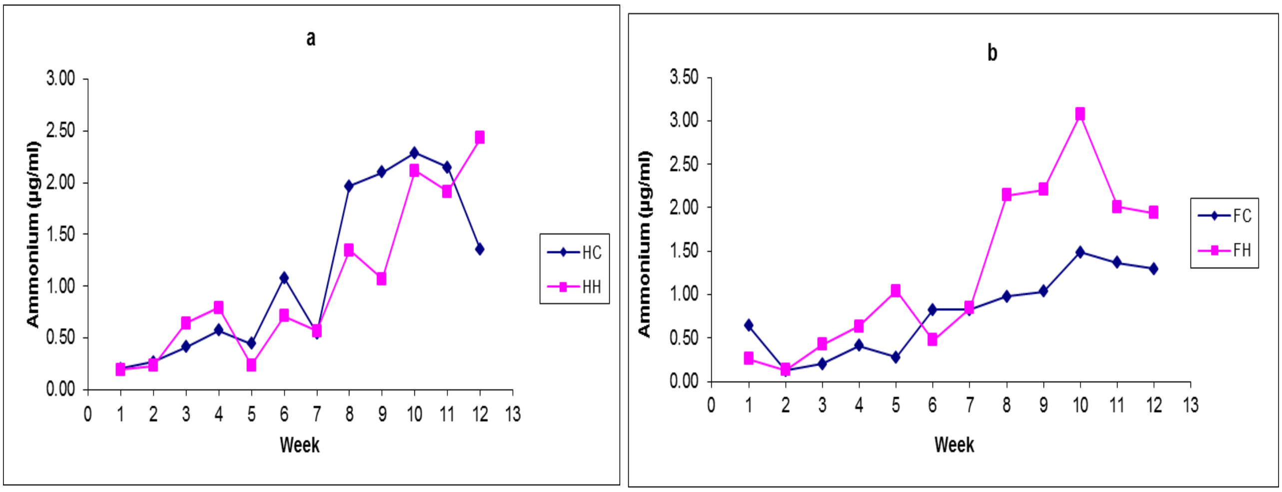


Fig. 3.4: Change in ammonium-N concentrations (µg ml^{-1}) over the experiment in cores taken from (a) Holme Moss and (b) Featherbed Moss. C indicates unheated glasshouse, H indicates heated glasshouse.

Nitrate concentrations showed a rapid decrease from high concentration in weeks 1 and 2, when the cores were outside, and before the experimental treatments started (Fig 3.5). After week 2, similarly low concentrations were recorded in all weeks and treatments.

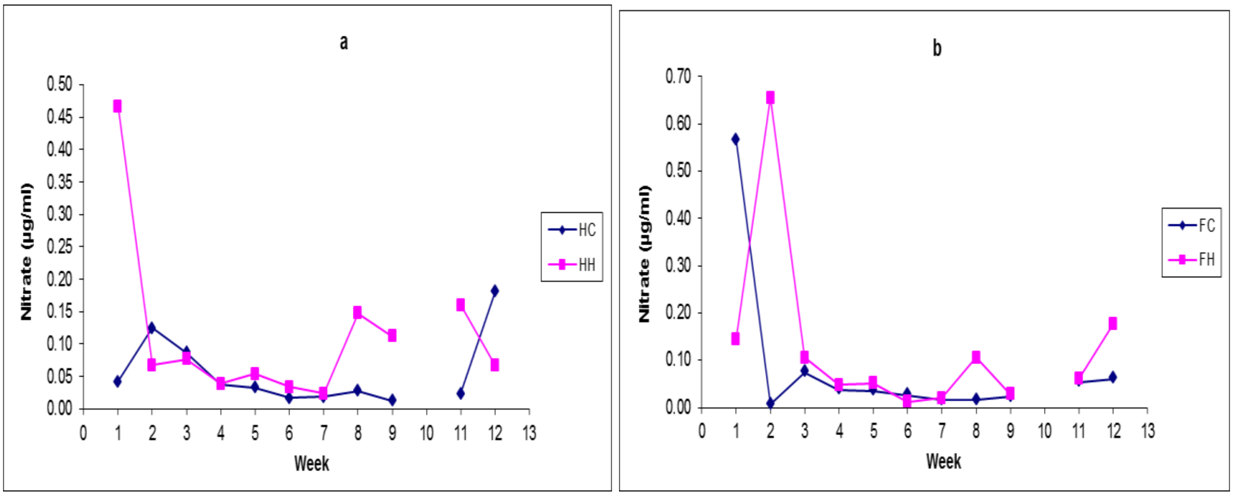


Fig: 3.5: Change in nitrate-N concentration (µg ml^{-1}) over the experiment in cores taken from (a) Holme Moss (H) and (b) Featherbed Moss. C indicates unheated glasshouse, H indicates heated glasshouse.

3.3.4 Changes in metal concentrations over time

Pore water concentrations of four metals from the different cores and treatments over the course of the experiment are presented in Figures 3.6-3.9. Concentrations of Cd were often close to instrumental detection limit and quite uncertain, so no further data analysis was undertaken for this metal. Little difference in Ni concentrations between treatments over time was evident in the Featherbed Moss cores (Fig. 3.6), but in the cores from Holme Moss, the unheated treatment cores, but not the heated treatment cores, showed a small but steadily increase in concentration towards the end of experiment. By week 12, concentrations of Ni were three times higher in the unheated cores that in the heated Holme Moss cores.

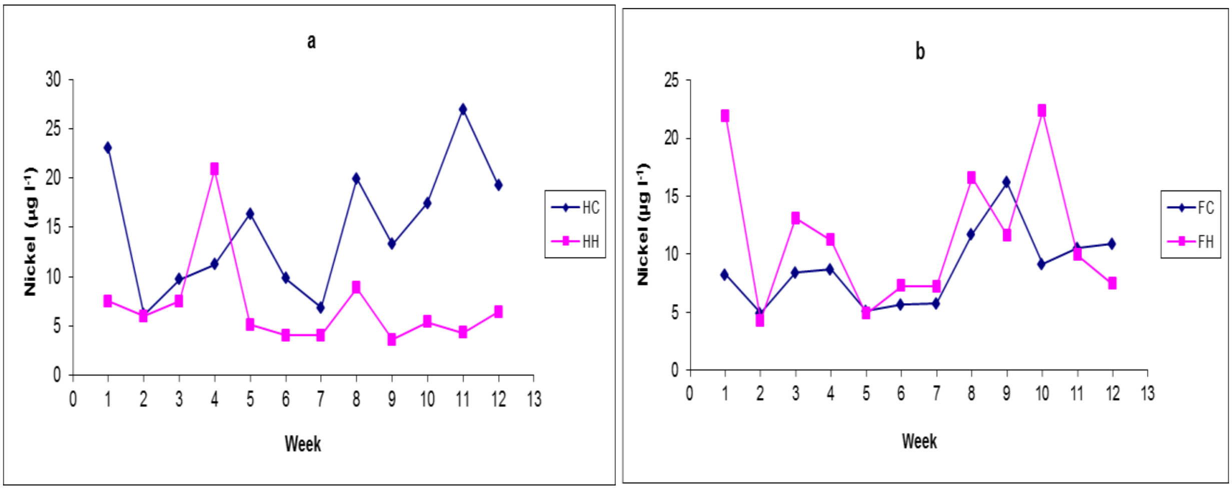


Fig. 3.6: Change in nickel concentration (µg l^{-1}) in pore water over the experiment in cores taken from (a) Holme Moss (H) and (b) Featherbed Moss. C indicates unheated glasshouse, H indicates heated glasshouse.

There was a clear trend of increasing Pb concentrations over time (Fig 3.7). As for Ni, there was little difference in the trends over time between the heated and unheated cores from Featherbed Moss, but there was a greater increase in unheated cores from Holme Moss, especially after week 8, with the final Pb concentrations in unheated cores being four times higher in the heated cores.

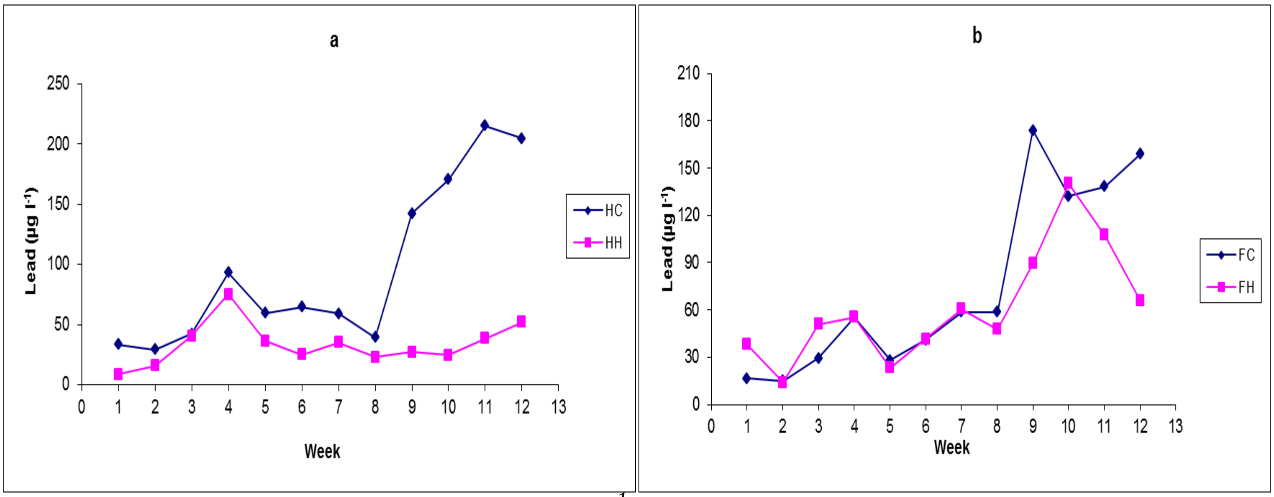


Fig. 3.7: Change in lead concentration (µg l^{-1}) in pore water over the experiment in cores taken from (a) Holme Moss and (b) Featherbed Moss. C indicates unheated glasshouse, H indicates heated glasshouse.

For both sites, the highest concentrations of Cu were seen in week 1 (Fig 3.8), after which there was little trend over time. There were also no consistent differences between the two treatments in either the cores from Holme Moss or Featherbed Moss.

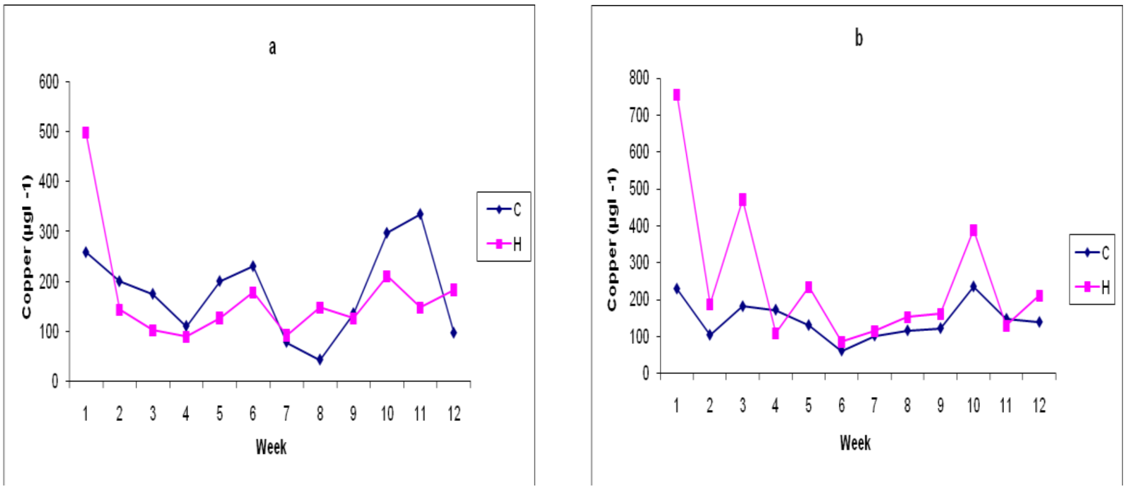


Fig 3.8. Change in copper concentration (µg l^{-1}) in pore water over the experiment in cores taken from (a) Holme Moss and (b) Featherbed Moss. C indicates unheated glasshouse, H indicates heated glasshouse.

The data quality for Zn concentrations was unreliable for the first four weeks of the experiment, and so data were only available for weeks 5-12. Although the concentrations varied over time (Fig. 3.9), there was little consistent trend and no consistent difference between unheated and heated cores.

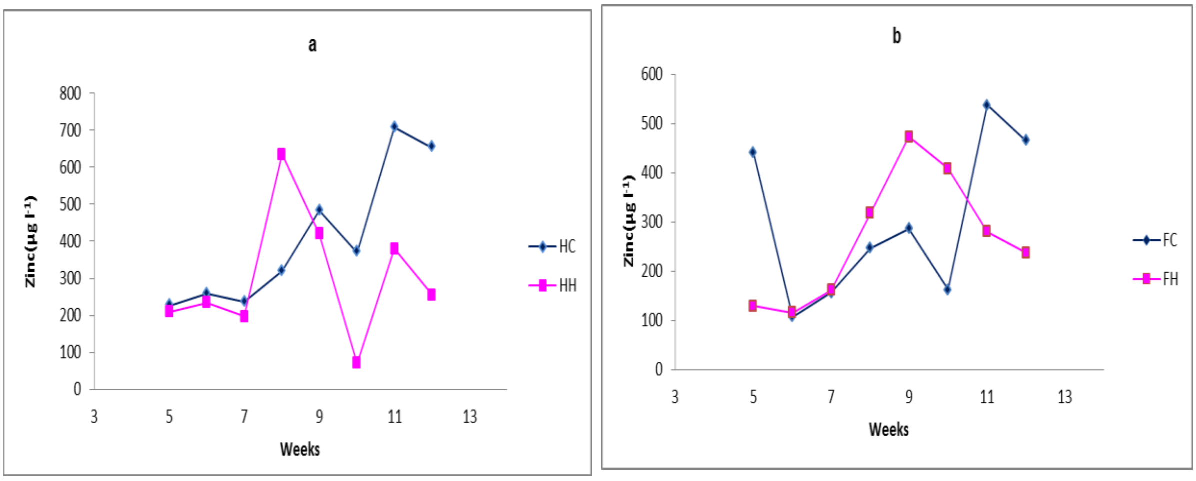


Fig. 3.9: Change in zinc concentration (µg l^{-1}) in pore water over the experiment in cores taken from (a) Holme Moss and (b) Featherbed Moss. C indicates unheated glasshouse, H indicates heated glasshouse.

3.3.5 Correlations between components of soil solution chemistry

The correlation coefficients, and their significance, between the different soil solution parameters (excluding metals), calculated for the mean weekly values separately for both sampling sites and glasshouses are shown in Table 3.4. This analysis identifies relationships between the changes through time in the values of the different parameters.

*Table 3.4: Correlation matrix between pore water chemical determinants (df=10), based on weekly mean values for each site and glasshouse. H=Holme Moss; F=Featherbed Moss. C= unheated glasshouse; H= heated glasshouse. * p<0.05; ** p< 0.01; *** p< 0.001.*

		pH	DOC	NH_4^+-N
DOC	HC	0.190		
	FC	-0.090		
	HH	0.020		
	FH	0.430		
NH_4^+-N	HC	0.020	0.500*	
	FC	0.010	0.588*	
	HH	0.800***	0.420	
	FH	0.62*	0.565*	
NO_3^--N	HC	-0.340	0.630**	0.218
	FC	-0.060	-0.490	-0.047
	HH	-0.040	-0.420	0.139
	FH	-0.150	0.660*	-0.350

Table 3.4 shows no significant relationship between soil solution pH and DOC, and no significant relationship between pH and NO_3^--N. However, there was a significant positive correlation of pH with NH_4^+-N in heated, but not unheated, glasshouses. Fig 3.10 shows the relationship between these two parameters; although the correlation coefficient was lower for Featherbed Moss, the value of the slope was greater, suggesting a greater increase in ammonium concentrations per unit change in pH. There was also a consistent positive correlation between DOC and NH_4^+-N concentrations, for both sites and glasshouses (Fig 3.11)

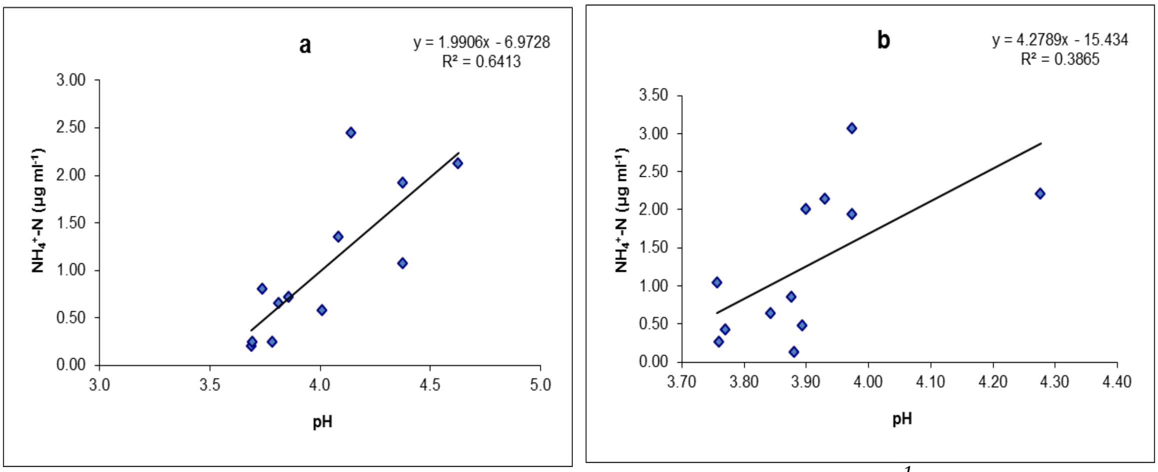


Fig. 3.10: Relationship between ammonium-N concentration (µg ml^{-1}) and pH, based on mean weekly values, in cores taken from (a) Holme Moss and (b) Featherbed Moss in the heated glasshouse.

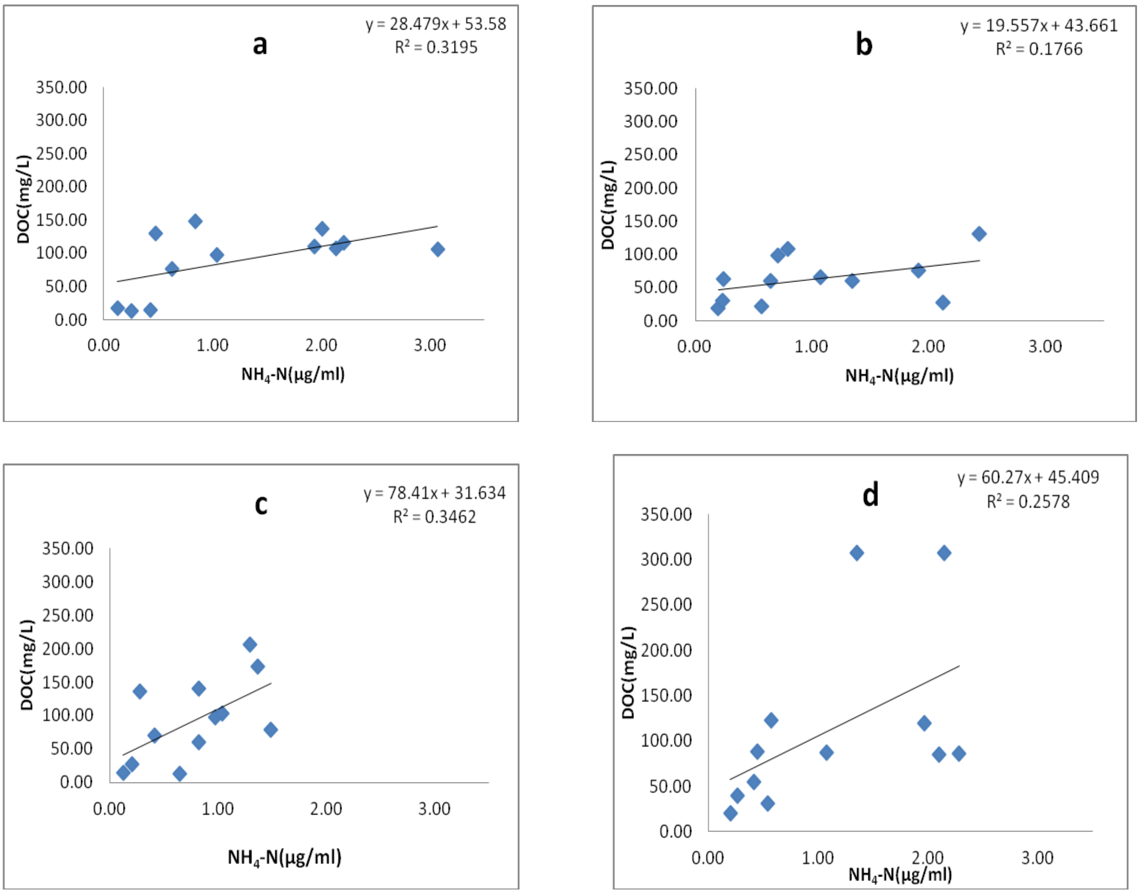


Fig. 3.11: Relationship between DOC and ammonium-N concentrations (µg ml^{-1}), based on mean weekly values, in (a) heated cores from Holme Moss; (b) heated cores from Featherbed Moss; (c) unheated cores from Featherbed Moss; (d) heated cores from Holme Moss.

These positive relationships between DOC and ammonium concentrations reflect the general trend for both determinants to increase over time during the experiment, possibly reflecting a change is some common underlying driver.

The analysis above assessed whether the changes through time in the mean values of the different variables in each group of cores were correlated. A second analysis was undertaken, for each site, to assess if there was a correlation between components of pore water across all individual cores at all measurement dates. This resulted in a total of 72 data points for each site, (2 treatments x 12 dates x 3 replicate mesocosms). The results of the analysis are shown in Table 3.5.

*Table 3.5: Correlation matrix between pore water chemical determinants (df=70) in cores from Holme Moss (H) and Featherbed Moss (F) based on all data. * p<0.05; ** p< 0.01; *** p< 0.001.*

		pH	DOC	NH4
DOC	H	-0.383***		
	F	0.137		
NH_4^+-N	H	0.024	0.517***	
	F	0.116	0.082	
NO_3^--N	H	0.42***	0.157	0.137
	F	0.128	0.175	0.017

Table 3.5 shows a strong negative correlation in Holme Moss cores between pH and DOC. pH was also significantly positively correlated with NO_3^-–N in Holme Moss cores, while ammonium-N and DOC were significantly positively correlated in Holme Moss cores. In contrast, the Featherbed Moss cores showed no significant correlations between any of the parameters. The different correlation structures in the Holme Moss and Featherbed Moss cores may be reflected in different relationships with pore water metal concentrations, an issue that is explored in the next section.

The significant correlations found in Holme Moss cores are illustrated in Figure 3.12. This suggests that relationship between pH and NO_3^--N is driven by a few outliers, and may be less reliable than that between pH and DOC, and DOC, and NH_4^+-N.

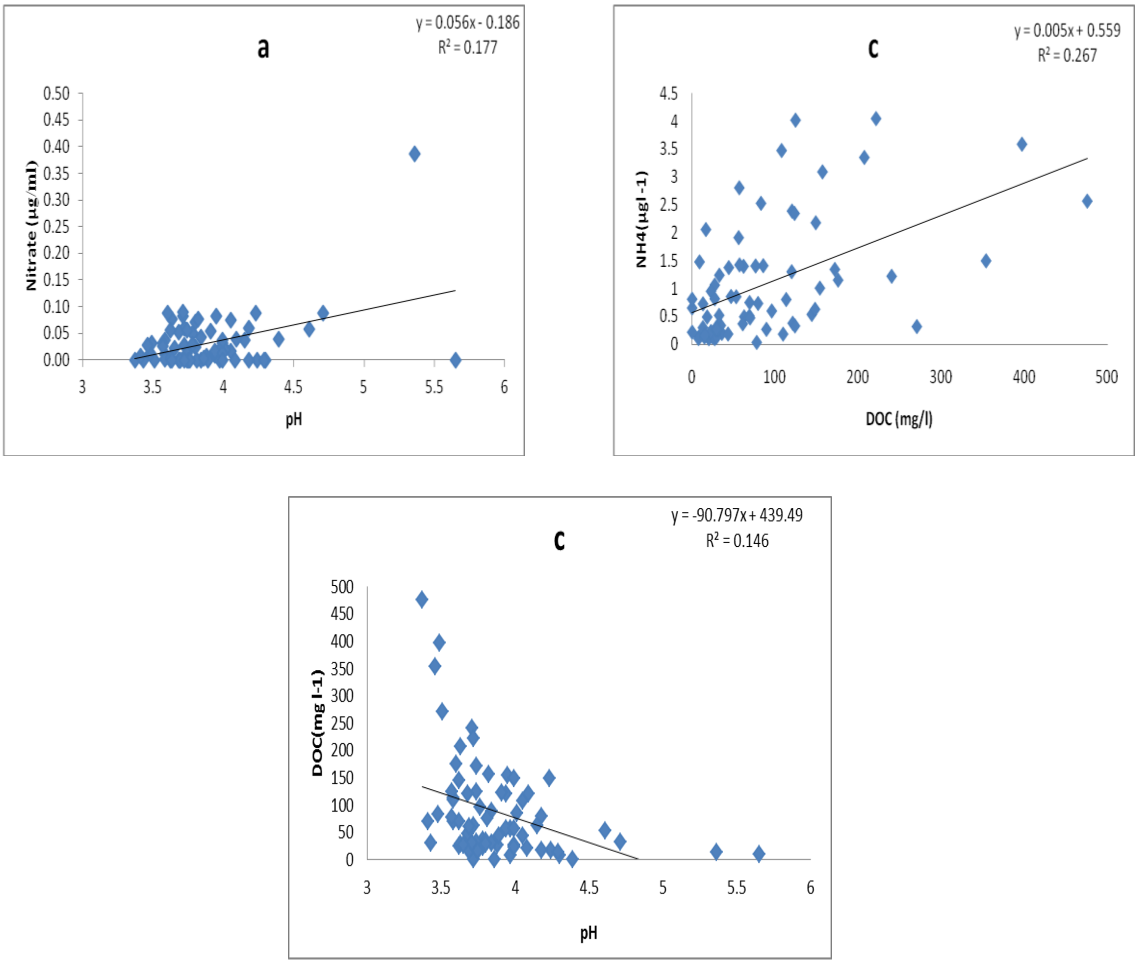


Fig. 3.12: Relationships between (a) pH & nitrate concentrations, (c) ammonium concentrations & DOC(c) pH & DOC in Holme Moss cores, based on all 72 individual values

3.3.6 Correlations between metal concentrations and soil solution parameters

The analysis of relationships between metal pore water concentrations and those of potential determinants (pH, DOC, NH_4^+, NO_3^-) followed the same structure as presented above for the non-metal parameters. Firstly, the data for the 12 mean values in each site and treatment were used, in order to focus on correlations between trends in time during the experiment. Secondly the data for the 72 individual measurements for the cores from each site were analysed, to identify overall associations within the whole dataset.

Table 3.6 shows the relationships between soil solution determinants and pore water metal concentrations for the 12 (8 for Zn for reasons explained earlier) data points of mean concentrations, i.e. for the changes over time during the experiment. The text below discusses these results for each metal in turn.

*Table 3.6: Correlation matrix between pore water determinants and metal concentrations, based on weekly mean values for each site and glasshouse. H=Holme Moss; F=Featherbed Moss. C= unheated glasshouse; H= heated glasshouse. * $p<0.05$; ** $p< 0.01$; *** $p< 0.001$. No correlation is shown for Zn and NO_3–N because of the limited number of data point.*

			pH	DOC	NH4	NO3
Ni (df=10)	HC		0.370	0.590*	0.480	0.070
	FC		0.570*	0.285	-0.070	0.042
	HH		0.370	0.330	-0.090	0.028
	FH		0.020	-0.230	-0.040	0.250
Pb (df=10)	HC		0.000	0.81***	0.70**	0.206
	FC		0.310	0.65*	0.81**	0.526*
	HH		-0.070	0.697*	0.260	0.273
	FH		0.500*	0.460	0.810***	0.501*
Cu (df=10)	HC		-0.080	0.080	0.138	0.151
	FC		0.130	-0.220	0.137	0.736**
	HH		-0.130	-0.330	-0.113	0.960***
	FH		-0.370	-0.680*	-0.182	0.091
Zn (df=6)	HC		-0.222	0.898**	0.560	-
	FC		-0.031	0.806**	0.060	-
	HH		-0.390	0.157	-	-
	FH		0.813**	-0.286	0.874**	-

* $p<0.05$; ** < 0.01; and *** < 0.001 level of significance.

