An assessment of the representation of moorland nitrogen sinks in static critical load models for freshwater acidity

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ABSTRACT

The context of the thesis is the requirement for critical load models for total acidity (sulphur plus nitrogen) to freshwaters. It has long been recognised that nitrogen leaching into acid-sensitive upland waters is widespread, and the contribution of nitrate associated acidity to freshwater acidification and the prevention of recovery is increasing in relative importance as sulphur deposition and leaching decline in response to international emissions reductions, while total nitrogen deposition remains relatively constant. What is less certain is that nitrate leaching could increase in absolute terms even under constant deposition. The widely-used static critical loads model for freshwater acidity, the First-order Acidity Balance (FAB) model, employs a number of default values for key nitrogen processes which provide sinks for deposition inputs of nitrogen. The model is used to determine critical loads of sulphur and nitrogen for use in international integrated assessment modelling to aid policy decisions on emissions reductions for sulphur and nitrogen within Europe.

Application of the FAB mass balance to catchments with known input-output budgets for nitrogen shows that there is a very large difference between current leaching of nitrate into surface waters, which is generally a small proportion of nitrogen inputs, and that predicted at steady-state by the FAB model. The aim of this thesis is to experimentally test the assumptions of the FAB model for four moorland catchments at which the major nitrogen fluxes have been measured, across a gradient of total nitrogen deposition in the acid-sensitive UK uplands. In particular, the degree to which the representation of nitrogen immobilisation and denitrification processes are appropriate for these ecosystems is assessed by a combination of field measurements and laboratory experiments. The relative importance of mineralisation and nitrification in catchment soils as controls on nitrogen retention and leaching is tested through laboratory incubations of soils, and the implications of their absence from the FAB model are discussed. Finally, two methods for the determination of nitrogen saturation status, using stable isotopes ($^{15}$N) and soil carbon/nitrogen ratios, are applied, in an attempt to determine the effects of nitrogen accumulation on the terrestrial catchments and evaluate the assumptions of nitrogen saturation and enhanced leaching of nitrate at steady-state which are implicit in the FAB model.
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SECTION I

INTRODUCTION AND CRITIQUE OF FAB MODEL
CHAPTER 1

CRITICAL LOADS FOR FRESHWATERS
AND THE NITROGEN PROBLEM
1. CRITICAL LOADS FOR FRESHWATERS AND THE NITROGEN PROBLEM

1.1 The impacts of acid deposition

Acid deposition originating mainly from the oxidised sulphur compounds released by fossil fuel combustion has long been recognised as an environmental problem on an international scale (e.g. Odén, 1967). The acidification of soils and waters, and subsequent damage to sensitive ecosystems, from forest die-back to the loss of fisheries, has been linked to atmospheric pollution in wide-ranging studies spanning many countries (e.g. Drabløs & Tollan, 1980; Mason, 1990; Howells, 1995).

Oxides of sulphur (S) and nitrogen (N) may dissolve in rainwater to form acids, which is deposited onto soils, vegetation and surface waters as acid precipitation. Cloudwater droplets may contain very high concentrations of these acid anions and their associated protons (H\(^+\)), which can contribute significantly to acid inputs in upland areas. Dry deposition of the acid gases NO\(_2\) and SO\(_2\) directly onto terrestrial surfaces may occur. Ammonia may dissolve in rain or be absorbed directly by plants, and may generate acidity through various biological processes.

Soil acidification may occur when H\(^+\) enters the soil via atmospheric deposition and displaces the base cations (calcium, magnesium, sodium and potassium) naturally present on the ion-exchange complex of soils. The base cations are then leached out of the soils, accompanied by the acid anions sulphate (SO\(_4^{2-}\)) and nitrate (NO\(_3^-\)), into groundwater or surface waters (lakes, rivers and streams). The store of base cations in soils, one measure of which is called base saturation, may decrease if base cations are removed more quickly than they are replaced by geological weathering processes, and this is a broad definition of soil acidification. Since base cations are required for plant growth, their removal and the accompanying acidification of soils can have adverse effects on plant health.

The acidification of lakes and streams occurs when soils can no longer buffer the incoming acids by exchanging H\(^+\) for base cations. As the base cation store is
depleted, H\(^+\) may instead mobilise aluminium, or may leach directly into surface waters with its associated acid anions. Both H\(^+\) and particularly dissolved aluminium species can be toxic to aquatic life and lead to the loss of certain acid sensitive organisms and a decline in biodiversity. Early work in Scandinavia was prompted by concerns about the decline in salmonid fish stocks in certain rivers and lakes which was attributed to the effects of acid deposition.

Although the loss of sensitive fish species like salmon and trout can be the most obvious impact of surface water acidification to the public, the definitive link between acid deposition and lake acidification in the UK was first demonstrated by the study of the microscopic algae, diatoms. These algae are ubiquitous in freshwaters and different species vary in their tolerance to acidity. They are also well preserved in lake sediments, so that the fossil species assemblage is indicative of historical lake water pH. Changes in the fossil diatom assemblage through time in dated lake sediments were convincingly used to demonstrate that the decline in pH of acidified lakes began during the early Industrial Revolution in the 19\(^{\text{th}}\) Century and continued to decline in parallel with the increasing use of the fossil fuels that are responsible for S deposition (Flower & Battarbee, 1983; Battarbee \textit{et al.}, 1985). Hence the major cause of surface water acidification was widely acknowledged by industry and by governments.

1.2 The transboundary nature of acid deposition and the critical loads concept

While many national governments recognised the need for measures to reduce atmospheric pollution, there was a specific problem in that acid emissions can travel great distances, which means that pollution generated in one country can potentially cause damage in other countries. Unilateral measures to reduce acid emissions will not necessarily confer equivalent reductions in acid deposition within national boundaries. The United Nations Economic Commission for Europe (UNECE) recognised that international co-operation was required to tackle the problem, and set up the Convention on Long-Range Transboundary Air Pollution (CLRTAP) in 1979.
Under the CLRTAP, international strategies were negotiated for the control and abatement of S emissions. The first international agreement brokered under the CLRTAP adopted an arbitrary, fixed reduction for emissions; in 1985 the first "Sulphur Protocol" proposed a reduction of S emissions by at least 30% from 1980 levels (UN, 1985). It was recognised that a problem with this approach was the arbitrary nature of emissions reductions; there was no indication of whether these reductions were sufficient to prevent acidification damage or whether they were being applied in the appropriate areas where such damage was a problem (Bull, 1991). The next step was the development of an "effects based" approach whereby acid deposition could be linked scientifically to measurable damage through the development of dose-response functions. Such an approach was used in the negotiation of the Second Sulphur Protocol, signed in Oslo in 1994 (UNECE, 1994). The Oslo Protocol was underpinned scientifically by critical loads models linking S deposition fluxes to selected measures of environmental damage (Bull, 1995). Critical loads have been defined in various ways, but the most widely used is that of Nilsson and Grennfelt (1988), who defined a critical load as:

"a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge."

The models require a defined dose-response function between the pollutant (here, acid deposition) and an indicator organism. If the pollutant load exceeds the critical load, then significant harmful effects will occur, although the timescale for damage is not specified. The critical load definition is therefore grounded in the concept of sustainability.

1.3 Empirical critical loads of S for freshwaters

Critical loads models for S were used to produce national and international maps of both critical loads, indicating the most acid sensitive areas, and critical load exceedances, showing the regions where acidification impacts on specified receptors were potentially greatest (e.g. Kämäri et al., 1992, 1993; Henriksen et al., 1992;
Langan and Wilson, 1994; Sverdrup et al., 1994; CLAG, 1994). The maps introduced an "effects-based" method for spatial targeting of emission abatement measures, thereby providing a useful policy tool at the international level.

For freshwaters, the Steady-State Water Chemistry (SSWC) model was used to generate critical load and exceedance maps of S for Scandinavia and the UK (Henriksen et al., 1992; CLAG Freshwaters, 1995). The SSWC model was employed because of its modest data requirements, needing only water chemistry data and an estimate of annual runoff to calculate the critical load, with an estimate of S deposition to determine whether the critical load was exceeded. The key to the simplicity of the model is the assumption that the SO$_4^{2-}$ anion is mobile in catchments (Seip, 1980; Henriksen, 1984), so that input fluxes of SO$_4^{2-}$ as acid deposition will equal output fluxes of SO$_4^{2-}$ in the runoff from a catchment. Empirical relationships are employed to derive the original, pre-industrial leaching rate of base cations from a lake or stream catchment using only measured water chemistry (Henriksen et al., 1992). If a critical acid neutralising capacity (ANC) value is selected from known relationships with biological response, it can be converted into a critical flux in catchment runoff and used to derive the long-term critical load (Henriksen et al., 1992). Details of the model are provided in Appendix 1.

1.4 The increasing importance of nitrogen

While S deposition declined dramatically through the 1970s and 1980s across much of Europe, scientists in various countries had begun to notice that NO$_3^-$ concentrations in acid-sensitive lakes appeared to be increasing from their very low "pristine" levels (e.g. Grennfelt & Hultberg, 1986; Brown, 1988; Henriksen & Brakke, 1988; Sullivan et al., 1997). During the same period, emissions of oxidised N from vehicles and industry along with ammonia emissions from intensive agriculture had at best remained constant, but in many areas had increased (INDITE, 1994). The S problem was being addressed through the negotiation of international agreements to reduce emissions, so concern was re-directed towards the potential effects of N deposition. Since both oxidised and reduced forms of N can contribute to acidification, in the latter case via nitrification, the concern was that if N deposition increased and led to
greater concentrations of NO$_3^-$, the chemical recovery of freshwaters could be slowed down or even reversed. Previously it had been assumed that most acid-sensitive upland catchments were N limited in the terrestrial ecosystem, so that significant NO$_3^-$ leaching was unlikely to become a problem (e.g. Henriksen et al., 1992) except perhaps during spring snowmelt (Grennfelt & Hultberg, 1986). However, the process of N saturation was beginning to be recognised and associated with increases in NO$_3^-$ levels observed in both long-term monitoring sites (temporal trends) and regional or national surveys (increasing spatial extent of significant NO$_3^-$ levels).

1.5 Temporal changes in N leaching

It had long been known that N leaching into surface waters followed a seasonal pattern partly because of leaching pulses associated with snowmelt and partly because of the biological demand for the nutrient N in the terrestrial ecosystem (INDITE, 1994; Reynolds & Edwards, 1995). However, in the late 1980s and early 1990s the concept of N saturation was described (Ågren & Bosatta, 1988; Skeffington & Wilson, 1988; Aber et al., 1989; Stoddard, 1990; Aber, 1992; Dise and Wright, 1995). The hypothesis states that in N limited terrestrial ecosystems, atmospheric N deposition can be assimilated in terrestrial biomass, but that as N accumulates in the system it becomes less and less limiting until a point is reached where biological N demand can be met entirely by internal supply and some other factor becomes limiting (see Section 1.9 below). In this situation, atmospheric N deposition is no longer retained within the terrestrial ecosystem, and N “breakthrough” occurs, so that NO$_3^-$ leaching into surface waters increases. This process does not necessarily occur suddenly; NO$_3^-$ leaching might increase gradually as biological demand declines, until the leaching of a large proportion of atmospheric inputs occurs.

Certain long term datasets in Scandinavia (Norway and Sweden - Dickson, 1986; Henriksen, 1988; Finland - Lepistö, 1995) and North America (Catskill Mountains, USA - Stoddard, 1991; Murdoch & Stoddard, 1992) showed increasing trends in surface water NO$_3^-$ concentrations, which seemed to support the idea of cumulative N saturation. The potential importance of N saturation and increased NO$_3^-$ leaching had therefore gained widespread recognition by the mid-1990s.
However, more recent analyses of trends in water chemistry at sites across Europe and North America in the International Co-ordinated Programme on Assessment and Monitoring of Acidification of Rivers and Lakes (ICP Waters) found that while earlier trend analyses had suggested a greater number of increasing NO$_3^-$ trends than decreasing, many of these had now ceased (Stoddard et al., 1999; Skjelkvåle et al., 2001a). A separate analysis of trends over the period 1982 – 1994 in north-eastern U.S. lakes found little evidence of trends in NO$_3^-$ (Stoddard et al., 1998), and no trends were found for NO$_3^-$ in acid sensitive headwater lakes in Finland over the period 1987-1998 (Mannio, 2001), or in a large number of acid-sensitive Canadian lakes monitored from 1988-1996 (McNicol et al., 1998). Decreasing trends in lakewater NO$_3^-$ over the period 1984-1995 were found in several Czech forest lakes (Veselý et al., 1998), and the only lake to show a trend in NO$_3^-$ over the period 1983-1996 in a study of Californian high mountain lakes showed a decrease (Sickman & Melack, 1998).

Similarly varied trends in NO$_3^-$ were found across Europe within the RECOVER 2010 project (Germany - Alewell et al., 2001; UK Acid Waters Monitoring Network - Evans et al., 2001; Galloway, Scotland – Ferrier et al., 2001; Scotland - Harriman et al., 2001; Central Europe - Kopáček et al., 2001; Plynlimon, mid-Wales – Neal et al., 2001; Scandinavia - Skjelkvåle et al., 2001b; European streams - Wright et al., 2001). As many sites were found with decreasing trends as with increasing trends since the 1980s. Only in some Italian lakes was clear evidence of rising NO$_3^-$ concentrations found (Mosello et al., 2001; Rogora et al., 2001). This overall lack of a general trend was suggested to be the result of opposing factors; the increasing N saturation status of catchments in the context of declining N deposition in some parts of Europe (Wright et al., 2001).

In a review of evidence for temporal trends of NO$_3^-$ leaching in UK upland waters, no increasing trends were found in the limited datasets collated (INDITE, 1994). However, a later analysis of 10 years of water chemistry data from 1988-1998 in the UK Acid Waters Monitoring Network (AWMN) showed increased NO$_3^-$ trends at 6 out of 22 sites, potentially indicating the possible onset of N saturation at these sites (Evans & Monteith, 2000; NEGTAP, 2001). Climatic factors linked to the North
Atlantic Oscillation (NAO) have been suggested as the possible causes of the observed patterns in NO$_3^-$ leaching, so the trends have to be interpreted with caution (Monteith et al., 2000). The roughly decadal timescale of this climatic driver means that data series of more than 10 years duration may be required to detect trends in water chemistry. Indeed, a further trend analysis with an extra 2 years of monitoring data for the same set of AWMN sites found that only 1 of the 6 sites which showed positive NO$_3^-$ trends was still doing so, although 2 different sites were now showing this trend (Evans & Monteith, 2001). A similar caveat related to possible climatic influences on water chemistry was attached to the positive trends in NO$_3^-$ found after 5 years of monitoring in the UK Environmental Change Network (ECN: Miller et al., 2001).

It is evident that there are very few datasets of sufficiently long timescales to say with any certainty whether there are real long-term trends in surface water NO$_3^-$ that are related to changes in atmospheric deposition rather than other drivers, such as climatic effects. There is, however, evidence that NO$_3^-$ can be an important acid anion in surface waters, and this becomes even more apparent when wider, regional datasets are considered. Furthermore, it is possible that N deposition may exacerbate climatically driven variations in surface water acidity.

### 1.6 Spatial extent of N leaching

With the growing concerns about the possibility of increased N leaching, national datasets were analysed in several countries and it was found that while SO$_4^{2-}$ was by far the most important acid anion in upland water bodies, NO$_3^-$ leaching was also significant. In a UK study, Allott et al. (1995) found that NO$_3^-$ concentrations were significant in many upland catchments where non-atmospheric sources could be ruled out, while a Norwegian study found that lake NO$_3^-$ concentrations in the Sørlandet region of southern Norway had doubled between surveys in 1974/75 and 1986 (Henriksen et al., 1988). It was apparent that if SO$_4^{2-}$ concentrations declined substantially in line with emission reductions of S, and if N emissions increased or N saturation occurred, then NO$_3^-$ could potentially become the most important acid anion in many surface waters.
1.7 The need for critical loads models for S and N

The Oslo Protocol ensured that measures would be adopted for the abatement of S emissions across Europe, but more recent efforts were directed towards the negotiation of a multi-pollutant, multi-effect protocol which would also account for the potential acidifying effects of N deposition. The importance of increased NO$_3^-$ leaching had been demonstrated in the studies described above.

While the assumed mobility of the SO$_4^{2-}$ anion in catchments enables the conversion of deposition fluxes into surface water runoff fluxes, the same assumption cannot be made for N species. The complex biological interactions affecting the transport of N (and hence its associated H$^+$) through catchments, and the presence of mechanisms for the removal or long-term storage of N in catchment soils and vegetation (Aber et al., 1989; Stoddard, 1994), mean that it is not possible to use a single set of empirical relationships to link deposition and surface water chemistry. Instead, a process-oriented, mass-balance approach is required which can be used to quantify the proportion of total N deposition which is transported through the terrestrial part of a catchment into surface waters along with its associated acidity, therefore leading to a reduction in ANC.

One model adopting this approach which has been used for the derivation of linked critical loads for N and S is the First-order Acidity Balance (FAB) model (Posch et al., 1997; UBA, 1996; Henriksen, 1998). The FAB model employs a simple charge balance for N and S, along with the original base cation leaching rate from the SSWC model (see Appendix 1), to construct a "critical load function" (CLF) which quantifies the deposition reduction requirements for either N or S. FAB is a steady-state model with very modest input data requirements compared with dynamic models. A detailed description of the model and its applications is provided in Chapter 2.
A requirement for the FAB model is the parameterisation of steady-state rates of the key N retention processes, and this has largely been based on simplified models and literature default values for several processes. Application of the FAB model to regional and national datasets using these literature-based default terms (e.g. Curtis et al., 1998, 2000; Kaste et al., 2002) indicates that NO\textsubscript{3} concentrations are predicted to increase substantially from current levels, and could significantly offset any recovery anticipated from reductions in S deposition.

The FAB model provided linked critical loads for S and N deposition which were used in the negotiation of the multi-pollutant, multi-effect protocol for emissions reductions under the auspices of the UNECE, signed in Gothenburg on 1\textsuperscript{st} December 1999 and thereafter known as the Gothenburg Protocol. The importance of the model and its predictions is therefore clear, but there are large uncertainties associated with the simplified representation of N sinks and export fluxes within the model. These uncertainties form the focus of this thesis.

1.8 Overview of the terrestrial N cycle

Before looking in detail at the structure of the FAB model it is useful to consider the overall terrestrial N cycle in a simplified upland ecosystem. There have been many attempts to schematically represent the N cycle, with varying degrees of complexity or simplification and with different emphases depending on the aspect of the system which is of primary interest (e.g. Skeffington & Wilson, 1988; van Miegrot & Johnson, 1993; INDITE, 1994; Stoddard, 1994; Ferrier et al., 1995). A general schematic for moorland systems is presented in Figure 1.1.

The focus in Figure 1.1 is on the inorganic N pools (NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-}) which are, at least superficially, of greatest interest to critical loads modellers. Inputs to the NO\textsubscript{3}\textsuperscript{-} pool include direct deposition inputs and nitrification, while outputs potentially include immobilisation (storage as organic N in soil organic matter), denitrification, plant uptake or leaching below the rooting zone. Of these fluxes from the NO\textsubscript{3}\textsuperscript{-} pool, only denitrification and leaching provide direct routes for export of inorganic N from the catchment.
Figure 1.1: Schematic diagram of the terrestrial N cycle

(Adapted from van Miegrot and Johnson, 1993 and INDITE, 1994)
Inputs to the NH$_4^+$ pool may include direct deposition inputs, mineralisation of organic N from litter and the soil organic pool and inputs from livestock or other animals in urine and faeces. Fluxes out of the NH$_4^+$ pool include immobilisation in soil organic matter, plant uptake, volatilisation and leaching, although the latter two fluxes are assumed to be negligible for upland systems (INDITE, 1994). Nitrification transfers N from the NH$_4^+$ pool to the NO$_3^-$ pool, although some may be lost from the system in oxidised, gaseous forms during this process.

The N cycle entails the continual transformation of N from inorganic to organic forms and back again, but at any particular time the fluxes into and out of the organic N pool will not involve the same N atoms. The organic N pool is generally much larger than the inorganic pool (Ågren & Bosatta, 1988; INDITE, 1994). Plant uptake of NH$_4^+$ and NO$_3^-$ may be a major flux, while microbial and abiotic immobilisation of inorganic N can also entail very large gross fluxes. Fixation of atmospheric N$_2$ can be important in certain systems, but is assumed to be negligible in the British uplands; a flux of just 1 kgN ha$^{-1}$ yr$^{-1}$ has been suggested for such systems without leguminous plants or alder trees (INDITE, 1994).

Much organic N stored in plant biomass is physically returned to the soil organic N pool via plant death and litterfall, but some is also mineralised during microbial decomposition, converting organic N back into NH$_4$-N and closing a loop in the N cycle. Of course the schematic separation of the plant and soil pools is artificial, since plant roots are physically contained within the soil matrix; root death therefore entails a notional transfer from the plant to the soil organic N pool without requiring any physical movement of organic N.

Fluxes of organic N out of the system are not of intrinsic interest for modelling acidification and critical loads, but they may provide indirect routes for the export of inorganic N inputs which have been assimilated into biomass. In a forest system, logging and timber removal would represent a flux of organic N out of the system or catchment, but in moorland systems there is no harvesting of this type. However, grazing can represent a net flux of N out of the system, since only a proportion of the N consumed is returned in urine and faeces; some is also assimilated by the animal as it grows and gains weight. Removal of animals therefore entails a net removal of
organic N from the system; the loop is only closed if the animal dies in the catchment and decomposes. In moorland systems, management practices which involve burning (e.g. grouse moors) remove organic N in smoke and ash; only a proportion is redeposited within the catchment.

While this representation of the N cycle seems rather complex, gross simplifications are still required. For example, the representation of soil organic matter as a single box omits the distinctions that could be made between, say, the microbial biomass and refractory organic matter in soil humus. Organic N pools could be subdivided into labile and refractory components (e.g. Cosby et al., 1997) that participate in different processes to different degrees. Figure 1.1 does, however, provide an overview of the complexities involved in the N cycle and a context for the derivation of mass balance models for N. For modelling acidification of surface waters, it is the processes that control the leaching of inorganic N, primarily as NO$_3^-$, that are of greatest interest.

1.9 The concept of N saturation

1.9.1 Definitions of N saturation

While N budgets indicate that a large proportion of inorganic N inputs to terrestrial ecosystems is generally retained within the plant-soil system (e.g. Aber, 1992; Murdoch & Stoddard, 1992; Stoddard, 1994), the quantity of N in the system cannot increase indefinitely. In N limited systems, biological demand for N as a nutrient may be primarily responsible for its retention, but there has to be a theoretical limit of N inputs above which supply exceeds biological demand (i.e. some other factor becomes limiting; Skeffington & Wilson, 1988; Ågren & Bosatta, 1988). This theory leads to the concept of N saturation.

There have been several definitions of N saturation, based on absence of growth response to N inputs from vegetation, the initiation of NO$_3^-$ leaching into surface waters, and the equivalence of N inputs and losses (no N retention) (Aber, 1992). All describe stages of declining N retention within an ecosystem. Many of the processes in Figure 1.1 may contribute to N retention, including plant uptake, immobilisation via microbial
uptake and incorporation into soil organics, cation exchange, and abiotic incorporation of mineral N into soil organics.

N saturation has been defined as:

“the availability of ammonium and nitrate in excess of total combined plant and microbial nutritional demand (excluding the use of nitrate as a substrate for denitrification)” (Aber et al., 1989).

By this definition N saturation could be identified simply by the accumulation of mineral N in soils or by increased leaching below the rooting zone. This definition was proposed as the accepted working definition for critical loads work (Grennfelt & Thörmelöf, 1992). According to Aber et al. (1989), N saturation is therefore reached when its availability (from deposition and mineralisation) exceeds the biotic uptake capacity of the system, and this usually results from water, phosphorus or sometimes light limitation.

Kämäri et al. (1992) state that N saturation is often taken to mean a permanent change in the functioning of the N cycle, from virtually closed internal cycling to a more or less open cycle where the excess N is leached from the system. However, elevated NO$_3^-$ in streams does not necessarily imply N saturation in the terrestrial ecosystem, and N saturation may not always be accompanied by elevated NO$_3^-$ leaching. Furthermore, the onset of N saturation may not be sudden, but may occur as a gradual set of changes in ecosystem processes (Aber, 1992).

Other definitions have been discussed by Tamm (1991) and in the INDITE Report (INDITE, 1994). The definition of Nilsson (1986) states that N saturation occurs when primary production is not further increased by increased N supply. Ågren and Bosatta (1988) use the term to describe ecosystems where N losses approximate or exceed inputs measured over long periods, indicating an excess of N. They further differentiate between “saturation of the uptake capacity”, whereby very large fertiliser N applications exceed the immediate uptake capacity of the system, and cumulative N saturation, whether by small or large inputs, in which the internal cycling of N becomes saturated. Skeffington and Wilson (1988) pointed out that such definitions do not account for
potential changes in vegetation communities which may be regarded as deleterious effects.

