Novel studies of the dynamics of mineral N species transformations and their mobilities relevant to assessing risks to drainage water.

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Finally I pay homage to the University of York where I utilized and enjoyed my stay as a PhD student and I will cherish the memories during the rest of my life.

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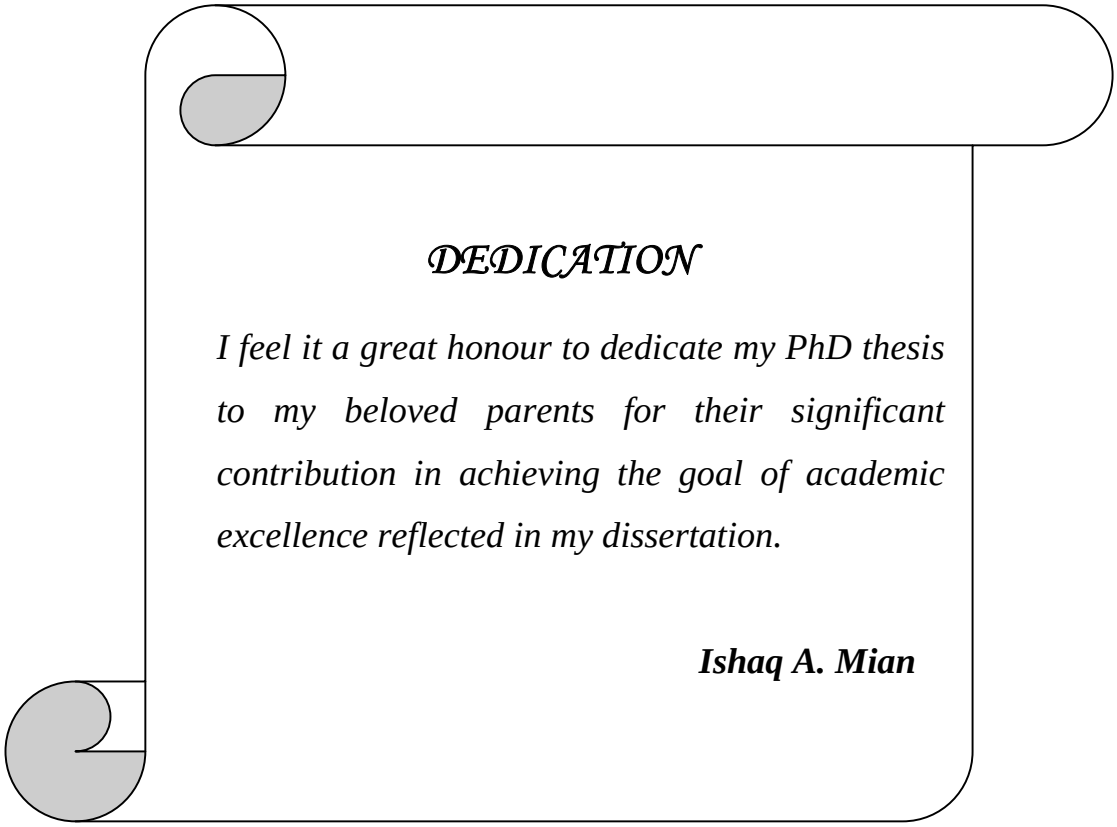

DEDICATION

I feel it a great honour to dedicate my PhD thesis to my beloved parents for their significant contribution in achieving the goal of academic excellence reflected in my dissertation.

Ishaq A. Mian

ABSTRACT

The biogeochemical cycling of nitrogen has been studied in detail at a predominantly acid grassland nature reserve, Hob Moor, just outside the city of York in the UK. Because of the risk of more frequent and/or extended summer droughts as a potential consequence of climate change, particular attention was paid to the influence of drying and rewetting upon the mobility of mineral N species. It was found that ammonification proceeds after net nitrification has ceased, and that subsequently nitrate is immobilized when the drying period is protracted. It is suggested that this is probably due to uptake by drought-tolerant micro-organisms. The experimental work showed that much of the first flush of nitrate after a period of drying is therefore attributable to stored nitrate, as long as the drying out period is not excessively long.

The mobility of ammonium-N in soils from Hob Moor was studied to test the hypothesis that in the heavily N-impacted soils at the site it would be more mobile than most soil scientists would predict, by measuring adsorption/desorption characteristics. The absorption isotherms confirmed that ammonium in these soils is potentially mobile, and when mobilized below the rooting depth may pass to the adjacent stream around the edge of the site. This helps explain the high ammonium-N and nitrate-N concentrations observed in this stream.

A developing interest in the Gaia hypothesis prompted the author to make a brief preliminary investigation of the idea that deciduous trees have evolved naturally to provide a close match between the dynamics of N release by litter decomposition and the dynamics of plant N requirement. The experiment showed that initially the fresh litter with a high C/N ratio immobilized nitrate especially in the forest soil. Under the relatively warm conditions of the experiment decomposition was rapid, and the immobilization was not sustained as would be predicted. Further evaluation of this concept is advocated.

The extent of immobilization by litter prompted a study of long-term seasonality of trends in nitrate concentration throughout the River Derwent in North Yorkshire using data obtained from the Environment Agency. It was thought that a ban on straw burning in 1993 might have reduced winter annual nitrate concentration peaks and possibly increased summer minima. The data partly supported this idea, but the timing match was not perfect, and it was thought that the foot and mouth disease impact and farmers' responses to environmental concerns and policy and to increasing fertilizer and energy costs were probably also important.

Finally a study was made of the importance of storage conditions upon extractable ammonium and nitrate concentrations in soils. Surprisingly nitrification was not sufficiently inhibited in some soils stored under refrigerated conditions overnight, and it is concluded that volumetric sampling and immediate extraction in the field may be a preferred option.

List of Papers and Manuscripts.

Chapter 1: Cresser, M. S., Aitkenhead, M.J. and **Mian, I. A. 2008**. A reappraisal of the terrestrial nitrogen cycle: What can we learn by extracting concepts from Gaia theory? ***Science of The Total Environment, 400, 1-3, 344-355.***

Chapter 3: **Mian, I.A.,** Riaz, M. and Cresser, M.S. **2010.** Potential effects of drying on N cycling in soil under Hob Moor acid grassland: A preliminary investigation. ***Submitted.***

Chapter 4: **Mian, I.A.,** Riaz, M. and Cresser, M.S. **2008**. What controls the nitrate flush when air dried soils are rewetted? ***Chemistry and Ecology*, 24, 259-267.**

Chapter 5: **Mian, I. A.,** Riaz, M. and Cresser, M.S. **2009.** The importance of ammonium mobility in nitrogen-impacted unfertilized grasslands: A critical reassessment. ***Environmental Pollution, 157, 4, 1287-1293.***

Chapter 6: **Mian, I. A.,** Stephens, C., Riaz, M. and Cresser, M.S. **2010**. Is the low C:N ratio of forest litter an evolutionary strategy to help conserve ecological niche? ***Submitted.***

Chapter 7: **Mian, I. A.,** Riaz, M. and Cresser, M.S. **2010.** How stable are soils for the determinations of available N? ***Communications in Soil Science & Plant Analysis. In press.***

Chapter 8: **Mian, I.A.,** Begum, S.,Riaz, M., Ridealgh, M., McClean, J.C. and Cresser, M. S. **2010**. Spatial and temporal variations in nitrate concentrations in the River Derwent, North Yorkshire and its need for NVZ status. ***Science of The Total Environment, 408, 702-712.***

List of Joint Publications

Muhammad Riaz, **I.A. Mian,** and M.S. Cresser. **2008**. Extent and causes of 3D spatial variations in potential N mineralization and the risk of ammonium and nitrate leaching from an N-impacted permanent grassland near York, UK. ***Environmental Pollution, 156:1075-1082.***

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Muhammad Riaz, **I.A. Mian,** and M.S.Cresser. **2010.** Litter effects on ammonium dynamics in an acid soil under grassland. ***Geoderma, 159:198-208.***

Muhammad Riaz, **I.A. Mian,** and M.S. Cresser. **2010.** How important is plant litter to the regulation of mineral-N leaching to streams in winter? An observations-led experimental approach. ***Soil Use and Management, In press.***

Muhammad Riaz, **I.A. Mian,** and M.S. Cresser. **2010.** A microcosm study from an N-impacted acid grassland soil shows that litter alters N dynamics and reduces potential risk of N leaching in winter. ***Water, Air and Soil Pollution, In press.***

Muhammad Riaz, **I.A. Mian,** and M.S. Cresser. **2010.** Litter controls on DIN, DON and DOC leaching in highly N-impacted freely draining grassland soils. **S*ubmitted.***

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CHAPTER 1: A REVIEW OF PAST RESEARCH INTO THE FACTORS CONTROLLING THE RATES OF TRANSFORMATION OF NITROGEN SPECIES IN SOIL

1.1 Introductory preface

Literally many thousands of papers have been published on every aspect of the nitrogen cycle. Critically reviewing them all would (even if possible) have produced an excessively long thesis introductory chapter, and then have resulted in potentially wasteful repetition later, in the more selective and focused introductions in subsequent chapters. The author therefore decided to make this first chapter relatively concise, and use his literature survey selectively to introduce where the ideas that form the basis of subsequent chapters emerged from. More detailed literature surveys of the literature underpinning the research in individual chapters are included in those chapters, and excluded from this one, with the hope that this would make the thesis more readable.

1.2 Forms and sources of plant-available nitrogen

The forms in which N occurs are very important as their responses to environmental factors vary markedly. Inorganic nitrogen occurs in four main chemical forms in the soil, alongside the massively predominant organic N. The main inorganic N forms, apart from N_2, are as follows:

1. Ammonium (NH_4^+)
2. Ammonia (NH_3)
3. Nitrate (NO_3^-)
4. Nitrite (NO_2^-)

All the above forms of inorganic N are available to plants to a greater or lesser extent. In addition, there will be traces of gases such as N_2O in the soil atmosphere and of course N_2. On the other hand organic N includes compounds like amino acids, proteins and more complex N compounds of humus. The organic N forms are not available to plants directly.

The N cycle is very complex and includes many transformations. It is actively biotic in nature and therefore organisms influence it directly. There are also abiotic transformations and processes in the cycle which include, for example, ionic adsorption of NH_4^+ to clay particles, transformations attributable to fires, and oxidation of N_2 to

NO_3^- by lightning. In most ecosystems (e.g. streams, lakes, coniferous forests, prairies, salt marshes) the processes in the basic nitrogen cycle are similar but specific organisms play different vital roles in transformations and the relative importance of individual processes varies.

In soils the availability of nutrient elements depends upon a range of biotic and abiotic factors. These include soil moisture, acidity, salinity, soil particle size, nutrient input and activity of roots and microbes (Binkley and Vitousek, 1989; Mengel and Kirkby, 2001). Fitter and Hay (2002) emphasised that the availability of N is largely under biological control while that of other nutrients is determined predominantly by inorganic equilibria. The key procedures governing the formation and mobility of nitrogen species are mineralization, volatilization, nitrification, immobilization and denitrification (Black, 1968; Hellebrand, 1998). Figures 1.1 and 1.2 are typical schematic representations of the N cycle, and indicate its highly dynamic nature.

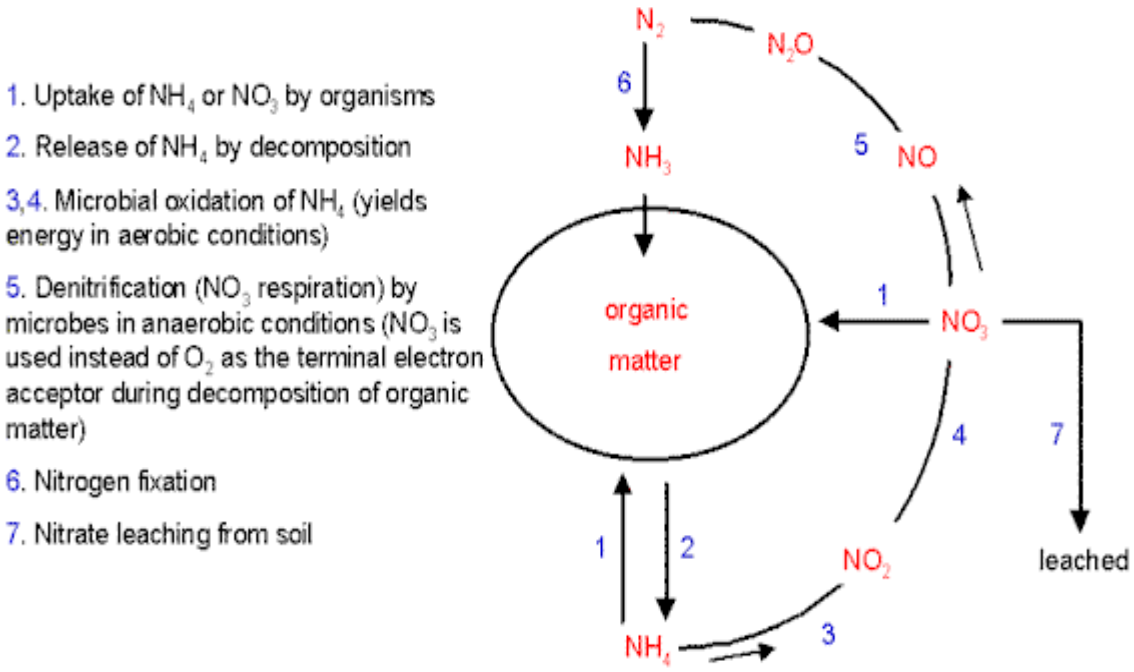


Fig: 1.1 Key processes in the natural nitrogen cycle.

Source: http://www.biology.ed.ac.uk/research/groups/jdeacon/microbes/nitrogen.html

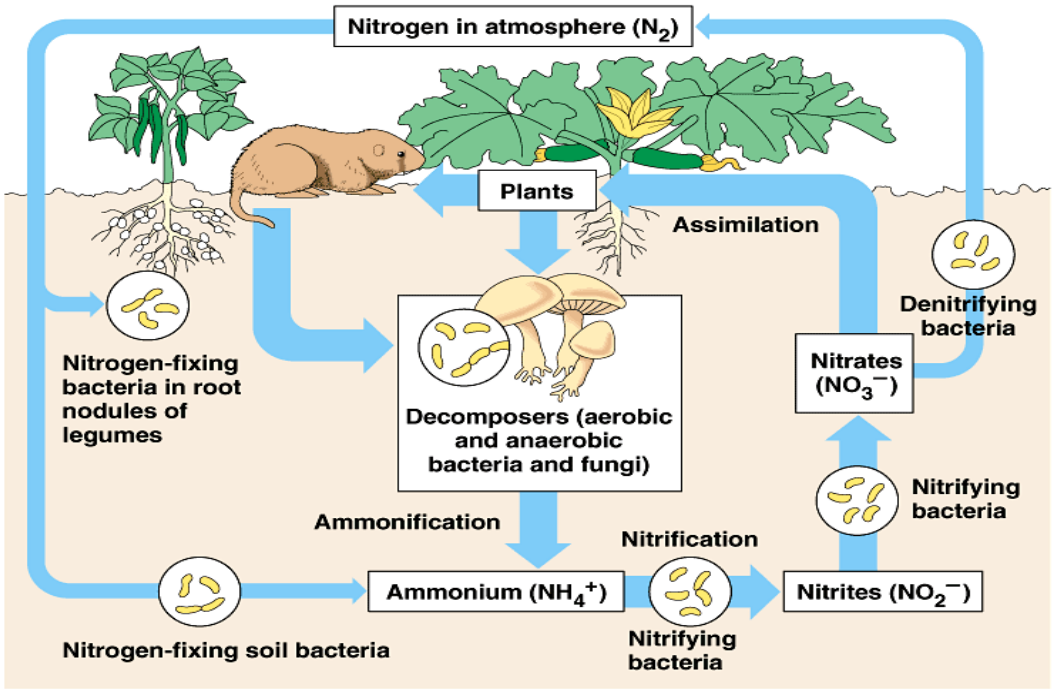


Fig. 1.2 A more pictorial representation of the terrestrial nitrogen cycle, drawn from a localised and predominantly microbiological perspective.

Source: http://www.cdli.ca/courses/sci2200/unit01_org02_ilo04/b_activity.html

1.2.1 Nitrification

In 1890 Winogradsky discovered that nitrification was a two-step process and that ***Nitrosomonas*** and ***Nitrobacter*** were the organisms involved in this oxidative reaction (Stevenson and Cole, 1999). Nitrification (part of the overall mineralization process) is the microbially mediated oxidation of NH_4^+ to NO_3^- carried out by autotrophic bacteria (Simek, 2000). According to Prosser (1989) the two steps are performed by different groups of the ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). The equations may be represented as follows (Brady and Weils, 1999).

Step 1) $NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O + 275$ kJ energy

Step 2) $NO_2^- + 1/2\ O_2 \rightarrow NO_3^- + 76$ kJ energy

Several researchers have reported results showing that acid coniferous forest soils nitrify, as discussed later in section 1.7. The main ammonia-oxidizing bacteria have been reported to be *Nitrosomonas* and the main nitrite-oxidizing bacteria are *Nitrobacte*r in soils (Bock et al., 1991). Nitrification is a soil acidifying process (van Miegroet and Cole, 1984). These bacteria play important roles in the nitrogen cycle because of the high bioavailability and relatively high mobility of nitrate. Apart from

autotrophic nitrification, aerobic oxidation of ammonia can also be performed by various heterotrophic fungi and bacteria (Prosser, 1989; Killham, 1990). Several studies have noted that heterotrophic nitrifiers are responsible for nitrification in some acidic coniferous soils (Killham, 1987 and 1990; Papen and von Berg, 1998; Brierley et al., 2001; Jordan et al., 2005). The results from a large number of studies applying specific inhibitors of autotrophic nitrification have revealed that heterotrophic nitrification does not play an important role in acidic coniferous forest soils (Stams et al., 1990; Stark and Hart, 1997; Paavolainen and Smolander, 1998; Rudebeck and Persson, 1998; Pedersen et al., 1999; Laverman et al., 2000; Ross et al., 2004; Burns and Murdoch, 2005). Many active ammonia-oxidizing Archaea were found in grassland soils (Leininger et al., 2006).

1.3 The importance of nitrogen to plants

Nitrogen is one of the most abundant elements in the Earth's biosphere and a major constituent of living cells. It is a key element in the metabolism of plants and essential for plant growth as a constituent of proteins, nucleic acid, chlorophyll and growth hormones. It is required by plants in larger amounts than other nutrients and its deficiency in soil results in lower crop yields (Buresh et al., 1993; George et al., 1993). Soil nutrient availabilities, more often than any other environmental factor, limit the growth of agricultural plants and forest trees, but are also essential to soil microorganisms. As a result, soil nutrients have wide-ranging and often surprising effects on ecosystem function, for example, by changing plant community composition when supplies are either deficient or excessive (Vitousek et al., 1997). Nutrient deficiencies may limit the potential growth stimulation response of an ecosystem to elevated CO_2 (Zak et al., 2000). Human-induced global change is likely to affect soil nutrient availability, but much remains to be learned about the magnitude and even the direction of these effects. To the author, climate change, and particularly potential disrupting effects of increased incidence and extent of droughts, therefore seemed a worthwhile research topic. Hence the effects of drying and re-wetting are considered in some detail in Chapters 3 and 4.

The amounts of N required by plants differ greatly from one species to another, and, for any given species, with genotype characteristics and the environment (Viets and Hageman, 1971). This suggests that, for a PhD study, it is sensible to concentrate on a single ecosystem type such as the grassland discussed in Chapter 2.

1.4 Importance of N transformations in soil

Many scientists, for example Stevenson and Cole (1999), Compton and Boone (2002), Templer et al. (2003) and Grenon et al. (2004), have reported that soil N transformations, and hence N bioavailability to plants, are microbially mediated processes influenced by composition and diversity of the soil microbial community, substrate quality and quantity, and environmental conditions. These concepts are universally accepted and must therefore underpin the work in this thesis. Several researchers (e.g. Tilman, 1987; Aerts and Berendse, 1988; Wedin and Tilman, 1996; Vitousek et al., 1997) have also concluded that changes in N availability can lead in changing dynamics of plant populations and their primary consumers and ultimately all species that depend on plants. This, and my supervisor's interest in discussing the Gaia hypothesis (Cresser et al., 2008), led to the idea behind Chapter 6, that the N cycle may be the heart of James Lovelock's Gaia, and plants have evolved to have a low C:N ratio at senescence and litter fall so that litter decomposes slowly to conserve nutrient N until needed later by the same plant. Increased N deposition from the atmosphere may modify the relative importance of several processes in the N cycle. Subsequently this may influence other elemental biogeochemical cycles (Aber et al., 1989; Vitousek et al., 1997; Aber et al., 1998; Tietema et al., 1998; Lovett et al., 2000). In this context it could clearly damage ecological niche by providing bio-available N too early, giving competitor plants a competitive edge.

According to several researchers, excess nitrogen can lead to eutrophication or levels of ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^-) potentially toxic to humans, livestock and wildlife in aquatic systems (Cairns et al. 1990, Carpenter et al. 1998, Marco et al., 1999). Lovelock (2006) would regard this as part of the "revenge of Gaia".

1.5 Nitrification in drainage waters and its links to ammonium

Nitrifying bacteria are important in both soil and drainage water because they oxidize ammonium-N (NH_4^+-N) to nitrate-N (NO_3^--N), which is generally accepted to be a more mobile chemical species in soil (Sprent, 1987). Tank et al. (2000) and Webster et al. (2003) investigated what influences NH_4-N dynamics along streams. They found uptake of ammonium by nitrifying bacteria negligible compared with its removal by other processes such as heterotrophic metabolism in-stream. Others though have described nitrification as a quantitatively important in streams (Mulholland et al., 2000; Findlay and Sinsabaugh, 2003). Despite these unresolved contrary opinions, few studies

have been conducted of potential variations in nitrification rate and what controls it (Bernhardt et al., 2002; Strauss et al., 2002). Generally though, temperature, NH_4^+-N availability, dissolved oxygen (DO), and pH have been perceived as the potential regulators of nitrification rates (Wild et al., 1971; Kuenen and Robertson, 1987). Strauss et al. (2002) examined 13 variables that might affect nitrification rates in sediments in 36 streams, NH_4^+-N availability and pH predicted nitrification rates most effectively. These studies, and the findings of Cresser et al. (2004) that NH_4^+-N is more mobile in N-impacted upland soils in the UK than the vast majority of soil scientists believe, stimulated the author in Chapter 3 to investigate ammonium mobility in, and hence potentially from, acid soils under grassland near York.

1.6 Disruptions of the natural N cycle

The key processes that interact to regulate N species concentrations in soils include: nitrification, immobilization, nitrogen fixation, atmospheric deposition, mineralization, denitrification and leaching **(**Stoddard, 1994). Nitrogen is the most abundant element in the atmosphere as molecular dinitrogen (N_2), but only after N_2 is converted into ammonium and/or nitrate is it available to plants and microbes (Galloway et al., 2003). Ammonium has been found to be the preferred form of N for assimilation by microbes in many cultivated soils (Azam et al., 1993). Nitrogen deficiency frequently limits forest productivity (Binkley and Hart, 1989; Paul and Clark, 1989; Reich et al., 1997), in spite of its great abundance in the atmosphere. Human activities over the past 100 years have more than doubled the rate of mineral-N production on the planet (Vitousek et al., 1997). This is because of industrial production of fertilizers atmospheric emission of N species from fossil fuel combustion and cultivation of symbiotically N- fixing crops (Smil, 2001).

1.7 Forest soils

There have been numerous reports of nitrification in acidic forest soils (e.g. Killham, 1990; De Boer and Kowalchuk, 2001; Laverman et al., 2002; Bäckman and Klemedtsson, 2003; Bottomley et al., 2004; Laverman et al., 2005; Hart, 2006). For a long time nitrification was thought to be largely insignificant to nitrogen cycling in coniferous forest soils (Mintie et al., 2003) because several soil factors were regarded as suboptimal for nitrifying microorganisms. High soil acidity, high C/N ratio, low nitrogen availability and/or the presence of chemical compounds from coniferous litter could all impede net nitrate production (de Boer and Kowalchuk, 2001; Kowalchuk and

Stephen, 2001). The cycling of forest litter therefore seemed an important research topic to the author, and features at least to some extent in Chapter 6.

1.8 Minimally managed/natural ecosystems

Nitrogen is very often the most limiting nutrient in terrestrial ecosystems (Stark, 2000) and often limits their biological production (Schlesinger, 1997). However, surplus nitrogen can have harmful effects. For example, surplus N can facilitate increased losses of nutrient cations and increase soil and water acidity in forest ecosystems (Vitousek et al., 1997), while in aquatic ecosystems it may cause eutrophication (Carpenter et al., 1998; Marco et al., 1999). Almost all the N that enters a terrestrial ecosystem by natural processes is derived from biological nitrogen fixation and atmospheric deposition (Stevenson and Cole, 1999).

1.9 Importance of measuring nitrification rates

To better understand soil fertility and ecosystem function it is necessary to be able to accurately assess nitrification rates in soils. It is important to know how nitrogen is transformed from one nitrogenous compound to another and what factors regulate the transformation dynamics.

1.9.1 Methods for measuring nitrification rates

There are several possible approaches to quantifying nitrification rate. Laboratory incubations under controlled moisture and temperature conditions are often employed. Net inorganic nitrogen species accumulation is then monitored after a selected time period from days to several months (Laverman et al., 2005; Kanerva et al., 2006). *In situ* incubations of enclosed soils at field sites are regarded as more realistic by some researchers. Again net inorganic nitrogen species accumulation is measured at the end of a few weeks or a few months (Vestgarden et al., 2003; Jussy et al., 2004; Fenn et al., 2005; Hart, 2006). However, the extent to which "realism" is enhanced if the soil has been removed from its associated vegetation must be regarded as questionable.

Laboratory- or field-based incubations using isotopic labeling, in which changes in a ^{15}N-labeled ammonium-N pool are measured over 1-3 days of incubation may be preferable (e.g. Scowcroft et al., 2004; Perakis et al., 2005). Some authors just measure net nitrification rates over a short period (typically 1 day) in the laboratory (e.g. Bäckman and Klemedtsson, 2003; Ross et al., 2006).

1.9.2 Overview on methods

The major criterion for choosing the suitable method for each study is based on the objectives of the study, particularly on the selected element of the nitrogen cycle under focus. According to Binkley and Hart (1989), aerobic incubation under controlled environmental conditions is the most commonly employed method for assessment of nitrification. They further concluded that none of the methods gives an exact, accurate assessment of the nitrification rates in a forest soil (Binkley and Hart, 1989).

1.9.3 Factors affecting nitrification

Under appropriate environmental conditions (e.g., electron acceptor and sabstrate), and in the presence of oxygen and NH_4^+, the process of nitrification takes place extensively. Numerous rate-regulating variables have been suggested to be potentially influencing activities of nitrifying bacteria, including: NH_4^+ availability, the competition for NH_4^+ from other sinks (Jones et al., 1995; Verhagen et al., 1995; Strauss and Dodds, 1997), soil pH (Sarathchandra, 1978), soil temperature (Paul and Clark, 1989), oxygen concentration in the soil atmosphere (Wild et al., 1971; Stenstrom and Poduska, 1980; Kuenen and Robertson, 1987), and the availability (quantity and quality) of organic carbon (Verhagen and Laanbroek, 1991; Strauss and Dodds, 1997; Butturini et al., 2000).

Bianchi et al. (1999) could explain > 74% of the variability in nitrification in a single area by variation in NH_4^+ concentration. However, it is improbable that such a single factor would control net nitrification over a large scale because other environmental factors would then be much more variable.

One regulatory factor that potentially could strongly affect net nitrification is the availability of organic carbon. Carbon availability is both highly dynamic and spatially variable in streams, but high carbon availability favours microbial immobilization of N. Its concentration varies with the abundance of wetland zones in a drainage basin (Kortelainen, 1993; Gergel et al., 1999), with water retention times (Sedell and Dahm, 1990), with catchment slope characteristics (Rasmussen, 1989),with drainage area contributing to discharge at different times during a storm event (Engstrom, 1987; Kortelainen, 1993), with discharge (and whether it is rising or falling (Sedell and Dahm, 1990), and with litter deposition and the subsequent leaching of organic molecules (McDowell and Fisher, 1976; Meyer et al., 1998). In-stream nitrification is of interest to the author because of his interest in controls on seasonality trends for nitrate concentrations in the River Derwent in North Yorkshire, as discussed in Chapter 8.

1.10 Immobilization

The conversion of inorganic N (NH_4-N and NO_3-N) to organic forms is biotic immobilization. This is the reverse process of mineralization, although the two processes must generally occur simultaneously in soils.

1.11 Denitrification

The process of NO_3^- conversion to gaseous forms of N such as N_2O and N_2 by facultative and obligate anaerobes in soil is termed denitrification (Brady and Weil, 1999). Some N_2O is also evolved during nitrification (Sutka et al., 2006). Denitrification is important not only because it results in a loss of available N for plants, but also because N_2O is a greenhouse gas 230-fold more potent than CO_2 at trapping infrared radiation and it survives in the atmosphere 3-5 times longer than CO_2 (Powlson, 1993). Besides denitrification, leaching of NO_3^--N is regarded as another way that NO_3^--N is lost from terrestrial ecosystems. It occurs because NO_3^- ions are not adsorbed significantly by negatively charged surfaces that occur in soils (Brady and Weil, 1999).

Nitrate leaching is a concern in the present context not just because it decreases available N supply for plants and may acidify freshwaters and cause eutrophication of estuaries and coastal waters (Murdoch and Stoddard, 1992; Henriksen and Hessen, 1997). It also may contribute to denitrification in sub-soils and sediments. The heterotrophic process of denitrification and can itself be limited by the availability of organic C in some aquatic environments however (Seitzinger, 1988).

Nitrogen mineralization (the release of ammonium from decomposing organic matter) is thought by many scientists to be controlled by the C:N ratio of the environment, although it should not be assumed that soil organic matter (and hence also C:N ratio) is homogeneous because dinitrification may also occur in subsoils and river sediments. Under high C:N ratio conditions nitrogen is more likely to be imobilized in microbial biomass, whereas under low C:N ratio conditions a net flux of ammonium into the environment is more likely (Schlesinger, 1997).

1.12 Identification of Gaps in Knowledge

To know the impacts of nitrogen on all biological systems it is necessary to understand how nitrogen is transformed from one nitrogenous compound to another and what factors regulate the dynamics of these transformations. The key processes may be the same or different, their relative importance changing according to different stages and/or different conditions. A firm understanding of the biogeochemical nitrogen cycle is needed to address all the environmental challenges associated with assessing the importance of anthropogenically induced imbalances in ecosystem N cycling, such as those induced by global climate change (Houghton, 1997) or by acid rain (Driscoll et al., 2001).

A specific way to quantify changes brought in the soil N cycle by enhanced N availability is to measure rates of N mineralization and nitrification, two important microbial processes that govern the availability of N to plants and micro-organisms. These processes, usually measured as net N mineralization and net nitrification, can provide an accurate benchmark as to where the system is in terms of saturation, a condition where N availability exceeds biotic demand. Although several researchers have been working on the different aspects of the N cycle (e.g. Vitousek et al., 1997, Smil, 2001, Galloway et al., 2003), a fully comprehensive study allowing the quantification of nitrogen processes, is still one of the greatest challenges in N research. Other scientists (e.g. Jarvis et al., 1996; Powlson, 1997) have reported that mechanistic approaches of N processes may improve our understanding of the relationship between soil organic matter and N mineralization.

The soil organic matter pool may be split into an 'active' pool and a 'passive' pool (Jansson, 1958). Indeed, mechanistic models (Smith et al. 1997; Jansson and Karlberg, 2001; Kätterer and Andrén, 2001) often divide the organic matter pool into a whole series of organic C pools, like a stabilized soil organic matter C pool, a microbial biomass C pool and organic C pool from crops. Each pool has a different turnover rate and assumes a characteristic C:N ratio (Rijtema and Kroes, 1991; Hansen et al., 1991). Each C pool is treated as a homogenous substrate following first order kinetics to simplify the model production. The turnover rate of each individual pool may be modified by abiotic factors such as temperature, soil moisture or soil texture, generally by using empirically based relationships. The C:N ratio of the organic matter in the individual pools determines whether net N mineralization or net N immobilization occurs.

Validating models precisely is not possible, as the different pools of soil organic matter, in reality, can never be measured directly and they are therefore conceptual . A model by Bosatta and Ågren (1985) and Ågren and Bosatta (1996) considers the decomposition of soil organic matter as progressing through a continuum, so organic matter is assumed to progress down a quality scale. The mechanism of this approach is very complicated. However, the concept is used to some extent in Chapter 6. There it is assumed that forest litter, because of its high C:N ratio, initially immobilizes mineral N, but as the decomposing litter component C:N ratio progresses to lower values, eventually mineral N starts to become progressively more available.

1.13 The research in this thesis

As discussed in this chapter, because of the complexity of the N cycle and the timescale and resources available to complete a PhD in the Environment Department at the University of York, the author decided to concentrate his efforts upon unfertilized soils close to York and predominantly on N cycling under acid grassland at Hob Moor, as discussed in Chapter 2. Chapters 3 and 4 were stimulated by the thoughts that climate change is likely to lead to increased occurrence of periods of drought in the UK, and hence more drying and re-wetting cycles that will disrupt the N cycle.

Chapter 5 was triggered by the desire to explain my supervisor's observation that ammonium-N seemed to be imobilized at unexpectedly high concentrations into a stream that runs beside Hob Moor. It seemed that measuring ammonium absorption/desorption characteristics in soils over a range of depth was the most appropriate way to answer this question. This was made possible by allowing the author to use groups of second year environmental science students for two days to process the large number of samples in a short period of time, an essential requirement for this study. The author designed and tested the experimental protocol, and closely supervised the students in the laboratory. He performed all the ammonium determinations himself, however.

Chapter 6 was prompted by a developing interest in viewing the N cycle from the perspective of the Gaia hypothesis. The nature of this chapter and its results are unusual for a PhD thesis since several aspects remain speculative as it was a very preliminary evaluation of a set of novel ideas. The author nevertheless thinks that this is an exciting contribution to understanding how atmospheric N pollutant deposition may be causing biodiversity change.

Chapter 7 was stimulated by observations of higher than anticipated variation in ammonium and nitrate concentrations some experiments, and the need to know more about the sample preparation and storage condition constraints to the widely used operationally defined procedures for assessing mineral N species in field moist soils. This experiment too was only possible because my supervisor arranged for me to get access to a group of second year environmental science students for a 2-day practical session so that very tight processing time constraints could be met. They were supervised closely throughout by the author.

Chapter 8 looks at spatial and temporal variations in nitrate-N concentrations in the River Derwent in North Yorkshire from the perspective of its having been declared a nitrate vulnerable zone, and to see if any of the preceding research helped to explain temporal trends found in 20-year runs of Environmental Agency data.

Finally, Chapter 9 briefly summarizes the conclusions from all the research and discusses their significance, but also suggests some possible future research avenues.

CHAPTER 2: SITE DESCRIPTIONS AND ANALYTICAL METHODS

2.1 Introduction

This chapter introduces the soils used in this study, and the analytical procedures employed in one or more subsequent chapters to measure soil properties that were thought probably to be regulating soil nitrogen species transformations and mobilities. It describes the major characteristics of the experimental sites used during the experimental studies.

2.2 Site description

2.2.1 Site selection rationale

Keeping in mind the hypotheses and research objectives of the thesis outlined at the end of Chapter 1, Hob Moor was selected as an experimental field site. Hob Moor has been affected for decades by anthropogenic N and S deposition, but no synthetic fertilizer additions have been made to the site for at least several decades (Claire Suddaby, personal communication). Therefore any changes in N status down soil profiles and current biochemical N transformations occurring are almost certainly responses to high levels of N deposition in this part of the UK (Hornung et al., 1995). Little research has been done on the site, though a poorly draining stagno-gley argillic brown earth profile from Hob Moor has been shown to contain 12.5 tonnes of N per hectare to 36 cm depth in a study of soil profile nitrogen storage (Crowe et al., 2004), the C:N ratio in that profile was <10 at all soil depths studied inspite of the fact that no fertilizer has been applied. The site has the advantage of being rapidly accessible from the Environment Department at the University of York and having soils with a diverse range of pH values and textures. Ready and rapid access was deemed to be important, because it was desirable to minimize N species transformations during transport back to the laboratory.

2.2.2 Hob Moor

The study site selected, Hob Moor, is just to the south of the city walls at York in the UK (53º57'30"N & 1º4'48"W, see Fig. 2.1). Hob Moor is a Local Nature Reserve covering an area of 36.4 ha of predominantly unfertilized grassland (see plates 2.1 and

2.2). The soils vary between naturally slowly permeable, seasonally wet, clay loams and more freely draining (and more acidic) very fine sandy loams and loamy sands (Plates 2.3- 2.5 show a typical example). The area is thus dominated by seasonally wet pastures but has small peripheral patches of deciduous woodland.

It has been cultivated in the distant past, and in some areas medieval ridge and furrow is also still visible (Smith, 2000). This needs to be considered when examining organic matter distributions in such soil profiles. In other areas residual impacts of ploughing in the Napoleonic era may be seen, usually though as parallel lines only on misty mornings or when the sun is very low or after heavy rainfall. Several grass tracks cross the moor (Fig. 2.1) and in 2002 a cycle track was established around the circumference. The management plan aims to maintain low nutrient status and high biodiversity of flora and fauna, birds and small mammals. The pasture land is grazed by cattle for more than six months every year, in an attempt to help reduce nutrient status, though this was not the case in 2009 because of the controls on animal movement. The site is dominated by perennial grasses, and currently the spreading of an area of thistles is becoming problematic. An attempt has been made to control this by hand weeding or hoeing by volunteers (Claire Suddaby, pers. comm.). Over the winter of 2007-08 cattle remained on the site because of restrictions in animal movement imposed by DEFRA.

Plate 2.1: A general view across the moor in summer looking N.E. towards the city of York. York Minster is visible in the distance. Vegetation has been kept short by grazing cattle.

Plate 2.2: A view of the freely draining acid grassland area in the N.E. corner of the moor. The area is mown infrequently to encourage biodiversity.

Plate 2.3: Typical acid grassland brown earth soil profile at Hob Moor. This is one of the acid brown earth profiles sampled for the ammonium absorption/desorption experiments described in detail in Chapter 5.

Plate 2.4: Exploring acid grassland brown earth soil profile at Hob Moor. From Right to Left: Malcolm Cresser, Ishaq A. Mian and Muhammad Riaz.

Plate 2.5: Sampling typical acid grassland brown earth soil profile at Hob Moor.

The Holgate Beck runs along two sides of the site, and the management strategy should reduce the risk of nitrate leaching into the surface water. However the beck is very prone to eutrophication, often supporting excessive aquatic plant species to the extent that its role in helping maintain local drainage can be restricted. The Beck is part of the flood protection scheme of this part of the city of York. It is maintained by Marston Moor Drainage Board, who, from time to time, apply herbicide to the stream channel. The killed vegetation is generally, however, simply deposited on the bank near

the acid grassland area. There it decomposes, returning nutrients to the stream and encouraging regrowth of aquatic plants.

Nitrate-N concentrations in the Beck have been monitored in February each year since 2001, and ranged from 0.2 up to 7.8 mg l^{-1} (Cresser, unpublished results), However, some of this almost certainly comes from gardens and a sports field upstream of the moor. There can be little doubt that much N enters to the stream in water draining from Hob Moor however. In the context of this thesis it will be shown that ammonium mobility down profiles may contribute to the stream N concentration and potentially to ground water contamination. Riaz et al. (2010b) have very recently reported that ammonium-N concentrations in the Beck frequently exceeded those of nitrate-N.

The climate is highly changeable, having sunny summer months (June-September) with an average temperature of 18-21 ºC. November to January are the coldest and wettest months. The precipitation pattern also has high temporal variation and annual rainfall is 639 mm. October-November receives 34.7% of total rainfall. The variation in temperature is also prominent around the 13.5 ºC mean monthly temperature. June and July are the hottest two months with a 21 ºC average monthly temperature. The mean monthly relative humidity is 80.3%. Source: (www.metoffice.gov.uk).

Figure 2.1: A simplified location map and map of Hob Moor. Ordinance survey © Crown copyright 2007.

2.3 Analytical methods used for studying soil N transformations and their dynamics

2.3.1 Determination of soil moisture content

In the laboratory on the same day that samples were collected, soils were spread temporarily on polythene sheets and carefully but quickly hand sorted wearing thin pre-washed (with deionized water) rubber gloves to remove remaining roots as far as possible and any remaining small stones and then mixed thoroughly. Approximately 10-g duplicate sub-samples of each field moist soil were weighed into pre-weighed, dried foil dishes or porcelain basins, oven dried over night at 105 °C, cooled in desiccators, and reweighed to determine the moisture content from the loss on mass, expressed on an oven-dry weight basis.

2.3.2 Measurement of soil C, N and C:N ratio

The oven-dried soil residues obtained as described in section 2.3.1 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 carry over 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 uses data from glutamic acid check standards, usually run after every 8 samples, to compensate for small amounts of instrument response drift. Because C and N concentrations were usually quite low, 150-200 mg samples were often used for analysis even though this gives a higher ash residue in the instrument.

2.3.3 Extractable ammonium-N and nitrate-N

Ten or 20-g sub-samples of field moist soil were weighed into labelled bottles. To each bottle 50 ml of 0.5 molar potassium chloride solution (0.5M KCl) was added. The bottles and duplicate reagent blanks were shaken intermittently by hand for one hour, and the contents filtered through Whatman No. 42 filter papers. The extracts were stored at < 4°C until analysis for ammonium-N and nitrate-N using a standard AutoAnalyser protocol with matrix-matched standards, as soon as possible (generally the next day) after extraction.

2.3.4 Ammonium absorption characteristics

Sub-samples of 10 +/- 0.1 g of each field moist soil (for which absorption isotherms were to be measured) weighed to an accuracy of +/- 0.01 g into nine labelled 120-ml plastic bottles.

Nine stock solutions of ammonium chloride were prepared, containing the following concentrations of ammonium-N: 0.0, 0.2. 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 and 50.0 μg ammonium-N ml^{-1}. To each bottle in each series of 9, 50 ml of the appropriate ammonium-N solution was added. The bottles were shaken vigorously by hand for 10 minutes and then immediately filtered through Whatman No. 42 papers. Extracts were refrigerated until the samples were analysed for ammonium-N using an AutoAnalyser (within 3 days). When calculating the amounts of ammonium-N in solution, the volume of water contained in the field moist soil was added to the volume of solution added (50 ml). The above spike sizes were selected after a preliminary trial and error experiment using two soils. These demonstrated significant differences between isotherms when ammonium was added as sulphate rather than chloride. This was attributed to suphate absorbtion reducing the mobile anion concentration.

2.3.5 Measurement of soil pH

The pH of soil was measured in duplicate, generally in both water and in 0.05 molar calcium chloride solutions, by adding 20 ml of water or $CaCl_2$ to 10 g sub-samples of field-moist soil. The mixtures were equilibrated with periodic agitation for 30 minutes prior to measurement of the soil pH with a glass/calomel combination electrode using a pH meter 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.

2.3.6 Cation exchange capacity and exchangeable base cations

Exchangeable base cations and cation exchange capacity were measured by leaching 10-g sub-samples of field moist soil held in a 50 ml plastic syringe barrel with 100 ml of 1 molar ammonium acetate, washing out the non-absorbed ammonium with 100 ml of 80% ethanol, and then leaching the absorbed ammonium with 1 molar sodium chloride, all from inverted 100-ml volumetric flasks (Marr and Cresser, 1983). The final leachate was diluted back to 100 ml with the sodium chloride solution prior to determination of its desorbed ammonium content. In Chapter 5 these preparations were performed by a

group of second year BSc Environmental Science students closely supervised by the author, using the instructions given on the following page which were prepared by the author for the class use. This was necessary because a large number of samples had to be processed within a few hours for this chapter. The author performed all the ammonium determinations on an AutoAnalyser, however.