Ni concentrations correlated positively with DOC in unheated glasshouses for Holme Moss (Fig. 3.13). A high negative correlation was also observed between pH and Ni concentrations (Fig. 3.13) in the Featherbed Moss cores in the unheated glasshouse, but the effects of pH and DOC were not significant for the cores in the heated glasshouse, and no significant correlations were found between Ni concentrations and either NH_4^+ or NO_3^- concentrations.

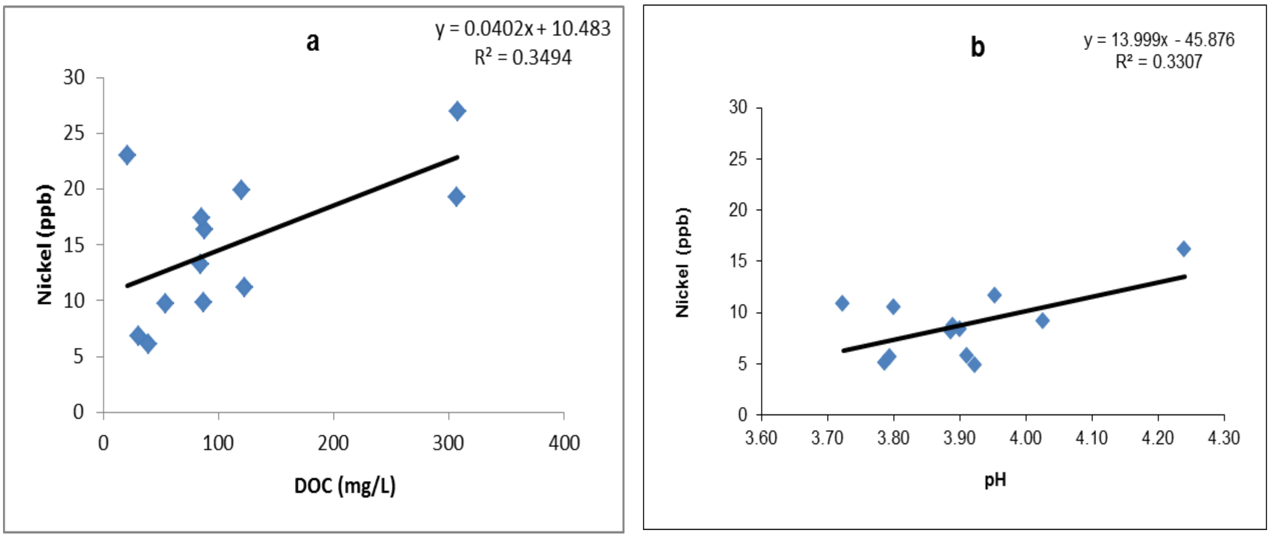


Fig. 3.13: Relationships between nickel concentrations (µg l^{-1}) & (a) DOC for Holme Moss cores in the unheated glasshouse and (b) pH for Featherbed Moss cores in unheated glasshouse.

Lead concentrations were consistently positively correlated with both DOC and ammonium concentrations (Table 3.6). Figure 14 shows these relationships for DOC; the slopes of the fitted line were greater in the unheated than the heated glasshouse for cores from both sites, reflecting the higher pore water concentrations in this treatment. The positive relationships between lead and ammonium concentrations most likely reflect the close association between DOC and ammonium concentrations (Table 3.4).

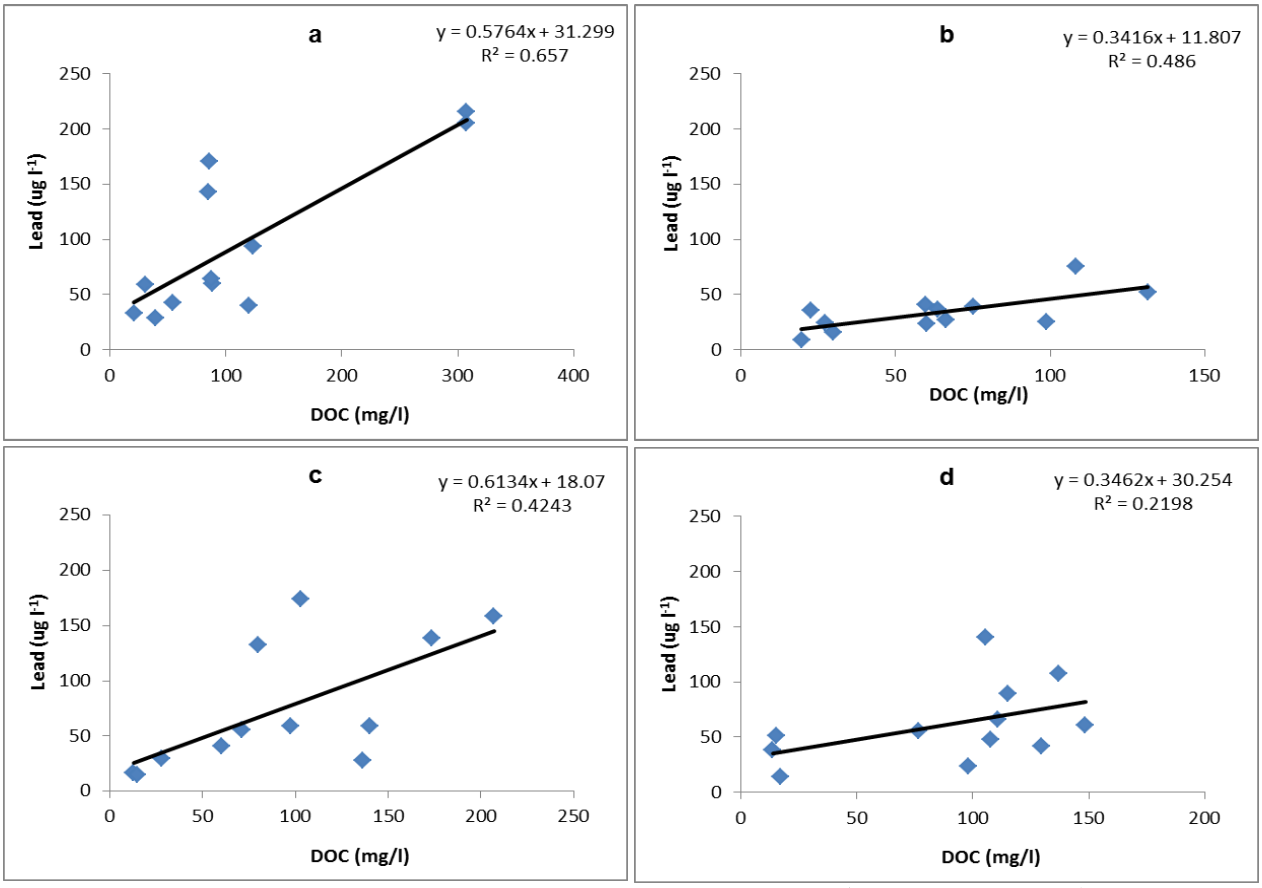


Fig. 3.14: Relationship between lead concentrations (µg l^{-1}) & DOC (mg l^{-1}) (a) for Holme Moss cores in unheated glasshouse; (b) for Holme Moss cores in heated glasshouse; (c) for Featherbed Moss cores in unheated glasshouse; (d) for Featherbed Moss cores in heated glasshouse.

Cu concentrations were positively correlated with NO_3-N concentrations in the heated glasshouse for Holme Moss cores, and in the unheated glasshouses for Featherbed Moss cores (Table 3.6; Fig 3.15). Although the correlations are highly significant, it is clear that this is due to one point with very high Cu values – without this point, no significant associations were found.

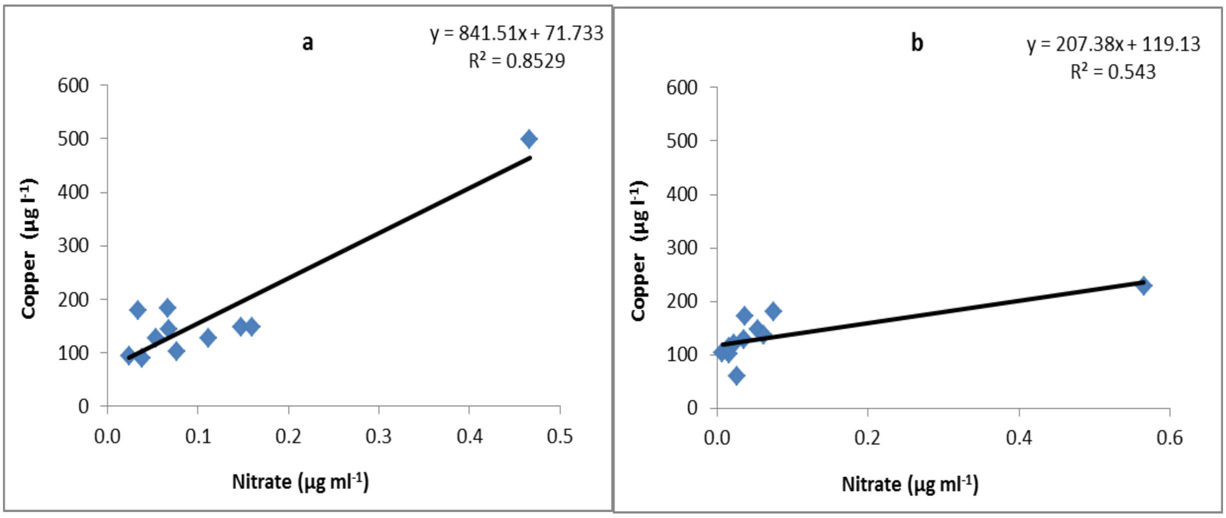


Fig. 3.15: Relationships between copper concentrations (µg l^{-1}) and nitrate-N concentrations (µg ml^{-1}) in (a) Holme Moss cores in heated glasshouse and (b) Featherbed Moss cores in unheated glasshouse.

Zinc concentrations correlated positively with DOC (Table 3.6; Figure 3.16) in unheated glasshouse cores from Holme and Featherbed Moss. Zinc concentrations were generally correlated negatively with pH, but the only significant correlation was a positive one in the Featherbed Moss heated glasshouse cores. Zinc concentrations also significantly correlated negatively with NH_4-N concentrations in heated Featherbed Moss cores.

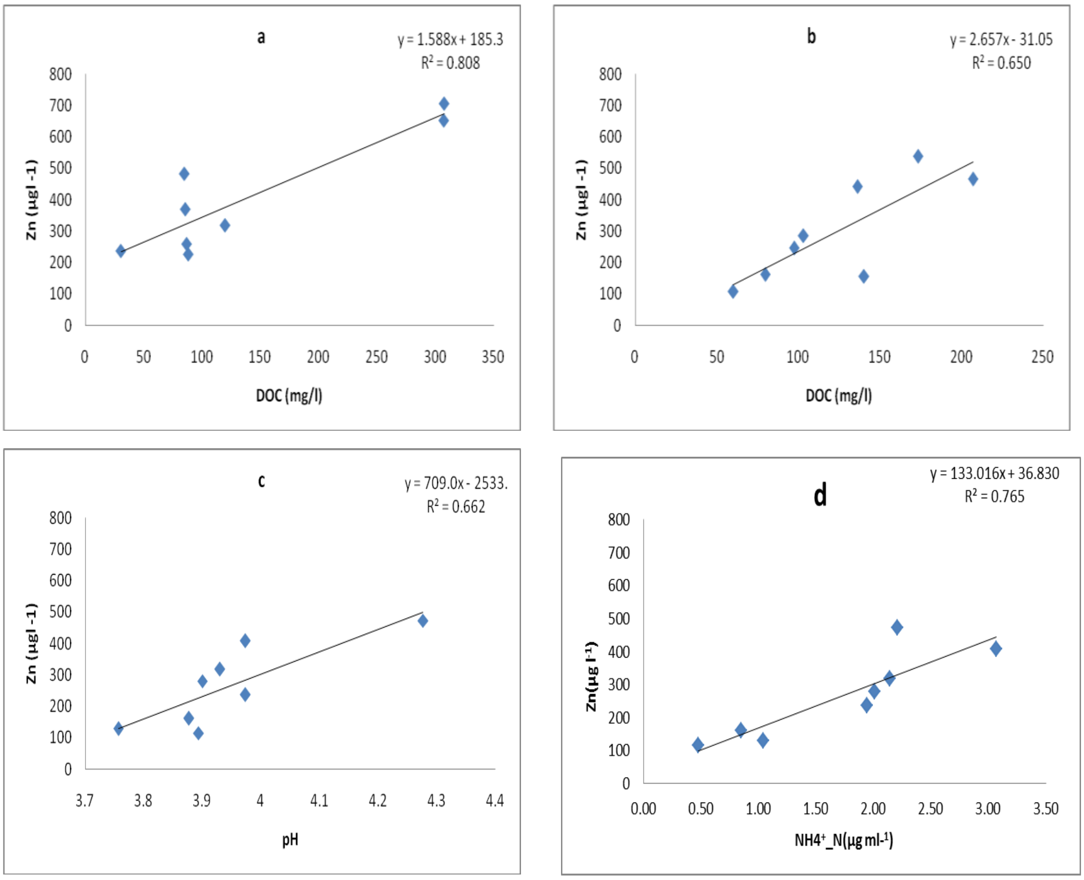


Fig. 3.16: Relationships between zinc concentrations (µg l^{-1}) and (a) DOC in unheated cores from Holme Moss, (b) DOC in unheated cores from Featherbed Moss (c) pH in heated cores from Featherbed Moss and (d) NH_4¯N concentrations (µg ml^{-1}) in heated cores from Featherbed Moss.

Table 3.7 shows the correlation matrix based on all values for each site’s cores (i.e. 72 values for Pb, Ni, and Cu; 48 values for Zn). The following text discusses the findings for each metal in turn, highlighting differences and similarities with the analysis based on the 12 point time-course (Table 3.6).

Table 3.7: Correlation matrix between pore water chemical determinants and metal concentrations in cores from Holme Moss (H) and Featherbed Moss (F) based on all data. $^{*}\,p<0.05$; $^{**}p<0.01$; $^{***}\,p<0.001$.

		pH	**DOC**	**NH_4**	**NO_3**
Ni(df=70)	H	-0.339**	0.515***	0.364***	0.076
	F	0.116	0.244*	0.443***	0.017
Pb(df=70)	H	-0.383***	0.796***	0.517***	0.106
	F	0.137	0.589***	0.082	0.121
Cu(df=70)	H	-0.164	0.044	0.022	0.131
	F	0.048	0.223	0.005	0.144
Zn(df=46)	H	-0.204*	0.389**	0.01	0.076
	F	0.094	0.236*	0.404***	0.017

Ni showed a significant negative correlation with pH in Holme Moss cores (Table 3.7; Fig. 3.17), but not Featherbed Moss cores. This may reflect the much greater range of pH values in the Holme Moss cores (cf. Fig. 3.3). In contrast, the analysis based on time-course (cf. Fig.3.13) showed a positive relationship with pH at Featherbed Moss, although this was largely due to one value.

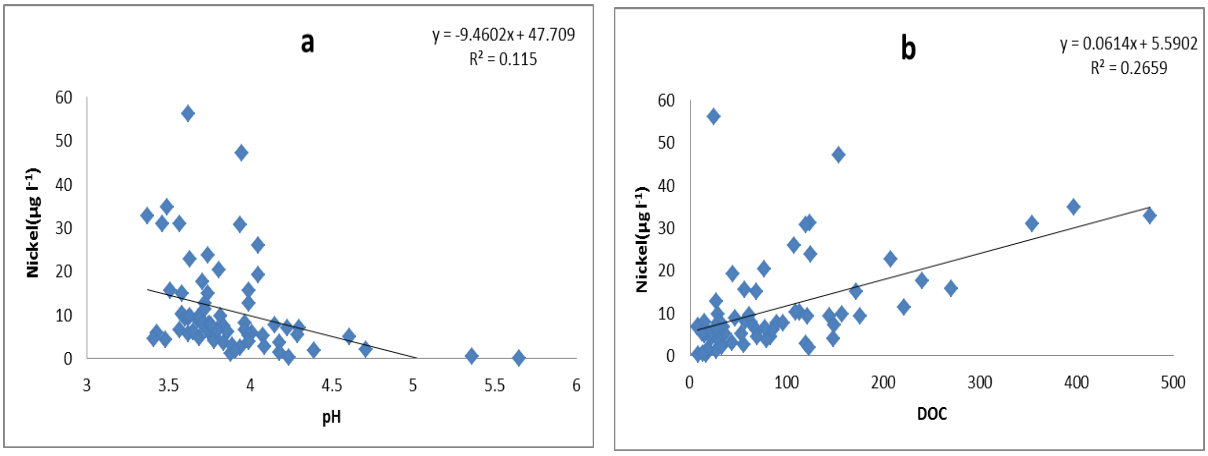


Fig. 3.17: Relationship between nickel concentrations (µg l^{-1}) and (a) pH and (b) DOC (mg l^{-1}) in Holme Moss cores based on 72 points.

Positive correlations were also found between nickel concentrations and DOC in cores from both Holme Moss and Featherbed Moss (Fig. 3.18; Table 3.7). A similar relationship, with a similar slope, was only found for the unheated Holme Moss cores in the time course analysis (Table 3.6; Figure 3.13); these were the cores that showed the greatest variation in DOC over time (Fig. 3.2). Positive relationships between Ni and ammonium concentrations were also found in cores from both sites (Table 3.7; Fig 3.18).

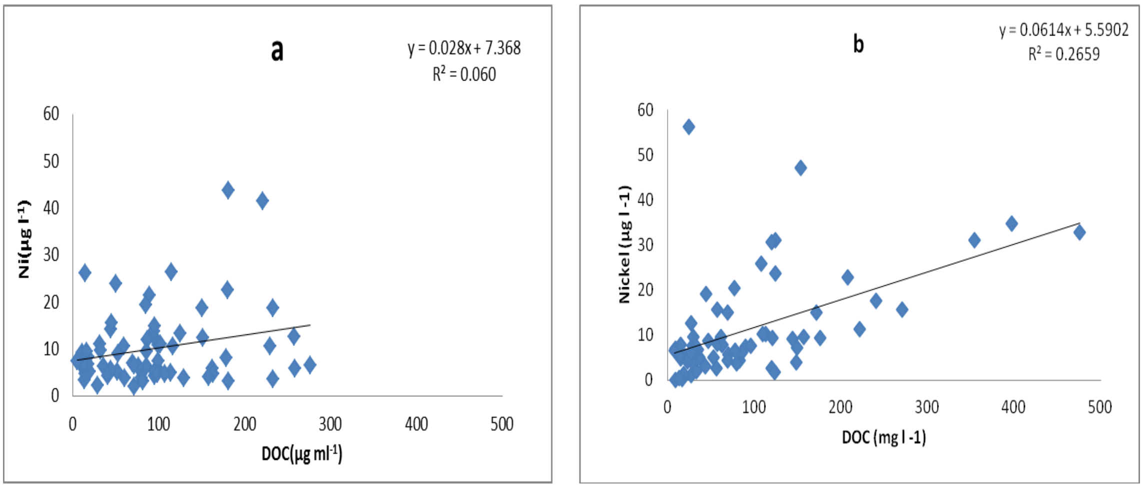


Fig. 3.18: Relationship between nickel concentrations (µg l^{-1})and (a) DOC in Holme Moss cores; (b) DOC in Featherbed Moss cores;

The results show a strong positive correlation between Pb concentration and DOC values for cores at both sites. The relationships in Figure 3.19 show the tighter fit to the data at Holme Moss, but also that the Holme Moss data covers a greater range of values of both DOC and Pb concentrations. Nevertheless, the slopes of the fitted lines were similar for the two sites. The time course analysis showed the same positive relationships (Table 3.6; Figure 3.14). It is thus highly likely that this consistent positive relationship between Pb concentration and DOC reflects a real causal association.

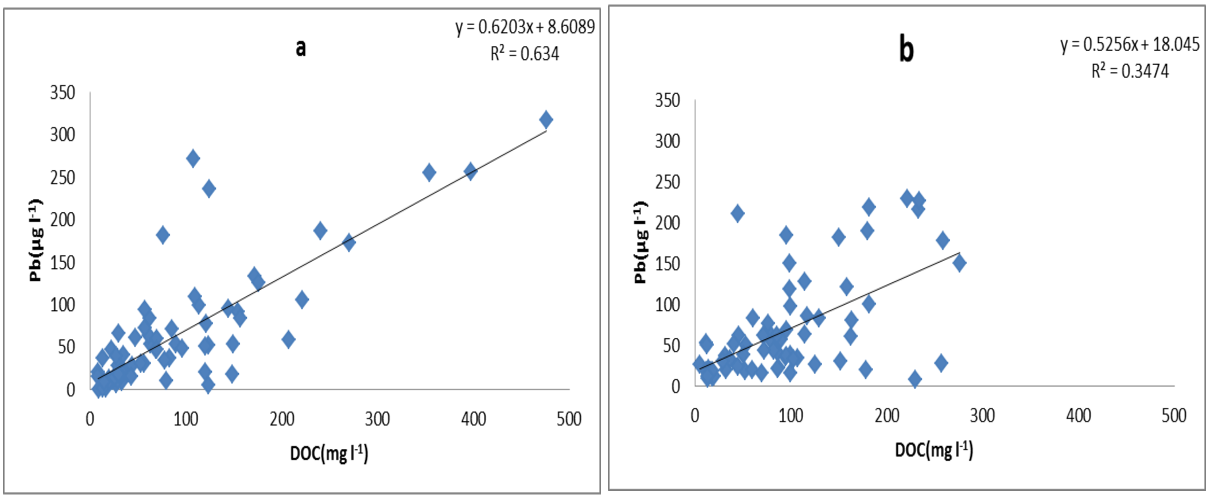


Fig. 3.19: Relationship between lead concentrations (μg l-^{1}) and DOC (mg l^{-1}) in (a) Holme Moss cores and (b) Featherbed Moss cores based on 72 points.

Table 7 also shows a significant negative relationship between Pb concentrations and pH, and a positive relationship between Pb and ammonium concentrations in the Holme Moss cores, but not the Featherbed Moss cores (Figure 3.20). Since DOC values were significant correlated in the 72 point analysis with pH and NH_4^+ in Holme Moss cores, but not Featherbed Moss cores (Table 3.5), it is likely that these, and the correlations over time, are not causal relationships with Pb concentrations but reflect the association of high DOC levels with lower pH values and higher NH_4^+-N concentrations.

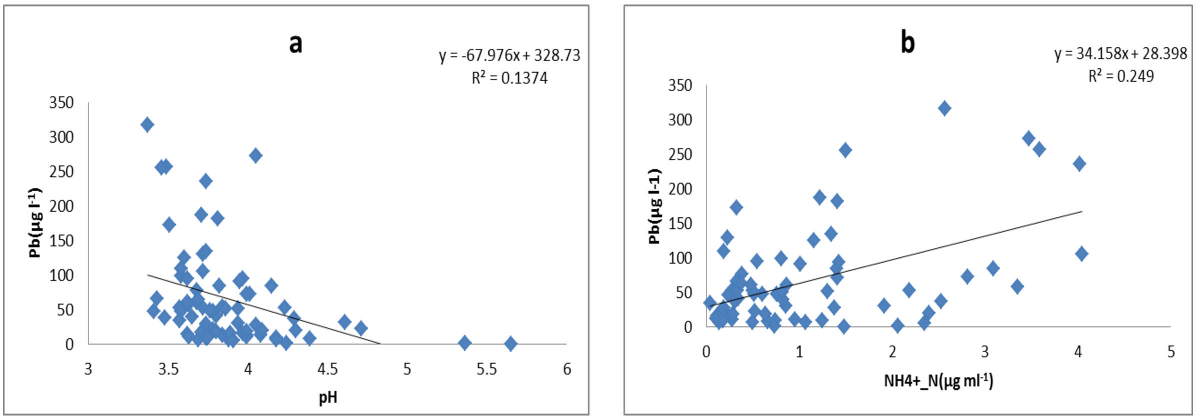


Fig. 3.20: Relationship between lead concentrations (µg l-^{1}) and (a) pH in Holme Moss cores and (b) NH_4^+-N concentrations (µg ml^{-1}) in Holme Moss cores, based on 72 points.

Copper concentrations showed no significant relationships with any of the variables (Table 3.7). The only consistent significant relationships found for copper in the time-course analysis was with nitrate concentrations, but these appeared to be related to a single point, related to the elevated concentrations at the start of the experiment (cf. Figure 3.8). Therefore, little of the variation in copper concentrations is likely to reflect responses to the experimental treatments.

Zinc concentrations were, like those of Ni and Pb, positively correlated with DOC concentrations in cores from both sites (Table 3.7; Figure 3.21). As for Pb, the slopes of the relationships were similar for both sites. Zinc also showed a negative correlation with pH in cores from Holme Moss and a positive correlation with ammonium concentrations in Featherbed Moss cores. Similar relationships with DOC (although only in unheated cores) and with ammonium in Featherbed Moss cores, were shown in the 8 point analysis (Table 3.7). However, the significant negative trends with pH were not found in the time course analysis of Holme Moss cores.

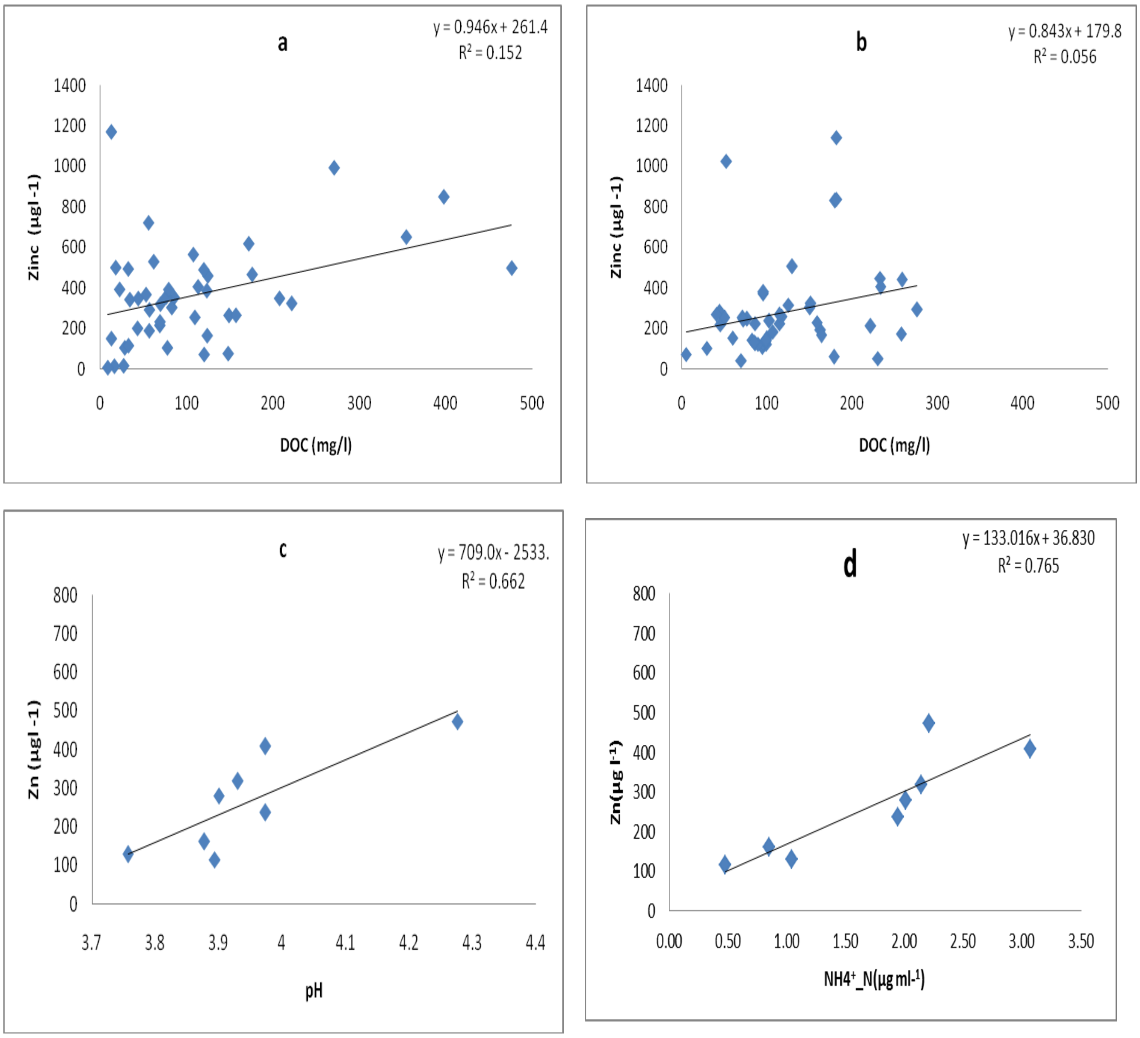


Fig. 3.21: Relationships between zinc (μg l^{-1}) concentrations and (a) DOC in Holme Moss cores (b) DOC in Featherbed Moss cores; (c) pH in Holme Moss cores; and (d) NH_4^+-N concentrations (μg ml^{-1}), based on 48 data points.