Tamm (1991) noted that the temporal variations in NO$_3^-$ leaching usually observed in forest streams make the use of a definition based on export relative to inputs problematic. Furthermore, a saturated system may lose an appreciable proportion of its excess N through denitrification rather than leaching to surface waters. He therefore concurred with the assertion of Skeffington and Wilson (1988) that authors should always provide their own definition. He considered N saturation to require both the satisfaction of the physiological N demand of primary producers and elevated NO$_3^-$ leaching.

The cumulative process of N saturation described by Ågren and Bosatta (1988) has been broken down into arbitrary stages by various authors, and two of the most widely used are summarised below.

1.9.2 Stages of terrestrial N saturation

Stages in the development of N saturation for forest ecosystems were described by Aber et al. (1989); brief descriptions are given below.

1.9.2.1 Stage 0: N cycling under N limitation
In the least impacted sites, N cycling continues under N limitation, and small N additions lead to increased net primary production and litter decomposition rates.

1.9.2.2 Stage 1: initial effects of chronic N deposition
Forests that are repeatedly harvested or burned (i.e. continual removal of N) continue to assimilate N as a fertiliser, but unmanaged forests show slow increases in the total N content of the vegetation, litter and soils, with faster decay rates. There is little change in the form of N taken up or in dissolved losses.
1.9.2.3 Stage 2 - N saturation

N saturation is reached when availability exceeds the biotic uptake capacity of the system, i.e. some other factor becomes limiting (see above). The effects of N saturation may include increased foliage N and a resultant decrease in both litter and soil organic matter C:N ratio (see Chapter 8). Increases in soil ammonium (NH$_4^+$) induce nitrification, resulting in increased NO$_3^-$ leaching from soils and gaseous losses through nitrification or denitrification, with a large reduction in biomass. Overall, the system no longer functions as a sink for N, but converts N inputs into surface or groundwater NO$_3^-$, possibly without visible forest decline.

1.9.2.4 Stage 3: forest decline

Excess NH$_4^+$ leads to forest decline through several possible mechanisms, including nutritional imbalances, foliar yellowing, reduced fine-root biomass and increased drought damage. Total net photosynthesis and productivity are reduced. Loss of the forest canopy will increase mineralisation, liberating accumulated pools of N, potentially to the extent that the catchment becomes a net source of N.

1.9.3 Stages of catchment N saturation

The forest N saturation stages of Aber et al. (1989) were re-interpreted in the context of catchment scale N saturation and surface water leaching of NO$_3^-$ by Stoddard (1994). A later scheme was developed for application to water chemistry data, in which NO$_3^-$ concentration thresholds were suggested for sites with at least monthly data (Stoddard and Traaen, 1995). A modified scheme was also provided by the same authors for sites with less frequent water chemistry data. These catchment oriented stages, which assume that NO$_3^-$ leaching (rather than denitrification) is the major export route for excess N, are summarised below.

1.9.3.1 Stage 0

The dominance of forest/microbial uptake results in a seasonal pattern of NO$_3^-$ leaching, with very low peak concentrations (less than that in deposition) occurring only during...
snowmelt or spring rainstorms. NO$_3^-$ concentrations are <3 $\mu$eql$^{-1}$ for more than 3 months of the growing season, with a peak value of <20 $\mu$eql$^{-1}$.

1.9.3.2 Stage 1
The seasonal pattern of NO$_3^-$ leaching is amplified and prolonged. Surface water NO$_3^-$ concentrations during leaching episodes may exceed those in deposition through preferential elution from melting snow or elevated mineralisation and nitrification in soils. NO$_3^-$ concentration is <3 $\mu$eql$^{-1}$ for up to 3 months of the growing season, or if below this threshold for more than 3 months, has a peak value of >20 $\mu$eql$^{-1}$.

1.9.3.3 Stage 2
Further delays in the onset of terrestrial N limitation result in a greater amplification and extension of the seasonal NO$_3^-$ leaching period. Baseflow NO$_3^-$ concentrations are elevated, approaching those found in deposition. Year round leaching of NO$_3^-$ is therefore observed, but a strong seasonal pattern is still apparent. NO$_3^-$ concentration is never <3 $\mu$eql$^{-1}$ but is < 50 $\mu$eql$^{-1}$ for more than 3 months of the growing season.

1.9.3.4 Stage 3
The catchment becomes a net source of N with elevated mineralisation and nitrification leading to surface water NO$_3^-$ concentrations that often exceed those in deposition. The seasonal pattern in NO$_3^-$ leaching breaks down as there are no longer any strong terrestrial sinks for N. High NO$_3^-$ concentrations are therefore observed throughout the year. NO$_3^-$ concentration is <50 $\mu$eql$^{-1}$ for less than 3 months of the growing season.

1.9.4 N saturation and critical loads

The transition between the stages of N saturation in the two schemes described above may have two elements. First, increasing N deposition levels could lead to movement from one stage to the next (or beyond), depending on the magnitude of increase and the characteristics of the N cycle in the impacted system. With high enough deposition inputs, the immediate saturation of the uptake capacity described by Ågren and Bosatta (1988) could, in theory, occur. In practice, though, upland systems in the UK
are unlikely to experience sufficiently high inputs of inorganic N for this process to occur.

The major importance of increases in N deposition is much more likely to be an accelerated accumulation of N in the system, which has implications for the other aspect of the N saturation process. This second element of N saturation is the gradual accumulation of N in the system even under a constant (anthropogenically elevated) N deposition load, which leads to the cumulative progression through the stages of N saturation described above. It is this latter process which is of greatest relevance to acidification modelling in upland systems. For static critical loads modelling, the eventual, steady-state leaching rate of acid anions (here, NO$_3^-$) under a given deposition load has to be quantified. A further requirement for dynamic models of acidification is an understanding of the rate at which the N saturation process will occur for a given level of deposition and the timescale to steady-state.

It is clear that an understanding of the processes that control terrestrial N saturation is fundamental for modelling the fate of N deposition and its impact on surface water quality. The assumptions which have to be made about the steady-state rates of key N cycling processes in models such as FAB provide the key issues which this thesis will attempt to address.

Most research on terrestrial N saturation has focused on forests because of concerns about productivity (e.g. Aber et al., 1989; Aber, 1992; see section 1.9.2 above), but these systems are relatively scarce in the British uplands, where acid grassland and heather moorlands are much more widespread. Furthermore, pan-European studies on the N dynamics of managed forest systems have been carried out, such as NITREX, which produced a special issue of the journal Forest Ecology and Management (see overview in Wright & van Breemen, 1995), but few similar studies have been carried out on moorland systems. The commercial importance of managed forests and the broad evidence for potential adverse effects on productivity prompted this wide body of research, but no such concerns have been raised about moorland systems, where the main issues are more related to terrestrial ecological change and conservation. Hence it was decided that this thesis should focus on moorland systems which are
more representative of the acid-sensitive UK uplands and less well understood than forests in terms of N dynamics.

1.10 Aims and structure of the thesis

While evidence for the progression of surface waters through stages of N saturation is equivocal, NO$_3^-$ is already an important acid anion in some areas. Furthermore, the assumption of N saturation at steady-state is implicit in the critical loads model of acidity for freshwaters, FAB. In general terms, the aims of this thesis are therefore:

1. to critically evaluate the terrestrial component of the FAB model approach using process-based studies,
2. to propose modifications to existing models if necessary,
3. to ascertain the value, utility and limitations of steady-state critical load models for total acidity, and
4. to compare the approach adopted in dynamic models of surface water acidification.

The thesis is structured to address these general aims by ending Section I with a detailed description and critical review of the FAB model formulation in published applications (Chapter 2). Comparison of predicted with measured leaching fluxes of acid anions into surface waters reveals the magnitude of current and modelled sinks for inorganic N inputs, which are then compared with values from similar studies in the literature. Perceived uncertainties in the FAB model formulation are highlighted, relating to the relative importance of denitrification as a sink for N, immobilisation, mineralisation and nitrification as controls on N leaching, and the dynamics of the N saturation process in moorland catchments. The questions posed by these uncertainties form the basis of the experimental work in Sections II - IV, where actual measurements of N fluxes and assessments of key processes and indices of N saturation are provided through a combination of field and laboratory experiments.

In Section II, four study catchments are characterised, and N input-output budgets over the course of an experimental “budget year” in the field are described. Chapter 3 explains the criteria for the selection of study catchments and soils across an apparent
gradient of N deposition, N saturation and NO₃⁻ leaching. Input-output budgets for N are established through the following exercises: measurement of bulk deposition inputs, comparison with modelled deposition inputs from national datasets, an assessment of the importance of grazing outputs and the calculation of leaching fluxes of N in surface waters. Soilwater chemistry, soil moisture and soil temperature are measured and their links to the overall catchment N budgets assessed. These data provide the basis for the comparison of FAB model predictions of leaching outputs with current measurements, and are crucial for the interpretation of other data on N cycling and saturation. Chapter 4 provides an assessment of the current and potential importance of denitrification as a sink for N in catchment soils. Field measurements of N₂O fluxes provide estimates of current denitrification rates. These field data are complemented by the experimental testing of potential denitrification rates in the laboratory under various conditions of moisture, temperature and N availability. Thus it is possible to compare the FAB model representation of denitrification with actual and potential rates in study catchment soils.

In Section III, the roles of other key N cycling processes which might exert some control on current and future inorganic N leaching but are not explicitly specified in the FAB model are established through the testing of catchment soils in the laboratory. Potential mineralisation and nitrification rates in incubated soil cores are assessed as indicators of denitrification and NO₃⁻ leaching in Chapter 5. The roles of these processes in explaining the current behaviour of inorganic N in catchment soils are discussed. Chapter 6 describes the use of a stable isotope tracer (¹⁵N) to establish the immediate fate of atmospheric N inputs and estimate short-term rates of N uptake and immobilisation during the course of the budget year. These data provide an indication of the degree to which deposition inputs interact with, and are controlled by, the soil-vegetation system in the short-term.

Section IV deals with the process of N saturation and its measurement, as an explanation of the discrepancies between current observations and future predictions of N fluxes. In Chapter 7, the distribution and natural abundance of the stable isotope ¹⁵N is quantified and assessed as an indicator of the N saturation status of the different soils and their associated vegetation. In Chapter 8, the C:N ratio of organic surface
soils is assessed as an indicator of N saturation for moorland systems, and its potential use in modelling applications is considered.

Finally, in Section V, Chapter 9 synthesises the key findings of previous chapters and provides a general qualitative assessment of the FAB model in the context of the current fate of N deposition inputs, measures of N saturation, and the probability of FAB model predictions being realised. Recommendations for the representation of key N processes in static and dynamic models are provided, and the key areas of uncertainty requiring further work are highlighted.

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CHAPTER 2

CRITIQUE OF PROCESS-ORIENTED APPROACHES
2. CRITIQUE OF PROCESS-ORIENTED APPROACHES

2.1 The requirement for a process-oriented, mass balance approach

With critical loads models for S it was assumed that the $\text{SO}_4^{2-}$ anion is mobile in catchments, and therefore S deposition would quickly reach steady-state with leaching into surface waters (Seip, 1980), taking anything from weeks to decades in soils with the greatest adsorption capacity (Reuss & Johnson, 1986). The concentration of non-marine $\text{SO}_4^{2-}$ in the lake or stream water could, in theory, be linked directly to the deposition loading. While certain processes might cause time-lags in reaching steady-state, most notably S adsorption in soils, these processes were considered short-term and ignored over the longer timescale in critical load models (Henriksen et al., 1992). In general, measurements of non-marine $\text{SO}_4^{2-}$ leaching fluxes should be reasonably closely correlated with deposition input fluxes. This assumption has been verified in several studies, for example Curtis et al. (1998).

For S deposition, the response in $\text{SO}_4^{2-}$ leaching to surface waters is therefore relatively fast. This does not mean that damage occurs immediately, because cation-exchange processes in soils generally provide a degree of buffering against surface-water acidification. While the $\text{SO}_4^{2-}$ anion is mobile, the accompanying protons can displace base cations from exchange sites in the soil, thereby causing soil acidification but lessening the degree of water acidification (Reuss & Johnson, 1986). In acid deposition, non-marine $\text{SO}_4^{2-}$ is accompanied by an equivalent quantity of protons, but after undergoing cation exchange in the soil, a proportion of the protons are replaced by base cations. Some of the base cations removed from the soil exchange complex are replaced by weathering. It is the pre-industrial leaching flux of base cations from weathering (plus a background non-marine deposition flux) that forms the basis of the SSWC critical load, since these are the only sustainable source of base cations in the long term. Hence soils with low weathering rates will be drained by surface waters with low SSWC critical loads and will also be susceptible to rapid acidification since base cations are removed faster than they are replenished.

Following cation exchange in soils, only the remaining proportion of protons leached into surface waters contribute to an immediate decline in ANC, while base cation
concentrations are actually increased. It may take several decades for this ion-exchange process to be exhausted (Reuss & Johnson, 1986) and the degree of freshwater chemical change predicted by, for example, the SSWC model, to be realised, but at least the response in terms of \( \text{SO}_4^{2-} \) leaching is much more rapid. Only dynamic acidification models can provide a timescale for the decline in soil base saturation and the corresponding decrease in surface water ANC under a constant deposition load and leaching rate of S.

When only sulphur-based acid deposition is considered, there are therefore two responses as a steady-state between deposition and leaching is achieved:

1. \( \text{SO}_4^{2-} \) may be partially adsorbed onto the soil complex, but this is usually considered a small and short term response, so that the \( \text{SO}_4^{2-} \) leaching flux quickly reaches the value of the deposition flux.

2. Exchange of protons from acid deposition with base cations from the soil exchange complex leads to a reduction in the leaching flux of protons, but an increase in the leaching flux of base cations. This process occurs over a finite timescale which is largely independent of the steady-state between S inputs and outputs. Dynamic modelling usually indicates a timescale of the order of decades for the attainment of steady state between acid inputs and proton / base cation leaching outputs (Cosby et al., 1985a).

For N the situation is much more complex, since deposition inputs enter the terrestrial N cycle (see Chapter 1). Terrestrial processes can remove or immobilise N deposition over very long timescales or even permanently. For example, denitrification returns N to the atmosphere as \( \text{N}_2 \text{O} \), NO or \( \text{N}_2 \) and permanently neutralises the associated acid inputs. Microbial cycling of N can lead to immobilisation in refractory organic matter, again retaining the associated protons.

Catchment input-output budgets generally indicate that only a small proportion of N deposition is leached into surface waters with its associated protons (e.g. Curtis et al., 1998). If the retention of a fixed proportion of N deposition represented a steady-state situation then there would be no problem modelling the effects of N deposition and setting a critical load for N. For example, if the current retention rate for N is 90%, it might be assumed that as the N deposition load changes, 10% of the new load will
still be leached into surface waters, and this would be very easy to model. The SSWC model was adapted to take account of NO$_3^-$ leaching by using measured NO$_3^-$, converted into a flux using runoff, as a measure of the contribution of N deposition to critical load exceedance (Kämäri et al., 1992). This method makes no reference to actual N deposition, and hence cannot take account of possible changes in NO$_3^-$ leaching under a different N deposition scenario. Another critical load model, the diatom model for total acidity, assumes that the ratio of N to S in deposition dictates the ratio of N to S in surface waters (Allott et al., 1995), thus allowing the model to take account of changes in N deposition, but making the baseless assumption that the relative proportion of NO$_3^-$ leached would not change.

However, studies of terrestrial N dynamics indicate that a process of N saturation can occur, whereby N accumulates in the soil-vegetation system until it is present in excess of biological demand and leaching increases, until potentially almost all N deposition is leached (see Chapter 1). It cannot therefore be assumed that N leaching under a fixed deposition load will remain constant. Furthermore, it cannot be assumed that if the N deposition load changes, the proportion of N leaching will remain constant. This process of N saturation takes place over a timescale which is largely independent of the processes which affect S leaching and its associated cation exchange processes, except for the contribution of the protons associated with N to cation exchange reactions in the soil. N therefore adds a further dimension to the problem of defining a steady-state situation.

A steady-state critical loads model for total acidity which can be used for scenario testing therefore requires the quantification of N retention processes over the very long term. If the sustainable (as opposed to short-term) rates of N retention or removal can be quantified, then the critical load can be determined. In the formulation of process-oriented models such as FAB, best available knowledge (in terms of published data) has been used to derive a steady-state mass balance for N. Several key retention processes have been identified: denitrification, N immobilisation, N removal in vegetation (grazing, forestry, burning) and in-lake retention. The long-term, steady-state rates at which all these key processes operate for a given deposition load of N (and the equivalent terms for S) must be quantified if a critical load of total acidity is to be defined.
2.2 General description of the FAB Model

The basic outline of a mass balance model for freshwater critical loads was provided in the first draft manual for critical loads prepared by the UNECE (Anonymous, 1990). It was proposed that the potential acidifying effects of N deposition should be accounted for by the introduction of a term \((\Delta C_N)\) for the net acidity produced by N uptake and nitrification. These processes were included in the MACAL and PROFILE models for forest soils and the leachate from them (Anonymous, 1990).

The first published derivation of a mass-balance model for the definition of critical loads of S and N specifically for freshwaters was presented at the Lokeberg workshop in 1992 (Kämäri et al., 1992) and was based on an earlier formulation from 1991, which was not in print until after the workshop (Posch et al., 1993; Kämäri et al., 1993). The 1991 formulation of the model, called the “steady-state mass balance model for lakes”, employed separate mass balances for S and N in order to provide a critical load for each. The definition of separate critical loads of S and N relied upon the calculation of the “sulphur factor”, called “\(\sigma\)”, which is the proportion of the net flux of acid anions to the lake provided by S. Hence the separate critical loads for S and N could only be calculated for a given level of S and N deposition, i.e. the critical load was deposition-dependent. In this precursor to the FAB model, denitrification and N immobilisation were ignored (Posch et al., 1993; Kämäri et al., 1993), while the vegetation uptake of S and base cations were explicitly included.

By the time of the Lokeberg workshop, the mass balance for N had been expanded to incorporate a term for N immobilisation in humus \((N_{imm}\) - not described further) and a rate-dependent term for denitrification. While this improved model, now called the First-order Mass Balance (FMB) Model, still relied upon the use of the “sulphur factor” to determine separate critical loads for S and N, an alternative formulation was also provided which considered the sum of S and N balances together in order to avoid the deposition-dependence of the critical load (Posch et al., 1993). The FMB model reformulation could not provide individual critical loads for S or N; instead it provided the basis of what later became known as the critical load function, and was renamed the First-order Acidity Balance (FAB) model (UBA, 1993; Henriksen et al., 1993). These
first descriptions of the FAB model included a term for organic N export, which was to disappear from the model when it was described in a journal publication (Posch *et al.*, 1997a). In fact during the same year as the FAB model was first published, the formulation excluding organic N export was published in the CCE Status Report on critical loads mapping in Europe (Downing *et al.*, 1993).

With the FAB model, a charge balance incorporating the major processes affecting the acid anion budget for the lake and catchment is invoked (Posch *et al.*, 1997a):

\[ N_{\text{dep}} + S_{\text{dep}} = \left\{ f N_{\text{upt}} + (1-r)(N_{\text{imm}} + N_{\text{den}}) + r(N_{\text{ret}} + S_{\text{ret}}) \right\} + AN_{\text{leach}} \]

\( N_{\text{dep}} = \) total N deposition  
\( S_{\text{dep}} = \) total S deposition  
\( N_{\text{upt}} = \) net growth uptake of N by forest vegetation (removed by harvesting)  
\( N_{\text{imm}} = \) long term immobilisation of N in catchment soils  
\( N_{\text{den}} = \) N lost through denitrification in catchment soils  
\( N_{\text{ret}} = \) in-lake retention of N  
\( S_{\text{ret}} = \) in-lake retention of S  
\( AN_{\text{leach}} = \) acid anion leaching from catchment  
\( f = \) fraction of forested area in the catchment  
\( r = \) lake:catchment area ratio

All units are expressed in equivalents (moles of charge) per unit area and time. Braces enclose "internal" catchment processes, i.e. those terrestrial and in-lake processes which operate on acid anion inputs to control the net export in catchment runoff.

The charge balance equates the deposition inputs of acid anions with the sum of processes which control their long term storage, removal and leaching exports. Several major assumptions are made in this formulation:

1. long term sinks of S in the terrestrial part of the catchment (soils and vegetation) are negligible,
2. there are no significant N inputs from sources other than atmospheric deposition, i.e. no fertiliser application in the catchment,
3. \( \text{NH}_4^+ \) leaching is negligible because any inputs are either taken up by the biota, adsorbed onto soils, or nitrified to \( \text{NO}_3^- \).

The FAB mass balance for N is shown schematically in Figure 2.1. Comparison of this diagram with the schematic diagram of the N cycle in Figure 1.1 shows the degree to which the FAB model simplifies the N cycle and considers only the major input and export fluxes.

The acid anion balance of the FAB model can provide the critical leaching rate of acid anions (critical \( \text{AN}_{\text{leach}} = L_{\text{crit}} \)) which will depress ANC below the pre-selected critical value (\( \text{ANC}_{\text{crit}} \)) as in the SSWC model (Henriksen et al., 1992; see Appendix 1). Therefore, at critical load, \( \text{AN}_{\text{leach}} \) can be substituted as:

\[
L_{\text{crit}} = B_{\text{crit}} - \text{ANC}_{\text{crit}}
\]

which gives the same formulation of the FAB model as described in the latest version of the mapping manual (UBA, 1996). Note that the correct formula for the charge balance is given here, rather than the erroneous balance in Posch et al. (1997a) where \( \text{Alk}_{\text{leach}} \) (the equivalent of \( \text{ANC}_{\text{crit}} \) here) is added to rather than subtracted from the right hand side of the equation.

The internal catchment processes affecting acid anion budgets introduced by the FAB model are linked to vegetation cover, soil type and catchment morphology. In order to calculate critical loads for S and N, the sink terms for acid anions have to be quantified, as described below.

2.2.1 \( \text{N}_{\text{apt}} : \text{net growth uptake (in forest)} \)

The only permanent sinks for N in the biomass occur when there is some form of harvesting or removal of biomass from the catchment. Short term, seasonal processes which lead to biological uptake of N during the growing season do not form a net sink
for N over the long term, since the N content of biomass is subject to biological
cycling. There is no net assimilation of N within catchment biomass over the long
term, because it is released on the death of plants through decomposition and
mineralisation.

Figure 2.1: Schematic representation of the FAB model mass balance for N
(arrows = major fluxes, boxes = N pools, 3D boxes = long term N sinks)

The main route for the removal of biomass from upland catchments is through the
harvesting of forest and the removal of wood from the catchment. If there are
estimates available for the N content of the relevant tree species in a catchment, and if
the time period of the harvesting cycle is known, a long-term figure for the export of
N can be derived. Ideally, forestry data (species, age, Yield Class) should be used to
determine N removal rates on a local basis.

There are other possible routes for the removal of biomass from catchments; in grazed
or burned moorland catchments there may be small net losses of N from other types
of catchment vegetation in terms of weight gain of removed livestock and losses in
smoke. If figures are available for N losses through these processes, they can be incorporated into the long-term N mass balance (e.g. INDITE, 1994).

Since N uptake and biomass removal processes are specific to certain vegetation types, the derivation of the mass balance requires both the N removal/loss rate per unit area, and the area of each specific vegetation type within the catchment. In the standard formulation of the FAB model, only forest harvesting is considered so the proportion of the catchment covered in forest (f) is required.

2.2.2 \( N_{\text{imm}} \): long term immobilisation of N in catchment soils

Estimates of the long term immobilisation of N in different soil types have been derived by the analysis of total N content of soil profiles, which is divided by the age of the profile (often assumed to be approximately 10,000 years since the last glaciation) to determine the annual immobilisation rate. A suggested long-term range of net immobilisation rates (including N-fixation) is 2-5 kgN ha\(^{-1}\) yr\(^{-1}\) (Downing et al., 1993, UBA, 1996), although it is noted that under present conditions with high growth due to elevated N deposition this range may extend to a much higher figure. Since N immobilisation rate varies with soil type, the relative proportion of the catchment covered by each soil type is required to determine the mean value for soils in the whole (terrestrial) catchment. Note that the term ‘I-r’ is used to weight the mean immobilisation rate by the terrestrial part of the catchment only, because in-lake retention processes are considered separately.

2.2.3 \( N_{\text{den}} \): input-dependent denitrification

It is assumed that N immobilisation and growth uptake are faster processes than denitrification (Posch et al., 1997a), and since denitrification is assumed to be linearly related to the net input of N into the soil system, it can be estimated as a fraction of net input as follows:

\[
N_{\text{den}} = f_{\text{de}} (N_{\text{dep}} - N_{\text{imm}} - fN_{\text{upt}})
\]
where $f_{de}$ is the "denitrification fraction", with a value between 0 and 1, ascribed to the catchment soils.