2.3.7 Procedure for determination of exchangeable base cations and CEC

- Insert 3.0 g of cotton wool into the bottom of a labelled leaching tube for each soil.
- Weigh out 10 g of field moist soil (weighed to the nearest 0.01 g; record the weight) on a plastic boat. Do the next step at the same time at the same balance.
- Immediately tip the soil carefully into the tube above the cotton wool plug. If any soil adheres to the boat, reweigh the boat and adhering soil without re-taring, and subtract the residue weight from the original weight. Hand in the weight of soil that actually went into the tube (in g) to Ishaq Mian.
- Add another 3.0 g of cotton wool above the soil.
- Lightly compact the cotton wool plugs and soil.
- Leach 100 ml of 1 molar ammonium acetate from an inverted 100-ml volumetric flask (as will be demonstrated to you) through the soil to make sure every cation exchange site is occupied by an ammonium ion (NH_4^+). Leaching should take at least 30 minutes. If it is too fast, it may be necessary to compact the soil more. Collect the ammonium acetate that leaches through into a 100-ml volumetric flask, and if necessary dilute to the 100-ml mark with more ammonium acetate. Transfer this solution to a clean labelled plastic bottle (labelled with soil No and Amm. Ac.). Save this solution as it contains exchangeable Ca^{2+}, Mg^{2+}, K^+ and Na^+, which will eventually be determined by AAS. Hold the labelled bottles to Ishaq Mian for checking.
- Wash the initial 100-ml volumetric flask (the one that contained the leaching solution before it went through the soil) very carefully, by rinsing 4 times with tap water and 3 times with deionized water. Fill it to the 100-ml mark with 80% ethanol solution and leach this through the soil. This washes out any residual ammonium in the soil which was not held on cation exchange sites. Collect the leachate in the empty collecting flask that you just emptied into a 125-ml plastic

bottle. When the tube stops dripping, discard the wash solution down the sink, and wash the flask very carefully as before.

- Add 100 ml of 1 molar sodium chloride solution to the flask that previously contained the 80% pure ethanol used for washing. Leach this through the soil, collecting the leachate in another 100-ml volumetric that you have just carefully washed out.
- Dilute the leachate to exactly 100 ml with sodium chloride.
- Transfer the diluted solution to another clean labelled 125-ml plastic bottle (labelled with soil No and NaCl leachate), and hand the labelled sample to Ishaq Mian for checking.
- Thoroughly wash and rinse the empty volumetric flasks 4 times with deionized water and leave to drain.

2.4 Conclusions

To avoid unnecessary detail repetition, this chapter has described the site in some depth and analytical methods in detail. In future chapters, only brief descriptions are therefore used unless extra chapter specific detail is necessary.

CHAPTER 3: POTENTIAL EFFECTS OF DRYING AND REWETTING ON N CYCLING IN SOIL UNDER HOB MOOR ACID GRASSLAND: A PRELIMINARY INVESTIGATION

3.1 Introduction

The influence of natural drying and rewetting cycles in soils is currently a topic of considerable interest globally, because of possible effects that climate change may have on carbon exchange, especially as the greenhouse gases carbon dioxide and methane, between soils and the atmosphere (Wu et al., 2010). Although C and N mineralization are thought to be triggered in semi-arid and arid ecosystems by periodic pulses of water availability (McIntyre et al., 2009), rather less attention apparently has been paid, to the impacts of such rewetting upon the nitrogen cycle, although comparable impacts are likely for N biogeochemical cycling.

Baldwin and Mitchell (2000) reviewed the potential impacts of drying and rewetting of floodplain sediments in Australia. They suggested that partial drying of previously very wet sediments could reduce N availability by providing adjacent local zones for nitrification and subsequent denitrification. They also commented that complete desiccation could lead to "death of bacteria" and subsequent mineralization of N and P. Their paper included a diagram of the N cycling for such systems, which suggested that they perceived the N released from dead bacteria became available to biota in the overlying water column, rather than to regeneration of the microbial biomass, which the author thinks is likely to be of comparable importance.

Bottner (1985) found that rapid drying of soils for 8 – 10 days at 40 °C apparently destroyed a third to a quarter of the biomass in 4 successive drying and rewetting cycles. The "dead" biomass is likely to constitute a substrate for subsequent regeneration of biomass. Gordon et al. (2008) found that microbial biomass N was reduced significantly as a consequence of drying and rewetting. They observed that the stress induced by drought stimulated greater nutrient leaching from improved grassland soil compared with unimproved grassland soil, and suggested that the greater abundance of fungi in the latter ecosystem was significant in terms of its greater resistance to drying impacts. Landesman and Dighton (2010), based upon a two year water-exclusion experiment in New Jersey Pinelands, concluded that, to a substantial degree,

microbial populations were adapted to tolerate often substantial periods of natural drought.

It has been well known for decades that soil drying-rewetting cycles result in an increase in the DOC concentration in soil solution, and hence potentially in drainage water (Kieft et al., 1987; Lundquist et al., 1999). Williams and Xia (2009) investigated the nature of the organic matter that became soluble after soils had been subjected to drying at controlled moisture tensions and rewetting. They concluded that the flush of soluble organic C following rewetting was not heavily dominated by the microbial osmolytes such as glycerine, betaine, glycerol, mannitol, proline etc. that might be expected, and that more research was needed into the processes involved.

3.1.1 Effects of drying-rewetting cycle periods on soil C and N transformations

It is recognized that drying and rewetting cycles may severely stress soil microbial communities (Fierer and Schimel, 2002). Duration of the cycles clearly will be an important factor regulating the nature and extent of impact. Fierer and Schimel (2002) subjected soils to laboratory simulated wetting drying cycles, applying 0, 1, 2, 4, 6, 9 or 15 such cycles spread over a 2-month period. They conducted the experiment so that the mean soil moisture content was constant for all samples however. Respired CO_2 increased with number of cycles for soils from under oak wood, but not for a grassland soil. Extractable-ammonium concentrations were low in both soils, and not affected by treatment. They also found no significant effects of the stress treatments on the concentrations of 0.5M-extractable nitrate-N either 1 day or 7 days after application of the stress the final drying-rewetting stage, but a small but significant decrease after 6 weeks delay.

An example of an extreme drying period may be found in the work of Nobili et al. (2006), who examined the effect of rewetting soils that had been stored dry for 103 years, and compared soil microbial biomass activity and concentration after rewetting with the values for soils stored air dry for different periods. The CO_2 evolution on rewetting was more than doubled by extended storage. The ATP concentrations were lower in rewetted soils than values for corresponding field moist soils, but trends were variable and were not consistent. The authors did conclude, though, that loss of viability during long-term storage occurred mainly over the earlier years. Measurement of ATP has been used for some time as a useful index of recovery of microbial activity during rewetting (Ahmed et al., 1982). Working on much shorter time scales, Van-

Gestel et al. (1993) reported that drying soil at 40 °C for 27 days immediately following addition of plant residues had a very pronounced impact upon microbial activity on subsequent rewetting. They concluded that the actively growing part of the microbial biomass was hit hardest by periods of desiccation.

3.1.2 Effects drying-rewetting cycles on C and N mineralization of litter residues

Litter mineralization is a crucial part of the N cycle in natural and managed soil systems. Kruse et al. (2004) investigated the effect of drying and rewetting upon C and N mineralization in soils with a low organic matter content to which either compost or cotton leaf litter had been added. The moisture effect was not significant in the control (un-amended) soils). However, for the leaf litter-amended soil, N mineralization fell from 25 to 40% of the residue N being mineralised in continuously moist soil to -1.3 to 6.9% in soil under fluctuating moisture conditions. Decreases were smaller for the compost-amended soil.

The conditions of rewetting appear to be important to the effects on microbial activity observed. McIntyre et al. (2009) found that if soils became saturated as a consequence of rewetting, the rate of mineralisation was appreciably lower than when they were wetted up to a lesser extent.

Muhr et al. (2010) reported that for microcosms containing organic horizons of soils from a Norway Spruce forest in Germany, drying and rewetting reduced C and N mineralisation and that the reduction increased with simulated drought duration. Nitrate leaching was lowest from the microcosms subjected to the greatest drought, suggesting to the present author the possibility of at least partial nitrate immobilization during the drying period. They commented that net nitrification was close to zero during the drought period. It should be remembered, however, that effects on net nitrification response to drying/rewetting may be very different in surface soils and sub-surface soils (Xiang et al., 2008).

3.2 Aims of present experiment

It is interesting to speculate on what happens to the net nitrate and net ammonium present in soil during the drying process itself, as this does not appear to have been done comprehensively in the papers reviewed in the introduction. If a soil is ammonifying and nitrifying, it may be assumed that these processes will continue, especially within aggregates, during the early period of soil drying. If the soil has been removed from vegetation, this would be likely to lead to an initial increase in the extractable ammonium-N and nitrate-N concentrations in the soil. However, if the additional ammonium-N produced is nitrified, then ammonium-N concentration could remain initially quite similar. However, some mineralized N would also be recycled through microbial biomass. Thus both ammonium and nitrate could be incorporated into new biomass. Clearly at this stage it is conceivable that a mis-match could occur between rates of mineral-N species production and microbial biomass N immobilization, since both processes are likely to respond differently to the changing desiccation conditions.

As drying proceeds and some of the microbial biomass components that are more sensitive to desiccation start to die due to the desiccation stress, then substrate could become available to less susceptible components of the biomass. At this stage it may be hypothesised that mineral N species would be at least partially incorporated into any new microbial biomass growth. As soils become drier, however, the soil solution solute species concentrations become more concentrated, encouraging osmotic penetration of water through cell walls and microbial cell collapse.

If nitrate-N immobilization as hypothesised above does not occur during later stages of drying, then rewetting would lead to an instantaneous apparent nitrate-N flush as residual nitrate-N retained in the dry soil is released. If the flush is not instantaneous then it is necessary to invoke the slower process whereby the population of nitrifying organisms increases, followed by a delayed nitrate flush.

It was therefore decided to collect duplicate samples of a freely-draining, brown-earth, acid grassland soil from Hob Moor, just outside York, and allow them to dry out at room temperature. The KCl-extractable ammonium-N and nitrate-N concentrations were measured immediately and then at intervals over a 6-week period. The soils would be rewetted, and N mineralisation monitored over 7 days. For the drying experiment soils would be collected from five 20-cm depth increments, covering a total depth of 1 m, as papers discussed in the introduction suggest depth may significantly influence response of net mineral N to desiccation.

The experiment will test the following hypotheses:

- Nitrate-N and ammonium-N will increase over the early drying period.
- Nitrate-N concentration will reach a maximum when nitrification rate becomes insignificant compared with microbial sinks for nitrate.
- When nitrification becomes insignificant, ammonium-N concentration may undergo further increases for some time.
- If some microbial activity continues, nitrate concentration may start to fall as a consequence of microbial immobilization.
- If nitrate is immobilized, this will show up in a delay in any nitrate-N flush when the soil is rewetted.

3.3 Materials and methods

3.3.1 Field sampling

The characteristics of the Hob Moor site were described in detail in Chapter 2. The area sampled was in the part of the moor which is a freely draining acid grassland. This was selected because it is fenced off from cattle which graze the rest of the Moor for about 6 months each year. However, it does occasionally receive urine and faeces from dogs being walked in the area, although observations suggest this animal fowling is predominantly adjacent to the paths, which were a few metres away from the sampled areas.

Two soil pits, separated by about 10 m, were excavated to a depth of 1 m with the help of Riaz and Cresser in May 2007, taking care to leave one face undisturbed for sampling. At each pit the surface vegetation from this face was carefully removed with a sharp, stainless steel carving knife with a serrated blade. Samples were then collected to represent 20-cm depth increments from the bottom of the cleaned profile face upwards (to reduce risk of contamination from above) using a pointed stainless steel trowel. Samples were taken from either side of the exposed profile face, to give duplicate field samples. The samples were transferred to clean, pre-labelled polythene bags for immediate return to the laboratory and analysis.

3.3.2 Sample preparation and initial analysis

Stones and obvious root fragments were removed as quickly as possible by hand. Sub-samples were then analysed on the same day that they had been taken for 0.5 M KCl-extractable ammonium-N and nitrate-N (10:50 m:v), soil pH (in 0.5 M KCl at 2:1 soil:solution to minimise mobile anion effects on soil pH profiles), and soil moisture content, as described in Chapter 2. The residual dry soil, after measuring the weight-loss the next day after oven drying, was finely ground and used to measure soil C%, N% and C:N ratio, again as described in Chapter 2. All determinations were performed in duplicate, and means of the analytical duplicates were used to provide 4 mean values for the 4 field replicates (2 from each profile).

3.3.3 Drying experiment

The 4 field replicate residual soils from each of the 5 sampling depths increments were spread thinly (ca. 2 – 4 cm depth) to dry on sheets of heavy duty polythene in a laboratory which was not otherwise being used at the time. After 5, 10, 15, 20 and 42 days of drying, subsamples were taken for duplicate determination of 0.5 M KCl-extractable ammonium-N and nitrate N using a Bran and Luebbe AutoAnalyser® -3 with matrix-matched standards, and soil moisture content, as described in Chapter 2. All results were blank-corrected, and expressed on an oven-dried soil basis, taking into account the dilution effect of the residual water in the soils.

3.3.4 Rewetting experiment

Subsamples of the 4 field replicate residual soils from the 0-20, 20-40 and 40-60 cm depth samples were rewetted by the addition of deionized water. Six sub-samples of 10 g of each of the soils from these depths that had been dried for 42 days were weighed into clean polythene bottles, and 2 ml of water was added to each. The bottles of moistened soil were shaken vigorously for 5 minutes. Two subsamples of each soil were extracted immediately with 0.5 M KCl, two were extracted after 3 days of incubation and the final pair of duplicates after 7 days of incubation at room temperature. The bottles of moistened soil were weighed, and during incubation deionized water was added to replenish any loss by evaporation. The ammonium-N and nitrate-N concentrations in the extracts were measured using an AutoAnalyzer as described in Chapter 2, and the masses of nitrate-N and ammonium-N per unit mass of soil were calculated, making a correction for the mass of added water.

3.4 Results

3.4.1 Changes in extractable nitrate-N and ammonium-N during drying

Figures 3.1 to 3.5 show how the extractable nitrate-N concentration changed over 42 days of drying at room temperature for the 0 – 20, 20 – 40, 40 – 60, 60 – 80 and 80 – 100 cm depth increments, respectively. The nitrate-N attained a maximum value at 5 – 10 days for all depths, and then declined to a very low residual value. A 3rd order polynomial equation was fitted in each case to the data over the first 20 days only. Although 4th order polynomials fitted the data well over 42 days, this resulted in some negative values of nitrate concentration between 20 and 42 days, so was deemed inappropriate.

Figures 3.6 to 3.10 show how the extractable ammonium-N concentration changed over 42 days of drying at room temperature for the 0 – 20, 20 – 40, 40 – 60, 60 – 80 and 80 –100 cm depth increments respectively. In this instance linear regression analysis suggested that ammonium-N concentration continued to increase linearly with time.

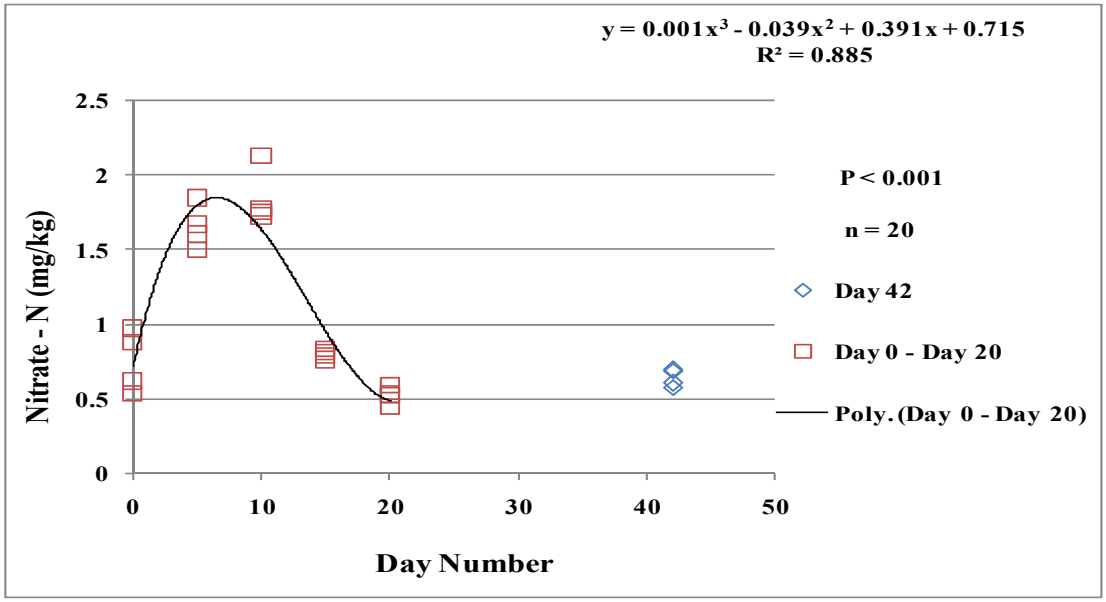


Fig. 3.1 Change in KCl-extractable nitrate-N concentration in soil from 0-20 cm depth during drying over 42 days. The 4 points at each time are means of analytical duplicates for the field duplicates from the duplicate soil profiles.

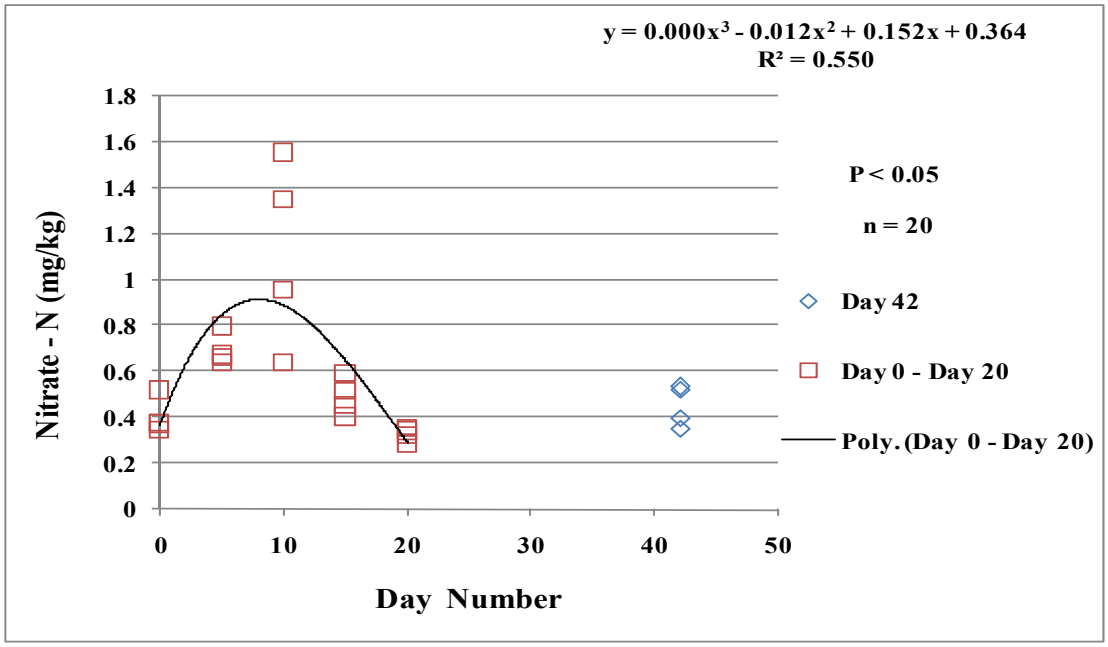


Fig. 3.2 Change in KCl-extractable nitrate-N concentration in soil from 20-40 cm depth during drying over 42 days. The 4 points at each time are means of analytical duplicates for the field duplicates from the duplicate soil profiles.

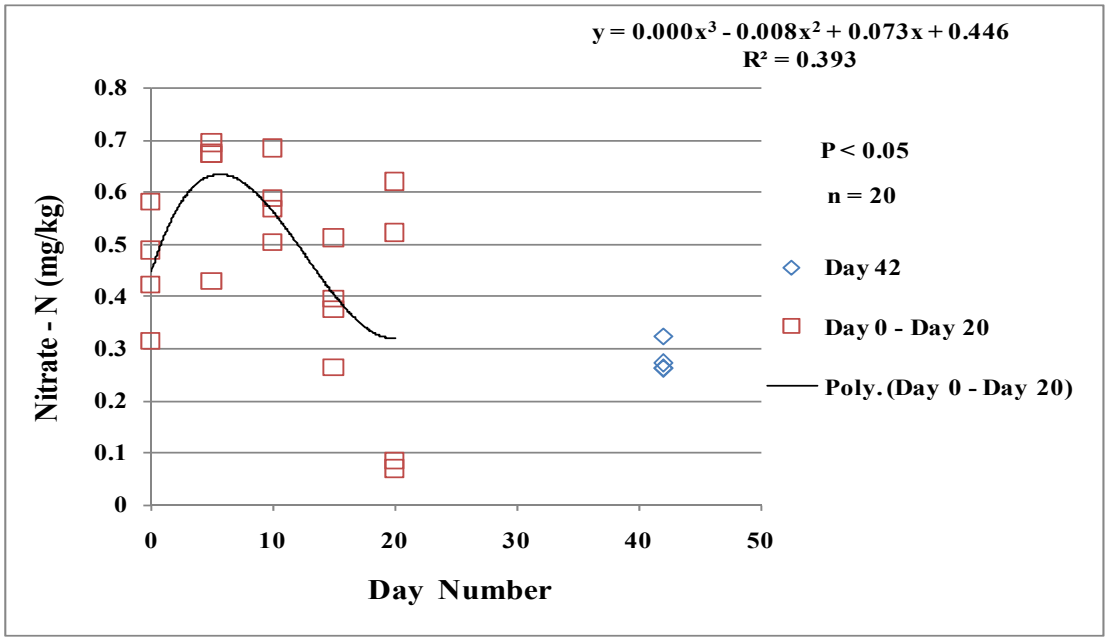


Fig. 3.3 Change in KCl-extractable nitrate-N concentration in soil from 40-60 cm depth during drying over 42 days. The 4 points at each time are means of analytical duplicates for the field duplicates from the duplicate soil profiles.

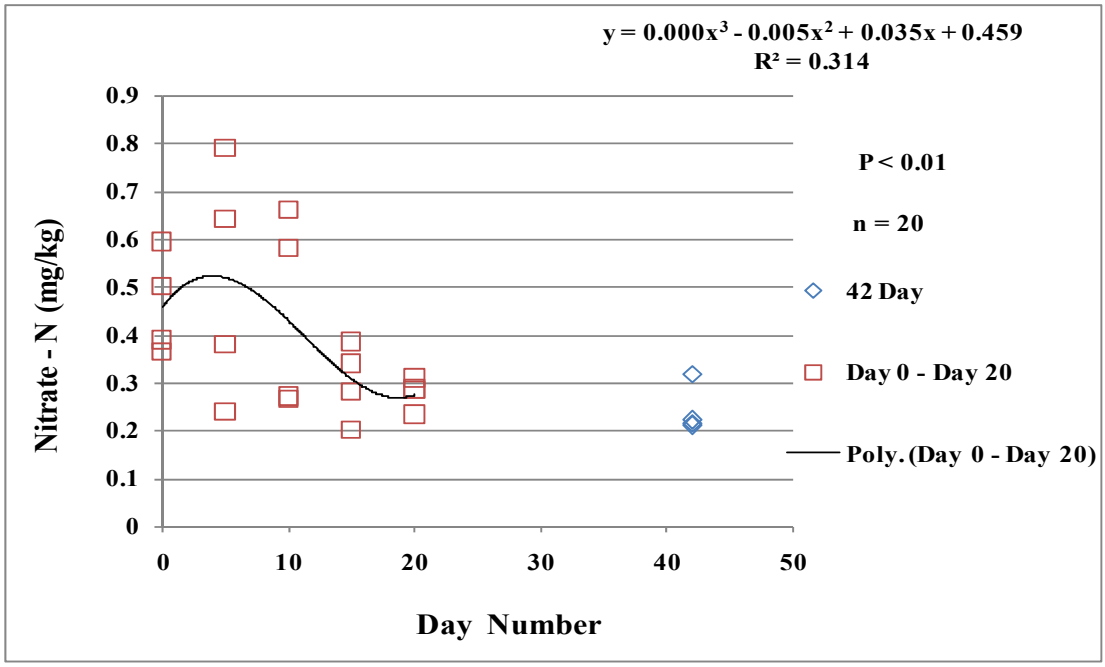


Fig. 3.4 Change in KCl-extractable nitrate-N concentration in soil from 60-80 cm depth during drying over 42 days. The 4 points at each time are means of analytical duplicates for the field duplicates from the duplicate soil profiles.

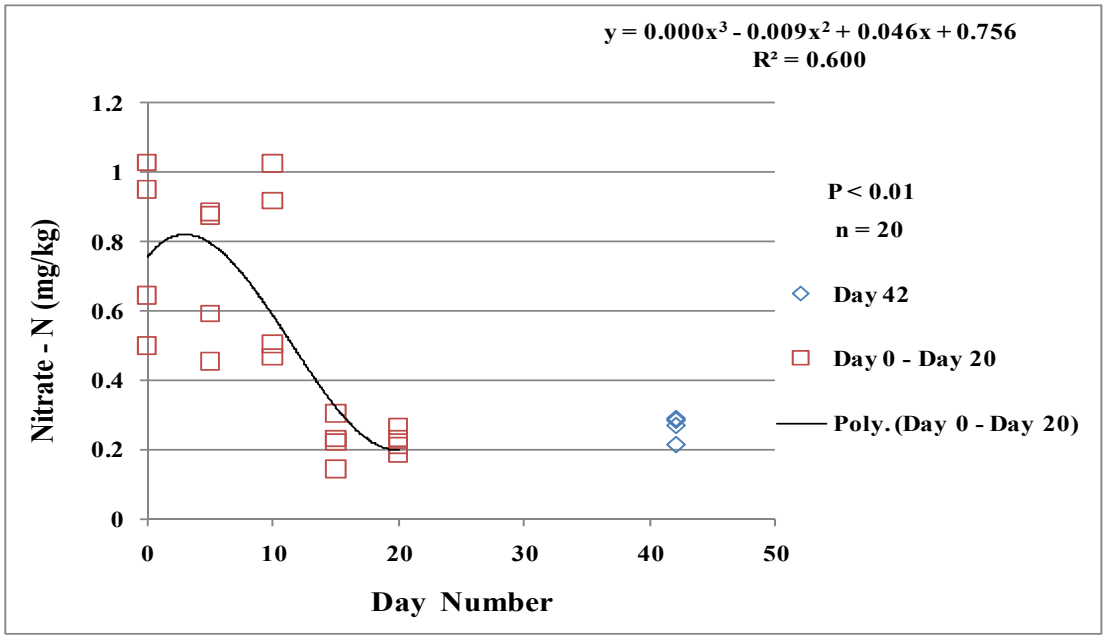


Fig. 3.5 Change in KCl-extractable nitrate-N concentration in soil from 80-100 cm depth during drying over 42 days. The 4 points at each time are means of analytical duplicates for the field duplicates from the duplicate soil profiles.

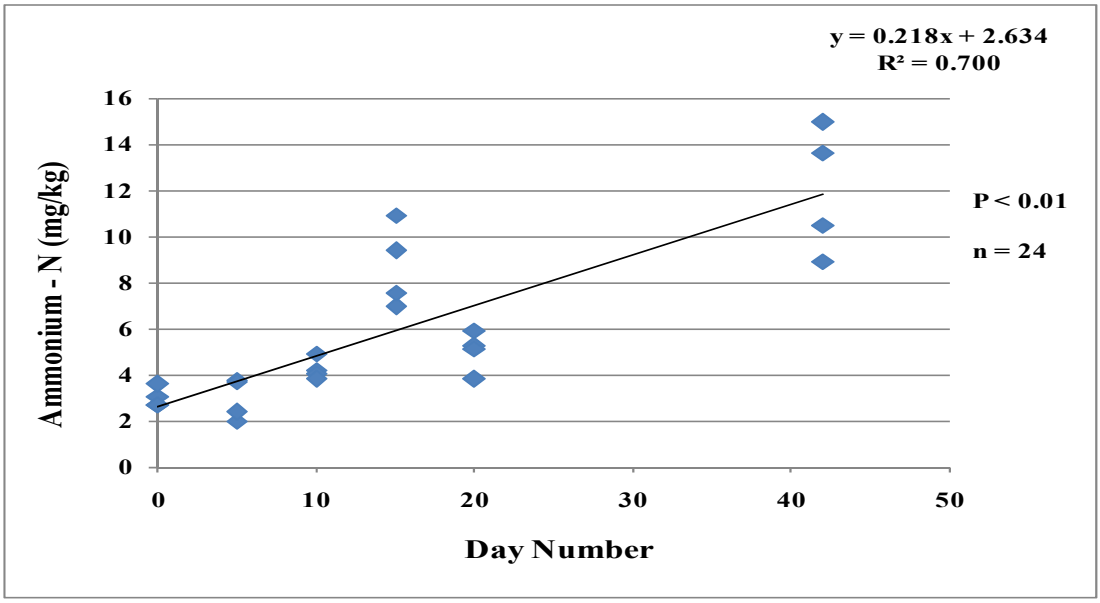


Fig. 3.6 Change in KCl-extractable ammonium-N concentration in soil from 0-20 cm depth during drying over 42 days. The 4 points at each time are means of analytical duplicates for the field duplicates from the duplicate soil profiles.

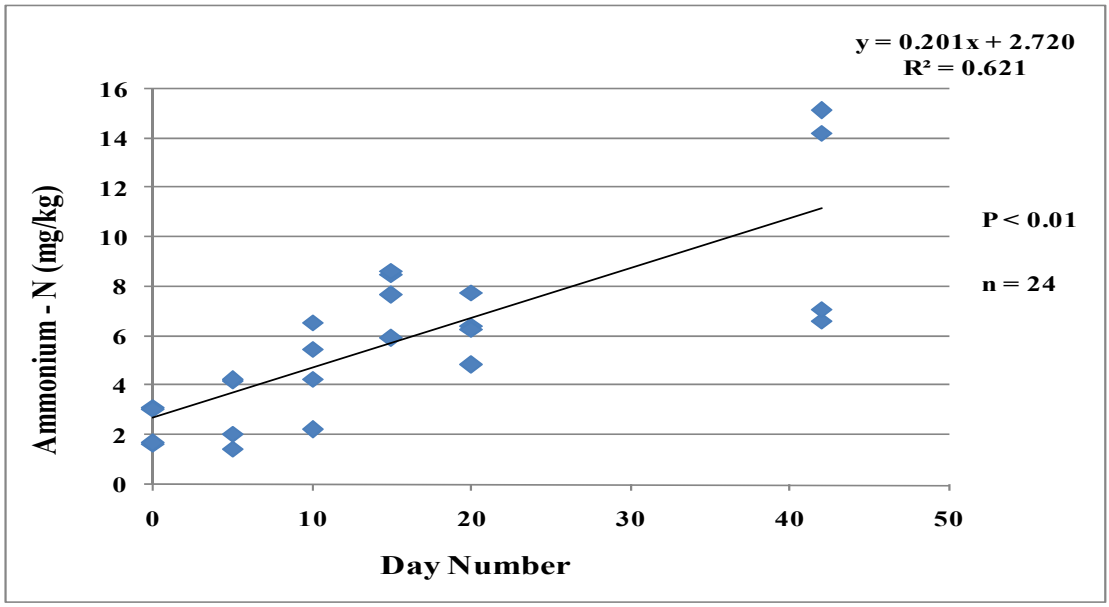


Fig. 3.7 Change in KCl-extractable ammonium-N concentration in soil from 20-40 cm depth during drying over 42 days. The 4 points at each time are means of analytical duplicates for the field duplicates from the duplicate soil profiles.

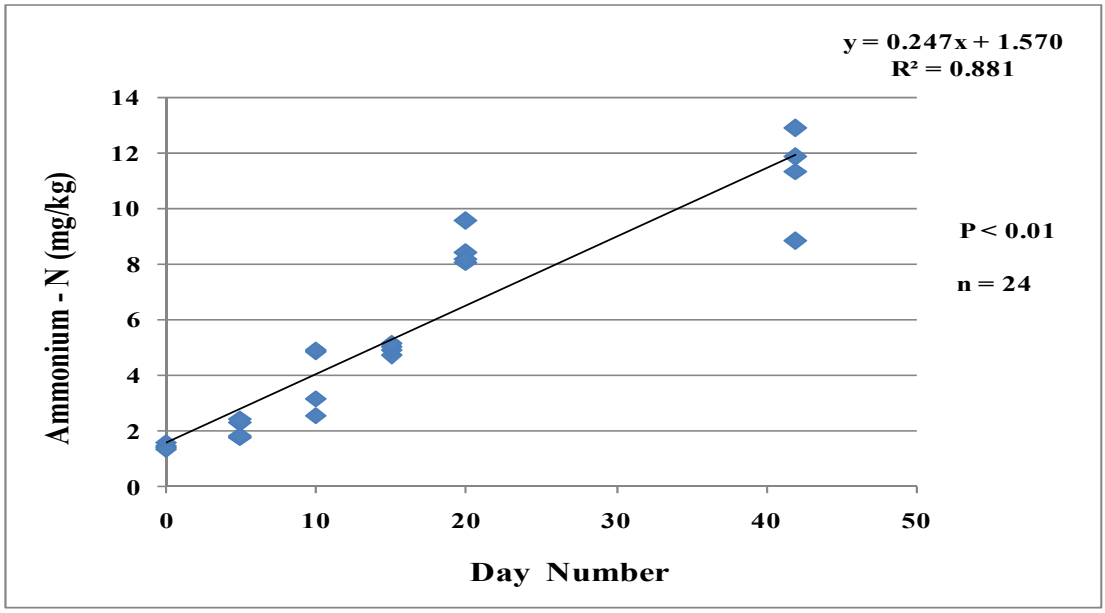


Fig. 3.8 Change in KCl-extractable ammonium-N concentration in soil from 40-60 cm depth during drying over 42 days. The 4 points at each time are means of analytical duplicates for the field duplicates from the duplicate soil profiles.

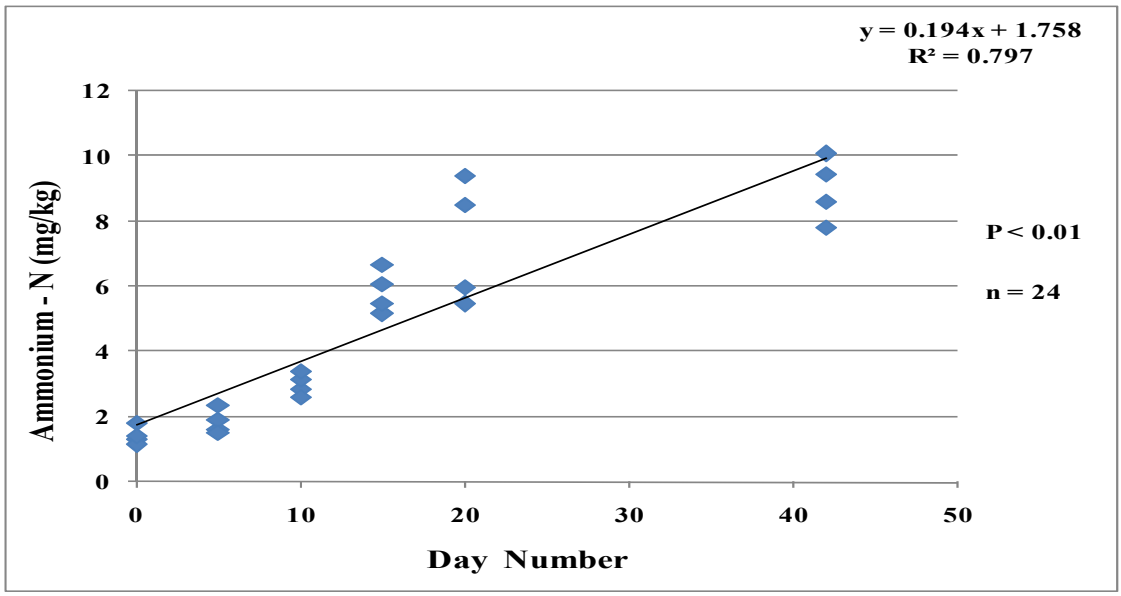


Fig. 3.9 Change in KCl-extractable ammonium-N concentration in soil from 60-80 cm depth during drying over 42 days. The 4 points at each time are means of analytical duplicates for the field duplicates from the duplicate soil profiles.

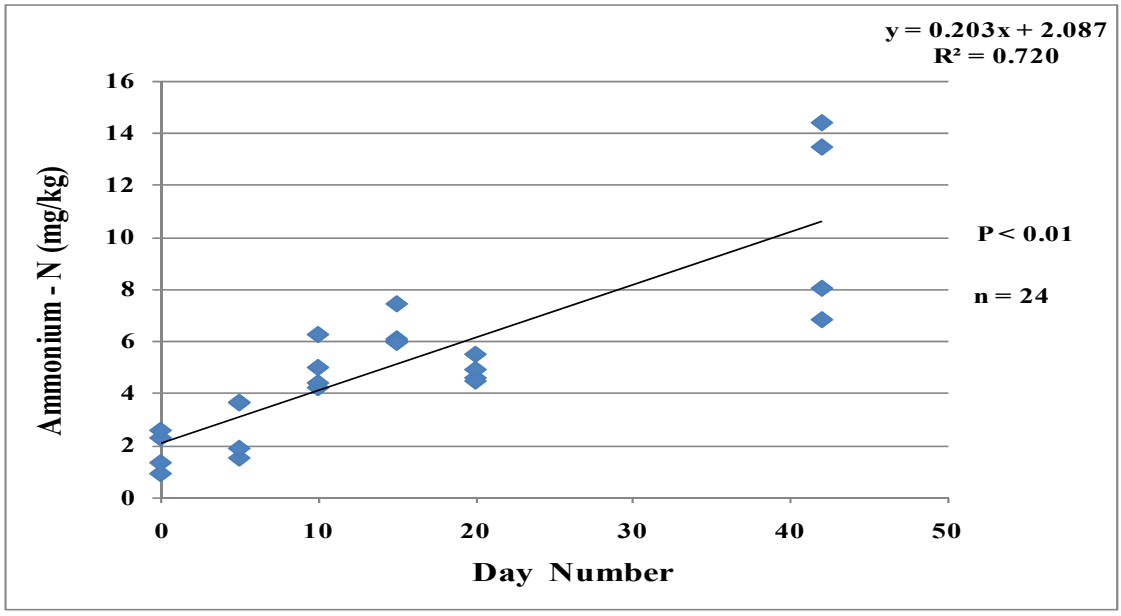


Fig. 3.10 Change in KCl-extractable ammonium-N concentration in soil from 80-100 cm depth during drying over 42 days. The 4 points at each time are means of analytical duplicates for the field duplicates from the duplicate soil profiles.

3.4.2 Changes in extractable ammonium-N and mineral-N after rewetting

Figures 3.11 to 3.13 show the effects of rewetting (after drying for 42 days) on the concentrations of KCl-extractable ammonium-N and total mineral-N after 1, 3 and 7 days, respectively. The results are plotted separately for soils from profiles A and B in each figure to facilitate comparison of the two soil profiles. Figures 3.11 to 3.13 show results for soils from 0-20, 20-40 and 40-60 cm depths respectively.

Ammonium-N and mineral N concentration values were very similar in almost every soil at most sampling dates. It is immediately obvious therefore that nitrate-N concentrations were almost negligible compared with ammonium-N concentrations for both profiles at all 3 depth increments, except after 3 days (R3) for profile B at 40-60 cm depth.

Profile B had higher initial amounts of ammonium-N and mineral-N than profile A, and this was also the case one day after rewetting (R1) for soil from 0-20 and 20-40 cm depths (Figs. 3.11 and 3.12). However by 3 and 7 days the soil from 0-20 cm from profile A (Fig. 3.11) had more ammonium-N and mineral-N than the corresponding soil from profile B. In marked contrast, soils from 20-40 cm gave much more ammonium-N and mineral-N in profile B than profile A at day 3 (Fig. 3.12). Ammonium-N and nitrate-N concentrations had increased significantly by day 3 compared to day 1 in both

profiles, but then decreased significantly by day 7 for soils from 0-20 cm. The trend was similar for 20-40 cm but the decrease from day 3 to day 7 was not significant for profile A (Fig. 3.12).

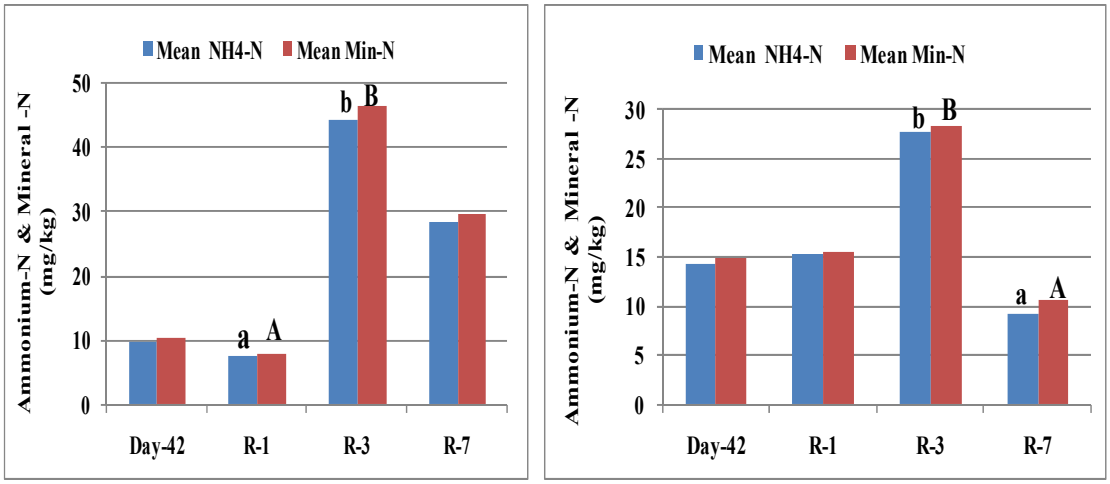


Fig. 3.11 Changes in ammonium-N and mineral-N concentrations in soils from 0 - 20 cm depth from profile A (left chart) and profile B (right chart) between the end of the 42-day drying period and following 1 (R1), 3 (R3) and 7 (R7) days of rewetting. Each bar is the mean of 4 replicate determinations (2 analytical replicates x 2 field replicates). Bars for ammonium-N or for mineral-N with different letters differ significantly at $P < 0.05$.