3.3.7 Comparison of pore water and leachate analysis

As described in the Methods section, 10 ml of deionized water was added regularly to all the mesocosms, After two weeks, a constant and stable water load was established within the mesocosms, The leached water was then collected on a weekly basis for analysis, starting from the third week of the experiment, after which the cores did not receive any additional rain water inputs.

Appendix 1 summarises the time courses of leached water composition (DOC, ammonium, nitrate and metal concentrations) over the 10 weeks. pH was not determined, as the leach watered remained for a week in the tray under the cores, and hence could have changed significantly over that period. Changes in temperature, and evaporation, might also have affected the concentrations of other determinants, and this needs to be considered in data interpretation.

The analysis in this section is focussed on a comparison of pore water and leachate composition, and an analysis of whether the relationships between metal concentrations and DOC, NO_3^--N and NH_4^+-N concentrations that were found for pore water were also found in leachate. The minimum, maximum and average values for pore water and leachate composition are given in Table 3.8. As for pore water, Cd concentrations were very low and were not analysed further, and Zn data were only available for weeks 5-12. The values were calculated for the two sampling sites (including both heated and unheated glasshouses) over the period of 10 weeks (weeks 3-12) when both leachate and pore water are collected. Since the pore water concentrations of many of these determinants showed significant variation over the time-course of the experiment, a detailed comparison based on both Table 3.8, and the time-courses in pore water and leachate, is provided below.

Table 3.8: The minimum, maximum, and average concentrations of the soil solution parameters in leachate collected from Holme Moss and Featherbed Moss cores, over the last 10 weeks of the experiment. Values from heated and unheated glasshouses are combined for cores from the two sites.

			DOC ($mg\ l^{-1}$)	**NH_4^+-N ($\mu g\ ml^{-1}$)**	**NO_3^--N ($\mu g\ ml^{-1}$)**	**Ni ($\mu g\ l^{-1}$)**	**Pb ($\mu g\ l^{-1}$)**	**Zn ($\mu g l^{-1}$)**	**Cu ($\mu g\ l^{-1}$)**
HOLME MOSS	**PORE WATER**	**min**	7.7	0.12	0.12	0.17	0.06	7.5	11.7
		max	475.9	4.05	0.39	47.1	316.1	1169	597.4
		average	104.1	1.24	0.04	11.0	73.5	353.1	155.4
		N	57	60	54	60	60	48	60
	LEACHATE	**min**	7.5	0.00	0.00	1.19	2.8	15.7	26.9
		max	150.3	6.66	1.88	26.4	120.7	668.5	521.7
		average	52.9	1.05	0.25	6.0	33.5	105.4	156.8
		N	54	60	60	60	60	48	60
FEATHERBED MOSS	**PORE WATER**	**min**	4.8	0.13	0.12	2.02	7.5	41.1	30.9
		max	276.3	6.34	0.50	43.6	319.0	1142	483.7
		average	109.6	1.19	0.02	10.0	78.3	282.6	156.5
		n	59	59	53	59	59	48	59
	LEACHATE	**min**	2.9	0.00	0.00	1.25	5.6	16.3	52.6
		max	274.0	7.53	1.18	15.5	233.2	685.4	499.0
		average	58.8	0.97	0.20	5.0	39.1	92.0	166.8
		n	60	60	60	60	60	48	60

Table 3.8 shows that mean DOC concentrations in pore water were about twice those in leachate. Figure 3.22 shows the time course of DOC concentrations; for each week, all the measured values for pore water and leachate in individual cores are plotted. While there were consistently higher DOC levels in pore water after week 3, the differences were particular marked in the final two weeks of the experiments, when the highest DOC concentrations were recorded.

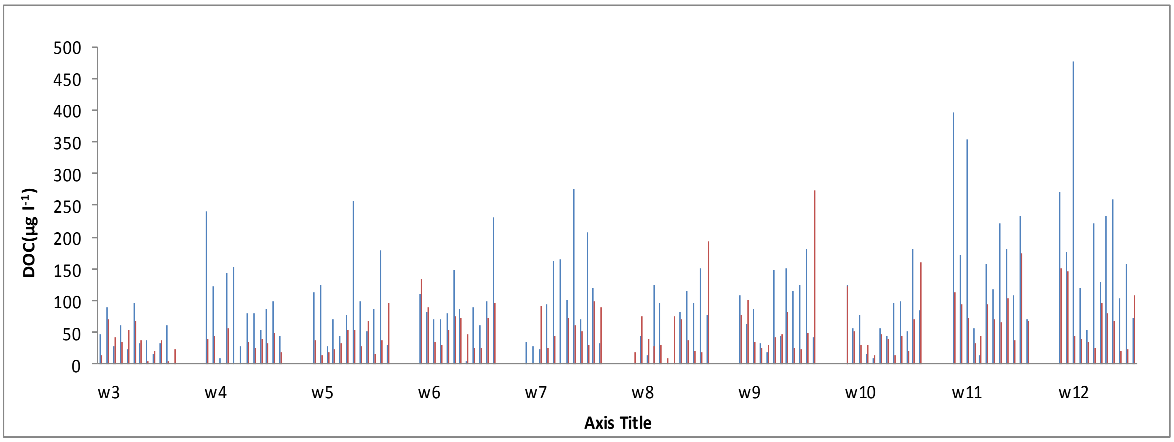


Fig. 3.22: DOC concentrations (mg l^{-1}) in leachate (L) and pore water (P) over weeks 3-12 of the experiment. For each week, the paired values from 12 individual cores are plotted.

There were higher values of NH_4^+-N concentrations in pore water than in leachate for both sites, but there were much lower values of NO_3^--N concentrations in pore water than in leachate at both sites (Fig. 3.23). When the sum of nitrate and ammonium concentrations was calculated, the values were very similar in leachate and porewater (1.30 and 1.28 µg ml^{-1} respectively for Holme Moss and 1.17 and 1.21 µg ml^{-1} respectively for Featherbed Moss). Hence it appears that nitrification of ammonium is the source of the higher nitrate concentrations found in leachate than in porewater.

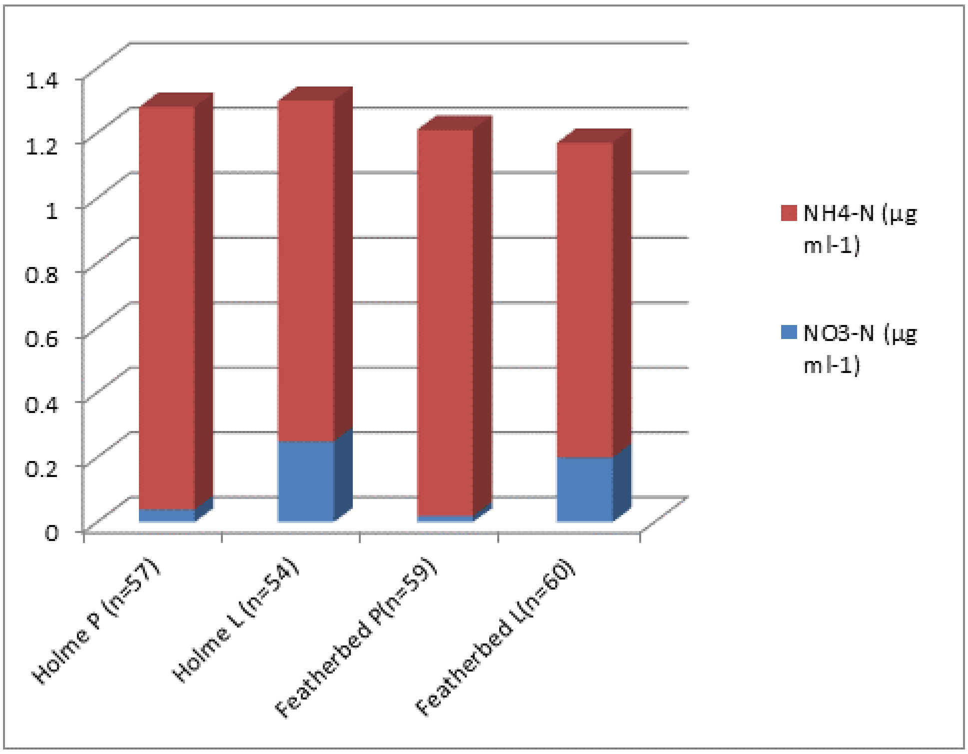


Fig. 3.23: Differences in mean NH_4_N and NO_3^--N concentrations (µg ml^{-1}) between leachate (L) and pore water (P) over weeks 3-12 of the experiment. .

Ni concentrations, like those of DOC, were approximately twice as high in pore water than in leachate. The time-course over the experiment (Fig. 3.24) clearly shows that these differences were more marked in the second half of the experiment that the first half, a pattern which is also broadly consistent with that for DOC.

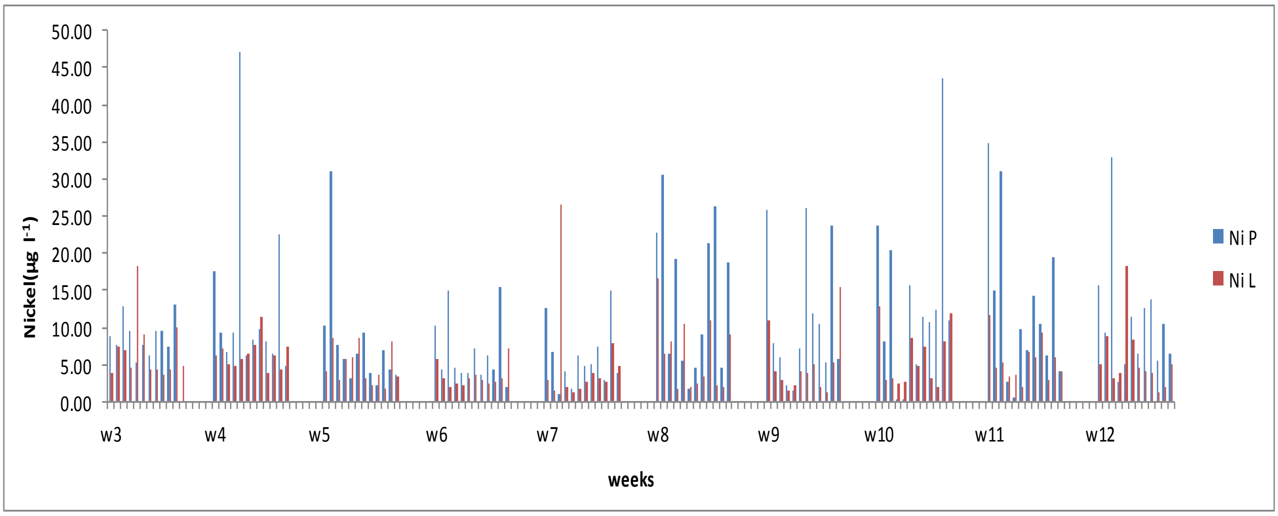


Fig. 3.24: Ni concentrations (µg l^{-1}) in leachate (L) and pore water (P) over weeks 3-12 of the experiment. For each week, the paired values from 12 individual cores are plotted.

The Pb concentrations showed a very similar difference to those of DOC and Ni, with concentrations in pore water being approximately twice those in leachate (Table 3.8). Fig 3.25 also shows a very similar pattern to that for DOC and Ni concentrations, with the differences in concentrations between pore water and leachate becoming greater over the course of the experiment.

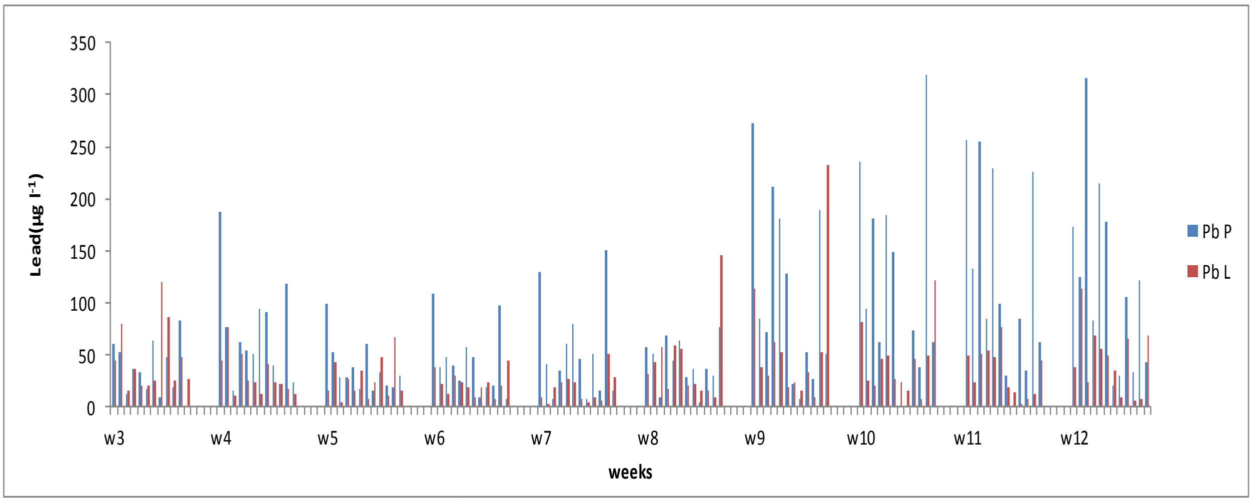


Fig. 3.25: Pb concentrations (µg l^{-1}) in leachate (L) and pore water (P) over weeks 3-12 of the experiment. For each week, the paired values from 12 individual cores are plotted.

In contrast, in the case of Cu concentrations, there was very little difference between the mean values in pore water and leachate (Table 3.8). This was a consistent effect over the course of the experiment, as shown in Fig. 3.26, as the concentrations in both pore water and leachate showed a very similar pattern over time, rising and falling together.

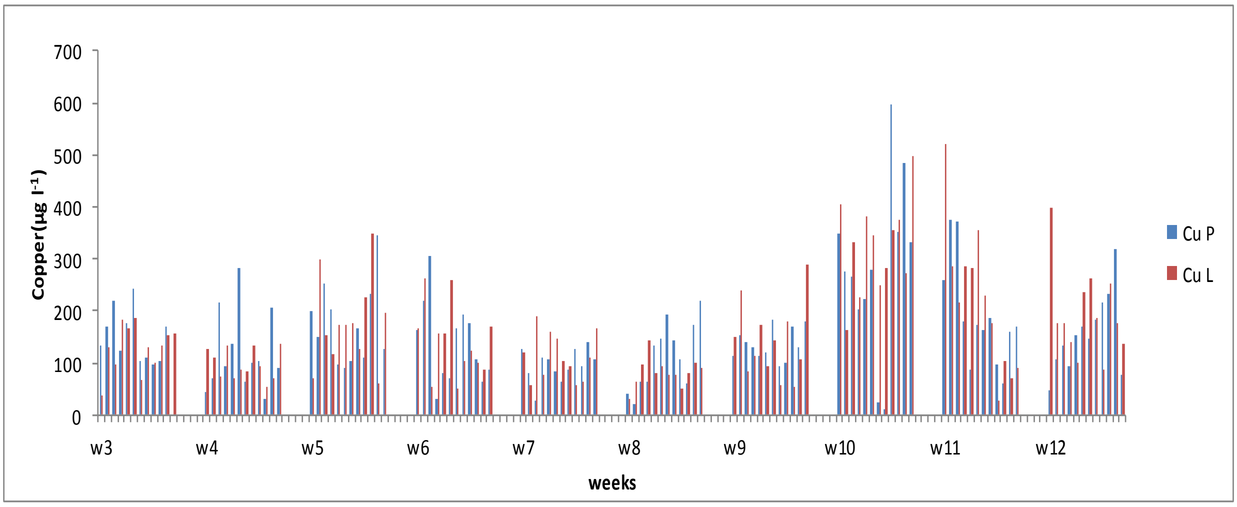


Fig. 3.26: Cu concentrations (µg l^{-1}) in leachate (L) and pore water (P) over weeks 3-12 of the experiment. For each week, the paired values from 12 individual cores are plotted.

The concentrations of Zn showed a greater contrast between pore water and leachate than any other analyte; the mean concentrations in pore water were approximately three times higher in pore water than in leachate. The pattern of the difference in concentrations becoming greater as the experiment progressed was also apparent for Zn concentrations; there was little change in leachate concentrations over time, but a gradual increase in porewater concentrations (Fig. 3.27)

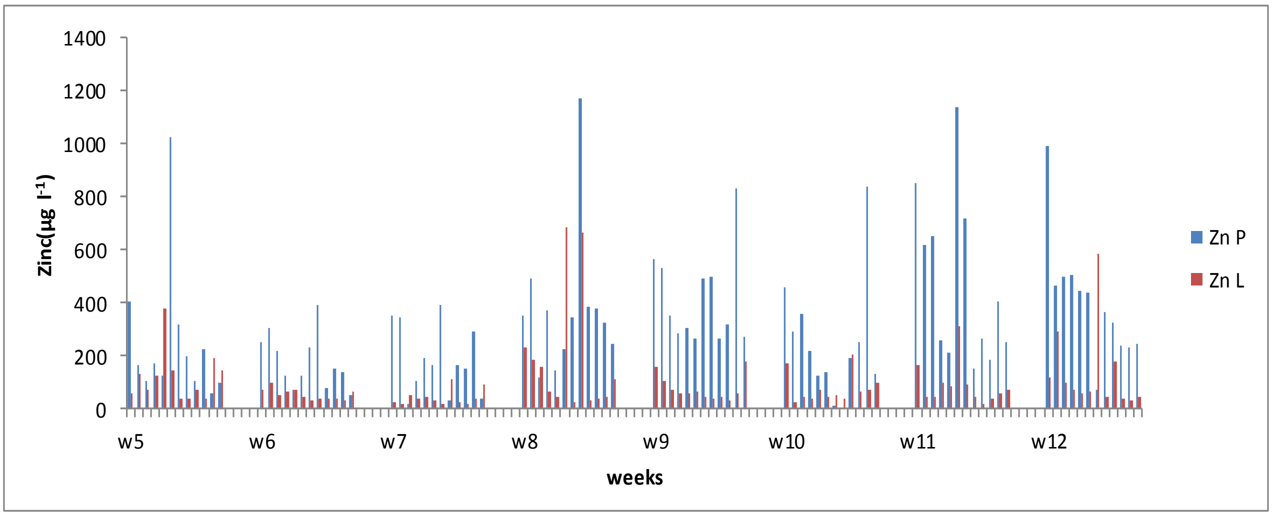


Fig. 3.27: Zn concentrations (µg l^{-1}) in leachate (L) and pore water (P) over weeks 3-12 of the experiment. For each week, the paired values from 12 individual cores are plotted.

3.3.8 Correlation analysis for leachate

This analysis assessed if there was a significant correlation between chemical components of leachate across all individual cores on all measurement dates. This resulted in a total of 60 data points (2 treatments x 10 dates x 3 replicate cores). This was reduced to 48 for Zn, as data were only available for 8 dates. The results of the analysis are summarized in Table 3.9. This corresponds to the same analysis for pore water data that was summarised in Tables 3.5 and 3.7, except that data were not available for pH or for the first two dates. A comparison of the findings with those for pore water is also provided.

*Table 3.9: Correlation matrix for composition of leachate in Holme Moss (H) and Featherbed Moss (F) based on all values recorded between weeks 3 and 12. * p<0.05; ** p< 0.01; *** p< 0.001. df=58, except for Zn, for which df = 46.*

		NH_4	**NO_3**	**Ni**	**Pb**	**Cu**	**Zn**
DOC	H	0.376**	0.166	0.226*	0.394**	0.432***	0.047
	F	0.624***	0.173	0.522***	0.777***	0.148	0.059
NH_4	H	-	0.139	0.276*	0.489***	0.312**	0.192
	F	-	0.151	0.590***	0.691***	0.148	0.026
NO_3	H	-	-	0.076	0.088	0.238*	0.098
	F	-	-	0.119	0.162	0.232*	0.078

Table 3.9 shows a strong positive correlation between NH_4^+–N and DOC concentrations in both Holme Moss and Featherbed Moss cores, but no significant relationships between either NH_4^+–N and DOC concentrations and those of NO_3^--N. These correlation patterns are consistent with those found for pore water (cf. Table 3.5), except that a significant positive relationship between DOC and NH_4^+–N concentrations was only found for Holme Moss cores for pore water.

Ni concentrations were significantly correlated in cores from both sites with DOC and NH_4^+_N concentrations, but not NO_3^--N concentrations. This was the same pattern that was found for pore water (cf. Table 3.7). Since the relationships between Ni concentrations and NH_4^+_N concentrations most likely reflects the high correlation between DOC and NH_4^+_N concentrations, quantitative comparisons of relationships in pore water and leachate concentrated on DOC (Fig. 3.27). Although the strength of the correlations differed for leachate from cores from the two sites, the slope of the fitted lines was very similar, suggesting consistent relationships across all the experimental cores. For Featherbed Moss, for which the range of DOC concentrations was comparable in pore water and leachate, the slope of the fitted line was also similar for pore water and leachate. However, for Holme Moss cores, the slop was twice as great in pore water than in leachate, possible reflecting the much smaller range of Ni concentrations in the leachate.

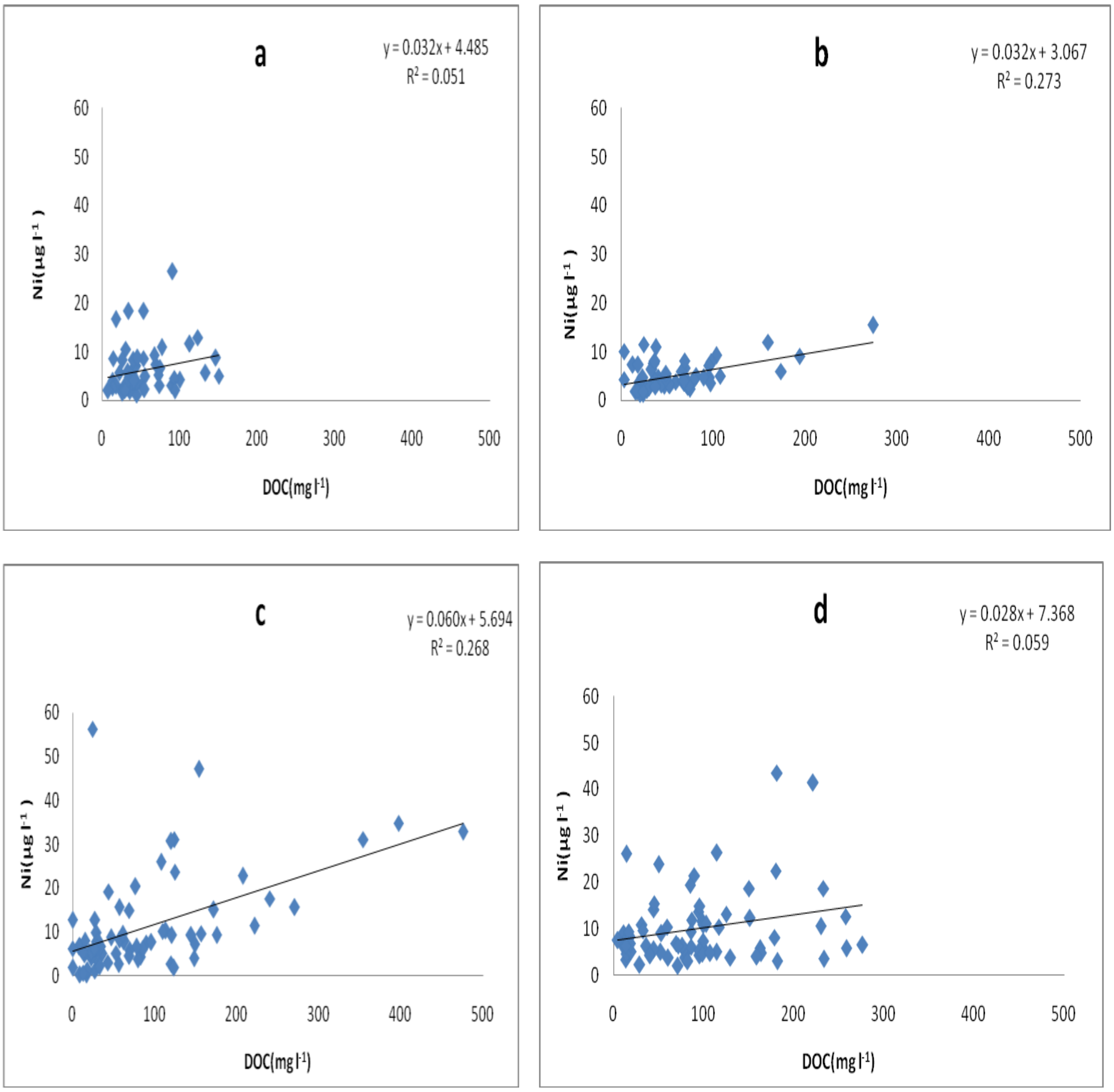


Fig. 3.28: Relationships between Ni concentration (μg l^{-1}) and DOC(mg l^{-1}),based on data from all cores in weeks 3-12, for (a) Holme Moss leachate; (b) Featherbed Moss leachate; (c) Holme Moss pore water; (d) Featherbed Moss pore water.

In the case of Pb concentrations, the same significant correlations with DOC and NH_4^+_N, but not NO_3^-N, concentrations for cores from both sites were found as for Ni. This was similar to the findings for the analysis of pore water, except that no significant relationship was found in that case for NH_4^+_N concentrations in Featherbed Moss cores (cf. Table 3.7). The positive relationships between Pb concentration and DOC concentrations for cores from both sites are shown in Figure 3.29, alongside those for pore water. These show very similar patterns to those found for Ni concentrations. The slope of the positive relationship between Pb and DOC for leachate was similar to that found for the pore water analysis at Featherbed Moss for which the range of DOC and Pb values was similar. However, the slope for leachate data was lower than that for pore water at Holme Moss, and that for leachate in Featherbed Moss cores, possibly reflecting the small range of both DOC and Pb concentrations in Holme Moss leachate samples.

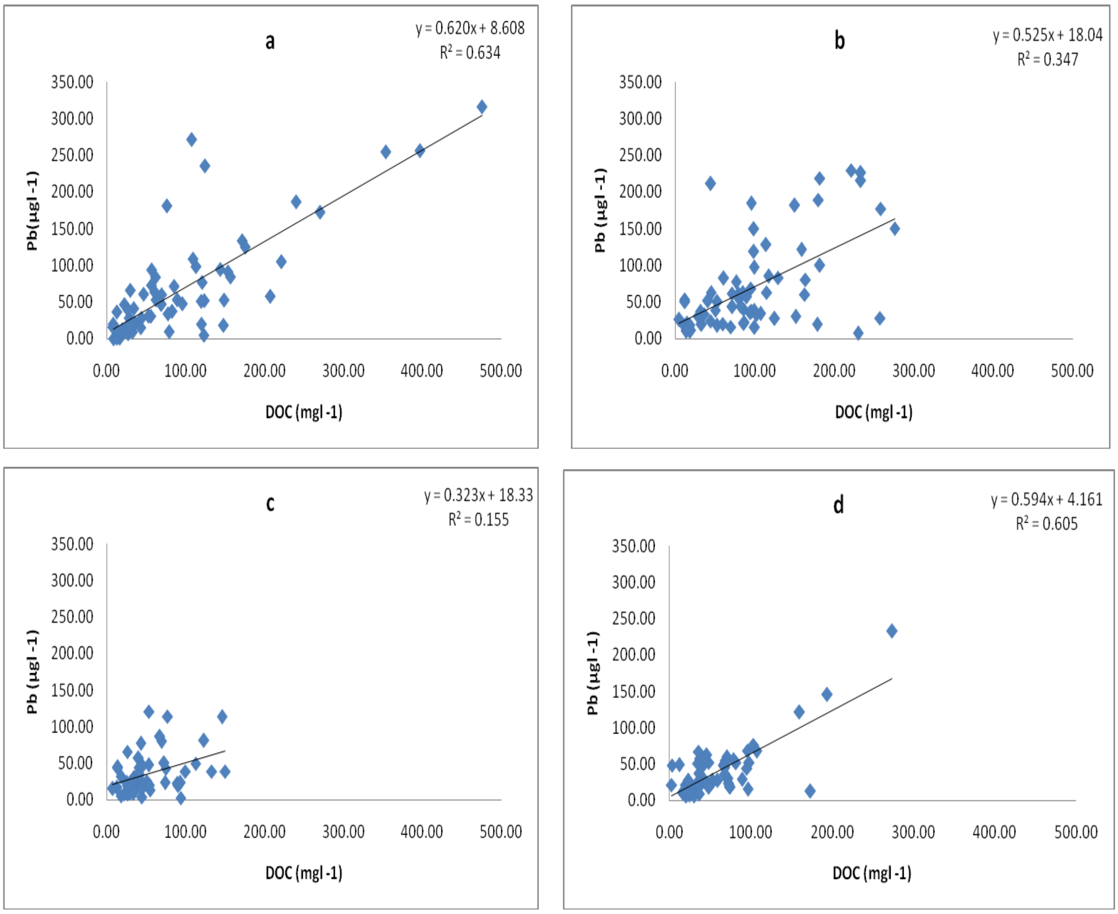


Fig. 3.29: Relationships between Pb concentration (µg l^{-1}) and DOC(mg l^{-1}),based on data from all cores in weeks 3-12, for (a) Holme Moss leachate; (b) Featherbed Moss leachate; (c) Holme Moss pore water; (d) Featherbed Moss pore water.