Posch et al. (1997a) have argued that peat soils are likely to provide the highest rates of denitrification, and proposed a method for the calculation of a "denitrification fraction". They assumed that in peat soils, denitrification of net N inputs could be 80%, while the minimum denitrification fraction for other soil types would be around 10%. The denitrification rate for a catchment can therefore be estimated by an interpolation of these figures:

$$f_{de} = 0.1 + 0.7f_{peat}$$

where $f_{de}$ is the denitrification fraction, and $f_{peat}$ is the proportion of peat soils within the catchment. Denitrification within any catchment therefore varies between 10% and 80% of net inputs. This approach has been used in several countries around Europe, as recommended by the CCE Mapping Manual (UBA, 1996). With this method, only the proportion of peat soils within the terrestrial catchment is required to determine denitrification fluxes. As for the soil-dependent N immobilisation process, the mean denitrification rate is weighted by the term $1-r$ to account for the terrestrial proportion of the catchment in which the process occurs. Although denitrification can occur within lake sediments, this process is implicitly accounted for in the in-lake retention term.

2.2.4 Input-dependent in-lake retention of N and S

The in-lake retention of acid anions is assumed to be a linear function of the net input of acidity. It is sequentially the final sink of acidity encountered by inputs which move through the catchment. Net in-lake retention of N is calculated as:

$$r_{N_{ret}} = \rho_N [N_{dep} - f_{upt} - (1-r)(N_{imm} + N_{den})]$$
The "in-lake retention fraction" for N (\( \rho_N \)) is calculated from a kinetic equation accounting for water retention time:

\[
\rho_N = \frac{S_N}{S_N + \frac{Q}{r}} = \frac{S_N}{S_N + \frac{Q}{r}}
\]

where \( S_N \) is the mass transfer coefficient for N, \( \zeta \) is mean lake depth, \( \tau \) is water residence time in the lake, \( Q \) is runoff and \( r \) is the lake to catchment area ratio (Kelly et al., 1987). A similar equation is used to calculate \( \rho_S \), the "in-lake retention fraction" for S.

Literature default values for the mass transfer coefficients of N and S are recommended by the Mapping Manual in the absence of site-specific data, with \( S_N = 5.0 \) m yr\(^{-1} \) and \( S_S = 0.5 \) m yr\(^{-1} \) (Kelly et al., 1987, Dillon and Molot, 1990). The appropriateness of these literature default values was explored in a literature review by Curtis (2001a).

It should be noted that for stream sites, the lake:catchment ratio \( r \) is zero (the surface area of catchment streams is ignored in the calculation of \( r \), since only in-lake processes are assumed to contribute to net retention of S and N). Hence both \( \rho_S \) and \( \rho_N \) are effectively zero.

Most published formulations of the FAB model do not take into account direct deposition onto the lake surface in lake catchments. Instead, it is assumed that all deposition falls onto the terrestrial catchment and is subject to terrestrial retention processes, so that only the non-retained portion of inputs reaches the lake. The significance of this assumption is discussed in Section 2.2.8 below. The most recently published version (Henriksen & Posch, 2001) does take into account direct deposition to the lake surface, but this more complex formulation is not discussed further since the focus of this thesis is on the terrestrial mass balance for N within FAB.
2.2.5 Critical load function for input-dependent denitrification

Using the above methods to derive the terms for “internal” catchment processes, the charge balance provides those combinations of S and N deposition for which $A_{N_{\text{leach}}} = L_{\text{crit}}$, thereby defining the critical load function (CLF).

$$A_N CL(N) + A_S CL(S) = b_1 N_{upt} + b_2 N_{\text{imm}} + L_{\text{crit}}$$

where:

$$A_N = (1-f_{de} (1-r)) (1-\rho_N) \quad b_1 = f(1-f_{de}) (1-\rho_N)$$

$$A_S = 1-\rho_S \quad b_2 = (1-r) (1-f_{de}) (1-\rho_N)$$

This formulation can be used to define the critical load function (Figure 2.2), with the following critical load constraints:

$$CL_{\text{max}}(N) = (b_1 N_{upt} + b_2 N_{\text{imm}} + L_{\text{crit}}) / A_N$$

$$CL_{\text{min}}(N) = (b_1 N_{upt} + b_2 N_{\text{imm}}) / A_N$$

$$CL_{\text{max}}(S) = L_{\text{crit}} / A_S$$

2.2.5.1 CLF for stream sites with input-dependent denitrification

The equations defining the CLF are simplified for stream sites where there is no input-dependent in-lake retention. For stream catchments, both the lake:catchment ratio $r$ and the retention factors $\rho_N$ and $\rho_S$ are zero. The immobilisation and denitrification sink terms apply to the whole catchment, not to the land fraction only $(1-r)$. It is assumed that there is no retention of either S or N within the stream.

Given that in FAB:

$$A_N = (1-f_{de} (1-r)) (1-\rho_N) \quad b_1 = f(1-f_{de}) (1-\rho_N)$$
\[ A_S = 1 - \rho_S \quad b_2 = (1-r) (1-f_{de}) (1-\rho_N) \]

then for streams:

\[ A_N = 1 - f_{de} \quad b_1 = f(1 - f_{de}) \]

\[ A_S = 1 \quad b_2 = 1 - f_{de} \]

which gives the following critical load constraints:

\[ CL_{min}(N) = \frac{(b_1N_{upt} + b_2N_{imm})}{A_N} \]

which becomes:

\[ CL_{min}(N) = fN_{upt} + N_{imm} \]

and

\[ CL_{max}(N) = \frac{(b_1N_{upt} + b_2N_{imm} + L_{crit})}{A_N} \]

which becomes:

\[ CL_{max}(N) = fN_{upt} + N_{imm} + (L_{crit} / (1-f_{de})) \]

For S:

\[ CL_{max}(S) = \frac{L_{crit}}{A_S} \]

becomes:

\[ CL_{max}(S) = L_{crit} \]

which is equivalent to the SSWC model critical load for S (see Appendix 1). The CLF for streams with input-dependent denitrification is illustrated in Fig.2.3. The gradient of the slope is \(1-f_{de}\).
Figure 2.2: Critical load function with deposition dependent denitrification

\[ \frac{L_{\text{crit}}}{A} = \frac{(b_1 N_{\text{sp}} + b_2 N_{\text{sm}})}{A} \]

Figure 2.3: Critical load function for streams with deposition dependent denitrification

\[ \frac{L_{\text{crit}}}{(1-f_{\text{red}})} = \frac{f N_{\text{sp}} + N_{\text{sm}}}{(1-f_{\text{red}})} \]
2.2.6 Critical load function for fixed denitrification rates

The problem with the 'fd' method for determining denitrification is that the resulting denitrification rate may be far higher than measured values, for example in UK soils (Curtis et al., 2000). For peat soils $f_{de} = 0.8$, which implies that 80% of net N inputs (after N uptake and N immobilisation) are denitrified. With total N deposition exceeding 30 kgN ha$^{-1}$ yr$^{-1}$ across some parts of upland Britain (RGAR, 1997), the denitrification rates for unafforested peat catchments, after subtracting the component immobilised in catchment soils (a figure of 1-3 kgN ha$^{-1}$ yr$^{-1}$ is used for Britain), equate to 80% of at least 27 kgN ha$^{-1}$ yr$^{-1}$, i.e. more than 21 kgN ha$^{-1}$ yr$^{-1}$. This value is an order of magnitude higher than observed denitrification values for peat soils in Britain (Emmett and Reynolds, 1996) and five times greater than the recommended maximum value for UK soils (Hall et al., 1997).

It has therefore been proposed that for the UK the FAB model is modified to include the denitrification component as a fixed value for certain soil types, independent of deposition (Curtis et al., 2000). If a fixed value of $N_{den}$ is to be used, then each soil type must be allocated a denitrification value and its proportional cover within the catchment must be quantified, in an identical way to the calculation of mean N immobilisation for the catchment (see British FAB applications described below).

It might, however, be argued that a possible reason for the low rates of denitrification observed in certain soils is that current high immobilisation rates reduce the supply of N for denitrification. If N immobilisation rates decline to the low values recommended by the Mapping Manual (UBA, 1996), the supply of N for denitrifiers would increase and the rate of N removal via this route could rise. The issue provides one of the key questions to be addressed in this thesis.

The new critical load constraints on the critical load function (Fig. 2.4) with a fixed denitrification term are:

$$CL_{\text{max}}(S) = L_{\text{crit}} / L_{\rho_S}$$
\[ CL_{\text{min}}(N) = f N_{\text{upt}} + (1-r)(N_{\text{imm}} + N_{\text{den}}) \]
\[ CL_{\text{max}}(N) = f N_{\text{upt}} + (1-r)(N_{\text{imm}} + N_{\text{den}}) + (L_{\text{crit}}/1-\rho_N) \]

This formulation effectively adds the denitrification component onto the \( CL_{\text{min}}(N) \) part of the CLF, so that the difference between \( CL_{\text{min}}(N) \) and \( CL_{\text{max}}(N) \) is dictated solely by the in-lake retention of N, the only input-dependent parameter for N.

2.2.6.1 The CLF with fixed denitrification for streams

The CLF for streams with a fixed catchment-weighted figure for denitrification is defined by the most simple set of equations:

\[ CL_{\text{max}}(S) = L_{\text{crit}} \]
\[ CL_{\text{min}}(N) = f N_{\text{upt}} + N_{\text{imm}} + N_{\text{den}} \]
\[ CL_{\text{max}}(N) = f N_{\text{upt}} + N_{\text{imm}} + N_{\text{den}} + L_{\text{crit}} \]

Hence:

\[ CL_{\text{max}}(N) = CL_{\text{min}}(N) + CL_{\text{max}}(S) \]

and the gradient of the slope on the CLF is 1 (Figure 2.5).

2.2.7 The CLF and the nature of critical load exceedance

It is not possible to define a single value to represent the critical load of total acidity using the FAB model, since the acid anions \( SO_4^{2-} \) and \( NO_3^- \) behave differently in the way they are transported with hydrogen ions; one unit of deposition of S will not have the same net effect on surface water ANC as an equivalent unit of N deposition. The above sets of equations effectively define the critical deposition loads for S and N individually. \( CL_{\text{max}}(S) \) defines the critical load for S when total N deposition is less than \( CL_{\text{min}}(N) \). In stream catchments this term is equivalent to the SSWC model critical load for S, and for lakes is modified slightly for the in-lake retention of a small
portion of inputs. When S deposition exceeds $CL_{\text{max}}(S)$, the critical load is exceeded by S alone, regardless of the level of N deposition.

**Figure 2.4: Critical load function with fixed denitrification**

**Figure 2.5: Critical load function for streams with fixed denitrification**
The contribution of N deposition to an increase in exceedance over that resulting from S alone is determined by the FAB charge balance. CL\text{min}(N) defines the deposition of total N (NH\text{X} + NO\text{Y}) at which terrestrial catchment processes effectively remove all N, so that deposition loads lower than CL\text{min}(N) result in no net leaching of NO\text{3}. The terrestrial sinks for N are fixed by soil type and forest cover. An important assumption here is that all N deposition is transported through the terrestrial part of the catchment, i.e. in lake catchments there is negligible deposition directly onto the lake surface (see below). CL\text{max}(N) defines the critical load for total N deposition when S deposition is zero. When total N deposition exceeds CL\text{max}(N) the critical load is exceeded by N deposition alone, although critical load exceedance may be further increased by S deposition.

While the precise definition of the various CLF thresholds will vary depending on whether lakes or streams, or fixed or input-dependent denitrification rates, are used, the interpretation of the critical load function is identical. The nature of critical load exceedance for any given pair of S and N deposition values is illustrated in Figure 2.6.

In practice, neither S nor N deposition will ever be zero, so the critical load for the deposition of one species is fixed by the deposition of the other, according to the line defining the unshaded area of the CLF in Figure 2.6 (Posch et al., 1997a). For pairs of S and N deposition values which are located in the unshaded (white) area of the CLF, the site is protected. If the deposition values fall above the CLF, the critical load is exceeded. The options for protecting the site, in terms of deposition reductions, are then dictated by the location of the given deposition values in a particular segment of the CLF (Figure 2.6). The colour coding of the CLF in this way lends itself to the mapping of deposition reduction requirements. However, the use of the CLF in this way can only provide qualitative information, in terms of whether reductions in either N or S deposition, or both, are optional or compulsory to attain non-exceedance. There is no quantitative indication of the amount by which the deposition of either species must be reduced, and indeed this is not possible because of the interdependence of the two species in jointly causing critical load exceedance. The requirement to reduce deposition of either species depends on the status of the other species, i.e. whether it remains constant or changes to a different, known value.
There are only two specific cases where the deposition reduction requirement for a species can be quantified. If the deposition of S is zero, the critical load exceedance and reduction requirement for N can be quantified. Such a situation is, however, extremely unlikely to occur because of the ubiquitous nature of atmospheric S pollution. A more feasible case in this formulation of the FAB model is that N deposition may be less than $CL_{\text{min}}(N)$, implying that all deposited N is retained within the catchment. In such a case, the FAB model would provide a critical load exceedance value for S. This particular case only holds true for the assumption that N deposition directly onto the lake surface is negligible; otherwise some N leaching is inevitable and there is again a situation where both S and N are contributing to exceedance.

Despite the impossibility of quantifying the deposition reduction requirement for either N or S independently, except in the special circumstances above, the FAB model can provide a numerical value for critical load exceedance (see below). This value can only be interpreted as the amount by which the total acid flux (from both S and N) exceeds the critical load, and provides no indication of how site protection (non-exceedance) might be attained.

While the CLF diagram provides a visual indication of the nature of critical load exceedance for a site given any pair of deposition values for S and N, the FAB model actually provides a prediction of steady-state leaching of base cations, acid anions and hence ANC, which determines whether the critical load is exceeded and by how much.

2.2.8 FAB model predictions and quantifying critical load exceedance

The charge balance which forms the basis of the FAB model and the “critical leaching of acid anions” ($L_{\text{crit}}$) term calculated with the SSWC model are based on assumed long-term, steady-state conditions. The FAB model outputs therefore indicate the status of freshwater bodies under a steady-state condition between deposition and catchment processes. In this respect, FAB outputs using current deposition data cannot necessarily be expected to reflect the current chemical status of freshwaters,
because a steady-state cannot be assumed at present with respect to N (Curtis et al., 1998).

Figure 2.6: Schematic interpretation of exceedance on the CLF

While the FAB model is used to derive a critical load function for both S and N deposition, an exceedance of the critical load cannot be expressed in terms of a required reduction in both S and N deposition. It can, however, be calculated as the amount by which the acid anion leaching flux resulting from the specified deposition flux exceeds the critical load for a site.
Critical load exceedance is simply the difference between the predicted leaching fluxes of S+N and the critical leaching flux of acid anions ‘L_{crit}'. For the input-dependent denitrification method (UBA, 1996), exceedance can be calculated as:

\[
\text{Total exceedance} = A_N^N_{dep} + A_S^S_{dep} - b_1N_{upt} - b_2N_{imm} - L_{crit}
\]

Alternatively, for the fixed denitrification method:

\[
\text{Total exceedance} = A_S^S_{dep} + (1-P_n)(N_{dep} - fN_{upt} - (1-r)(N_{imm} + N_{den})) - L_{crit}
\]

An alternative interpretation is that the critical load exceedance calculated in this way indicates the amount by which the leaching flux of acid anions must be reduced, which could potentially be achieved by many different combinations of reductions in S and N deposition. Perhaps a more useful and relevant method of expressing exceedance with the FAB model is the prediction of steady-state ANC, since it is assumed that the biota are responding to this critical chemical parameter. This first requires the prediction of SO_4^{2-} and NO_3^- concentrations with the FAB mass-balance.

2.2.8.1 Prediction of SO_4^{2-} leaching

Since it is assumed that SO_4^{2-} is a mobile anion (Seip, 1980) and that deposition inputs are in steady-state with leaching outputs, it might be expected that measured and predicted fluxes of non-marine SO_4^{2-} will be similar, depending on the uncertainty associated with the measurement of deposition and leaching fluxes. Although there is a deposition-dependent sink for S in the FAB model through in-lake retention processes, this generally accounts for a very small percentage of the S budget and so the difference between measured and predicted fluxes under a given deposition load should be very small.

The predicted, steady-state non-marine SO_4^{2-} leaching flux (S_{leach}) is derived by subtracting the in-lake retention component from deposition inputs:

\[S_{leach} = (1 - \rho_S) S_{dep}\]
For stream sites, where $\rho_S=0$, the predicted leaching flux is therefore equal to the deposition flux. Division by the annual runoff converts the predicted leaching flux into a concentration, which can then be compared with measured values.

2.2.8.2 Predicted increases in $\text{NO}_3^-$ leaching

Most of the processes included in the FAB model are selected to determine the sustainable removal or retention rates of N. It is recognised that current N dynamics within catchments may be dictated by short term elevated rates of certain processes, notably soil immobilisation of N (Dise and Wright, 1995), but these transient processes must be excluded from the long term definition of critical loads (Curtis et al., 1998).

The mass balance employed in the FAB model provides the potential, future $\text{NO}_3^-$ leaching ($N_{\text{leach}}$):

a) Input-dependent denitrification method:

$$N_{\text{leach}} = A_N N_{\text{dep}} - b_1 N_{\text{upt}} - b_2 N_{\text{imm}}$$

which, for streams, reduces to:

$$N_{\text{leach}} = (1-f_{de}) (N_{\text{dep}} - f N_{\text{upt}} - N_{\text{imm}})$$

b) Fixed denitrification method:

$$N_{\text{leach}} = (1-\rho_N) (N_{\text{dep}} - f N_{\text{upt}} - (1-r)(N_{\text{imm}} + N_{\text{den}}))$$

which, for streams, reduces to:

$$N_{\text{leach}} = N_{\text{dep}} - f N_{\text{upt}} - N_{\text{imm}} - N_{\text{den}}$$

For any of the above methods, the leaching flux of $\text{NO}_3^-$ can be converted into a predicted concentration if no change in annual runoff is assumed.
2.2.8.3 Changes in ANC associated with acid anion leaching rates

If acid anion leaching does increase to a new steady-state level in the future, there will be an equivalent decrease in ANC associated with this change, and this is ultimately what the freshwater critical load models use to define exceedance. A site with a predicted ANC of less than 0 μeqL⁻¹ induced by a given acid deposition load is exceeding its critical load when the critical ANC is zero, even though current measured ANC may be greater than zero until steady-state is achieved (Curtis et al., 2001).

The charge balance of the FAB model can be employed to calculate the potential, future ANC at a site under a constant deposition load. Future SO₄²⁻ and NO₃⁻ leaching are derived as above, while the base cation leaching from sites exceeding their critical load is provided by the SSWC model (Appendix 1). The SSWC model assumes that the only permanent, sustainable source of base cations is provided by weathering, so that despite current elevated rates of base cation leaching through cation exchange processes with acid inputs, base cation concentrations under a constant deposition load and runoff will decline to the value of [BC]₀⁺ where the SSWC critical load is exceeded (see Appendix 1). In the FAB model, the potential, future ANC concentration ([ANCₚₒₚₜ]) for exceeded sites is then provided by the difference between future base cation and acid anion concentrations:

$$[\text{ANC}_\text{pot}] = [\text{BC}]_0^+ - (S_{\text{leach}} + N_{\text{leach}})/Q$$

where $S_{\text{leach}}$ and $N_{\text{leach}}$ are derived as above. If the critical load is not exceeded in the FAB model, then the predicted increase in acid anion leaching will be partially offset by increased base cation leaching according to the "F-factor" (Brakke et al., 1990). Such non-exceeded sites will, however, continue to maintain a value of ANC greater than the chosen critical value and are therefore not considered further.

At exceeded sites, [ANCₚₒₚₜ] could be used in conjunction with the dose-response function linking ANC to biological response (e.g. Lien et al., 1992), to predict the ultimate biological status for the indicator organism at steady-state. Used in this way, the FAB model exceedance value has a practical application (e.g. Juggins, 2001).
2.2.9 Direct deposition onto lake surfaces

In the formulations of the FAB model described above, which are based on published applications, no account is taken of direct deposition to the lake surface. This could be a significant problem in the application of the model to lakes where the surface area forms a large proportion of the catchment area.

For S there are no terrestrial retention processes included within the FAB mass-balance so there is no problem, but for N there is an important discrepancy in the model. It is assumed that N leaching into the lake will only occur if N deposition is greater than the value of \( CL_{\min}(N) \). Furthermore, the in-lake retention component of the model is input-dependent, with a fixed proportion (<100%) of net N inputs being retained. This proportion applies equally to direct inputs onto the lake surface. It is therefore evident that a proportion of direct inputs onto the lake surface is not retained within the lake, so some \( NO_3^- \) leaching must occur, because in-lake retention cannot reach 100% of inputs. The implication is that the FAB model should be reformulated to take this into account, and then the horizontal line at \( CL_{\max}(S) \) on the CLF attains a slope. The term \( CL_{\min}(N) \) becomes meaningless, because for any non-zero value of \( N_{dep} \) some leaching of N from the catchment will occur. A further consequence is that there is no region of the CLF where only \( S_{dep} \) needs to be considered, i.e. there is no "reduce S only" class of exceedance unless \( N_{dep} \) is zero. These adaptations were made in a recent reformulation of FAB (Henriksen & Posch, 2001), but are not discussed further since they apply only to the in-lake retention component.

2.3 FAB model applications

2.3.1 FAB applications in Britain

In Britain, it was decided that national critical loads data for freshwaters should be based on the FAB model so that they could be incorporated in the CCE mapping and modelling work at the European scale under the CLRTAP, and in 1995 work began on the collation of the necessary datasets. A first attempt used data collated for 527 "acid-sensitive" sites ([Ca\(^{2+}\)]<300 \( \mu \)eq\( l^{-1} \)) from a national freshwaters survey, where
all the relevant data were derived manually (C.Curtis, unpublished data). Catchment and lake areas were estimated from maps and gridded transparencies by eye, and soil proportions (for f_{peat}) were estimated by eye from soils maps alongside the topographic maps. Forest cover was estimated by eye from the landcover map held digitally on a GIS at ITE Monks Wood, while a fixed value was used for N_{imm}. Results from a FAB model application to this preliminary dataset were submitted to the CCE mapping programme (Posch et al., 1995).

In order to facilitate an application of FAB to all British mapping sites using automated GIS techniques (rather than manual and “by eye” methods) a catchment digitising programme was undertaken. At the same time, as a ‘pilot study’ prior to the national application, the model was to be tested on a small network of lake sites at which high quality chemistry data were being collected. These sites were referred to as the CLAG Nitrogen Network sites, and provided the first datasets on which the FAB model mass balances were tested in Britain (Curtis et al., 1998). Previously, FAB model applications had been published only for Scandinavia.

For both the Nitrogen Network pilot study and the full national mapping applications in Britain, the required catchment data were derived in the following ways. Since the paper by Curtis et al. (2000) was intended to provide a definitive description of the model application in Great Britain, this section draws heavily on the paper.

1. Site catchments and lake outlines were digitised from topographical maps at 1:25,000 scale, from which lake to catchment area ratio (r) was derived.
2. Leaching fluxes from catchments were calculated using catchment area weighted runoff data from the 1km grid annual mean values for the period 1992-94.
3. Soil type and percentage cover were extracted for each catchment using digital catchment outlines and digital soils maps at 1:250,000 scale. The soils data were used to provide catchment weighted estimates of N_{imm} and N_{den} using the values suggested for each soil type in Hall et al. (1997) (Table 2.1). The ‘fixed denitrification’ method was selected in preference to the ‘denitrification fraction method’ in the CCE Mapping Manual.
4. Coniferous forest cover (f) was estimated for each catchment using digital catchment boundaries with the ITE land cover map (Fuller et al., 1994) derived
from satellite imagery at 25m pixel resolution. In the Nitrogen Network study, none of the catchments contained forest and N uptake was therefore set to zero. For national mapping applications, data from published studies were used to provide a default value for N uptake. Emmett and Reynolds (1996) estimated the potential removal of N in thinnings and bolewood during harvesting of Sitka spruce stands of Yield Class 6 to 24 to be in the range 1.0-8.5 kgN ha⁻¹ yr⁻¹ for the UK. These figures assume harvesting at 40 years of age. It is recognised that the annual uptake of N will not be constant throughout the 40 years of forest growth, because there is a link between N uptake and tree age (Emmett et al., 1993, Reynolds et al., 1994).

For national mapping in Britain, a default value in the mid-range of these published data was employed (Nupt = 0.279 keq ha⁻¹ yr⁻¹ or c. 4 kgN ha⁻¹ yr⁻¹) for catchments containing areas of coniferous forest. Other possible routes for N export in biomass (grazing, burning) were assumed to be negligible in the acid sensitive upland catchments of Britain.

5. The national “standard” S and N deposition data were generated at the 20 km x 20km grid scale from measured and interpolated mean annual data in the UK (CLAG Deposition Fluxes, 1997; RGAR, 1997). For S, total wet plus dry, seeder-feeder enhanced, non-marine values are used. For N, total deposition is quantified as the sum of dry (NO₂⁺NH₃), cloud (NO₃⁻, NH₄⁺) and wet (NO₃⁻, NH₄⁺) deposition (CLAG Deposition Fluxes, 1997).