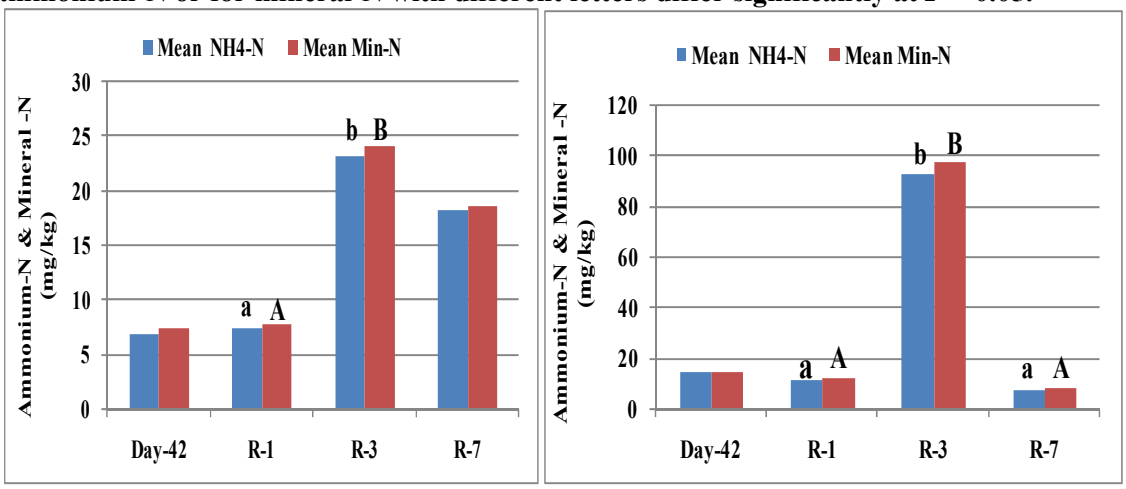


Fig. 3.12 Changes in ammonium-N and mineral-N concentrations in soils from 20 - 40 cm depth from profile A (left chart) and profile B (right chart) between the end of the 42-day drying period and following 1 (R1), 3 (R3) and 7 (R7) days of rewetting. Each bar is the mean of 4 replicate determinations (2 analytical replicates x 2 field replicates). Bars for ammonium-N or for mineral-N with different letters differ significantly at $P < 0.05$.

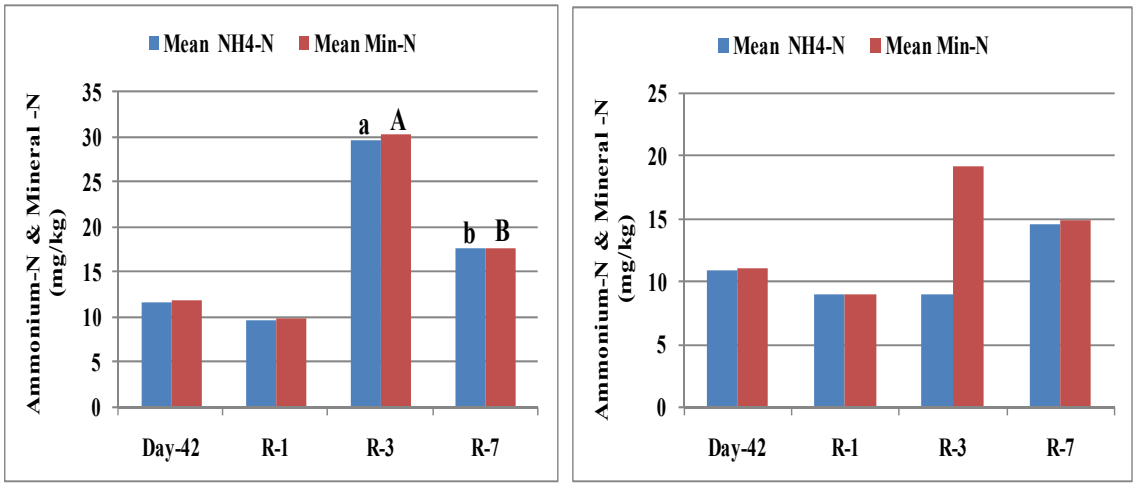


Fig. 3.13 Changes in ammonium-N and mineral-N concentrations in soils from 40 - 60 cm depth from profile A (left chart) and profile B (right chart) between the end of the 42-day drying period and following 1 (R1), 3 (R3) and 7 (R7) days of rewetting. Each bar is the mean of 4 replicate determinations (2 analytical replicates x 2 field replicates). Bars for ammonium-N or for mineral-N with different letters differ significantly at $P < 0.05$.

For soil from 40-60 cm depth, the extractable ammonium-N and mineral-N for the 2 profiles did not differ significantly either initially or after 1 day (Fig 3.13). However, 3 days after rewetting, more mineralisation had occurred for profile A than for profile B and profile B was showing clear evidence for substantial nitrification, as mineral N >> ammonium-N (Fig. 3.13). There was significant ($P < 0.05$) evidence of immobilization for profile A, between day 3 and day 7, but not for profile B.

Seven days after rewetting, there were substantial declines in ammonium-N and mineral-N concentrations compared with those after 3 days at all depth increments studied for both profiles (Figs. 3.11-3.13). This immobilization of mineral N was however particularly marked for profile B at 0-20 and 20-40 cm.

3.4.3 Changes in extractable nitrate-N after rewetting

Changes in KCl-extractable nitrate-N concentrations following rewetting after a 42-day drying period are shown in Figs. 3.14 - 3.16 for soils of the two profiles from 0-20, 20-40 and 40-60 cm respectively. The nitrate data have been presented separately because of the very low-nitrate-N concentrations after drying, compared with the ammonium-N concentrations.

In the soils from 0-20 cm the two profiles initially had similar nitrate-N concentrations (Fig. 3.14). However nitrification proceeded more rapidly in soil from profile A (ca. 2.1 mg kg^{-1} compared with 0.7 mg kg^{-1} in profile B). At this depth it appeared that immobilization was occurring by day 7 in soil from profile A though the

decline was not significant, whereas nitrification was higher at day 7 than at day 3 for profile B (Fig. 3.14) but again the apparent effect was not significant. At 20-40 cm depth, on the other hand, both profiles gave their maximum nitrate-N concentrations at day 3, with marked immobilization by day 7 that was significant for profile B only (Fig. 3.15). The nitrate-N peak was at a much higher concentration for profile B than for profile A, as indicated by the difference between ammonium-N and total mineral-N for this profile at day 3, mentioned in the previous section. Trends with time after rewetting were superficially similar for soils from 40-60 cm depths to those for soils from 20-40 cm depths for both profiles, but again although nitrification appeared more substantial in profile B, no differences were significant.

Strictly speaking, of course it should be said that net nitrate production was higher in soil from profile B, because the extractable nitrate-N concentration depends upon initial nitrate-N concentration, nitrification rate, nitrate-N immobilization rate and any denitrification (though the latter was thought to be small as the soils were maintained under aerobic conditions).

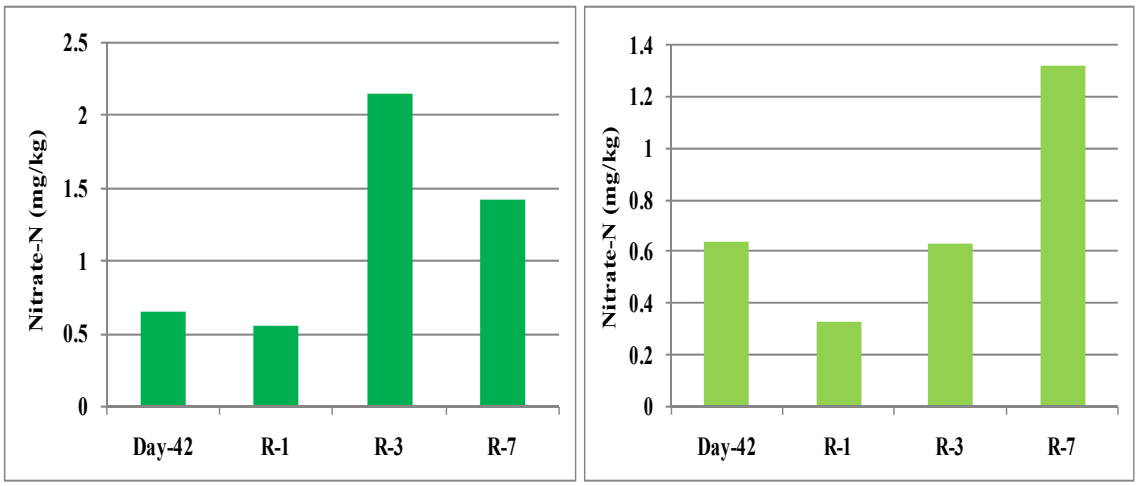


Fig. 3.14 Changes in nitrate-N concentrations in soils from 0 - 20 cm depth from profile A (left chart) and profile B (right chart) between the end of the 42-day drying period and following 1 (R1), 3 (R3) and 7 (R7) days of rewetting. Each bar is the mean of 4 replicate determinations (2 analytical replicates x 2 field replicates). No differences between times were significant at $P < 0.05$ at this depth.

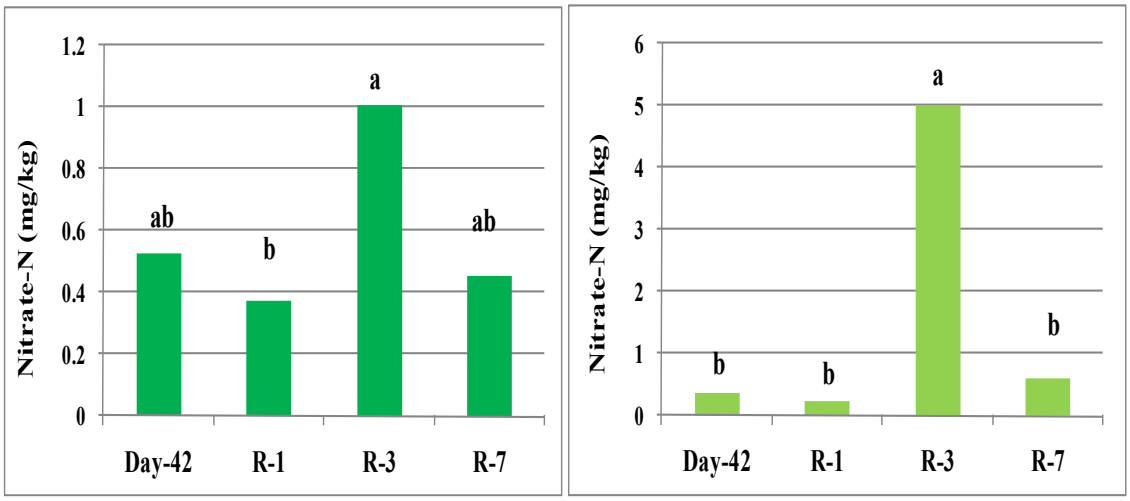


Fig. 3.15 Changes in nitrate-N concentrations in soils from 20 - 40 cm depth from profile A (left chart) and profile B (right chart) between the end of the 42-day drying period and following 1 (R1), 3 (R3) and 7 (R7) days of rewetting. Each bar is the mean of 4 replicate determinations (2 analytical replicates x 2 field replicates). Bars with different letters only differ significantly at $P < 0.05$.

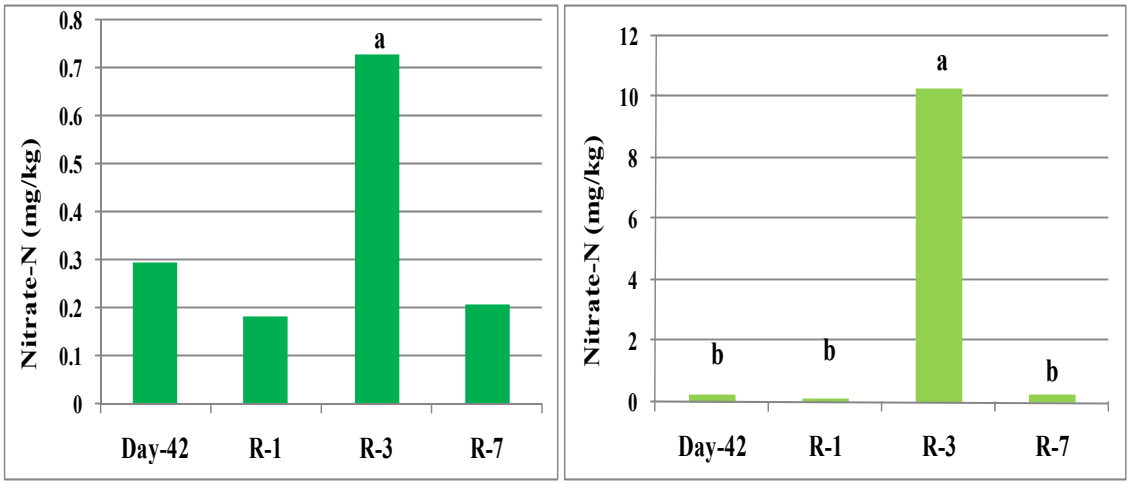


Fig. 3.16 Changes in nitrate-N concentrations in soils from 40 - 60 cm depth from profile A (left chart) and profile B (right chart) between the end of the 42-day drying period and following 1 (R1), 3 (R3) and 7 (R7) days of rewetting. Each bar is the mean of 4 replicate determinations (2 analytical replicates x 2 field replicates). Bars with different letters only differ significantly at $P < 0.05$.

3.4.4 Variation in extractable ammonium-N after rewetting with depth

Changes with soil depth in KCl-extractable ammonium-N concentrations following rewetting after a 42-day drying period are shown for the 2 soil profiles in Figs. 3.17 and 3.18. For profile A, 1 day after rewetting, extractable ammonium-N had decreased except at 20-40 cm. For profile B it had decreased at 20-40 and 40-60 cm. Profile B at some depths had significantly more ammonium-N than profile A.

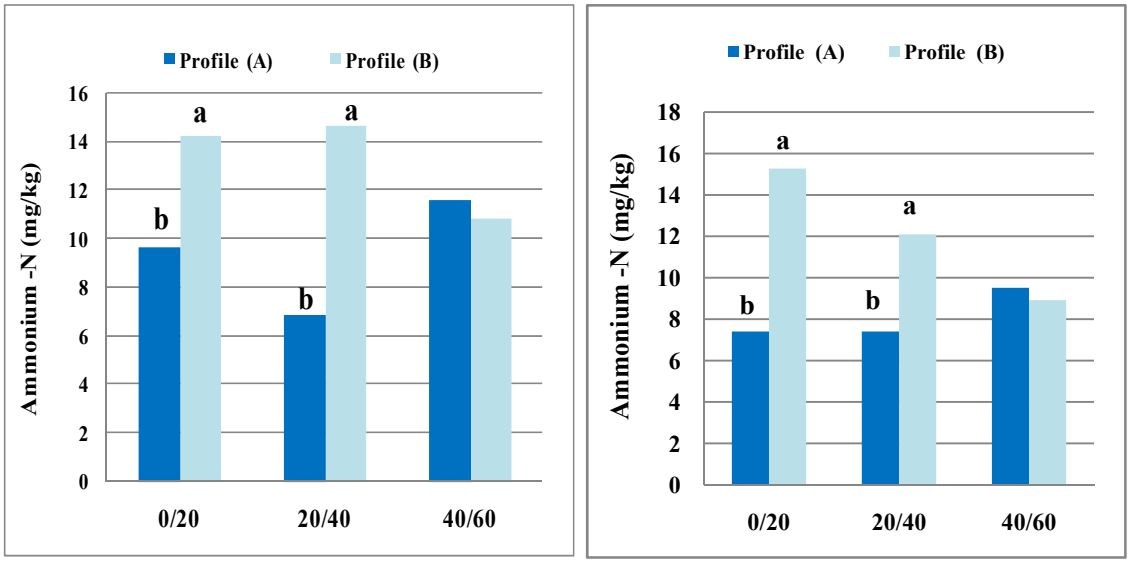


Fig. 3.17 Changes in ammonium-N concentration in soils from different depths (0-20, 20-40 and 40-60 cm) for profile A and profile B after 42 days of drying (left chart) and 1 day after subsequent rewetting (right chart). Bars are means of 4 replicates. Where adjacent bars in a pair have different letters, there is a significant difference between profile A and B.

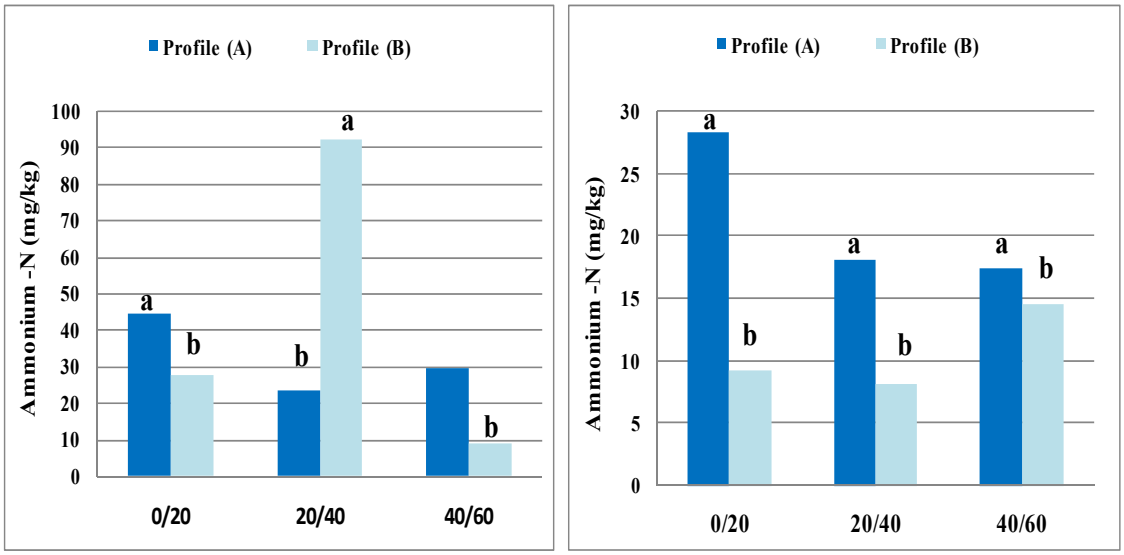


Fig. 3.18 Changes in ammonium-N concentration in soils from different depths (0-20, 20-40 and 40-60 cm) 3 days (left chart) and 7 days (right chart) after rewetting following 42 days of drying for profile A and profile B. Bars are means of 4 replicates. Where adjacent bars in a pair have different letters. there is a significant differences between profile A and B.

Between 1 and 3 days after rewetting, net N mineralization had increased substantially for profile A at all depths. There were consistent statistically significant differences between profile A and B at days 3 and 7 (Fig. 3.18), with more ammonium-N in profile A at all depths. Soils from profile B also showed net mineralization between days 1 and 3, especially at 20-40 cm. They then showed net immobilization of ammonium-N between 0-20 and 20-40 cm by day 7, but net ammonification at 40-60 cm (Fig. 3.18).

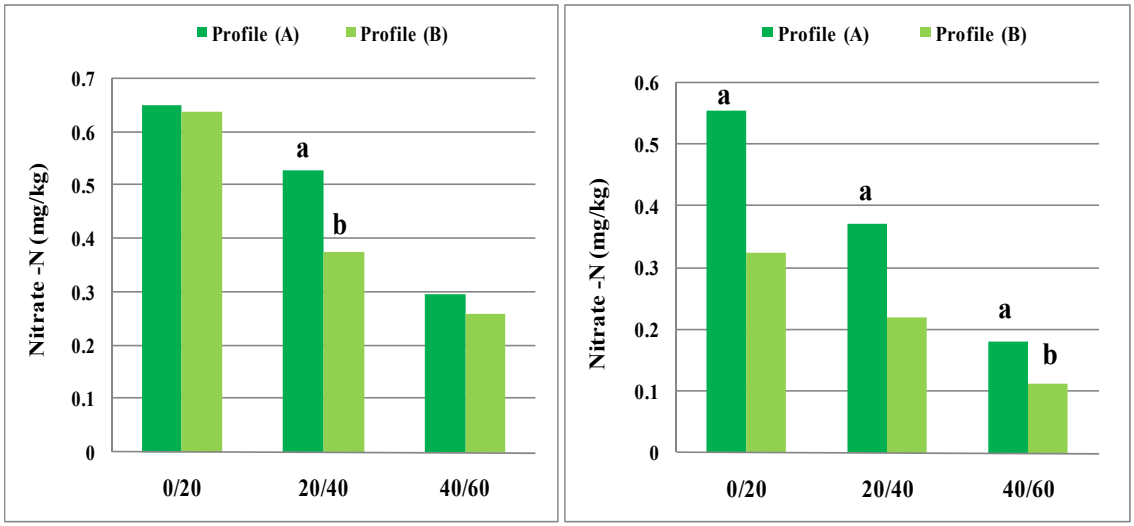


Fig. 3.19 Changes in nitrate - N concentration in soils from different depths (0-20, 20-40 and 40-60 cm) after 42 days of drying (left chart) and 1 day after subsequent rewetting (right chart) for profile A and profile B. Bars are means of 4 replicates. Where adjacent bars in a pair have different letters, there is a significant difference between profile A and B.

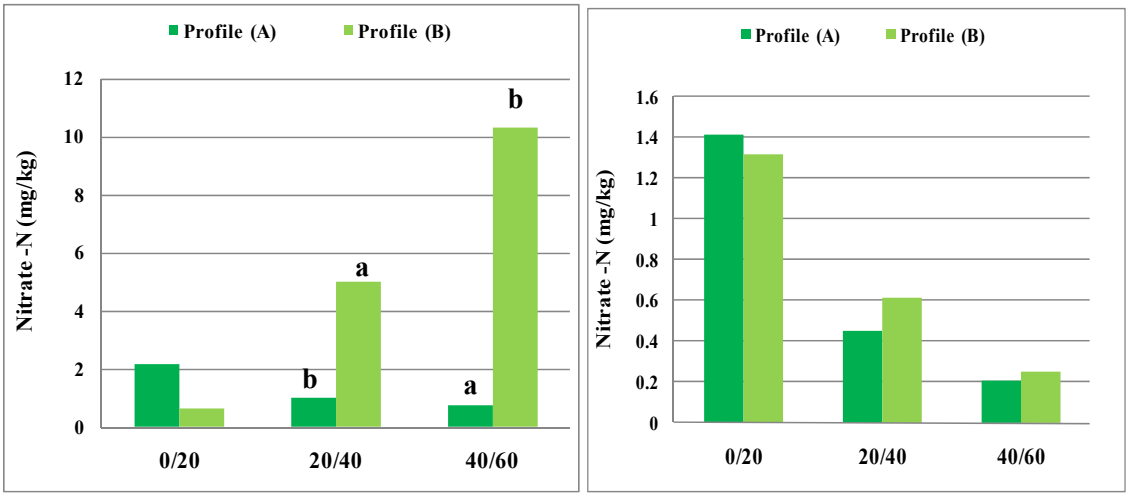


Fig 3.20 Changes in nitrate - N concentration in soils from different depths (0-20, 20-40 and 40-60 cm) 3 days (left chart) and 7 days (right chart) after rewetting following 42 days of drying for profile A and profile B. Bars are means of 4 replicates. Where adjacent bars in a pair have different letters, there is a significant difference between profile A and B.

Figure 3.19 illustrates the effects of soil depth upon the extractable-nitrate-N concentration for soils from the two profiles at the end of 42 days of drying and 1 day after rewetting. For both sampling events the two soils followed a similar trend. Soil nitrate concentration declined consistently and significantly with depth. Net nitrate-N immobilization was more in evidence than any nitrate flush. Figure 3.20 illustrates the effects of soil depth upon the extractable-nitrate-N concentration for soils from the two profiles 3 days and 7 days after rewetting. Soils from both profiles show evidence for nitrification at day 3, especially for profile B. Perhaps surprisingly, this showed the

highest nitrification in soil from 40-60 cm depth at day 3. The reason for this is not obvious. By day 7 (Fig. 3.20), there was strong evidence for nitrate-N immobilization, especially in the deeper soils.

3.5 Discussion

As stated in section 3.2, this preliminary experiment was set up to test the following five hypotheses:

1. Nitrate-N and ammonium-N will increase over the early drying period.
2. Nitrate-N concentration will reach a maximum when nitrification rate becomes insignificant compared with microbial sinks for nitrate.
3. When nitrification becomes insignificant, ammonium-N concentration may undergo further increases for some time.
4. If some microbial activity continues, nitrate concentration may start to fall as a consequence of microbial immobilization.
5. If nitrate is immobilized, this will show up in a delay in any nitrate-N flush when the soil is rewetted.

Conclusions related to each of these hypotheses are briefly discussed in turn in the following subsections.

Hypothesis 1: Nitrate-N and ammonium-N will increase over the early drying period

Net nitrate production did appear to increase consistently over the first 5 days of the drying period for soils at all depths. (Figs. 3.1-3.5). Generally, the maximum nitrate-N concentration value tended to decline with soil depth, except for soil from 80-100 cm. It is not possible here to say whether nitrification cessation is the cause of the maxima alone or nitrate starts to be immobilized by microbial cells (that are more drought resistant) more quickly than it is produced. Intuitively the former seems more probable, but the subsequent declines in nitrate concentration tend to suggest that immobilization is perhaps more important.

Ammonium-N concentration apparently increased significantly with depth in soils from all soil depths, but there was no consistent trend with depth. However, close examination of Figs. 3.6-3.10 seems to suggest that between days 10 and 15, ammonium concentration was starting to increase exponentially once net nitrification was zero or negative, at least for soils from 0-80 cm. However, because of variation associated with

differences between the two soil profiles further experiments with more soils and greater replication would be needed to test this idea.

Hypothesis 2: Nitrate-N concentration will reach a maximum when nitrification rate becomes insignificant compared with microbial sinks for nitrate

The author had initially expected to see a plateau in nitrate *versus* drying time graphs, rather than distinct maxima. However, there is no reason to suppose that there should be a close match between the soil moisture contents at which nitrification and microbial immobilization of nitrate-N cease. If, as Landesman and Dighton (2010) suggest, microbial populations become adapted to drought, it seems probable that at least part of the microbial population is likely to be able to use nitrate under drought conditions. Possibly this part of the population might make use of osmolytes originating from less drought-tolerant organisms, which might explain why such chemical species do not accumulate in soil solution to the extent that might be anticipated, helping to explain the findings of Williams and Xia (2009). However, this must remain speculative without further research.

Hypothesis 3: When nitrification becomes insignificant, ammonium-N concentration may undergo further increases for some time

The results appear to strongly support this hypothesis, which originated from the author's preconception that at least part of the microbial biomass capable of contributing to ammonification would be more drought-tolerant than the nitrifier population.

Hypothesis 4: If some microbial activity continues, nitrate concentration may start to fall as a consequence of microbial immobilization

Before the experiment commenced, it was anticipated that this hypothesis would probably be disproved, because the experiment described in the next chapter was set up to test the idea that nitrate stored in soil after drying makes a substantial contribution to the well-documented nitrate flush on rewetting. However it appears conclusive from the present experiment that nitrate-N is immobilized in later stages of drying. It is possible that this reflects the important role being played by fungi in these acidic grassland soils. As mentioned in the introduction, Gordon et al. (2008) suggested that fungi were more

drought tolerant than bacteria. It is not clear though whether "tolerance" in this context refers to survival or remaining metabolically active.

Hypothesis 5: If nitrate is immobilized, this will show up in a delay in any nitrate-N flush when the soil is rewetted

In the rewetting experiment, at day 1 the soils showed, if anything, a decline in nitrate-N concentration compared with values obtained immediately after the drying period. However by the third day there was clear evidence of a nitrate flush in all soils. This was short lived, however, and by day 7 nitrate-N concentration had fallen considerably in most soils compared with values observed 3 days after rewetting.

3.6 Conclusions

The experimental results supported all of the hypotheses listed in the introduction. The extent of immobilization of nitrate in later stages of drying was greater than anticipated prior to the experiment, but undoubtedly for such acid grassland soils would contribute to the delay in the nitrate-N flush when dried soils are rewetted.

The changes that occurred with the duration of the drying period were substantial, and present a problem when attempting to interpret the results of many of the earlier drying/rewetting experiments where data are gathered after one or a series of fixed-period drying cycles.

The experiment showed that ammonium and nitrate production occurred in these soils at depths down to 100 cm, which is well below the rooting zone at this site. In periods of drought, when plant growth, and hence plant N uptake, is likely to be severely restricted, it seems highly probable for these soils that ammonium-N will continue to be produced within the soil. Under these conditions there is a high risk of such ammonium being mobile down the soil profile and possibly out of the profile into drainage waters. However, the mobility on rewetting in early rainfall will presumably be dependent upon associated mobile anion concentrations.

This experiment suggests that, for the acid soils under grassland at Hob Moor, net nitrate-N production continues for up to 10 days as soils dry out. It was therefore decided to conduct a further experiment, which is described in the next chapter, to see if soils dried out for 6 days, when rewetted, release this stored nitrate, rather than a flush of nitrate emanating from a very rapid flush of nitrification within hours of rewetting.

CHAPTER 4: WHAT CONTROLS THE NITRATE FLUSH WHEN AIR DRIED SOILS ARE REWETTED?

4.1 Introduction

Soils are regularly subjected to drying/rewetting cycles in many parts of the world, including the UK, and mineral-N flushes have often been documented from air-dried or partly-dried soils when rewetted, reputedly due to changes in microbial activities and diversity (Fierer et al., 2003). For example, reducing the water content of a coniferous forest litter layer material to 10% of dry weight for 12 days reduced microbial biomass C by 67% and markedly reduced respiration (Pulleman and Tietema, 1999). Subsequent rewetting to 340% resulted in significant flushes of respiration, soluble C and mineral N within a few hours. Gordon et al. (2008) investigated the effects of drying and rewetting on the concentrations of inorganic N species in leachate from improved and unimproved grassland soils. Nitrate leaching was increased by the stress from drying, especially in the improved soil. Thus soils that may be prone to extended drought periods often give a nitrate flush in the next precipitation event. In Israel a rapid increase in nitrate concentration was noted in a mineral soil under shrub-land when the first winter rainfall rewetted dried soil, although in an adjacent forest soil an unexpected increase in nitrite concentration accompanied by only a small increase in nitrate concentration was observed (Gelfand and Yakir, 2008). It was suggested that this might be due to different changes in microbial populations in response to summer stress for the two ecosystems.

Such mineral N fluxes are of interest in both natural ecosystems and in some managed ecosystems. Nitrate flushes may become a potential environmental issue when soil/vegetation mesocosms are used as bio-filters for the removal of inorganic N pollution from urban runoff. For example, when such filters were subjected to simulated drought periods of 4 to 6 weeks, nitrate concentrations always increased sharply in the subsequent first flush of outlet water (Hatt et al., 2007a, 2007b). The flushes were only short lived, however which could support the concept of intracellular cell solute release following cell lysis in response to osmotic shock upon rewetting (Fierer et al., 2003).

However, although nitrate might make a contribution to osmo-regulation in microbial cells, its contribution is likely to be very small as the intracellular solutes released subsequent to dilution stress are predominantly thought to be organic compounds or potassium (Halverson et al., 2000). Therefore the author started to consider other possible mechanisms that might produce a nitrate flush and decided that

the initial nitrate flush could partly be due to removal of nitrate accumulated in soil but not taken up by plants during drying rather than just cell lysis. Ford et al. (2007) recently studied the effects of rewetting on mineralization in semi-arid grassland soils from Western Australia. The concentration of nitrate initially extractable from their air-dried soil was small (ca.1 mg kg^{-1}) compared with that subsequently produced when the soil was rewetted and incubated over 4 weeks. At the 40°C incubation temperature that they used, nitrate accumulated in the soil quite rapidly over the first 2 to 3 days. This could produce a delayed flush via nitrification. Other researchers have concluded that microbial cells killed during soil desiccation were not major contributors to N flushes on rewetting (Van Gestel et al., 1991). The results of Ford et al. (2007) highlight the need to distinguish between the nitrate flux available for immediate mobilization from rewetted air-dried soils, and the nitrate subsequently produced in the soil once it has been rewetted.

It seems probable that, as soils dry, water and associated solute species migrate to progressively smaller and smaller pores, so both the water and the solute that it contains might become unavailable to plants. Such retained nitrate could be removed quite quickly during a subsequent rain storm event. Any nitrate flush from a sudden burst of activity of nitrifiers and possibly also ammonifiers is only likely to occur later in rewetted air-dried soil.

In an attempt to improve understanding of how extended drought periods might influence the dynamics of nitrate leaching to an adjacent stream from soils that have been heavily N-impacted by atmospheric deposition, it was decided to:

(1) Compare how nitrate production rates in a rewetted air-dried soil and the corresponding field moist soil change over time after first flushing out any stored residual nitrate or nitrate from cell lysis with a deionised water wash. However, because the results in Chapter 3 suggested nitrate immobilization after ca. 5+10 days drying, probably by drought-resistant fungi, it was decided only to use a 6 - day drying period in the study in this chapter.

(2) Determine if ammonium substrate availability limits the initial rate of nitrification in a heavily N-impacted, dried or field-moist soil by testing whether ammonium spiking enhances the nitrification rate.

(3) Conduct a nitrate spiking experiment to confirm that, if the net nitrate production rate after rewetting appears to be slow, this is not due to microbial immobilization of nitrate and/or localized denitrification.

4.2 Materials and methods

4.2.1 Experimental Site

The soil used was from Hob Moor, taken from the permanent acid grassland near York, as described in Chapter 2. The area is often quite dry, mean annual rainfall in the area being 639 mm. To recap briefly, the site is a Local Nature Reserve with a management plan to maintain low nutrient status and high biodiversity. Small streams close to the moor edges have been shown in occasional analyses over seven years to contain variable nitrate concentrations up to 27 mg l^{-1}, a high concentration for an unfertilized site that has received no synthetic fertilizer for at least six decades (Riaz et al., 2008). However, there is some input of drainage water from nearby houses, gardens and a sports field. For the soils though, any ammonium or nitrate mobilized within the profiles would be from natural element cycling/recycling and/or from deposition of atmospheric pollution. The moor is used for grazing cattle over the summer months, as part of the plan to maintain a low nutrient status, but they are excluded from the area sampled by fencing and a cattle grid.

The soil chosen was a freely draining, sandy loam, as shown in plate 2.3, and therefore appropriate for comparison with soils likely to be used in biofilters. In shallow layers of incubated moist soil it would not be prone to denitrification over planned incubation periods of up to 9 days.

4.2.2 Soil preparation and moisture content measurement

A bulk soil sample was collected on 14/12/2006 from the upper 20 cm of the soil profile, below approximately 1 cm of litter. The soil was carefully, but quickly, hand sorted, using pre-washed rubber gloves to minimize risk of contamination, to remove any obvious root material and the few stones present. To standardise conditions and make results from field moist soils directly comparable to those from air-dried soils, half of the thoroughly mixed bulk sample was air dried as a shallow layer (2-3 cm) on plastic trays for 6 days, and the other half was stored at 4ºC in a refrigerator. The moisture contents of the field moist and air-dried samples were then determined in duplicate by oven drying at 105ºC overnight.

4.2.3 Incubation experiment

To standardize conditions as much as possible, the masses of moist and air-dried soil equivalent to exactly 10.0 g of oven-dry soil were calculated and found to be 12.34 and 10.28 g respectively. Two series of sub-samples with these masses of moist and air-dry soil were packed into series of 50-ml syringe tubes to serve as leaching tubes, each being plugged at the bottom with 0.40 g of cotton wool. The cotton wool was weighed 10 $\pm$ 0.01g to allow precise compensation for possible contamination by using blanks. Duplicate blank tubes were also prepared with no soil. All tubes and contents, and the blanks, were immediately leached over 2-3 h with 100-ml portions of deionised water to remove native nitrate-N, and then left to drain for 24 h. The wash solutions were discarded.

After 24 h, one third of each set of the tubes containing previously air-dried or field moist soil was treated with 1 ml of deionised water, one third with 1 ml of solution containing 50 μg of ammonium-N (as ammonium sulphate), and the remaining third with 1 ml of solution containing 50 μg of nitrate-N as potassium nitrate. Thus 10 tubes of moist soil and 10 tubes of air-dry soil received each N treatment. The N spike size was selected to give an N species N concentration value comparable to that of the native nitrate-N and ammonium-N concentrations.

From each of these sets of 10 tubes, two tubes were leached with 100 ml of 0.5 molar potassium chloride immediately after the N or water spike additions, and then further duplicates were leached after intervals of exactly 1, 2, 5 and 9 days.

4.2.4 Soil analyses

Soil analyses were performed as described in detail in Chapter 2, and are summarised only briefly here.

4.2.4.1 Soil pH

Soil pH was measured using a glass/calomel electrode and a pre-calibrated Thermo Orion pH meter at a 1:5 field-moist soil: deionised water ratio. The solution was stirred thoroughly and allowed to stand for 30 minutes to equilibrate, then stirred again and pH was measured to the nearest to 0.1 pH unit.

4.2.4.2 Soil C:N ratio

The soil C%, N% and C/N ratio were determined in duplicate using finely ground, oven dry soil sub-samples with an Elementar Vario Macro C & N analyser. The oven dry soil samples were first ball milled into fine powder (as described in Chapter 2), and approximately 100-mg sub-samples were weighed to the nearest 0.1 mg into tin foil sample cups. The samples were tightly wrapped to avoid any loss, and analysed for C (%), N (%) and C/N ratio, using glutamic acid as a calibration standard.

4.2.4.3 Extractable ammonium-N and nitrate-N

Extractable ammonium- and nitrate-N in the 0.5 molar KCl extracts were measured using a standard Bran and Luebbe two channel Auto Analyser® -3 with matrix-matched standards. Whenever necessary, sample extracts were appropriately diluted off line with the 0.5 molar KCl extractant solution to give a final concentration below 2 mg l^{-1} for measurement. All results were corrected for reagent blanks.

4.2.5 Statistical analyses

To investigate the significance of spike treatment effects on extractable ammonium-N and nitrate-N concentrations at individual times (T0 to T9), Tukey HSD multiple comparison was employed, taking treatment as grouping variable. Treatment effects were assumed significant at $P<0.05$. To assess significance of differences over time, one-way ANOVA was used to compare means of extractable nitrate-N and ammonium-N for distilled water- (DW), nitrate- and ammonium-spiked soil samples. Tukey HSD multiple comparison ($\alpha = 0.05$) was used as post hoc test using time as grouping variable.

4.3 Results

The soil used was a very fine sandy silt loam with a pH value of 4.45. The electrical conductivity of a saturated paste was 80.5 μS cm^{-1}. The mean concentrations of C and N were 4.03% and 0.361%, respectively, and the mean CN ratio was 11.5. Figure 4.1 shows how nitrate-N concentrations changed over the 9-day incubation period following the deionised water flush and subsequent spiking with either deionised water (the control), 5 mg of nitrate-N kg^{-1} of soil, or 5 mg of ammonium-N kg^{-1} of soil, for the air dried soil (upper chart) and for the field moist soil (lower chart). Table 4.1 summarises

the results of one-way ANOVA to compare differences over time in mineral-N species concentrations for each treatment.

Comparison of the results for the air-dried and field moist soils treated only with deionised water shows clearly that there was a substantial delay in the onset of nitrification following air drying and the soil wash (Fig. 4.1, upper chart, white bars), but nitrification was already very rapid in the field moist soil by T0, only 24 h after the water rinse (Fig. 4.1, lower chart, white bars), bearing in mind the fact that there was a period of 24 h between flushing with deionised water and the subsequent KCl leaching of ammonium and nitrate. By day 5 and day 9, nitrate concentration had fallen significantly in the field-moist soil (Fig. 4.1, lower chart, white bars) compared with the initial concentration at time zero.

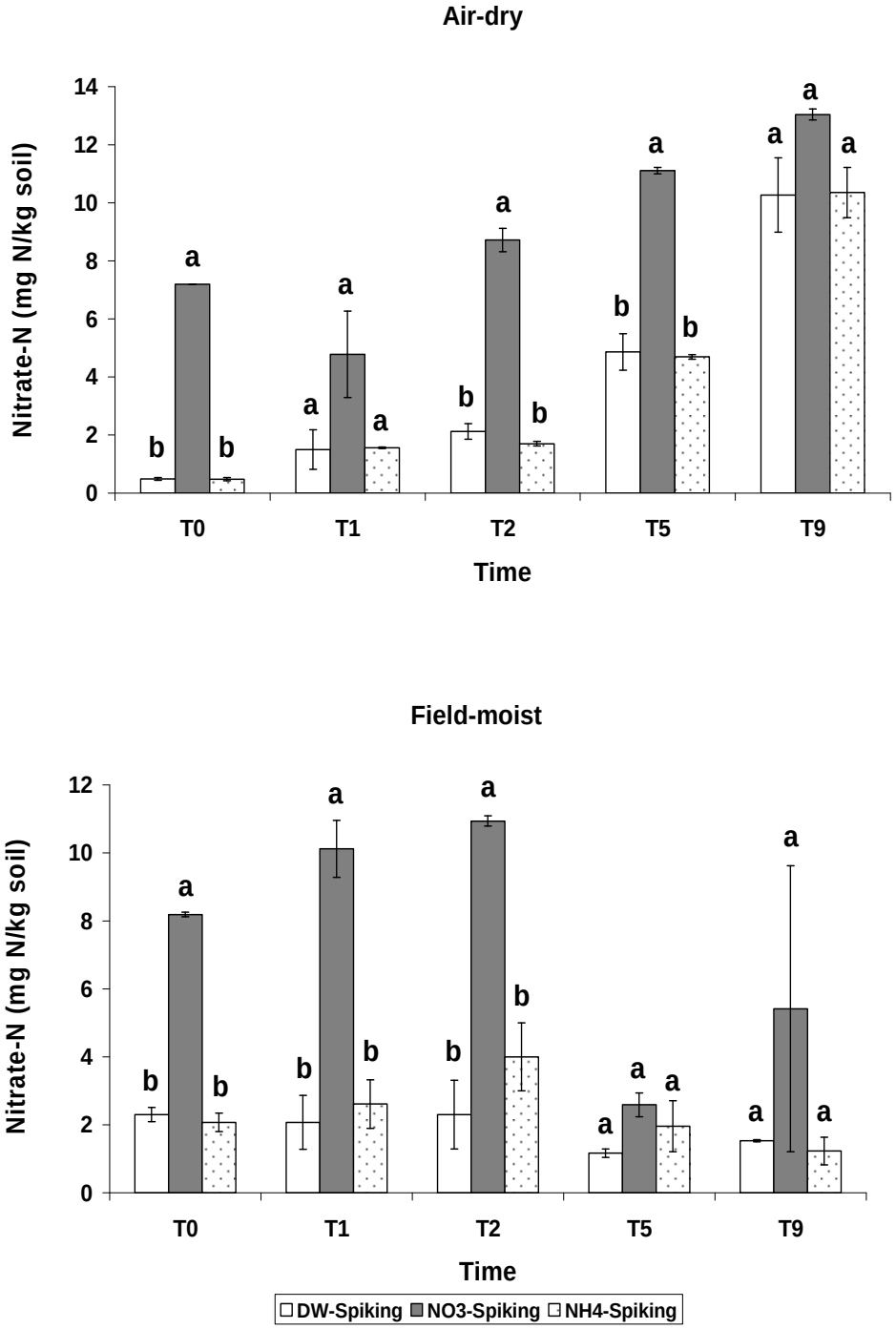


Fig. 4.1: Changes in concentration of extractable nitrate-N in air dry (upper bars) and field moist (lower bars) soils after spiking with deionised water, nitrate-N or ammonium-N over a 9 day incubation period at room temperature. All values are means of two replicates. Error bars indicate standard errors of means (+ 1 s.e.).

Comparison of the results for the air dried and field moist soils spiked with 5 mg kg^{-1} nitrate-N shows that over the first five days of incubation, net nitrate-N for the air-dried soil increases by approximately this amount (Fig. 4.1, upper chart, compare grey and white bars). There is a clear sign in Fig 4.1 of immobilization/loss of nitrate-N by T9 for the air-dry soil however. In the field moist soil (Fig. 4.1, lower chart) substantial nitrate loss occurred by T5 and nitrate loss was still marked at T9.

Ammonium spiking of the air-dried soils confirmed the slow recovery of nitrification seen for the distilled water control soils (Fig. 4.1, upper chart, compare spotted and white bars). It also showed that nitrate production was not ammonium substrate-limited throughout the 9-day incubation period as ammonium addition did not stimulate additional nitrification.

Nor did ammonium spiking stimulate substantial additional nitrate production in the ammonium-spiked field-moist soils (Fig 4.1, lower chart, compare spotted and white bars).