Cu concentrations were significantly correlated DOC and NH_4^+_N concentrations in leachate from Holme Moss, but not Featherbed Moss, cores (Table 3.9). Significant positive correlations with nitrate concentrations were found in leachate from both cores. These results differ from those in pore water (cf. Table 3.7), although positive relationships with nitrate concentrations in pore water were found in the time course analysis (cf. Table 3.6). Fig 3.30 shows the relationships between copper and nitrate concentrations in pore water and leachate. The positive slopes were comparable in leachate in both Holme Moss and Featherbed Moss cores. In pore water, it appears that a few outliers of high Cu concentrations confound the relationships, but these isolated high values are not found in leachate.

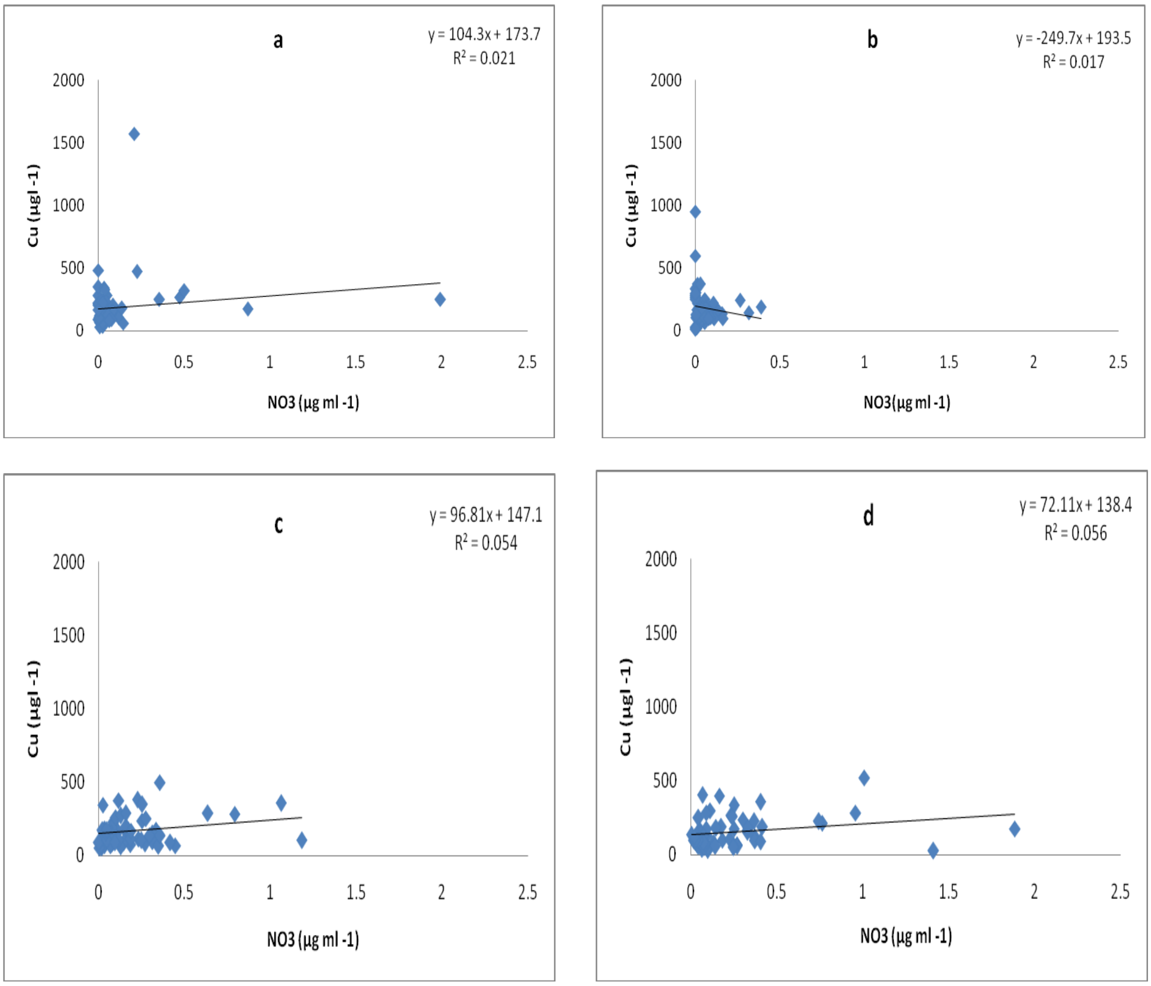


Fig. 3.30: Relationships between Cu concentration (µg l^{-1}) and nitrate N (µg ml^{-1}) ,based on data from all cores in weeks 3-12, for (a) Holme Moss leachate; (b) Featherbed Moss leachate; (c) Holme Moss pore water; (d) Featherbed Moss pore water.

In the case of Zn, no significant correlations were found for the leachate (Table 3.9). This is contrast to the results from the pore water analysis, for which significant positive correlations were found with DOC and (in Featherbed Moss cores) with ammonium concentrations. One reasons for this may be the very large reductions in Zn concentrations in the leachate, leading to a much lower degree of variation between the replicate cores.

3.3.9. Soil Analysis

The mean metal concentrations, and the range, in acid digests, KCl extracts and water extracts are summarised in Appendix 2 & 3. The analysis in this section focuses on whether there was any effect of treatment on soil metal concentrations, and whether the concentrations found in pore water and leachate were related to those in soil KCl extracts.

Table 3.10 shows the results of an analysis of variance of the effects of temperature, depth in the core, and site for the four metals that were successfully analysed in pore water and leachate. There were no significant effects on the acid digest metal concentrations, except for an effect of site in the case of zinc (Appendix 4). There were no significant effects of depth (at P=0.05) of the sample within the core for any of the three extracts. However, there were significant effects of temperature, site and a site-temperature interaction for the KCl extracts of Ni and Zn, while there was also a significant site-temperature interaction in the case of the water extractable Ni. There were no significant effects on concentrations of Cu or Pb. Table 3.10 also shows the analysis of variance results for soil pH. There was a significant effect of temperature on the pH measured in KCl, and a significant effect of site and temperature for water extract pH..

Table 3.11 shows the effects of site and temperature on the mean values. Focussing on the effect of temperature, heating significantly decreased the pH in KCl extracts in Holme Moss cores, but not in those from Featherbed Moss, but had no significant effect in water extracts. In contrast, KCl extractable concentrations of nickel and zinc were increased significantly, by a factor of 2, by heating in the Featherbed Moss cores, but there was no significant effect of temperature in the Holme Moss cores.

Table 3.10: Summary of analysis of variance of effects of temperature, core depth and site on metal concentrations and pH in KCl and water extracts(corrected df=11)(error df=24).

		KCl Extract					Water extract				
Treatment		Ni	Pb	Cu	Zn	pH	Ni	Pb	Cu	Zn	pH
Temperature	F	23.15	3.46	1.75	13.47	6.36	1.24	1.19	0.36	0.65	0.75
	P	0.00	0.08	0.20	0.00	0.02	0.28	0.29	0.56	0.43	0.40
Soil Level	F	2.13	1.75	0.40	0.17	0.07	1.63	2.87	0.34	0.35	2.44
	P	0.14	0.20	0.68	0.84	0.93	0.22	0.08	0.71	0.70	0.11
Site	F	16.74	0.12	0.12	28.10	0.02	0.96	1.47	1.73	2.37	3.53
	P	0.00	0.74	0.73	0.00	0.88	0.34	0.24	0.20	0.14	0.07
Site × Temperature	F	6.67	0.15	0.57	9.55	1.04	5.44	0.26	0.47	1.29	5.80
	P	0.02	0.70	0.46	0.01	0.32	0.03	0.62	0.50	0.27	0.02
Site × Soil Level	F	0.55	0.36	1.30	1.38	0.02	1.37	1.44	0.58	0.06	1.77
	P	0.58	0.70	0.29	0.27	0.98	0.27	0.26	0.57	0.94	0.19
Temperature × Soil Level	F	2.41	0.13	0.67	3.83	0.16	0.15	0.86	0.47	1.00	0.50
	P	0.11	0.88	0.52	0.04	0.85	0.86	0.44	0.63	0.38	0.61

Table 3.11: Summary of mean water and KCl extractable metal concentrations (µg g^{-1}) and pH from different sites and glasshouses Means in each column showing different letters differ significantly from each other at $p<0.05$. H: Holme Moss, F: Featherbed Moss(n=9)

Treatment	Ni		Pb		Cu		Zn		pH	
	KCl	Water	KCl	Water	KCl	Water	KCl	Water	KCl	Water
H heated	56.46^{a}	4.76^{a}	2026^{a}	68.12^{a}	82.99^{a}	153.76^{a}	436.85^{a}	97.92^{a}	2.93^{a}	4.45^{ab}
H unheated	41.96^{a}	5.54^{a}	1386.61^{a}	60.33^{a}	98.61^{a}	151.18^{a}	394.65^{a}	122.22^{a}	3.41^{b}	4.29^{a}
F heated	99.85^{b}	6.87^{a}	1819.02^{a}	91.12^{a}	52.75^{a}	95.98^{a}	1046.55^{a}	92.92^{a}	3.09^{ab}	4.4^{ab}
F unheated	51.78^{a}	4.68^{a}	1400.19^{a}	69.74^{a}	109.94^{a}	132.95^{a}	555.33^{b}	88.75^{a}	3.29^{ab}	4.73^{b}

Since both pH and metal concentrations showed significant effects of site and temperature, the relationships between these values were examined. No significant correlations were found between pH and KCl or water extractable concentrations of all four metals (n=36), except in the case of copper, which showed a significant positive correlation (r=0.355; P<0.05) with pH in the KCl extract (Fig. 3.31).

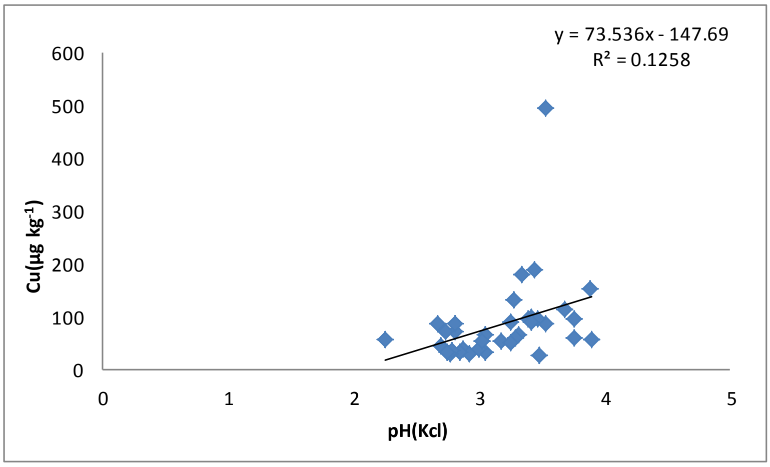


Fig. 3.31: Relationship between KCl extract pH and KCl extractable soil concentrations of copper (µg kg^{-1}).

However, when the values from the heated and unheated glasshouses were assessed separately, significant correlations were found, as shown in Table 3.12, which shows values based on 18 data points (2 sites*3 cores*3 soil levels) for each treatment. No significant correlations were found in the water extracts. However, in the KCl extracts, there was a tendency for the correlation coefficients between pH and metal concentrations to be negative in the unheated glasshouse and positive in the heated glasshouse. For Pb, both the negative correlation in the unheated glasshouse and the positive correlation in the heated glasshouse were significant, with Pb concentrations changing by a factor of 2 over a pH range of 1-1.5 (Fig 3.32). Negative correlations were also found in the unheated glasshouse cores for Ni and Zn, but these were only significant at P=0.10.

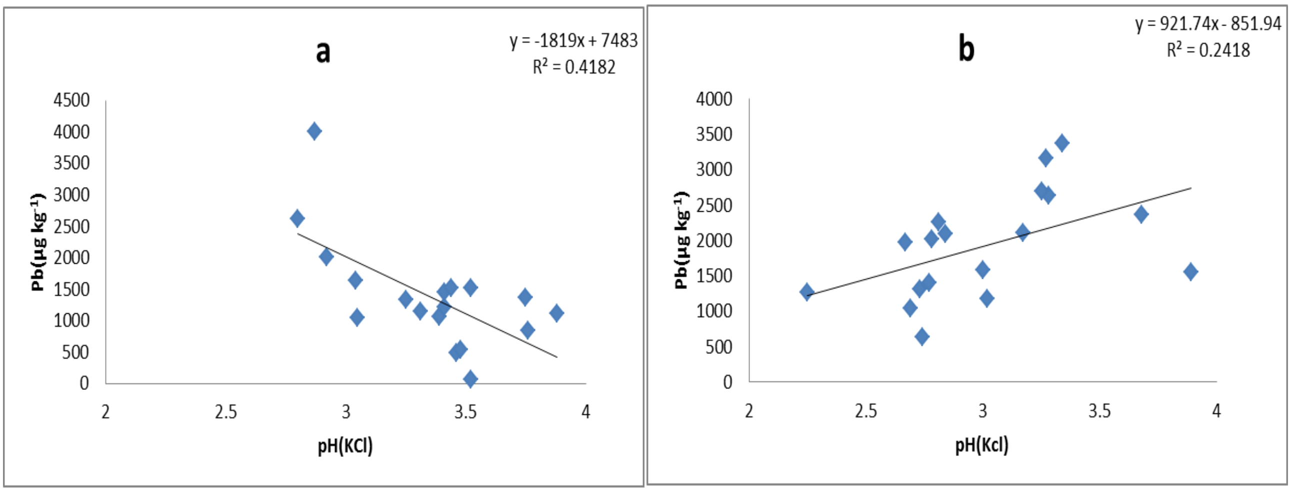


Fig. 3.32: Relationship between KCl extract pH and KCl extractable soil concentrations of Pb (µg kg $^{-1}$) (*a) in the heated and (b) unheated glasshouse.*

Table 3.12: Correlation coefficients between KCl and water extractable concentrations of metals and extract pH in samples from heated and unheated glasshouses

		KCl extract pH		water extract pH	
N=9		unheated	heated	unheated	heated
Ni	R	-0.42	0.19	-0.26	0.08
	P	0.08	0.45	0.29	0.74
Pb	R	-.647	.492	-0.27	-0.25
	P	0.00	0.04	0.27	0.308
Cu	R	0.33	0.38	0.16	0.39
	P	0.19	0.12	0.52	0.10
Zn	R	-0.43	0.10	0.045	0.26
	P	0.08	0.68	0.86	0.29

Table 3.13 shows the correlations between metal concentrations in the KCl extracts, averaged over the three depths, and the metal concentrations in porewater and leachate that was collected in Week 12. Rather than the positive correlations between the concentrations in porewater (and leachate), the results showed a trend towards negative relationships, even if none of these were significant at P=0.05.

Table 3.13: Correlations between metal concentrations in KCl extracts and pore water and leachate metal concentrations (µg l^{-1}) in week 12 (n=12)

	N=12	Pore water(µg l^{-1})	Leachate(µg l-1)
Ni KCl extract (µg Kg^{-1})	Pearson Correlation	-0.261	.-0.272
	Sig. (2-tailed)	0.412	0.392
Pb KCl extract (µg Kg^{-1})	Pearson Correlation	0.232	-0.466
	Sig. (2-tailed)	0.468	0.127
Cu KCl extract (µg Kg^{-1})	Pearson Correlation	-0.095	-0.341
	Sig. (2-tailed)	0.768	0.278
ZnKCl extract (µg Kg^{-1})	Pearson Correlation	-0.552	-0.342
	Sig. (2-tailed)	0.063	0.277

These relationships are shown for porewater in Figure 3.33. There was no obvious correlation between the values in the different cores, although there was a clear negative correlation for Zn, which was significant at P=0.10.

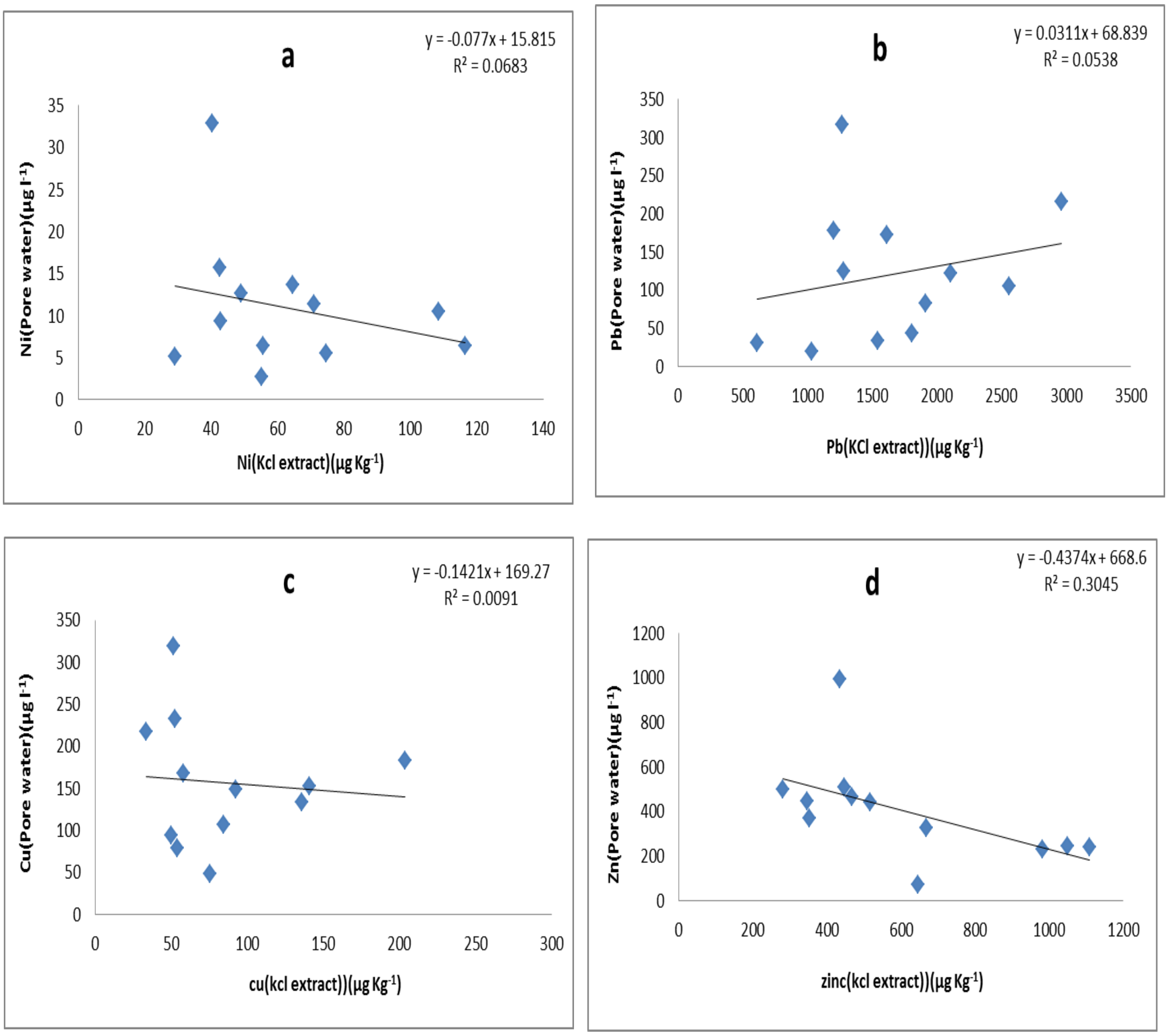


Fig. 3.33: Relationships between soil KCl extractable metal concentrations (µg kg $^{-1}$) and concentrations in pore water (µg l $^{-1}$) collected in Week 12.

3.4 Discussion

3.4.1 The influence of temperature on metal concentrations

Most chemical reaction rates are highly sensitive to temperature changes and thus effects metal speciation (Elder, 1989). As (noted in the Introduction) an increase of 10°C cans double biochemical reaction rates and it also affects rates of element uptake by an organism, because biological process rates (Luoma, 1983; Prosi, 1989). However, the experimental results did not show a consistent effect of temperature on soil solution metal concentrations. In particular, in the Featherbed Moss cores there was no consistent difference between the cores in the heated and unheated glasshouses throughout the 12 week period for any of the metals, even if, in the case, for example, of lead, there was a trend of increasing concentration over time for both treatments. In contrast, the Holme Moss cores showed a clear difference between the heated and unheated glasshouses. For nickel and lead, in particular, the concentrations in the unheated glasshouse were about four times higher than those in the heated glasshouse at the end of the 12 week experimental period. Zinc showed a similar trend, although less clearly, while there was no clear trend for copper.

Comparing these broad trends with those for pH, and for DOC, NH_4^+ and NO_3 concentrations, there are some clear similarities in the observed patterns. For DOC and pH, like the metals, there were no consistent differences between the unheated and heated glasshouses for the Featherbed Moss cores, but there were clear differences in the Holme Moss cores by the end of the experiment, with pH values being higher by the end of the experiment, but DOC values being lower, in the heated glasshouse. This suggests that effects of the temperature treatments on Pb and Ni pore water concentrations, and possibly also those of Zn, might be driven mainly by the changes in pH and DOC. However, NH_4^+ concentrations showed a different pattern, with little difference between heated and unheated treatments in Holme Moss cores but a higher concentration in the Featherbed Moss cores in the heated treatments.

Interpretation of the differences between the two treatments is complicated by the nature of the differences in temperature between them. While the weekly average temperatures, estimated from the max-min thermometers, were about 10°C higher in the heated glasshouse, this disguises some important differences between the maximum and minimum temperatures. The daily maximum temperatures in the unheated glass houses often reached temperatures that were similar to those in the heated glasshouse. In contrast, the minimum temperatures were quite different; those in the heated glasshouse never fell below 15^{o}C, but those in the unheated glasshouse showed much more fluctuation, falling below freezing point on some days. There are therefore significant uncertainties in understanding whether the observed treatment effects were due to the higher average temperature in the heated glasshouse or due to the lower minimum temperatures, and greater temperature range, in the unheated glasshouse. This is impossible to resolve with the current study design.

Several factors of importance to the mobility of metals have been found to be influenced by climate, including concentrations of DOC and, SO_4, pH, chemical weathering and acid neutralizing capacity (ANC) production (Veselý *et al.* 2003). However, very few studies have directly connected the climate-induced changes in any of these parameters to the secondary effects on metal mobility. There are uncertainties in the understanding of metal behaviour with respect to the change in temperature in this experiment, but it is of interest that greater metal concentrations were in general observed in the unheated, rather than the heated, glasshouses. For nickel and lead in particular, the concentrations were about four times higher than those in the heated glasshouse at the end of the 12 week experimental period in the Holme Moss cores. Laboratory studies have shown that simulated freeze/thaw cycles do cause DOM release (reviewed by Zsolnay, 1996), with the amount released being a function of the water content of the soil before freezing. DeLuca *et al.* (1992) suggest that freeze/thaw events in soil disrupt microbial tissues. Hence, one explanation for the higher concentrations of some metals in the unheated cores may be the greater release of DOC caused by the greater temperature ranges, rather than any inhibition of biological processes.

Despite these limitations, the treatments did have effects on metal concentrations in pore water, as well as on pH and DOC, and the experimental results still provide an opportunity to assess factors controlling the changes in metal concentrations in pore water in response to environmental change in contaminated peats.

3.4.2 Relationship between pH and metal concentrations in pore water

Significant negative relationships between metal concentrations in pore water and pH were found for Pb, Ni, and Zn, but only for Holme Moss. This may reflect the greater range of individual values in Holme Moss cores (3.37-5.65) compared to Featherbed Moss cores (3.47-4.36). The Ni, Zn and Pb concentrations in porewater decreased with increasing pH. The literature also suggests, for reasons described in previous Chapters, that mobility of metals increases with decreasing pH. However, this may not only be a direct effect of pH. Ni and other metals make complexes with DOC in soil solution, and soil solutions with high concentration of DOC provide more organic ligands to form metal complexes. Hence these relationships may also reflect the association of high DOC levels with lower pH values in this study, despite the reported inhibition of DOC production at low pH (Kabata-Pendias & Mukherjee, 2007; Tye *et al*., 2004; McGrath, 1995).

3.4.3 Chemical control of metal partitioning

Before discussing the relationships between pH, DOC and metal concentrations in any further detail, it is useful to discuss the basic chemical processes of relevance. The original hypotheses and objectives for this experiment were formulated as if there were independent controls of metal solubility by pH and DOC, and this was reflected in the statistical analysis that was undertaken. Care must be taken, however, in. for example, considering the effect of pH on metal solubility in isolation, since other important factors, such as DOC concentrations, can also be affected by pH. Suave *et al.* (1998) reported that increase in Pb solubility with decreasing pH below pH 6.5, but above neutral pH, Pb complexed with DOC with an increasing amount in the soil solution.McBride and Blasiak (1979) also confirmed the same mechanism for Zinc and copper as they react similarly in soil when they complexed with DOC at high pH.

Similar trends have been observed for Ni. The speciation and physico-chemical state of nickel, like those of other metals is important in considering its behaviour in soils and porewater and its availability to biota. These interactions are summarised in Figure 33, which will also apply in broad principles to other metals. Ni can occur in soil solution complexed with DOM, as a free ion or as inorganic complexes, each of which interact with the soil matrix and each other.

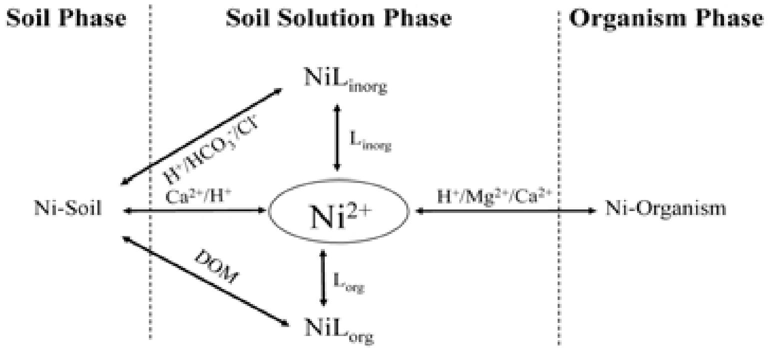


Fig. 3.34: Summary of interactions between nickel in soil solution phase, soil phase, and uptake by organisms. Source: Allen (2001).

Nickel takes part in nitrogen metabolism, and is essential for plants supplied with urea. At one well-studies site "low pH enhances the mobility of Nickel (Giusti *et al.* 1993); another study postulates that, the sulphate concentration and the surface area of soil iron oxides effected nickel adsorption" (Richter & Theis 1980). Lead, copper, and zinc are strongly adsorbed by soil, then Nickel .(Rai & Zachara 1984 Tyler & McBride (1982) found higher adsorption in organic soil than in mineral soils. Humus also plays an important role However adsorption of nickel by soil is site specific. (Richter and Theis, 1980;Giusti *et al.*,1993). "when one mole of nickel is adsorbed by iron and manganese oxide or for aluminum oxides,releases 1–1.5 and 2-2.5 moles H^+ respectively. Rai and Zachara (1984), and Mustafa and Haq (1988)

The high mobility of Ni is reported to be dependent to their affinity with soluble soil organic matter. Amrhein *et al.* (1992) also showed the increased solubility of Cu, Ni, and Pb in the presence of DOC. There are very few reports in the literature on the speciation of Ni in contaminated soil pore waters. Sanders and Adams (1987) examined free Ni^{2+} activities in 0.01 mol l^{-1} $CaCl_2$ extracts of sludge-amended soils using an ion exchange procedure (Sanders, 1983). Extractions were performed over a range of pH values between 4 and 8. They found that Ni in the extracts was predominantly Ni^{2+} at solution pH values less than 6, and above this pH, Ni^{2+} was increasingly complexed by soluble organic matter. More recently, Weng *et al.* (2003) also examined Ni speciation in 0.01 mol l^{-1} $CaCl_2$ extracts of soil and found similar results.