2.3.1.1 CLAG Nitrogen Network sites

Thirteen paired lake and inflow stream monitoring sites were selected along gradients of N deposition and lake-water calcium concentration (as a measure of sensitivity to acidification). All sites were located in non-forested catchments with minimal human disturbance other than atmospheric deposition and low level extensive sheep grazing.

The presence of a major inflow stream for monitoring was a prerequisite for site selection. Lake and stream-water chemistry were sampled monthly over two years, and provided high quality data on mean concentrations of major ions. The sites are listed in Table 2.2.
Table 2.1 CLAG recommended $N_{imm}$ and denitrification rates (Hall et al., 1997)

<table>
<thead>
<tr>
<th>Mapping Soil code</th>
<th>General Soil description</th>
<th>Immobilisation</th>
<th>Denitrification</th>
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<td>$keq , N , ha^{-1} , yr^{-1}$</td>
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Table 2.2: CLAG Nitrogen Network Sites

<table>
<thead>
<tr>
<th>Site number, code and name</th>
<th>Region</th>
<th>N Dep.</th>
<th>[Ca(^{2+})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CZNG83: Lochan Dubha</td>
<td>NW Scotland</td>
<td>0.90</td>
<td>111</td>
</tr>
<tr>
<td>2 CZNN68: Loch Caoldair</td>
<td>Central Scotland</td>
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</tr>
<tr>
<td>3 CZNO16: Loch Beanie</td>
<td>Grampians</td>
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</table>

N deposition units in keq ha\(^{-1}\) yr\(^{-1}\); Calcium concentration in µeq l\(^{-1}\)

Chemical and catchment data (derived as above) from the Nitrogen Network sites were used to test the S and N mass balances in the FAB model. While a near one-to-one relationship was found between non-marine S deposition estimates and leaching outputs, significant N retention was observed (Curtis et al., 1998).

The Nitrogen Network sites show a wide range in N deposition, N leaching and percent N retention, which differ between species (Table 2.3). While reduced N is generally greater than oxidised N in deposition, a tiny fraction of reduced N is leached (average retention 96%), but leaching of oxidised N is very variable. If oxidised N is considered alone, then the percentage retention varies from 96% to -93% (i.e. net source of NO\(_3^-\) in the lake).
Table 2.3: N leaching and retention at Nitrogen Network sites (kgN ha\(^{-1}\) yr\(^{-1}\))

<table>
<thead>
<tr>
<th>Site No.</th>
<th>Deposition</th>
<th>Output</th>
<th>Retention %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH(_x)</td>
<td>NO(_x)</td>
<td>Tot. N</td>
</tr>
<tr>
<td>1</td>
<td>2.9</td>
<td>4.6</td>
<td>7.6</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>3.2</td>
<td>5.8</td>
</tr>
<tr>
<td>3</td>
<td>5.6</td>
<td>5.6</td>
<td>11.2</td>
</tr>
<tr>
<td>4</td>
<td>22.8</td>
<td>14.4</td>
<td>37.2</td>
</tr>
<tr>
<td>5</td>
<td>16.5</td>
<td>9.8</td>
<td>26.3</td>
</tr>
<tr>
<td>6</td>
<td>21.3</td>
<td>12.3</td>
<td>33.6</td>
</tr>
<tr>
<td>7</td>
<td>11.8</td>
<td>5.9</td>
<td>17.6</td>
</tr>
<tr>
<td>8</td>
<td>13.7</td>
<td>8.0</td>
<td>21.7</td>
</tr>
<tr>
<td>9</td>
<td>16.6</td>
<td>11.1</td>
<td>27.6</td>
</tr>
<tr>
<td>10</td>
<td>3.5</td>
<td>6.0</td>
<td>9.5</td>
</tr>
<tr>
<td>11</td>
<td>18.6</td>
<td>11.8</td>
<td>30.4</td>
</tr>
<tr>
<td>12</td>
<td>12.8</td>
<td>6.9</td>
<td>19.7</td>
</tr>
<tr>
<td>13</td>
<td>11.5</td>
<td>6.6</td>
<td>18.1</td>
</tr>
</tbody>
</table>

Mean: 12.3 8.2 20.5 0.5 5.1 5.6 96 42 76 34

At 3 sites, retention is negative (there is a net source of NO\(_3^-\)) and this could be due to nitrification of reduced N, which is almost all retained. If total inorganic N (TIN) is considered, retention varies between 30 and 96%, with a mean value of 76% for all sites.

The FAB model predicts a reduced retention of TIN at all sites, with a mean of 34%. If retention at steady-state were to decline to these levels, the effects on ANC of the enhanced NO\(_3^-\) leaching would be severe: mean NO\(_3^-\) concentration would increase from 17 to 65 \(\mu\)eq l\(^{-1}\), resulting in a mean decrease in ANC of 48 \(\mu\)eq l\(^{-1}\) (Curtis et al., 1998). The potential effects of such a decrease in ANC are very severe; it is therefore of utmost importance to ascertain the probability of this N breakthrough occurring.
2.3.1.2 National FAB mapping application in Britain

For FAB mapping in Britain, a national network of 1470 freshwater sites was selected to represent the most sensitive water body on a 10 km square grid basis (20 km in non-sensitive lowland areas), with lakes selected in preference to streams where possible (Kreiser et al., 1993, Curtis et al., 1996). One-off dip samples were taken during autumn or early spring over the period 1992-94, and provided water chemistry which is assumed to approximate to flow-weighted mean chemistry (Forsius et al., 1992). Chemical analysis was carried out according to the methodology of Harriman et al. (1990). All catchment-specific data were derived as described above and the study is reported fully in Curtis et al., 2000. Since this model application employed the national deposition data which were contemporary with the water sampling period, it provided a comparison of predicted with measured chemistry at the national level.

For non-marine $\text{SO}_4^{2-}$, FAB predictions of leaching under the 1992-94 deposition load correlated well with measured values in sensitive upland areas (Curtis et al., 2000). For $\text{NO}_3^-$, however, leaching was predicted to increase significantly from measured values in the uplands, with a corresponding decrease in ANC leading to critical load exceedance ($\text{ANC} < 0 \mu\text{eq}l^{-1}$) at more than a quarter of sampled sites. The contribution of N to critical load exceedance was much greater for England and Wales than for Scotland, where S deposition was the major cause of exceedance.

As in the study at the Nitrogen Network sites, the national picture of severe future $\text{NO}_3^-$ leaching predicted by the FAB model results from the small size of available long-term N sinks in the model relative to current N deposition rates (Curtis et al., 1998, 2000). The national map of predicted $\text{NO}_3^-$ leaching therefore closely reflects the theoretical distribution of $\text{NO}_3^-$ concentrations resulting from the conversion of total N deposition flux into concentrations using runoff data. The conclusion of the national study, that N emissions reductions are required in addition to S reductions in order to prevent critical load exceedance, depends upon the realisation of the FAB model predictions of $\text{NO}_3^-$ leaching, again highlighting the importance of establishing whether the N saturation and leaching processes will actually occur.
2.3.1.3 FAB and HARM predictions for UK

The FAB model has also been applied to the national freshwaters database for Britain using three deposition scenarios generated with the Hull Acid Rain Model (HARM) (Curtis et al., 1999). Critical load exceedance and changes in three important chemical indicators (NO$_3^-$, non-marine SO$_4^{2-}$ and ANC) were assessed for 1990 baseline deposition levels, planned emissions reductions under existing international commitments (REF scenario), and a potential stringent deposition reduction scenario under the so-called “EU acidification strategy” (E10 scenario). Model outputs indicated that the proportion of sampled sites exceeding their critical load would be reduced by sixty and seventy-three percent respectively under the two future deposition scenarios. Planned reductions in emissions under the REF scenario would protect most Scottish freshwaters, but substantial areas of the English and Welsh uplands would remain exceeded. Most of the required reductions in acid deposition for both Scotland and Wales would be met by the more stringent E10 scenario. In the most sensitive areas of northern England, even greater reductions in both S and N emissions than those described under the E10 scenario are required. While S remained the most important source of acid deposition even after future reductions, freshwaters in sensitive areas could not be protected by abatement of S emissions alone. The study showed the clear need for a strategy to reduce N deposition if British freshwaters in sensitive areas are to be protected.

Since the publication of this work, the Gothenburg Protocol was signed under the auspices of the UNECE CLRTAP. This protocol committed signatory nations to reductions in both S and N deposition, and its effects on critical load exceedances for freshwaters in Britain are assessed in Curtis (2001b).

2.3.2 Scandinavian applications

A Scandinavian exercise in critical loads modelling with FAB was the first to be published (Henriksen et al., 1993) and built on a distinction conceived at the Løkeberg Workshop of 1992, that for a given critical load there was a ‘present exceedance’ and a ‘potential exceedance’. The ‘present exceedance’ is calculated using measured NO$_3^-$ leaching to represent the proportion of N not retained in the
catchment (the SSWC model for total acidity – see Appendix 1), while the ‘potential exceedance’ is based on a consideration of all relevant processes acting as net sinks for S and N, i.e. it employs a process-oriented mass-balance (now called the FAB model). The distinction made here is that current water chemistry represents a steady-state for both S and N and so provides an exceedance for current deposition loads, but the N leaching rate is assumed to change with a change in N deposition so that current values cannot be used. Instead, the ‘potential exceedance’ requires use of the mass-balance approach for testing deposition scenarios where N deposition changes from the present value, to provide the new N leaching rate. This distinction failed to acknowledge the process of N saturation, whereby a deposition load of N maintained at current levels could lead to increased N leaching through time, rendering the concept of ‘present exceedance’ with the SSWC model meaningless.

The mass-balance modelling exercise employed data from Finland (1450 lakes sampled 1987-89), Norway (2305 lakes sampled during surveys in 1986-1990) and Sweden (760 lakes sampled January-March 1990).

In this early application of the mass-balance approach, the N balance incorporated a term for organic N:

\[ N_{\text{leach}} = N_{\text{dep}} - N_{\text{den}} - N_{\text{upt}} - N_{\text{imm}} - N_{\text{ret}} - N_{\text{exp}} \]

where \( N_{\text{leach}} \) is total inorganic N leaching and \( N_{\text{exp}} \) is organic N export. \( N_{\text{exp}} \) was obtained by linear regressions from chemical oxygen demand (COD: N. Finland and Lapland) or total organic carbon (TOC: Norway), or as the difference between total N and total inorganic N (Sweden and the remainder of Finland).

The denitrification fraction method was used to determine \( N_{\text{den}} \), but since no catchment soils data were available for the study, \( f_{\text{peat}} \) was derived from empirical relationships. For parts of Finland, mainly in Lapland, \( f_{\text{peat}} \) was derived from a linear regression with COD; elsewhere it was obtained from a non-linear relationship between \( f_{\text{peat}}, \) TOC and latitude.
For \( N_{\text{lim}} \), data from chronosequence studies of Swedish forest soils were used to derive the range \( 0.2-0.5 \, \text{kgN ha}^{-1} \, \text{yr}^{-1} \), with the higher value being used in this model application. National forest inventory data were used to provide \( N_{\text{upt}} \). In-lake retention was calculated using the default mass-transfer coefficients described above for Britain. A version of the SSWC model modified slightly from the 'standard' of Henriksen et al. (1992) for the determination of pre-acidification \( \text{SO}_4^{2-} \) and \( F \) was used to provide the critical base cation (and hence acid anion) leaching rate. The selected value of \( \text{ANC}_{\text{crit}} \) was 20 \( \mu\text{eq}l^{-1} \).

The study utilised deposition data which were approximately contemporary with the water sampling programmes; Finland used modelled data for 1990, while Sweden and Norway used data for 1992. In this respect, the study could be considered comparable to the British national study (Curtis et al., 2000) where contemporary deposition and chemistry data were used to compare measured with predicted leaching fluxes of acid anions. The Scandinavian study was, however, restricted to the assessment of ‘present’ and ‘potential’ exceedance and deposition reduction requirements, instead of providing a comparison between present and potential \( \text{NO}_3^- \) leaching.

The contribution of measured \( \text{NO}_3^- \) to ‘present exceedance’ was mostly very small, with concentrations of \(<2 \, \mu\text{eq}l^{-1} \) in all regions except southern Norway and southern Sweden, where concentrations exceeding \( 10 \, \mu\text{eq}l^{-1} \) were found. The FAB modelling results show a clear regional pattern in the relative importance of \( S \) or \( N \) deposition. In northern Scandinavia and almost all of Finland, non-exceedance of critical loads could be achieved by reductions in \( S \) alone, while in southern Sweden and particularly southern Norway, additional reductions in \( N \) deposition are also required.

A repeat of the FAB modelling exercise was carried out for Norway in 1998 (Henriksen, 1998), using water chemistry data for Norway from the Nordic Lakes Survey of 1995 (5690 lakes; Henriksen et al., 1998), the Norwegian critical loads database (2315 lakes), a series of monitored rivers (16) and calibrated catchments (6, plus 200 lakes sampled annually) and 6 lakes from the REFISH project. This exercise used the variable-ANC method (Henriksen et al., 1995) whereby \( \text{ANC}_{\text{lim}} \) varies in the range 0-50 \( \mu\text{eq}l^{-1} \) depending on runoff. All other FAB model parameters were
derived as in the wider Scandinavian study described above. Deposition data were weighted means for the period 1988-92, plus a deposition scenario for 2010 based on the Second (Oslo) Sulphur Protocol.

The study concluded that after the second sulphur protocol, N would be the critical factor in determining future acidification. While lakes in forested areas did not yet show any significant N leaching, at lakes in non-forested areas leaching of up to 40-50% had been recorded (Henriksen, 1998). As in the previous Scandinavian study, comparisons of “current” exceedance with present levels of NO$_3^-$ leaching (29% of lakes according to the SSWC model) and “potential” or “future” exceedance at maximum N leaching (44% according to the FAB model) were made.

The FAB model was used to predict potential NO$_3^-$ leaching from Norwegian soft water lakes by Kaste et al. (2002) in an identical way to that used by Curtis et al. (1998). For N$_{imm}$, a rate of 0.5 kgN ha$^{-1}$ yr$^{-1}$ was used as in previous Scandinavian applications of FAB. Uptake of N in forests was based on national inventories which showed a gradient in maximum N$_{upt}$ from north (7 kgN ha$^{-1}$ yr$^{-1}$) to south (3.5 kgN ha$^{-1}$ yr$^{-1}$). Mean N$_{upt}$ was calculated from a regression line based on the relationship between N$_{upt}$ and latitude, giving values of c. 2.1 kgN ha$^{-1}$ yr$^{-1}$ in southernmost Norway and 1.1 kgN ha$^{-1}$ yr$^{-1}$ in northernmost Norway. For in-lake retention, default values of mass transfer coefficients were used as in other Scandinavian applications ($S_N = 5$ m yr$^{-1}$ and $S_S = 0.5$ m yr$^{-1}$). As in an earlier application of FAB to Canadian lakes (Hindar et al., 2000; 2001), different approaches were explored for modelling retention in chains of lakes, treating them either as one “big-lake” or as a “lake system”, and comparing results with those obtained if only the catchment outlet lake was considered (Kaste et al., 2002).

2.3.3 Other published FAB applications

The FAB model was applied to European surface waters included in the ICP Waters programme by Henriksen and Posch (1998). For N$_{upt}$ data were obtained at the European Monitoring and Evaluation Programme (EMEP) 50km grid scale as the mean value for all known within the corresponding grid cell. A fixed N$_{imm}$ value of 1
kgN ha\(^{-1}\) yr\(^{-1}\) was used for all sites, while the peat fraction method was used to calculate denitrification. Default values were used for in-lake retention mass transfer coefficients (\(S_N = 5\) m yr\(^{-1}\) and \(S_S = 0.5\) m yr\(^{-1}\)). The same values for all parameters were used in a similar application of FAB to lakes in the Killarney Provincial Park, Ontario, Canada, except that in this area forestry operations were not carried out, so \(N_{\text{upt}}\) was set to zero (Hindar et al., 2000; 2001).

Ye et al. (2002) applied the FAB model to lakes in China, using the same default values for in-lake retention mass transfer coefficients as in the above studies. The values used for \(N_{\text{upt}}\) and the method used to calculate denitrification were not specified, but a fixed mean value of the lake:catchment ratio of 0.08, based on other studies, was used.

2.3.4 Role of FAB in the European context

The UNECE Working Group on Effects (WGE) employs critical loads and exceedances for use in European integrated assessment modelling carried out under the Convention on Long Range Transboundary Air Pollution (Posch et al., 1999). Critical loads and exceedance maps are also used directly in protocol negotiations. Critical loads data are synthesised into European maps and databases by the Coordination Centre for Effects, based at the National Institute of Public Health and the Environment (RIVM) in Bilthoven, the Netherlands. In preparation for the multi-pollutant, multi-effect protocol which was signed in Gothenburg in December 1999, standard methodologies were developed for the provision of critical loads data, and for freshwaters these required the application of the FAB model.

The FAB model data submitted to the CCE mapping programme over the last 5 years are listed in Tables 2.4 and 2.5 (\(F_{\text{de}}\) = denitrification fraction, \(N_{\text{imm}}\) = immobilisation, \(N_{\text{den}}\) = denitrification and \(N_{\text{upt}}\) = net forest uptake). As of 1999, only Sweden, Finland, Norway, Britain and the Republic of Ireland had submitted FAB data to the CCE. It can be seen that methodologies were modified between the CCE Status Reports of 1997 (Posch et al., 1997b) and 1999 (Posch et al., 1999), with Ireland being the latest country to apply FAB.
Since FAB model outputs are being utilised at this international level for the development and negotiation of protocols to control transboundary air pollution, the need for assessment of model uncertainties is obvious.

2.4 Key uncertainties within the FAB model

The FAB model was developed specifically as a tool for the calculation of linked critical loads for S and N via the critical load function, so that the potential effects of different S and N deposition scenarios on critical load exceedance percentiles could be modelled. Scandinavian applications of the FAB model have tended to use it in this way only, distinguishing between ‘current’ and ‘potential’ exceedance through the comparison of SSWC model exceedance, which uses measured NO$_3^-$ leaching, and FAB model exceedance, which uses modelled NO$_3^-$ leaching. Some of the key uncertainties within FAB have, however, only been categorically demonstrated by its use for the prediction of potential, steady-state water chemistry in British and Norwegian modelling applications (Curtis et al., 1998, 2000; Kaste et al., 2002). Although the model was not developed explicitly as predictive tool for water chemistry, predicted changes in the leaching of base cations, acid anions and hence ANC are implicit in the model. In particular, the magnitude of the potential increase in NO$_3^-$ leaching and associated decrease in ANC from current values which the model predicts reveals the implications of the structure and assumptions of the N budget within the model.

Table 2.4: FAB model inputs into CCE mapping programme in 1997 (Posch et al., 1997b: fluxes in kgN ha$^{-1}$ yr$^{-1}$ where specified)

<table>
<thead>
<tr>
<th>Country</th>
<th>No. sites</th>
<th>Type</th>
<th>$N_{\text{imm}}$</th>
<th>$N_{\text{den}}$</th>
<th>$N_{\text{ap}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finland</td>
<td>1450 L</td>
<td>0.5</td>
<td>$f_{\text{le}}$</td>
<td>forest data</td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>2305 L/S</td>
<td>2</td>
<td>$f_{\text{le}}$: $f_{\text{pe}}$ from TOC /latitude</td>
<td>forest data</td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>3172 L</td>
<td>linked to $N_{\text{le}}$</td>
<td>Sverdrup &amp; Ineson method</td>
<td>balance with BC/P supply</td>
<td></td>
</tr>
<tr>
<td>GB</td>
<td>527 L/S</td>
<td>2</td>
<td>$f_{\text{le}}$: $f_{\text{pe}}$ from soil map by eye</td>
<td>2.5 (conf. by eye from GIS)</td>
<td></td>
</tr>
</tbody>
</table>

Type: L = lake/standing water, S = stream/river
Table 2.5: FAB model inputs into CCE mapping programme in 1999 (Posch et al., 1999: fluxes in kgN ha\textsuperscript{-1} yr\textsuperscript{-1} where specified)

<table>
<thead>
<tr>
<th>Country</th>
<th>No. sites</th>
<th>Type</th>
<th>N\textsubscript{limn}</th>
<th>N\textsubscript{den}</th>
<th>N\textsubscript{upt}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finland</td>
<td>1450</td>
<td>L</td>
<td>1.0</td>
<td>(f_{de})</td>
<td>forest data</td>
</tr>
<tr>
<td>Ireland</td>
<td>175</td>
<td>L</td>
<td>2 or 3 (by soils)</td>
<td>(f_{de})</td>
<td>forest data</td>
</tr>
<tr>
<td>Norway</td>
<td>2305</td>
<td>L/S</td>
<td>2</td>
<td>(f_{de}): (f_{pea}) from TOC/latitude</td>
<td>forest data</td>
</tr>
<tr>
<td>Sweden</td>
<td>2378</td>
<td>L</td>
<td>linked to N\textsubscript{dep}</td>
<td>(f_{de})</td>
<td>balance with BC/P supply</td>
</tr>
<tr>
<td>GB</td>
<td>1445</td>
<td>L/S</td>
<td>1 or 3 (by soils)</td>
<td>1,2 or 4 (by soil type)</td>
<td>4 (conif. only)</td>
</tr>
</tbody>
</table>

Type: L = lake/standing water, S = stream/river

The uncertainties peculiar to the FAB model, rather than those associated with, for example, cation exchange or S adsorption which are common to all freshwater critical load models, are related to steady-state representations of N dynamics. The magnitude of the predicted increases in N leaching from current measured values with the FAB model means that the quantification of uncertainty in the predictions is of great importance. There could be severe implications for prevention of recovery under reduced S deposition, or even a further decline in ANC, if NO\textsubscript{3}\textsuperscript{-} leaching increased. The Gothenburg Protocol incorporates reductions of N deposition as well as S, but while these would serve to delay the onset and magnitude of increased N leaching it is vital to know how effective these measures may be in facilitating long term recovery.

Some of the uncertainties are related to the quality of datasets employed in model applications, for example the use of a single value for N uptake in coniferous forest systems in the UK. It would be possible on a local basis to obtain much better data on, for example, tree species, rotation period and N content of the particular species. These uncertainties linked to data availability at the national scale are beyond the scope of this thesis; instead, the model structure and process descriptions are the main focus. The ability of the model to provide reliable predictions at the catchment scale is the key issue to be addressed. The greatest perceived uncertainties are listed below.
1. Which processes are responsible for the discrepancies between current and predicted N leaching? While it is assumed that very high N immobilisation rates may be occurring at the present time, leading to a decline in C:N ratio and eventually to N saturation, there is currently very little experimental evidence for this in moorland catchments. If N immobilisation does not account for the very high retention of N observed in upland catchments, is denitrification being underestimated? Or is there some other process in operation which has yet to be identified as significant?

2. Is the N immobilisation process adequately understood, particularly for non-forest systems? The dynamics of the N saturation process are not well understood for forest systems, and even less so for moorland systems. Ecological changes can be induced by increased N availability, such as changes in vegetation type and cover. Since it is not possible for the ecosystem to assimilate N indefinitely, there must be a finite, long-term rate of N immobilisation, but how feasible is it to quantify a steady-state figure for this, and are chronosequence studies an appropriate method? Does N immobilisation rate increase with N supply, and if so, is the elevated rate sustainable?

3. How important is the denitrification process and how should it be described? It has been argued that the denitrification fraction method, which can lead to 80% removal of net N supply, is not appropriate for the UK because observed rates are very low. However, this may simply be due to low supply rates if N is being removed or retained elsewhere. If N saturation leads to increased N supply for denitrifiers, will denitrification rates increase? Should the denitrification fraction method be employed for the UK but with revised figures, or is the current method (rate fixed by soil type) the most appropriate? Is there a need to employ a more sophisticated model of denitrification, taking into account temperature and soil moisture?

4. Is the in-lake retention process adequately described? All published FAB model applications have used the same method and default values for modelling in-lake retention, yet the literature figures were derived for a small number of large Canadian lakes. What is the fate of N (and S) “retained” in lakes - sedimentation of refractory organic matter, denitrification or leaching of organic N? If net N supply to lakes increased with N saturation of the terrestrial ecosystem, would in-lake retention become a major sink for N and S? This uncertainty is not addressed further in this thesis, which focuses on the terrestrial mass balance for N.

5. Is the problem for modelling N processes one of great spatial (within-catchment) heterogeneity? It is known that certain processes, for example denitrification, can
occur in soil microsites at greatly elevated rates, so that spatially limited studies could significantly underestimate actual mean rates for a whole catchment. The role of the riparian zone is also not well understood. Similarly, there is some evidence that N content of organic matter, whether in soils or vegetation, can vary greatly over very small spatial scales. The limited spatial data which can be obtained experimentally for modelling may be insufficient for the calculation of realistic acid anion budgets for a whole catchment. This issue is distinct from that of data availability at the national scale, where excellent, high-resolution experimental data may be available locally but cannot be extrapolated nationally.