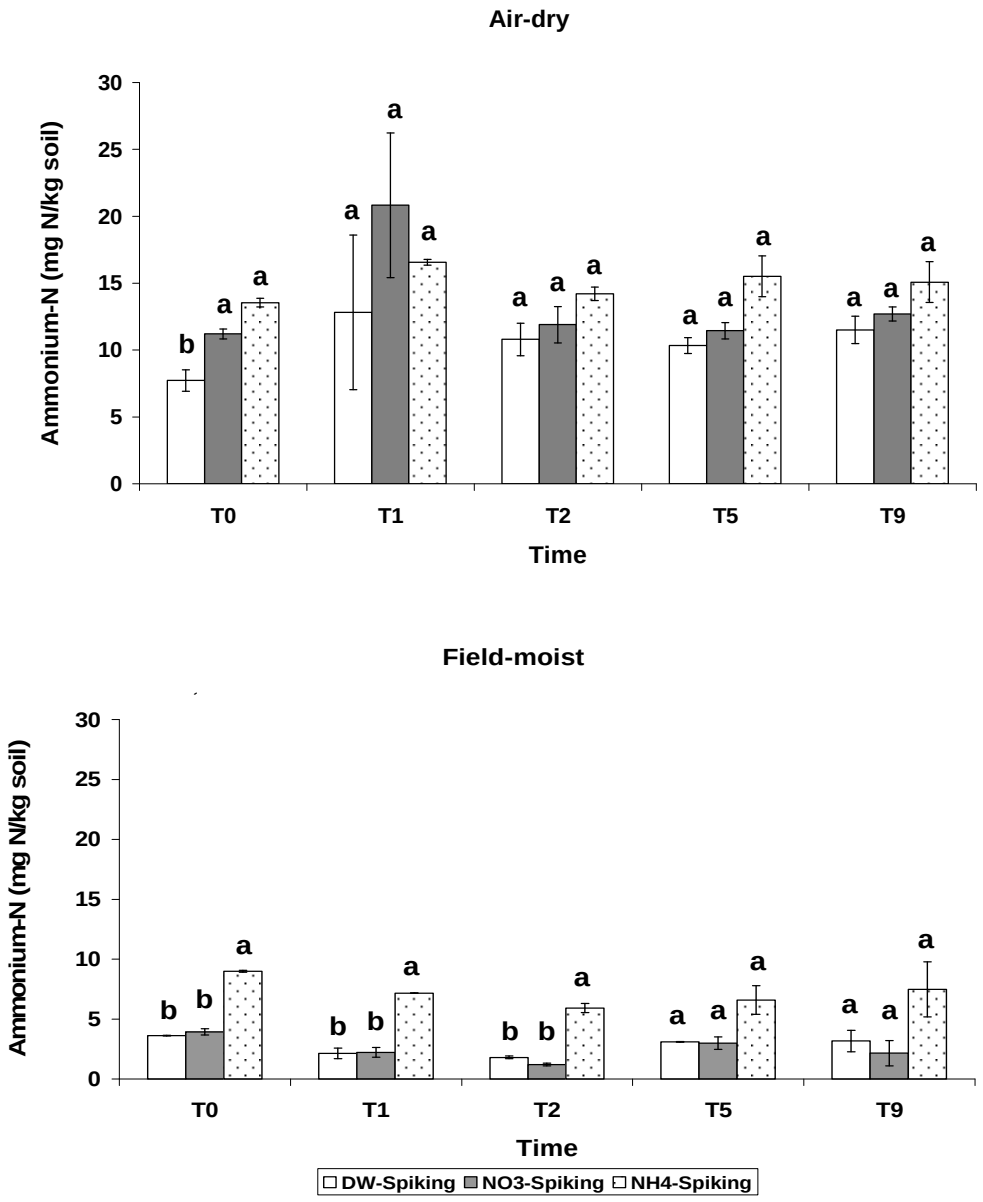


Fig 4.2: Changes in concentrations of extractable ammonium-N in air dry (upper bars) and field moist (lower bars) soils after spiking with deionised water, nitrate-N or ammonium-N over a 9 day incubation period at room temperature. All values are means of two replicates. Error bars indicate standard errors of means. (± 1 s.e.).

To confirm that ammonium substrate is not limiting nitrate production also requires evidence that the ammonium added during spiking has not been used by, or immobilised substantially in, microbial biomass. Figure 4.2 shows how ammonium-N concentration changed over the 9-day incubation period following the deionised water flush and subsequent spiking with either deionised water (the control), 5 mg of nitrate-N kg^{-1} of soil, or 5 mg of ammonium-N kg^{-1} of soil, for both air-dried (upper chart) and field moist (lower chart) samples. Figure 4.2 (note change of scale on the ammonium-N axes between upper and lower charts) suggests that most of the ammonium-N spikes added to field moist soil is still KCl-extractable throughout the incubation period.

It may be assumed that the nitrate production buildup over the 9 days seen in Fig. 4.1 (upper chart) for the rewetted air-dry soils (white bars) has arisen as a consequence of ammonium oxidation. It appears that this oxidation has restricted significant ammonium accumulation within the rewetted air-dried soil after T1 (Fig. 4.2, upper chart, white bars). Similar behaviour may be seen for the ammonium-N and nitrate-N spiked soils (i.e. build up of nitrate over the first day's incubation, but subsequently much more constant ammonium-N concentration (Fig. 4.2, upper chart, grey and spotted bars). In the field-moist soil, however, there was a significant decline in ammonium concentration between T0 and T2, regardless of whether or not an ammonium-N or nitrate-N spike had been added (Fig. 4.2, lower chart).

Table 4.1: One-way ANOVA for time to compare means for extractable nitrate-N and ammonium-N concentrations in soil for DW-, nitrate and ammonium-spiked soil samples. Tukey HSD multiple comparison (α = 0.05) was used as post hoc test using time as grouping variable.

Time (Days)	Air Dry					
	Nitrate-N (mg N/kg soil)			Ammonium-N (mg N/kg soil)		
	DW-Spiked	NO_3-Spiked	NH_4-Spiked	DW-Spiked	NO_3-Spiked	NH_4-Spiked
T0	0.485 c	7.190 bc	0.475 c	7.720 a	11.200 a	13.535 a
	(0.045)	(0.010)	(0.055)	(0.800)	(0.380)	(0.325)
T1	1.495 bc	4.775 c	1.560 c	7.805 a	20.830 a	16.545 a
	(0.685)	(1.485)	(0.020)	(5.785)	(5.410)	(0.215)
T2	2.115 bc	8.720 b	1.700 c	10.790 a	11.895 a	14.205 a
	(0.265)	(0.400)	(0.080)	(1.210)	(1.365)	(0.495)
T5	4.860 b	11.105 ab	4.690 b	10.335 a	11.440 a	15.505 a
	(0.630)	(0.105)	(0.080)	(0.585)	(0.610)	(1.525)
T9	10.620 a	13.040 a	10.345 a	11.500 a	12.685 a	15.080 a
	(1.280)	(0.190)	(1.223)	(1.020)	(0.742)	(1.530)
	Field Moist					
	Nitrate-N (mg N/kg soil)			Ammonium-N (mg N/kg soil)		
	DW-Spiked	NO_3-Spiked	NH_4-Spiked	DW-Spiked	NO_3-Spiked	NH_4-Spiked
T0	2.300 a	8.190 a	2.070 a	3.615 a	3.935 a	8.985 a
	(0.210)	(0.070)	(0.270)	(0.035)	(0.255)	(0.075)
T1	2.070 a	10.120 a	2.605 a	2.140 a	2.215 a	7.175 a
	(0.790)	(0.840)	(0.715)	(0.450)	(0.405)	(0.025)
T2	2.030 a	10.770 a	3.995 a	1.810 a	1.210 a	5.915 a
	(1.010)	(0.150)	(1.005)	(0.110)	(0.120)	(0.375)
T5	1.165 a	2.590 a	1.960 a	3.095 a	2.995 a	6.585 a
	(0.125)	(0.350)	(0.750)	(0.035)	(0.515)	(1.205)
T9	1.525 a	5.420 a	1.230 a	3.165 a	2.155 a	7.465 a
	(0.035)	(4.210)	(0.410)	(1.265)	(1.055)	(2.295)

All values are means of duplicate samples. Standard errors of means are enclosed in parentheses. Means in any column sharing only different letters at two times differ significantly at $P<0.05$.

4.4 Discussion

The nitrate-N concentration results for the air-dried and field moist soils very clearly showed a marked delay in the onset of nitrification following air drying (Fig. 4.1, upper chart), but that nitrification was very rapid in the field moist soil over the 24 h after the deionised water flushing (Fig. 4.1, lower chart). In the latter, nitrate concentration was >2 mg kg^{-1} at T0, only 24 h after the deionised water flushing. As noted in the results section, by day 5 and day 9, nitrate-N concentration had dropped significantly in the field-moist soil compared with the nitrate-N concentration at T0. It could be suggested that this decline is indicative of denitrification, but the soil layers in the bottles were shallow (<1 cm), bottles were loosely capped, and in the field the soil was very freely drained so it probably would not have had a population of anaerobes. It is therefore extremely unlikely that the soils in the experiment were anaerobic. There was no such trend in the air-dried soils, which were incubated under similar conditions, although these samples had slightly lower overall moisture content, and would in any case be less likely to become anaerobic. It seems more likely therefore that the effect is due to the warmer temperature during the incubation, which could favour increase in microbial biomass and biomass turnover, and thus microbial immobilisation of nitrate-N. Such an effect, if it does occur, might be expected to occur later post air-drying, as the build up of microbial biomass (not just that involved in mineral N production) would be delayed. This is supported by the loss of nitrate after nitrate spiking of the air-dried soil by day 9 (T9), which is clearly apparent in Fig. 4.1 (upper chart, comparing grey and white bars).

An important conclusion from this study is that rapid nitrate flushes following rewetting of dry soils that have often been attributed to surges in soil microbial activity are, in practice, more likely due to flushing out of residual nitrate from the soil that has been produced and stored during the drying phase or nitrate from cell lysis, as discussed in the introduction. This is only clear here because of careful selection of a 6–day drying period. This was short enough to make risk of net nitrate immobilization prior to rewetting very small.

The results for air-dried and field moist soils spiked with 5 mg kg^{-1} nitrate-N over the first five days (Fig. 4.1, comparing grey and white bars) provided little evidence of immobilisation/loss of nitrate-N for air-dried soil (Fig. 4.1, upper chart), but for the field-moist soil nitrate loss occurred by T5, and was still marked at T9 (Fig. 4.1, lower chart). However, it should be pointed out that the soil used had a quite low C: N ratio, which would lower the risk of microbial immobilisation. It is well known that

nitrate additions to soils with high C:N ratios may result in immobilization of nitrate in the short term.

Nitrate production in the previously air-dried soil was not ammonium-substrate limited throughout the 9-day incubation because ammonium spiking of the soil did not stimulate additional nitrification (Fig. 4.1, upper chart, compare spotted and white bars). Ammonium spiking also failed to stimulate nitrate production significantly in the field-moist soils (Fig. 4.1, lower chart). This ties in well with the observation that ammonium added during spiking of the soil used was not noticeably immobilised into microbial biomass. As outlined in the results, Fig. 4.2, lower chart, shows that the added ammonium-N spike was still KCl-extractable over the 9-d test period, especially from the field-moist soil. Atmospheric pollution of the Hob Moor soils by N deposition is high, and the soils there consequently have a low C:N ratio.

For the air-dry soils, the increasing (over time) oxidation of ammonium to nitrate (Fig. 4.1, upper chart) prevented ammonium accumulation within the re-wetted soil (Fig. 4.2, upper chart). In the field-moist soil, however, regardless of whether or not an ammonium-N or a nitrate-N spike had been added, ammonium concentration declined significantly between T0 and T2. For the ammonium- and nitrate-spiked soils, this could be at least partially due to nitrification over the corresponding time period.

One of the most conspicuous effects of the drying/rewetting cycle is the very rapid rise (by T0) in ammonium-N concentration following drying/rewetting (Fig. 4.2, compare white bars and note change of scale on ammonium-N concentration axes). It is almost certain, after the highly significant increases shown in Chapter 3, that ammonification continues for a while during the drying stage, possibly after nitrification has slowed or stopped, and not all residual ammonium would have been leached out by the soil washing stage with deionised water. Subsequently it appears that ammonium production and nitrification rates approximately match in the rewetted soils. For Californian oak wood and grassland soils it has been found that ammonium concentrations remained low and unaffected by wetting/drying stress cycles, but it was concluded that effects of repeated wetting/drying cycles on nitrate concentrations in soil could last for several weeks (Fierer and Schimel, 2002). Appel (1998) reported a mineral N flush following drying/rewetting cycles from an arable soil in Germany, which he related to mobilization of non-biomass organic N**.** Van Gestel et al. (1993) suggested that non-biomass organic residues contributed to flushes of mineral N after drying/rewetting, but thought that at least part of the flush was due to microbial biomass killed by drying.

Because of the sharp rise in ammonium N noted above following drying in this experiment, the net result here too was a mineral N flush, but a delay of a few days will occur before this manifests itself as an enhanced nitrate-N concentration. It should be remembered that the field moist soils had been stored at < 4°C for six days while half of the soil was drying, and this would have slowed the production of ammonium-N in the stored soil during this period.

4.5 Conclusions

The results of this experiment support the hypothesis for the acid soil used here that any rapid initial flush of nitrate from rewetted soil would have to have originated either from lysis of microbial cells or from residual nitrate stored in soil as it dried out. There is then be a second nitrate flush as the nitrifier population re-establishes. At this stage, ammonium accumulated in the soil during the drying stage may be nitrified, further contributing to the delayed nitrate flush. Further work is needed however to assess the relative importance of these mechanisms. In the soil from this site, ammonium substrate was not limiting nitrification. This was as expected, bearing in mind the high level of N deposition at the site, which would also include redistributed inputs from manure from the cattle grazing the site over the summer and early autumn months. It must be remembered, though the experiment gave clear results because the preliminary study in Chapter 3 allowed selection of an appropriate droughting period of 6 days.

CHAPTER 5: IS AMMONIUM MOBILE IN NITROGEN-IMPACTED UNFERTILIZED GRASSLANDS? A CRITICAL REASSESSMENT

5.1 Introduction

Although the previous chapters indicated that, under dry conditions, ammonium may accumulate in soils, becoming potentially mobile when soils are rewetted, it is generally assumed that ammonium-N deposition to soils and/or ammonium-N produced insitu in soil is immobile, especially when compared to nitrate-N. Brady (1990), for example, discusses nitrate leaching from soils comprehensively, but lists only five fates for ammonium, namely appropriation by micro-organisms, plant uptake, inter-layer fixation, volatilization to the atmosphere, and nitrification; he makes no mention of ammonium leaching; Nitrate leaching is also extensively considered in texts on soil management (e.g. Fullen and Catt, 2004), but potential ammonium losses by leaching are again generally ignored. They are occasionally considered when agricultural soils are being studied, however. For example, Page et al. (2003) investigated whether ammonium leaching might explain the high ammonium concentrations they had observed below 1 m depth in vertisols near Warra in Queensland, Australia, but from the soil Q/I characteristics concluded that it was unlikely. Microbial inoculation experiments by the same group suggested that the elevated ammonium pool at depth was attributable primarily to a low nitrification rate (Page et al., 2002). However, Matschonat and Matzner (1995), after studying Q/1 relationships for acid forest soils, concluded that although ammonium absorption was not a major risk for N in the long term, it was an important consideration when assessing seasonal N species dynamics and transport.

Leaching losses of nitrogen from agricultural soils are widely perceived as being "mainly NO_3" (Burt and Haycock, 1993). According to Hornung and Langan (1999), when comparing effects of inputs of pollutant N as NH_y and NO_x to forests, soil systems largely retain ammonium inputs, whereas nitrate inputs are essentially leached. Wilson and Emmett (1999) noted that forest soils are a key sink for ammonium-N inputs as ammonium applications were retained whereas nitrate was leached. Williams and Anderson (1999) reviewed the extensive literature showing retention of ammonium-N inputs to soils. They reported very little evidence for significant ammonium mobility except from litter horizons, but concluded that such ammonium was generally in any case retained lower in the soil profile. However, they did note that some leaching of

ammonium was observed in the CORE project when soil cores from 6 forest sites along an N and S pollution gradient were reciprocally transplanted between sites (Raubach et al., 1995).

In the context of the assessment of the impacts of deposition of atmospheric N pollution, significant nitrate leaching from an unfertilized catchment is regarded as the most appropriate indicator of nitrogen saturation (Aber et al., 1989; Henrikson and Posch, 1995). However Heathwaite et al. (1990, 1993) have suggested that ammonium-N also may make a significant contribution to leaching losses from heavily grazed grasslands. Wachendorf et al. (2008) have recently demonstrated significant concentrations of ammonium-N in water seeping from zero-tension lysimeters containing soil exposed to cattle urine. Ammonium-N concentrations in soil may become very high under animal dung and urine patches. Ammonium-N leaching was included conceptually, but not mechanistically, in the process-based MERLIN model when it is used for prediction of retention and losses of inorganic N from catchments (Ferrier et al., 1995). Land use distribution is used in the INCA model to predict the concentrations of ammonium-N and nitrate-N in waters of major rivers (Whitehead et al., 1998a; 1998b).

In rivers draining unfertilized upland catchments in Scotland, the marked dominance of inorganic N in river water by nitrate rather than ammonium may be greatly reduced and distinctive seasonal trends in ammonium-N concentration observed that reflect trends in biological activity (Clark et al., 2004). For the heavily N-impacted River Etherow upland-moorland catchment in England, Cresser et al. (2004) showed that elevated ammonium-N concentrations in river water were associated with periods of high discharge and acid flush events. Highest concentrations were observed in tributaries in which water originating from surface, organic-rich soil horizons made a greater relative contribution to total discharge. This possibly ties in with the high ammonium production in litter horizons as discussed earlier in this Chapter. They showed also that in the N and S pollution-impacted organic surface soils of the catchment the biological immobilization/transformation of ammonium-N inputs was so slow that conditions approached cation-exchange equilibrium, making ammonium leaching much more probable. The latter observation prompted Riaz et al. (2008) to quantify N-species transformation rates in seven soil profiles at Hob moor, near York, down to depths below the rooting zone to see if they could confirm the likelihood of ammonium being mobilized down unfertilized soil profiles in an N-impacted lowland area; they showed that such mobilization could be occurring, and that the ammonium-N

could subsequently be nitrified at depth, thereby contributing to the nitrate load in an adjacent stream under base-flow conditions.

In the present study, the ammonium-N absorption characteristics of surface and sub-surface grassland soils from five profiles in the same locality as that studied by Riaz et al. (2008) were investigated further, (1) to see whether they provided additional confirmation of the probable mobility of ammonium to depth and (2) to examine, and attempt to explain, any spatial variation seen in the ammonium-N absorption characteristics in a range of unfertilized grassland soils. It was decided to adopt two complementary experimental approaches, absorption isotherm methodology and intact core leaching. The latter approach was used by Riaz as part of a joint experimental study. It was recognized from the outset that spatial variations in rates of soil organic N mineralization and microbial immobilization processes during the author's absorption experiments might complicate the interpretation of the absorption behaviour following additions of known ammonium-N spikes, especially as the experimental sites are intermittently grazed by cattle, but it was envisaged that this would primarily be problematical at low ammonium input concentrations, and less of a relevant problem with the intact core leaching experiments.

5.2 Materials and methods

5.2.1 Sampling date and site description

Soil samples and cores were taken on 30/04/2008 from Hob Moor in York, England (53º57'30"N & 1º4'48"W), a 36.4 ha local nature reserve predominantly covered by unfertilized grassland. The site and its location were fully described in Chapter 2. Soils used in this study vary from poorly permeable, seasonally wet, clay loams that are near neutral, to freely draining, and much more acidic, fine sandy loams and loamy sands. To recap briefly, the reserve management plan aims to maintain a high biodiversity of flora and fauna, birds and small mammals by keeping the soils at low nutrient status. Cattle are brought in to graze for ca. six months each year in an attempt to reduce nutrient status. The site is dominated by perennial grasses.

Although no synthetic fertilizer has been applied for at least several decades, the site has been affected heavily by atmospheric N and S deposition, which will influence N cycling in soil profiles (Hornung et al., 1995). Crowe et al. (2004) have shown that a stagno-gley argillic brown earth profile from the site contains 12.5 tonnes of N per

hectare to 36 cm depth, and the soil C:N mass ratio consistently was <10 as mentioned in Chapter 2.

5.2.2 Soil sampling

Five pits were sampled in duplicate below any litter horizon (from opposite sides of the pits), at 2 soil depths, 0-15 cm and 15-30 cm. The bulk of the roots and any stones were removed before bagging field replicate samples of ca. 500 g, to give 4 bags of soil from each profile, and 20 soil samples in total.

In addition on the same day, 2 intact soil cores were collected by Riaz from each profile to 30 cm depth in 33-cm long, square section tubes. The soil was cut flat at the bottom of each tube with a sharp knife and a polythene bag was attached to the tube to retain the soil during transfer back to the laboratory.

5.2.3 Soil analyses

5.2.3.1 Soil moisture content

In the laboratory on the same day that samples were collected, the soils were carefully, but quickly, hand sorted to remove the rest of the roots as far as possible and any remaining small stones and mixed thoroughly, with assistance from a group of undergraduate students supervised by the author to speed up the process. Approximately 10-g duplicate sub-samples of each soil were oven dried at 105 °C to determine the moisture content.

5.2.3.2 Measurement of soil C, N and C:N ratio

The oven-dried soil residues obtained as described above were individually finely ground with a ball mill for 3 minutes at 25 Hz (See Chapter 2) 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.

5.2.3.3 Initial extractable ammonium-N and nitrate-N

For each of the 5 soil profiles, 20-g sub-samples of each field moist soil were weighed into a series of 8 labelled bottles (2 depths x 2 field replicates x 2 analytical replicates). To each bottle 50 ml of 0.5 molar potassium chloride solution (0.5M KCl) was added. The bottles and duplicate reagent blanks were shaken for one hour, and the contents filtered through Whatman No. 42 filter papers. The extracts were stored at < 4°C until

analysis for ammonium-N and nitrate-N using a standard AutoAnalyser protocol with matrix-matched standards (See Chapter 2).

The residual field-moist soils were stored in clearly labelled bags in a fridge (to minimize microbial transformations of N species within the soil) until used for measurement of ammonium-N absorption characteristics two days later.

5.2.3.4 Ammonium absorption characteristics

Sub-samples of 10 +/- 0.1 g of each field moist soil were weighed to an accuracy of +/- 0.01 g into one of 20 series (2 field replicates x 2 depths x 5 profiles) of nine labelled 120-ml plastic bottles. These were used to assess absorption characteristics by the procedure outlined in Chapter 2.

Nine stock solutions of ammonium chloride were prepared, containing the following concentrations of ammonium-N: 0.0, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 and 50.0 μg ammonium-N ml^{-1}. To each bottle in each series of 9, 50 ml of the appropriate ammonium-N solution was added. The bottles were shaken vigorously for 10 minutes and then immediately filtered through a Whatman No. 42 papers. Extracts were refrigerated until analysis using an AutoAnalyser (within 3 days). When calculating the amounts of ammonium-N in solution, the volume of water contained in the field-moist soil was added to the volume of solution added (50 ml). Blanks with no soil were put through an identical procedure.

5.2.3.5 Measurement of soil pH

The pH of each soil was measured in duplicate in both water and in 0.05 molar calcium chloride solution, adding 20 ml of water or $CaCl_2$ to 10 g sub-samples of field-moist soil. The pastes were equilibrated with periodic agitation for 30 minutes prior to measurement of the soil pH with a glass/calomel combination elctrode.

5.2.3.6 Cation exchange capacity

Cation exchange capacity was measured by leaching 10-g sub-samples of field moist soil with 100 ml of 1 molar ammonium acetate (ammonium ethanoate), washing out the non-absorbed ammonium with 100 ml of 80% ethanol, and then leaching the absorbed ammonium with 1 molar sodium chloride. The final leachate was diluted back to 100 ml with the sodium chloride solution prior to determination of its desorbed ammonium

content. Results were corrected for mean ammonium concentration in triplicate blanks. As described in Chapter 2, to obtain results immediately after sampling, these determinations were performed by a group of undergraduate environmental science students under close supervision by the author.

5.2.4 Intact core experiments

The intact cores used were 65 mm x 65 mm in cross section by 33 cm in length. Because of the importance of mobile anion concentration to cation mobility, they were subjected to simulated rainfall containing 10 mg l^{-1} sodium chloride rather than deionized water. This part of the experiment was designed and performed by Riaz as part of his PhD thesis research. The simulated rain was applied in 76 ml doses, corresponding to 20 mm rain per application. Six applications were made over 10 days, and drainage water was collected and removed and analysed the day following application. However, for profile D there was no drainage at zero tension, so a MOM Eijkelkamp rhizon soil solution sampler, 10 cm long and 2.5 mm in external diameter and attached to 60 ml syringe, was inserted at that base of each soil core, and the water was sampled with the syringe (Riaz, pers. Comm.).

5.3 Results

5.3.1 General soil properties

Selected analytical data for soils from the five profiles are summarised in Table 5.1. The results are listed individually for analytical replicates (denoted by 1 & 2 in the sample No.), and the field replicates (denoted by a & b). The sites were selected to give 2 acid grasslands (profiles A and B) and 3 near-neutral grasslands (C – E). The results of the ammonium N absorption studies are presented in a slightly unconventional format in Figs. 5.1 and 5.2. Results are plotted separately for the individual field replicates to provide an indication of variability for individual profiles. The forms of the plots are unusual and reasons for these are discussed in section 5.4.3. Figures 5.3 to 5.7 show the same data plotted as conventional absorption isotherms.

Table 5.1: Selected physico-chemical properties of soils from the 5 profiles (A – E). Under "Soil No." for individual determinations a and b denote field replicate results and 1 and 2 denote analytical replicate results. Thus data for a1, a2, b1 and b2 can only be compared across rows for C%, N% and C:N mass ratio and for initial ammonium-N and initial nitrate-N, as a1 across arrow involves 4 individual sub-samples.

Profile, Soil No. and Depth		pH (water)	pH ($CaCl_2$)	Initial ammonium-N mg/kg	Initial nitrate-N mg/kg	C%	N%	C:N mass ratio
A	0-15a1	4.3	3.05	3.43	0.65	4.75	0.336	14.1
A	0-15a2	4.32	3.00	3.16	0.66	3.92	0.277	14.2
A	0-15b1	4.42	3.05	2.48	1.06	3.20	0.245	13.1
A	0-15b2	4.41	3.12	2.86	0.86	3.08	0.236	13.0
A	15-30a1	4.33	3.23	1.47	0.72	2.73	0.197	13.9
A	15-30a2	4.36	3.20	1.86	0.72	2.50	0.194	12.9
A	15-30b1	4.32	3.26	2.93	0.68	2.78	0.195	14.3
A	15-30b2	4.32	3.23	2.45	0.74	2.30	0.179	12.8
B	0-15a1	4.81	3.32	4.93	0.78	7.69	0.438	17.6
B	0-15a2	4.78	3.31	7.80	1.10	5.45	0.347	15.7
B	0-15b1	4.55	3.22	5.78	0.57	4.75	0.324	14.7
B	0-15b2	4.56	3.25	4.08	0.53	4.91	0.329	14.9
B	15-30a1	4.32	3.33	1.28	0.64	2.25	0.170	13.3
B	15-30a2	4.35	3.32	1.50	0.85	2.24	0.165	13.5
B	15-30b1	4.40	3.33	2.15	0.75	1.88	0.159	11.9
B	15-30b2	4.33	3.29	1.84	0.84	2.42	0.173	14.0
C	0-15a1	5.98	4.32	7.06	0.47	4.92	0.418	11.8
C	0-15a2	5.99	4.36	6.41	0.21	4.89	0.416	11.8
C	0-15b1	6.06	4.47	2.39	0.94	5.10	0.432	11.8
C	0-15b2	6.04	4.48	1.99	0.48	5.12	0.444	11.5
C	15-30a1	5.53	4.07	5.79	0.74	2.13	0.209	10.2
C	15-30a2	5.54	4.04	6.17	0.79	2.24	0.215	10.4
C	15-30b1	5.47	4.06	4.35	0.53	2.52	0.236	10.6
C	15-30b2	5.46	4.03	5.49	0.48	2.81	0.259	10.9
D	0-15a1	6.40	4.84	4.80	0.34	3.76	0.35	10.8
D	0-15a2	6.56	4.76	8.60	1.10	3.79	0.348	10.9
D	0-15b1	6.39	4.86	4.66	0.88	3.84	0.35	11.0
D	0-15b2	6.34	4.80	4.02	0.63	3.67	0.341	10.8
D	15-30a1	6.49	5.06	4.96	0.50	2.82	0.275	10.3
D	15-30a2	6.57	5.11	4.87	0.34	2.76	0.272	10.2
D	15-30b1	6.64	5.09	3.95	0.92	2.97	0.293	10.2
D	15-30b2	6.84	5.09	3.35	0.42	3.14	0.295	10.6
E	0-15a1	5.86	4.47	1.51	0.35	4.11	0.319	12.9
E	0-15a2	5.86	4.30	1.53	0.35	4.35	0.338	12.9
E	0-15b1	6.16	4.36	1.46	0.50	4.86	0.386	12.6
E	0-15b2	6.05	4.37	1.30	0.83	4.51	0.353	12.8
E	15-30a1	6.01	4.17	1.52	0.36	2.41	0.198	12.2
E	15-30a2	5.84	4.34	1.60	0.42	2.42	0.200	12.1
E	15-30b1	6.28	4.44	1.58	0.55	2.07	0.179	11.6
E	15-30b2	6.19	4.50	1.39	0.84	1.99	0.169	11.8

Table 5.2: Mean CEC values and associated standard deviations and soil textures of soils from the 5 profiles (A – E) at 0 - 15 cm and 15 - 30 cm depths.

Profile	Depth (cm)	Mean CEC ($mmol^{+}\ kg^{-1}$)	Std. dev.	Mean Carbon (%)	Texture
A	0-15	86.8	25.4	3.74	Fine sandy loam
A	15-30	84.6	30.7	2.58	Very fine sandy loam
B	0-15	90.5	4.3	5.70	Very fine sandy loam
B	15-30	53.1	4.9	2.20	Fine sandy loam
C	0-15	137.6	41.4	5.01	Silty clay loam
C	15-30	74.1	6.9	2.43	Silty clay loam
D	0-15	62.8	23.3	3.77	Silty clay loam
D	15-30	63.6	11.2	2.92	Silty clay loam
E	0-15	56.3	11.9	4.45	Fine sandy loam
E	15-30	72.1	27.0	2.22	Fine sandy silt loam

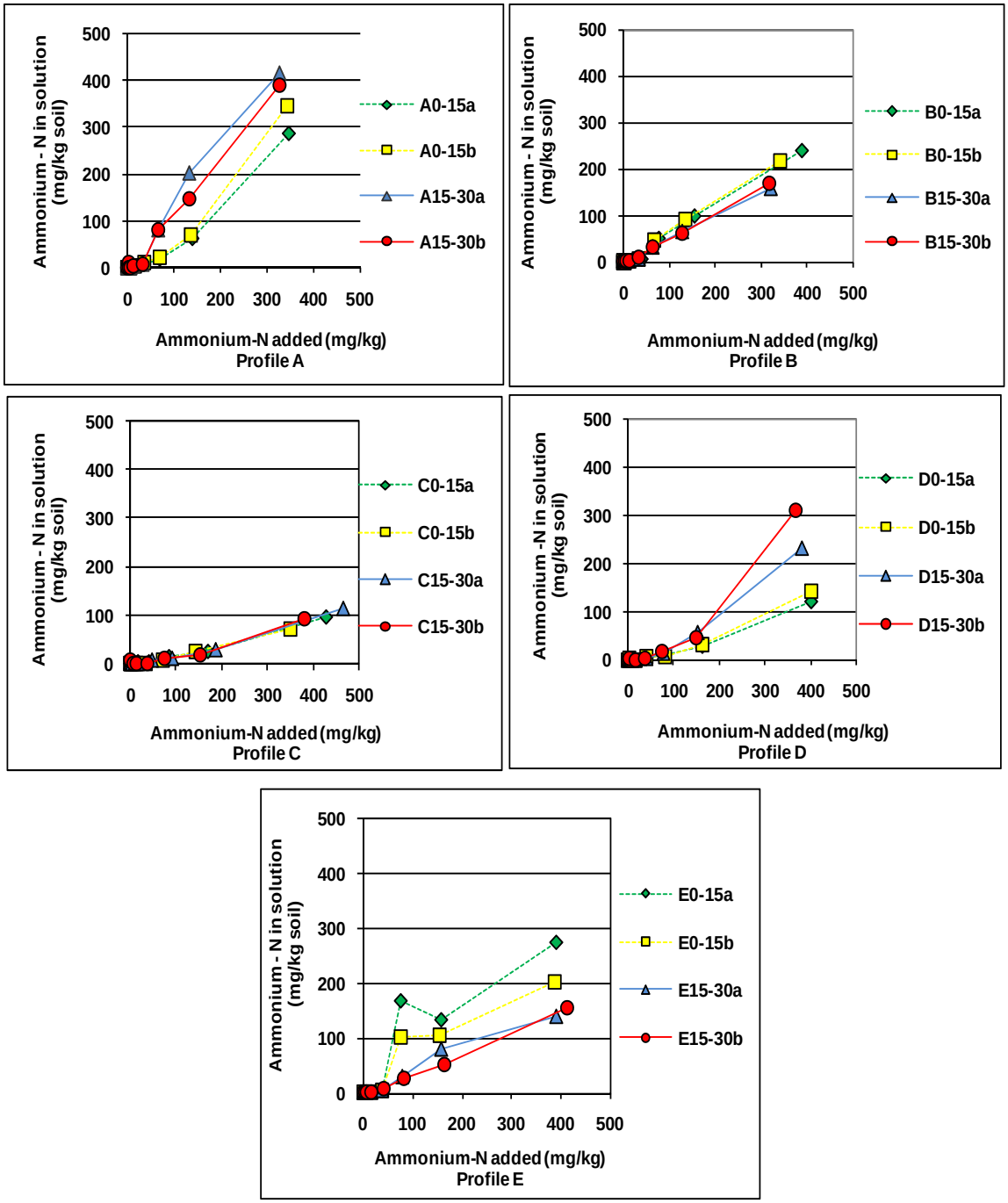


Figure 5.1: Relationships between ammonium-N remaining in solution and ammonium-N added, both expressed as mg per kg of oven-dry soil, for duplicate field samples from 0-15 cm (broken lines, diamond and square symbols) and 15-30 cm (solid lines, circle and triangle symbols).

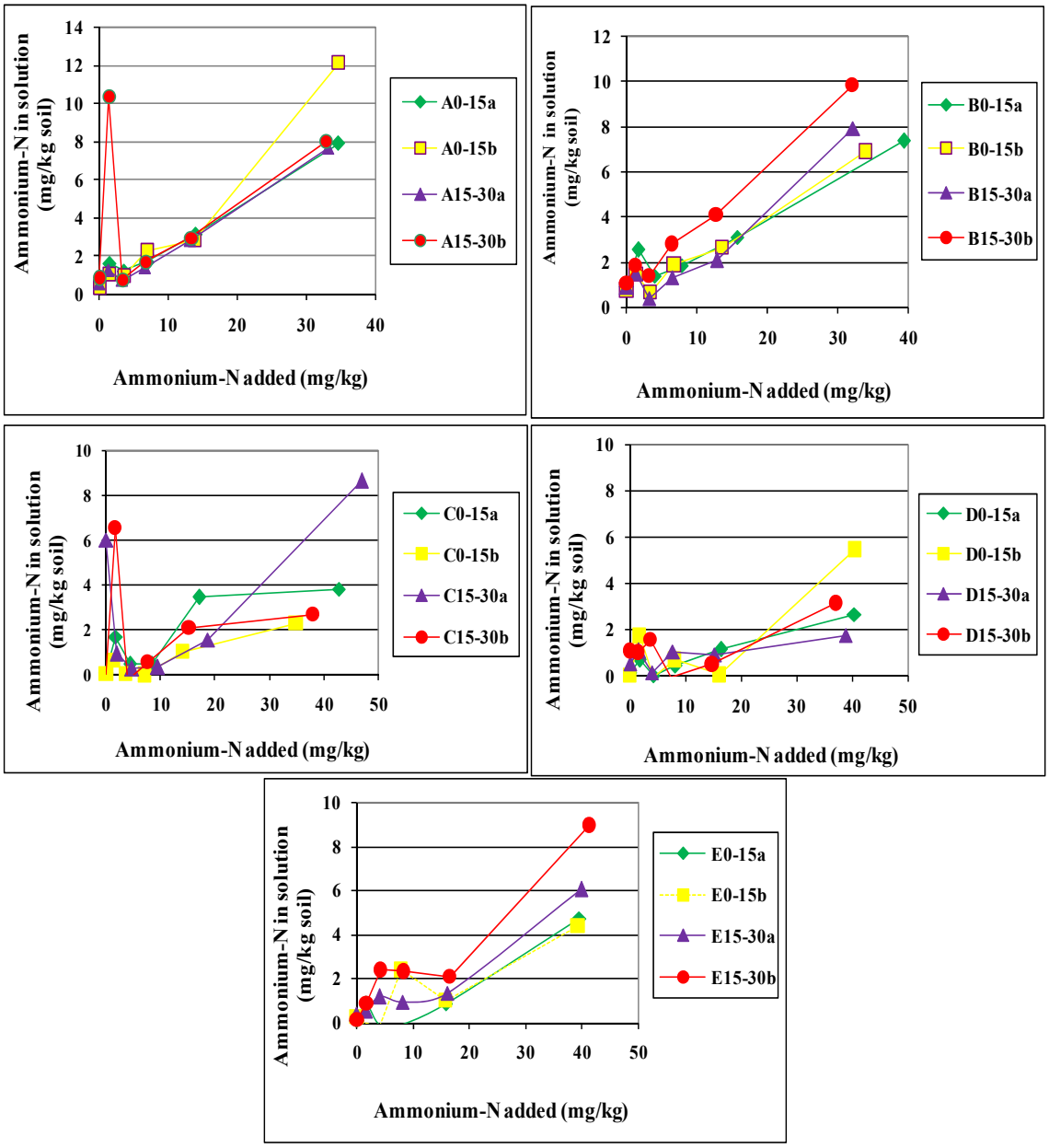


Figure 5.2: Relationships between ammonium-N remaining in solution and ammonium-N added, both expressed as mg per kg of oven-dry soil, for duplicate field samples from 0-15 cm (broken lines, diamond and square symbols) and 15-30 cm (solid lines, circle and triangle symbols), plotted on enlarged scale for additions < 50 mg kg^{-1}.

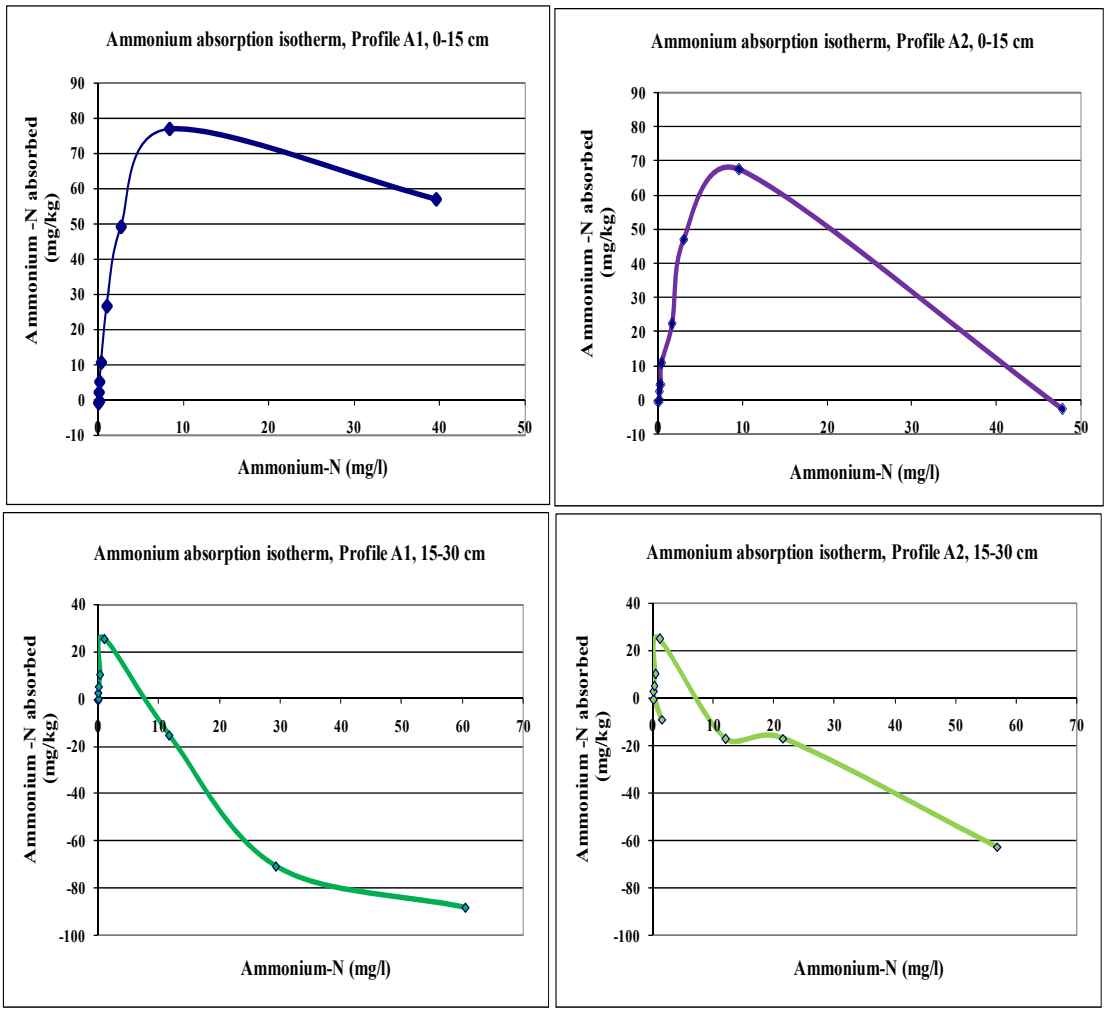


Figure 5.3: Relationships between ammonium-N absorbed and in solution for duplicates field samples from profile A (0-15 cm and 15-30 cm).