The higher concentrations of Ni when both hydrogen ion and DOC concentrations increase is thus readily explained by the model in Figure 33. However, it is clear that pH and DOC are themselves not independent, that the analysis in this study has not accounted for inorganic complexation, and that the competition with binding to organic matter in soils is also a significant factor. Hence, the changes in temperature in this experiment, may not simply affect metal concentrations in soil solution through changes in pore water pH and DOC concentrations.

3.4.4 Effects of temperature on concentrations of dissolved organic carbon and metals in pore water

In summer higher DOC concentrations are linked to temperature-dependent as accelerated biological activity enhances litter decomposition (McDowell and Likens, 1988). However, there was no consistent treatment effect across the cores from the two sites on DOC concentrations in pore water, although both treatments showed strong differences in their DOC concentrations in pore water over time, The sharp increase in DOC concentrations from the start of the experiment until week 4 could be due to an initial decomposition. It may also partly be due the fact to make mesocosms, soils were disturbed from natural conditions which could result in the system being much more dynamic and organic matter turning over relatively faster (Tipping et al, 2003; Michalzik *et al.*, 2003). The increased concentrations in the final weeks of the experiment may be associated with higher temperatures, and partially humified organic matter contributing to more DOC production (Michalzik *et al.*, 2003), as many studies have shown the highest DOC absorptions in leachates occur during late summer, when soil temperatures are highest (e.g. Kaiser *et al.*, 2002; Kalbitz *et al.*, 2000; Yano *et al.*, 2000).

The rate of mineralization of soil organic carbon might have been artificially increased in this study by adding 200 ml water every day until the end of 12th week. This might be expected to trigger the activity of the microbial biomass such that the rate of mineralization increased. The very high DOC concentrations at the end of the experiment may thus be explained by a combination of elevated temperatures, regular supply of water, the disturbance of the cores, and the lack of any vegetation to exploit the increased DOC concentrations.

The solubility behaviour with respect to DOC differed among the four metals. In general, concentrations of Pb and Zn paralleled the behaviour of DOC, approving the significance of DOC in metal mobility from contaminated peats, and similar relationships were found for nickel. The Pb and Zn concentrations in solution increased with increasing DOC concentrations, indicating that Pb and Zn are complexing with non-labile soluble organics. Yuan (2009) also reported that Ni and Zn formed complexes with dissolved organic matter. Through a series of soil types, soil solution Zn was present as the free ion more than 50 % (Lorenz *et al.*, 1997). According to (Reddy *et al.*, 1995) soil solution Zn was also found to be organically complexed, dependent on soil pH. Hence, DOC, while significant, does not tend to be as important feature as pH in influencing Zn bioavailability (Elrashidi and O'Connor, 1982). However, in highly organic soils, Zn may compete for dissolved organic carbon more effectively for soluble ligands. It has also been suggested that Pb and Zn bind to DOC of different molecular weights or to different functional groups of the DOC pool (Stevenson, 1976).

In general, concentrations of Cu, unlike those of Ni, Zn and Pb, did not parallel the behaviour of DOC (or pH) in this experiment. According to Stevenson (1991), copper ions form complexes with organic matter. Therefore, Cu is generally bound to the organic matter in the soil (Fotovat *et al.*, 1997). The level of Cu contamination is another important factor determining the fate of Cu (Pampura *et al.*, 1993). Reddy et al. (1995) found the "proportion of Cu bound to organic matter in the soil solution increased from 37 to 95% as the pH decreased (McBride *et al.*, 1997 (Jeffery and Uren, 1983).

It has been stated in literature that most of DOC is comprised of high molecular weight organics. It is likely that molecular weight differed and fractions of DOC react with metals were not similar and acted with trace elements in a special way. Hence competition between metals such as Zn and Pb for DOC can be important if concentrations in solution are high, (Stevenson, 1976).

3.4.5. Relationships between leachate and pore water concentrations of DOC and metals

Both the metal concentrations and DOC concentrations in the collected leachate were lower than those in porewater. This provides further evidence of the importance of DOC to metal mobilisation in these soils. The reasons for the lower concentrations in leachate are less clear, although DOC concentrations in leachate are known to be a result of decomposition rates and adsorption capacity versus transportation with percolating water.

There was a general homogeneity and lack of distinct horizons with depth in the cores, as is expected in these peat systems. This was also reflected in the lack of any significant effect of soil depth on metal concentrations or on soil pH. However, significantly higher DOC concentrations in pore water may have occurred in the upper zone, where the Rhizon samplers were installed, due to decomposition of residual fallen leaf litter at the core surface creating a small zone of partially decomposed organic material in the upper 0–10 cm. This would lead to a decrease in total water-soluble carbon with depth, Furthermore, leaching of DOC also plays an important role. (Kalbitz *et al.* 2000). “This leads to a important increased retention period of C in the soil” (Kalbitz & Kaiser, 2008). The same process could also result in transport of metals between soil horizons if they are tightly associated with the DOC. This process would be consistent both with the lower DOC and metal concentrations in the leachate and the lack of any significant effect of depth on soil metal concentrations.

Another cause may be differences in the source of the pore water and leachate metals and DOC. For leachate, this was more likely to be the larger macropores, as opposed to intermediate pores for Rhizon extracted water. The exact source of leachate DOC is, however, unclear and leachate water may also be contributed through micropores. DOM is distributed among different pore sizes within soils and different methods are probably therefore sampling different fractions of the soil DOC. (Kalbitz *et al.*, 2003; Zsolnay, 2003)

However, the experimental design may also influence the interpretation of these data. Leached water was collected from the bottom of the mesocosms, and therefore it is possible that water-extractable organic carbon from the soils is contributing to the leached water. This typically has a different chemical composition to DOC, with a high molecular weighed content of carbohydrates (Kalbitz *et al.*, 2003). Thus, the question arises as to whether the chemical characteristics of DOC collected in the Rhizon sampers and in the leachate was the same. However, the fact that all samples were filtered to 0.45um means that DOC should dominate in both sets of samples. In addition, the time and methods employed for the pore water extractions and leachate collection were not identical. The fact that leachate was left in trays for one week, when it was exposed to temperature changes and evaporation may have occurred, could have led to changes in DOC concentrations.

3.4.6. Effect of treatment on soil metal concentrations

As expected, there were no treatment effects on the total pool of metals in the cores, as represented by the acid digest concentrations. However, there were treatment effects on the NaCl extractable metal concentrations. In general, these concentrations were higher in the heated glasshouse for Pb, Zn, and NI, but were lower in the heated glasshouse for Cu. Although this would be consistent with greater pore water concentrations in pore water in the unheated glasshouse, at least for Holme Moss cores, this is unlikely to represent a simple difference in the loss rate of metals, since Pb, Zn and Ni concentrations in pore water showed no effect of treatment in Featherbed Moss cores, but there was still an effect on NaCl extractable metal concentrations from this site. Furthermore, there was not the negative association between NaCl extractable metal concentrations and those in pore water or leachate that would be expected if this were the case.

A more likely explanation lies changes in chemistry within the cores. The soil pH was significantly lower in NaCl extracts in the heated glasshouse for Pb, Zn and Ni, and this might increase the available fraction of the soil metal pool. However, no significant correlations were found between pH and NaCl extractable metal concentrations, except in the case of Cu, for which a positive relationship was found, while the relationships for Pb concentrations appeared to differ in heated and unheated glasshouses. Most likely, the changes in NaCl extractable metal concentrations are associated with another change in soil chemistry or microbial activity which was not assessed in this study.

3.4.7 Effects of temperature on ammonium, nitrate and metal concentrations

Concentrations of N in porewater were dominated by NH_4^+-N for the majority of the 12 weeks in both treatments. Higher NH_4^+-N concentrations occurred in the final weeks of the experiment in both treatments, which might be attributed to increased temperature which generally activates decomposition (Hedin *et al.*, 1995). As for DOC, this effect signifies changes in core chemistry and microbial activity over time, which are not necessarily associated with rising temperature, although NH_4^+-N concentrations tended to be higher in the weeks with high temperature. Cresser *et al.*, (2004) explained that transformation of ammonium to organic N or nitrate in the UK are very slowly. Therefore, ammonium can accumulate on the cation exchange sites; at some point ammonium leaching may then start to occur, and elevated ammonium concentrations are found in adjacent surface waters.

In contrast to ammonium, there was a reduction in nitrate concentrations over time in all treatments that may indicate an increase in temperature-dependent events rather than productive processes. Although it could be suggested that this decline is indicative of denitrification (Groffman *et al.* 1999), and nitrate can be partially removed by microbial assimilation and denitrification (Groffman *et al.* 1992; Lowrance 1992; Hill *et al.* 2000). It seems more likely that the effect is due to the warmer temperatures favouring an increase in microbial biomass and biomass turnover, and thus microbial immobilisation of nitrate-N (Luo *et al*., 2001). Warming has been reported to increase below-ground carbon allocation and consequently enhance microbial nitrogen immobilization and reduce net N mineralization (Wan, 2005).

The concentrations of ammonium in leachate were lower than those in porewater, while those of nitrate were higher. infact N deposition occurs in upper soil horizons (Aber *et al.* 1989), since the concept was presented not much attention has been paid Krug and Winstanley (2002) though sub soils are now getting higher Nitrogen inputs (Cresser *et al.* 2008; Riaz *et al.* 2008).

There were consistently significant positive correlations between NH_4^+-N concentrations and concentrations in particular of Zn and Pb. However, no study in the literature has previously reported a role for ammonium in metal mobility and soil- porewater partitioning. One possibility is that the results in this study do not reflect a causal relationship, but rather reflect the high correlations between ammonium and DOC in porewater.

However, there are possible interactions between temperature, N deposition and DOC production. It is possible to relate the effects of accumulated NH_4^+ in peats to changes in decomposition rates at higher temperatures for material accumulated under higher atmospheric N supplies, which would also lead to increased DOC release. Hence increased N availability as a result of atmospheric deposition favours increased microbial decomposition, because peatlands are exclusively nurtured by atmospheric deposition, which in turn has effects on the DOC released during litter breakdown. Accordingly, an understanding of the Processes of soil responses to changing N status, and the consequences for metal mobility, is essential to better understand any independent effects of NH_4^+-N and DOC on metal mobility. This requires a controlled experimental manipulation of N inputs rather than the correlative analysis described in this Chapter. Chapter 4 describes such a study of the effects of changing deposition of both nitrate and ammonium on pore water chemistry and metal mobility.

Chapter 4 – Nitrogen induced heavy metal leaching; the effects of different forms of nitrogen

4.1 Introduction

4.1.1. Background

The global nitrogen cycle has dramatically changed since the discovery of industrial nitrogen fixation by Haber and Bosch in 1909 (Galloway et al., 1995). Total nitrogen deposition to non-forest ecosystems in the UK range now shows a large spatial variation, ranging from high (25-35 kg N ha^{-1} yr^{-1}) to very low (3-5 kg N ha^{-1} yr^{-1}). Over the 20 years since 1988, N deposition in the UK has fallen by about 15%, but it is unclear if soil processes are recovering due to decrease in N deposition, as highest rates of Nitrogen emissions and deposition in the 20th century which were instantly headed by four decades (Fowler *et al.*, 2004b; RoTAP, 2012). This decline seems to be late to prevent and fully converse the destruction caused in the most N-polluted regions of the UK by the inputs of 3000–5000 kg N ha^{-1} from 1900 to 2000 (Fowler *et al.,* 2004b). Also, in spite of the considerable recent decreases in releases, according to Matejko *et al.,* 2009, the future deposition rates modelled in 2020 suggests that critical loads will continue to be exceeded in about half the area of the most sensitive ecosystems in the UK (RoTAP, 2012).

However, it is important that nitrogen is deposited in two main forms, a reduced (mainly ammonia, NH_3 or ammonium, NH_4^+) and an oxidized (mainly nitrate NO_3^-) form. While emissions of oxidised nitrogen in the UK declined by 58% in the period 1988-2008, those of reduced nitrogen only declined by 21% (RoTAP, 2012). Furthermore, high emissions of reduced nitrogen are associated with regions of intensive agriculture, while regions of high emissions of oxidised nitrogen are associated with high vehicle density or industrial activity. Hence the proportion of nitrogen deposition that is in the oxidised or reduced form can vary spatially across the UK, and is also changing over time. For example, the UK shows a large spatial range in NH_4^+/NO_3^- deposition ratios, from 0.3 mol mol^{-1} to 3 mol mol^{-1} (Fowler *et al.*, 2004). Improved understanding of the effects of different forms of N deposition on soil biogeochemical processes, toxicity mechanisms and ecosystem function is important because it would allow sites and habitats at greatest risk to be more reliably identified, and hence provide a greatly improved evidence base for protection measures. Furthermore, these different nitrogen forms may have differential effects on vascular plants and bryophytes as a result of preferential uptake mechanisms, direct toxicity and indirect effects through changes in soil chemistry.

Soil acidification and soil buffering capacity, measured as the acid neutralizing capacity (ANC), are thought to play an important role in this. In addition, nitrogen mobility is partly driven by DOC (Kaiser 2001), which is a potentially important component of carbon export from the soil system under highly organic conditions. Atmospheric nitrogen deposition may be linked to increased DOC production through increased microbial activity, DOC is particularly significant in the transport of metals as metals form complexes with DOC. Hence increased nitrogen deposition may result in increased metal transport to deeper ground water layers. In particular, in soils with high metal concentrations, and which are slightly acidified and depleted of base cations, and consequently in the aluminium buffer range, may be expected to experience elevated levels of metals in soil solution through both the decreased pH and the increased DOC concentrations (Jones 1998; Tipping & Hurley 1992).

4.1.2. Introduction to the long-term experiment

A large mesocosm experiment at the University of York was specifically designed by Dr Leon van den Berg to test the differential effects of different dominant nitrogen forms in atmospheric deposition on nutrient-poor species-rich wet heathlands. In a full factorial design, the effects of nitrogen load, nitrogen form and liming on floristic and biogeochemical parameters were tested to compare effects of reduced and oxidized nitrogen deposition; specifically, the same nitrogen deposition load was applied with ammonium/nitrate ratios varying from 1:9 to 9:1 (mol:mol). Yearly changes in vascular plant and bryophyte cover and composition were recorded and pore water was analysed monthly. This experiment lasted for over 3 years until the final plant and soil harvest was done.

The pore water samples from the first two years of the experiment were analysed by Dr van den Berg (pers.comm.) and the results showed little effect of total N load on mean pore water DOC concentrations or pH. However the DOC concentration and pH were significantly higher when nitrate was the dominant nitrogen form in deposition, rather than ammonium. Application of base cations in the form of lime also resulted in an increase in pH and DOC concentration, especially in those treatments where ammonium was dominant.

Hence the evidence obtained by Dr Van den Berg showed that the nitrate/ammonium ratio affected both pH and DOC concentration in pore water. Both of these, as shown in Chapters 2 and 3, are likely to affect metal concentrations in soil solution. Furthermore, these mesocosms were taken from the Isle of Skye, Scotland in an area with underlying serpentine rock. Serpentine rock is known for its elevated concentrations of heavy metals such as copper, nickel, chromium and zinc (Alexander, 2004). This therefore provided a unique experimental set-up to test the hypothesis that ammonium and nitrate deposition have differential effects on heavy metal leaching through their differential effects on DOC and on pH. The work described in this chapter involved analysis of pore water samples that were taken in the final 15 months (from June 2009 to September 2010) of the experiment, and an analysis of soil metal concentrations at the end of the experiment.

4.1.3 Aims and Hypotheses

The broad aim of this study was to determine if different nitrogen deposition loads and ratio of ammonium to nitrate altered porewater concentrations of metals. The following specific hypotheses were tested:-

1. Total nitrogen deposition load has a significant effect on (Ni, Cu, Pb, Zn and Cd concentrations in pore water.
2. The ratio of ammonium to nitrate in deposition has a significant effect on metal concentrations in pore water.
3. The application of lime has a significant effect on metal concentrations in pore water.
4. The effects of load, ratio and liming on metal concentrations in porewater can be explained by the changes in soil solution pH and DOC
5. Mobilisation of Cu and Pb is primarily associated with increased DOC concentrations and therefore Cu and Pb concentrations in porewater are significantly higher in cores where nitrate is dominant in deposition.

6. Mobilisation of Cd, Ni and Zn is primarily associated with increased acidity, and therefore Cd, Ni and Zn concentrations in porewater are significantly higher in cores where ammonium is dominant in deposition.

4.2 Materials and Methods

4.2.1 Overview of overall study design

As described above, the work described in this chapter is part of a long-term mesocosm experiment that began in July 2007. The experiment was originally set up to address two main hypotheses:-

(1) High NH_4^+/NO_3^- ratios in atmospheric N deposition have a significant effect on biogeochemical processes and species composition which is independent of total N deposition

(2) Soil acid neutralizing capacity (ANC) modifies the effects on biogeochemical processes and species composition response of high NH_4^+/NO_3^- deposition.

This section summarizes the major features of the overall experiment that are relevant to the specific work described in this chapter. The next section then describes the methods used in the specific work described in this chapter in more detail.

4.2.2. Sampling and experimental set-up

The intact monoliths, including both the soil and vegetation, were taken in July 2007 from a wet-heath site (NVC M15 wet heath) on the Isle of Skye, UK (grid ref: GR 408224), a site with long history of low N deposition (estimated at 1-3 kg N ha^{-1} yr^{-1}). A uniform patch of M15 vegetation was selected. Within this area, 96 PVC cores (25 cm height and a diameter of 20 cm) were pushed in to the peat while saving the vegetation. These were then dug up to obtain intact peat cores including vegetation. They were transferred to greenhouse facilities at the University of York, where the experiment took place. The glass house selected followed outside fluctuations in temperature but was generally warmer. The evaporation was, as far as possible, controlled for by watering (with additional watering in the summer) with deionised water. The water level in the cores was controlled for by tubes outside the cores that were cut at 5 cm below the surface. Soil monoliths, with intact vegetation, with a diameter of 20 cm and a height of 25 cm, were fitted with a plastic bottom. This included an outlet to maintain pore water levels at 5cm below field level. Each core was fitted with two Rhizon samplers (Eijkelkamp, The Netherlands), one at 10 cm below the surface of the core and one at 20 cm depth. More details of the use of Rhizon samplers were provided in Chapter 2. The sides of the monoliths were covered with bubble aluminum foil to prevent large effects on soil temperature.

N deposition was simulated using artificial rainwater (deionised water with macro- and micro-nutrients at background concentrations) with varying NH_4Cl and $NaNO_3$ concentrations (Van den Berg *et al.*, 2008) applied 3 times per week. Five different NH_4^+/NO_3^- ratios in simulated atmospheric deposition were tested (1:9, 1:5, 1:1, 5:1 and 9:1 (mol:mol)) representing a gradient in ammonium dominance to nitrate dominance at two total N deposition loads (16 and 32 kg N ha^{-1} yr^{-1}), corresponding approximately to respectively the critical N load and the highest ambient N deposition rates in the UK for this ecosystem. These five different NH_4^+/NO_3^- ratios were also tested at a fixed N deposition load of 32 kg N ha^{-1} yr^{-1} with a lime ($CaCO_3$ 80% $MgCO_3$ 10%) addition (100 kg m^{-2}) applied as a powder to the soil surface at the start of the experiment. Lime addition was only added once, assuming that enough lime was added to compensate for base cation depletion throughout the experiment without changing the soil pH too much. These treatments started on 18th August 2007. There were six replicates of each treatment, resulting in 90 intact monoliths (Table 4.1).

Table 4.1: Summary of the experimental treatments, providing different NH_4^+ and NO_3^- ratios at two fixed total N deposition loads, with an additional lime application at the higher deposition load. Nnumbers in columns represent replicates in each case.

NH_4^+/NO_3^- Ratios	**32 kg N ha^{-1}yr^{-1} with lime**	**32 kg N ha^{-1}yr^{-1}**	**16 kg N ha^{-1}yr^{-1}**
1 : 9	6	6	6
1 : 5	6	6	6
1 : 1	6	6	6
5 : 1	6	6	6
9 : 1	6	6	6

4.2.3 Detailed methods

Pore water samples for detailed analysis were selected from those taken in the final 15 months of the experiment, from June 2009 to Sep 2010, from both 10cm and 20 cm depths. Those selected for metal analysis were from June 2009, September 2009, January, 2010, April 2010, July 2010, and September 2010, only for the top 10 cm. This gave approximately 600 samples. These samples were analysed for pH, DOC concentrations, nitrate and ammonium concentrations, as follows, following the procedures described in Chapter 2.

Soil solution samples were analysed for dissolved organic carbon concentration using a TOC elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Samples were analyzed as soon as possible after collection. Samples to be analysed for DOC were first filtered through 0.45 µm Whatmann. From a basic stock solution of 500 mg l^{-1} TIC + 500 mg l^{-1} TOC (using sodium carbonate as the TIC standard and potassium phthalate as the TOC standard), intermediate stock solutions were produced for calibration. Calibration was done at concentrations of 1, 2.5, 5, 10, 20, and 50 mg l^{-1} DOC.

The extracts and pore water were stored at 4^{o}C until analysis for ammonium-N and nitrate-N using a standard Auto Analyser (Bran+Luebbe Auto Analyser 3 digital colorimeter) protocol with matrix-matched standards, as soon as possible (generally the next day) after extraction. Ammonium nitrate standards for KCl (for KCl extracts of soils) and H_20 (for pore water and water extracts of soil) at 2 ppm, 1.5 ppm, 1.0 ppm, 0.5 ppm and 0 ppm were made in a 100 ml volumetric flask. The samples were then run through the Auto-Analyser.

Pore water samples for metal analysis were acidified before storing at 4^{o}C, with concentration of 100 µl 70% HNO_3 to every 10 ml sample to prevent metals from settling.

Soil samples from the 90 cores were taken in October 2010 and frozen. These soil samples were taken by cutting at least 70 g fresh soil from the top 15 cm of peat in each core. These samples were then homogenized by hand, roots were removed, and they were dried. Homogenized portions of 200 mg dry soil were digested with 4 ml HNO_3 (65%) and 1 ml H_2O_2 (30%), using an Ethos D microwave labstation (Milestone srl, Sorisole, Italy) as described in Chapter 2. Digests were diluted and concentrations of metals in these samples and pore water samples were determined by ICP-MS and ICP-OES. Zn concentrations were measured using an ICP Spectrometer (IRIS Intrepid II, Thermo Electron Corporation, Franklin, MA), and those of other metals were determined using ICP Spectrometer (X series; Thermo Fisher Scientific, Waltham, MA, U.S.A). Further details of analytic methods were given in Chapter 2.

4.2.4. Statistical Analysis

The effects of load and ratio, and their interactions, on soil solution parameters, averaged for each core over the six monthly samples, using a two way-analysis of variance. A similar analysis was conducted for the effects of liming and ratio, and their interaction. This design meant that the data for the 32 kg N ha^{-1} yr^{-1} treatment were included in both anovas, but it was the most effective way of testing the original hypotheses. Tukey's student range tests were used to identify significant differences between individual ratio treatments. A similar analysis of variance was conducted for metal concentrations in acid digests at the end of the experiment. Bivariate correlations between metal concentrations in pore water and pH, DOC, and ammonium and nitrate concentrations were also tested across the 15 treatments. All statistical analysis was carried out in SPSS 19.

4. 3. Results

4.3.1 Overview of analysis of variance

Table 4.2 shows the results of the anova including load and ratio, and their interaction, while Table 4.3 shows the results of the anova including lime and ratio, and their interaction. Table 4.2 shows more significant effects of ratio than load, clearly demonstrating the importance of the ammonium/nitrate ratio, while Table 4.3 shows a range of significant effects of both liming and ratio. There no significant effects of load, liming, ratio, or their interaction for nickel and lead concentrations, or for ammonium concentrations. As in the previous experiment, Cd concentrations were close to the instrumental detection limit and were not analysed further.

Table 4.2: Analysis of variance of effects of load, ratio and their interactions, on pH, the mean metal concentrations, and those of DOC, NH_4^+ and NO_3^- over the period from June 2009 to September 2010. Level of significance is indicated as: * $p<0.05$, ** $p< 0.01$ *and* *** $p< 0.001$.

Treatment		pH	DOC	Ni	Cu	Zn	Pb	NH_4	NO_3
Load	F	0.400	0.868	0.072	3.83	0.480	1.147	1.875	2.468
	p	0.527	0.353	0.788	.051	0.489	0.285	0.172	0.118
Ratio	F	4.638	6.57	0.154	2.44	3.896	0.213	1.054	2.046
	p	0.001 ***	0.000 ***	0.961	0.048 *	.004 **	0.931	0.380	0.380
Load*Ratio	F	1.617	3.395	0.661	2.961	0.722	1.196	1.969	2.939
	p	0.170	0.01 **	0.619	0.021 *	0.578	0.313	0.100	0.022 *

*Table 4.3: Analysis of variance of effects of liming, ratio and their interactions, on pH, the mean metal concentrations, and those of DOC, NH_4^+ and NO_3^- over the period from June 2009 to September 2010. Level of significance is indicated as: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.*

Treatment		pH	DOC	Ni	Cu	Zn	Pb	NH_4	NO_3
Lime	F	24.009	8.302	0.067	2.787	10.085	0.048	1.684	2.688
	p	0.000 ***	0.004 ***	0.796	0.096	0.002 ***	0.826	0.196	0.102
Ratio	F	5.021	5.390	0.995	0.240	9.033	0.477	2.761	1.035
	p	0.001 ***	0.000 ***	0.411	0.915	0.000 ***	0.752	0.280	0.396
Lime*Ratio	F	1.199	1.926	1.030	4.658	0.437	0.842	0.752	2.042
	p	0.312	0.107	0.393	0.001 ***	0.782	0.500	0.139	0.089

Before discussing the effects of the ammonium/nitrate ratio specifically, the effects of load and liming are considered in the following section.

4.3.2 Effect of load and liming on soil pore water parameters and metal concentrations

As this study was part of a long-term mesocosm experiment, in which the analysis of metal concentrations started two years after the experiment commenced, it is useful to have an overview of how the values of pH, DOC, NH_4-N and NO_3-N in soil solution in the first twenty months compared with those during the later part of the experiment, for which data were collected and analysed in this chapter. Figure 4.1 shows the effects of load and lime during these two periods.