6. How significant is the problem of temporal variability? The processes which control N leaching are known to vary at scales ranging from decadal or more (climatic) to sub-diurnal. Seasonal variations in NO\textsubscript{3}\textsuperscript{-} leaching related to biological activity are well known, but there are processes operating at much shorter timescales which provide a logistical challenge to measure experimentally. For example, denitrification is known to vary diurnally, being dependent on soil temperature and moisture and therefore driven by both weather and solar radiation. There is a resultant uncertainty in scaling up spot measured data to annual budgets.

7. Can data obtained from studies in forest systems be used for modelling moorland systems? Historically, most terrestrial work on the effects of N deposition has focused on the most economically important component of upland catchments, forests. There are several journals dedicated to the study of forest management and nutrient dynamics have been very widely researched. For example, relationships between C:N ratio (forest floor) and NO\textsubscript{3} leaching have only been observed for forest soil waters and not for surface waters or moorland systems. In the UK, the majority of sensitive upland catchments are not forested and there is much doubt as to the applicability of relationships or data from forest systems. Also, since N uptake in forests is included in FAB, should grazing and burning regimes be taken into account in moorlands?

2.5 The role of N processes in dynamic models for critical loads

While dynamic modelling is beyond the scope of this thesis, which aims to determine the feasibility of defining steady-state critical loads (not dynamic target loads) of total acidity for freshwaters, there is some overlap between the two approaches because
dynamic models are necessarily process-oriented. The static models simply ignore the
temporal aspect of the relevant processes.

The most widely used dynamic model for both soil and freshwater acidification
modelling in the UK is MAGIC (Model of Acidification of Groundwater in Catchments:
Cosby et al., 1985a, b, c). Although the model was developed in the early 1980s, N
dynamics were not incorporated into the model until the mid-1990s, with the first
version to include N being called MAGIC-WAND (MAGIC With Additional N
Dynamics; Ferrier et al., 1995; Jenkins et al., 1997). For forested catchments N uptake
proved to be the most significant process. Unlike the static models which required a
single value for N uptake, a non-linear, dynamic process, dependent upon available
NH$_4^+$ and NO$_3^-$ as well as forest age, had to be described. N immobilisation was
excluded from this first version of the model for N, but was later included as a function
of C:N ratio in MAGIC7 (Cosby et al., 2001), whereby N leaching varies linearly
between an upper threshold of C:N (above which no N leaching occurs) and a lower
threshold (below which 100% N leaching occurs).

The key questions concerning the representation of processes like N immobilisation and
denitrification are therefore just as relevant for dynamic modelling as for static critical
loads modelling. Although dynamic models do not employ a steady-state rate for such
processes and are not used to calculate critical loads (since sustainability and therefore
absence of timescale for damage is inherent in the critical load concept ), they are
becoming more widely used for the assessment of potential timescales of recovery and
target loads to achieve a given chemical state within a specified timescale. The
challenges for all dynamic models of acidification which incorporate N processes are
even greater than for the static models, but the conclusions of this thesis should also
contribute to their development and refinement.

2.6 References

(1995) An empirical model of critical acidity loads for surface waters based on
palaeolimnological data. In: M. Hornung, M.A. Sutton and R.B. Wilson (Eds.)


SECTION II

FIELD AND LABORATORY MEASUREMENTS OF N FLUXES
CHAPTER 3

SITE DESCRIPTIONS AND FIELD MEASUREMENT OF N FLUXES
3. SITE DESCRIPTIONS AND FIELD MEASUREMENT OF N FLUXES

3.1 Introduction: the CLAM N budget sites

3.1.1 Site selection rationale

Given the complexity of N cycling processes and the requirement for highly instrumented, intensively sampled study catchments, it was evident that financial and logistical constraints would greatly restrict the number of sites. Since much previous work had already been done on forested catchments (e.g. NITREX – see Wright & van Breemen, 1995), which are much less widespread in the UK than moorland catchments, and since the controls on N leaching from the latter are poorly understood, it was decided to focus solely on non-forested, moorland catchments. The purpose of the study is therefore to identify and model the processes controlling N saturation and leaching from moorland catchments into surface waters under different deposition regimes.

A maximum of four sites could be included in the intensive N-budget study (budget sites) along gradients of N deposition and N saturation (Table 3.1, Figure 3.1). The availability of existing data was therefore a pre-requisite for the identification of N saturation status and in 1998, suitable sites were selected from the Acid Waters Monitoring Network (Patrick et al., 1995). In addition to the availability of water chemistry data, sites were co-located with bulk deposition collectors serviced as part of the DETR funded Acid Deposition Network (Hayman et al., 2001). Available modelled data for total N deposition ranged from low levels of 5.2 kgN ha\(^{-1}\) yr\(^{-1}\) at the Allt a’Mharcaidh, Cairngorms, up to 31.6 kg ha\(^{-1}\) yr\(^{-1}\) at the River Etherow in the Pennines, one of the regions of highest deposition of both S and N in the country.

The various stages of N saturation, as defined using seasonal patterns in water chemistry (Stoddard, 1994 – see Chapter 1), are represented in these four sites (Figure 3.1). In the Allt a’Mharcaidh very little NO\(_3\) is seen at any time, with just occasional leakage in mid-winter or early spring, indicating strong N limitation in the terrestrial catchment (Stage 0). In the Afon Gwy (Plynlimon) elevated rates of NO\(_3\) are observed annually through the winter and spring months, but levels decline to near
zero during the summer period of maximum terrestrial N demand (Stage 1). At Scoat Tarn in the Lake District NO₃⁻ leaching follows a distinct seasonal pattern but occurs all year round, never declining to zero and indicating advanced N saturation (Stage 2). In the Pennine site experiencing the highest deposition levels, severe N saturation is indicated by the very high NO₃⁻ leaching year round and the breakdown of the seasonal pattern (Stage 3). Mean chemistry data for 1997 are shown in Table 3.2.

Table 3.1: Modelled deposition data (ITE annual mean data for 1992-94, catchment weighted)

<table>
<thead>
<tr>
<th>Deposition (kg ha⁻¹ yr⁻¹)</th>
<th>Allt a’Mharcaidh</th>
<th>Afon Gwy</th>
<th>Scoat Tarn</th>
<th>River Etherow</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (non-marine):</td>
<td>6.4</td>
<td>19.0</td>
<td>20.3</td>
<td>39.7</td>
</tr>
<tr>
<td>Total N:</td>
<td>5.2</td>
<td>23.7</td>
<td>23.8</td>
<td>31.6</td>
</tr>
<tr>
<td>Runoff (mm):</td>
<td>1093</td>
<td>2476</td>
<td>2526</td>
<td>1192</td>
</tr>
</tbody>
</table>

All four sites have non-forested, acid-sensitive catchments with a range of soil types in order to ensure that plot-based experiments on major N sink processes could cover a variety of major upland soil types.

The current formulation of the FAB model was applied to the four N-budget sites using the mean 1997 chemistry data (Figure 3.2). The critical load is exceeded if the point marking the values of N and S deposition lies above the solid line of the critical load function (CLF – see Chapter 2). Figure 3.2 shows that according to the most recent deposition data available at the time of site selection (1992-94) three of the four sites exceed their critical load - only the Allt a’Mharcaidh is not exceeded. At the Gwy, either S or N deposition could be reduced to prevent critical load exceedance, while at Scoat Tarn and the River Etherow both S and N deposition are sufficiently high to cause critical load exceedance on their own, so that both must be reduced significantly to protect the sites. A gradient of critical load exceedance (represented
by distance from the CLF in terms of N and S deposition) is therefore covered by the four sites. (Table 3.3).

### Table 3.2: Selected water chemistry data (AWMN annual mean data for 1997)

<table>
<thead>
<tr>
<th></th>
<th>Allt a’Mharcaidh</th>
<th>Afon Gwy</th>
<th>Scoat Tarn</th>
<th>River Etherow</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.52</td>
<td>5.35</td>
<td>5.05</td>
<td>4.58</td>
</tr>
<tr>
<td>( \Sigma \text{BC}^* ) (\text{\textmu{eq}/l})</td>
<td>93</td>
<td>71</td>
<td>45</td>
<td>300</td>
</tr>
<tr>
<td>( \text{SO}_4^{2-}^* ) (\text{\textmu{eq}/l})</td>
<td>33</td>
<td>45</td>
<td>42</td>
<td>206</td>
</tr>
<tr>
<td>( \text{NO}_3^- ) (\text{\textmu{eq}/l})</td>
<td>2</td>
<td>15</td>
<td>18</td>
<td>57</td>
</tr>
<tr>
<td>ANC (\text{\textmu{eq}/l})</td>
<td>58</td>
<td>12</td>
<td>-15</td>
<td>37</td>
</tr>
<tr>
<td>Cl(^-) (\text{\textmu{eq}/l})</td>
<td>107</td>
<td>149</td>
<td>174</td>
<td>323</td>
</tr>
<tr>
<td>TOC (mg/l)</td>
<td>2</td>
<td>2.6</td>
<td>0.98</td>
<td>8.3</td>
</tr>
</tbody>
</table>

* non marine

### Table 3.3: FAB critical loads and exceedance (based on AWMN annual mean data for 1997 and 1992-94 deposition data as above; keq ha\(^{-1}\) yr\(^{-1}\))

<table>
<thead>
<tr>
<th></th>
<th>Allt a’Mharcaidh</th>
<th>Afon Gwy</th>
<th>Scoat Tarn</th>
<th>River Etherow</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{CL}_{\text{max}S} )</td>
<td>1.00</td>
<td>1.53</td>
<td>0.98</td>
<td>1.37</td>
</tr>
<tr>
<td>( \text{CL}_{\text{min}N} )</td>
<td>0.28</td>
<td>0.30</td>
<td>0.14</td>
<td>0.29</td>
</tr>
<tr>
<td>( \text{CL}_{\text{max}N} )</td>
<td>1.27</td>
<td>1.83</td>
<td>1.19</td>
<td>1.66</td>
</tr>
<tr>
<td>Exceedance</td>
<td>Not exceeded</td>
<td>1.05</td>
<td>1.71</td>
<td>3.08</td>
</tr>
</tbody>
</table>

3.1.2 Site descriptions

Catchment topography and the locations of study plots within catchments are shown in Figures 3.3-3.6. Plots are located on the major representative soil types as far as possible, to allow the scaling-up of plot based results to the catchment scale. Best available soil maps were used to select up to four major soil types within each catchment (Figs. 3.3-3.6). Each soil type constitutes a study area on which replicated experimental plots were installed (Table 3.4).
Figure 3.1: Patterns of NO$_3^-$ leaching at the 4 AWMN sites selected for N budget studies
Figure 3.2: FAB model critical load function for the 4 N budget sites (values of N and S deposition are marked with a circle)

- **a:** Allt a'Mharcaidh (1997 mean chemistry)
- **b:** Afon Gwy (1997 mean chemistry)
- **c:** Scoat Tarn (1997 mean chemistry)
- **d:** River Etherow (1997 mean chemistry)
Table 3.4: Experimental areas at N budget sites (n=13)

<table>
<thead>
<tr>
<th>Site</th>
<th>Soil code</th>
<th>Soil type</th>
<th>Altitude (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allt a’Mharcaidh</td>
<td>M1</td>
<td>Peaty ranker</td>
<td>700</td>
</tr>
<tr>
<td>Allt a’Mharcaidh</td>
<td>M2</td>
<td>Valley peat</td>
<td>570</td>
</tr>
<tr>
<td>Allt a’Mharcaidh</td>
<td>M3</td>
<td>Peaty podsol</td>
<td>495</td>
</tr>
<tr>
<td>Allt a’Mharcaidh</td>
<td>M4</td>
<td>Shallow peat</td>
<td>490</td>
</tr>
<tr>
<td>Afon Gwy</td>
<td>G1</td>
<td>Hilltop peat</td>
<td>570</td>
</tr>
<tr>
<td>Afon Gwy</td>
<td>G2</td>
<td>Peaty gley</td>
<td>550</td>
</tr>
<tr>
<td>Afon Gwy</td>
<td>G3</td>
<td>Podsol</td>
<td>480</td>
</tr>
<tr>
<td>Afon Gwy</td>
<td>G4</td>
<td>Valley peat</td>
<td>390</td>
</tr>
<tr>
<td>Scoat Tarn</td>
<td>S1</td>
<td>Podsol</td>
<td>750</td>
</tr>
<tr>
<td>Scoat Tarn</td>
<td>S2</td>
<td>Peaty gley</td>
<td>650</td>
</tr>
<tr>
<td>Scoat Tarn</td>
<td>S3</td>
<td>Deep peat</td>
<td>590</td>
</tr>
<tr>
<td>River Etherow</td>
<td>E1</td>
<td>Deep peat (recently burnt Calluna)</td>
<td>455</td>
</tr>
<tr>
<td>River Etherow</td>
<td>E2</td>
<td>Deep peat (unburnt Calluna)</td>
<td>445</td>
</tr>
</tbody>
</table>

In addition to the presence of representative upland soil types for plot-based work, the primary requirement for study catchments was for high quality deposition input and runoff output flux measurements.

3.1.2.1 Acid deposition measurements (catchment inputs)

In order to provide the best possible N input data the sites were co-located with Acid Deposition Network primary or secondary sites with, as a minimum, bulk deposition collectors within the catchments which were serviced at least 2-weekly. At the Afon Gwy and Allt a’Mharcaidh, weekly samples were collected. Samples were analysed by AEA according to standard methods used within the Acid Deposition Network (Hayman et al., 2001).
3.1.2.2 Water chemistry (catchment runoff outputs)

The four selected sites were all included within the UK Acid Waters Monitoring Network (AWMN) which provided historical water chemistry data (at least 10 years). However, the sampling frequency was increased from monthly (streams) or quarterly (Scoat Tam) to 2-weekly to improve surface water leaching estimates. At the River Etherow, water samples from two tributary streams (Rose Clough and Swan Clough – Fig. 3.6) draining the soil plot study areas were also taken 2-weekly for chemical analysis. The two main tributaries of Scoat Tarn (Fig. 3.5) were sampled in the same way, to provide data without the influence of in-lake retention processes at the site. Samples were analysed for major base cations (Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, NH<sub>4</sub><sup>+</sup>), acid anions (SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>), pH, alkalinity, conductivity, aluminium species and dissolved organic N, C and P, at the SOAFD Freshwater Fisheries Laboratory, Pitlochry, Scotland, according to the methods of Harriman et al. (1990) (see Appendix 2).

At the Scoat Tarn outflow, the only site without flow gauging, a pressure-transducer flow gauge was installed. Stage measurements obtained were calibrated to discharge through periodic dilution gauging. The stage data obtained indicate a high degree of temporal flow variability at this site.

3.1.3 Primary experimental plots

In each of the 13 soil study areas (Table 3.4), 3 replicated primary plots (Figure 3.7) were installed (39 primary plots in total). Each plot (dimensions 3×1m) comprised:

1. soil water suction sampler located below rooting zone
2. adjacent grazing exclosure
3. static denitrification chamber (Chapter 4)
4. location of 5 soil cores for laboratory incubation experiments (Chapters 4-5)
5. ¹⁵N additions area (Chapter 6)
Figure 3.3: The Allt a’Mharcaidh study catchment and plot locations

SOIL PLOTS
M1: Peaty ranker
M2: Valley peat
M3: Peaty podsol
M4: Shallow peat
Figure 3.4: The Afon Gwy study catchment and plot locations

SOIL PLOTS
G1: Hilltop peat
G2: Peaty gley
G3: Podsol
G4: Valley peat
Figure 3.5: The Scoat Tarn study catchment and plot locations

SOIL PLOTS
S1: Podsol
S2: Peaty gley
S3: Deep peat
Figure 3.6: The River Etherow study catchment and plot locations in Rose Clough / Swan Clough subcatchment

SOIL PLOTS

E1: Peat under burnt Calluna  E2: Peat under mature Calluna
The primary plots formed the basis of the N budget work in the 13 soil study areas. While deposition inputs and surface water leaching outputs were assumed to be representative at the catchment scale (see above), all other soil-specific measurements and experiments were carried out at the plot level.

3.1.3.1 Soil water samples
Porous cup suction samplers were assembled at UCL during spring 1999 and installed in June/July that year. After a “settling-in” period during which the samplers were emptied but the samples discarded, soil water sampling commenced in early September 1999. Sampling was done fortnightly and the samples were analysed for pH, NH$_4^+$, NO$_3^-$ and organic N at MLURI, Aberdeen (see Appendix 2). Sampling was carried out over a 13 month period to ensure that sufficient data were available for a “nitrogen budget year” which ran from 19th October 1999 to 4th October 2000. Samples obtained during this period were assumed to be representative for the one year period 5th October 1999 to 4th October 2000, i.e. each sample represented the preceding two-week period.

3.1.3.2 Grazing exclosures
In each study area, three grazing exclosures (1m$^2$) were installed adjacent to the soil experimental plots in March 1999 and these remained in situ throughout the 1999 growing season. In September 1999, six quadrats (0.5 × 0.5m) were destructively sampled within each study area; three from within the grazing exclosures and the remainder from unfenced locations adjacent to the exclosures. All vegetation was cut at ground level and sorted into major vegetation components. Calluna was divided into current year shoots and the remainder. Grass and moss components were divided into ‘live’ and ‘dead’, while surface litter was collected separately. Where a particular component represented a major proportion of the total biomass it was collected separately. The biomass of each sampled component was determined after oven drying at 40°C.

One study area was excluded from this part of the study; M1 (peaty rankers) at the Allt a’Mharcaidh, because the shallow depth of soil prevented the installation of fencing posts. However, this site is thought to be the least impacted by grazing since
the area is protected (Cairngorm National Nature Reserve) and no livestock are present.

Figure 3.7: General layout of individual experimental plots at N budget sites

3.1.3.3 Soil moisture measurements
Since soil moisture is known to be a control on many biological processes involved in N cycling, measurements were made at the time of sampling for soil waters (i.e. 2-weekly). Soil moisture was measured at three random points within each soil plot using a hand-held theta-probe (Delta-T Devices, Type ML2).
3.1.3.4 Soil temperature

It was not considered necessary (or feasible on a cost basis) to attempt to measure soil temperature within each of the 39 primary plots, since the close proximity of replicated plots (by definition) ensured that temperature variations between plots in a study area would be minimal. Furthermore, the proximity of two of the study areas at the Afon Gwy (G1 and G2) meant that measurements were carried out at only one of them (at G1) and G2 was excluded.

In each of the remaining 12 study areas a Tiny Talk temperature datalogger (Gemini Tiny Talk Range G: -40 to +85°C) was buried adjacent to the set of three replicate plots at 5-10cm depth. Each logger was sealed in a small water-tight plastic bottle to prevent moisture from reaching the internal circuits. The loggers were installed in early September 1999 when soil water chemistry and denitrification measurements commenced. They were programmed to take soil temperature measurements at two-hourly intervals on even numbered hours (1200, 1400, 1600 etc.), which meant that with a capacity of 1800 readings they had to be downloaded three times during the study period.

3.1.4 Secondary experimental plots

Secondary experimental plots (identical to the primary plots) were paired with the primary plots in seven selected study areas (Table 3.5), providing another 21 plots in total. The purpose of the secondary plots was two-fold; they included soil water suction samplers which served as emergency replacements for samplers in primary plots in case of damage or failure to collect a sample, and they contained static chambers for additional denitrification experiments using N additions (see Chapter 4). None were installed at the Scoat Tarn site where the relatively remote location precluded the carrying of extra N solutions on routine trips and the extra field time that would be required.
3.2 Measured and modelled deposition inputs

The results for the budget year (5th October 1999 to 4th October 2000) are presented below.

3.2.1 Bulk deposition inputs

The acid anion, pH and rainfall data are presented in Figures 3.8a-d. Annual mean data, raw and volume weighted, are presented in Tables 3.6a-b, while budget year mean data are presented in Tables 3.7a-b.

Tables 3.6a and b show that 2000 was a wetter year than 1999 for all sites except the Allt a'Mharcaidh, and acidity deposition was also greater in 2000 at all sites. Both acid anion and base cation concentrations were greater in 1999 for all sites.

Mean data for the budget year (19th October 1999 to 4th October 2000) show a significant difference in the loading of acid anions at each site, with lowest concentrations of non-marine $\text{SO}_4^{2-}$, $\text{NO}_3^-$ and $\text{NH}_4^+$ at the Allt a'Mharcaidh, followed by the Afon Gwy, Scoat Tarn and then largest for all acid anions at the River Etherow (Tables 3.7a-b). The pH of bulk deposition is, however, surprisingly constant at all sites (c. 4.9) except the Etherow, where bulk deposition is much more acid (pH = 4.5).
Table 3.6a: Annual raw mean bulk deposition chemistry  
(NMS = non-marine SO$_4^{2-}$; SBC = sum of base cations)

<table>
<thead>
<tr>
<th>SITE</th>
<th>Period</th>
<th>n</th>
<th>Rain mm</th>
<th>pH</th>
<th>SO$_4^{2-}$</th>
<th>NMS</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>Cl</th>
<th>SBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHAR</td>
<td>1999</td>
<td>39</td>
<td>799</td>
<td>4.92</td>
<td>24.0</td>
<td>12.6</td>
<td>10.8</td>
<td>8.2</td>
<td>150.5</td>
<td>170.0</td>
</tr>
<tr>
<td>MHAR</td>
<td>2000</td>
<td>34</td>
<td>673</td>
<td>4.70</td>
<td>21.0</td>
<td>12.7</td>
<td>12.1</td>
<td>5.7</td>
<td>79.6</td>
<td>101.4</td>
</tr>
<tr>
<td>MHAR</td>
<td>Overall</td>
<td>73</td>
<td>736</td>
<td>4.80</td>
<td>22.6</td>
<td>12.6</td>
<td>11.4</td>
<td>7.0</td>
<td>93.2</td>
<td>131.1</td>
</tr>
<tr>
<td>GWY</td>
<td>1999</td>
<td>41</td>
<td>2178</td>
<td>4.93</td>
<td>29.4</td>
<td>20.2</td>
<td>17.1</td>
<td>19.1</td>
<td>88.0</td>
<td>137.3</td>
</tr>
<tr>
<td>GWY</td>
<td>2000</td>
<td>37</td>
<td>2253</td>
<td>4.83</td>
<td>25.7</td>
<td>15.4</td>
<td>11.4</td>
<td>11.2</td>
<td>103.7</td>
<td>137.3</td>
</tr>
<tr>
<td>GWY</td>
<td>Overall</td>
<td>78</td>
<td>2216</td>
<td>4.88</td>
<td>27.7</td>
<td>17.9</td>
<td>14.4</td>
<td>15.4</td>
<td>95.5</td>
<td>126.1</td>
</tr>
<tr>
<td>SCOAT</td>
<td>1999</td>
<td>23</td>
<td>2110</td>
<td>4.80</td>
<td>37.6</td>
<td>28.2</td>
<td>22.5</td>
<td>28.1</td>
<td>86.2</td>
<td>128.5</td>
</tr>
<tr>
<td>SCOAT</td>
<td>2000</td>
<td>20</td>
<td>2307</td>
<td>4.78</td>
<td>31.4</td>
<td>23.1</td>
<td>18.6</td>
<td>21.9</td>
<td>80.8</td>
<td>94.6</td>
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<tr>
<td>SCOAT</td>
<td>Overall</td>
<td>43</td>
<td>2209</td>
<td>4.79</td>
<td>34.7</td>
<td>25.8</td>
<td>20.7</td>
<td>25.2</td>
<td>83.7</td>
<td>112.8</td>
</tr>
<tr>
<td>ETHR</td>
<td>1999</td>
<td>22</td>
<td>851</td>
<td>4.57</td>
<td>53.2</td>
<td>45.3</td>
<td>39.1</td>
<td>41.7</td>
<td>73.3</td>
<td>115.5</td>
</tr>
<tr>
<td>ETHR</td>
<td>2000</td>
<td>20</td>
<td>983</td>
<td>4.48</td>
<td>46.3</td>
<td>40.2</td>
<td>31.1</td>
<td>34.7</td>
<td>62.4</td>
<td>76.9</td>
</tr>
<tr>
<td>ETHR</td>
<td>Overall</td>
<td>42</td>
<td>917</td>
<td>4.52</td>
<td>49.9</td>
<td>42.9</td>
<td>35.3</td>
<td>38.4</td>
<td>68.1</td>
<td>97.1</td>
</tr>
</tbody>
</table>

Table 3.6b: Annual mean bulk deposition chemistry (volume weighted)  
(NMS = non-marine SO$_4^{2-}$; SBC = sum of base cations)