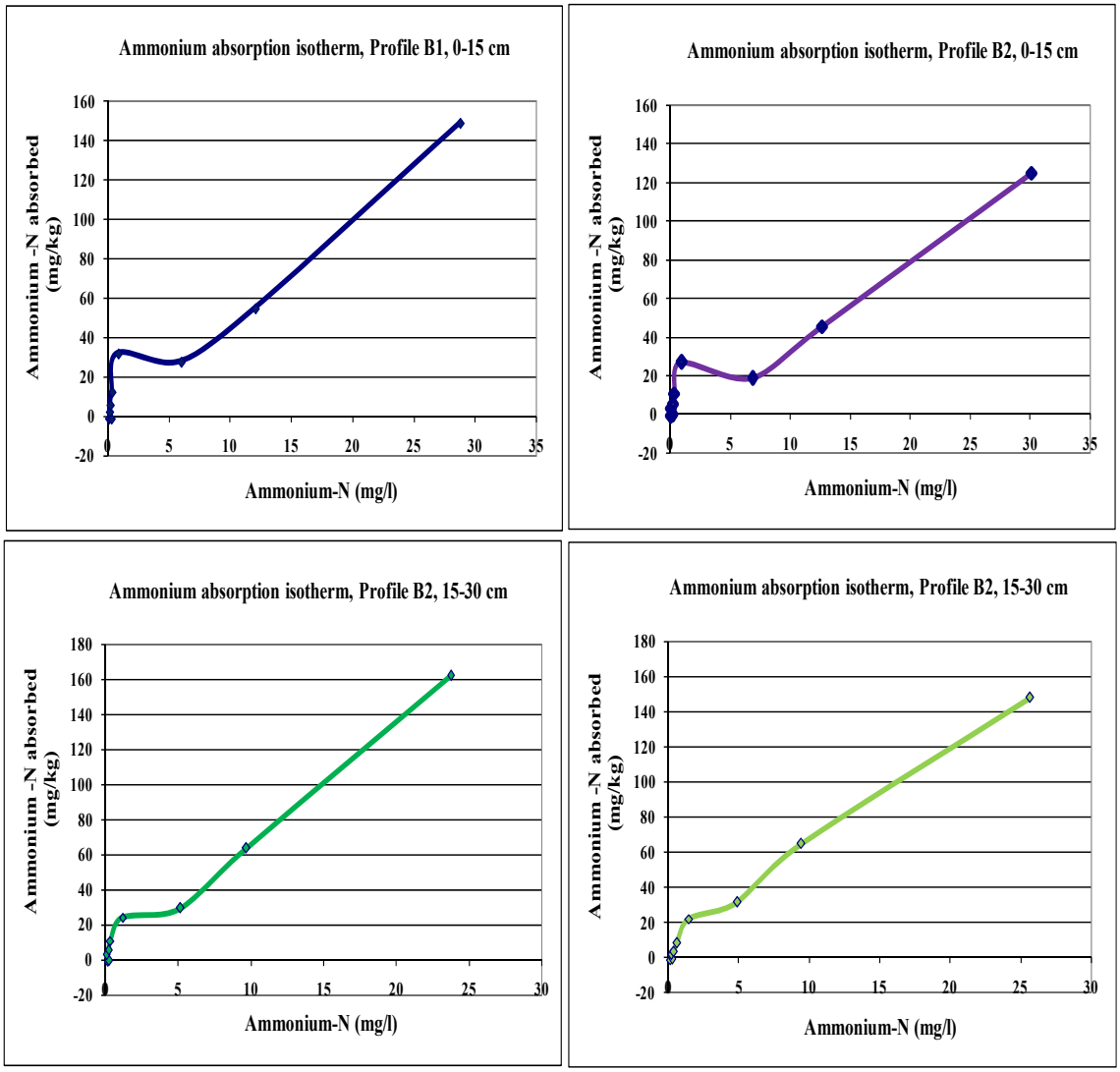


Figure 5.4: Relationships between ammonium-N absorbed and in solution for duplicate field samples from profile B (0-15 cm and 15-30 cm).

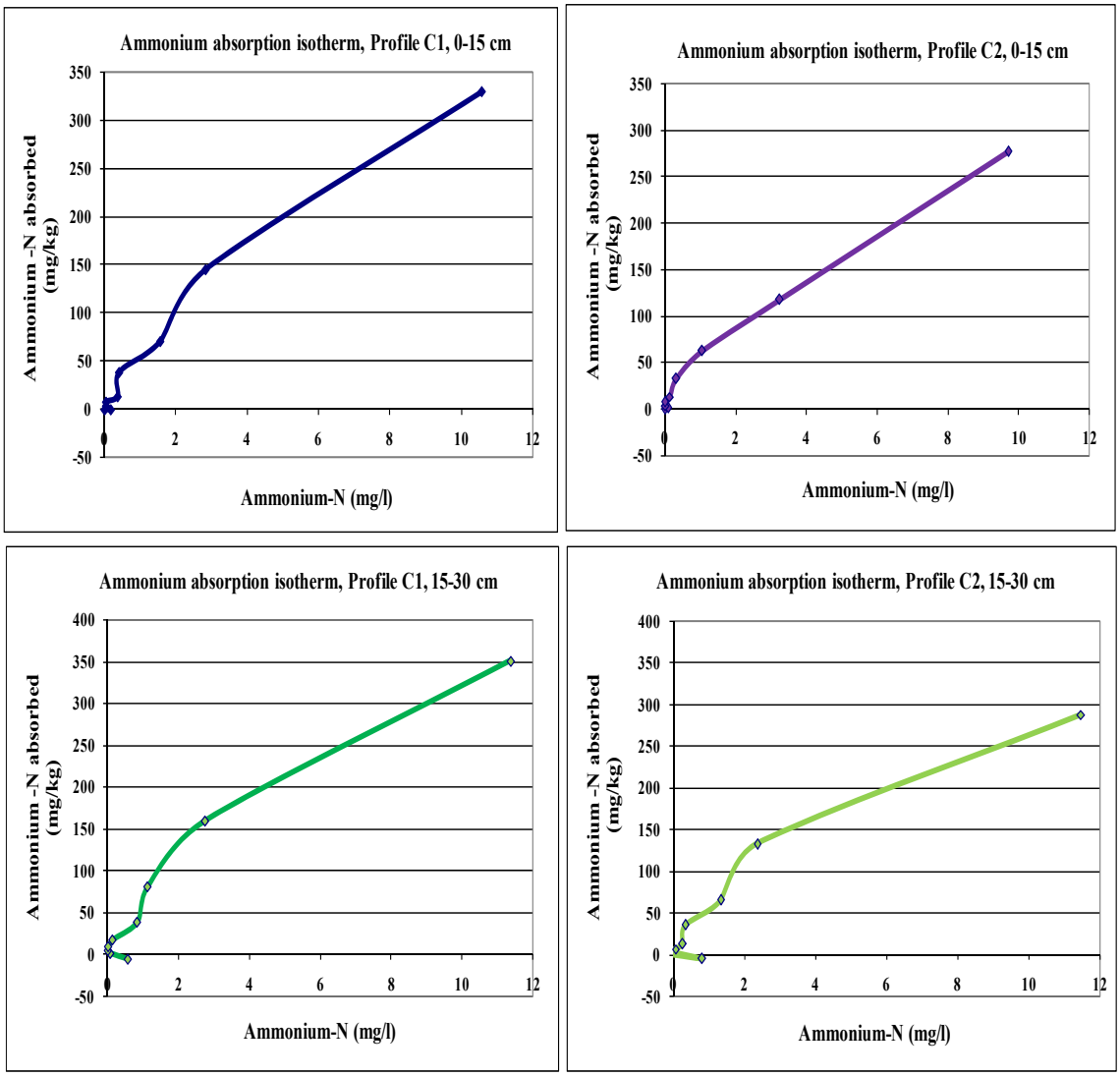


Figure 5.5: Relationships between ammonium-N absorbed and in solution for duplicate field samples from profile C (0-15 cm and 15-30 cm).

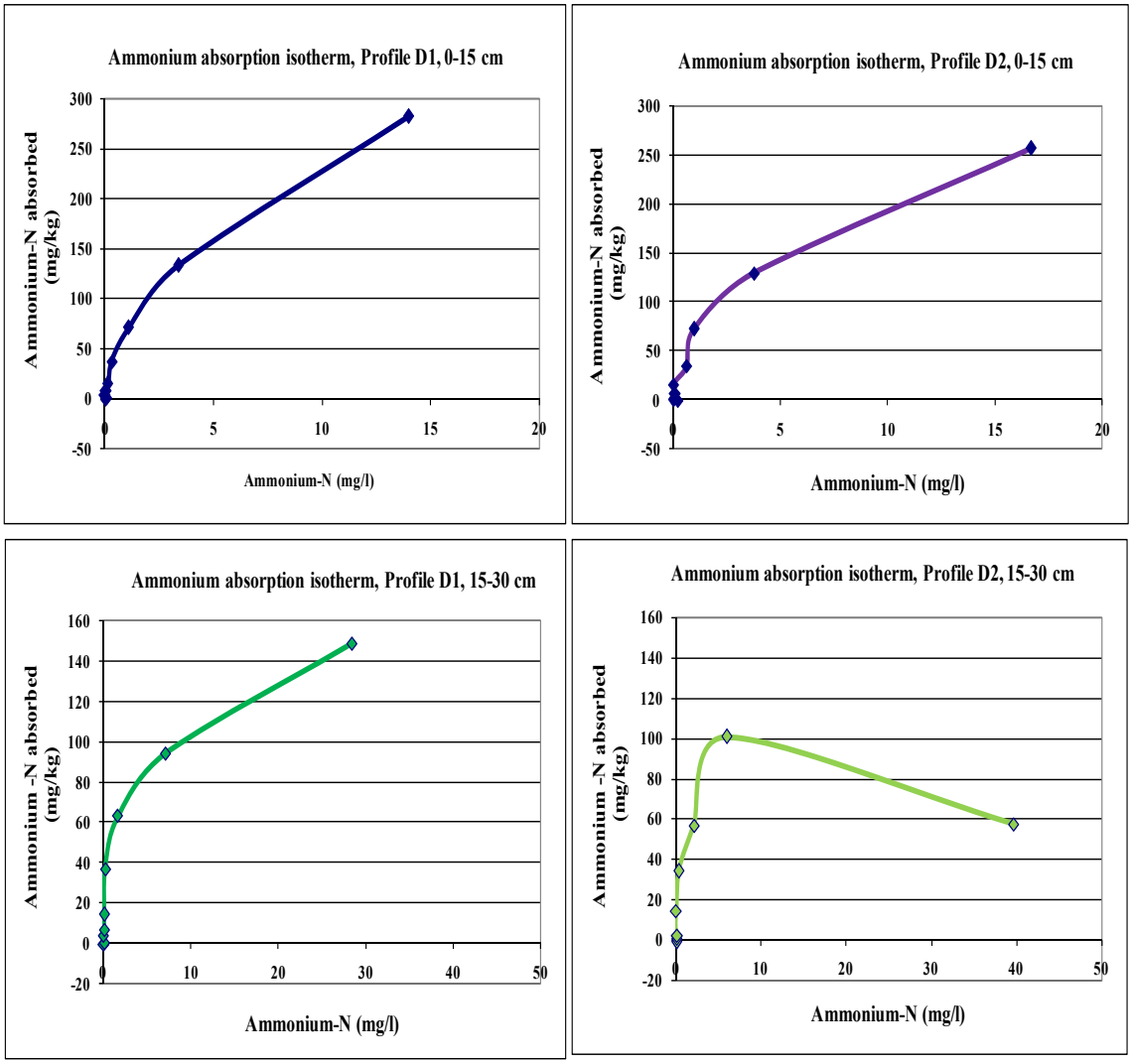


Figure 5.6: Relationships between ammonium-N absorbed and in solution for duplicates field samples from profile D (0-15 cm and 15-30 cm).

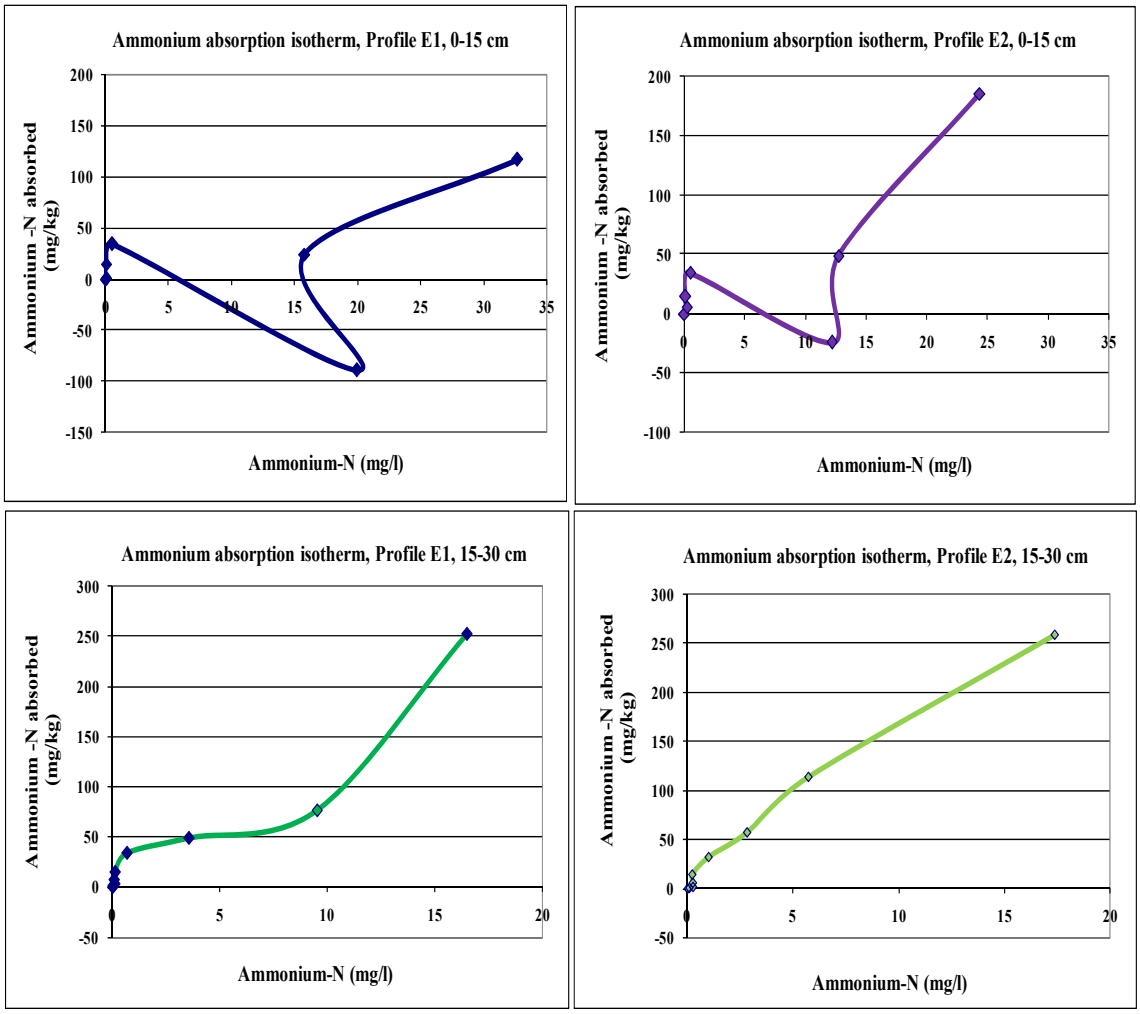


Figure 5.7: Relationships between ammonium-N absorbed and in solution for duplicate field samples from profile E (0-15 cm and 15-30 cm).

As might be expected, agreement was much closer between analytical replicates than between field replicates, and was generally good considering determinations for pH and initial ammonium-N and nitrate-N were conducted on field-moist soil sub-samples. The C, N and C:N values were obtained from 10-g sub-samples of field-moist soil after oven drying and grinding. The pH data confirmed the acidic status of profiles A and B. These more acidic profiles displayed higher C:N ratios; they were observed in the field to have more pronounced litter layers, but the soils sampled under the litter horizon did not have significantly higher carbon concentrations than the less acidic soils sampled except perhaps for profile Ba, (Table 5.1). The soil textures are shown in Table 5.2, together with the mean values of cation exchange capacity (CEC) and their associated standard deviations, and mean % carbon values to see if these indicate to what extent soil organic matter content dictates CEC. The standard deviations for CEC were quite high, which probably reflects the limitation of using 10-g sub-samples of field moist soil.

5.3.2 Absorption at high ammonium-N additions

Figure 5.1 shows the relationships for the 5 profiles between the amount of ammonium-N remaining in solution and the amount of ammonium-N added under the experimental conditions used. The results were also presented in this way and with constant scales for all profiles to clearly visually indicate what proportion of added ammonium remained potentially mobile (in solution). Especially for additions above ca. 50 mg of ammonium-N per kg of soil there are marked differences both with depth in the profile and between the soil profiles in their ammonium-N absorption characteristics. Profile C showed consistently the strongest absorption of ammonium-N at both depth increments for high ammonium-N inputs. Hand texturing indicated all soils from this profile were silty clay loams, and the 0 – 15 cm soil from profile C had the highest CEC value (Table 5.2).

Profiles D and E generally showed less absorption than profile C, although the silty clay loam from 0 – 15 cm at profile D showed only slightly less absorption than the silty clay loam at the corresponding depth from profile C (Fig. 5.1, broken lines). Absorption was greater in the surface soil of profile D (i.e. less ammonium remained in solution) than in the sub-surface soil, whereas the opposite was true for profile E. The silty clay loam surface horizon of profile D absorbed more ammonium than the sub-surface silty clay loam, in spite of their very similar mean CEC values; for profile E,

where the surface soil was a fine sandy loam, the sub-surface soil, which showed greater absorption, was a slightly more finely textured fine sandy silt loam.

The most acid soil profile, (A), behaved differently from all other soil profiles in that at high ammonium-N inputs absorption was negligible, and in fact desorption occurred from the sub-soils for both field replicates so more was left in solution than had been added (Fig. 5.1). The soils of this profile were a fine sandy loam at the surface above very fine sandy loam. This textural trend was reversed in the other acidic soil profile (B), as was the relative extent of the absorption at high ammonium-N inputs, but desorption was not apparent (Fig 5.1).

5.3.3 Absorption/desorption at low ammonium-N additions

Figure 5.2 shows the absorption/desorption relationships for the 5 profiles on an enlarged scale for additions of below 50 mg of ammonium-N per kg of soil. Again there are marked differences both with depth in the profile and between the soil profiles in their ammonium absorption characteristics. However, on comparing Figs 5.1 and 5.2 profile by profile, the relative trends with depth were often reversed at low concentrations. As might be expected when using biologically active, field moist soils there was more pronounced variation at the lower concentrations. In spite of this it is clear that often desorption is observed below 2 mg ammonium-N kg^{-1} additions. Even when significant ammonium-N absorption is occurring at inputs of around 10 - 20 mg ammonium-N kg^{-1}, it is important to note that ca. 1- 2 mg kg^{-1} remains in the solution phase.

5.3.4 Intact core leachates

Figure 5.8 shows the ammonium-N and nitrate-N concentrations measured by Riaz (pers. comm.) in leachates from the duplicate intact core mini-lysimeters for profiles A to C and E and from rhizon samplers inserted close to the base of cores from profile D. The use of rhizon samplers proved essential for profile D because of the negligible drainage from its intact cores. Ammonium was detected in every sample analyzed. Nitrate-N was leached from cores from profiles A, B and C, and for A and B nitrate-N concentration always exceeded that of ammonium-N. For profile C, however, ammonium-N concentration sometimes exceeded that of nitrate-N and for profiles D and E ammonium-N-concentration was always higher. Indeed, no nitrate was detected in the rhizon samples from profile D or in 5 of the 12 samples from profile E.

5.4 Discussion

5.4.1 Evidence for mobility of ammonium-N

At low input concentrations of ammonium-N in the absorption experiments a substantial proportion of the ammonium input was absorbed by the soil (Fig. 5.2), but nevertheless a significant amount of ammonium-N remained in solution and must be regarded as potentially mobile. For example, from Fig. 5.2, at an input of 10 mg ammonium-N (kg of soil)$^{-1}$, ca. 1 – 2 mg kg^{-1} remains in the solution phase. At higher input concentrations (Fig. 5.1), progressively more ammonium-N remains in solution, and hence potentially mobile. Even when no ammonium-N was added, in almost every instance some water-soluble ammonium-N was found.

5.4.2 What controls differences in ammonium-N absorption characteristics?

Inputs of ammonium-N at this site will come from the atmosphere and from *in-situ* mineralization of organic matter, including inputs in plant litter, animal faeces and urine. In another study of the acidic grasslands at this site very high rates of ammonium production in the litter horizons were found but with negligible nitrification (Riaz, pers. comm.). Thus the mineral soils sampled in the present study below the litter layer at profiles A and B would be receiving a high ammonium-N input via infiltrating precipitation. The mechanism for this is discussed in the next section. The absorption characteristic plots for profiles A and B, when compared with those for the other profiles (Fig. 5.1), show much weaker absorption (or sometimes desorption) compared with the plots for profiles C and D, which had much less pronounced litter layers.

The soils in profiles C and D were silty clay loams, whereas those in A and B were fine/very fine sandy loams (Table 5.2). Profile E was a fine sandy loam overlaying a fine sandy silt loam, and its absorption characteristics were generally closer to those of profiles A and B than those of profiles C and D. Thus texture seems to be very important. However the CEC data (Table 5.2) do not indicate that this is due to CEC differences. CEC was measured in this study using 10-g sub-samples of field moist, hand-sorted soil, so the standard deviation was, not surprisingly, quite high, as mentioned in section 5.3.1. Nevertheless, the effect of texture seems not to be due simply to CEC, and could instead reflect drainage influences. For profile D especially, the very low drainage rate suggests that any external nitrate input, or any nitrate

produced *in situ,* is denitrified, providing a nitrogen sink which facilitates subsequent ammonium-N retention. This may be compounded by vegetation N removal by grazing by cattle, which was conspicuous at profiles C, D and E but absent at profiles A and B.

The other factor that must be important to the mobility of cations through soils is the associated mobile anion concentration. In a recent study of mineral N leaching from sub-tropical soils treated with ammonium hydrogen carbonate, Qian and Cai (2007) found that nitrification resulted in sufficient leaching of nitrate as a mobile anion to stimulate ammonium leaching from their freely draining soils. Figure 5.8 clearly shows high nitrification rates for profiles A and B in the present study. Although samples were stored in a fridge when not being used immediately, it is very probable that nitrate production contributes to the greater ammonium-N mobility for soils from profiles A and B.

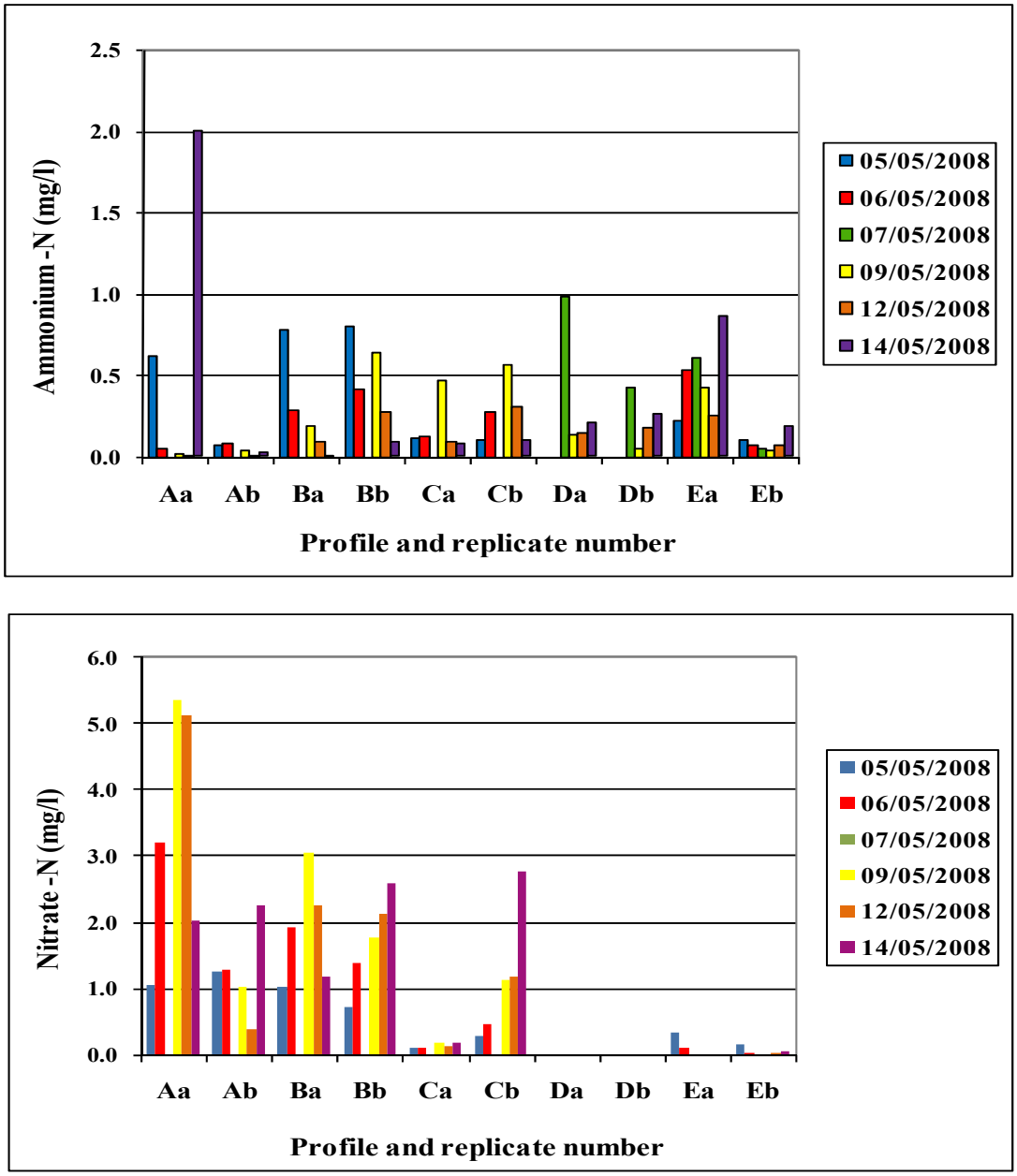


Figure 5.8: Changes in ammonium-N (upper chart) and nitrate-N (lower chart) concentrations in core leachates over 10 days from duplicate (denoted by a and b) intact 30-cm cores from the 5 profiles (A – E). Rhizon samplers were inserted to extract samples from Da and Db from 07/05/2008 because of the complete lack of drainage at zero tension. Samples were generated by Riaz and analyzed jointly by him and the author.

5.4.3 Conventional Absorption Isotherms

For ease of interpretation, and to demonstrate the potential mobility of the ammonium ions within the N deposition-impacted grassland soil profiles, results were initially presented and discussed using plots of ammonium-N in solution versus ammonium-N added, both being expressed in terms of mg of ammonium-N per kg of soil. For completeness they are also presented (Figs. 5.3 – 5.7) in more conventional absorption isotherm format (Bohn et al., 1979).

In this experiment soils were sampled from two depth increments below the litter horizon. This means that, under field conditions, the upper layer especially would have received inputs from ammonium-N produced by mineralization in the overlying litter horizon, and this would be especially pronounced for the most acidic profiles, A and B, which had distinct litter layers. Thus part of the initial ammonium-N (Table 5.1) could have been derived from this source, and part from in-situ mineralization of soil organic N. However, the ammonium produced by ammonification in the litter would not initially be mobile.

The equation usually given for ammonification is:

$$RNH_2 + H_2O + H^+ \leftrightarrow ROH + NH_4^+$$

It may be assumed that the protons consumed would effectively originate from the cation exchange sites, where they would be replaced subsequently by the ammonium. It may be hypothesised here that these ammonium cations would have very limited mobility initially, because of the lack of associated mobile anions. However, ammonium under field conditions could subsequently be displaced by inputs of either Na^+ and Mg^{2+} from sea-salts in precipitation or in dry deposition, or H^+ in acid deposition, which would be accompanied by Cl^-, SO_4^{2-} and NO_3^-. It is suggested that these processes together help explain, for the most acid soils of profile A, why ammonium absorption is relatively weak, and desorption occurs for the three highest spike additions in soil from 15-30 cm depth (Fig. 5.3). In this absorption isotherm experiment, additional chloride has been added, which would facilitate desorption compared to what would happen naturally under field conditions. The KCl-extractable ammonium-N concentrations in acidic litter horizons at Hob Moor are an order of magnitude or more higher than those in the underlying mineral soil horizons (Riaz, pers. comm.). The solution concentrations in the isotherm experiments are substantially higher for the 0-15 cm layers of profiles A and B than they are for the 0-15 cm layers of profiles C and D. Profiles A and B had coarser textures, so might be expected to have lower CEC values. The low pH of profile A would also reduce its CEC. Therefore it might be expected that ammonium could be more readily displaced from these soils, and especially from profile A, as was observed in practice.

As mentioned earlier, the equilibrated solution concentrations of ammonium-N are appreciably lower for profiles C and D than for profiles A and B (compare x axis scales in Figs. 5.5, 5.6 and 5.3 & 5.4). The former soils had higher pH values and more earthworm activity was observed at the time of sampling (though this was not quantified). Thus they did not have well developed litter horizons as the litter would

have undoubtedly been more mixed throughout the sampling depth. Therefore they would not have received the same elevated input of ammonium flushed from an overlying litter layer. Greater soil uniformity is the probable explanation for the greater similarity between results at 0-15 and 15-30 cm for profiles B and C than was seen for the most acid soil of profile A. As mentioned in section 5.3.1, profile B had a higher organic C% than profile A, and also had a higher C:N ratio at 0-15 cm, suggesting possibly greater mixing of organic matter in profile B. The difference in absorption isotherms between the two sampling depths is much less pronounced for profile B than for profile A.

In spite of their heavier textures and higher pH values, soils from profiles C and D did not in fact appear to have significantly higher CEC values (Table 5.2). They probably have been experiencing greater plant N uptake, as vegetation was conspicuously more lush at profiles C and D than on the more acid soils of profiles A and B. It was noticeable that soils from profiles C and D had significantly lower C:N ratios than the soils from the other profiles, which probably reflects greater decomposition rate of the organic matter in the soil over most of the year. However, grazing cattle would have removed vegetation (and the N it contains) from profiles C and D for at least a decade, so, in spite of the low C:N ratio, ammonium-N production may be occurring at a lower rate down these profiles, thus facilitating ammonium-N absorption. At profiles A and B grazing is only by rabbits, as fences prevent the cattle from reaching these sites.

The absorption isotherms for profile E differed from those for all the other profiles, especially for soils from 0-15 cm. Following addition of 10 mg l^{-1} ammonium-N, strong desorption occurred in both replicates, but some absorption occurred for more concentrated spike additions (Fig. 5.7). The reasons for this are unclear, and the effect may be due to chance variations in the sub-samples of field moist soil used, especially as profile E was from an area grazed by cattle. However the curious effect is reproducible between the replicates. Further work would be needed to see if it is reproducible in adjacent soils and over time. Up to the additions of 10 mg l^{-1} ammonium-N, soils from 0-15 cm of profile E behave moderately similarly to soils from 15-30 cm of profile A, and have a quite similar texture to these soils (Table 5.2), but the profile E soils are much less acidic.

5.4.4 Does C:N ratio influence soil ammonium concentration?

The results in Table 5.1 show that soil C:N mass ratio is consistently significantly higher in the two more acidic soil profiles, A and B compared with the other three profiles, especially compared to profiles C and D, and generally decreases with increasing soil pH; this is more readily apparent when the results are compared graphically, as in Figs. 5.9 – 5.10.

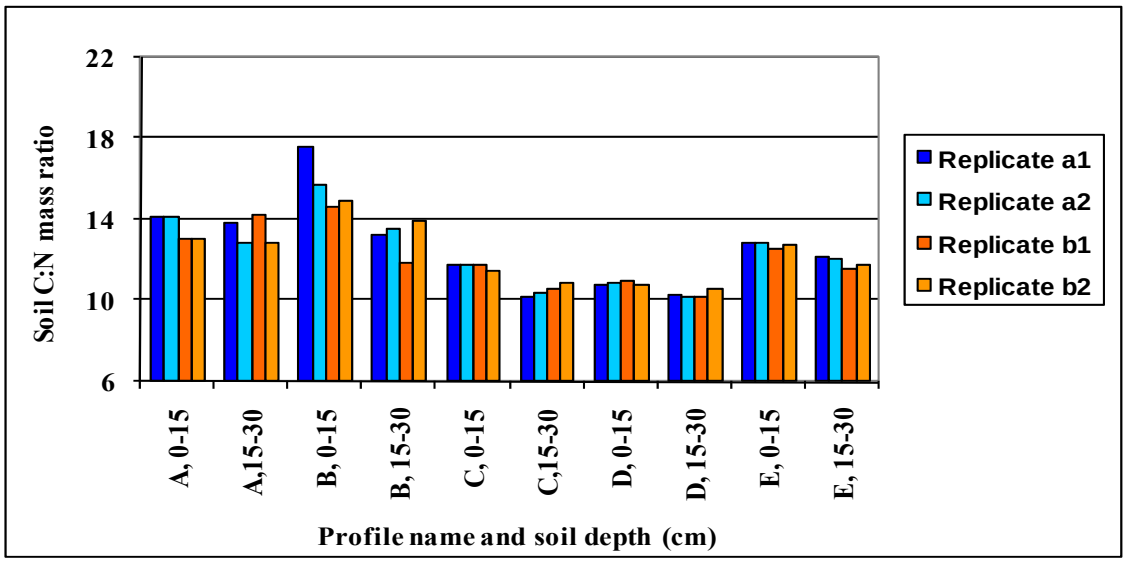


Figure 5.9: Change in soil C:N mass ratio for the five profiles (A – E) between 0- 15 cm and 15-30 cm for duplicate field samples (a and b) analysed in duplicate (a1 & a2 and b1 & b2).

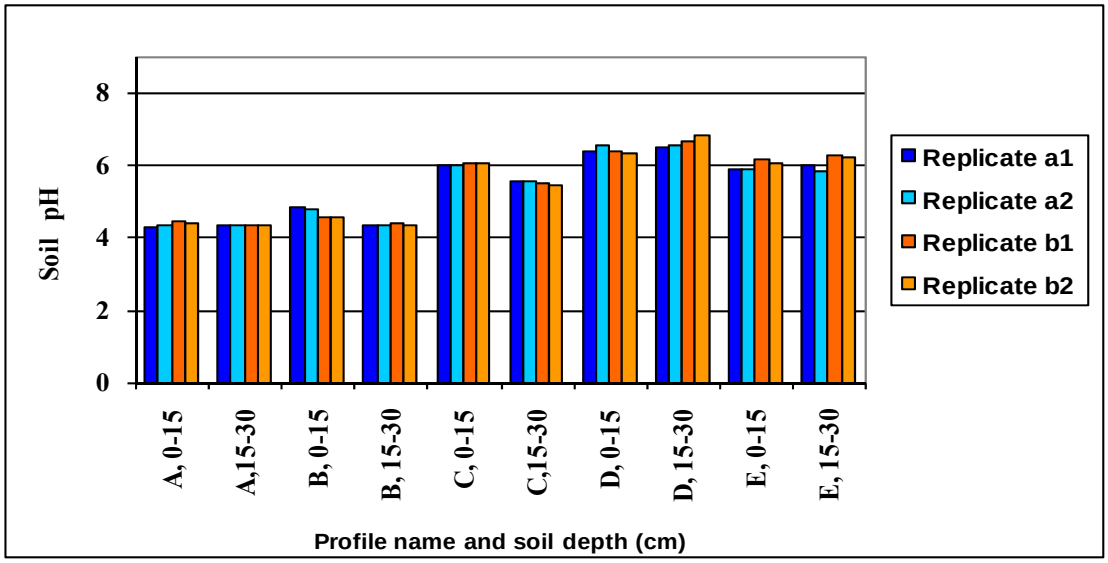


Figure 5.10: Change in soil pH (in water) for the five profiles (A – E) between 0-15 cm and 15-30 cm for duplicate field samples (a and b) analysed in duplicate (a1 & a2 and b1 & b2).

This trend might be expected from the lower decomposition rates expected in the more acidic grassland soils. However, although the highest mean soil C% was for the 0 – 15 cm soil from profile B, Tables 5.1 and 5.2 provide no consistent indication of greater

organic matter accumulation in the more acidic profiles (A and B) compared with the other three profiles, so at this site quality rather than quantity of soil organic matter is apparently influenced by pH.

It was thought that the soil N:C ratio, together with CEC and nitrification rate, might be a factor regulating the initial soil ammonium-N concentration, and hence also important to the soils' responses to subsequent ammonium-N additions. When the data were analysed together (i.e. for both soil depths), no significant correlation was found between soil mean initial ammonium-N concentration and N:C mass ratio. However a statistically significant correlation did exist for the mean values for the 15-30 cm soils (Fig. 5.11). Only mean values can be plotted here because replicates a and b differ for the CEC determination and the determinations of C and N%. More data would be needed to see if this relationship has any predictive value.

It is highly probable that in the most acidic 0 – 15 cm soils, (profiles A and B) leached ammonium inputs from litter decomposition are playing a dominant role in the N cycling in the underlying soils, in contrast to the other profiles where faunal mixing of organic matter would play a greater role at the higher pH values. At the time of sampling it was noted that profiles A and B both had very clear and distinctive litter horizons because of the poor mixing of organic litter by the limited soil fauna at the very low pH, and this would account for the lack of obvious or consistent increase in soil C% (Table 5.1) in the samples taken for these experiments from below the litter layer. Earthwork activity was conspicuous in profiles C – E.

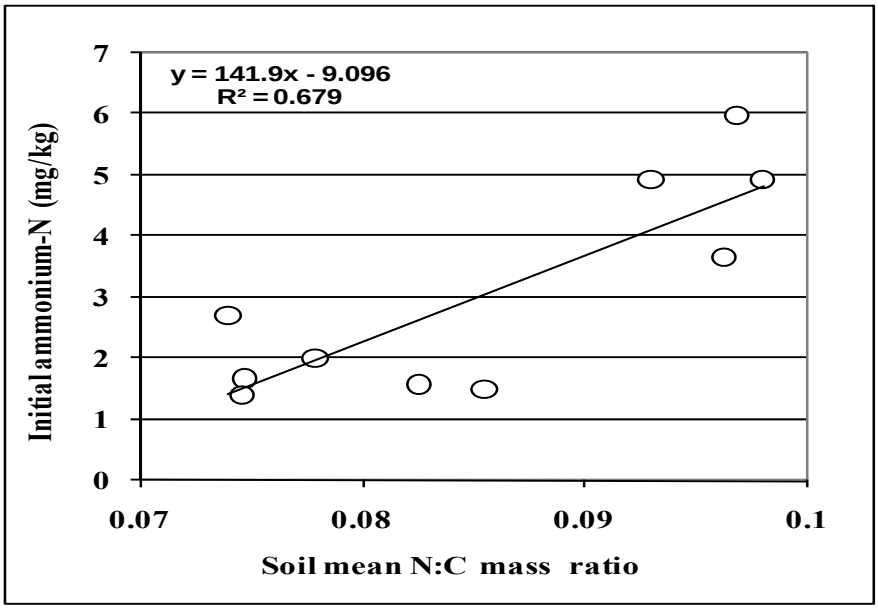


Figure 5.11: Relationship between mean initial ammonium-N in soils from 15-30 cm at sites A to E and mean soil N:C mass ratio.

5.5 Conclusions

The results from both the author's experimental approaches and the microcosm leaching experiment of Riaz clearly indicate that ammonium in N-impacted, unfertilized grassland soils may be considerably more mobile than previously thought, and ammonium translocation down profiles may be playing an important role in N cycling in such soils. This is certainly true at the Hob Moor site and will be especially the case where high local ammonium inputs arise from animal faeces and urine. If ammonium leaches to below the rooting zone it is likely to be nitrified in sub-soils and contribute to nitrate leaching to surface waters and/or groundwater, although some amelioration may occur as a consequence of denitrification at poorly drained sites. When designing experiments to quantify N species transformation dynamics in isolated soil samples, it is important to consider the possibility that initial N status may be critically dependent in the ammonium production in, and translocation from, overlying soil horizons.

CHAPTER 6: IS THE LOW C:N RATIO OF FOREST LITTER AN EVOLUTIONARY STRATEGY TO HELP CONSERVE ECOLOGICAL NICHE? A PRELIMINARY ASSESSMENT

6.1 Introduction

In a recent co-authored reappraisal of the terrestrial nitrogen cycle with a view to understanding pollutant N impacts, Cresser et al. (2008) suggested that plant evolution may have been driven by the requirement for a locally sustainable match of the dynamics of soil N supply with those of plant N requirement. They hypothesised that atmospheric pollutant N deposition may be adversely affecting plant biodiversity by inducing a dynamic mis-match between plant N needs and soil N supply. Following on from this, it could be further hypothesised that, in pristine environments where N supply is invariably limiting to plant growth, deciduous tree leaf litter needs to decompose slowly, or possibly not at all, at low winter temperatures when plant N uptake requirements are very low. It would therefore be beneficial for leaf litter immediately following litter-fall to have a high C:N ratio, thereby favouring N immobilization by biomass associated with the slowly decomposing litter. As litter starts to be mineralized, the C:N ratio of the decomposing residue would decrease, eventually reducing the probability of further N immobilization by microbial biomass and thereby eventually increasing plant bio-available N supply.

Recent experimental studies of the potential responses of soils from sub-arctic heath to the enhanced litter inputs from birch (*Betula pubescens* ssp. *Tortuosa*) that might be anticipated in response to global warming showed that net mineralization of P and microbial growth rate both increased with litter addition, whereas the inorganic N pool in soil cores incubated under field conditions decreased (Rinnan et al., 2007). Moreover, ammonium concentrations did not change significantly in response to litter additions and nitrate was not detectable (Rinnan et al., 2008). This, and their observation that in this relatively clean environment mineralized N was immobilized in biomass for all treatments, including the controls (Rinnan et al., 2007), suggest that fresh litter inputs do not lead to immediate increase in inorganic N pools, supporting the idea of immobilization at least in the short to medium term. Potthast et al. (2010) recently compared the mineralization of *Setaria sphacelata* grass with a C:N ratio of 33

with that of Bracken with a ratio of 77 in pasture soils of southern Equador and reported reduction in the pools of available organic carbon and nitrogen.

Mungai et al. (2006) investigated the effects of added litter quality on soil C and N dynamics. They compared effects of tree litter from Pecan, Silver Maple, Chestnut and Black Walnut (with C:N ratios of 42.3, 33.6, 59.3 and 33.1 respectively) with those of bluegrass, maize and soybean litters (C:N 19.1, 28.3 and 19.7 respectively). While soybean litter enhanced net N mineralization, all other litter types immobilized N for various periods of time. Although litter from the crop species gave significantly higher soil C mineralization rates, attributed to their lower lignin concentrations and C:N ratios and higher N concentrations, than the tree litter types, there were no obvious differences in N dynamics.

If litter C:N ratio is a major factor regulating the recycling of N, it raises the interesting question as to whether litter in higher N deposition areas has a lower ratio, making it potentially more readily mineralizable. Studying the effect of N deposition on litter decomposition from oak (*Quercus robur* L), Månsson and Falkengren-Grerup (2003) concluded that litter quality had been modified in a way that increased C and N mineralisation. They pointed out that although there was a lower C:N ratio in litter from the more N-impacted area, this was not reflected in differences in microbial biomass C:N ratio, and suggested that this might reflect a relatively small contribution of biomass attacking fresh litter to the total biomass present.

The above papers support the concept generally that high a C:N ratio in plant residues initially would favour immobilization of nitrogen. It is reasonable to suggest, however, that as litter started to decompose the C:N ratio would fall, until eventually mineral nitrogen would become progressively more bio-available. In regions with a climate similar to that of the UK, with warm summers but cold winters, plants would benefit in evolutionary terms if the seasonality of mineral-N production from plant litter decomposition was closely matched to the seasonality of the same plant species mineral-N requirements. In this chapter, therefore, my hypotheses are:

- There is naturally a dynamic match between plant N requirements and mineral N production in soil.
- Plant continued occurrence is favoured when this dynamic match is good (i.e. close dynamic match favours creation of an ecological niche).
- Diffuse N pollution could adversely affect biodiversity by inducing a dynamic mis-match between soil mineral N supply and plant N requirements.

- ❖ Deciduous litter therefore has evolved naturally, with a high C:N ratio, to help retain mineral N species produced during winter months when tree N uptake is low.
- ❖ As litter decomposes, its C:N ratio falls until microbial N immobilization is less favoured. Plant-available N then increases.

If the hypotheses can be proved to be correct it would be of high relevance to agencies responsible for formulation of pollution abatement policies. If a high level of diffuse pollution induces such a dynamic mis-match, biodiversity change would be an inevitable consequence, and inorganic N leaching would be likely to be more substantial in winter, and even more likely to occur in summer in N-impacted areas.