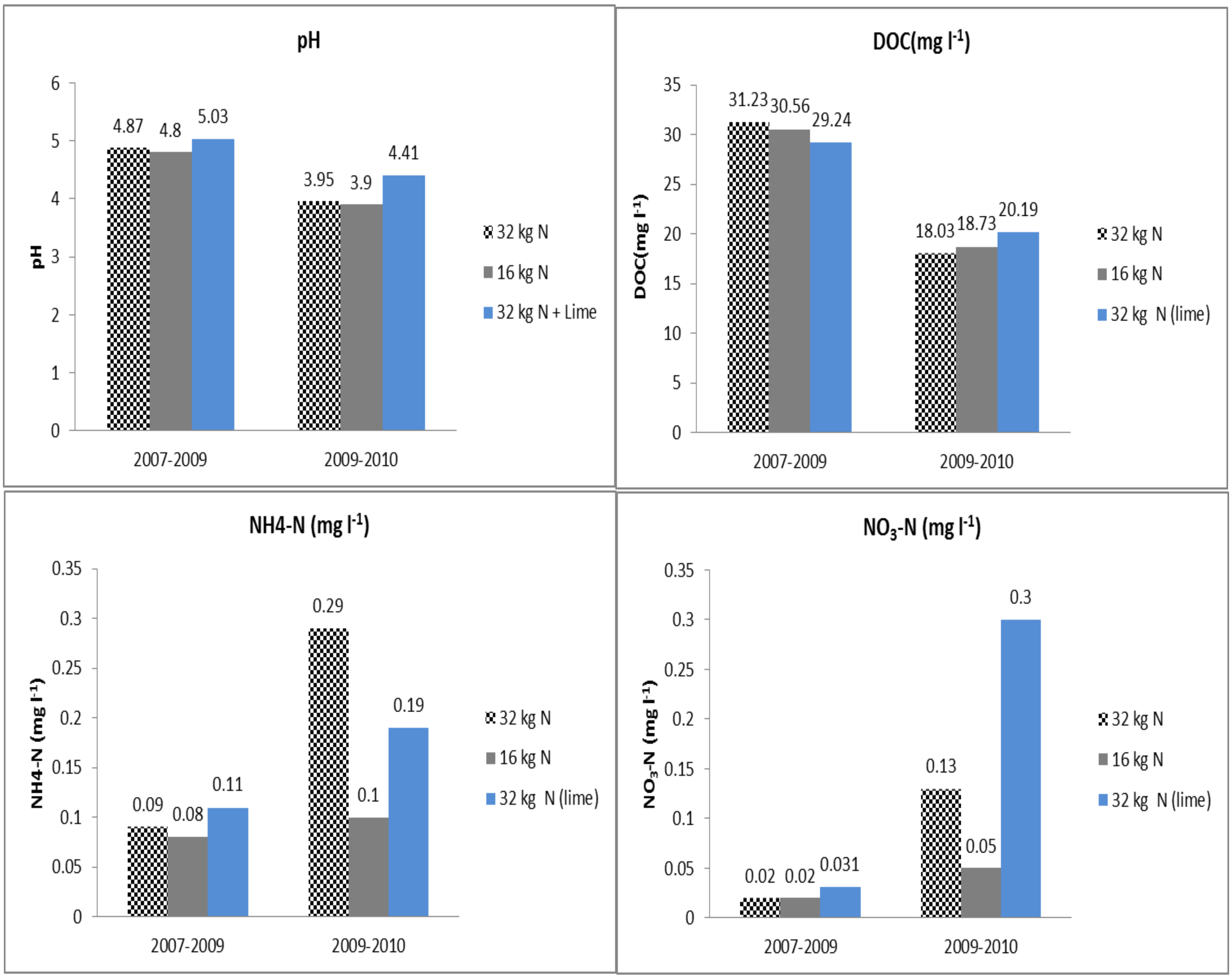


Fig. 4.1: Mean pH, and concentrations of DOC, NH_4-N and NO_3-N (mg l^{-1}) in the three load and lime treatments for the first 20 months of the study (Aug 2007 to Apr 2009) and the final 15 months (June 2009 to Sept 2010).

In general terms, the effects of the treatments were more marked in the experimental period than in the earlier twenty months. As expected, concentrations of NH_4^+-N and NO_3^--N were higher in the later period, reflecting the cumulative effects of N deposition over time, while pH was lower, reflecting a cumulative acidification of the experimental mesocosms. However, DOC concentrations were lower in the later period than in the first 20 months.

Figure 4.2 shows the effects of load and liming on these parameters in more detail for the study period. As indicated in Table 4.2, there was little overall effect of the N deposition load on any of the soil solution parameters. Both ammonium and nitrate concentrations were, as expected, higher in the 32N (32 kg ha^{-1} yr^{-1}) than in the 16N (16 kg ha^{-1} yr^{-1}) treatment, but these effects were not significant even at P=0.1 (Table 2). However, liming did have a significant effect on the mean soil solution pH, increasing it from about 3.9 to about 4.4. As shown in Table 4.3, this effect was significant at P=0001. There was also a significant effect of liming on DOC, with a small increase, from about 18 mg l^{-1} in the 32N treatment to about 21 mg l^{-1} in the 32NL (32 kg ha^{-1} yr^{-1} plus lime) treatment. Although liming approximately doubled the mean ammonium concentrations and trebled the mean nitrate concentration in soil solution, these effects were not significant, reflecting the high variation in these concentrations (Fig 4.2).

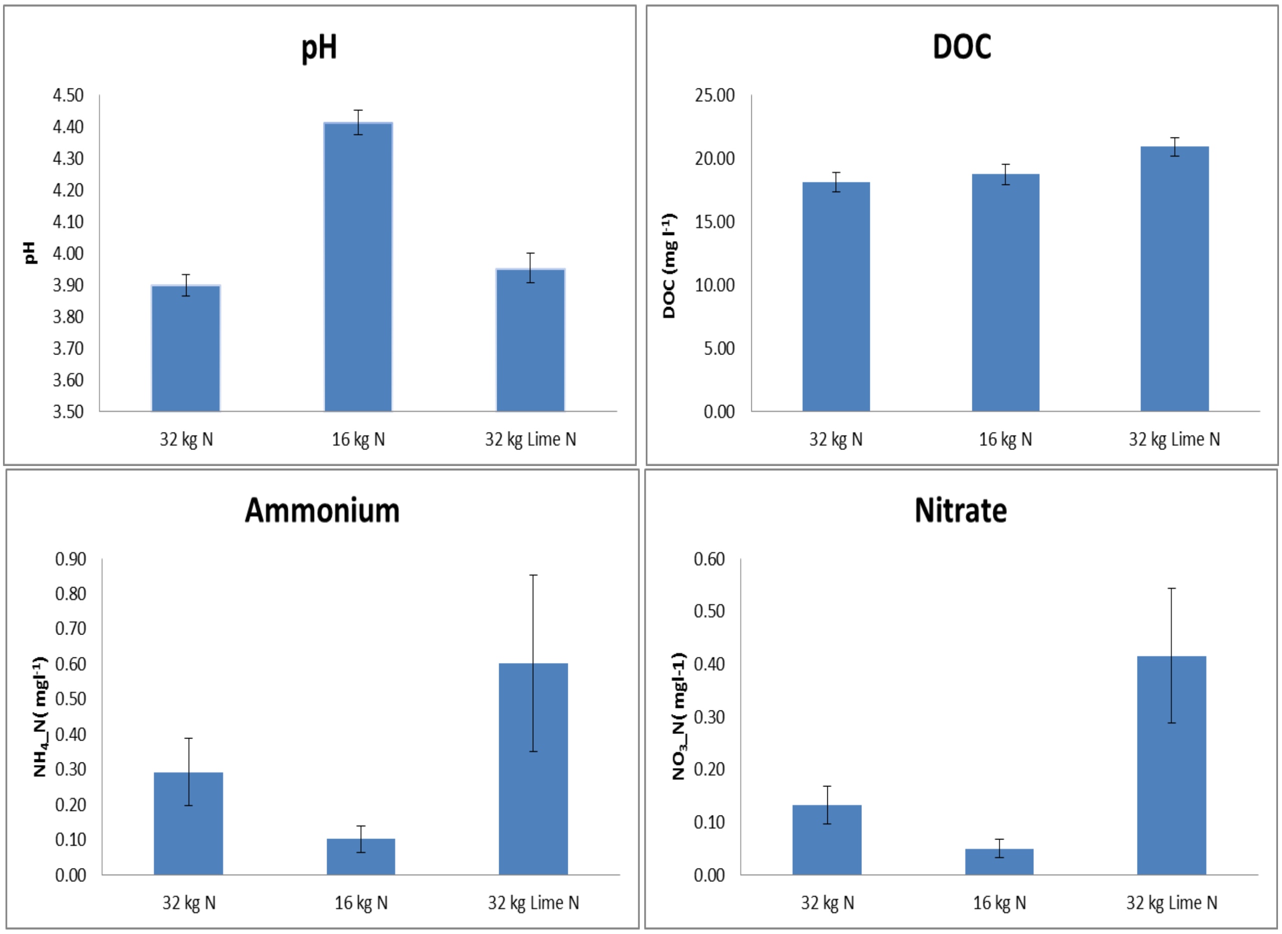


Fig. 4.2: Mean values in the three load and lime treatments of pH, and concentrations of DOC NH_4-N, and NO_3-N (mg l^{-1}), over the period June 2009 to Sept 2010. Values are the mean and the error bars, Error bars indicate standard errors of means (±1s.e)

Figure 4.3 shows the mean pore water concentrations of metals in the different load and lime treatments over the course of the experiment. Pb and Ni showed no significant effect of either liming or load (Table 4.2 and 4.3); Pb concentrations in soil solution were uniformally low, while the mean Ni concentrations differed by less than 3% between the three treatments (Fig. 4.3). However, there was a significant effect of liming, but not load, on

Zn concentrations, which were about 15% lower in the 32NL treatment than in the 32N treatment. In contrast, liming increased Cu concentrations significantly, by about 30%. The Cu concentrations in the 32N treatment were 25% higher than in the 16N treatment, although this effect was only significant at $P<0.1$.

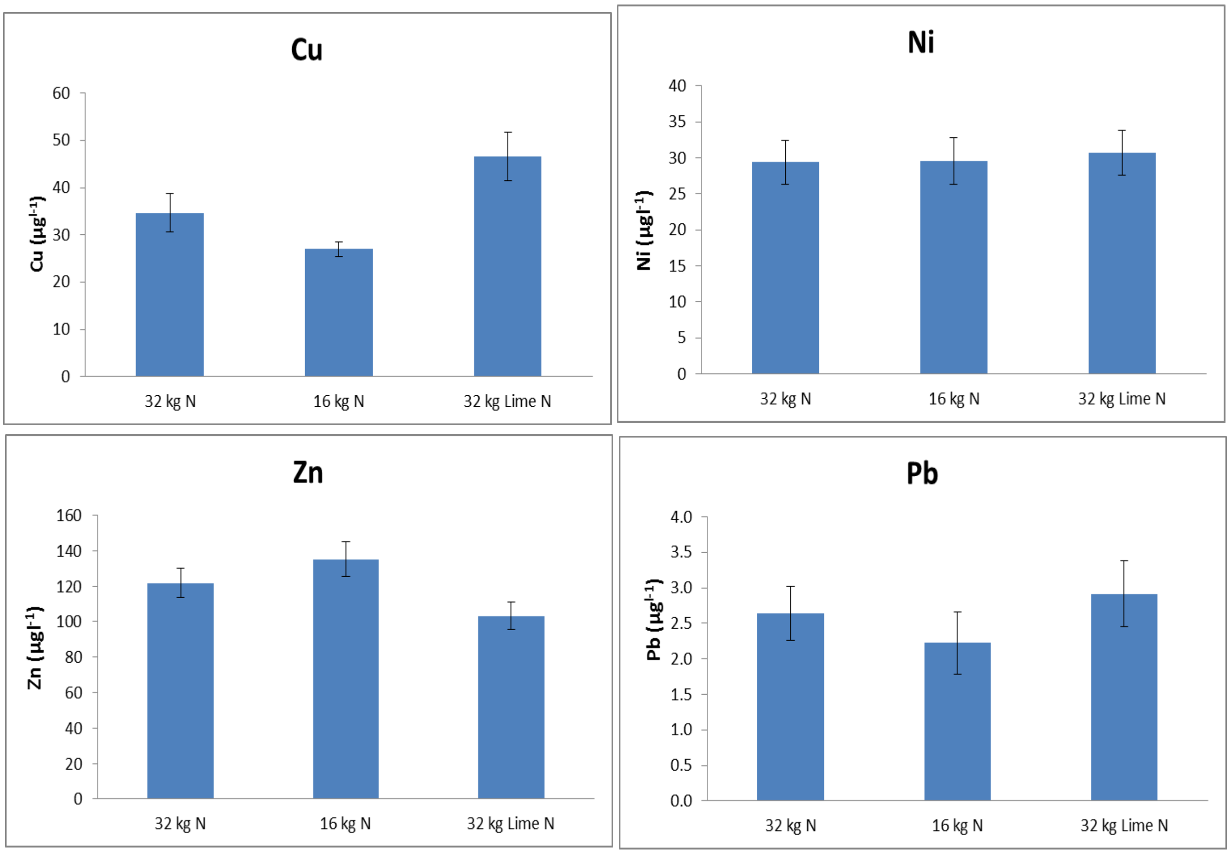


Fig. 4.3: Mean concentrations of metals (µg l^{-1}) in pore water in the three load and lime treatments, over the period June 2009 to Sept. 2010. Error bars indicate standard errors of means (±1s.e)

4.3.3 Effect of ammonium/nitrate ratio in combination with two loads

Table 4.4 shows the mean values of all the measured parameters in soil solution, averaged over the two nitrogen loads. The results which showed significant effects of ratio, or a significant load/ratio interaction, are discussed in more detail below.

Table 4.4: Mean of the values of each parameter in the five ammonium/nitrate ratio treatments, averaged for the 16N and 32N treatments.

Parameter	ratio	9:1	5:1	1:1	1:5	1:9
pH		3.34	3.53	3.63	3.98	4.08
DOC (mgl^{-1})		14.1	16.0	18.2	21.4	20.9
Ni ($\mu g\ l^{-1}$)		27.0	27.8	28.5	31.3	28.7
Cu ($\mu g\ l^{-1}$)		25.9	26.2	27.8	26.7	46.0
Zn ($\mu g\ l^{-1}$)		135.0	135.9	129.4	95.7	105.4
Pb ($\mu g\ l^{-1}$)		2.40	2.51	2.10	2.53	2.27
NH_4^+-N ($\mu g\ ml^{-1}$)		0.05	0.33	0.25	0.05	0.23
NO_3^--N ($\mu g\ ml^{-1}$)		0.02	0.07	0.20	0.13	0.10

The ratio of ammonium to nitrate affected pH significantly (P=0.001; Table 4.2) but there was no significant interaction with deposition load. Figure 4.4 shows that the pH decreases with increasing ammonium dominance, with a mean value of 3.34 at a NH_4^+/NO_3^- ratio of 9:1, compared to a mean value of 4.08 at a NH_4^+/NO_3^- ratio of 1:9.

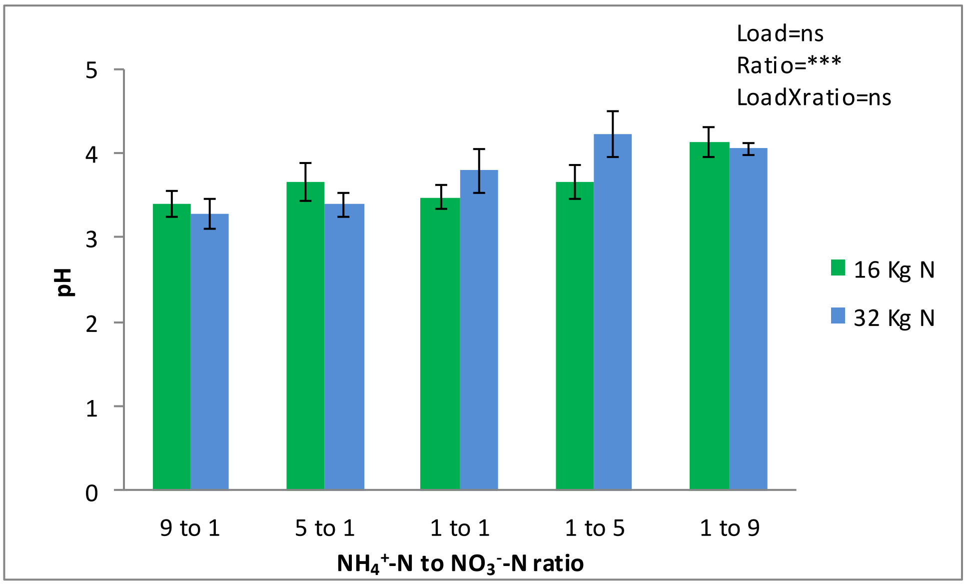


Fig. 4.4: Effect of ammonium/nitrate ratio on soil solution pH over the period June 2009 to Sept. 2010. Values are the means across the 16N and 32N treatments. Error bars indicate standard errors of means (±1s.e)

Table 4.2 shows that there was a highly significant effect of load on DOC concentrations, but that there was also a significant interaction with deposition load. The mean DOC concentration increased with increasing nitrate dominance, from 14.1 mg l^{-1} at a NH_4^+/NO_3^- ratio of 9:1 to a mean value of 20.9 mg l^{-1} at a NH_4^+/NO_3^- ratio of 1:9. This finding is consistent with the hypothesis that DOC production is inhibited by the lower soil solution pH when ammonium is dominant. Figure 4.5 shows that the effect of nitrate dominance was much stronger at the higher deposition load than at in the 16N treatment, as might be expected if the ratio effect on DOC was dose-dependent.

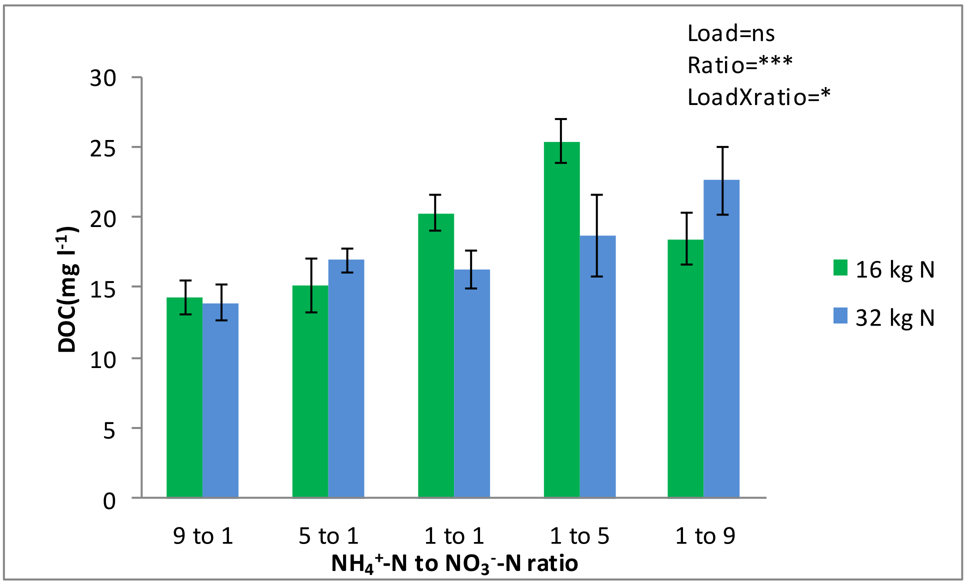


Fig. 4.5: Effect of ammonium/nitrate ratio on soil solution DOC over the period June 2009 to Sept. 2010, in the 16N and 32N treatments. Error bars indicate standard errors of means (±1s.e)

There was no significant effect of load or ratio, or their interaction, on Ni or Pb concentrations in soil solution (Table 4.2), although there was a slight trend for higher Ni concentrations when nitrate was the dominant ion (Table 4.4). However, significant effects of the ammonium/nitrate ratio were found for Cu and Zn.

Figure 4.6 shows the effect of ammonium/nitrate ratio on Zn concentrations. This effect was significant at P=0.01, with no significant interaction with load (Table 4.2). The mean Zn concentration was 135 µg l^{-1} over the two treatments with a NH_4^+/NO_3^- ratio of 9:1 and 5:1, but when nitrate was dominant (NH_4^+/NO_3^- ratios of 1:9 and 1:5), it fell to 100 ug l^{-1}. Zn partitioning between the soil and soil solution is thought to be mainly controlled by pH, which is consistent with the higher Zn concentrations that were observed at the lower soil solution pH that was found when ammonium dominates (Figure 4.4).

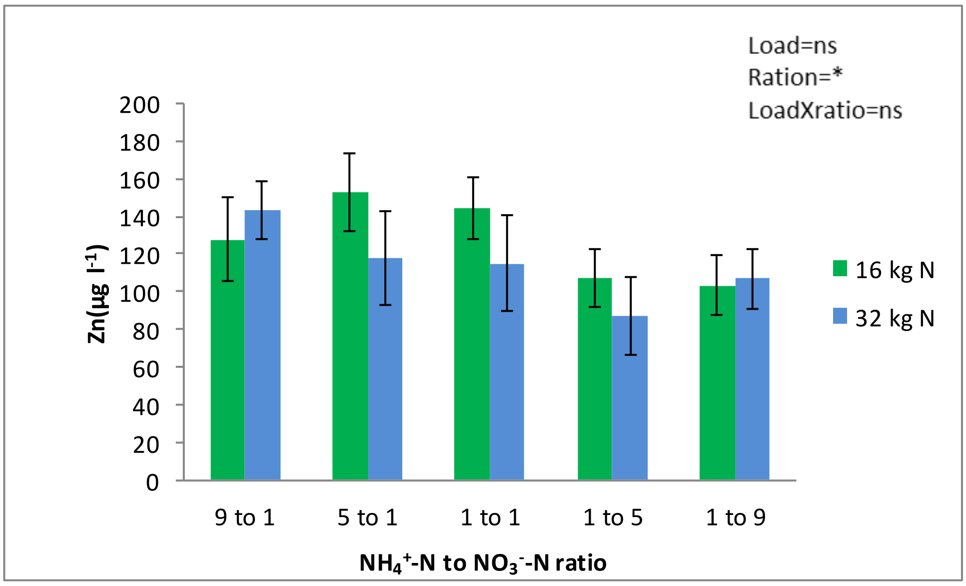


Fig. 4.6: Effect of ammonium/nitrate ratio on soil solution Zn concentrations over the period June 2009 to Sept. 2010. Values are the means across the 16N and 32N treatments. Error bars indicate standard errors of means (±1s.e)

In the case of Cu, the effect of ratio, which was significant at P=0.05 (Table 4.2), was opposite to that on Zn. The mean Cu concentration was 26 µg l^{-1} over the two treatmentr with a NH_4^+/NO_3^- ratio of 9:1 and 5:1, but when nitrate was dominant (NH_4^+/NO_3^- ratios of 1:9 and 1:5), it increased to 36 µg l^{-1}. Most of this increase occurred in the 1:9 ratio treatment. This effect is consistent with the hypothesis that mobilization of Cu is primarily driven by DOC concentrations, as DOC concentrations were also higher when nitrate was dominant (Figure 4.5).

As for DOC, there was a significant ratio/load interaction for Cu concentrations in soil solution at P=0.05 (Table 4.4). As shown in Figure 4.7, the effects of ratio on Cu concentrations was more marked in the 32N treatment than in the 16N treatment, a similar effect to that found for DOC (Figure 4.4). However, whereas the effect of nitrate dominance increased gradually for DOC, it was only strongly marked in the 1:9 treatment for Cu.

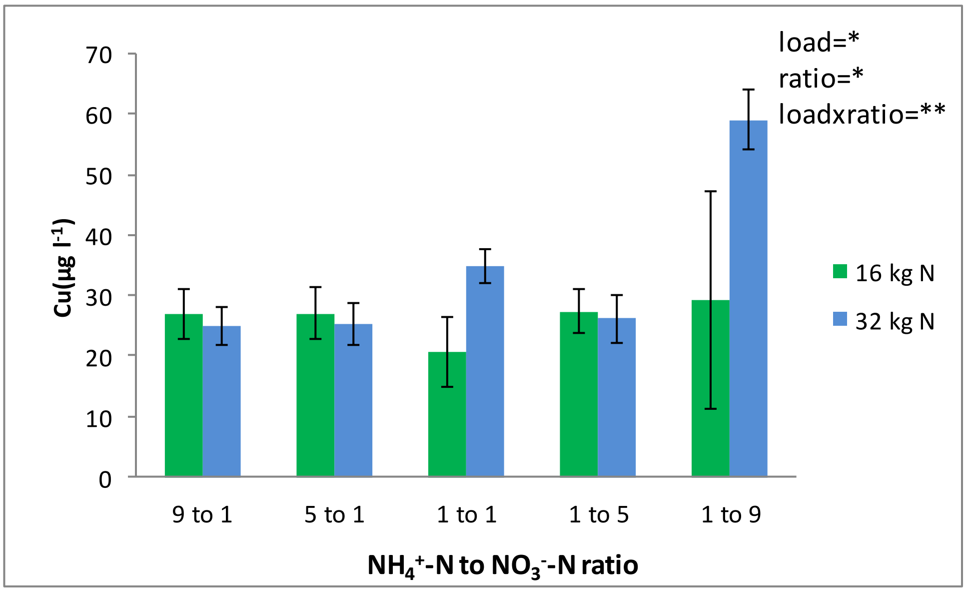


Fig. 4.7: Effect of ammonium/nitrate ratio on soil solution Cu concentrations over the period June 2009 to Sept. 2010, in the 16N and 32N treatments. Error bars indicate standard errors of means (±1s.e)

4.3.4 Effect of ammonium/nitrate ratio in combination with two liming

Table 4.5 shows the mean values of all the measured parameters in soil solution, averaged over the two nitrogen loads. The results which showed significant effects of ratio, or a significant load/ratio interaction, are discussed in more detail below.

Table 4.5: Mean of the values of each parameter in the five ammonium/nitrate ratio treatments, averaged for the 32 kg N ha^{-1} yr^{-1} limed and unlimed treatments.

Parameter ratio	9:1.	5:1.	1:1	1:5	1:9
pH	3.75	3.78	3.95	4.31	4.30
DOC (mgl^{-1})	17.6	16.5	18.6	20.1	23.2
Ni (µg l^{-1})	24.8	28.2	32.8	33.1	27.4
Cu (µg l^{-1})	33.9	40.8	41.5	39.5	41.9
Zn (µg l^{-1})	134.9	119.3	95.2	74.1	95.0
Pb (µg l^{-1})	2.52	2.76	2.88	2.31	3.05
NH_4^+-N (µg ml^{-1})	0.17	0.64	1.18	0.06	0.14
NO_3^-N (µg ml^{-1})	0.55	0.39	0.29	0.19	0.08

Figure 4.8 shows the effects of liming and ammonium/nitrate ratio on soil solution pH. As shown in Table 4.3, there was a significant effect of lime and ratio, both at P=0.001, but no significant lime/ratio interaction. The pH decreases with increasing ammonium domination, with a mean value of 3.75 at a NH_4^+/NO_3^- ratio of 9:1, compared to a mean value of 4.3 at a NH_4^+/NO_3^- ratio of 1:9. There was no significant lime-ratio interaction, although Figure 4.8 suggests that the effect of liming was stronger when ammonium was dominant than when nitrate was dominant.

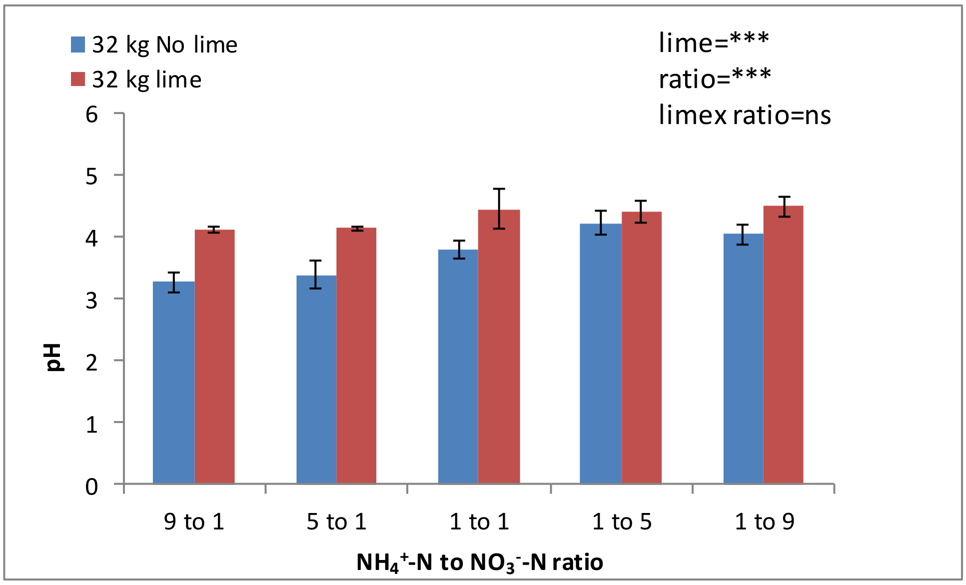


Fig. 4.8: Effect of ammonium/nitrate ratio on soil solution pH over the period June 2009 to Sept. 2010, in the 32N and 32NL treatments. Error bars indicate standard errors of means (±1s.e)

Figure 4.9 shows the effects of liming and ammonium/nitrate ratio at 32 kg ha^{-1} yr^{-1} on DOC concentrations. As for pH, there was a significant effect of lime and ratio, both at P=0.001, but no significant lime/ratio interaction (Table 3). As for pH, the DOC concentration increased with liming, and increased with increased nitrate dominance. The mean DOC concentration increased with increasing nitrate dominance, from a mean value of 17.6 mg l^{-1} at a NH_4^+/NO_3^- ratio of 9:1, to a mean value of 23.2 mg l^{-1} at a NH_4^+/NO_3^- ratio of 1:9. The similarity in the effects of liming and ammonium/nitrate ratio on pH and DOC is consistent with the hypothesis that acidity inhibits DOC production.