<table>
<thead>
<tr>
<th>SITE</th>
<th>Period</th>
<th>n</th>
<th>Rain mm</th>
<th>pH</th>
<th>SO$_4^{2-}$</th>
<th>NMS</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>Cl</th>
<th>SBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHAR</td>
<td>1999</td>
<td>39</td>
<td>799</td>
<td>4.99</td>
<td>19.2</td>
<td>9.2</td>
<td>8.0</td>
<td>6.5</td>
<td>92.9</td>
<td>137.0</td>
</tr>
<tr>
<td>MHAR</td>
<td>2000</td>
<td>34</td>
<td>673</td>
<td>4.74</td>
<td>18.1</td>
<td>9.4</td>
<td>8.8</td>
<td>4.3</td>
<td>82.3</td>
<td>101.9</td>
</tr>
<tr>
<td>MHAR</td>
<td>Overall</td>
<td>73</td>
<td>736</td>
<td>4.86</td>
<td>18.7</td>
<td>9.3</td>
<td>8.3</td>
<td>5.5</td>
<td>88.1</td>
<td>120.9</td>
</tr>
<tr>
<td>GWY</td>
<td>1999</td>
<td>41</td>
<td>2178</td>
<td>5.01</td>
<td>23.2</td>
<td>13.4</td>
<td>9.8</td>
<td>11.8</td>
<td>96.6</td>
<td>140.5</td>
</tr>
<tr>
<td>GWY</td>
<td>2000</td>
<td>37</td>
<td>2253</td>
<td>4.91</td>
<td>22.8</td>
<td>11.6</td>
<td>7.6</td>
<td>7.5</td>
<td>112.5</td>
<td>122.4</td>
</tr>
<tr>
<td>GWY</td>
<td>Overall</td>
<td>78</td>
<td>2216</td>
<td>4.96</td>
<td>23.0</td>
<td>12.5</td>
<td>8.7</td>
<td>9.6</td>
<td>104.7</td>
<td>131.3</td>
</tr>
<tr>
<td>SCOAT</td>
<td>1999</td>
<td>23</td>
<td>2110</td>
<td>4.86</td>
<td>32.9</td>
<td>22.6</td>
<td>16.0</td>
<td>20.8</td>
<td>95.1</td>
<td>135.3</td>
</tr>
<tr>
<td>SCOAT</td>
<td>2000</td>
<td>20</td>
<td>2307</td>
<td>4.83</td>
<td>25.8</td>
<td>16.9</td>
<td>11.9</td>
<td>13.7</td>
<td>86.8</td>
<td>98.9</td>
</tr>
<tr>
<td>SCOAT</td>
<td>Overall</td>
<td>43</td>
<td>2209</td>
<td>4.84</td>
<td>29.2</td>
<td>19.6</td>
<td>13.9</td>
<td>17.1</td>
<td>90.7</td>
<td>116.3</td>
</tr>
<tr>
<td>ETHR</td>
<td>1999</td>
<td>22</td>
<td>851</td>
<td>4.61</td>
<td>45.9</td>
<td>38.6</td>
<td>30.6</td>
<td>34.1</td>
<td>69.0</td>
<td>105.8</td>
</tr>
<tr>
<td>ETHR</td>
<td>2000</td>
<td>20</td>
<td>983</td>
<td>4.49</td>
<td>39.9</td>
<td>33.5</td>
<td>24.2</td>
<td>26.3</td>
<td>64.1</td>
<td>76.7</td>
</tr>
<tr>
<td>ETHR</td>
<td>Overall</td>
<td>42</td>
<td>917</td>
<td>4.54</td>
<td>42.7</td>
<td>35.9</td>
<td>27.2</td>
<td>29.9</td>
<td>66.4</td>
<td>90.2</td>
</tr>
</tbody>
</table>

Table 3.7a: Budget year bulk deposition mean chemistry (raw data)  
(NMS = non-marine SO$_4^{2-}$; SBC = sum of base cations)

<table>
<thead>
<tr>
<th>SITE</th>
<th>Rain mm</th>
<th>pH</th>
<th>SO$_4^{2-}$</th>
<th>NMS</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>Cl</th>
<th>SBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHAR</td>
<td>36</td>
<td>868</td>
<td>4.85</td>
<td>19.4</td>
<td>10.2</td>
<td>9.4</td>
<td>5.1</td>
<td>87.0</td>
</tr>
<tr>
<td>GWY</td>
<td>37</td>
<td>1983</td>
<td>4.82</td>
<td>25.9</td>
<td>17.0</td>
<td>13.3</td>
<td>12.6</td>
<td>92.5</td>
</tr>
<tr>
<td>SCOAT</td>
<td>20</td>
<td>2202</td>
<td>4.81</td>
<td>34.3</td>
<td>23.5</td>
<td>18.3</td>
<td>21.2</td>
<td>100.8</td>
</tr>
<tr>
<td>ETHR</td>
<td>23</td>
<td>984</td>
<td>4.50</td>
<td>49.5</td>
<td>42.0</td>
<td>32.1</td>
<td>35.8</td>
<td>74.5</td>
</tr>
</tbody>
</table>
Table 3.7b: Budget year bulk deposition mean chemistry (volume weighted)
(NMS = non-marine SO\textsubscript{4}\textsuperscript{2-}; SBC = sum of base cations)

<table>
<thead>
<tr>
<th>SITE</th>
<th>n</th>
<th>Rain mm</th>
<th>pH</th>
<th>SO\textsubscript{4}\textsuperscript{2-} \textmu eq l\textsuperscript{-1}</th>
<th>NMS \textmu eq l\textsuperscript{-1}</th>
<th>NO\textsubscript{3}^- \textmu eq l\textsuperscript{-1}</th>
<th>NH\textsubscript{4}^+ \textmu eq l\textsuperscript{-1}</th>
<th>Cl \textmu eq l\textsuperscript{-1}</th>
<th>SBC \textmu eq l\textsuperscript{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHAR</td>
<td>36</td>
<td>868</td>
<td>4.88</td>
<td>16.5</td>
<td>7.4</td>
<td>6.5</td>
<td>3.9</td>
<td>85.0</td>
<td>112.0</td>
</tr>
<tr>
<td>GWY</td>
<td>37</td>
<td>1983</td>
<td>4.90</td>
<td>21.1</td>
<td>12.6</td>
<td>8.9</td>
<td>8.0</td>
<td>90.5</td>
<td>107.7</td>
</tr>
<tr>
<td>SCOAT</td>
<td>20</td>
<td>2202</td>
<td>4.87</td>
<td>28.6</td>
<td>17.7</td>
<td>11.9</td>
<td>13.2</td>
<td>103.1</td>
<td>125.5</td>
</tr>
<tr>
<td>ETHR</td>
<td>23</td>
<td>984</td>
<td>4.48</td>
<td>45.6</td>
<td>38.1</td>
<td>27.3</td>
<td>28.7</td>
<td>74.8</td>
<td>95.2</td>
</tr>
</tbody>
</table>

Note that while acid anion concentrations at Scoat Tarn are around half those at the Etherow, rainfall at Scoat Tarn is more than double, indicating that for bulk deposition inputs, acid anion fluxes are of similar magnitude at both sites. At the Allt a'Mharcaidh, the lowest concentrations of acid anions coincide with the lowest rainfall, leading to fluxes in bulk deposition which are much lower than at the other three sites (Table 3.8).

Table 3.8: Bulk deposition input fluxes (kg ha\textsuperscript{-1} yr\textsuperscript{-1})

<table>
<thead>
<tr>
<th>Site</th>
<th>NM SO\textsubscript{4}\textsuperscript{2-}-S</th>
<th>NO\textsubscript{3}^- -N</th>
<th>NH\textsubscript{4}^+ -N</th>
<th>TIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHAR</td>
<td>1.5</td>
<td>1.1</td>
<td>0.7</td>
<td>1.8</td>
</tr>
<tr>
<td>GWY</td>
<td>5.6</td>
<td>3.5</td>
<td>3.1</td>
<td>6.6</td>
</tr>
<tr>
<td>SCOAT</td>
<td>8.1</td>
<td>4.8</td>
<td>5.3</td>
<td>10.1</td>
</tr>
<tr>
<td>ETHR</td>
<td>6.8</td>
<td>4.2</td>
<td>4.5</td>
<td>8.7</td>
</tr>
</tbody>
</table>
3.2.2 Comparison with modelled deposition

It is known that bulk deposition collectors provide only approximate data for wet deposition inputs (and rainfall) and completely neglect dry deposition inputs. For comparison with the bulk deposition fluxes, total deposition figures (wet+dry) from the CEH national database for the most recent available period (1995-97) are provided in Table 3.9. While emissions of both S and N may have decreased since 1995-97, the very large difference in fluxes between these data and those for bulk deposition in 1999-2000 will be due largely to gross underestimation of cloud deposition and the omission of dry deposition from the latter.

Table 3.9: total deposition input fluxes from CEH national data (kg ha\(^{-1}\) yr\(^{-1}\)) - mean values for 1995-97

<table>
<thead>
<tr>
<th>Site</th>
<th>NM SO(_4^{2-})-S</th>
<th>NO(_3^{-})-N</th>
<th>NH(_4^{+})-N</th>
<th>TIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHAR</td>
<td>6.7</td>
<td>3.1</td>
<td>4.2</td>
<td>7.3</td>
</tr>
<tr>
<td>GWY</td>
<td>18.7</td>
<td>9.2</td>
<td>17.8</td>
<td>27.0</td>
</tr>
<tr>
<td>SCOAT</td>
<td>24.0</td>
<td>11.2</td>
<td>22.4</td>
<td>33.6</td>
</tr>
<tr>
<td>ETHR</td>
<td>34.1</td>
<td>12.2</td>
<td>21.6</td>
<td>33.7</td>
</tr>
</tbody>
</table>

These figures are very similar to those for 1996 reported in the AWMN 10 Year Report (Monteith & Evans, 2000), and were derived in the same way.

The magnitude of the difference between measured bulk deposition and modelled total deposition illustrates the potential uncertainty associated with modelled deposition estimates and the inherent difficulties in calculating and interpreting input-output budgets for deposited pollutants in upland areas. For example, intensive measurements of precipitation and cap cloud chemistry at Holme Moss, just a few kilometres to the north of the Etherow, revealed the opposite pattern: measured deposition of N species was around double the figure for the corresponding 5km grid square of the national deposition dataset in 1996 (Dore et al., 2001). This problem is partly due to large variations in elevation within a given grid square for which a single deposition value is given, when it is known that deposition can increase with altitude.
due to seeder-feeder effects. Hence modelled deposition may give an under- or an over-estimate, depending on site location relative to the mean elevation of the grid square. These uncertainties apply equally to critical load exceedances estimated from modelled deposition data.

Compared with the data for 1992-94 used in site selection to ensure that a gradient of N deposition was covered (Table 3.1), the 1995-97 data show an increase in TIN deposition at all sites. While the four sites are still ranked in the same order of increasing N deposition, estimated deposition at Scoat Tarn has increased by a much greater proportion, so that while it was deemed to experience a similar level of N deposition to the Afon Gwy during site selection (see Table 3.1), more recent data indicate that it seems to experience a higher deposition load than the Gwy, and is much more similar to that found at the highest deposition site, the River Etherow (Table 3.9).

Acid anion deposition fluxes are shown in Figure 3.9. It can be seen that deposition input fluxes occur in a pattern of pulsed events, which is particularly evident at the Etherow. These large flux events do not coincide with concentration peaks in bulk deposition (Figure 3.8a-d) and therefore it is rainfall that drives these large fluxes, causing seasonality, with the highest fluxes generally occurring in the spring.

3.3 Surface water chemistry and leaching fluxes

3.3.1 Mean surface water chemistry

Mean water chemistry values for the budget year (19th Oct. 1999 – 18th Oct. 2000) were calculated for all four sites (not flow weighted) and associated streams (Table 3.10). The data show a gradient of acidity, from the highest pH (geometric mean) at the Allt a’Mharcaidh, decreasing through the Afon Gwy and Scoat Tarn to a minimum at the River Etherow.

The acidity gradient across the four catchments follows the gradient in acid anion concentrations and sea-salt inputs (as chloride), with lowest values of non-marine
SO$_4^{2-}$ and NO$_3^-$ at the Allt a’Mharcaidh and the highest values in the River Etherow and its tributaries. Similarly, alkalinity and DOC decline with increasing acidity, except at the Etherow, which has by far the most coloured of the waters sampled (much greater DOC and dissolved organic N).

Although organic N was not measured at the Afon Gwy, there are obvious differences between the other sites in the relative importance of dissolved organic N (DON) leaching relative to total dissolved N on an annual basis. Almost all leached N at the Mharcaidh is organic, while in most other waters inorganic N leaching is more important. Rose Clough is the only site other than the Mharcaidh streams where organic N makes up the greater proportion of leached N, but it is only slightly more than a half.

The mean chemistry data for the Afon Gwy and Scoat Tarn are very similar for all determinands, the major difference being the higher NO$_3^-$ and lower TOC values at Scoat Tarn, the latter reflecting its very high clarity (low colour and organic content).

Dissolved organic N (DON) was not analysed at the Gwy, but the data show very similar concentrations for the Mharcaidh and Scoat Tarn, while the Etherow streams have much higher values.

The two sampled tributaries of the Allt a’Mharcaidh (Mhar2 and Mhar3 – see Figure 3.3 for sampling locations) have, as expected, very similar, dilute chemistry to the main river, the main difference being their slightly lower pH, base cation and alkalinity concentrations.

Of the two sampled inflow streams to Scoat Tarn, Red Pike stream has almost identical chemistry to the lake, except for lower organic content (organic N and DOC), while Scoat Fell stream is slightly less acid and has a lower NO$_3^-$ concentration and slightly higher alkalinity than the lake itself. Total base cation concentrations are lower in Red Pike stream, particularly the non-marine component. The difference in chemistry between the two streams, while not great, probably reflects differences in physical characteristics and soil types of their subcatchments.
Figure 3.8a: Bulk deposition chemistry and rainfall at the Allt a’Mharcaidh
Figure 3.8b: Bulk deposition chemistry and rainfall at the Aren Cwy.
Figure 3.8c: Bulk deposition chemistry and rainfall at Scoat Tarn
Figure 3.8d: Bulk deposition chemistry and rainfall at the River Etherow

[Graphs showing NM Sulphate (meq/l) and pH over time, with concentrations of NO3- and NH4+ also displayed.]
Figure 3.9: Acid anion deposition fluxes

Allt a’Mharcaidh

Afon Gwy

Scoat Tarn

River Etherow

Flux (eq ha$^{-1}$)

1 Jan-99  26 Feb-99  28 Mar-99  29 Apr-99  1 May-99  25 Jun-99  28 Jul-99  29 Aug-99  5 Sep-99  26 Sep-99  5 Oct-99  24 Nov-99  23 Dec-99  14 Jan-00  22 Feb-00  21 Mar-00  14 Apr-00  12 May-00  22 Jun-00  21 Jul-00  20 Aug-00  19 Sep-00  18 Oct-00  17 Nov-00  16 Dec-00
Table 3.10: Budget year mean water chemistry (SBC = sum of base cations, *denotes non-marine component)

<table>
<thead>
<tr>
<th>Site</th>
<th>Alk.</th>
<th>SBC</th>
<th>SBC*</th>
<th>SO₄²⁻</th>
<th>(SO₄²⁻)*</th>
<th>DOC</th>
<th>Cl</th>
<th>NH₄⁺</th>
<th>NO₃⁻</th>
<th>DON</th>
<th>Total N</th>
<th>Org.N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µeqL¹</td>
<td>µeqL¹</td>
<td>µeqL¹</td>
<td>µeqL¹</td>
<td>µeqL¹</td>
<td>µeqL¹</td>
<td>µeqL¹</td>
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<td>µeqL¹</td>
<td>µeqL¹</td>
<td>µgL¹</td>
<td>µgL¹</td>
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<tr>
<td>Mharcaidh</td>
<td>41</td>
<td>6.25</td>
<td>233</td>
<td>118</td>
<td>38</td>
<td>28</td>
<td>3.0</td>
<td>103</td>
<td>0.1</td>
<td>1</td>
<td>115</td>
<td>126</td>
</tr>
<tr>
<td>Mhar2</td>
<td>33</td>
<td>5.89</td>
<td>209</td>
<td>108</td>
<td>36</td>
<td>27</td>
<td>3.2</td>
<td>91</td>
<td>0.0</td>
<td>1</td>
<td>127</td>
<td>138</td>
</tr>
<tr>
<td>Mhar3</td>
<td>23</td>
<td>6.08</td>
<td>195</td>
<td>96</td>
<td>38</td>
<td>28</td>
<td>2.7</td>
<td>89</td>
<td>0.1</td>
<td>1</td>
<td>105</td>
<td>118</td>
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<tr>
<td>Afon Gwy</td>
<td></td>
<td>5.47</td>
<td>205</td>
<td>61</td>
<td>52</td>
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<td>132</td>
<td>0.0</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Scoat Fell.</td>
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<td>79</td>
<td>57</td>
<td>41</td>
<td>1.3</td>
<td>158</td>
<td>0.6</td>
<td>11</td>
<td>113</td>
</tr>
<tr>
<td>Red Pike str.</td>
<td>-5</td>
<td>5.10</td>
<td>243</td>
<td>61</td>
<td>57</td>
<td>40</td>
<td>1.0</td>
<td>163</td>
<td>0.4</td>
<td>17</td>
<td>98</td>
<td>318</td>
</tr>
<tr>
<td>Scoat Tarn</td>
<td>-6</td>
<td>5.10</td>
<td>260</td>
<td>78</td>
<td>53</td>
<td>36</td>
<td>1.5</td>
<td>162</td>
<td>0.5</td>
<td>15</td>
<td>125</td>
<td>329</td>
</tr>
<tr>
<td>River Etherow</td>
<td>22</td>
<td>4.72</td>
<td>704</td>
<td>290</td>
<td>191</td>
<td>152</td>
<td>8.0</td>
<td>373</td>
<td>0.5</td>
<td>42</td>
<td>317</td>
<td>860</td>
</tr>
<tr>
<td>Swan Clough</td>
<td>74</td>
<td>4.72</td>
<td>608</td>
<td>420</td>
<td>225</td>
<td>207</td>
<td>9.1</td>
<td>169</td>
<td>0.5</td>
<td>58</td>
<td>437</td>
<td>1174</td>
</tr>
<tr>
<td>Rose Clough</td>
<td>-83</td>
<td>4.11</td>
<td>389</td>
<td>219</td>
<td>191</td>
<td>176</td>
<td>17.7</td>
<td>153</td>
<td>2.0</td>
<td>43</td>
<td>639</td>
<td>1160</td>
</tr>
</tbody>
</table>

Even more marked is the difference between the chemistry of the two tributaries to the Etherow, Swan Clough and Rose Clough, which drain the soil plot experimental area (see Fig. 3.6). The two tributaries have very different chemistries from the main body of the Etherow, which is perhaps to be expected given that the catchment of the Etherow is very large and includes much improved pasture and a major road, while Swan Clough and Rose Clough drain a relatively undisturbed area of deep peat.

Of greater interest is the major difference between the chemistry of the two tributaries, which are separated by only a few hundred metres and drain similar terrain either side of the small interfluve on which the terrestrial experimental plots are located. The difference in alkalinity between the streams is more than 150 µeqL¹ and more than half a pH unit (a difference in H⁺ concentration of 60 µeqL¹). The more acid stream, Rose Clough, has a very high organic content, in particular DOC which is almost double the level found in Swan Clough (which itself has a high DOC content). Base cation concentrations are much lower in Rose Clough. It is also the only water body sampled which regularly has measurable concentrations of NH₄⁺, while its mean NO₃⁻ concentration, though high, is less than that in Swan Clough.
These major differences are presumably due to hydrological factors which could account for dramatic changes in water chemistry, depending on flow routing through the very acid deep peats. Large soil pipes are present in the study area, and pipeflow can contribute a significant proportion of streamflow in Pennine deep peats (Holden & Burt, 2002). Saturation-excess overland flow can dominate the catchment response, while a proportion of this flow can drain directly into pipes. Major localised differences in either of these modes of water flow could presumably account for the large differences in runoff chemistry.

The chemical time series data are shown in Figures 3.10 – 3.13 and discussed for each catchment below.

3.3.1.1 Allt a’Mharcaidh

The pH of the Mharcaidh and its tributaries is relatively constant at around pH 6.5 but is subject to periodic depressions down to pH 5.0-5.5, associated with high flow events. Base cation concentrations are also depressed during these episodes, which are sufficient to depress alkalinity to near zero from a mean value of 41 μeq l⁻¹ in the main river, and small, negative alkalinity values are occasionally observed in Site 2 (Fig. 3.10a). Chloride concentrations are relatively stable at all sample points, with some flow-associated depressions observed.

While mean NO₃⁻ levels are very low in the Mharcaidh system (c. 1 μeq l⁻¹) a seasonal pattern in leaching can still be observed (Fig. 3.10b). Concentrations up to 2.5 μeq l⁻¹ occur during the winter months, decreasing to c. 1 μeq l⁻¹ or less for the rest of the year. Concentrations of NH₄⁺ are almost all zero, with just a few very low values observed during the period of monitoring. Concentrations of organic N peak in late autumn / early winter and late summer. At the Mharcaidh, the very low levels of inorganic N mean that the pattern in total N is driven almost entirely by organic N (Fig. 3.10c).

Total SO₄²⁻ concentrations are low (mean 38 μeq l⁻¹) and are depressed in high flow events (Fig. 3.10c). Non-marine SO₄²⁻ is probably quite close to background levels
(mean 28 μeq/l). TOC values are generally low (c. 2 mg/l) but the mean is increased to 3 mg/l by high values due to flushing at high flow events.

3.3.1.2 Afon Gwy

While the mean pH of the Gwy (mean 5.47) is lower than that of the Mharcaidh, it varies over a similar range from pH 6.5 down to pH 5.0. Mean alkalinity is only 5 μeq/l and can be negative for periods of several weeks (Fig. 3.11a). Chloride looked like it was following a declining trend through the budget year, but increased again in the last few samples. Total base cation concentrations are relatively stable, with some flow associated depressions, while non-marine base cation concentrations follow a similar pattern to pH and alkalinity.

A seasonal trend in NO$_3^-$ is clearly seen at the Gwy, with concentration peaks of up to 15-20 μeq/l in winter declining to near zero through the summer (Fig. 3.11b). Concentrations of NH$_4^+$ are below detection limits. Total SO$_4^{2-}$ values are relatively constant, but appear to be slightly lower in the later months of the budget year. DOC at the Gwy is variable but generally quite low and similar to the Mharcaidh, with a mean of c. 3 mg/l. While there is no strong seasonal pattern, DOC does seem to follow the opposite cycle to NO$_3^-$, being lowest in winter. Organic N was not analysed at the Gwy.

3.3.1.3 Scoat Tarn

The pH of Scoat Tarn is quite constant at around pH 5.0, and is closely mirrored in Red Pike stream, but not in Scoat Fell stream (Fig. 3.12a). Alkalinity at Scoat Tarn is negative throughout almost all of the monitoring period and reaches a maximum of only 2 μeq/l, while Red Pike stream, which again closely mirrors the lake, has a higher peak of almost 15 μeq/l. In Scoat Fell stream, negative alkalinity values in the winter and spring become positive throughout the summer, following the same seasonal pattern as non-marine base cations and the pH data. Chloride, which reflects sea-salt inputs and presumably storminess and/or prevailing wind direction, follows a distinct seasonal pattern, with highest values in mid-winter declining to a minimum in late summer, reflecting the pattern in total base cations of which c. 70% are associated with marine inputs.
Figure 3.10a: Allt a’Mharcaidh streams water chemistry (pH, alkalinity and chloride)
Figure 3.10b: Allt a’Mharcaidh streams water chemistry (N species)
Figure 3.10c: Allt a’Mharcaidh streams water chemistry (total N, total organic carbon and sulphate)
Figure 3.10d: Allt a’Mharcaidh streams water chemistry (sum of base cations, non-marine base cations and non-marine sulphate)
Figure 3.11a: Afon Gwy water chemistry (pH, alkalinity and chloride)
Figure 3.11b: Afon Gwy water chemistry (NO$_3^-$, sulphate and dissolved organic carbon)
Figure 3.11c: Afon Gwy water chemistry (sum of base cations, non-marine base cations and non-marine sulphate)
Figure 3.12a: Scoat Tarn and inflow streams water chemistry (pH, alkalinity and chloride)
Figure 3.12b: Scoat Tarn and inflow streams water chemistry (N species)

- **NO₃** (μeq l⁻¹):
  - Red Pike Stream
  - Scoat Fell Stream
  - Scoat Tarn

- **NH₄** (μeq l⁻¹):
  - Red Pike Stream
  - Scoat Fell Stream
  - Scoat Tarn

- **Dissolved organic N (μg l⁻¹):
  - Red Pike Stream
  - Scoat Fell Stream
  - Scoat Tarn
Figure 3.12c: Scoat Tarn and inflow streams water chemistry (total N, total organic carbon and sulphate)
Figure 3.12d: Scoat Tarn and inflow streams water chemistry (sum of base cations, non-marine base cations and non-marine sulphate)
Figure 3.13a: Etherow streams water chemistry (pH, alkalinity and chloride)
Figure 3.13b: Etherow streams water chemistry (N species)
Figure 3.13c: Etherow streams water chemistry (total N, total organic carbon and sulphate)
Figure 3.13d: Etherow streams water chemistry (sum of base cations, non-marine base cations and non-marine sulphate)
The pronounced seasonal pattern in NO$_3^-$ at Scoat Tarn is very highly correlated with chloride (Fig. 3.12b). The mean value of 15 μeq/l in the lake is high for an acid-sensitive, upland site in the UK, and NO$_3^-$ does not decline to zero even during the summer, indicating a degree of N saturation. The seasonal pattern in the inflow streams is slightly larger, but very similar, with a sharp reduction in concentrations occurring in early May. Very low levels of NH$_4^+$ are recorded on several occasions, with a peak just after the May reductions in NO$_3^-$, but concentrations are mostly near zero throughout the sampling period. No strong seasonal pattern is apparent in organic N, but maximum values seem to occur in the summer, peaking at the same time as NH$_4^+$. A seasonal pattern is more obvious in total N, which is driven largely by NO$_3^-$ (Fig. 3.12c). As mentioned above, Scoat Tarn has very low levels of both organic N and TOC, with concentrations of the latter rarely exceeding 1.5 mg/l.