For this preliminary assessment of the hypothesis, it was decided to conduct an experiment using foliage from a single tree species, collected over a period of several months to obtain a range of C:N ratios, to assess how litter C:N ratio of litter amendments to a local woodland soil influenced net mineral-N production in that soil. The hypothesis therefore was that net mineral N production rate would be more rapid in soils with a litter amendment with a low C:N ratio compared with the rate in the same soil when the litter had a high C:N ratio. The experiment, designed by the author, would be partially conducted by an undergraduate environmental science student, Claire Stephens, for her honours project in 2009-2010, under close supervision by the author. The analyses, and this interpretation of the results, were, however, all performed by the author.

6.2 Experimental design

6.2.1 Foliar sampling and preparation

Leaves from Hazel (*Corylus avellana*) were sampled by the author in June (22.06.2009), August (22.08.2009) and October (24.10.2009) to get "litter" with a range of C:N ratios. It was anticipated that the C:N ratio would be highest in the October litter and lowest in the litter from the younger leaves collected in June. Leaves were cut at random from a single tree growing on Little Hob Moor, immediately adjacent to the main Hob Moor site that is fully described in Chapter 2. It was decided that using foliage all taken from a single tree would minimise the risk of other potential sources of variation in the litter chemical composition apart from C:N ratio, although C, O and H concentrations of the litter would also vary with age, as indeed would concentrations of other major and trace nutrients (Marr and Cresser, 1983).

Because they had to be stored for extended periods over the summer and early autumn months, the foliar samples were all dried at 70°C and homogenized by pushing the crumbly, dried material by hand through a 4.75-mm stainless steel sieve. A 4.75-mm sieve was used, rather than a finer mesh size, because it was thought that excessive shredding of the foliar material would expose too much surface area, making the simulated litter decomposition conditions even less realistic than they already were.

Sub-samples were oven dried and ball-milled for C and N analysis using a C & N analyzer, as described in Chapter 2.

6.2.2 Soil sampling, preparation and analysis

A mineral soil was sampled from a woodland area at the eastern edge of the University of York Heslington campus. The soil was collected from under beech trees, from the upper 10 cm depth below the thin litter layer present in October, and taken back to the laboratory, sieved through a 2.8-mm sieve to remove any stones and roots, and thoroughly mixed. A 2.8-mm sieve was chosen, rather than the more normal 2-mm sieve used in soil science, to allow some small aggregates to pass through and minimise the amount of soil disturbance at least to some extent.

Sub samples of the soil were analysed for moisture content by oven drying, so that results could all be calculated on an oven dry weight of soil basis, and the oven dry soils were ball-milled to fine powders and analysed in duplicate for C%, N% and C:N mass ratio, as described fully in Chapter 2.

6.2.3 Experimental set-up

On 17.10.2009, a 15-g sub-sample of field-moist soil was added to each of sixty 250-ml screw-cap glass jars. Either 0, 0.5, 1.0 or 2.0 g of dried litter and 4 ml of de-ionized water were added in triplicate to the soils in the loosely capped glass jars. During incubation at room temperature, caps were balanced on the tops of jars to maintain aerobic status and minimise water losses. Jars were weighed and water was added as necessary to maintain constant weight, approximately every 2 to 3 days.

KCl-extractable ammonium-N and nitrate-N were measured after 7 days and 14 days, as described in Chapter 2. Results were corrected for the moisture content of the initial soil and the added water.

6.3 Results

6.3.1 Seasonal changes in litter C:N ratio

Figure 6.1 shows the results of the duplicate analyses of soil and litter samples for C:N ratio. Litter C:N ratio was always higher than soil C:N ratio and increased consistently with age at sampling time. Differences between pairs of results were all significant at $P<0.001$ (Tukey post hoc test).

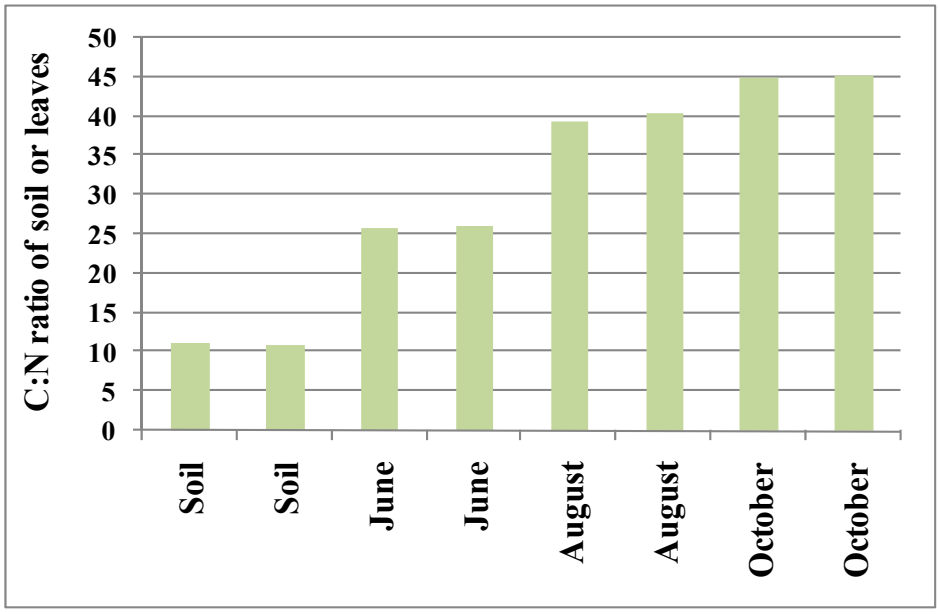


Fig. 6.1. The C:N ratio of the woodland soil and the hazel foliar samples in June, August and October 2009.

6.3.2 Effects of treatments on extractable ammonium-N and nitrate-N in soil

Figure 6.2 shows the mean values from analysis of triplicate samples for extractable ammonium-N and nitrate-N concentrations at the end of 7 days of incubation. Only one set of triplicate controls was prepared for, and sampled at, the first sampling, so in this Fig. the bars for controls for June, August and October litters are, in fact, identical. It is very obvious when the results for samples with litter added are compared with the results for the controls that both ammonium-N and nitrate-N are immobilized to a very substantial extent by the presence of small amounts of litter, and that, in percentage terms, nitrate-N is immobilized more strongly than ammonium-N. It is clear also in this figure that, in the litter-amended samples, in marked contrast to the control samples,

there was less nitrate-N than ammonium-N, regardless of the date on which the foliage was sampled to produce litter.

It appears that the amount of litter in the amendment is important. For the June and August litter, soils with 2 g of litter apparently contained more extractable ammonium-N than soils with 1 or 0.5 g of litter added in their amendments. However, there was only significant immobilization of ammonium-N with 1 g of June litter or 0.5 g of August or October litter. Although it appeared that for the October litter, the treatment with 1 g of litter added produced more ammonium-N than the 2 g litter treatment or the 0.5 g litter treatment, the difference was not significant.

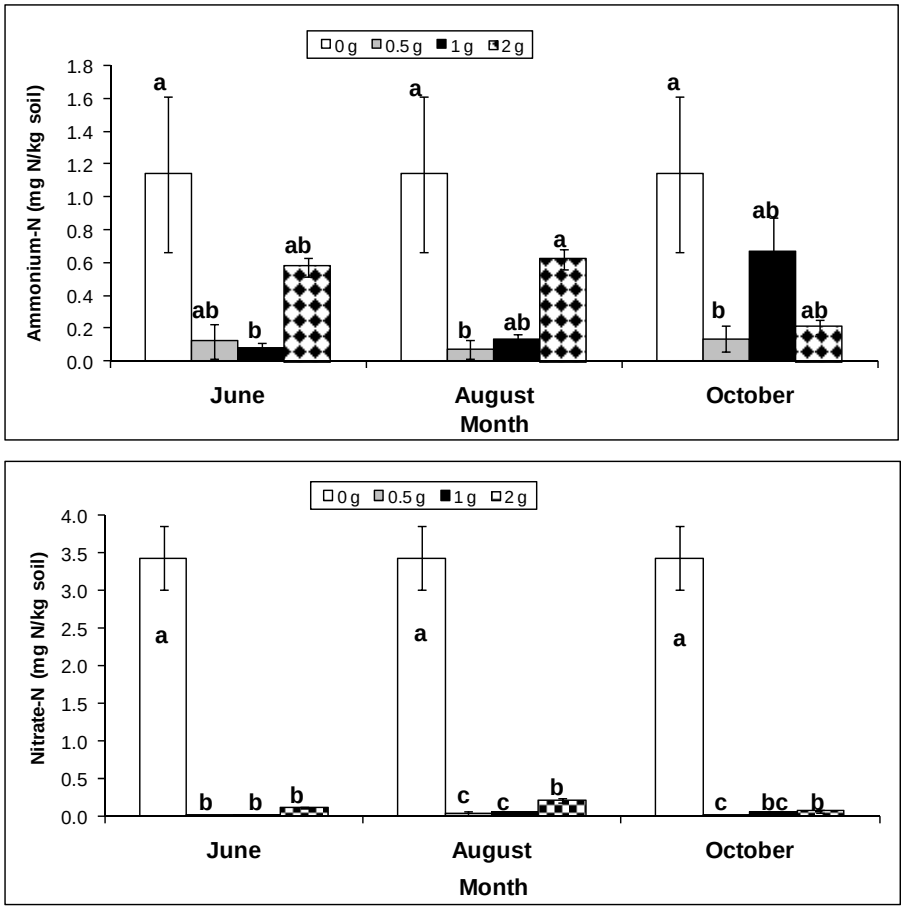


Fig. 6.2. Effect of litter treatments on ammonium-N (upper chart) and nitrate-N (lower chart) concentrations in soil compared with the controls after 7 days of incubation. The x axis shows the month the leaves were sampled and the keys show the mass of the litter in grams. In each chart bars in a group with only different letters differ significantly (P<0.05).

Evidence for immobilization of soil N by litter was much stronger for nitrate-N. The 0.5, 1 and 2 g litter treatments all produced significantly less nitrate-N than the control treatments, regardless of when the litter was collected. For the treatment with litter collected in June, there was no significant effect of the amount of litter added. For the

August litter treatment, the effect of litter amount on nitrate-N concentration was significant, with both the 0.5 g treatment and the 1 g treatment giving significantly lower nitrate concentration than the 2 g treatment, suggesting that when sufficient litter was present nitrification rate started to exceed immobilization rate. For the October litter treatment, 2 g of litter resulted in a significantly higher nitrate-N concentration that 0.5 g of litter (result not shown).

The effects of litter were much more variable after 14 days of incubation compared with those after 7 days. Nitrate-N concentration still exceeded ammonium-N concentration for the controls, as after 7 days. However, after 14 days, nitrate-N concentration consistently exceeded ammonium-N concentration, except for the soil amended with 0.5 g of June litter. This is in marked contrast to the results after 7 days.

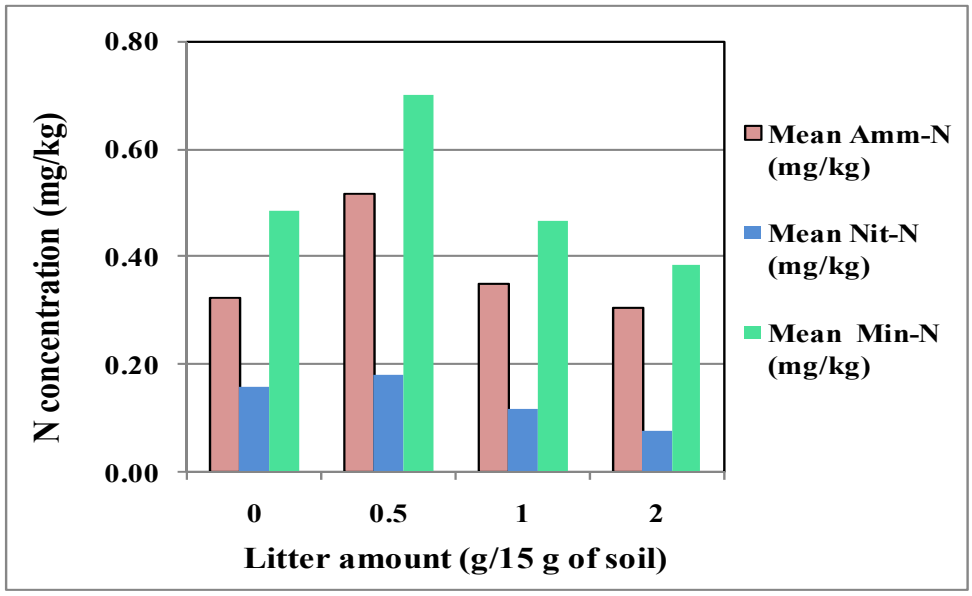


Fig. 6.3. Effect of litter treatments on mean ammonium-N, nitrate-N and mineral-N concentrations in soil compared with the controls after 14 days of incubation. The x axis shows the mass of the litter. There was no significant effect of litter sampling date or mass of litter used for any litter sampling date.

There were no significant differences attributable to either size of litter amendment or litter sampling date for either soil ammonium-N concentration or soil nitrate-N concentration after 14 days.

6.4 Discussion

6.4.1 Seasonal changes in litter C:N ratio

The change over time in the foliar C:N ratio was a significant decline over time, as hypothesised. It may be concluded then that, to a first approximation, the 3 "litter" samples subsequently used were reasonable starting materials for the purposes of the present experiment. It could be argued that concentrations of all major and trace nutrient elements could also have been varying between the 3 sets of litter (Marr and Cresser, 1983) and not just C:N ratio. The author, however, was of the opinion that this possibility was acceptable, and the approach he adopted was almost certainly more realistic than alternative experiments in which, for example, a selection of organic compounds with a range of C:N ratios was used instead of plant material. If litter could have been collected from along an N deposition gradient, this probably would not have given sufficient variation in C:N ratio to meet the needs of the experiment. In the present experiment, even the June "litter" had a C:N ratio as high as 25:1, and this was clearly high enough to favour mineral N immobilization.

An alternative in future experiments might be to use pot-grown species treated with variable rates of N fertilizer, and collect genuine, fallen, litter. Even that though might not be more appropriate, and indeed probably wouldn't work. In field experiments with fertilized cereal crops, for example, it has been shown that there is only a period of about two months during the growing season when plant tissue N concentration is significantly related to initial soil N supply (O'Neill et al., 1983 a and b), and by the time senescence sets in, any such relationship is lost. The purpose of the experiment was to assess the concept that plants have evolved naturally so that litter has a high C:N ratio to conserve N from litter over winter as it is first stored, then decomposes, in soil, and the author believes that his approach is the most appropriate starting point to test this idea.

6.4.2 Effects of treatments on extractable ammonium-N and nitrate-N in soil

As stated in the introduction, the aim of this chapter was to make a simple preliminary evaluation of 5 hypotheses, namely:

- There is naturally a dynamic match between plant N requirements and mineral N production in soil.
- Plant continued occurrence is favoured when this dynamic match is good (i.e. close dynamic match favours creation of an ecological niche).
- Diffuse N pollution could adversely affect biodiversity by inducing a dynamic mis-match between soil mineral N supply and plant N requirements.
- Deciduous litter therefore has evolved naturally, with a high C:N ratio, to help retain mineral N species produced during winter months when tree N uptake is low.
- As litter decomposes, its C:N ratio falls until microbial N immobilization is less favoured. Plant-available N then increases.

Each of these will now be briefly discussed in turn to assess to what extent each hypothesis is supported by the experimental data produced.

Hypothesis 1: There is naturally a dynamic match between plant N requirements and mineral N production in soil

This stated that there is naturally a dynamic match between plant N requirements and mineral N production in soil. Clearly this hypothesis was not comprehensively tested in this experiment. However, immobilization of a large part of the N mineralized from decomposing litter is a crucial prerequisite for this hypothesis to be true. The experiment provided very clear evidence for immobilization in the first week, especially for nitrate, and the extent of this is likely to be attributable to a large degree to the high litter C:N ratio, based upon the references cited in the introduction. That said, when the litter to soil ratio was higher it was clear that N retention was generally slightly less efficient. When however 2 g of the oldest (October) litter was used, it is interesting to observe that there was less extractable ammonium-N than for 2 g of the June and August litters. This supportive point might be countered by the point that 1 g of October litter gave higher extractable ammonium-N than either 1 g of June litter or 1 g of August litter. However, it must be realised that net ammonification is being

considered in this set of data, and clearly more ammonium could be being produced but subsequently more immobilized and re-immobilized for the 1 g of October litter.

It might be concluded that the results after 14 days incubation do not support the hypothesis. Unfortunately no low temperature incubation facility was available to the author and Claire Stephens, so the preliminary experiment had to be performed at room temperature. This was around 18-20˚C, in spite of the fact that windows were left open all day to try to keep temperature low. If I was performing further experiments on this topic, I would undoubtedly use ambient outdoor temperatures and work on a far larger scale with sampling intervals being more frequent over winter months. Suffice to say here that the high levels of ammonification and nitrification at this sort of temperature in the second 7 days actually support the idea that as soils warm up, eventually bio-available mineral-N starts to be produced in the soil.

Hypothesis 2: Plant continued occurrence is favoured when this dynamic match is good (i.e. close dynamic match favours creation of an ecological niche)

This hypothesis was that plant continued occurrence is favoured when the dynamic match between N supply and N requirement is close (i.e. close dynamic match favours creation of an ecological niche). Clearly this must remain speculative, since in this preliminary evaluation, no attempt was made to assess likely dynamics of plant N uptake. At least it is possible to say that the experiment provided evidence that under the warm conditions likely to stimulate plant growth mineral-N would become more available.

Hypothesis 3: Diffuse N pollution could adversely affect biodiversity by inducing a dynamic mis-match between soil mineral N supply and plant N requirements

Diffuse N pollution could adversely affect biodiversity by inducing a dynamic mis-match between soil mineral N supply and plant N requirements, must again remain speculative. Further experiments, involving external ammonium-N or nitrate-N additions and under more realistic temperature regimes, would be needed to test the idea, to see if mineral-N became more available earlier in areas of high N deposition. If it did, this could clearly provide a more competitive edge to plant species that came into active growth earlier in the year. Longer term experiments with mixed plant

communities would be needed to show that the outcome was biodiversity change however.

Hypothesis 4: Deciduous litter therefore has evolved naturally, with a high C:N ratio, to help retain mineral N species produced during winter months when tree N uptake is low

It was hypothesised that deciduous litter has evolved naturally, with a high C:N ratio, to help retain mineral N species produced during winter months when tree N uptake is low. Although litter does indeed clearly immobilize mineral N, the dynamics are more complex than the author initially envisaged. Figure 6.4 shows the raw data for the triplicate nitrate-N concentration determinations for all the foliar samples ("litter") collected in June, August and October. It was hoped that less nitrate would be found for the October litter, which had the highest C:N ratio, because of greater immobilization. However, this was only true when 2 g of litter was added to 15 g of soil. Under those conditions the October litter gave the lowest nitrate-N concentration, but, on the other hand, the August litter gave a higher concentration than the June litter. When 1 g of litter was used, it appeared that June litter was most efficient at immobilizing nitrate-N. However, this merely serves to indicate the complexity of the system, since only net nitrate-N production could be measured in this preliminary study. The possibility cannot be ruled out that if the June litter is more readily degradable because of its more favourable C:N ratio, the litter will be colonized more rapidly, giving a greater biomass to potentially immobilize N. Unfortunately biomass C and N were not measured in this experiment.

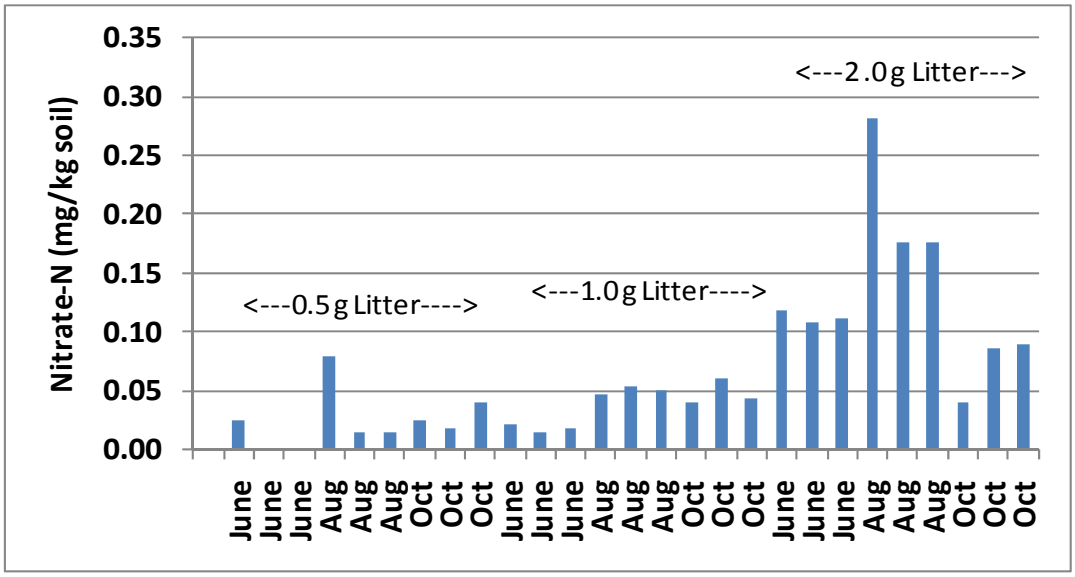


Fig. 6.4 Raw data after 7 days of incubation, grouped according to litter amount and as triplicates for each litter sampling date (plotted as sampling month).

Although the N immobilization effect of litter is very clear overall therefore, the hope that October litter with the highest C:N ratio would immobilise N from the native pool of N in the soil and from litter early mineralization more effectively than August and June litters was optimistic. The importance of litter-to-soil ratio is perhaps clearest of all data are presented in a different format for the 7-day incubation. This has been done in Fig. 6.5. It is clear that as litter mass is increased there is a tendency towards greater production of mineral-N (here as nitrate-N) out-competing mineral-N immobilization.

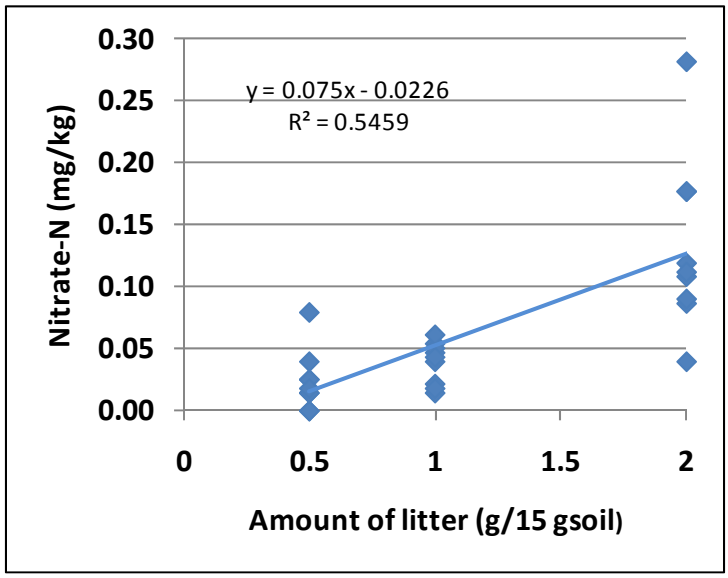


Fig. 6.5 Effect of sample mass, using all data regardless of leaf sampling date, on nitrate-N concentration after 7 days.

Hypothesis 5: As litter decomposes, its C:N ratio falls until microbial N immobilization is less favoured. Plant-available N then increases

Direct evidence to support the hypothesis that, as litter decomposes, its C:N ratio falls until microbial N immobilization is less favoured is not attainable directly from this experimental design. In future experiments litter contained in litter bags should be used to test this hypothesis directly, to study how plant-available N increases as litter C:N ratio falls. However, it may be concluded, as mineralized soil and litter N were so readily immobilized, that there must have been at least a small decrease in the system C:N ratio as decomposition progressed.

6.5 Conclusions

This preliminary experiment, run at room temperature, showed dramatic mineral N immobilization by litter initially, favouring mineral N retention. However, it also showed strong competition between mineral N species production rates and immobilization rates at the temperature used. More experiments are needed over longer time scales and at more appropriately seasonal temperatures to properly test the hypotheses further. One aspect that emerged very strongly was the role of litter in nitrate immobilization in soils in winter. This will be investigated further in a later chapter and in a paper by the author (Mian et al., 2010a) on possible causes of changes in seasonal trends in nitrate concentrations over 20 years in the River Derwent in North Yorkshire. This is thought to be especially important as it seems likely that ammonium-N is more mobile than previously thought, as indicated in Chapter 5 (Mian et al., 2009).

In 1992, straw and stubble burning was banned in UK, resulting in incorporation of litter into soil post 1993. In Chapter 8 the author will investigate whether this led to a reduction in seasonal peaks in winter nitrate-N concentrations in the River Derwent in North Yorkshire.

CHAPTER 7: HOW STABLE ARE SOILS FOR THE DETERMINATION OF AVAILABLE N?

7.1 Introduction

The assessment of soil nitrogen availability has long been a topic of great interest in both agricultural and natural environmental contexts, and over many years much effort has gone into evaluating procedures for assessing N availability (Bremner, 1965; Keeney and Bremner, 1966; Fox and Piekielek, 1978).

Typically, ca. 95% of the nitrogen in soils is present in the form of organic nitrogen, almost totally in the soil solid phase and/or in biomass. In the absence of recent fertilizer or pollution inputs, only ca. 5% of the total N or less is present in inorganic forms following organic-N mineralization to ammonium, nitrite or nitrate. Most of the ammonium produced will be held as the NH_4^+ cation on negatively charged cation-exchange sites in the soil, whereas nitrate (NO_3^-) is relatively mobile in soil solution and drainage water. To assess the amounts of N in these inorganic, bio-available forms, either soil solution is extracted directly (e.g. Ross and Bartlett, 1990) or fresh, field moist soil is extracted with potassium chloride solution (Bremner, 1965).

It is well known that drying and rewetting of soils modifies the composition of soil solutions (e.g. Walworth, 1992) and Chapters 3 and 4 of this thesis and references therein. It is therefore generally claimed that the determination of extractable ammonium and nitrate should be conducted on fresh, field-moist soils if the results are to be meaningful (Bremner, 1965), because if the soil is dried and rewetted a surge of mineralization may occur (Bremner, 1965; Fierer et al., 2003; Gordon et al., 2008; Mian et al., 2008). Recently, though, Mian et al. (2008) and Chapter 4 demonstrated that the first flush of nitrate from rewetted soil after 6 days of drying was stored nitrate, and that it took a few days for new nitrate production by microbial activity, possibly supporting the idea that nitrate might be better determined in rapidly air-dried soil. Westfall et al. (1978) suggested drying within 12 hours of collection as an acceptable compromise when measuring extractable nitrate as a basis for fertilizer recommendations, as changes in concentration would be small.

Once soil is removed from the plant root environment, plant uptake of inorganic N species is eliminated, so mineralized N species may start to accumulate in the moist soil, or N species may be immobilized microbially or chemically. It has long been recognized that how, and how long, soils are handled prior to analysis may have a large impact upon the concentrations of extractable mineral N species found. For example,

nitrate concentrations over seven days increased dramatically in seven soils when incubated at 30°C, stored at room temperature or left on a sunny window ledge, and by almost 50% even when they were refrigerated (Westfall et al., 1978). Ross and Bartlett (1990) observed large increases in nitrate concentrations in soil solutions from forested spodosols within 24 h when soils were stored in a refrigerator at 3°C. Bremner (1965) reviewed early studies which demonstrated that even rapid freezing of stored soil samples at -15°C could result in significant changes in concentrations of subsequently extracted mineral N species.

For results to be comparable between soils and between sampling dates, the results must be expressed on an oven-dry soil mass basis. Therefore while one sub-sample of a fresh moist soil is being extracted with molar KCl, a second, weighed sub-sample of moist soil is oven dried at 105°C so that the amounts of extractable ammonium-N and nitrate-N per kg of oven dry soil may be calculated. Correction must be made for the moisture present in the soil, which will slightly dilute the KCl extract. It was decided, in the interests of future researches and because of the time it take to process large numbers of samples to study how stable mineral-N concentratons are in field moist soils.

The purpose of this chapter is to assess how important it is to use really freshly sampled soils if extractable ammonium and nitrate are determined on field moist soils. To do this on 12 soil samples, 12 pairs of experienced, but closely supervised, undergraduate students were each asked to collect and analyse a soil sample in duplicate so that all the soils could be prepared, and extraction commenced, within 30 minutes of collection from the field. Sufficient soil was collected for each pair to start to extract subsamples of the fresh soil and measure soil moisture content within this time frame, and also to store further subsamples in a refrigerator or left out at room temperature overnight prior to extraction, to test the hypotheses that any changes seen would be small after refrigerated storage, but more significant after room temperature storage. Very little seems to have been published on the need for speed of analysis, which is difficult to achieve in practice when a substantial number of samples is to be analysed.

7.2 Materials and methods

7.2.1 Field Sampling

To ensure that samples could be extracted as rapidly as possible after collection, three permanent grassland sites and three deciduous woodland sites were selected immediately adjacent to the University of York campus. Twelve pairs of closely supervised students each collected duplicate samples of soil, each about 1 kg, with stainless steel trowels from 0-6 cm or 6 – 12 cm depths below the surface at their allocated site. As much vegetation as possible and any obvious stones were removed at the time of sampling by spreading briefly on clean polythene sheets. The samples were then placed in clean, labelled polythene bags for transport back to the laboratory. At one grassland site the root growth and branching was so extensive that rapid sampling near the surface proved impractical, so this soil was sampled instead at 10-20 and 20-30 cm depths.

7.2.2 Sample Preparation and Analysis

Back in the laboratory, the soil was again spread on polythene sheets, and any remaining obvious roots or stones were removed as quickly as possible and each sample was thoroughly mixed (ca. 10 minutes for all samples). Immediately 10-g sub-samples of each soil were weighed in duplicate into pre-weighed and labelled foil dishes, and two further 10-g sub-samples weighed in duplicate into labelled 120-ml plastic bottles. The soils in foil dishes were placed into an oven at 105°C to dry for at least 8 h, cooled in desiccators and reweighed to quantify the moisture contents of the field moist soils. To the subsamples in plastic bottles 100 ml of 0.5M KCl was added, and the bottles were capped and shaken intermittently for 1 h. The mixtures were then filtered through Whatman No. 42 papers into clean, dry labelled bottles, and the extracts were stored in a refrigerator until the following day. Duplicate KCl blanks were also filtered so that a blank correction could be applied.

Each of the residual soil samples was divided into two subsamples and each was transferred to a pre-labelled sealed polythene bag. One set of samples was stored overnight at room temperature, and the other set was transferred immediately to a refrigerator for storage at 2-4 °C overnight. The following morning, 16 h later, duplicate 10-g sub-samples from all samples from both sets of storage conditions were extracted with 100 ml portions of 0.5M KCl as described above for the fresh samples.

7.2.3 Determination of N Species

The concentrations of ammonium-N and nitrate-plus-nitrite-N in the soil extracts were determined by automated colorimetric analysis, using a standard Bran and Luebbe AutoAnalyzer®-3 manifold and matrix-matched standards. The masses of ammonium-N and nitrate-N in 100 ml of KCl extract plus the volume (ml) of soil water (calculated from the loss in weight of separate 10-g sub-samples that had been oven dried) were calculated, and used to quantify the mass of each N species per kg of oven dry soil.

7.3 Results

The amounts of ammonium-N, nitrate-plus-nitrite N, and total mineral-N, were calculated in mg per kg of oven-dry soil, for fresh field-moist soil, for moist soil after storage for ca. 16 h in a refrigerator, and for moist soil after storage for ca. 16 h at room temperature. Figures 7.1 and 7.2 show the effects of storage conditions on the N species results for the three grassland soils for the upper and lower soil layers respectively; Figures 7.3 and 7.4 show the corresponding results for the three sets of mixed deciduous woodland soils.

7.3.1 Differences in nitrate-plus-nitrite-N concentrations with storage conditions

For grassland surface soils, storage in a refrigerator for 16 h prior to extraction had no significant effect upon nitrate-plus-nitrite-N concentration, but significantly enhanced its concentration in all three grassland sub-soil samples. Storage for 16 h at room temperature caused no further statistically significant enhancement compared with refrigerated storage for these three sub-soils (Fig. 7.2) or for the surface soils however (Fig. 7.1). On the other hand, for G2 and G3 near-surface soils, room temperature storage gave higher nitrate-plus-nitrite concentration than immediate extraction (Fig. 7.1). It should be remembered that G1 was only sampled from below the surface at 10-20 cm rather than at 0-6 cm, and it behaves like the grassland sub-soil with no significant difference between refrigerated and room temperature storage (Figs. 7.1 and 7.2). For the woodland soils the variation in nitrate-plus-nitrite-N concentration was higher, and changing storage conditions therefore had no statistically significant effect on the results (Figs. 7.3 and 7.4).

7.3.2 Differences in ammonium-N concentrations with storage conditions

Refrigerated storage for 16 h prior to extraction had no significant effect upon ammonium-N concentration for grassland surface soils, nor did it significantly change its concentration in all three grassland sub-soil samples. Although ammonium-N concentrations generally declined slightly during storage of the grassland soils at room temperature for 16 h, there was no statistically significant change compared with refrigerated storage for the three sub-soils (Fig. 7.2) or for two of the near-surface soils, an exception being G3 from 0-6 cm (Fig.7.1).

For the woodland soils, although ammonium-N concentration generally fell following storage, the effect was not statistically significant for the near-surface soils (Fig. 7.3) or for the W3 sub-soil regardless of storage temperature. However, the decline following storage prior to extraction was significant at $P<0.05$ for woodland sub-soils W1 and W2 (Fig. 7.4). There was no effect of storage temperature for these two soils, however.

7.3.3 Differences in total mineral-N concentrations with storage conditions

For woodland near-surface soils, moist soil storage in a refrigerator or at room temperature for 16 h before extraction had no significant effect upon mineral-N concentration for 5 of the 6 soils, but significantly reduced mineral-N concentration in one woodland lower layer soil. For this soil there was no statistically significant change after room temperature storage compared with refrigerated storage (Fig. 7.4).

7.3.4 Differences in N species concentrations with depth

For all soils, nitrate-plus-nitrite concentrations were significantly ($P<0.05$) higher closer to the surface, However ammonium-N did not vary significantly with depth.

7.3.5 Differences in N species concentrations between sites

Nitrate-plus-nitrite-N concentrations were significantly higher in the grassland soils than in the woodland soils at both depths. Although the ammonium concentrations looked slightly higher in the woodland soils, an independent-t test showed that the difference was not significant.

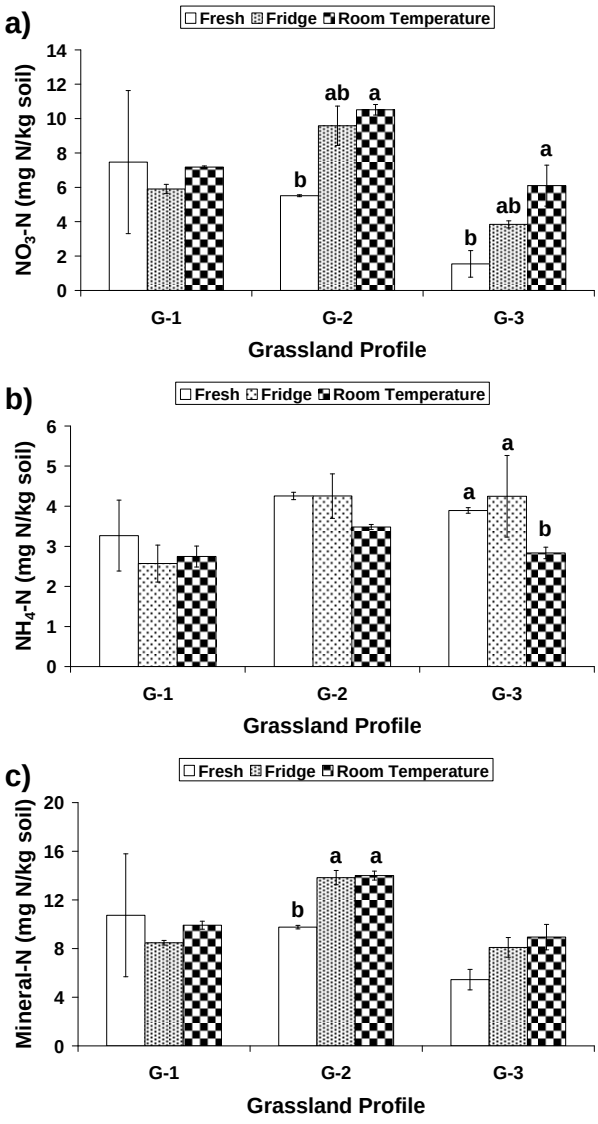


Figure 7.1: Changes in a) NO_3-N, b) NH_4-N and c) Mineral-N (mg N/kg soil) at 0-6 cm depth (except G-1 where depth was 10-20 cm) in 3 grassland profiles. All values are means of duplicate soil samples. Error bars are standard error of means. Bars with different letters differ significantly at $P < 0.05$.

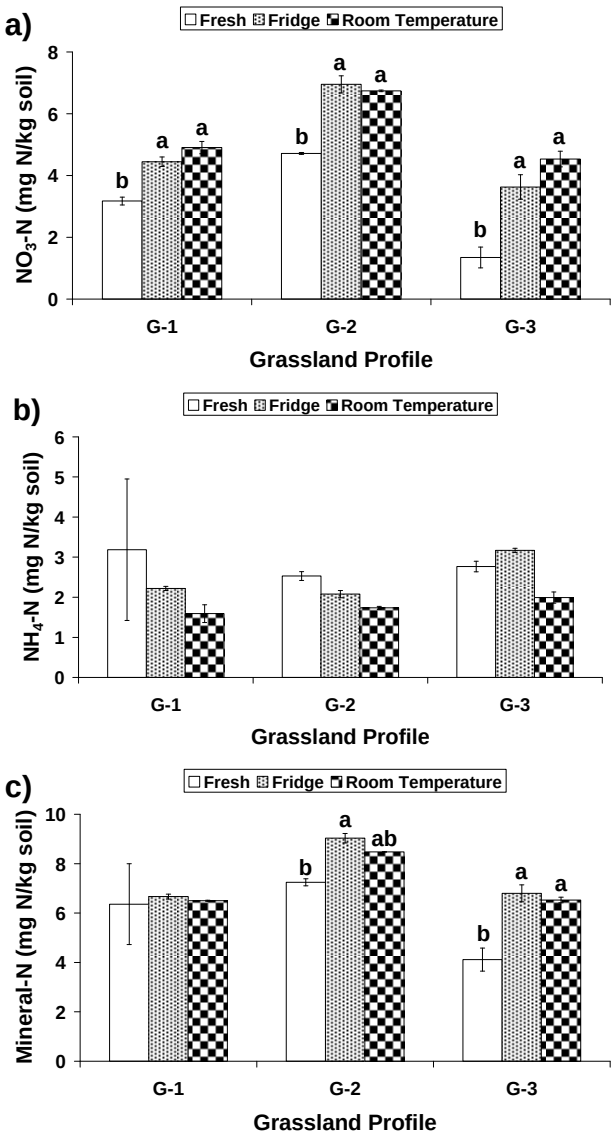


Figure7.2: Changes in a) NO_3-N, b) NH_4-N and c) Mineral-N (mg N/kg soil) at 6-12 cm depth (except G-1 where depth was 20-30 cm) in 3 grassland profiles. All values are means of duplicate soil samples. Error bars are standard error of means. Bars with different letters differs significantly at $P<0.05$.

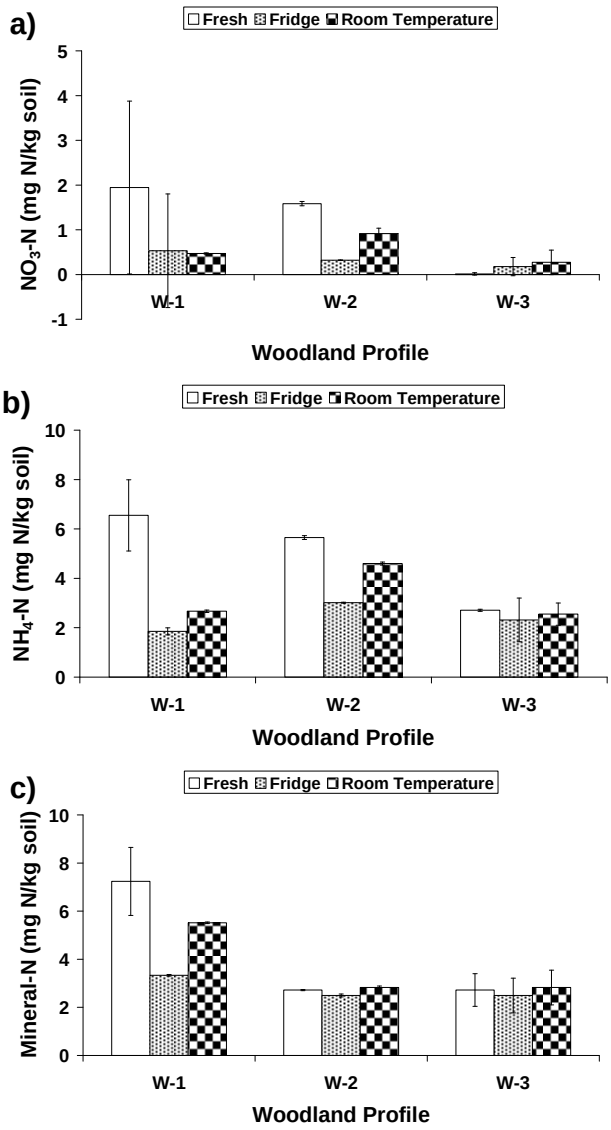


Figure7.3: Changes in a) NO_3-N, b) NH_4-N and c) Mineral-N (mg N/kg soil) at 0-6 cm depth in 3 woodland profiles. All values are means of duplicate soil samples. Error bars are standard error of means. Bars with different letters differ significantly at $P<0.05$.

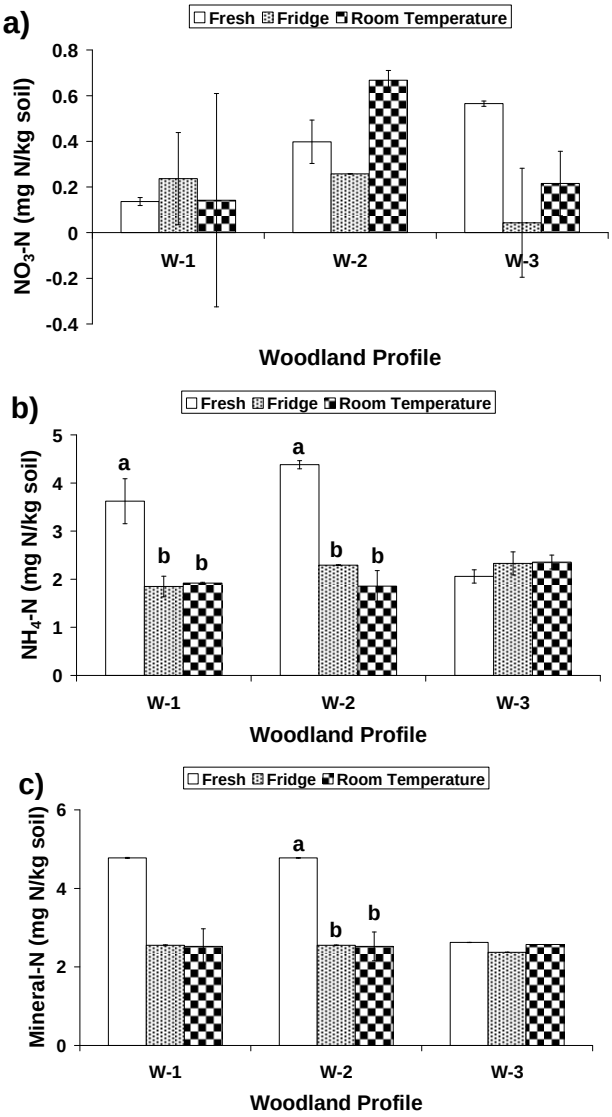


Figure 7.4: Changes in a) NO_3-N, b) NH_4-N and c) Mineral-N (mg N/kg soil) at 6-12 cm depth in 3 woodland profiles. All values are means of duplicate soil samples. Error bars are standard error of means. Bars with different letters differ significantly at $P<0.05$.