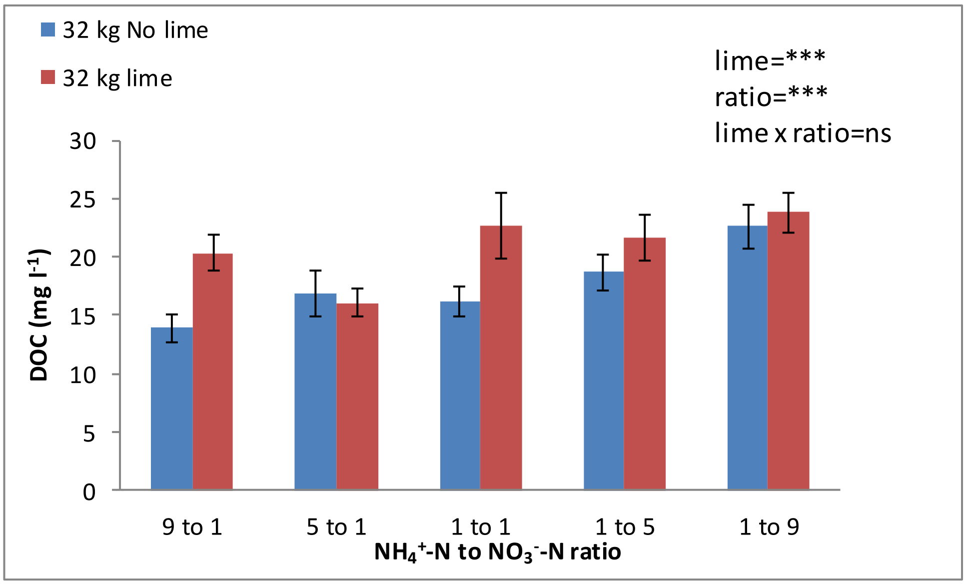


Fig. 4.9: Effect of ammonium/nitrate ratio on mean soil solution DOC concentration (mg l^{-1}) over the period June 2009 to Sept. 2010, in the 32N and 32NL treatments. Error bars indicate standard errors of means (±1s.e)

As for the analysis of load and ratio, no significant effects were found for lead and nickel, although there was a slight trend for high Ni concentrations with increased nitrate dominance. However, significant effects were found for effects of lime and ratio in the case of copper and zinc (Table 4.3).

In the case of Zn, there was a significant effect of liming and ratio at P=0.001. As shown in Figure 4.10, liming decreased Zn concentrations, as did increased nitrate dominance. The mean Zn concentration decreased from a mean value of 135 µg l^{-1} at a NH_4^+/NO_3^- ratio of 9:1, to a mean value of 74 µg l^{-1} at a NH_4^+/NO_3^- ratio of 1:5, and a mean value of 95 µg l^{-1} at a NH_4^+/NO_3^- ratio of 1:5. There was no significant lime/ratio interaction (Table 4.3), although the effect of the ratio appeared to be greater when lime was added

(Figure 4.10). These effects of ratio and liming are again consistent with the hypothesis that Zn mobility is greater with increasing acidity.

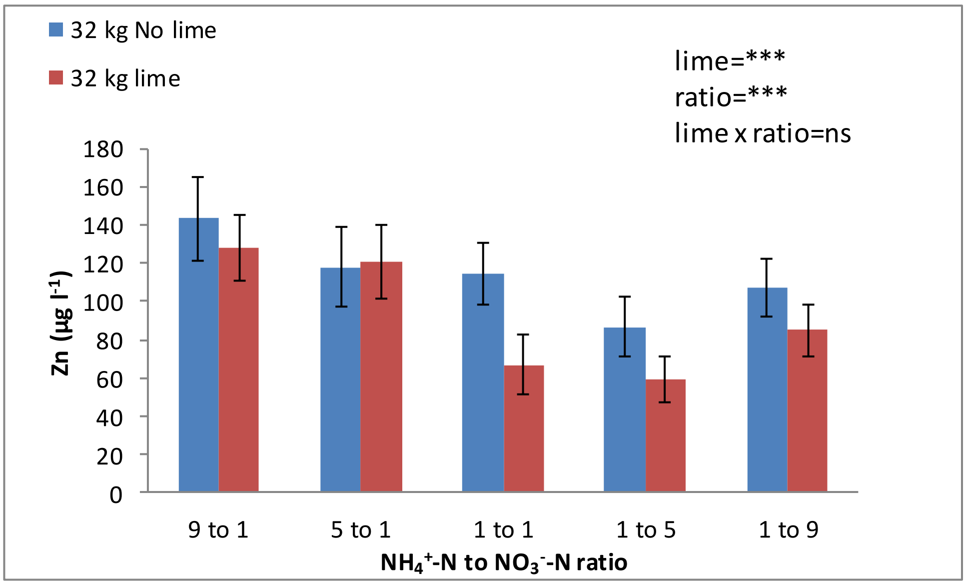


Fig. 4.10: Effect of ammonium/nitrate ratio on mean soil solution Zn concentration (µg l^{-1}) over the period June 2009 to Sept. 2010, in the 32N and 32NL treatments. Error bars indicate standard errors of means (±1s.e)

In the case of Cu, there was no significant effect of liming or ratio overall, but the lime/ratio interaction was significant at P=0.001 (Table 4.3). As shown in Figure 4.11, this interaction relates to the fact that liming greatly increased Cu concentrations, except in the 1:9 treatment, with high nitrate dominance, in which liming decreased Cu concentrations. This interaction effect cannot be readily explained from the observed effects of liming on ratio on either pH or DOC concentrations.

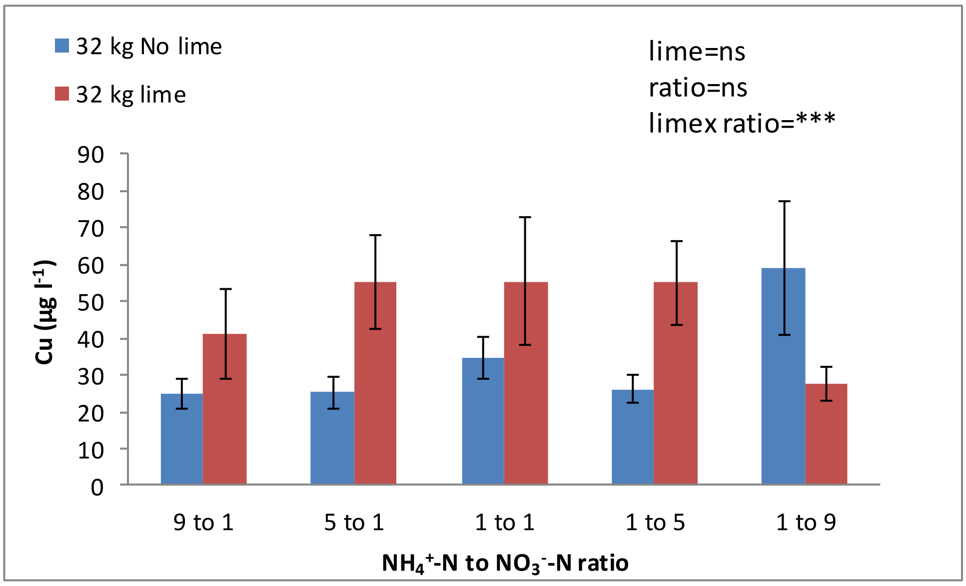


Fig. 4.11: Effect of ammonium/nitrate ratio on mean soil solution Cu concentration (µg l^{-1}) over the period June 2009 to Sept. 2010, in the 32N and 32NL treatments. Error bars indicate standard errors of means (±1s.e)

4.3.5. Regression analysis

The analysis described above shows significant effects of liming, load, and ratio on pH and DOC, and also on concentrations of Cu and Zn, but not Pb and Ni. In order to test whether the mean concentrations of the different metals were significantly associated with mean pH or DOC concentrations across the 15 different treatments (5 ratios within each of the 16N, 32N and 32NL treatments, a bivariate correlation analysis was carried.

The results of this analysis are shown in Figure 4.12 and Fig 4.13. No significant correlations were found for lead, as expected given the low overall concentrations and the lack of any significant treatment effect. There was tendency for Ni and Cu concentrations to increase with increasing DOC concentrations, but the correlations were weak and non-significant. In contrast, Zn concentrations showed a significant decrease with both increasing pH and increasing DOC concentrations. As noted above, the effects of the treatments on both pH and DOC concentrations showed similar trends, making it difficult to separate the effects of the two components of soil solution.

Figures 4.14 and 4.15 show the results of a similar analysis for NH_4^+-N and NO_3^--N concentrations. These are consistent with those for pH and DOC – only Zn showed a relationship with NH_4^+-N and NO_3^--N concentrations, decreasing with ammonium concentrations and increasing with nitrate concentrations.

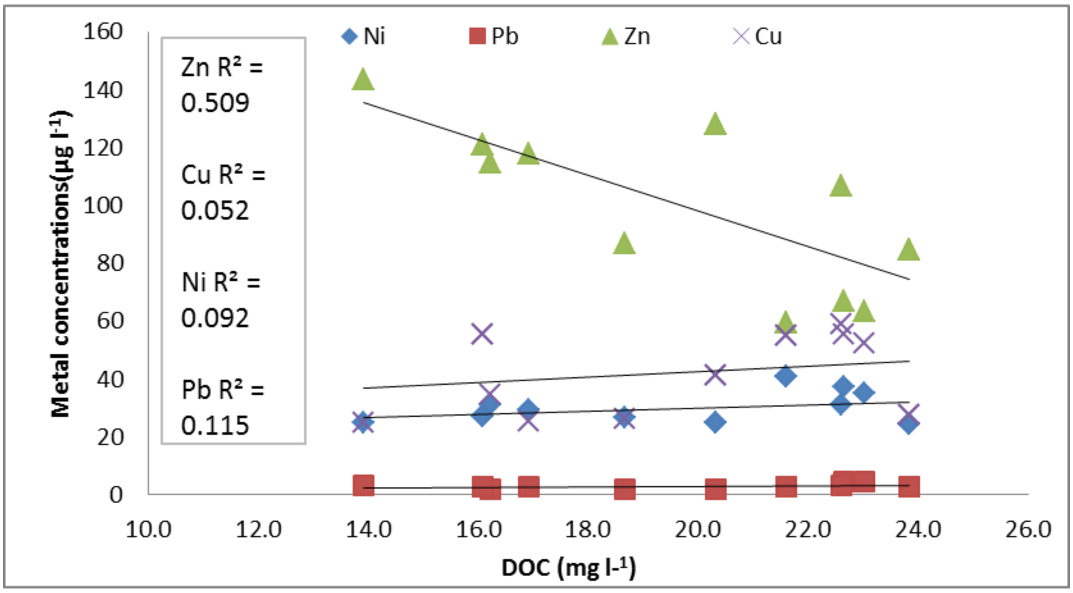


Fig. 4.12: Relationship between DOC(mg l^{-1}) and metals (µg l^{-1}), based on 15 different treatments in soil pore water (5 ratios within each of the 16N, 32N and 32NL treatments)..

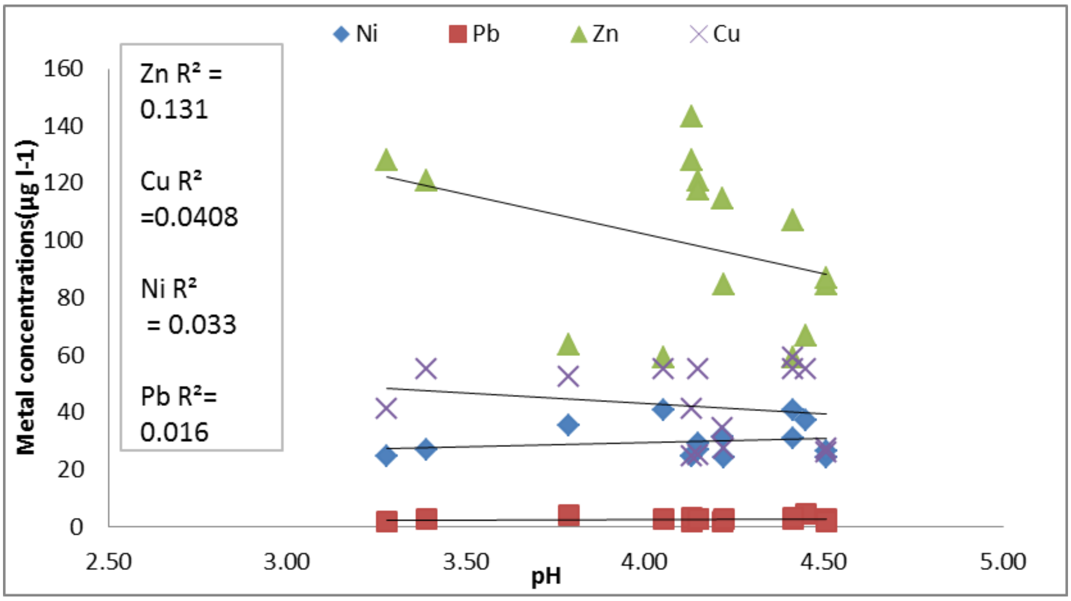


Fig. 4.13: Relationship between pH and metals (µg l^{-1}), based on 15 different treatments in soil pore water (5 ratios within each of the 16N, 32N and 32NL treatments)

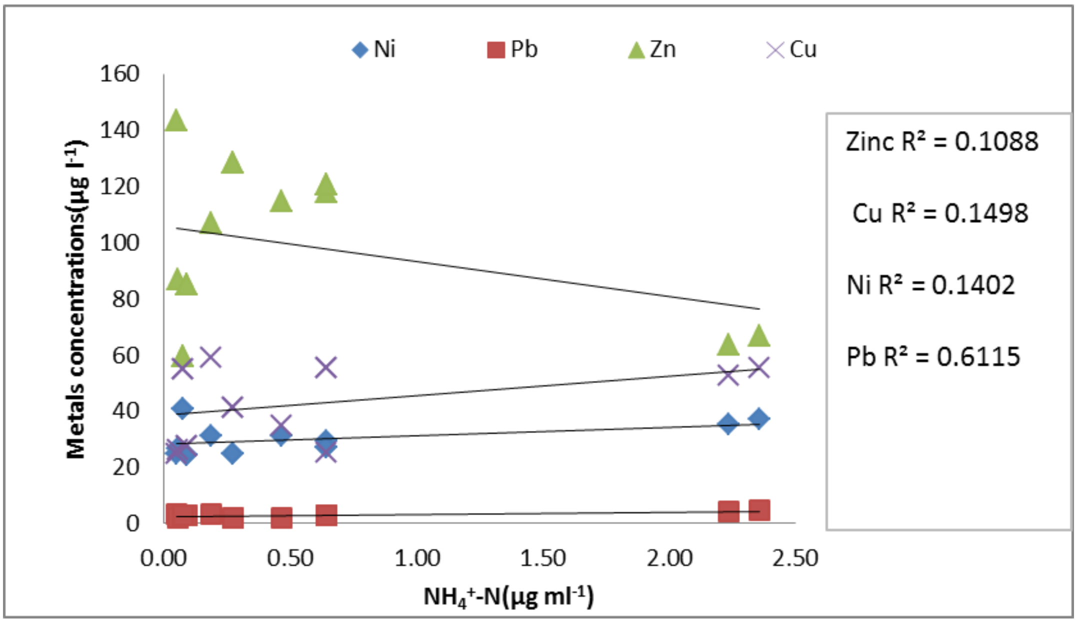


Fig. 4.14: Relationship between NH4+-N concentrations (mg l^{-1}) and metal concentrations (µg l^{-1}), based on 15 different treatments in soil pore water(5 ratios within each of the 16N, 32N and 32NL treatments)

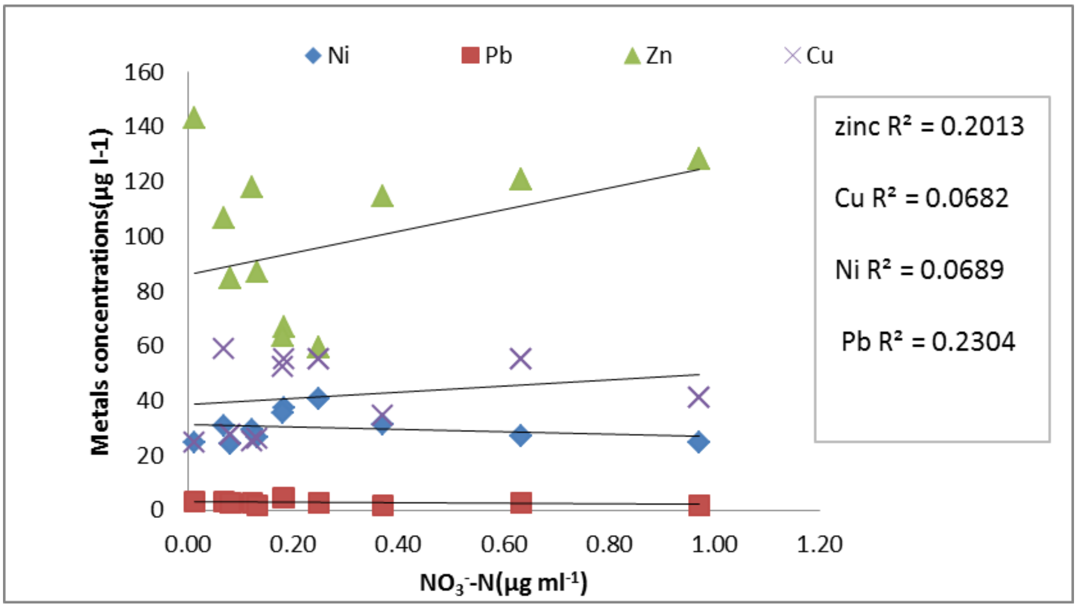


Fig. 4.15: Relationship between NO3--N concentrations (µm l^{-1}) and metal concentrations (µg l^{-1}), based on 15 different treatments in soil pore water(5 ratios within each of the 16N, 32N and 32NL treatments)

4.3.6 Soil analysis

It was assumed in the analysis above that the random assignment of cores to the experiment treatment would avoid any possibility of metal concentrations in the soil matrix affecting those observed in the soil solution as the experiment progressed. However, it was also possible that the cumulative effects of the experimental treatments would affect the metal concentration differentially, hence influencing the concentrations observed in the second half of the experiment in soil solution. This was checked by soil analysis of the cores receiving the highest and lowest ammonium/nitrate ratios. The mean concentrations in the 16N, 32N and 32NL treatments at these two ratios are shown in Figure 4.15.

These results show some differences between the 9:1 and 1:9 treatments for individual metals and load/lime treatments, but there was no overall pattern. This was confirmed by an analysis of variance, testing the effects of load and ratio together, and lime and ratio together, as was done for soil solution analysis. The results, which are summarised in Tables 4.6 and 4.7, show no significant effects of ratio, load, liming or their interaction on acid digest concentrations of any of the four metals. It was therefore concluded that any treatment-related variation in soil metal concentrations had no significance for the interpretation of the soil solution data.

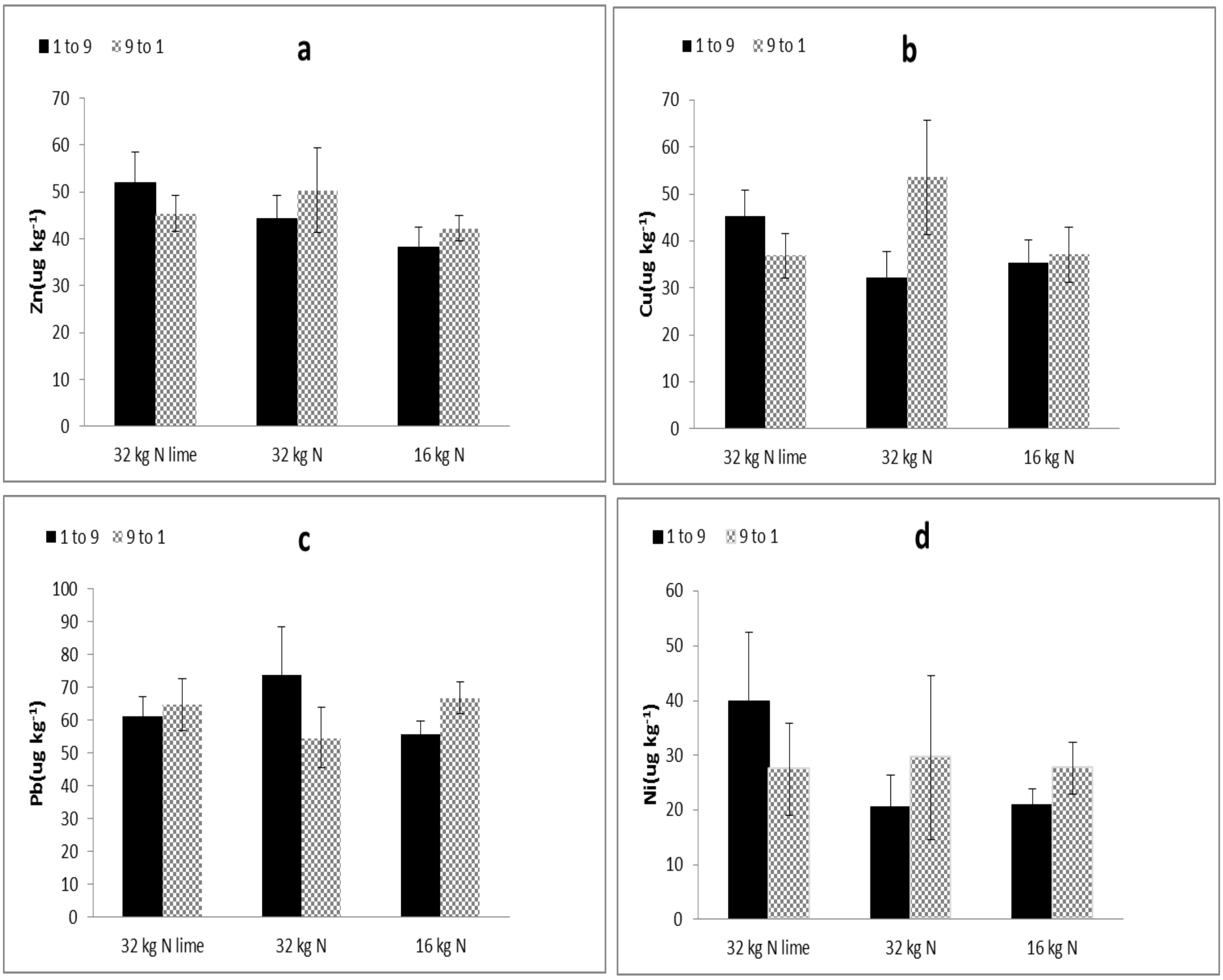


Fig. 4.16: Effect of ammonium/nitrate ratio (9 to 1)and (1 to 9) on HNO3 soil extract concentrations of (a) zinc), (b) copper, (c) lead, and (d) nickel at the end of experiment in the 16 N, 32N and 32NL treatments. Error bars indicate standard errors of means (±1s.e)

Table 4.6: Analysis of variance of effects of load, ratio and their interactions, on metal concentrations in HNO_3 extracts of soils at the end of the experiment.

Treatment		Ni	Cu	Zn	Pb
Load	F	0.01	0.651	1.378	0.084
	p	0.922	.432	0.259	0.776
Ratio	F	0.955	1.974	0.682	0.170
	p	0.343	0.180	0.422	0.686
Load*Ratio	F	0.018	1.442	0.032	2.359
	p	0.895	0.248	0.861	0.145

Table 4.7: Analysis of variance of effects of liming, ratio and their interactions, on metal concentrations in HNO_3 extracts of soils at the end of the experiment.

Treatment		Ni	Cu	Zn	Pb
lime	F	0.608	0.060	0.045	0.014
	p	0.447	0.810	0.834	0.906
Ratio	F	0.026	0.747	0.002	0.647
	p	0.873	0.399	0.968	0.432
lime*Ratio	F	0.941	3.908	0.990	1.383
	p	0.346	0.065	0.334	0.256

4.4 Discussion

In this experiment, the effects of total N load, and the ammonium/nitrate ratio, on metal concentrations in porewater, and their association with changes in pH and DOC concentrations, were analysed. In this discussion, the effects on pH and DOC are first considered, and then the effects on metal concentrations.

4.4.1 Effects on pore water pH

Elevated N deposition is frequently reported to increase acidification and decrease soil buffering capacity (e.g. Vogt *et al.*, 2006; Lu *et al.*, 2009). For example, Aber *et al.* (1998) reported that N additions significantly enhanced soil acidification, however Fan *et al.* (2007b) found that exchangeable base cations ,decreased with increasing N addition after three years of N manipulation (60–240 kg N ha^{-1} yr^{-1}). The effects of N deposition on soil chemistry may be cumulative. For example, Zhang *et al.* (2008) reported that 3 years of N addition (0–640 kg N ha^{-1} yr^{-1}) caused increase in soil NH_4^+–N, NO_3^-–N concentrations, Similarly, in this experiment, the porewater concentrations of ammonium and nitrate increased in the second phase of experiment from 2009 -2010.

In this study, the ratio of ammonium to nitrate also affected pore water pH; with a high ammonium to nitrate ratio, pH was lower than when nitrate was dominant. This effect of a high ammonium to nitrate ratio has been reported by other workers, and is more pronounced under acid conditions; it is explained by the greater release of H+ in soils thus decreasing the soil pH (Lucassen *et al.*, 2003; Van den Berg *et al.*, 2005a). This is because during the nitrification process, two H+ ions are liberated, which can accumulate and significantly reduce the pH of the soil. The ammonium therefore decreases soil pH to a greater extent due to the nitrification process (Booth *et al.*, 2005).

As expected, liming increased pH, although this effect was somewhat lower when ammonium dominated, as shown by the significant liming/ratio interaction. This was probably because liming was done only once, at the start of the experiment. The higher nitrification rates caused by a high ratio of NH_4:NO_3 in deposition, and the fact that nitrate is less likely to be retained in the soils, is likely to lead to greater soil acidification, and a greater reduction in the initial effect of the applied lime over time (Lazof *et al.*, 1994; Ghnaya *et al.*, 2007).

4.4.2 Effects on porewater DOC concentrations

Previous studies (e.g. Luo *et al.* 2011) have reported that nitrogen deposition increased concentrations of DOC. Findlay (2005) also suggested that changes in pH and nitrogen deposition were potential mechanisms to explain observed increases in DOC export from ecosystems. The underlying mechanism remains hypothetical, although there are several reports of decreased activity of oxidative enzymes in soils receiving N amendments (e.g. Sinsabaugh *et al.* 2004). The net result would be greater availability of intact phenolic compounds in the bulk DOC pool, which are potentially available for export. One consequence of such a change in carbon degradation patterns in soils would be a decrease in decomposability for this pool of dissolved organic matter. However, no effects of N load on DOC concentration were found in this study.

DOC concentration were, however, higher when limed. Curtin and Smillie (1983) also found that liming increased the amount of organic matter in the soil solution, as did Anderson *et al.* (1994) in a soil incubation experiment. Gottlein *et al.* (1991) also determined that DOC increased after liming. This finding is consistent with the idea that acidity inhibits DOC production. The reasons for this are (a) that the solubility of DOM increases with pH because of the increasing the negative charge of the molecule (Tombacz and Meleg, 1990), and (b) microbial activity increases with increasing pH, leading to an increased DOC concentration (Kreutzer, 1995) because of an increase in the organic matter degradation rate.

This link to pH can also explain the significant effect of ammonium/nitrate ratio on pore water DOC concentrations, in which DOC concentrations increased with increased dominance of nitrate. This was consistent with the increased acidification when ammonium was dominant, for reasons explained above. Recent evidence also suggests that chronic nitrate deposition can increase the amount of dissolved organic carbon (DOC) exported, although the mechanisms eliciting this response are not clear; for example nitrate addition may increase DOC leaching from litter or lead to increased C adsorption to mineral surfaces thus facilitating SOM formation (Deforest *et al.*, 2006; Findlay, 2005).

4.4.3. Effects on porewater metal concentrations

As discussed in previous chapters, the chemistry of metals in soils is readily affected by pH, which influences the availability and plant uptake of micronutrients. It is clear that both the complexing capacity of organic acids and low pH are major factors related to mobilization of metals in soil. The literature cites Zn partitioning between soil and porewater to be mainly controlled by pH. As hypothesised, the results from this experiment clearly showed higher Zn concentrations at the lower pH values that were found when ammonium dominated. Likewise, the addition of lime decreased Zn concentrations in pore water, consistent with the reduced solubility at higher pH values. Other mechanisms may be important: increased NH_4^+ deposition affect the concentration of essential base cations, such as K+, Mg+ and Ca+, by exchanging them on the cation exchange sites in the soil, and at low pH by decreasing base cation uptake by plants. (Boxman *et al.*, 1991; Gloser & Gloser, 2000), and similar effects may apply to Zn.