Sulphate concentrations in Scoat Tarn and Red Pike stream follow a seasonal pattern, only partly driven by sea-salt inputs (Figs. 3.12c-d). When non-marine SO$_4^{2-}$ is compared with NO$_3^-$ and chloride, it becomes obvious that SO$_4^{2-}$ follows the opposite seasonal pattern to the other anions. This is not, however, true for Scoat Fell stream, where non-marine SO$_4^{2-}$ concentrations are remarkably constant compared with Red Pike stream and the lake.

3.3.1.4 River Etherow

The most striking feature of the River Etherow and at least one of its tributaries, Swan Clough, is that it is subject to very severe episodic acidification, with pH falling from almost circumneutral levels by up to 3 pH units to around pH 4.0 on regular occasions (Fig. 3.13a). These acid episodes are also reflected in very large variations in base cations and alkalinity, the latter ranging from 150 μeq/l down to less than -200 μeq/l in Rose Clough. Chloride in the Etherow itself is much higher and more variable than the monitored tributaries to the south, but this may be attributed at least partly to road salt inputs into the main Etherow channel, which follows a major road. In Rose Clough and Swan Clough, which should not be affected by road salt, chloride is tightly correlated and varies in the range 100-200 μeq/l.
Concentrations of NO$_3^-$ in the Etherow streams are the highest of all sites in this study and the wider AWMN, with levels in Swan Clough being higher than the Etherow and Rose Clough (Fig. 3.13b). However, Rose Clough stands out as one of the few upland, acid-sensitive sites in the UK where significant NH$_4^+$ concentrations are regularly found, up to 9 μeq l$^{-1}$. These streams are also distinctive in having much higher levels of organic N and TOC than the other study sites (Figs. 3.13b-c). In particular, mean TOC levels in Rose Clough are very high (c. 18 mg l$^{-1}$) but can rise much higher to c. 45 mg l$^{-1}$. A seasonal pattern of NO$_3^-$ leaching is notably absent in the Etherow streams, with very high concentrations year round indicating severe N saturation. Conversely, there does appear to be a seasonal pattern in SO$_4^{2-}$ concentrations, as observed also at Scoat Tarn, with higher concentrations in summer.

### 3.3.2 Comparisons with deposition data

The mean chemistry of surface waters and bulk deposition is compared below for the Mharcaidh, Gwy, Red Pike stream (closest to lake chemistry for Scoat Tarn but without problem of in-lake processes) and the two Etherow tributaries (Rose Clough and Swan Clough) in Figures 3.14a-d. Deposition input fluxes are also plotted for comparison, and are discussed further below. Bulk deposition mean concentration and (wet) flux was calculated as described in Section 3.2.1 above (Tables 3.7 - 3.8).

It is apparent in Figure 3.14a that non-marine SO$_4^{2-}$ concentrations are higher in surface waters than in bulk deposition for all sites. This will be partly due to concentration by evaporation/transpiration in surface waters, but is also likely to be due to unquantified dry deposition inputs and possibly to inputs from weathering or mineralisation sources. The same pattern is present in both surface water and bulk deposition, increasing from very low levels at the Mharcaidh, through intermediate levels at Gwy and Red Pike stream (Scoat Tarn) to much higher values in the Etherow streams.

The same pattern is followed by total inorganic N concentrations, except that surface water concentrations are less than those in bulk deposition. At the Mharcaidh and
Gwy there is a large difference between the two, while at Red Pike and particularly the Etherow streams, surface water concentrations of TIN are much larger as a proportion of bulk deposition (Fig. 3.14b).

If NO$_3^-$ and NH$_4^+$ are considered separately it can be seen that NH$_4^+$ levels are so low in surface waters that it is almost entirely NO$_3^-$ that determines the link between TIN and bulk deposition (Figs. 3.14c-d). While NH$_4^+$ concentrations in bulk deposition increase across the four catchments, it is barely detected in surface waters even at high (wet) deposition inputs. The pattern is very different for NO$_3^-$; as bulk deposition concentration increases, the surface water concentration increases disproportionately.

In the Mharcaidh, NO$_3^-$ concentration is much lower in the stream than in bulk deposition, while in the Gwy NO$_3^-$ reaches almost half the level in bulk deposition. The two values are very close for Red Pike stream, but in Rose Clough and Swan Clough, NO$_3^-$ is much greater than in bulk deposition. This could indicate significant dry deposition at the Etherow or nitrification of wet deposited NH$_4^+$. For oxidised N, dry deposition tends to make up around 25% of the total (RGAR, 1997), which would not be enough to account for the near doubling of NO$_3^-$ in Swan Clough compared to bulk deposition. Hence it is likely that nitrification of NH$_4^+$ accounts for a proportion of the NO$_3^-$ in Swan Clough.

**Figure 3.14a: Non-marine sulphate concentrations and fluxes**

![Non-marine sulphate concentrations and fluxes](image)
Figure 3.14b: Total inorganic N concentrations and fluxes

Figure 3.14c: Nitrate concentrations and fluxes
3.3.3 Runoff fluxes

Flow was measured at all locations, using existing gauges at the Allt a’Mharcaidh, Etherow and Gwy, and a newly installed pressure transducer gauge at the outflow of Scoat Tarn. Problems with siltation at the Etherow weir meant that surrogate data had to be used, as described in Curtis et al. (2001). Discharge at the time of sampling was used to generate volume weighted mean concentrations, which were then converted to annual fluxes using total mean annual discharge. Uncertainties in discharge estimates mean that flux estimates must be considered approximate.

Estimated sulphur fluxes (Table 3.11) vary as a function of deposition, from 5 kgS ha\(^{-1}\) yr\(^{-1}\) at the Allt a’Mharcaidh to 52 kgS ha\(^{-1}\) yr\(^{-1}\) at the Etherow. At all four sites, S output fluxes exceed wet deposition inputs, confirming the importance of dry deposition. However there are marked discrepancies between 1995-97 estimated total inputs and estimated outputs. At the Etherow, export is greater than input, consistent with a weathering or mineralisation source of S, which is believed to be present in this catchment. However at the other sites only around a half of inputs are exported in
runoff, and since the decline in S deposition between 1995-97 and 1999/2000 is unlikely to be sufficient to explain this imbalance, the implication of these observations is either that (i) output fluxes are under-estimated; (ii) 1995-97 input fluxes are over-estimated; or (iii) these catchments are retaining some S input.

Estimates of N output fluxes vary considerably between sites, as a function primarily of variations in concentration (Tables 3.12-3.13). The smallest inorganic N output flux, 0.1 kgN ha\(^{-1}\) yr\(^{-1}\), was observed at the Allt a'Mharcaidh, where virtually all deposited N is retained. Intermediate levels of leaching were observed at the Afon Gwy (1.8 kgN ha\(^{-1}\) yr\(^{-1}\)) and Scoat Tarn (4.9 kgN ha\(^{-1}\) yr\(^{-1}\)), and a maximum level of 11.0 kgN ha\(^{-1}\) yr\(^{-1}\) at the River Etherow. The Etherow is the only site at which NH\(_4^+\) contributes measurably to outputs, but even here it represents only around 3% of the inorganic N total.

There is clearly a correlation between inorganic N leaching and deposition, but the relationship appears non-linear, with the proportion of N leached increasing with deposition. This pattern is more obvious for NO\(_3^-\) than for TIN because of the retention of almost all the NH\(_4^+\) at all sites. The percentage of N leached (based on 1995-97 total deposition) rises from 1% at the Allt a'Mharcaidh to 33% at the River Etherow. At the Etherow, it is notable that of an estimated 34 kgN ha\(^{-1}\) yr\(^{-1}\) total deposition, only 12 kgN ha\(^{-1}\) yr\(^{-1}\) occurred as NO\(_x\). Since the NO\(_x\) deposition figure from 1995-97 could be an overestimate for the sampling period, the figure of 87% export may be an underestimate for NO\(_3^-\), indicating a near-zero net retention of deposited NO\(_x\) at this site.

Table 3.11: Total S fluxes in deposition and runoff (kgS ha\(^{-1}\) yr\(^{-1}\))

<table>
<thead>
<tr>
<th>Site</th>
<th>Runoff</th>
<th>Bulk (wet)</th>
<th>Total 1995-97</th>
<th>% leached</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mharcaidh</td>
<td>4.7</td>
<td>3.31</td>
<td>10.4</td>
<td>45.2</td>
</tr>
<tr>
<td>Gwy</td>
<td>14.7</td>
<td>9.41</td>
<td>28.8</td>
<td>51.0</td>
</tr>
<tr>
<td>Scoat</td>
<td>17.8</td>
<td>13.1</td>
<td>31.5</td>
<td>56.5</td>
</tr>
<tr>
<td>Etherow</td>
<td>51.7</td>
<td>8.11</td>
<td>35.7</td>
<td>144.9</td>
</tr>
</tbody>
</table>
Table 3.12: Fluxes of NO$_3$-N and NH$_4$-N (kg N ha$^{-1}$ yr$^{-1}$)

<table>
<thead>
<tr>
<th>Site</th>
<th>Runoff</th>
<th>Bulk</th>
<th>Tot.1995-97</th>
<th>% out</th>
<th>Runoff</th>
<th>Bulk</th>
<th>Tot.1995-97</th>
<th>% out</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mharcaidh</td>
<td>0.1</td>
<td>1.1</td>
<td>3.1</td>
<td>2.6</td>
<td>0</td>
<td>0.7</td>
<td>4.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Gwy</td>
<td>1.8</td>
<td>3.5</td>
<td>9.2</td>
<td>19.6</td>
<td>0</td>
<td>3.1</td>
<td>17.8</td>
<td>0</td>
</tr>
<tr>
<td>Scoat</td>
<td>4.9</td>
<td>4.8</td>
<td>11.2</td>
<td>43.4</td>
<td>0</td>
<td>5.3</td>
<td>22.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Etherow</td>
<td>10.6</td>
<td>4.2</td>
<td>12.2</td>
<td>87.1</td>
<td>0.4</td>
<td>4.5</td>
<td>21.6</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 3.13: Fluxes of Total Inorganic N (kg N ha$^{-1}$ yr$^{-1}$)

<table>
<thead>
<tr>
<th>Site</th>
<th>Runoff</th>
<th>Bulk</th>
<th>Tot.1995-97</th>
<th>% out</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mharcaidh</td>
<td>0.1</td>
<td>1.8</td>
<td>7.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Gwy</td>
<td>1.8</td>
<td>6.6</td>
<td>27.0</td>
<td>6.7</td>
</tr>
<tr>
<td>Scoat</td>
<td>4.9</td>
<td>10.1</td>
<td>33.6</td>
<td>14.5</td>
</tr>
<tr>
<td>Etherow</td>
<td>11.0</td>
<td>8.7</td>
<td>33.8</td>
<td>32.5</td>
</tr>
</tbody>
</table>

3.4 Soil water chemistry

3.4.1 Suction samplers

Mean soilwater N species data for 2-weekly samples throughout the budget year are presented in Tables 3.14a-d. While these tables indicate the spatial variability of soilwater chemistry within each soil type, the major differences occur between soil types and between sites. An inter-site comparison is provided in Table 3.15.

The most striking feature of the data in Table 3.15 is the complete absence of NO$_3^-$ in the soilwaters of the Mharcaidh and Gwy, over a range of soil types. At Scoat Tarn, however, NO$_3^-$ is present in all soil types, but particularly the podsol and peaty gley soils. Table 3.14c shows that high levels of NO$_3^-$ were measured in all three replicate plots on these soils. At the Etherow sites, the highest NO$_3^-$ values found within the whole study occur in the deep peats under burnt *Calluna* (Soil E1), but the very large
mean value is driven by inexplicably high measurements at one particular plot (E1D3 – see Table 3.14d). This is an order of magnitude greater than the mean value for the nearby plot E1D1, which otherwise has the highest NO₃⁻ concentrations of all the remaining study plots.

Table 3.14a: Nitrogen species in soilwaters at the Allt a’Mharcaidh (mg l⁻¹)

<table>
<thead>
<tr>
<th>Soil</th>
<th>Plot</th>
<th>Total N</th>
<th>NH₄-N</th>
<th>NO₃-N</th>
<th>Org.N</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 Peaty ranker</td>
<td>M1D1</td>
<td>0.43</td>
<td>0.03</td>
<td>0.00</td>
<td>0.40</td>
</tr>
<tr>
<td>M1 Peaty ranker</td>
<td>M1D2</td>
<td>0.32</td>
<td>0.02</td>
<td>0.00</td>
<td>0.30</td>
</tr>
<tr>
<td>M1 Peaty ranker</td>
<td>M1D3</td>
<td>0.39</td>
<td>0.02</td>
<td>0.00</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>M1 average</strong></td>
<td></td>
<td><strong>0.38</strong></td>
<td><strong>0.02</strong></td>
<td><strong>0.00</strong></td>
<td><strong>0.36</strong></td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>M2D1</td>
<td>0.65</td>
<td>0.03</td>
<td>0.00</td>
<td>0.63</td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>M2D2</td>
<td>0.65</td>
<td>0.03</td>
<td>0.00</td>
<td>0.62</td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>M2D3</td>
<td>0.51</td>
<td>0.01</td>
<td>0.00</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>M2 average</strong></td>
<td></td>
<td><strong>0.60</strong></td>
<td><strong>0.02</strong></td>
<td><strong>0.00</strong></td>
<td><strong>0.57</strong></td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>M3D1</td>
<td>0.31</td>
<td>0.02</td>
<td>0.00</td>
<td>0.28</td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>M3D2</td>
<td>0.57</td>
<td>0.03</td>
<td>0.00</td>
<td>0.53</td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>M3D3</td>
<td>0.40</td>
<td>0.02</td>
<td>0.00</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>M3 average</strong></td>
<td></td>
<td><strong>0.43</strong></td>
<td><strong>0.02</strong></td>
<td><strong>0.00</strong></td>
<td><strong>0.39</strong></td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>M4D1</td>
<td>0.27</td>
<td>0.03</td>
<td>0.00</td>
<td>0.23</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>M4D2</td>
<td>0.79</td>
<td>0.09</td>
<td>0.00</td>
<td>0.67</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>M4D3</td>
<td>0.46</td>
<td>0.01</td>
<td>0.00</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>M4 average</strong></td>
<td></td>
<td><strong>0.51</strong></td>
<td><strong>0.04</strong></td>
<td><strong>0.00</strong></td>
<td><strong>0.45</strong></td>
</tr>
</tbody>
</table>

Table 3.14b: Nitrogen species in soilwaters at the Afon Gwy (mg l⁻¹)

<table>
<thead>
<tr>
<th>Soil</th>
<th>Plot</th>
<th>Total N</th>
<th>NH₄-N</th>
<th>NO₃-N</th>
<th>Org.N</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 Hilltop peat</td>
<td>G1D1</td>
<td>1.77</td>
<td>0.10</td>
<td>0.00</td>
<td>1.67</td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>G1D2</td>
<td>0.88</td>
<td>0.07</td>
<td>0.00</td>
<td>0.80</td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>G1D3</td>
<td>0.73</td>
<td>0.04</td>
<td>0.00</td>
<td>0.69</td>
</tr>
<tr>
<td><strong>G1 average</strong></td>
<td></td>
<td><strong>1.13</strong></td>
<td><strong>0.07</strong></td>
<td><strong>0.00</strong></td>
<td><strong>1.05</strong></td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>G2D1</td>
<td>0.61</td>
<td>0.04</td>
<td>0.00</td>
<td>0.57</td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>G2D2</td>
<td>0.58</td>
<td>0.03</td>
<td>0.00</td>
<td>0.55</td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>G2D3</td>
<td>0.66</td>
<td>0.04</td>
<td>0.01</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>G2 average</strong></td>
<td></td>
<td><strong>0.62</strong></td>
<td><strong>0.04</strong></td>
<td><strong>0.00</strong></td>
<td><strong>0.58</strong></td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>G3D1</td>
<td>0.38</td>
<td>0.05</td>
<td>0.01</td>
<td>0.32</td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>G3D2</td>
<td>0.42</td>
<td>0.02</td>
<td>0.00</td>
<td>0.40</td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>G3D3</td>
<td>0.25</td>
<td>0.03</td>
<td>0.00</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>G3 average</strong></td>
<td></td>
<td><strong>0.35</strong></td>
<td><strong>0.03</strong></td>
<td><strong>0.00</strong></td>
<td><strong>0.32</strong></td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>G4D1</td>
<td>2.10</td>
<td>0.05</td>
<td>0.00</td>
<td>2.05</td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>G4D2</td>
<td>2.07</td>
<td>0.05</td>
<td>0.00</td>
<td>2.02</td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>G4D3</td>
<td>0.81</td>
<td>0.05</td>
<td>0.00</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>G4 average</strong></td>
<td></td>
<td><strong>1.66</strong></td>
<td><strong>0.05</strong></td>
<td><strong>0.00</strong></td>
<td><strong>1.61</strong></td>
</tr>
</tbody>
</table>
Unlike NO$_3^-$, NH$_4^+$ was found in all 39 plots, especially in peats. At the Allt a’Mharcaidh, mean NH$_4^+$ concentrations are very similar for all soils and plots (mean 0.02 mg l$^{-1}$), with just one plot in the shallow peat (M4D2) having a higher value and leading to a higher mean for the shallow peat soil there (M4). In the Gwy soils, soilwaters have higher NH$_4^+$ concentrations than at the Mharcaidh, but values are still low. Plots on the hilltop (G1) and valley (G4) peats consistently show the highest NH$_4^+$ concentrations at the Gwy (Table 3.14b). At Scoat Tarn very similar NH$_4^+$ concentrations are observed in all three soil types studied there (Table 3.14c), with values similar to the peaty gley and podsol soils (the lowest) at the Gwy (G2 and G3). Again, the extreme values are found at the Etherow, although not in the same study area. The high NO$_3^-$ concentrations were found in the deep peat under burnt vegetation, and while the NH$_4^+$ levels there are much higher than at the other three

| Soil Plot Total N | NH$_4^-$ N NO$_3^-$ N Org.N |
|------------------|-----------------|----------------|---------------|
| S1 Podsol S1D1   | 0.61            | 0.04           | 0.15          | 0.42          |
| S1 Podsol S1D2   | 0.44            | 0.03           | 0.13          | 0.28          |
| S1 Podsol S1D3   | 0.41            | 0.03           | 0.07          | 0.30          |
| **S1 average**   | **0.49**        | **0.03**       | **0.12**      | **0.34**      |
| S2 Peaty gley S2D1 | 0.74        | 0.04           | 0.17          | 0.53          |
| S2 Peaty gley S2D2 | 0.50        | 0.03           | 0.19          | 0.28          |
| S2 Peaty gley S2D3 | 0.46        | 0.02           | 0.09          | 0.35          |
| **S2 average**   | **0.56**        | **0.03**       | **0.15**      | **0.39**      |
| S3 Deep peat S3D1 | 0.53          | 0.03           | 0.12          | 0.37          |
| S3 Deep peat S3D2 | 0.44          | 0.02           | 0.01          | 0.41          |
| S3 Deep peat S3D3 | 0.38          | 0.04           | 0.00          | 0.35          |
| **S3 average**   | **0.45**        | **0.03**       | **0.04**      | **0.38**      |

Unlike NO$_3^-$, NH$_4^+$ was found in all 39 plots, especially in peats. At the Allt a’Mharcaidh, mean NH$_4^+$ concentrations are very similar for all soils and plots (mean 0.02 mg l$^{-1}$), with just one plot in the shallow peat (M4D2) having a higher value and leading to a higher mean for the shallow peat soil there (M4). In the Gwy soils, soilwaters have higher NH$_4^+$ concentrations than at the Mharcaidh, but values are still low. Plots on the hilltop (G1) and valley (G4) peats consistently show the highest NH$_4^+$ concentrations at the Gwy (Table 3.14b). At Scoat Tarn very similar NH$_4^+$ concentrations are observed in all three soil types studied there (Table 3.14c), with values similar to the peaty gley and podsol soils (the lowest) at the Gwy (G2 and G3). Again, the extreme values are found at the Etherow, although not in the same study area. The high NO$_3^-$ concentrations were found in the deep peat under burnt vegetation, and while the NH$_4^+$ levels there are much higher than at the other three

| Soil Plot Total N | NH$_4^-$ N NO$_3^-$ N Org.N |
|------------------|-----------------|----------------|---------------|
| E1 Peat (burnt) E1D1 | 1.16          | 0.09           | 0.28          | 0.79          |
| E1 Peat (burnt) E1D2 | 3.90          | 0.48           | 0.01          | 3.41          |
| E1 Peat (burnt) E1D3 | 8.29          | 0.28           | 3.59          | 4.41          |
| **E1 average**   | **4.45**        | **0.28**       | **1.29**      | **2.87**      |
| E2 Peat (unburnt) E2D1 | 6.96          | 2.32           | 0.02          | 4.62          |
| E2 Peat (unburnt) E2D2 | 8.49          | 2.83           | 0.04          | 5.61          |
| E2 Peat (unburnt) E2D3 | 9.59          | 2.81           | 0.07          | 6.71          |
| **E2 average**   | **8.35**        | **2.66**       | **0.04**      | **5.65**      |
sites, they are an order of magnitude greater still under the mature *Calluna* (E2). These extreme values, observed in all three E2 plots (Table 3.14d) are almost two orders of magnitude greater than all soils at other sites (Table 3.15).

Table 3.15: Nitrogen species and acidity in soilwaters – comparison of mean concentrations by site

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Total N mg/l</th>
<th>Org.N mg/l</th>
<th>NH4-N μeq/l</th>
<th>NO3-N μeq/l</th>
<th>pH</th>
<th>Org. N %</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 Peaty ranker</td>
<td>0.38</td>
<td>0.36</td>
<td>1.4</td>
<td>0.0</td>
<td>3.4</td>
<td>93.7</td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>0.6</td>
<td>0.57</td>
<td>1.4</td>
<td>0.0</td>
<td>3.7</td>
<td>94.9</td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>0.43</td>
<td>0.39</td>
<td>1.4</td>
<td>0.0</td>
<td>4.0</td>
<td>91.4</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>0.51</td>
<td>0.45</td>
<td>2.9</td>
<td>0.0</td>
<td>3.9</td>
<td>87.7</td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>1.13</td>
<td>1.05</td>
<td>5.0</td>
<td>0.0</td>
<td>3.4</td>
<td>93.6</td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>0.62</td>
<td>0.58</td>
<td>2.9</td>
<td>0.0</td>
<td>3.6</td>
<td>93.2</td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>0.35</td>
<td>0.32</td>
<td>2.1</td>
<td>0.0</td>
<td>4.1</td>
<td>90.2</td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>1.66</td>
<td>1.61</td>
<td>3.6</td>
<td>0.0</td>
<td>3.8</td>
<td>96.8</td>
</tr>
<tr>
<td>S1 Podsol</td>
<td>0.49</td>
<td>0.34</td>
<td>2.1</td>
<td>8.6</td>
<td>3.9</td>
<td>69.2</td>
</tr>
<tr>
<td>S2 Peaty gley</td>
<td>0.56</td>
<td>0.39</td>
<td>2.1</td>
<td>10.7</td>
<td>3.8</td>
<td>68.4</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>0.45</td>
<td>0.38</td>
<td>2.1</td>
<td>2.9</td>
<td>3.9</td>
<td>83.3</td>
</tr>
<tr>
<td>E1 Deep peat (burnt)</td>
<td>4.45</td>
<td>2.87</td>
<td>20.0</td>
<td>92.1</td>
<td>3.0</td>
<td>64.6</td>
</tr>
<tr>
<td>E2 Deep peat (unburnt)</td>
<td>8.35</td>
<td>5.65</td>
<td>190.0</td>
<td>2.9</td>
<td>3.3</td>
<td>67.6</td>
</tr>
</tbody>
</table>

Organic N concentrations follow the same pattern as NH₄⁺, being low at the Mharcaidh and Scoat Tarn, higher at the Gwy, especially the peats at G1 and G4, and highest by a large margin at the Etherow. The pattern in organic N is linked to peat soils more closely than for NH₄⁺, the only exception being the deep peat at Scoat Tarn (S3).