7.4 Discussions

The effect of soil storage conditions

7.4.1 Grassland soils

For the near-surface grassland soils stored in a refrigerator, an apparent nitrification effect on nitrate-plus-nitrite-N concentration for G2 or for G3 was not significant. However, the effect became statistically significant for all three of the lower horizon soils under refrigerated storage for 16 h (Fig. 7.1). This probably reflects a higher population of nitrifiers or more favourable conditions for nitrification in the lower soil layers. Changes in the nitrate concentrations found after storage represent the combined overall effect of nitrification and microbial immobilization, so it is also possible that immobilization may be higher in the soil nearer the surface which visibly appeared at the time of sampling to have a higher density of roots.

Storage at room temperature overnight compared with refrigerated storage did not cause any significant additional enhancement in nitrate-plus-nitrite-N concentration for any of the grassland soils (Figs. 7.1 and 7.2). However, for G3 surface soil from 0-6 cm, the significant decline in ammonium during room temperature storage compared with refrigerated storage could be indicative of enhanced nitrification, perhaps combined with immobilization.

In the near-surface grassland soils, total mineral N was significantly enhanced by 16-h storage only for G2, and to the same extent regardless of storage temperature. This reflects the nitrification during storage in this soil, although the enhancement in nitrate-plus-nitrite-N concentration was significant only after storage at room temperature (Fig. 7.1). Total mineral N increased during storage in a refrigerator in two of the lower layer grassland soils (G2 + G3, Fig 7.2), again reflecting the consistently significant nitrification in the lower layer soils. It is noticeable in Fig. 7.2 that nitrification induced enhancement was greater for these G2 and G3 soils. Storage at room temperature compared to refrigerated storage caused no further significant enhancement in total mineral N.

7.4.2 Woodland soils

The woodland soils behaved differently from the grassland soils, with no significant net nitrification effect during storage for either near-surface or lower-layer soils during either refrigerated storage or room temperature storage. Following ideas from Chapter

6, however, it is possible, looking at the consistent decline in NO_3-N concentration (Fig. 7.3) that immobilization exceeds nitrate production. The KCl-extractable ammonium decreased significantly for the W1 + W2 lower-layer soils, probably via microbial immobilization of N because of the lack of any evidence for significant nitrification. Other studies in the author's laboratory have clearly shown significant immobilization of ammonium during incubation of grassland soils at ambient outdoor winter temperatures (Bhatti and Cresser, unpublished results). This was attributed to elimination of atmospheric pollutant N inputs during incubation, which shifts the relative dynamics of N species transformation processes.

7.4.3 The factors likely to be regulating the amounts of N species found

In this experiment, pairs of trained undergraduate students were used to speed up the fresh field moist soil extraction process as much as possible. In reality, often individual workers bring substantial numbers of soils back to their laboratory in cool boxes, and analyse soils as quickly as possible on the same day or after refrigerated over-night storage. These experiments have shown that modest, but statistically significant, amounts of nitrification may occur during 16 h of refrigerated storage for grassland sub-soils, and perhaps even more surprisingly that refrigeration did not improve the sample stability significantly compared to storage for 16 h at room temperature. Remaining visible roots and stones were removed as quickly as possible in the laboratory by hand sorting prior to analysis and/or storage. There can be little doubt that, during this period of about 10 minutes, the soil warms up significantly. Although the soils were then immediately returned to the refrigerator, cooling would have taken a significantly longer time, and much of the nitrification may have occurred during this phase. This may be facilitated by the thorough mixing of the soils, again by hand, prior to sub-sampling, which could redistribute pockets of higher ammonium concentration and the population of nitrifiers.

There was evidence in this experiment of ammonium immobilization in two woodland lower-layer soil samples. This too occurred to similar extents during refrigerated storage and room-temperature storage. As mentioned above, other studies have demonstrated significant immobilization of ammonium during incubation of soils at ambient outdoor winter temperatures, attributed to removal of atmospheric pollution ammonium- and ammonia-N inputs (Bhatti and Cresser, unpublished results). These were shown to exceed in-situ ammonium production by mineralisation of soil organic N

at winter temperatures. This would explain why ammonium-N immobilization is favoured in these forest soils even at low temperature, especially as any forest litter brought down into the mineral soil by soil fauna has a high C:N ratio, which would again favour N immobilization. Early work on ammonium-N immobilization, and the difficulty it presents for assessing available mineral N in soils, has been thoroughly discussed by Bremner (1965).

7.5 Conclusions

The results highlight both practical constraints in, and more general limitations of, operationally-defined procedures for assessing the amounts of available N in soils**.** The main practical constraint is the speed with which samples need to be prepared and extracted, which is a potentially serious problem if a substantial number of samples is returned to the laboratory in a cool box for subsequent extraction and N species determination. It appears that the most reliable approach might be to sample soil by volume in the field and add the measured volume of soil to a known volume of molar KCl solution, bringing other sub-samples of moist soil back to the lab to measure moisture content. This is currently being investigated by Bean, an MSc student in the author's department. Refrigerated storage, either during transit or in the laboratory, will not be sufficient to stop N speciation change, as other workers have found (Westfall et al., 1978; Ross and Bartlett, 1990). Moreover, the extent of change will vary from soil to soil, depending upon land use and probably a range of soil characteristics.

The N cycle is complex, because it involves interactions of anthropogenic and soil-derived N species inputs, their transformations in soil, and the dynamics of plant N uptake. Often too it involves very dynamic soil hydrological conditions. This brings into question how useful operationally defined procedures as commonly practised really are for assessing bioavailability, or potential bioavailability, of N. Measuring potentially mineralizable N (Haney et al., 2008; Kadono, 2009; Nyiraneza et al., 2009) or extractable organic N (MacLean, 1964; Stanford, 1968; Michrina et al., 1982) are probably more meaningful approaches. On the other hand, with careful randomization of sample handling time experiments such as those conducted by the author in earlier chapters are still valid for providing quantitative indicators of relative treatment effects.

CHAPTER 8: SPATIAL AND LONG-TERM TEMPORAL TRENDS IN NITRATE CONCENTRATIONS IN THE RIVER DERWENT NORTH YORKSHIRE AND ITS NEED FOR NVZ STATUS

8.1 Introduction

Although ammonium-N leaching has been shown in this thesis to be significant for soils from Hob Moor, nitrate leaching is more widely seen as a potential problem in both fertilized and semi-natural, minimally managed, soils. Since the concept of nitrogen saturation of soils was introduced by Aber et al. (1989) there has been growing concern in areas not impacted directly by fertilizer use about the long-term trends in nitrate concentration in surface waters. Tipping et al. (2008) pointed out that steep slopes, areas of outcropping or bare rock and limited soil depth, together with lower plant N-uptake, facilitate N leaching in upland catchments in the UK and northern Europe that are subject to high levels of N deposition; however, they reported that no consistent pattern is being shown in time-series trend plots for sensitive areas. They supported this statement by reference to the conclusions in the comprehensive study by Stoddard et al. (1999) for the period from 1980-1995 for sites across N. America and Europe, and in a later study by Evans and Monteith (2001), based upon a similar geographical area, which found no change for 35 of 56 sites, but either increases or decreases in the remainder. Assembling data collected over 50 years, Tipping et al. (2008) were able, however, to demonstrate highly significant trends for increasing nitrate concentration over time ($P<0.001$) for two lakes in Cumbria in N.W. England. They also demonstrated that the nitrate fluxes from feeder streams were significantly ($P<0.02$) correlated to the area of rankers soil in the stream catchments; they concluded that these soils were responsible for the increase in nitrate over time because of the limited capacity of the highly organic soils to retain atmospheric N inputs, and that nitrate leaching from the other dominant soil type, brown podzols, was not significant.

It has been known for fifteen years that peat soils in areas with even quite modest N deposition may have a very limited capacity to retain nitrate when compared with adjacent podzols, so stream-water nitrate concentrations increase in streams wherever peat is extensive in the adjacent riparian zone (Black et al., 1993). Thus limited abundance of peat soils and rankers may be one factor influencing the lack of significant increases in nitrate concentration over time in some semi-natural catchments.

Another factor may be the time taken for soils to become N saturated. This is notoriously difficult to predict because of the complexity of predicting the spatial variation in soil properties, especially those related to soil N concentration, at a landscape scale (Crowe et al., 2004).

One of the key factors controlling whether or not atmospheric N deposition is retained within the soils of a catchment is the rate at which any incoming N species (ammonia, ammonium and/or nitrate) is converted to other forms. Cresser et al. (2004) used laboratory incubation experiments to demonstrate that ammonium inputs to heavily N-impacted organic soils in the UK were transformed to organic N or nitrate very slowly. As a consequence ammonium could accumulate on the cation exchange sites until equilibrium was attained. At this point ammonium leaching may start to occur, and ammonium is found in adjacent surface waters (Cresser et al., 2004). The same paper also demonstrated that nitrate transformations were very slow in acidified organic soils, making nitrate susceptible to leaching, especially during storm events, even over the summer months.

Recently Riaz et al. (2008) have been testing the hypothesis that if ammonium is now leaching to adjacent freshwaters in heavily N-impacted areas, then it is likely to be leaching down soil profiles too at many sites. It may thus pass below the rooting zone to lower soil horizons, and subsequently be nitrified at depth in the soil profile thus becoming susceptible to leaching to ground water and/or to surface waters as a ground water end member. It was suggested here and in Chapter 5 that this mechanism may further enhance nitrate concentrations in streams draining acid grasslands.

More recently, there has been growing concern too about nitrate contamination of both surface waters and ground waters from agricultural practices. This led, in 2002, to substantial regions of the UK being declared Nitrate Vulnerable Zones (NVZ), largely in response to the European Implementation of Nitrates Directive 91/676/EEC. The subsequent legislation limited quantities of fertilizer and manure that could be applied and the times of year over which applications could be made, as well as regulating manure storage conditions and amounts and spreading procedures. In agricultural catchments this would be expected to lead to a relatively rapid beneficial response in late autumn/winter nitrate concentration peaks, as well as a possible slower (and less dramatic) response as the riparian zone soil N cycle changed over several years.

Changes in land use and management apart from changes in fertilizer application may have a strong impact upon nitrate concentrations in river waters over longer

timescales. Tetzlaff et al. (2007), for example, studied changes in water chemistry over 26 years in two small streams at the Loch Ard catchment in central Scotland. In one, partial clear felling of the Sitka Spruce (*Picea sitchensis*) resulted in a rapid ca. 6-fold increase in nitrate concentration, and the effect was still noticeable (though at a reduced level) several years later. In the other, clear felling had a much smaller effect, because the felling was in smaller areas and brash was collected and removed. From a study of 14 catchments distributed across the UK, Gagkas et al. (2008) concluded that, under high flow conditions, nitrate concentration was significantly correlated with the percentage broadleaf woodland cover. Thus any change in woodland cover within the time frame of a time series plot should be taken into account when interpreting long-term trends, especially if the woodlands are in, or close to, the riparian zone. For rivers in the Humber Basin, including the Derwent which is considered in detail in this study, Davies and Neal (2004) showed a significant negative curvilinear relationship between nitrate concentration and the percentage upland semi-grass cover, though this could simply reflect both the higher point source N pollution inputs from urban areas and diffuse and point source pollution from arable agriculture where they dominate over upland grassland. Suffice it to say here that even quite small increases in urban development may mask potential long-term trends attributable to pollution impacts. Koo and O'Connell (2006) demonstrated the importance of using % arable, grassland and woodland data when modelling nitrate concentrations in the River Kennet in southern England with a view to designing a management strategy for the chalk catchment.

There is a strong tendency to consider the biogeochemical cycling of nitrogen in isolation in the context of assessing pollution impacts, except perhaps occasionally in the context of soil and freshwater acidification (Stoddard et al., 1999) and interactions with the carbon cycle. However, the strong pH dependence of many steps in the nitrogen cycle should not be overlooked. Especially when a liming subsidy was available to UK hill farmers, large amounts of lime were applied to British uplands (Goulding and Black, 1998). These undoubtedly would have resulted in the mineralization of very large quantities of organic matter within previously more acidic upland soils, leading, via ammonification and nitrification, to nitrate production and transfer to surface waters and ground waters. The effects of liming are slow where mixing by soil fauna is limited, and it may take several years for liming to take effect even a few centimetres below the surface. Conversely, return to more acidic conditions is also likely to be slow, which could result in a slow decline in nitrate concentrations in

associated streams over a decade or longer. In arable agricultural areas, nitrate inputs are invariably associated with applications of nitrogen fertilizers by most authors, and the effects of liming to optimise soil pH for agriculture are generally ignored. However, with the current expansion of the use of organic manures and composts in organic agriculture, this should probably not be the case in future.

It also may be advisable to consider more closely the use of composts and lime in urban gardens in some areas, alongside the use of nitrogenous fertilizers by gardeners. The risk of over-watering to prevent scorching of lawns if weather remains too dry is common practice in the author's experience. Moreover the water being applied is frequently tap water, which may itself contain much higher concentrations of nitrate than rain water. This nitrate, with use of a sprinkler, may have inadequate time to be taken up by biomass before passing below the rooting zone and on to ground water.

Very recently it has also been demonstrated that use of road salt in winter may seriously disrupt the nitrogen cycling in soils down slope of roads, again predominantly via the increase in soil pH that it causes (Green and Cresser, 2008 a and 2008b; Green et al., 2008). The pH of organic-rich upland soils may increase by as much as 2 pH units, resulting in dramatic decreases in soil organic matter content. The pH shift is caused by cation exchange, with retention of Na^{+}, Ca^{2+} and Mg^{2+} from the road salt and loss of ammonium and H^{+}. It was shown that the enhanced mineralization and nitrification especially can cause a highly significant increase in nitrate concentrations in rivers down slope of, and running parallel to roads (Green and Cresser, 2008a). The consequences of ammonium displacement by road salt depend upon the drainage patterns in the catchment.

A further confounding factor when attempting to interpret long-term trends found for nitrate concentrations in river water is the influence of ground water draining from permeable bedrock. This has been eloquently discussed by Neal et al. (2006), when they were considering the temporal trends in, and influence of discharge upon, nitrate concentrations in tributaries of the upper Thames. Jackson et al. (2008) have warned that the delay in response of nitrate concentrations in ground waters to current agricultural practices may mean that further rises may be unavoidable even if the most stringent constraints are imposed upon agriculture. Wade et al. (2006) found it difficult to decide which was most robust between a model in which nitrate concentration was controlled by soil- and ground-water inputs, and one in which in-stream processes were important. Another crucial factor is the strong positive curvilinear relationship often

found linking nitrate concentration to discharge, as reported by Jarvie et al. (2005) for the River Avon tributaries in Hampshire in southern England.

Several studies have noted the strong seasonal trend in nitrate concentrations in surface waters, with nitrate concentrations peaking in winter and falling, sometimes to values below the detection limit, in summer when biotic uptake is maximal (e.g. Johnes and Burt, 1993; NEGTAP, 2001; Smart et al., 2005; Neal et al., 2006; Tetzlaff et al., 2007; Tipping et al., 2008). Indeed models are available for predicting such seasonal variation (Casey and Clarke, 1979; Walling and Webb, 1984; Burt et al. 1988; Smart et al., 2005).

Based upon this review of the literature, the author hypothesises that a consequence of N saturation of minimally managed soils often associated with headwater catchment areas is likely to be a shift in summer minima to higher values as a consequence of N saturation. Such a shift should be detectable visibly in long-term (decadal) time series plot data. In more agricultural areas it might be anticipated that peaks in nitrate concentrations in late autumn/winter would decline as more efficient use is made of N fertilizers and manures through growing farmers' awareness of seasonal losses and high energy and fertilizer costs over recent years. The dramatic effects of litter on nitrate-N retention, as reported in Chapter 5, could also mean that the ban on straw and stubble burning introduced in England and Wales late in 1992 (Turley et al., 2003) would have reduced winter nitrate leaching. An early study a year after the ban, using porous cup tension lysimeters, confirmed the feasibility of this idea (Nicholson et al., 1997).

It was decided to use a 20-year run of data for 9 sampling sites along the River Derwent in North Yorkshire to test the following hypotheses, while bearing in mind all the potential causes of spatial and temporal variation in nitrate concentration highlighted earlier. It is hypothesised that (1) there would be a tendency for nitrate concentration to increase relatively (i.e. in % terms) over time most markedly at sites in which the land use was predominantly moorland and rough, unimproved grazing, because in areas with more arable agriculture farmers would automatically tend to be applying less nitrogen fertilizer and/or manure than hitherto to meet crop requirements; and (2) in more agriculturally-impacted zones of the catchment, nitrate concentrations would be higher overall than in more upland areas, but winter maxima should have become less pronounced over time, especially post 1993, reflecting the ban on straw and stubble burning, and again post 2002, reflecting both legislative impacts and growing awareness

of the nitrate pollution issue, increased use of buffer strips, better timing of manure and fertilizer applications, and higher fertilizer prices over recent years.

However, further factors may need to be considered for the Derwent. The catchment is susceptible to occasional flooding in places, and has flood plains that may be regularly inundated. After such occurrences nitrate concentrations may fall as a consequence of denitrification in soil and/or the flood sedimentary deposits (Forshay and Stanley, 2005). Moreover, even before the introduction of NVZ status to part of the catchment in 2002, the outbreak of Foot and Mouth disease in 2001 led to the slaughter of many animals in North Yorkshire and the East Riding of Yorkshire, that might have had a beneficial impact by reducing the manure load in the area, and hence nitrate leaching to surface waters.

8.2 Methods

Data were provided by the Environment Agency for nitrate-N concentrations for 9 sites along the River Derwent in North Yorkshire in England. The site locations are shown in Fig. 8.1. GIS was used to calculate the area and the land cover distribution of the catchment upstream of each sampling point. The land cover data used was the CEH 1990 data. To avoid having too many classes and making interpretation unnecessarily complex, several of the original CEH land cover classes present were combined for simplicity. Grass moor, Open dwarf shrub moor, Dense dwarf shrub moor, Bracken, Scrub/orchard, Upland bog, Inland bare ground, Lowland bog and Open dwarf shrub heath were combined to give a land use class termed “Moorland”. Grass heath, Mown/grazed turf and Meadow/verge/semi-natural were classed as “Grassland”. Suburban/rural development and Continuous urban were termed “Urban/infrastructure”. The small amount of Felled forest was incorporated into Evergreen woodland as “Evergreen woodland”. “Deciduous mixed woodland” and “Fresh water” were as in the original data set. A small area was “Unclassified”. Thus 8 classes were used for this assessment.

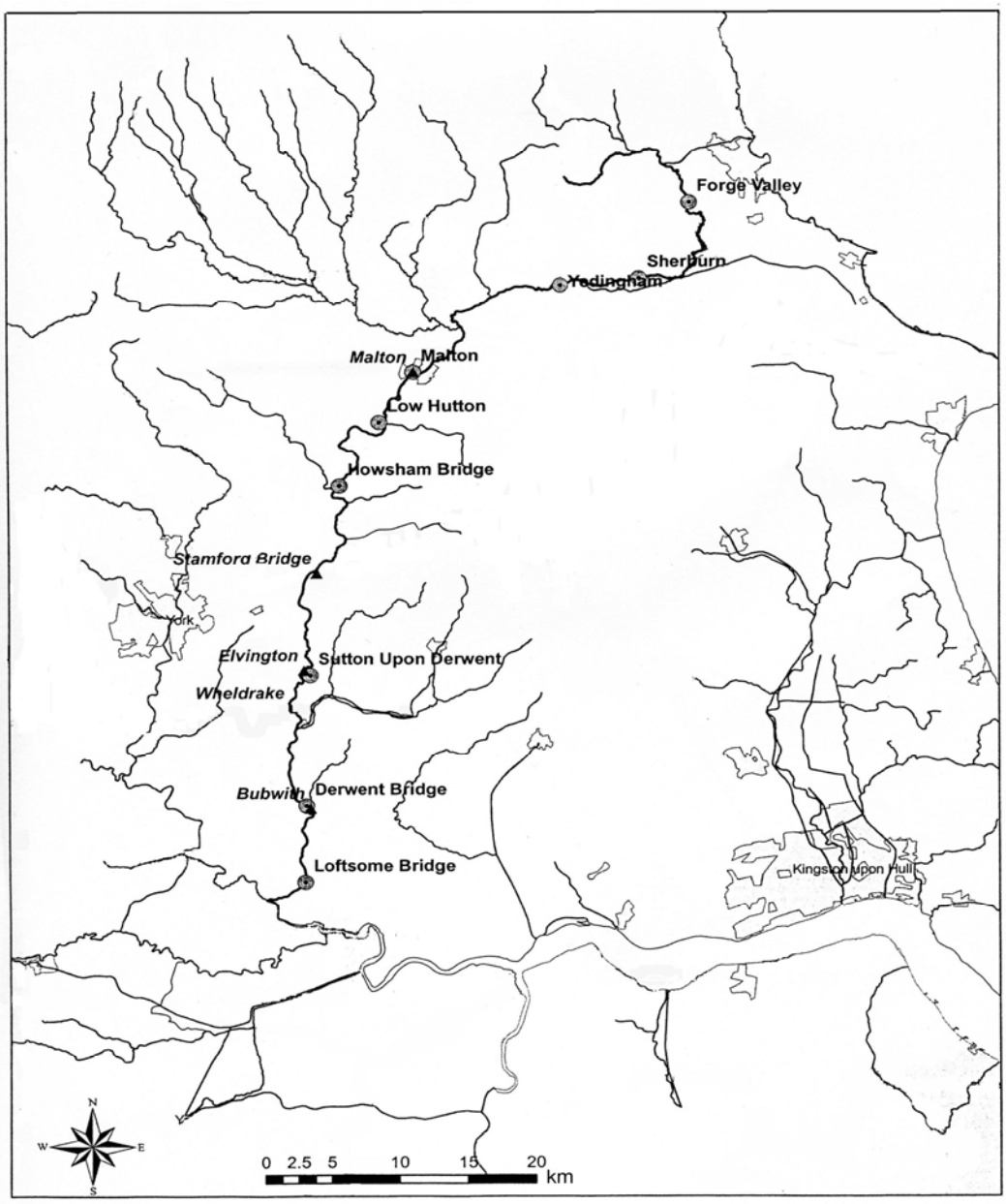


Fig. 8.1. Map showing river water sampling sites along the River Derwent (circles) and sewage treatment works (triangles).

Details of the terrain within each sub-catchment are included where appropriate in the discussion. Suffice it to say here that preliminary visual analysis suggested that two catchments, Forge Valley and Malton, would be most likely to show N saturation effects because their Moorland and grassland were visually around 50%. The agricultural and urban impact just downstream of Malton was thought likely to be insufficient to mask an increase in mean nitrate concentration because of the network of tributaries draining a relatively very large area of moorland feeding into the Derwent just upstream of Malton (Fig. 8.1).

Data on animal numbers for the East Riding of Yorkshire and North Yorkshire were obtained from statistics on the DEFRA web site. These data are not available at farm level, so can only provide a general indication of likely relative animal losses subsequent to the Foot and Mouth outbreak in the catchment.

In addition to using Environment Agency data for this evaluation, the river system was also sampled and analysed for N species at 29 points during a dry period from 19-23 March, 2009. Sampling points were selected to give good coverage of both moorland-dominated and agriculture-dominated land use. Nitrate was determined by both ion chromatography and automated colorimetric analysis using a standard manifold. There was no significant difference between results obtained by the two techniques.

8.3 Results

Table 8.1 shows the sub-catchment area and distribution of land use upstream of each of the first eight sampling points. No digitised data were available below Derwent Bridge, so the corresponding information is not reported for the Loftsome Bridge sampling point. As this is only 6.5 km south of the Derwent Bridge sampling point, and the surrounding land is agricultural and quite flat, this omission is not important. Table 8.2 shows the % area distribution of land use in the additional catchment area on progressing down stream from one sampling point to the next. Table 8.3 shows changes in animal numbers over time in N. Yorkshire and the East Riding of Yorkshire. These provide a general indication of losses from fertilizer and manure in the region only, as data were not available at farm or catchment level.

In addition to using EA data, 29 points on the river system were sampled by Begum and Ridealgh during a dry period from 19-23 March, 2009, as mentioned in section 8.2, and the waters analysed for N species. The results are shown in Table 8.4. Figures 8.2 to 8.4 show the time series plots of nitrate concentration for the 9 monitoring sites, moving progressively from Forge Valley near the top of the catchment down to Loftsome Bridge. As expected, the nitrate-N concentrations are overall lowest at Forge Valley (Fig. 8.2), and have increased steadily since 1988 over the next 15 years (to month 180) but now annual mean values appear to be attaining a plateau. The Pearson correlation coefficient for the relationship between annual mean nitrate concentration and year (plot not shown) was 0.482, which is significant at the 0.01 level. The seasonality is indistinct, suggesting precipitation pattern and changes in hydrological pathway are at least as important as seasonality.

Classification Group	Forge Valley SE987860	Sherburn SE962796	Yedingham SE893797	Malton SE787715	Low Hutton SE765677	Howsham Bridge SE732625	Sutton Lock SE704475	Derwent Bridge SE707364
Unclassified	1.28	1.12	1.19	0.99	1.00	1.03	1.15	1.53
Urban/urban infrastructure	1.60	5.24	6.19	4.81	4.84	4.77	4.60	4.54
Fresh water	0.00	0.02	0.01	0.01	0.01	0.02	0.02	0.02
Evergreen woodland	26.94	14.65	11.04	6.37	6.32	6.01	5.50	4.97
Deciduous/ mixed wood	9.79	6.46	5.57	5.66	5.64	5.67	5.29	5.55
Arable	6.83	28.01	35.86	31.94	32.14	33.32	35.91	37.39
Moorland	33.18	21.00	17.21	24.44	24.27	23.41	21.54	19.61
Grassland	20.37	23.49	22.92	25.78	25.80	25.78	26.01	26.40
Upstream area (km^2)	120.8	250.8	337.5	1414.8	1428.7	1511.3	1679.4	1881.0

Table 8.1. Percentage distribution of land use class at the top 8 of the 9 sampling sites along the River Derwent.

Classification Group	% Cover of Extra Area						
	Forge Valley to Sherburn	Sherburn to Yedingham	Yedingham to Malton	Malton to Low Hutton	Low Hutton to Howsham Bridge	Howsham Bridge to Sutton Lock	Sutton Lock to Derwent Bridge
Unclassified	1.0	1.4	0.9	2.0	1.6	2.2	4.7
Urban/urban infrastructure	8.6	8.9	4.4	7.7	3.6	3.1	4.0
Fresh water	0.0	0.0	0.0	0.0	0.2	0.0	0.0
Evergreen woodland	3.2	0.6	4.9	1.2	0.6	0.9	0.6
Deciduous/mixed wood	3.4	3.0	5.7	3.5	6.2	1.9	7.7
Arable	47.7	58.6	30.7	51.4	53.8	59.1	49.8
Moorland	9.7	6.2	26.7	6.8	8.5	4.7	3.5
Grassland	26.4	21.3	26.7	27.3	25.5	28.0	29.7

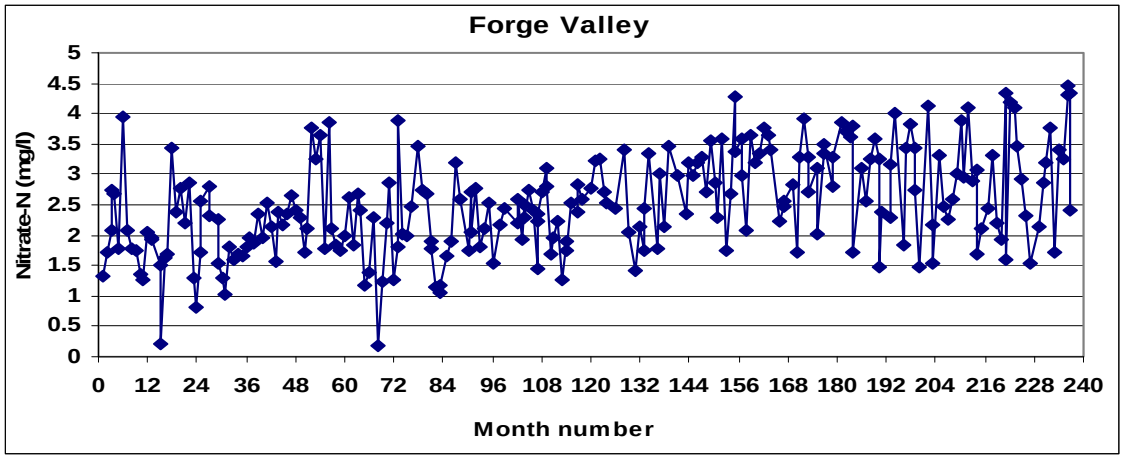
Table 8.2. Percentage distribution of land use class in the additional catchment area on progressing downstream between the 9 sampling sites along the River Derwent.

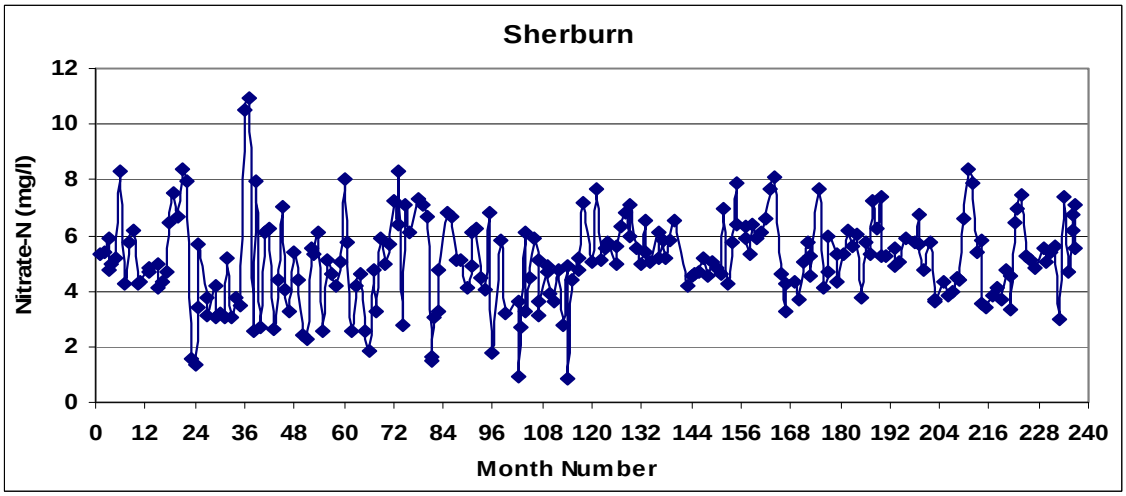
YEAR	ER	NY	ER	NY	ER	NY	ER	NY
2000	56,329	413,096	619,990	783,817	110,654	2,077,156	1,184,676	2,979,517
2001	56,476	385,548	575,690	700,098	105,164	1,793,702	1,321,990	3,028,308
2002	51,679	368,676	528,283	658,079	91,389	1,675,339	1,454,922	3,058,479
2003	52,896	383,150	449,968	588,897	85,920	1,746,518	1,920,756	3,029,876
2004	55,088	388,658	508,286	597,152	83,782	1,798,769	1,767,898	3,265,715
2005	52,771	379,033	504,837	561,430	85,715	1,817,514	1,496,277	3,099,391
2006	50,797	366,720	528,968	598,174	92,890	1,772,569	1,138,099	3,937,094
2007	52,939	396,344	481,340	569,306	87,934	1,761,878	1,039,559	3,531,634

Table 8.3. Changes in animal numbers in the East Riding of Yorkshire (ER) and North Yorkshire (NY) over the period of the foot and mouth outbreak in 2001, based upon DEFRA statistics available from the DEFRA web site.

Sampling point	Grid Ref	Upstream Catchment Land Use	Nirate	NH_4-N
			mg/l	mg/l
Un-named chalk stream	SE 957 777	Flat area of high quality grassland & arable; sheep on bank.	68.1	0.097
Ellis Beck	SE 900 777	Flat arable and grassland	62.1	0.177
Drainage channel north of Yedingham	SE 896 796	Arable & grassland.	61.3	0.026
Marrs Beck (tributary to Holbeck)	SE 670 766	Mainly agriculture, some woodland.	45.1	0.023
South channel parallel to Derwent at Sherburn	SE 954 794	Agricultural, including much arable.	44.4	0.032
Derwent at Yedingham	SE 891 795	Approximately 36% arable plus mix of moorland, grassland and woodland.	43.2	0.065
Derwent at Sherburn	SE 954 795	Approximately 30% arable plus mix of moorland, grassland and woodland.	36.4	0.086
Sherburn Cut	SE 954 784	Mixture of flat arable & grassland and sloping grassland.	33.5	0.042
Holbeck	SE 669 773	Agriculture, but woodland & moorland on steeper slopes; includes community at Ampleforth.	27.0	0.033
Ruston Beck at Sherburn Ings	SE 954 796	Flat arable & grassland.	22.0	0.066
Etton Gill at Helmsely (tributary to Rye)	SE 613 835	Arable, riparian woodland & improved grassland.	20.2	0.006
Derwent at Forge Valley	SE 987 860	Predomimantly moorland, grassland & woodland; ca. 7% arable.	19.3	0.022
Sea Cut Malthorpe Farm	SE 979 883	As Forge Valley.	19.2	0.012
Welldale Beck at Yedingham	SE 896 796	Predominantly grassland and some arable; minor urban.	19.1	0.064
Derwent at West Ayton	SE 987 848	Predominantly moorland, grassland and woodland; ca. 7% arable.	18.8	0.008
Derwent at Cockran Foot	SE 968 892	Similar to Forge valley, but slightly less agriculture.	18.2	0.012
Rye at South of Helmsley	SE 613 835	Moorland by headwaters on upper slopes; wooded riparian zones; some grassland & arable.	16.0	0.008
Black Beck near Langdale End	SE 942 908	Predominantly forestry plus some agriculture/improved grassland on gentle slopes.	10.5	0.004
Derwent at Langdale End	SE 943 909	Predominantly moorland and woodland; patches of arable.	9.4	0.009
Hodge Beck	SE 677 856	Large area of sloping moorland & riparian woodland; grassland & arable in low, flatter areas.	9.3	0.034
Bilsdale Beck at Chop Gate	SE 558 993	Moorland & improved grassland; some agriculture.	7.8	0.017
Rye above Ladhill Beck south of Hawnby	SE 543 893	Large area of moorland & woodland; some improved grassland.	7.8	0.008
Seph at Chop gate (confluence of Bilsdale Beck & Raisdale Beck)	SE 558 992	Predominantly moorland and improved grassland; some woodland.	7.5	0.044
Dove at Kirkbymoorside	SE 708 863	Predominantly sloping moorland; some riparian woodland; some grassland & arable.	7.0	0.011
Raisdale Beck at Chop gate	SE 558 992	Moorland, woodland & improved grassland.	7.0	0.038
Rye at Shaken Bridge	SE 559 883	Large areas of moorland; some woodland, including in riparian zone.	6.7	0.009
Ladhill Beck (tributary to Rye)	SE 546 899	Moorland with small patches of riparian zone woodland; some grassland;some grassland & minor arable.	6.0	0.008
Seph at Laskill	SE 562 908	Large area of moorland; some woodland; some grassland on lower slopes.	5.4	0.014
Ledge Beck at Grange	SE 573 961	Extensive sloping riparian woodland; regenerating burned moorland.	1.6	0.007

Table 8.4. Nitrate and ammonium-N concentrations in water samples across the upper Derwent catchment during a dry period from 19-23/03/2009.





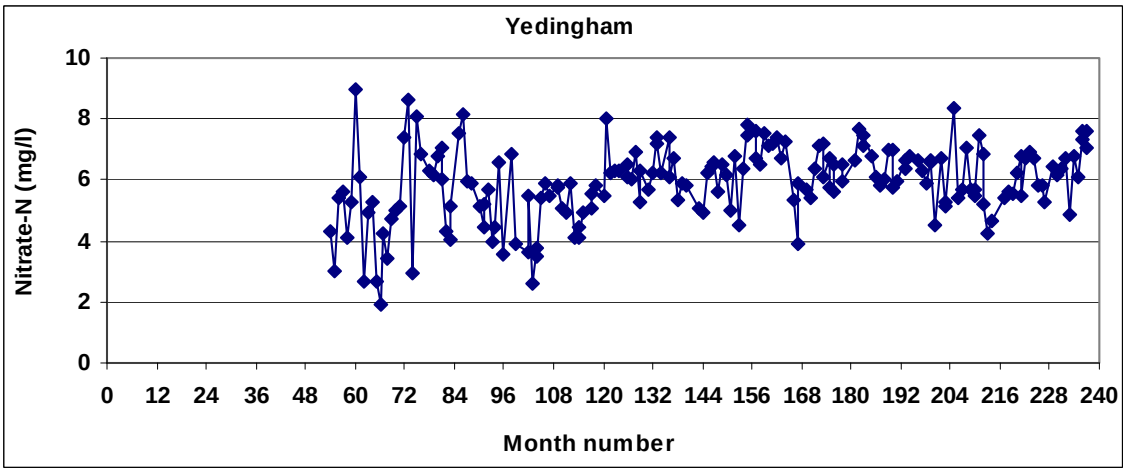
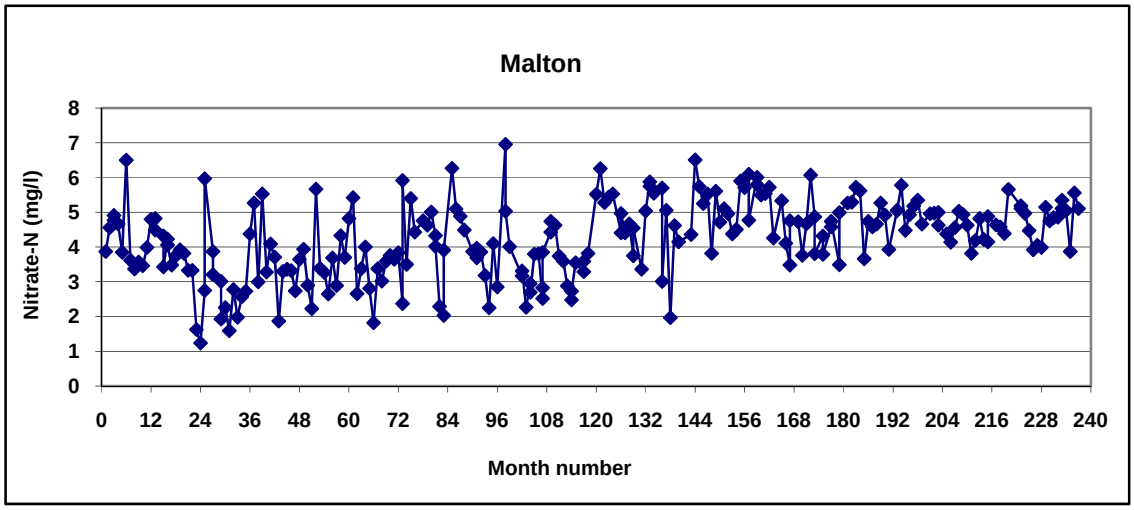
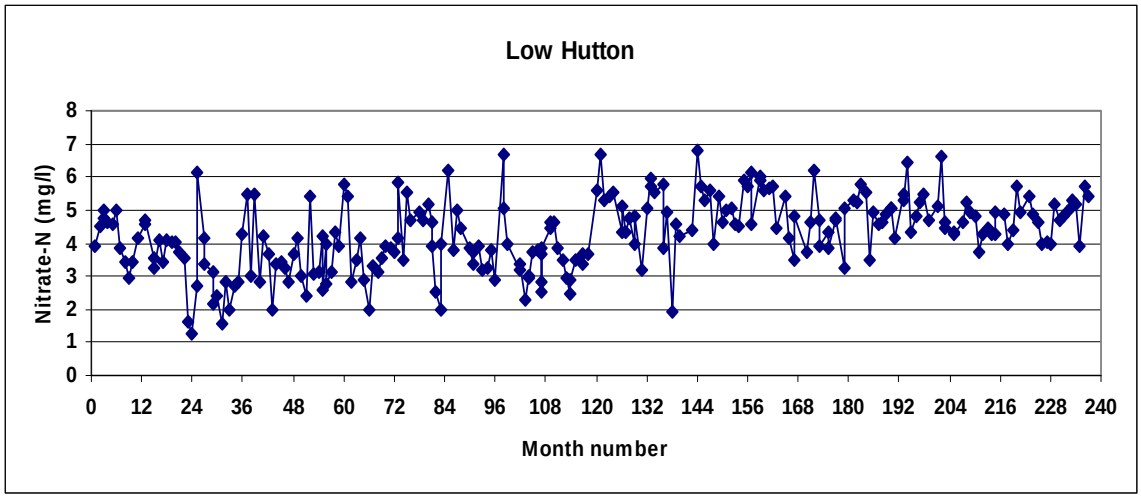


Fig: 8.2 Time series plots from 1988-2008 for Forge Valley, Sherburn and Yedingham.