Nickel, in contrast, showed no significant effect of nitrogen load, liming or ratio, and there was no association between changes in either DOC concentrations or pH and changes in Ni concentrations. Serpentine soils are rich in iron and magnesium and interactions between these elements and nickel, altering uptake and suppressing toxicity symptoms, have been reported by Slingsby (1974) and Proctor & McGowan (1976); further references to such effects are given by Mishra & Kar (1974) and Proctor & Woodell (1975). The nature of such factors is not clear, although McLean (1966) showed that nickel is not only chelated by humus, but may be associated with non-exchangeable sites on clay particles or precipitated as insoluble silicates, alumino- silicates or phosphates, all of which have the effect of lowering its availability to plants. Therefore, the particular chemistry of serpentine soils, compared to the peats examined in Chapter 3, may explain the lack of response of Ni to changes in pH and DOC concentrations.

The presence of DOC provides a mobile ligand that has been shown to facilitate mobility of trace elements. According to the initial hypotheses, mobilization of Pb and Cu is primarily driven by DOC concentrations. Cu been reported on number of occasions to form stronger complexes with organic ligands (Florence, 1982) than other metals in soil solution (Milne *et al.*, 2003). Cu concentrations in pore water were significantly higher in cores where nitrate was dominant in deposition, and in which there were higher concentrations of DOC, in the 16N and 32N treatments. However, the same effect of DOC was not found for Pb, for which no significant effect of load, liming or ratio was observed, despite the associations between DOC and Pb concentrations in pore water that were reported in the previous Chapters of this thesis. This may be because the concentrations of both Pb and DOC were relatively low in this experiment. Furthermore, the effect of ratio on Cu concentrations when liming was carried out was modified, and there was no longer a positive effect of nitrate dominance. The reasons for this are unclear.

Overall, although varying the ammonium/nitrate ratio did significantly affect both pH and DOC concentrations in pore water, these were not reflected in the expected changes in pore water concentrations of metals, except in the case of Zn. The reasons for the differences between these findings and those reported in Chapters 2 and 3 are discussed further in Chapter 5.

Chapter 5: Final Discussion

5*.1 Comparison of experimental and field conditions*

Due of the different range of soil properties and several forms of metals in the soil, assessing the range of metal availability in a soil is site and soil type specific. However, these variations can be explained by the key solubility mechanisms for metals in soil, as described in earlier chapters, including adsorption of the metal by the soil solid surfaces and absorption in the soil solution. Alongside to soil properties, attention needs also to be given, for example, to the type of metal and its concentration, complexing ligands, and pH. (Pulse *et al*., 1991). These common mechanisms should lead to responses to environmental perturbations that are consistent with the underlying mechanisms.

In the field survey and experiments described in this thesis, metal concentrations in pore water have been related in a more empirical way to properties such as pH, and DOC, ammonium and nitrate concentrations. However, the relationships found in the different studies were not consistent; for example, Pb concentrations in pore water showed a strong association with DOC concentrations in the temperature experiment described in Chapter 3, but no such relationship was found in the N deposition experiment described in Chapter 4. Before considering the mechanistic basis for these differences, it is useful to compare the different soil and pore water characteristics in the three studies which might have influenced the measured metal concentrations in pore water. These are summarised in Table 5.1. Note that the values for soil pH and LOI for Chapter 4 were measured at the start of the experiment, and not during the measurement period.

Table 5.1: Summary of soil and pore water characteristics in the studies described in Chapters 2, 3, and 4. The mean values are shown, alongside the range of values in brackets.

Parameter	Chapter 2 field study	Chapter3 experiment	Chapter 4 experiment
HNO_3ext. Pb (µg kg^{-1})	84 (4.2-391)	766 (221-1589)	63(23-124)
HNO_3ext. Ni (µg kg^{-1})	3.4(0.6-11.4)	11.1(4.4-21.0)	31.5(3.9-102)
HNO_3ext. Zn (µg kg^{-1})	22(3.3-120)	64(37-92)	47(29-89)
HNO_3ext. Cu (µg kg^{-1})	6.5(1.3-23)	77(6.1-132)	41(9.2-97)
Soil pH	3.9(2.6-6.7) NaCl extract	3.2(2.2-3.9) (KCl extract)	3.87(3.61-4.05) Nacl extract
Soil LOI (%)	44 (8-94)	75 (73-77)	83(62-95)
Porewater Pb (µg l^{-1})	20.5(0-125)	66.7(0.06-319)	2.9(1.9-4.7)
Porewater Ni(µg l^{-1})	3.8(1.2-14.4)	10.5(0.17-56.3)	30(24-41)
Porewater Zn (µg l^{-1})	278(33-956	300(7.5-1026)	99(59-143)
Porewater Cu (µg l^{-1})	79(41-147)	165 (11.7-597)	42(25-59)
Porewater pH	4.9(3.2-7.5)	3.9(3.4-5.6)	4.1(3.3-4.5)
Porewater DOC (mg l^{-1})	32(6.1-81)	92(4.8-476)	20(14-24)
Porewater NO_3^--N (mg l^{-1})	-	0.07(0.12-2.0)	0.32(0.01-0.97)
Porewater NH_4^+-N (mg l^{-1})	-	1.05(0.04-6.3)	0.54(0.05-2.4)

This data summary clearly highlights some major differences between the three studies described in this thesis. The field study, as expected, showed a greater range of soil variables than the two experimental studies, which only used samples from one or two sites. While the field survey included the highly organic soils of low pH that were used in the glasshouse experiments, it also extended to soils with a relatively low organic content and close to neutral pH. These differences were reflected in pore water pH values, for which the field study included samples with a similar pH to the experimental studies, but also included samples with pore water pH values that were 2-3 pH units higher than in the experimental studies.

The results for pore water DOC concentrations, in contrast, showed the greatest contrast between the two experimental studies. In the temperature study described in Chapter 3, the DOC concentrations covered a range of two orders of magnitude, from 5 to 500 mg l^{-1}. In complete contrast, in the nitrogen deposition study described in Chapter 4, the range was very small, from 14-24 mg l^{-1}. Values in the field study, taken under field conditions, ranged from 6-80 mg l^{-1}, intermediate between the two experimental studies. This range was comparable to that reported from the wider range of sites sampled by van den Berg *et al.* (2012). As discussed in Chapter 3, the very high DOC concentrations likely reflect the artificial nature of the experimental set-up. Concentrations of nitrate and ammonium in pore water were comparable in the two experimental studies.

LOI – DOC relationships were quite different in the soils examined in the experiment described in Chapter 4 compared to other two studies; in particular, despite the similarly high LOI values, the DOC concentrations in the Chapter 4 experiment were much lower than in the temperature experiment described in Chapter 3, despite the fact that soil organic matter decomposition is a major factor determining pore water DOC concentrations, as shown in Chapter 2. An important different is that the cores in Chapter 3 were separated from their vegetation and sealed on the surface, possibly leading to artificially high rates of DOC formation. The presence of vegetation in the cores in Chapter 4 may have reduced DOC concentrations; DOC concentrations were higher at the start of experiment, and then declined, reflecting the increased plant growth in the cores over time.

The metal concentrations found in acid digests and in pore water also showed important differences, as did the relationships between the two values. These are discussed in more detail in the next section, in which the factors influencing the measure pore water concentrations are discussed in more detail.

5.2. What explains the different pore water concentrations of metals and the effects of different treatments and soil parameters on them?

This section summarizes the outcomes of the studies reported in Chapters 2-4, and evaluates if the results are consistent, and, if not, which factors might influence the different findings. In order to structure the discussion, the results are discussed in turn for the four metals, although there will be some common themes between them.

5.2.1. Lead

In the case of lead, as expected, the concentrations in acid digests were much higher in the south Pennine sites used in Chapter 3, and this was also true for pore water concentrations. The lowest pore water concentrations were found in the nitrogen deposition experiment. The ratio between pore water and acid digest concentrations was higher in the two experimental sites than in the field study, perhaps reflecting the greater availability of lead in the less organic soils that were sampled in the field survey. This is consistent with the strong effect of LOI in increasing Pb concentration in acid digests, and the negative relationships between Pb concentrations in pore water and LOI, which were described in Chapter 2.

Although the analysis of data in Chapter 2 showed no significant effects of DOC on pore water concentrations, a strong relationship between DOC concentrations and Pb concentrations in pore water and leachate was reported in Chapter 3. This likely reflects the very high DOC concentrations that were induced by the experimental treatments in this study. Although effects of decreasing pH in increasing Pb concentrations in pore water were found in Chapter 3, this likely represents an effect of the negative association between DOC and pH, as no effect of similar shifts in pH on Pb concentrations was found in Chapter 4. Another important difference between the results in Chapters 3 and 4 is the lack of any effect of DOC on lead concentrations in the latter study, which may reflect the much lower DOC concentrations, which in turn may explain the relatively low Pb concentrations found in pore water. There was also no evidence from the study described in Chapter 4 that increasing NH_4^+ deposition increased lead concentrations in pore water, and thus the significant correlations with NH_4^+ concentrations that were found in Chapter 3 are most likely to reflect the similar effects of temperature on DOC and NH_4^+ concentrations

The fact that the ratio of Pb concentrations in pore water to that in acid digests of soil varied, an effect which is also apparent for Ni and Cu, indicates that, in many cases, weak relationships were observed between soluble metals and total soil metal. In the field survey described in Chapter 2, Pb was the only metal to show significant correlations between concentrations in acid digests and in pore water. This illustrates why the study of soil–water partitioning of metals has become an important issue (Carlon & Marcomini, 2004; Tipping, 2003). In the case of Pb from this study, the strong effects of DOC on soil solution metal concentrations indicate that the solubility behaviour of Pb parallels the behaviour of DOC, confirming the importance of DOC in Pb release from soils. DOC is also reported to be a significant carrier of soluble Pb in surface waters (Kerr et al., 2008**;** (Dawson et al., 2010).

5.2.2 Zinc

For zinc, there were smaller differences in concentrations between the three studies than for lead. As for lead, the ratio of pore water to acid extractable metal concentrations was higher in the field study. Acid extractable Zn concentrations in the field survey showed a strong positive correlation with pH, suggest that Zn is less available on less acid sites. Consistent with this, negative relationships between pore water pH and Zn concentrations were found in Chapter 3, while the effects of ratio and liming on pore water Zn concentrations in Chapter 4 were also consistent with a effect that was mediated through pH. However, an important role for DOC in Zn partitioning is also suggested by the results of Chapter 3, for which a significant positive relationship was found in pore water, but not leachate. This might reflect a stronger competition for binding by Zn at the higher DOC concentrations in pore water, although it should also be noted that the correlation between pore water Zn concentrations and the relatively low DOC concentrations in the N addition experiment was closer than that with pH, despite the very low DOC concentrations.

The finding that Zn is mainly influenced by soil and solution pH is consistent with other reports (e.g. Nolan *et al*., 2003; Japony & Young, 1994; Catlet *et al*., 2002). However, it has been reported that in peat soils, Zn may compete for dissolved organic carbon more effectively than for soluble ligands (Stevenson, 1976), and forms complexes with non-labile soluble organics (Aldrich *et al*, 2002; Krishnamurti & Naidu, 2002; Almas *et al*., 2000).

5.2.3 Nickel

In contrast to Pb and Zn, the pore water to acid extractable nickel ratios were very similar in the work described in all three chapters, even though the highest mean Ni concentrations were found on the serpentine-derived material used in the N addition experiment. This is surprising given the quite different ranges of soil and pore water properties in the different studies. As for Zn, acid extractable Ni concentrations in the field survey showed a strong positive correlation with pH, suggest that Ni is less available on less acid sites. However, the significant shifts in pH that were caused by liming and ratio in the N addition experiment had no effects on pore water Ni concentrations. Furthermore, the models fitted in the field survey described in Chapter 2 showed stronger positive effects of DOC and negative effects of LOI on pore water concentrations than of pH, suggesting adsorption by organic matter in soil and soil solution was a more important factor influencing Ni partitioning between soil and soil solution. This is consistent with the findings in Chapter 3, in which the high DOC concentrations in pore water were associated with higher Ni concentrations in both pore water and leachate. For Ni, as for Zn, the organic phase becomes more significant for partitioning of metals between soil and soil solution in organic-rich soils. This is contrast to other studies which have reported that pH was more important than any other single property for Zn and Ni solubility, although DOC also showed some effect (Anderson & Christensen, 1988; Nolan *et al*., 2003).

Although positive correlations were also found between nickel concentrations and variation in DOC concentrations over time in Chapter 3, in Chapter 4, Ni behaved differently, possibly because of the lower DOC concentrations. Minerals of the serpentine group are common examples of highly Ni-enriched soils. Despite the high contents of total nickel, British serpentine soils show low availability of nickel (Slingsby & Brown, 1977). It is contended that this is because that nickel is bound in different fractions in serpentine soils, which has the effect of rendering it much less available.

As for Pb, increasing NH_4^+ deposition did not increase Ni concentrations in pore water, and thus the significant correlations with NH_4^+ concentrations that were found in Chapter 3 likely reflect the similar effects of temperature on DOC and NH_4^+ concentrations.

5.2.4 Copper

For copper, like lead, the ratio between pore water and acid digest concentrations was higher in the two experimental studies than in the field study, perhaps reflecting the greater availability of copper in the less organic soils that were sampled in the field survey. This is consistent with the strong effect of LOI in increasing Cu concentration in acid digests, although no consistent relationships were found between LOI and Cu concentrations in pore water in the models that were fitted in Chapter 2. Furthermore, the very high DOC concentrations that were evident in the Chapter 3 experiment did not show a clear association with high pore water concentrations of Cu; the concentrations of this metal tended to decrease through time, while those of DOC tended to increase over time. This may suggest a much stronger affinity of Cu for organic binding sites in the soil matrix than in DOC in these systems. The lack of effect of pH on pore water concentrations of Cu is consistent with the known insensitivity of Cu solubility to pH (McBride et al, 1997; Sauvé et al, 1997). However, Cu has been reported on number of occasions to form stronger complexes with organic ligands (Florence, 1982) than other metals in soil solution (Milne et al., 2003). The strongest associations observed for pore water Cu concentrations in the heating experiment described in Chapter 3 were with nitrate concentrations, which also tended to decrease with time. This is consistent with the effect of elevated nitrate/ammonium ratios increasing pore water concentrations in unlimed cores in the N addition experiment described in Chapter 4. However, the mechanistic basis for this association with nitrate is unclear.

5.3 Implications of the findings

Although metal emissions to the atmosphere have fallen drastically over the past fifty years, many UK soils have accumulated a large burden of metals, as organic matter can bind large amounts of metals, and hold it for decades, even centuries (RoTAP, 2012). It is uncertain whether current metal concentrations in soils are increasing or decreasing, and the actual fate of those metals that are still emitted is uncertain. RoTAP (2012) reported higher rates of deposition for most of the metals than has been accounted for in the national emissions inventory, which is a further major uncertainty. This is a serious issue; if the fate of metals that are emitted and then accumulated in terrestrial ecosystems is unknown it becomes very important to find out the rate at which these metals are becoming available, so first identifying the scale of the problem. Knowledge of the soil/solution partitioning of these metals is therefore an important step towards using models to predict the metals are accumulating in, or being released from, these soil stores.

Furthermore, based on the results of this thesis, the availability of this store of metals, and their concentrations in drainage water, will change if other environmental factors influence key variables that determine soil/solution partitioning, such as soil acidity and release of DOC. Peats have long been known as unstable ecosystems where the degree of production of organic matter go beyond that of its breakdown. That's why it's of quite alarming and have been expressed that these stores of organic matter may get disturbed due to environmental changes (Gorham, 1991), by freeing their carbon. (Freeman *et al,* 2001; Worrall & Shedden, 2003). Those worries seem to be reinforced by interpretations of promptly rising dissolved organic carbon (DOC) concentrations in aquatic ecosystems (Freeman *et al*., 2001). Schindler *et al*. (1997) observed DOC concentrations in lakes to increase by 30–80% during one 20-year study in North America. Worrall & Burt (2007) also have shown that, out of 315 records in the United Kingdom, 68% showed a significant increase in DOC concentrations over timescales of between 9 and 42 years.

Turning to the chemical climate of the UK, as described by RoTAP (2012), this has been changed dramatically over the last 30 years. Acid rain is under control and soil and freshwater acidity is slowly recovering, which has implications for metal bioavailability and concentrations in drainage water; however, nitrogen deposition, and the associated effects on biodiversity, shows little sign of improvement. Furthermore, there is a potential link between recovering acidity and increasing DOC concentrations. The causes of the increasing export of DOC in surface waters are still a subject of debate, according to Evans *et al*. (2006) declining in acid deposition could be a trigger of DOC solubility which was suppressed by low pH. The experiment described in Chapter 4 has shown that liming can increase the concentrations of DOC in soil water, consistent with results reported for soils and pore water in other studies (Andersson *et al*., 1994; Curtin & Smillie, 1983).

Even though DOC in surface water is a concern in general, the managing of upland organic soils may play a role in defining the future C loss. Thurman 1985; Peterson 1990, suggested it as impact of a change in pH on DOC solubility is greatest in the pH 4 to 5 which is typical for upland organic soils in Britain, Tipping and Woof.(1990) described through titration experiments that an increase in soil water pH of 0.5 units could cause a 50% increase in DOC concentrations . Reducing liming in in water sources may reduce problems of water colour associated with organic matter.

The potential for increased pore water concentrations of DOC to be associated with increased efflux of Pb, and also Ni and Zn, was clearly shown in the experiment described in Chapter 3. The high DOC concentrations in this experiment could be the result of disturbing the soil cores. This implies that this DOC range could be artificial, and under field conditions, the result of changing temperatures on pore water DOC, and hence metal, concentrations, could be different. Furthermore, although the experiment described in Chapter 3 was designed to assess the effects of warming on metal mobility, it was of limited duration, and there are also significant uncertainties in understanding whether the observed treatment effects were due to the higher average temperature in the heated glasshouse or due to the lower minimum temperatures, and greater temperature range, in the unheated glasshouse. Nevertheless, the treatments did have a significant effect on metal concentrations in pore water, as well as those of pH and DOC, even if these effects were not consistent in cores from the two sites. This indicates the potential for climate change, in terms of the variations between maximum and minimum temperature, as well as mean temperature, to influence pore water metal concentrations.

Although several features of the effects of raised N deposition and N fertilization have been studied, but full mechanism is still unknown about the effects of N deposition on DOC and hence metal turnover. Additionally, it should be remembered that the enhancement of C mineralization in the peat soil is limited by the supply of inorganic nutrients such as nitrogen, although experimental studies suggest that N addition reduces DOC release rates. Elevated N deposition is reported to increase the acidification and decrease soil buffering capacity (e.g. Vogt *et al*., 2006; Lu *et al*., 2009) and, in a field study, Cronan *et al*. (1992) showed that the DOC release rate decreased by 20% after N fertilization (using NH_4Cl) of a forest soil. This could be due, in part, to decreasing pH and increasing ionic strength (Stuanes & Kjønass, 1998), a similar effect found in this study with ammonium dominance. Emmett *et al.* (1998)

found no changes in DOC or DON concentrations and fluxes in either the forest floor or the A horizon after N addition to conifer forests in Sweden and Wales, respectively. Since it is hypothesized that large volumes of labile C are essential to drive N immobilization (Aber, 1992), DOC concentrations and fluxes should, in theory, be high under N-limited environments and low under N-saturated conditions (Gundersen *et al.*, 1998). For arable soils, Chantigny *et al.* (1999) report that decreasing soil mineral N content was consistently associated with an increase in water-soluble organic carbon.

The experiment described in Chapter 4 also showed the potential importance of ammonium/nitrate ratio in modifying pore water pH and DOC concentrations, even though the only clear effect on metal concentrations in pore water was for Zn. Similar experimental studies are needed using cores taken from highly polluted peatland areas, such as the southern Pennines, to better understand the significance of this factor. Furthermore, this study simply assessed effects through changes in pore water chemistry. Uptake by vegetation of essential elements such as Cu, Zn and Ni was not considered but could have been an important factor that influenced pore water concentrations, especially as Ni, for example, is associated with plant assimilation of organic nitrogen.

The increased input of dissolved organic carbon is also a potential energy source for heterotrophic nitrifiers, and lime application caused a clear increase in nitrification towards the end of the experiment. Hildebrand & Schack (1998) found the slight initial nitrate pulse in the organic layer after adding lime, although Mersi et al. (1992) found no significant effects on microbiological parameters, despite an increased pH.

This thesis has focused on non-agricultural soils for which atmospheric nitrogen deposition is the major input and a significant threat to the environment in many situations. However, a full assessment of the importance of nitrogen pollution for metal availability needs to consider agricultural areas, especially where metal concentrations in soils are elevated by inputs from use of fertilisers or sewage sludge, as well as from deposition. Extensive analysis of soil, pore water and groundwater samples is needed to understand these linkages and hence to minimize any threat of increased metal bioavailability by proper management of agricultural systems

5.4 Future research needs

Despite intensive research in the last decade and many qualitative results on controls of DOC, nitrogen and metal speciation dynamics, quantitative prediction of DOC, nitrogen and metal fluxes at the field scale is still difficult. Because, in practice, they must be predicted under field conditions, the relationship between field and laboratory findings concerning controls is most critical. Soil cores never represent the heterogeneity of real ecosystems with regard to structures and processes. The results described in this thesis clearly identify the intimate relationships between changes in both temperature and nitrogen deposition, and changes in pore water pH, DOC concentrations, inorganic N concentrations and metal concentrations. The results may help to focus the attention to the key processes which must be quantified under more realistic conditions.

However, as discussed above, these were limited and short-term studies conducted mainly in artificial glasshouse environments. Therefore, future research should be focused on more comprehensive studies dealing with the relationship between DOC, pH, N and metal dynamics and environmental factors in the field, with supporting laboratory experiments. The aim of the next, practical, step in research should be to analyze if, and in that case how, historical metal flows from upland organic soils have varied. Also, more detailed ground and surface water characterization should be carried out with the aim to better understand the processes that affect selected metals. In terms of processes, the main objectives of such studies should be:-

(i) To better identify soil properties and environmental factors controlling pH, DOC concentrations, N availability and metals concentrations, and their interactions. The sources and sinks of DOM and the implications of different DOM composition also need assessment. For example, the different behaviour of Pb and Cu throughout this study, despite their known affinity for organic matter, suggests that the affinity of these metals may not be the same for all types of organic matter.

(ii) To better assess the relative importance of biological processes and. physico-chemical controls for the release and retention of metals in soils. Studies that block or eliminate key biological processes, e.g. through soli sterilisation, might be useful to compare the relative importance of these two controls.

(iii) The most important environmental controls on formation and removal of DOC and DON need to be confirmed and better quantified, since results of this study suggest that the quantity of released DOC/DON will affect metal re-mobilization in slightly acidic environments

(iv) The biodegradability of DOM in solution and adsorbed on minerals, including the effects of DOM properties under changing environmental conditions, needs to be better understood. The importance of hydrological conditions for the release and fate of DOC, e.g. through changes in groundwater levels and rainfall patterns through changes in the activity of the microbial biomass, need to be better understood and linked to metal partitioning between soil and soil solution.

(v) Nitrogen deposition in the forms of different ratios of ammonium and nitrate, should be applied in a wider research programme that includes sites with high accumulated loads of metals, in order to better understand the impacts of these different forms of deposited nitrogen through soil solution partitioning models.

(vi) More experiments are needed to understand the implications for UK soils, and in particular the fate of accumulated metals, of changes in both maximum and minimum temperatures, under summer and winter conditions. Warming of the soil is expected to have a direct effect only on the soil microbial biomass and activity, and to exert an indirect effect on the majority of the other soil variables.

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Appendix 1. The minimum, maximum, and mean (±standard errors) weekly mean values of soil solution parameters over the 12 week experimental period, in Holme Moss and Featherbed Moss cores in heated (H) and unheated (C) glasshouses. The first four weeks data for zinc are not included. N=12, except for zinc, for which n=8.

Site and treatment			**pH**	**DOC (mg l^{-1})**	**NH4-N (mg l^{-1})**	**NO3-N (mg l^{-1})**	**Ni (µg l^{-1})**	**Pb (µg l^{-1})**	**Cu (µg l^{-1})**	**Zn (µg l^{-1})**
HOLME MOSS	**C**	**min**	3.49	20.8	0.20	0.01	6.1	29.0	42.9	225.6
		max	4.07	307.7	2.29	0.18	27.0.	215.2	334.8	706.6
		average±SE	3.75±0.05	112.5±27.9	1.11±0.24	0.06±0.02	15.0±1.9	96.2±19.8	179.9±26.1	406.2±66.7
	H	**min**	3.69	19.6	0.20	0.02	3.6	8.5	89.7	70.5
		max	4.63	131.6	2.43	0.47	20.8	75.0	498.7	635.0
		average	4.02±0.09	63.6±10.3	1.02±0.22	0.11±0.04	7.0±1.3	33.5±5.1	170.8±31.7	300.0±61.3
FEATHERBED MOSS	**C**	**min**	3.72	12.8	0.13	0.01	4.9	14.9	60.8	106.9
		max	4.24	207.0	1.49	0.57	16.3	173.8	234.8	537.2
		average	3.90±0.04	93.6±17.9	0.79±0.13	0.08±0.05	8.8±0.95	75.5±16.8	144.6±14.9	300.1±57.3
	H	**min**	3.76	13.8	0.13	0.01	4.3	14.0	86.0	115.2
		max	4.28	148.5	3.07	0.66	22.3	140.1	755.1	472.6
		average	3.9±0.04	89.6±14.0	1.27±0.27	0.13±0.05	11.9±1.75	61.3±10.3	249.8±56.8	265.1±46.1

Appendix 2,shows the minimum, maximum and mean range in water extracts of the soil metals extracted from the three different levels of the 12 mesocosms of Holme Moss and Featherbed Moss(6 each).

level	N=12	Ni (μg/kg)	Pb(μg/kg)	Zn(μg/kg)	Cu((μg/kg)
upper	**min**	3.58	14.02	54.23	45.25
	max	9.36	156.90	153.20	202.30
	average	6.26	90.40	102.79	118.29
medium	**min**	2.45	19.16	54.84	43.92
	max	9.26	192.00	198.36	371.10
	average	5.13	75.11	105.40	147.46
lower	**min**	2.57	19.59	42.22	51.00
	max	8.36	96.59	154.25	359.30
	average	4.90	51.46	93.15	134.64
total	**min**	2.45	14.02	42.22	43.92
	max	9.36	192.00	198.36	371.10
	average	5.46	72.32	100.45	133.46

Appendix 3 ; shows the minimum, maximum and mean range of KCl Extractable Fractions. of the soil metals extracted from the three different levels of the 12 mesocosms of Holme Moss and Featherbed Moss(6 each).

		Ni (µg/kg)	**Pb(µg/kg)**	**Zn(µg/kg)**	**Cu((µg/kg)**
	min	44.23	1039.00	302.28	31.66
	max	94.52	2688.00	965.30	152.80
upper level	**average**	66.57	1860.08	579.21	71.79
	n=12				
	min	5.00	66.44	95.07	29.00
	max	129.90	3155.00	1521.00	495.30
medium level	**average**	53.05	1282.47	615.97	101.76
	n=12				
	min	25.82	538.20	202.90	25.13
	max	125.04	4017.00	1293.00	190.30
lower level	**average**	67.91	1831.30	629.84	84.67
	n=12				
	min	5.43	66.04	95.07	25.13
	max	129.70	4017.00	1521.00	495.30
total	**average**	62.51	1657.95	608.34	86.07
	n=12				

Appendix 4: Summary of analysis of variance of effects of temperature, core depth and site on metal concentrations and pH in KCl and water extracts.(corrected df=11)(error df=24)

		Acid Digest Metals			
Treatment		Ni	Cu	Zn	Pb
Temperature	F	0.12	0.06	3.02	1.59
	p	0.73	0.81	0.09	0.22
Soil Level	F	0.90	0.24	0.11	0.01
	p	0.42	0.79	0.90	0.99
Site	F	1.61	2.84	15.65	1.08
	p	0.22	0.10	0.00 ***	0.31
Site × Temperature	F	0.28	0.07	2.71	0.21
	p	0.60	0.79	0.11	0.65
Site × Soil Level	F	0.74	0.08	1.91	0.10
	p	0.49	0.93	0.17	0.91
Temperature × Soil Level	F	0.32	0.29	0.67	0.30
	p	0.73	0.75	0.52	0.74

* $p<0.05$, ** < 0.01 and ***< 0.001 level of significance