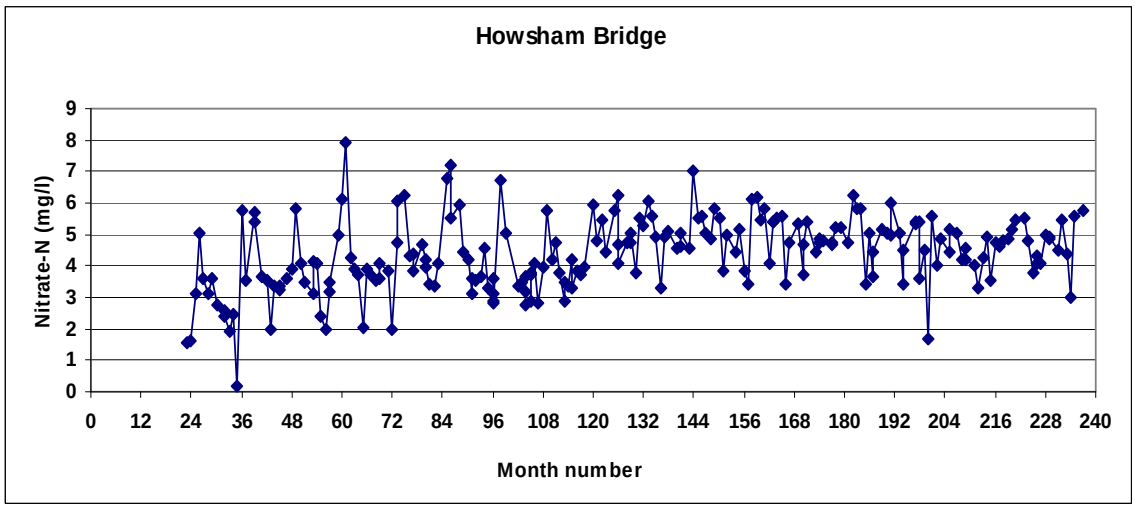
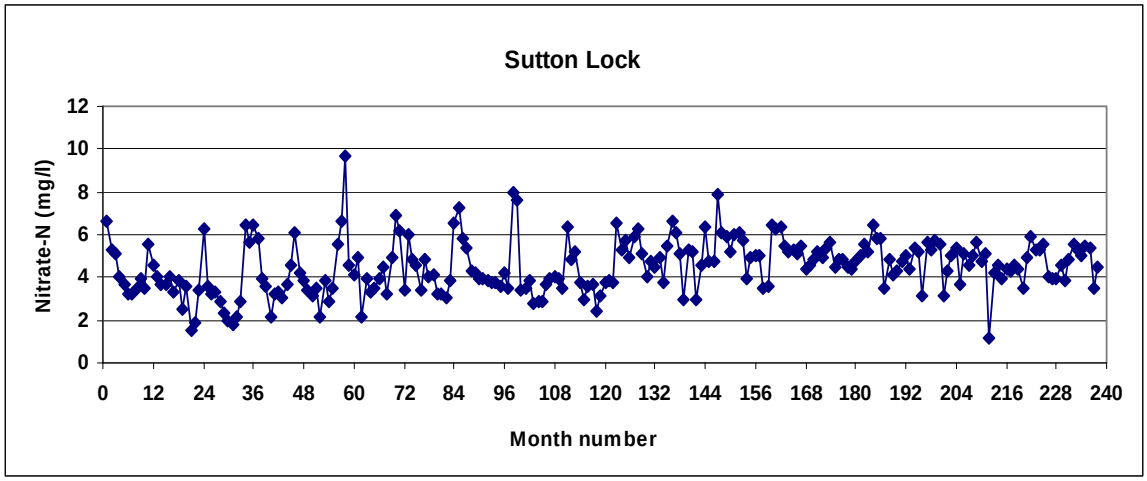
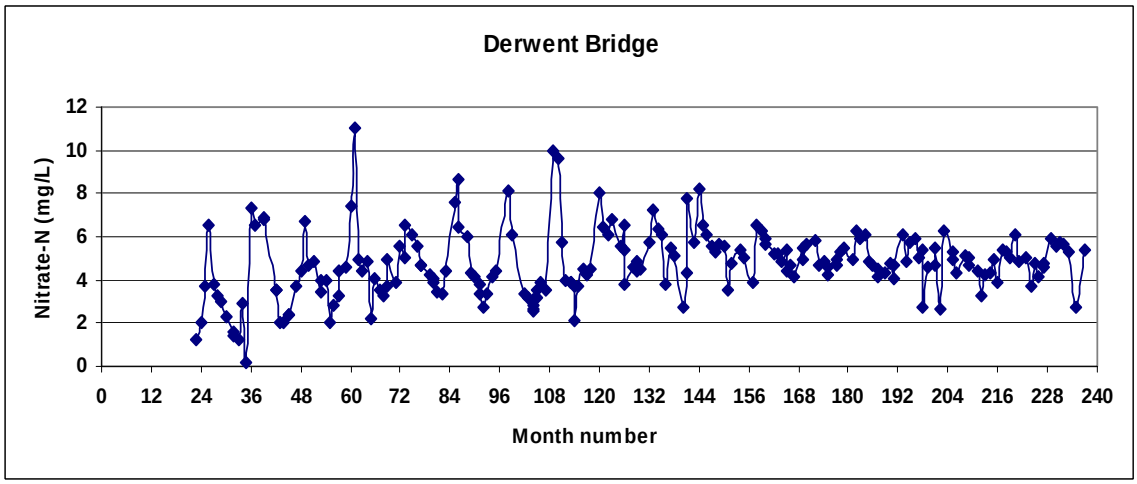


Fig. 8.3 Time series plots from 1988-2008 for Malton, Low Hutton and Howsham Bridge.





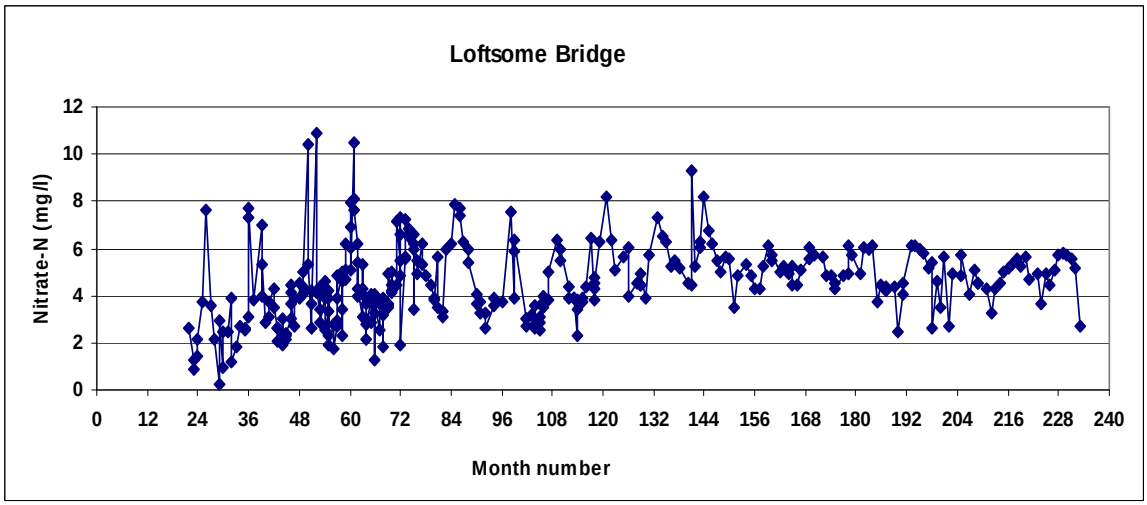


Fig: 8.4 Time series plots from 1988-2008 for Sutton Lock, Derwent Bridge and Loftsome Bridge.

Overall, nitrate-N concentrations are higher at Sherburn and Yedingam compared to those at Forge Valley (Fig. 8.2), reflecting the greater influence of agricultural land. The arable % land use increases from 6.83 to 28.01 and 35.86 on moving down stream between the sites (Table 8.1). However, three interesting trends are readily apparent. The first is that the clear increasing trend in mean nitrate-N concentration seen at Forge Valley is no longer discernable (not significant) at Sherburn, and less significant (Pearson coefficient = 0.357) at Yedingham (where no data were available over the first 4 years). The second is that clearer mid to late summer maxima (rather than minima) can be seen at Sherburn over the past 8 years. The third is that values in summer often showed some low concentration episodic minima over the first ten years but these are subsequently less noticeable, suggesting that summer leaching is now higher than it used to be a decade or longer ago, and winter leaching is lower. Trends in the time series plots are quite similar, but not identical, at Sherburn and Yedingham.

Compared with that above Yedingham, the catchment upstream of Malton has more moorland and grassland, and less arable land in percentage cover terms (Table 8.1). Nitrate concentration therefore falls slightly overall compared with that at Yedingham (Figs. 8.2 and 8.3), and there appears to be a steady increase between 1990 (month 25) and 2000 at Malton, followed by a plateau. The Pearson correlation at Malton was 0.443 between mean nitrate concentration and year, significant at the 0.01 level. The mid to late winter maxima are distinctive over the first 12 years, but subsequently become hard to detect. The timing of minima is variable over the first 12 years, between summer and autumn, and no distinctive minima can be seen over the past 8 years (Fig. 8.3).

The Low Hutton site is just below the Malton site, so the changes in land use and catchment area (Table 8.1), and thus also in water nitrate-N concentrations (Fig. 8.3), are negligible. Even at Howsham Bridge the small additional area of arable agricultural land has a very small impact. The concentration increase overall over time (the N-saturation effect) is still very obvious over the first eight years, and the late winter maxima are again clear over the first 10 years after 1990, but subsequently become hard to discern (Fig. 8.3). Overall, the mean annual nitrate increased (coefficient significant at $\leq$ 0.01) over the assessment period. Generally summer minima are at higher values over the second half of the period for which data were available for Howsham Bridge. Even here the nitrate-N

concentration is still lower than that at Yedingham because of the large input of water draining moorland and grassland just above Malton; moorland plus grassland is >49% at Howsham Bridge, compared with just over 40% at Yedingham.

With a growing relative influence of arable agriculture and grassland and a decline in relative influence of moorland (Table 8.1) on progressing down stream through the Sutton Lock and Derwent Bridge sites, there is a further slight increase in the overall nitrate-N concentration, and the N-saturation effect is still readily discernable (Fig. 8.4). The seasonality effect of agricultural use of fertilizers (strong late winter maxima in nitrate-N concentration) over the first 12 years of data collection at Derwent Bridge is extremely clear, and at both sites summer minima are higher but winter maxima have declined over the past 10 years. By the Loftsome Bridge site, 6.3 km further south, the time series plot is still quite similar to that for the Derwent Bridge site, though there is more variability over the first three years of monitoring (Fig. 8.4).

8.4 Discussion

8.4.1 Forge Valley

The Derwent rises in a valley to the south side of Fylingdales Moor, flowing initially east then south east. To the north is *Calluna* moorland, but on the south side of the valley lies the coniferous woodland of Langdale Forest. Tributaries join the Derwent from both sides of the valley, including Woof Howe Grain from the forest. The river then flows south between the eastern side of Langdale Forest and the west side of Broxa Forest (both coniferous), being joined by Jugger Howe Beck and Bloody Beck from the south eastern corner of Fylingdales Moor (High Moor), and Cowgate Slack draining Harwood Dale Forest (also coniferous). Several other tributaries join as the river flow south/south east towards Forge Valley, including Black Beck from Dalby Forest, White Beck through Deep Dale and Troutsdale Beck from Trouts Dale to the west, and Whisperdales Beck and Lowdales Beck from the east. The latter will be influenced by farming activity in the area. Minor roads run parallel to the River Derwent along approximately one third of its length to this point, so some road salting influence is possible, but this should be minor. Salting effects via pH effects would in any case depend upon hydrological conditions. There are a number of isolated dwellings, but their influence too should be small. Therefore evidence

for N saturation induced by atmospheric deposition might be expected at this site and is clearly visible in Fig. 8.2 as a steady increase in annual mean nitrate-N concentration.

The catchment area upstream of the sampling point at Forge Valley is 120.8 km^2, and much of the area is hilly (up to >290 m). Moderate to steep slopes are frequent. It has been documented that steep (20-40°) slopes facilitate nitrate transfer to rivers in British upland catchments (Smart et al., 2005). Rivers in catchments with steep slopes tend to be more flashy than lowland rivers, and nitrate concentration may increase with discharge (Jarvie et al., 2005). It is therefore reasonable to suggest that a strong hydrological pathway effect at Valley Forge is masking the strong seasonality effect often reported for nitrate-N in heavily N-impacted British upland catchments (Clark et al., 2004; Cresser et al., 2004; Smart et al., 2005). The high variability that this causes means that a large data set is needed over several years to detect long-term trends in such a catchment.

8.4.2 Sherburn

Below Forge Valley some influence on the Derwent is likely from East and West Ayton, and the small sewage treatment works (STW) to the west of Seamer, and this is reflected in the >3-fold increase in Urban/urban infra structure land use (Table 8.1). The river flows south through the Seamer Ings, and in this area, though relatively small compared with the upstream catchment area, the river is likely to be significantly influenced by agriculture. Arable agriculture increased from 6.83 % at Forge Valley to 28.01% at Sherburn. In fact, close to the Forge Valley sampling site, water is regularly re-routed from the Derwent via a sea cut to the North Sea as part of the flood protection system down-stream, so the effects of agriculture at Sherburn and Yedingham especially may be larger than would be anticipated. As the Derwent starts to flow towards the west through Sherburn Ings and Brompton Ings, the River has been re-routed, but again there should be a significant agricultural influence. This could be expected to partly reduce the impact of N saturation on winter nitrate concentration peaks if farmers are applying less N fertiliser over the past decade than over the one before in response to falling fertilizer need by crops because of increasing N deposition inputs and to increasing fertilizer prices. Declines in animal numbers, especially for pigs from 2001 (Table 8.3), could also be potentially beneficial, as could straw incorporation post 1993. Maxima are no greater over the past decade than they

were in the previous decade (Fig. 8.2). The catchment area upstream of the sampling point at Sherburn is 250.8 km^2, so the additional 130 km^2 compared with Forge Valley suggests that the agricultural influence should be substantial. It would be anticipated that the N-saturation impact on nitrate concentration would be less evident winter peaks at Sherburn, with reduced fertilizer inputs over time, though it should still be evident in higher summer minima from enhanced nitrate in ground water end members. Also, if straw incorporation reduces nitrate leaching in winter, as the residue litter C:N ratio falls over the late winter/early spring period, this retained N could start to be released in early summer. Higher summer minima certainly appear to be the case in Fig. 8.2. Overall though, the nitrate-N concentration should be higher at Sherburn than at Forge Valley because of the substantially greater agricultural impact, and it has approximately doubled on average compared with Forge Valley at any time over the 20-year sampling period. Change in catchment area would not be expected to give a pro-rata change in nitrate concentration because of differences in hydrological behaviour above and below Forge Valley. The area above Forge Valley has more steep slopes as mentioned earlier and almost certainly higher precipitation, and is more influenced by lower order streams. Also some water is periodically diverted via a sea cut.

8.4.3 Yedingham

The sampling site at Yedingham is only 6.9 km west of that at Sherburn, so only a slightly greater agricultural influence might be expected. Arable agriculture has increased from 28.01% at Sherburn to 35.86%. Sampling here commenced only in 1992, but between 1992 and 2007 it would be expected that the water chemistry at Yedingham and Sherburn would be very similar, with a slight further increase in average nitrate-N concentration at Yedingham, and further decline in the distinctive nitrate maxima for reasons given in the previous section. The catchment area upstream of the sampling point at Yedingham is only 337.5 km^2, an increase of only 86.7 km^2 compared to the area at Sherburn. Thus only a small further increase in nitrate concentration compared with Sherburn would be expected, and that is what is observed in practice. Minima have moved to higher values over the past 10 years, indicative of a breakthrough effect causing enhanced summer leaching. Nitrogen pollution occurs throughout the year, and leaching to subsoils is highly likely through late

autumn and winter. This N may be retained at least partially below the rooting depth, and then contribute to nitrate leaching from subsoils in the subsequent summer. In a totally unfertilized, but heavily N-impacted, grassland soil close to York, Riaz et al. (2008) have reported that both ammonium and nitrate may be mobilized down soil profiles to below the rooting depth where soil pH often increases, soil C:N ratio is reduced, and nitrification enhances nitrate mobilization from sub soils to adjacent streams via ground water end members. This is probably what is responsible for the lack of any conspicuous and consistent minima in nitrate-N concentration at Yedingham over the past decade.

8.4.4 Malton

Substantial differences might be anticipated in water chemistry between water samples taken at Yedingham and those taken at Malton, because of the influence of the River Rye that joins the Derwent just above Malton (Fig. 8.1). The impact of the agricultural area above this confluence point downstream of Yedingham will be smaller compared with the very large area of moorland and grassland, and lesser area of woodland that drains towards the south and into the Rye before it joins the Derwent. The river system includes Raisdale Beck and Tripsdale and Tamhole Becks, draining Bilsdale West Moor and Bilsdale East Moor into the River Seph as it flows south to join the Rye, Benfield Gill draining East Moors and Pockley Moor to join the River Ricall which, in turn, flows into the Rye. The Seph and the Rye will both be subject to some influence of road salting from the B1257 road, which runs parallel to the Seph for more than 10 km and could enhance nitrate concentration in the river through the year (Green and Cresser, 2008a). The town of Helmsley could significantly influence water quality in the Rye. Kikrbymoorside will have some influence on the River Dove before it joins the Rye. Overall though, the influence of the river system will undoubtedly be strongly dominated by water draining from the complex network of higher order rivers draining the moors, as the urban/urban infrastructure % is lower at Malton (4.81%) than at Yedingham (6.19%, Table 8.1). The catchment area upstream of the sampling point at Malton increases to 1414.8 km^2, an increase of 1077.3 km^2 compared with the 337.5 km^2 at Yedingham. Therefore it may be predicted that water from the Malton site would show as strong an influence, or even a stronger influence, of atmospheric N deposition and N saturation in time series plots than

water from the Forge Valley site, and the increase overall between 1990 and 2007 is very clear in Fig. 8.3. Because of the dilution with the influx of water derived from the moors and the N-impacted acid upland soils being drained, it might also be expected that a steady increase over the years in summer nitrate inputs would be noticeable at this site, as indeed observed (less conspicuous summer minima in Fig. 8.3), but late winter fertilizer or liming-derived nitrate peaks would be reduced even more as a consequence of the expected lower fertilizer inputs and possibly some straw incorporation and declining animal numbers. This input dilutes the overall nitrate-N concentration in the River Derwent at Malton compared to values at Yedingham. These effects are all visible comparing Fig. 8.3 and Fig. 8.2.

8.4.5 Low Hutton

The Low Hutton site is just 4 km to the south west of the Malton sampling site, and should provide a clear indication of any effect that the town of Malton has upon the quality of water in the River Derwent, but the overall Urban/urban infrastructure only increases from 4.81 to 4.84% (Table 8.1). The increase in upstream catchment area between the two sites, by 13.9 km^2 to 1428.7 km^2, is relatively very small, and tributaries joining the Derwent in this stretch are also minor, draining predominantly agricultural land. Little difference would be expected therefore between the two data sets for Malton and Low Hutton, and little difference is seen in Fig. 8.3. Because of the relative closeness of the sites and the small volume of additional water entering the main river, the Low Hutton water data also display evidence of N saturation over the 20-year sampling period with higher and less obvious summer minima and similar or lower winter maxima over the second decade of sampling, but an overall increase since 1990.

8.4.6 Howsham Bridge

The next sampling point at Howsham Bridge, 6 km south west of Low Hutton, also adds only modestly to the catchment area (an extra 82.6 km^2 compared with the total upstream area of 1511.3 km^2). The area is rather more hilly, but still predominantly agricultural. A number of small villages and their interlinking network of minor roads will have a small impact, as may the main A64 route running south west of Malton. The impact of these would be expected to be minor, because of the relatively small size of the additional

catchment area and the decline in the overall urban/urban infrastructure % (Table 8.1). Arable land % increases further, and the seasonal effect over the first 10 years is very noticeable in Fig. 8.3, but subsequently declines. The saturation effect can still be seen, but overall nitrate concentration has, if anything declined slightly over the past 10 years.

8.4.7 Sutton Lock

Agriculture (arable and grassland) increases further on moving down stream to Sutton Lock Table 8.1, and Fig. 8.4 shows very similar temporal trends for this site to those seen for Howsham Bridge in Fig. 8.3. The N saturation effect is still clear, but the overall increase seems to be predominantly due to greater nitrate leaching in summer, and again there is no increase in winter nitrate concentration maxima. Tributaries flowing into the Derwent from both east and west would benefit from NVZ status from 2002 onwards here and further downstream too, and this effect is marked.

8.4.8 Derwent Bridge

Agriculture (arable and grassland) increase again by Derwent Bridge, and moorland becomes less influential (Table 8.1). Seasonality becomes even more pronounced and consistent between 1990 and 2000 (Fig. 8.4), with sharp maxima initially in January/February and late summer/autumn minima. Both have become markedly less pronounced over the past 7-8 years. Here too the increasing trend from 1990 to 2000 is very clear, with no subsequent further increase. In the lower reaches of the catchment the NVZ constraints imposed in 2002 would start to come into effect.

8.4.9 Loftsome Bridge

The nitrate data at this southern-most site closely resemble those at Derwent Bridge, though with greater initial variability in the time series plot. It is interesting to note that although both the Derwent Bridge and the Loftsome Bridge sites have a greater % arable plus grassland land use than Yedingham, the latter still exhibits greater overall nitrate-N concentrations. This may well reflect loss of nitrate from the floodplains of the river (Forshay and Stanley, 2005) where the Ings are flooded quite regularly on the flat areas

adjacent to the river channel. The benefits of NVZ designation in 2002 should be greatest (in terms of improved sub-catchment areas) at this point.

8.5 Conclusions

The data presented here fit well on the plot of nitrate concentration versus % upland area in catchments published by Davies and Neal (2007). They also help to explain why such diverse long-term trends have been observed for the nitrate concentrations in rivers around the world in N-impacted regions (Stoddard et al., 1999; Evans and Monteith 2001; de Wit et al., 2008; Rogora et al., 2008; Tipping et al., 2008). At Forge Valley, the long-term trend in nitrate concentration was almost masked by the high variability attributable to diversity of hydrological pathways over time, which in turn relates to catchment topography. Nevertheless a long term trend attributable to a combination of direct leaching of nitrate and leaching of ammonium down profiles followed by nitrification below the rooting zone was clearly apparent. Further down stream at Sherburn, however, no such long-term trend was apparent, and it is suggested that this is due to farmers consciously or sub-consciously responding to the "free N fertilizer" associated with atmospheric N inputs and higher fertilizer and energy costs, straw incorporation post 1993, and the relative size of wooded, moorland and agricultural areas.

The downside of this "free" fertilizer is that it is applied throughout the year, and may thus be leached to depth in soil profiles when plant uptake is low, lowering the C:N ratio at depth in the soil. It is likely to lead also to some ammonium accumulation at depth, and subsequent nitrification of this will cause nitrate to leach to ground water or surface waters. In the chalky strata across part of the Derwent catchment, ground water inputs at low flow conditions may be very important, and help to cause the increase (i.e. decline in conspicuousness) over the past decade in the values of nitrate "minima" associated normally with summer months in both upland rivers and agricultural catchments.

Water from the Derwent at Malton is strongly influenced by input from the uplands and shows a clear increase in nitrate concentration over 10 years from 1990, followed by a plateau and markedly reduced variability. However, in the two previous years nitrate concentration seemed to be declining, a trend that was reflected downstream at Low Hutton and Sutton Lock. This means that the strength of the breakthrough relationship depends

upon the sampling period duration. An 18 year sampling period gives a strong relationship at almost all of the study sites, whereas a 10 year sampling period starting around 1998 would suggest no significant trend, or possibly even a slight declining trend. This suggests that it is important to look very carefully at catchment characteristics when interpreting results.

In terms of nitrate pollution, the Yorkshire River Derwent appears to be a relatively quite clean river compared with many in the UK, and farmers may well ask why in 2009 the whole catchment has been assessed to be a NVZ. In an attempt to answer this question, as described earlier, the river system was sampled and analysed for N species at 29 points during a dry period from 19-23 March, 2009. The results (Table 8.4) clearly indicate that many agricultural areas are making a significant contribution to the total nitrate load in the river, whereas upland areas provide relatively clean water that dilutes arable agricultural impacts. The whole area though contributes to the high nitrate concentrations at a point well down stream used to justify NVZ designation of the whole catchment. For example, the Derwent Bridge data over the 15 years from 1990 to 2004 (Fig. 8.4) may be used to predict the 95 percentile nitrate-N concentration in 2010 if no remedial action was taken; the result would exceed the critical value of 11.29 mg/l for nitrate-N which equates to 50 mg/l for nitrate. This would be sufficient to justify declaration of the whole catchment upstream as a NVZ. Such a modelling approach is clearly more sensible than using recent data if controls on nitrate leaching are to continue for water quality protection.

8.5.1 Should the River Derwent in N. Yorkshire be a NVZ?

It is easiest to understand how the imposition of NVZ status was decided by looking at a specific example. The sampling site at Sherburn has been selected here, as its catchment has typical mixed land use of a UK catchment on the edge of the uplands. Information obtained directly from the DEFRA Magic web site for the NVZ designation at Sherburn is as shown below.

Surface Water NVZ (England)

Type of NVZ in 2006	Id. of Lowest Catchment	Easting of Highest Nitrate in 2004	Northing of Highest Nitrate in 2004	Type of Catchment (1 or 2)	Nitrate Monitored in 2004 mg/l	Nitrate Predicted in 2010 mg/l	Nitrate Predicted by Model mg/l	Confidence of Model Prediction	Easting of Lowest Point	Northing of Lowest Point
SW	GB204027064270	470900	436400	1	60.4695	40.756	35.89976	CONFIDENT PASS	485489	423709

Although the model prediction is a confident pass, because the future predicted TON concentration would not exceed 11.29 mg N l^{-1} for 5% or more of the time at Sherburn, the catchment is still designated as a NVZ because of high monitored values (60.5 mg nitrate l^{-1}) downstream at the map reference given for the highest nitrate, namely 470900 434600. This grid reference corresponds to the EA sampling point at Derwent Bridge. Figure 8.5 shows a time series plot of the EA data for the Sherburn sampling point, made available to farmers who wished to appeal against implementation of NVZ status to their holding over the period 1990 to 2004. Here total oxidized N has been plotted, rather than nitrate-N, as there were a number of missing nitrate-N values in the nitrate data set in the earlier years. The highest TON value was 10.94 mg/l in January 1991. This corresponds to only 48.4 mg nitrate l^{-1}). Thus the 95 percentile value also must be < 50 mg nitrate l^{-1}) at Sherburn from direct measurement. The 60.5 mg nitrate l^{-1} does not apply at Sherburn itself. However, based upon EA monitoring data, it does not apply to any other Derwent monitoring site either, including Derwent Bridge.

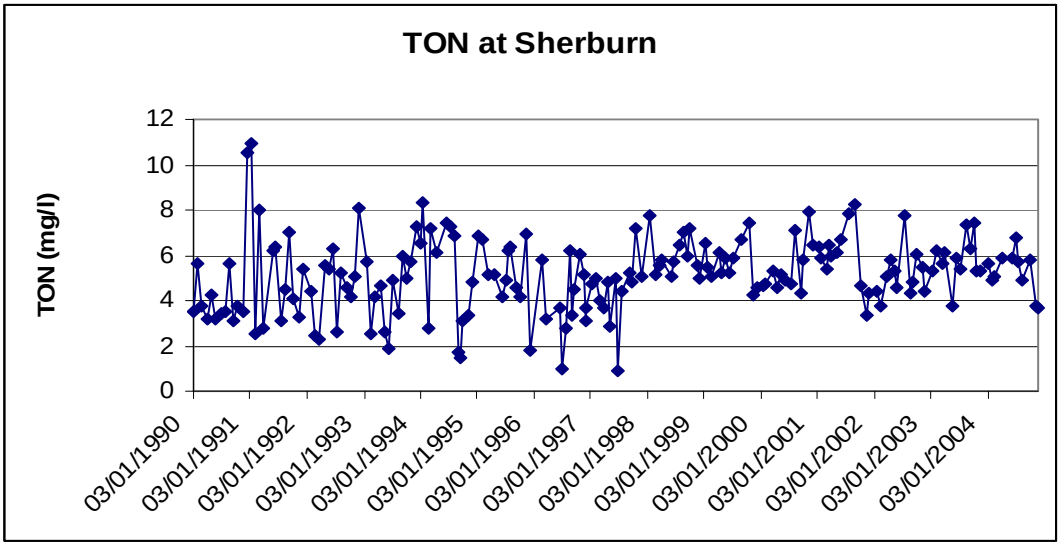


Fig. 8.5. Changes in TON from January 1990 to the end of 2004 at the Sherburn sampling site on the River Derwent, from the data made available from the Environment Agency to Farmers via DEFRA.

Figure 8.6 shows the time series plot of the EA data for the Derwent Bridge sampling point over the period 1990 to 2004, that were made available to farmers who wished to appeal against implementation of NVZ status to their holding. At no point over this period did the Derwent at Derwent Bridge exhibit a concentration above the critical concentration of 11.29 mg N l^{-1}, which corresponds to 50 mg nitrate l^{-1}. This implies that the Environment Agency extrapolation of the line to 2010 must have suggested to them that the 95 percentile value by 2010 would have reached or exceeded these critical concentration values. It should be remembered that the modelling approach employed by the Agency was assuming that the variation about the trend line would remain similar to that seen over the period 1990 to 2004. If this assumption is made, then recurrence of high winter peaks in nitrate concentrations such as those observed in 1995, 1996 and 1997 in or around 2010 could just lead to an unacceptably high 95 percentile value.

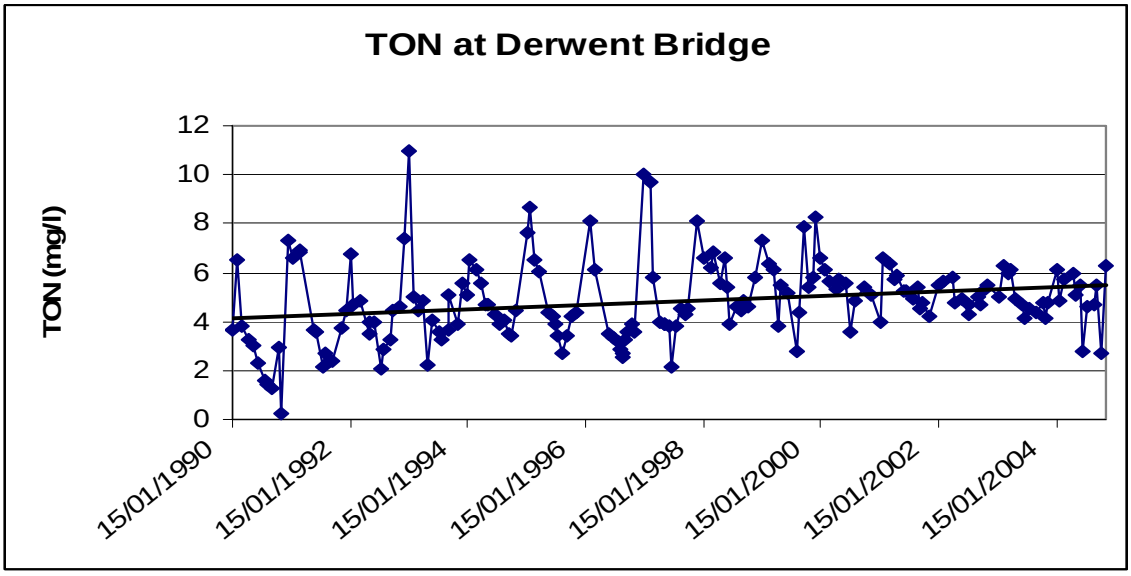


Fig. 8.6. Changes in TON from January 1990 to the end of 2004 at the Derwent Bridge sampling site on the River Derwent, from the data made available from the Environment Agency to Farmers via DEFRA.

Farmers might well argue that growing environmental awareness about nitrate pollution issues and the high costs of fertilizer and energy make such recurrences very improbable. If the above figure is compared with the time series plot presented earlier for the Derwent at Derwent Bridge which covers the period up to 2008, it does seem that high winter nitrate peaks are unlikely. However, it must be remembered that the lower reaches of the River

Derwent catchment have been in a nitrate vulnerable zone since 2002, so the consistent reduction in the severity of the annual winter nitrate peaks might simply be reflecting the success of the NVZ policy. The EA could therefore be justified in assuming that the substantial winter peaks in nitrate concentrations could resume if the imposition of NVZ status did not continue. However, the issue that remains unresolved in cases such as this one where the justification for failing status is questionable is whether oxidized N inputs from waste water treatment works and nitrate-N inputs originating from atmospheric pollutant deposition have been taken adequately and appropriately into account. There remains room for debate over these crucial points.

CHAPTER 9: SUMMARY OF MAIN FINDINGS OF THE THESIS, GENERAL CONCLUSIONS, AND SUGGESTIONS FOR FUTURE RESEARCH

9.1 The origins, aims, and successes of the research reported in this thesis

On moving to York to study for a PhD supervised by Professor Cresser, it seemed sensible to design a project that was complementary to the activities of other members of his research group who were devoting their time to improving understanding of the nitrogen cycle. The group was, and is, interested especially in the fate and impacts of atmospheric pollution on terrestrial ecosystems, and how impacts on soil systems subsequently have an impact also upon surface waters. This involved selecting topics, based upon an initial literature search and discussions with my supervisor, where it was hoped that I could make a significant contribution to understanding how the N cycle works without excessively overlapping the research of other group members. Therefore, in the following discussion, their research is also briefly mentioned where appropriate.

9.1.1 Chapters 1 and 2

As discussed in Chapter 1, a literature review rapidly revealed that the N cycle is highly complex, with many established facts known about it but sometimes apparently conflicting research results too. Therefore, with the resources available to complete a PhD at the University of York in the available timescale, it was essential to focus primarily of one ecosystem type close to York. Acid grassland at Hob Moor was selected, as discussed in Chapter 2, because it provided a diverse range of soil textures and hence drainage characteristics and pH values, because it was conveniently close for field sampling, and because it was known (Cresser, personal communication) that concentrations of ammonium-N in an adjacent small stream were unexpectedly high. However, later the author gave in to the need to do at least one study (in Chapter 6) using tree litter, because it has hoped that this would complement the research of another group member who was investigating the effect

on nitrate mobility of incorporation of grassland litter into soil (Riaz, personnal communication).

9.1.2 Chapter 3

No members of the group were investigating the effects of soil drying and rewetting, apart from Muhammad Riaz (Riaz, personal communication), who only planned drying periods of up to 3 days in some of his intact core microcosm experiments. As mentioned in Chapter 1, therefore, Chapter 3, and to some extent Chapter 4 which is discussed later, were instigated by the idea that climate change would increase occurrence, and probably extent, of periods of drought in the UK, and hence result in more drying and re-wetting cycles that would almost certainly disrupt the N cycle and potential transport of N to surface waters. A literature search on this topic suggested that previous studies had been based upon before-and-after measurements for one or more drying/wetting cycles, so it was decided that it would be worthwhile to measure how extractable N changed at intervals over an extended period of drying and then also at intervals over a period after rewetting, to fill a gap in knowledge.

This decision proved to be excellent, because the results for extractable ammonium, and even more so for extractable nitrate, were surprising. Before starting the experiment, the author thought it highly probable that ammonification and nitrification would continue for a few days, progressively slowing down and then stopping. It was thought that plots of extractable ammonium-N and nitrate-N versus time over the drying period would both reach plateaus, as stored mineralized-N species would be extracted but no more mineralization of N was occurring; however, it was thought that these plateaus would not necessarily coincide in time, if ammonium supply started to impact upon nitrification rate.

In practice, ammonification apparently continued over the 42-day duration of the experiment, and was still apparently occurring at a significant rate. It can only be speculated here that some components of the microbial population (but probably not nitrifiers!) were extremely tolerant of drought conditions, still functioning under near total desiccation conditions. These then could explain the immobilization of nitrate seen in the latter part of the drying phase of this experiment.

The significance of these results is that the results of other drying-wetting cycle experiments on the N cycle will change, possibly quite dramatically, with the duration of drying period. It would be interesting to see if the nitrate immobilization after extended drying periods occurred elsewhere. It could clearly be ecologically highly beneficial under desert conditions for maintaining N supply in such ecosystems.

9.1.3 Chapter 4

When the work for this thesis was being planned, Cresser had just returned from a short period of research at Monash University in Australia, and gave a seminar on work he had been doing there with Deletic and her colleagues on the use of large boxes of vegetated soil for treating urban runoff to remove nitrate, phosphorus and heavy metals. He had come up with the idea in Australia that nitrate flushes after prolonged dry periods were primarily due to sudden removal of nitrate stored in soils as they dried out, rather than an incredibly rapid flush of nitrification. He postulated that the latter would be a slower effect. He had been able to model this processes (Cresser, unpublished results), but had no experimental data to confirm that his assumptions about how the N cycle was functioning during drying out were correct when he was developing the model. The idea of Chapter 4 was to test this hypothesis. Fortunately, the results in Chapter 3 allowed selection of an appropriate drying period of 6 days for the study in Chapter 4, because at 6 days stored nitrate-N would be close to a maximum value for the test soil used.

The results confirmed the hypothesis that stored nitrate was flushed out fairly quickly, and would contribute significantly to the first flush of nitrate from urban runoff treatment systems seen when a heavy rain storm follows a few days of very dry conditions. The flush due to nitrification in Chapter 4 followed much more slowly; the ammonium-N spiking showed that the production of nitrate was not ammonium limited, and that the nitrifier activity was still increasing at day 9. The results in Chapter 4, and in the paper that resulted from it (Mian et al., 2008), confirmed the finding that the ammonium pool continues to increase over an extended period of drying, because the soil extractable ammonium-N concentrations were much higher from dried soils than from the field- moist soils. The author

believes that the findings are also important in more natural ecosystems, and should be considered in the development of models for predicting temporal variations in nitrate concentrations in catchment runoff, or indeed in models that attempt to predict nitrate passage to groundwater.

9.1.4 Chapter 5

As mentioned in Chapter 1, the research in Chapter 5 was triggered by a need to explain why ammonium-N was apparently being mobilized at unexpectedly high concentrations into a stream that runs beside Hob Moor. Measuring ammonium absorption/desorption characteristics in soils over a range of depths was an appropriate way to test the idea that ammonium-N might be more mobile in soil solution that the author and (based upon my literature survey at the start of Chapter 5, most soil scientists apart from my supervisor, believed.

The problem when the research idea was first envisaged was that, experimentally, it required a large number of samples and sub-samples to be processed extremely quickly, because it was widely perceived that N speciation in samples may change dramatically during storage, even for quite short periods. In the author's view, the results would only be meaningful, however, in terms of their intended use for assessing risk of ammonium leaching from soils to surface waters at Hob Moor, if the experiments were performed with field-moist Hob Moor soils. This was made possible in an innovative way by allowing the author to use groups of second year environmental science students for two days to collect and process the large number of samples in a short period of time. The author, however, started several weeks in advance of the time-tabled classes, so that he could design and test the experimental protocol. He subsequently closely supervised the students when they were performing the field sampling and the laboratory experimentation, before analyzing all the filtrates for ammonium-N and nitrate-N himself.

The results of the absorption isotherm experiments, which were published in Environmental Pollution (Mian et al., 2009), very conclusively demonstrated that ammonium is mobile within, and potentially from, the soils at Hob Moor, regardless of texture and potential drainage status, but that there were differences in mobility

between surface and sub-surface soils between the more acid and freely draining soils and the more poorly draining soils.

This finding is important in a number of contexts. At Hob Moor, from personal observations, it is clear that Marston Moor Drainage Board has difficulty in keeping the stream, which is part of the flood protection system for York, clear of aquatic vegetation. It appears that ammonium inputs to the stream are likely to be facilitating eutrophication, and these may be originating from atmospheric inputs of ammonia and ammonium and from animal excrement on the parts of the Moor used to graze cattle for part of the year under the Moor management scheme. The results also suggest that much more attention should be paid to the point made by Heathwaite et al., (1990, 1993) that ammonium leaching might be a potential problem from intensively grazed grasslands. It must be pointed out though that ammonium was still very mobile in the un-grazed, freely draining soils at Hob Moor.

9.1.5 Chapter 6

The research in Chapter 6 was stimulated by the author's developing interest in the potential research benefits of viewing the N cycle from the perspective of the Gaia hypothesis after he had contributed to a joint paper with his supervisor on this topic (Cresser et al., 2008). The results clearly showed the importance of litter with a low C:N ratio to the immobilization of nitrate in winter months. Although several hypotheses formulated in the chapter were, and must remain, speculative, the author nevertheless thinks that this a potentially important contribution to understanding how atmospheric N pollutant deposition may be causing biodiversity change, as mentioned in Chapter 1.

Even though not every hypothesis could be unequivocally proven in this brief preliminary experiment, when the results are considered alongside those of (Riaz et al., 2010 a and b) which have shown that incorporation of grass litter from Hob Moor into soil also dramatically reduces winter nitrate leaching, they become very relevant to policy makers who are trying to reduce nitrate concentrations in UK rivers. The ban on straw burning in England and Wales, introduced at the end of 1992 (Nicholson et al., 1997; Turley et al., 2003), may have played a not insignificant role

in reducing nitrate concentrations seen in Rivers such as the Derwent in North Yorkshire, as described in Chapter 8.

9.1.6 Chapter 7

With the wisdom of hindsight, the research in Chapter 7 should perhaps have been completed before any of the work in Chapters 2-6 was even started. In reality, however, it was stimulated by the author's observations of unexpectedly high variations in ammonium and nitrate concentrations between replicates for a few of his experiments, regardless of how careful he had been with pre-washing apparatus, and the desire to know more prior to doing any more research on N species transformation. This suggested the need to investigate how soil sample preparation and storage conditions impacted upon the results of determinations of mineral N species in field moist soils to gain insight into how stable soil N speciation was. In other words, how fast do samples need to be processed and how safe is it to store them overnight prior to analysis if they are, or are not, kept in a refrigerator. For this experiment too my supervisor arranged for me to get access to a group of second year environmental science students for a 2-day practical session. This was the only conceivable way that very tight processing time constraints could be complied with. The students were supervised closely throughout by the author.

The study showed that significant changes, especially in nitrate concentration, occurred over 16 h under refrigerated conditions, especially in grassland sub-soils, and these were reflected in total mineral-N levels. Surprisingly, storage at room temperature for the same time caused no significant additional net nitrate production. Extractable ammonium concentrations often fell significantly during storage, especially in woodland soil samples, probably via microbial immobilization as well as nitrification. In the paper that emerged from the study (Mian et al., 2010b), it was suggested that volumetric sub-sampling in the field and immediate addition to a known volume of KCl solution might be more reliable than methodology currently employed by many soil scientists. This was the most important conclusion from Chapter 7.

When the findings were first considered they caused the author momentarily to panic. This fortunately faded rapidly when the nature of the earlier experiments

was considered. For the time series plots in Chapters 3 and 4, storage of field moist soils was not an issue at all. For the work in Chapter 5, samples were all processed very rapidly because of the use of a large group of students, so changes during storage in mineral N speciation should have been minimal. Nor would storage time have been an issue in the simulation experiment in Chapter 6. Therefore the cautionary note that emerges from the research in Chapter 7 should be a take-away message to the author and others for future research.

9.1.7 Chapter 8

Chapter 8 reported the results of an investigation of the spatial and temporal variations in nitrate-N concentrations in the River Derwent in North Yorkshire from the perspective of its having been declared a nitrate vulnerable zone. It was hoped that research from preceding chapters would help to explain temporal trends found in 20-year runs of data that had kindly been supplied by the Environment Agency. In the Chapter, and the paper published in Science of the Total Environment that emerged from it (Mian et al., 2010a), some justification was found to support, or at least to explain, the Environment Agency and DEFRA decision to declare the whole of the Derwent catchment a nitrate vulnerable zone, though it would be easy to support a contrary viewpoint on the basis that farmers are unlikely to revert to previous poor practices, and, where their contribution to nitrate pollution is small, are being penalized for discharge of effluent from sewage treatment works.

It was found that winter nitrate leaching has declined over the past decade in agriculturally impacted parts of the catchment, but this could not be explicitly attributed to the ban on straw burning and increase subsequently in straw and residue incorporation. It is suggested that greater farmer awareness of environmental issues, better animal husbandry, higher fertilizer and energy costs, and the foot and mouth outbreak may all have contributed to the trends observed. Interestingly, in agriculturally impacted areas, summer minima are now higher than in past decades, and that could be due to greater retention of nitrate in winter enhancing nitrate leaching in the following summer.

9.2 Suggestions for future research

The results reported suggest a number of possible avenues for future research. The drying/re-wetting experiments described in Chapters 3 and 4 throw some light on what is occurring, but it would be interesting to investigate the changes in the nature of the microbial population over time. It is speculated in the thesis that some microbial species are much more tolerant of desiccation, and it would be interesting to test this idea further, possibly using soils from the edges of a range of desert regions to study the N cycle in such soils. In any case it would be important to extend the experiment reported in Chapter 4 to more soils, to see how variable the position of the nitrate maximum during drying was in other soils.

It would be interesting to extend the absorption isotherm experiments described in Chapter 5 to soils from areas which have been much less subject to atmospheric N deposition than North Yorkshire. It seems probable that decades of high levels of N deposition would change the nature of the soil organic matter, which could be modifying absorption desorption characteristics. No attempt was made in the present research to see whether the solubility of organic matter, assessed by measuring DOC, would help to explain differences in the absorption isotherms between soils. This could also be a fruitful avenue for research.

One aspect of my research that was very beneficial was the use of moderately large groups of closely supervised, but suitably experienced, students to allow the processing of large numbers of samples. This allowed some experiments to be performed which would otherwise have been impossible because of time constrains for sample preparation. The author found this to be extremely valuable in his research, and more consideration should be given to exploiting such a valuable resource in the University context in future.

Finally, after Chapter 7, it was suggested that volumetric sampling might be the best way forward for assessing potential plant-available N in soils. If this is done, moisture content can be measured on separate volumetric soil sub-samples, so results could still be expressed on an oven dry weight basis if deemed desirable. That said, plant roots explore volumes of soil, not masses of soil!

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