Soilwater pH is comparable at the Mharcaidh, Gwy and Scoat Tarn sites, with values generally close to pH 4.0. The more acid soils at the Mharcaidh and Gwy are also the
highest altitude soils (M1, G1), with average pH values around pH 3.5. The Etherow is again the odd site with much more acid soilwaters, especially under burnt vegetation where the mean value is pH 3.0.

3.4.2 Surface lysimeters

During a preliminary assessment of the existing data in March 2000 it had been noted that despite the presence of appreciable NO$_3^-$ concentrations in streamwaters at the Afon Gwy, 2-weekly soilwater samples almost all showed zero NO$_3^-$. It was initially feared that the suction samplers could have been installed too deep (generally at 20-30 cm) to detect NO$_3^-$, and that it was perhaps being denitrified or taken up microbially within the surface organic horizons.

It was therefore decided to install surface zero-tension lysimeters at selected plots in all study catchments to investigate whether this was indeed the case, and whether significant NO$_3^-$ concentrations occurred in near-surface soilwaters. These lysimeters were installed in April 2000 and after a short settling-in period, sampling commenced in May, continuing throughout the summer period until the end of the budget year in October. The results of the sampling are shown in Table 3.16.

Inspection of the data in Table 3.16 shows that there is no evident difference in soilwater chemistry between surface and deep soilwater samplers. Zero values are still obtained for NO$_3^-$ at the Mharcaidh and Gwy plots. For the deeper suction samplers used throughout the budget year, sampler depth does not therefore appear to be responsible for the lack of NO$_3^-$ in sampled soilwaters.

3.4.3 Soilwater fluxes

Soilwater chemistry data was not converted into annual fluxes because of the poor resolution of data on soil moisture and rainfall, and the complex hydrology of some sites where pipeflow appears to be significant. Differences in concentrations between soil and surface waters are therefore assumed to be due to processes below the rooting
zone, or to rapid in-stream processes. The main utility of the soilwater data therefore lies in the comparison of concentrations with surface water and bulk deposition chemistry.

3.4.4 Comparison of soilwater, surface water and bulk deposition

At the Mharcaidh, streamwater NH$_4^+$ is close to zero while mean NO$_3^-$ concentration is c. 1 µeq l$^{-1}$, which is the reverse of the soilwater chemistry. The small amount of NH$_4^+$ in soilwaters therefore appears to be nitrified either prior to or soon after entering the stream. Similarly, Gwy streamwater concentrations are zero for NH$_4^+$ and 6 µeq l$^{-1}$ for NO$_3^-$, while for the soilwaters the figures are 2-5 µeq l$^{-1}$ for NH$_4^+$ and zero for NO$_3^-$.

Again, this could be explained by nitrification of soilwater NH$_4^+$ either within the soils or in-stream. Concentrations of organic N are much higher in the Mharcaidh soilwaters than in streamwaters, ranging from 360-570 µgl$^{-1}$ and 105-127 µgl$^{-1}$ respectively. Organic N was not measured for Gwy streamwaters.

In the Scoat Tarn streams (rather than the lake, to avoid the potential influence of in-lake processes), mean NO$_3^-$ concentrations are 11 and 17 µeq l$^{-1}$, while NH$_4^+$ averages around 0.5 µeq l$^{-1}$. These figures are comparable to mean soilwater concentrations, especially if possible nitrification is considered, which vary from 3-11 µeq l$^{-1}$ for NO$_3^-$ and are close to 2 µeq l$^{-1}$ for NH$_4^+$. The concentrations of organic N in the streams (98-113 µgl$^{-1}$) are much lower than in the soilwaters (340-390 µgl$^{-1}$).

The high concentrations of NH$_4^+$ found in the Etherow soilwaters (20-190 µeq l$^{-1}$) are not reflected in the streams (0.5 – 2.0 µeq l$^{-1}$), while NO$_3^-$ is comparable in the soilwaters if the average for the burnt and unburnt plots is taken (3 µeq l$^{-1}$ under mature Calluna, 92 µeq l$^{-1}$ under burnt Calluna, compared with 43 and 58 µeq l$^{-1}$ in Rose Clough and Swan Clough).

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Table 3.16: Deep and surface lysimeter (SL) chemistry (mean values for the period May-October 2000)

<table>
<thead>
<tr>
<th>Code</th>
<th>Sample</th>
<th>NO$_3$-N mg/l$^1$</th>
<th>NH$_4$-N mg/l$^1$</th>
<th>Org.N mg/l$^1$</th>
<th>Tot.N mg/l$^1$</th>
<th>NH$_4$-N µeq/l$^1$</th>
<th>NO$_3$-N µeq/l$^1$</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>M4 Shallow peat</td>
<td>Deep</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>5.0</td>
<td>0.0</td>
<td>5.0</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>Deep</td>
<td>0.0</td>
<td>0.2</td>
<td>0.8</td>
<td>0.9</td>
<td>11.3</td>
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</tr>
<tr>
<td>M4 Shallow peat</td>
<td>Deep</td>
<td>0.0</td>
<td>0.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.4</td>
<td>0.0</td>
<td>4.0</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>Surface</td>
<td>0.0</td>
<td>0.0</td>
<td>0.5</td>
<td>0.6</td>
<td>1.9</td>
<td>0.0</td>
<td>4.2</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>Surface</td>
<td>0.0</td>
<td>0.0</td>
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<td>0.5</td>
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<td>M4 Shallow peat</td>
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<td>4.2</td>
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<td>4.0</td>
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<tr>
<td>G1 Hilltop peat</td>
<td>Deep</td>
<td>0.0</td>
<td>0.1</td>
<td>1.3</td>
<td>1.4</td>
<td>8.8</td>
<td>0.0</td>
<td>3.8</td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>Deep</td>
<td>0.0</td>
<td>0.1</td>
<td>1.0</td>
<td>1.1</td>
<td>6.3</td>
<td>0.0</td>
<td>3.7</td>
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<tr>
<td>G1 Hilltop peat</td>
<td>Deep</td>
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<td>0.0</td>
<td>0.8</td>
<td>0.8</td>
<td>3.4</td>
<td>0.0</td>
<td>3.9</td>
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<tr>
<td>G1 Hilltop peat</td>
<td>Surface</td>
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<td>0.1</td>
<td>1.1</td>
<td>1.3</td>
<td>9.0</td>
<td>0.0</td>
<td>3.7</td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>Surface</td>
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<td>0.1</td>
<td>0.9</td>
<td>1.0</td>
<td>5.7</td>
<td>0.0</td>
<td>3.9</td>
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<tr>
<td>G1 Hilltop peat</td>
<td>Surface</td>
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<td>1.0</td>
<td>5.2</td>
<td>0.0</td>
<td>3.9</td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>Deep</td>
<td>0.0</td>
<td>0.1</td>
<td>1.6</td>
<td>1.7</td>
<td>4.2</td>
<td>0.0</td>
<td>3.8</td>
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<tr>
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<td>0.1</td>
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<td>2.0</td>
<td>4.0</td>
<td>0.0</td>
<td>3.9</td>
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<tr>
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<td>0.1</td>
<td>0.8</td>
<td>0.8</td>
<td>4.8</td>
<td>0.0</td>
<td>4.4</td>
</tr>
<tr>
<td>G4 Valley peat</td>
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<td>1.1</td>
<td>1.2</td>
<td>7.5</td>
<td>0.0</td>
<td>4.0</td>
</tr>
<tr>
<td>G4 Valley peat</td>
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<td>0.1</td>
<td>1.5</td>
<td>1.5</td>
<td>5.8</td>
<td>0.0</td>
<td>4.4</td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>Surface</td>
<td>0.0</td>
<td>0.1</td>
<td>1.7</td>
<td>1.8</td>
<td>5.9</td>
<td>0.0</td>
<td>4.6</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>Deep</td>
<td>0.0</td>
<td>0.0</td>
<td>0.5</td>
<td>0.5</td>
<td>2.1</td>
<td>0.0</td>
<td>4.3</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>Deep</td>
<td>0.0</td>
<td>0.0</td>
<td>0.6</td>
<td>0.6</td>
<td>1.7</td>
<td>1.9</td>
<td>4.7</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>Deep</td>
<td>0.0</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>4.3</td>
<td>0.0</td>
<td>4.7</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>Surface</td>
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<td>0.1</td>
<td>0.7</td>
<td>1.0</td>
<td>4.7</td>
<td>1.3</td>
<td>4.8</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>Surface</td>
<td>0.0</td>
<td>0.0</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.0</td>
<td>4.6</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>Surface</td>
<td>0.0</td>
<td>0.0</td>
<td>0.5</td>
<td>0.5</td>
<td>2.7</td>
<td>0.1</td>
<td>5.0</td>
</tr>
<tr>
<td>E1 Peat (burnt)</td>
<td>Deep</td>
<td>0.6</td>
<td>0.1</td>
<td>0.9</td>
<td>1.6</td>
<td>4.5</td>
<td>44.8</td>
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</tr>
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<td>E1 Peat (burnt)</td>
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<td>0.3</td>
<td>2.1</td>
<td>2.5</td>
<td>22.1</td>
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<td>E1 Peat (burnt)</td>
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<td>0.3</td>
<td>0.8</td>
<td>4.6</td>
<td>22.3</td>
<td>246.6</td>
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<td>E1 Peat (burnt)</td>
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<td>1.1</td>
<td>1.4</td>
<td>3.0</td>
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<td>E1 Peat (burnt)</td>
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<td>0.3</td>
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<td>2.2</td>
<td>20.5</td>
<td>23.9</td>
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</tr>
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<td>0.4</td>
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<td>1.2</td>
<td>29.4</td>
<td>2.2</td>
<td>3.7</td>
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<tr>
<td>E2 Peat (unburnt)</td>
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<td>2.2</td>
<td>2.4</td>
<td>4.7</td>
<td>160.4</td>
<td>0.8</td>
<td>3.6</td>
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<td>2.7</td>
<td>2.9</td>
<td>5.6</td>
<td>194.3</td>
<td>3.1</td>
<td>3.4</td>
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<td>3.5</td>
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<td>180.3</td>
<td>3.0</td>
<td>3.5</td>
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<td>E2 Peat (unburnt)</td>
<td>Surface</td>
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<td>1.6</td>
<td>1.8</td>
<td>3.5</td>
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<td>5.9</td>
<td>3.8</td>
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<td>143.5</td>
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<tr>
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<td>2.5</td>
<td>3.9</td>
<td>89.8</td>
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</tbody>
</table>

3.5 Soil moisture

Annual mean soil moisture values for surface soils (0-10cm) are given in Table 3.17. Variations in soil moisture throughout the budget year are shown in Figures 3.15a-d.
Table 3.17: Annual mean soil moisture (m^3 m^-3)

<table>
<thead>
<tr>
<th></th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>E1</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.76</td>
<td>0.87</td>
<td>0.84</td>
<td>0.87</td>
<td>0.80</td>
<td>0.76</td>
<td>0.73</td>
<td>0.89</td>
<td>0.84</td>
<td>0.76</td>
<td>0.86</td>
<td>0.73</td>
<td>0.58</td>
</tr>
</tbody>
</table>

The driest soil at the Mharcaidh is at the highest altitude plots, the peaty ranker at M1, while the other Mharcaidh soils are among the wettest studied. However, it was not possible to obtain measurements for much of the winter at M1, since these plots tended to be covered by deep snow for long periods (Figure 3.15a). At the Gwy, the wettest soils are the hilltop peat at G1 and especially the valley peat at G4, while the podsol and peaty gley are much drier on average. The hilltop peat shows much more variable soil moisture throughout the winter and spring periods, while G2 and G3 soils become very dry during the summer months. The peaty gley is the driest soil at Scoat Tam, but all three soils measured follow very similar patterns. The soils at the Etherow are the driest studied, in particular the peat below the mature Calluna at E2 has a much lower soil moisture content than any other soil within the study. The Etherow soils also show the greatest variability between samples.

3.6 Soil temperatures

Seasonal variations in soil temperature throughout the budget year are shown in Figure 3.16a-d, while annual mean values are given in Table 3.18. Cumulative number of days above given temperatures are summarised in Table 3.19. To calculate cumulative temperature days, missing values which occurred in all Mharcaidh dataloggers (due to deep snow cover preventing downloads of some loggers, but never all at the same time) were derived from relationships with recorded temperatures from other loggers at the same site when two loggers were operating over the same period. Hence a small source of error has been introduced into the figures for M1 to M4.
Figure 3.15a: Soil moisture in Allt a’Mharcaidh soils

Figure 3.15b: Soil moisture in Afon Gwy soils
Figure 3.15c: Soil moisture in Scoat Tarn soils

Figure 3.15d: Soil moisture in River Etherow soils
The Mharcaidh site experiences the lowest mean, minimum (<-1°C) and maximum (12-15°C) soil temperatures, with several weeks below freezing point at M1 and M3. Note that the period of very low temperatures recorded for M1 uses interpolated data from other loggers, and will therefore have an associated error.

| Table 3.18: Annual mean soil temperatures (5-10cm depth) |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| M1        | M2        | M3        | M4        | G1        | G2        | G3        | G4        | S1        |
| 4.4       | 5.5       | 5.8       | 6.2       | 7.3       | 8.4       | 8.3       | 6.5       | 6.6       |
| S2        | S3        | E1        | E2        |           |           |           |           |           |
| 6.9       | 7.2       | 7.1       |           |           |           |           |           |           |

The Afon Gwy soils experience the highest mean (7.3 – 8.4°C) and minimum temperatures, never falling below 1°C at 5-10cm depth (Table 3.19). The soils at Scoat Tarn are somewhat cooler, with mean values of 6.5 – 6.9°C and 10-20 days below 1°C. While maximum temperatures are similar to those at the Gwy, expression of the data in “degree days” above given temperatures (temperature x time) reveals higher values for the Gwy soils than for Scoat Tarn (Table 3.20). A figure of 6°C is often used to define the growing season (Monkhouse & Small, 1979; Harrison et al., 1994), but this value refers to air temperature rather than soil temperature.

While the mean soil temperatures for the Etherow soils are very similar (Table 3.18) the bare soil at E1 experiences a more extreme temperature range than the soil under the mature Calluna at E2 because of the lack of surface cover at E1 (Table 3.18, Fig. 3.16d). The soils at E1 are colder during the winter, even freezing for a short period, and are warmer during the summer, reaching the highest maximum temperature of all soils at any site.

In addition to the obvious effects of weather and climate, soil temperature is affected by many non-climatic factors such as soil aspect, slope, shading, plant and litter cover (Paul & Clark, 1996). Factors which affect the rate of change of soil temperature include soil wetness (wetter soils have a higher specific heat capacity) and snow cover.
Table 3.19: Cumulative number of days above each degree Celsius for the budget year. Discrepancies from 366 days (leap year) above the minimum temperature are due to missing data.

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Table 3.20: Degree days above selected temperatures for the budget year

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<th>M3</th>
<th>M4</th>
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Figure 3.16a: Soil temperatures at the Allt a’Mharcaidh plots (some parts of curve derived from other dataloggers for periods of missing data)

Figure 3.16b: Soil temperatures at the Afon Gwy plots
Figure 3.16c: Soil temperatures at the Scoat Tarn plots

Figure 3.16d: Soil temperatures at the River Etherow plots
3.7 Grazing removal of N

The biomass of vegetation sampled in replicated quadrats inside and outside fenced exclosures is shown in Table 3.21. It can be seen that there is no significant difference between grazed and ungrazed biomass, due to the great spatial variability in biomass and small size of the quadrats sampled, i.e. spatial variability (indicated by the high standard deviations of the mean) is greater than any differences in biomass due to grazing pressure.

In a study of the N cycle in a montane grassland at Llyn Llydaw, Snowdonia, only 3.2 kgN ha\(^{-1}\) yr\(^{-1}\) were exported in sheep biomass, representing just 5% of the ingested N, although there was much localised redistribution of N in faeces and urine (Perkins, 1978, cited in INDITE, 1994). Batey (1982) suggested that the removal of N from upland pastures in sheep biomass (meat and wool) varies from 0.4-0.7 kgN ha\(^{-1}\) yr\(^{-1}\) at a stocking density of 0.5 ewes per hectare, up to 1.2-2.1 kgN ha\(^{-1}\) yr\(^{-1}\) at a high stocking density of 1.5 ewes per hectare. Hence it seems likely that the grazing export of N from the four study catchments is negligible and could not account for a major proportion of the N retention observed in these systems. Information on stocking density could, however, be used to obtain estimates for this minor flux. Grazing export will be close to zero at the Allt a'Mharcaidh, since the study area lies within a National Nature Reserve with no sheep and which is little used by deer.

3.8 Discussion: N retention and leaching from the study catchments

An overview of the N input-output status of the four study catchments is provided below, as a context for the following chapters which focus on more specific aspects of the N cycle and N saturation status.

3.8.1 Allt a'Mharcaidh

Measurements of bulk deposition inputs and leaching losses of N throughout the budget year confirm that the Allt a'Mharcaidh is still a very low input – low output...
site which is strongly N limited. Modelled inorganic N inputs are only 7.3 kgN ha\(^{-1}\) yr\(^{-1}\) (1.8 kgN ha\(^{-1}\) yr\(^{-1}\) in measured bulk deposition, with mean concentrations for NO\(_3^-\) and NH\(_4^+\) of 6.5 and 3.9 μeql\(^{-1}\) respectively) while just 0.1 kgN ha\(^{-1}\) yr\(^{-1}\) is leached into surface waters, indicating 99% retention. Soilwater concentrations of inorganic N are very low and only NH\(_4^+\) is present in measurable amounts, with the highest mean concentration of just 2.9 μeql\(^{-1}\) in the lowest altitude, shallow peat soil at M4. By comparison, streamwater concentrations of NO\(_3^-\) peak at 2.5 μeql\(^{-1}\) in mid-winter and average just 1 μeql\(^{-1}\), while NH\(_4^+\) is almost always below detection limits. Hence rapid nitrification of soilwater NH\(_4^+\) could account for observed streamwater NO\(_3^-\).

Table 3.21: Mean biomass (3 replicates) in grazed and ungrazed plots (g. dry weight per 0.25 m\(^2\) quadrat)

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<th>Status</th>
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<th>SD</th>
<th>Lichen &amp; moss Mean</th>
<th>SD</th>
<th>Non-heather (live) Mean</th>
<th>SD</th>
<th>Non-heather (dead) Mean</th>
<th>SD</th>
<th>Heather (woody) Mean</th>
<th>SD</th>
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<td>109.0</td>
<td>31.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E1</td>
<td>Grazed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>35.4</td>
<td>55.6</td>
<td>61.5</td>
<td>82.7</td>
</tr>
<tr>
<td>E1</td>
<td>Ungrazed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>30.4</td>
<td>8.5</td>
<td>35.1</td>
<td>15.2</td>
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<tr>
<td>E2</td>
<td>Grazed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>358.7</td>
<td>155.6</td>
<td>92.6</td>
<td>28.6</td>
</tr>
<tr>
<td>E2</td>
<td>Ungrazed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>356.3</td>
<td>92.1</td>
<td>121.8</td>
<td>7.1</td>
</tr>
</tbody>
</table>

The Mharcaidh soils, particularly the valley peat (M2) and the shallow peat (M4) are among the wettest studied on average, despite the low rainfall at the site. Mean soil
temperatures are the lowest of all sites, especially at the highest altitude plots on the peaty rankers (M1), where the annual mean temperature of 4.4°C is more than 2°C less than soils in other study catchments. The soils at M1 and to a lesser degree M3 appear to be frozen for periods of up to several weeks during the winter, much longer than at any other site.

3.8.2 Afon Gwy

While the Afon Gwy was selected as a moderate N deposition site, recent modelled and measured data suggest that it experiences rather high levels of total N deposition (27 kgN ha\(^{-1}\) yr\(^{-1}\)), while measured concentrations in bulk deposition average 9 \(\mu\)eql\(^{-1}\) for NO\(_3^-\) and 8 \(\mu\)eql\(^{-1}\) for NH\(_4^+\) (total flux 6.6 kgN ha\(^{-1}\) yr\(^{-1}\)). Despite these high inputs, leaching outputs are moderate, with an average NO\(_3^-\) concentration of only 6 \(\mu\)eql\(^{-1}\), peaking at around 15 \(\mu\)eql\(^{-1}\) in mid-winter. NH\(_4^+\) concentrations are always below detection limits in the stream. Retention of inorganic N is 93% of total inputs, or 80% of NO\(_3^-\) inputs. However, average soilwater NO\(_3^-\) is zero for all soils, while mean concentrations of NH\(_4^+\) range from 2-5 \(\mu\)eql\(^{-1}\), being highest in the hilltop peats at G1. Again, it seems that nitrification of soilwater NH\(_4^+\) is required to account for streamwater NO\(_3^-\) levels, but even then, soilwater concentrations of inorganic N at the depth sampled (c. 15-30cm) are lower than in the stream, so another source elsewhere in the soil must be present unless direct runoff of wet deposition is significant.

The valley peat (G4) is the wettest of all soils studied, while the hilltop peat (G1) also has high soil moisture content compared with the peaty gley and podsol soils at G2 and G3. Rainfall at the Gwy is very similar to that at Scoat Tarn, and more than double that at the Mharcaidh and Etherow catchments. Soil temperatures are higher at the Gwy that at any other site, in particular the lower altitude soils at G3 and G4 which both average more than 8°C. Furthermore, the soils are never frozen at a depth of 5-10cm, where the temperature does not fall below 1°C.
3.8.3 Scoat Tarn

Scoat Tarn was selected as a moderate N deposition site with high leaching outputs, but recent data suggest that it is a high deposition site, with inputs of inorganic N comparable to those at the Etherow according to modelled data (33.6 kgN ha\(^{-1}\) yr\(^{-1}\)), and even greater than the Etherow according to bulk deposition fluxes alone (10.1 kgN ha\(^{-1}\) yr\(^{-1}\)). Mean concentrations of NH\(_4^+\) and NO\(_3^-\) in bulk deposition are similar, at 13 and 12 μeql\(^{-1}\) respectively. Average NO\(_3^-\) concentrations in the inflow streams are 15-17 μeql\(^{-1}\), peaking at almost 30 μeql\(^{-1}\) during early spring in Red Pike stream, and represent a net retention of 85% of total inorganic N inputs, or 57% of NO\(_3^-\) inputs. Very low streamwater NH\(_4^+\) concentrations are occasionally recorded, but the annual mean is close to zero. In the soilwaters, NH\(_4^+\) concentrations are low at around 2 μeql\(^{-1}\), while high mean NO\(_3^-\) concentrations of 3-11 μeql\(^{-1}\) occur in all soils, but particularly in the podsol (S1) and peaty gley (S2). As in the previous sites, nitrification of either soilwater or direct runoff sources must be making a contribution to the observed surface water NO\(_3^-\) concentrations.

The rainfall is the highest of all sites at Scoat Tarn, and as a result the soils at S1 and S3 have a high average soil moisture, while S2 is somewhat drier. Soil temperatures are lower at Scoat Tarn (annual mean 6.5-6.9°C) than at the Gwy or the Etherow, but still much warmer than at the Mharcaidh. The soils rarely freeze at a depth of 5-10cm.

3.8.4 River Etherow

Although selected as the highest deposition site, it transpired during the budget year that total inorganic N deposition at the Etherow is in fact very similar to, and if anything slightly lower than, Scoat Tarn. While concentrations in bulk deposition are easily the highest at the Etherow, with means of 29 and 27 μeql\(^{-1}\) for NH\(_4^+\) and NO\(_3^-\), the low rainfall at the site means that inorganic N inputs are 33.7 kgN ha\(^{-1}\) yr\(^{-1}\) (total modelled) and c.9 kgN ha\(^{-1}\) yr\(^{-1}\) in bulk deposition. In contrast, streamwater concentrations of inorganic N are much higher at the Etherow than elsewhere, with mean NO\(_3^-\) concentrations of 43-58 μeql\(^{-1}\) and 2 μeql\(^{-1}\) for NH\(_4^+\) at Rose Clough, the
only site to show significant NH$_4^+$ leaching within the study. Unlike the other sites, there is no obvious seasonality in inorganic N leaching. Relative to other sites, the Etherow catchment leaches a large proportion of inorganic N (32.5%), but for NO$_3^-$ alone the proportion is extremely high at 87%.

The catchment is also noteworthy for showing extremely high (although spatially very variable) soilwater concentrations of inorganic N. The burnt Calluna plot at E1D3 is a particular hotspot for NO$_3^-$, while all three plots under the mature Calluna at E2 show mean soilwater NH$_4^+$ concentrations which are two orders of magnitude greater than at any other site (average 190 µeql$^{-1}$). It might be suggested from the bulk deposition data alone that nitrification of reduced N originating directly from deposition could account for surface water inorganic N concentrations, but consideration of the soilwater chemistry shows that there is little relation between bulk deposition, streamwater and soilwater, while a large sink for NH$_4^+$ must occur somewhere beneath the rooting zone. Furthermore, the soilwaters at the Etherow are much more acid than elsewhere, particularly under the burnt Calluna at E1 where mean pH is 3.0, perhaps reducing the potential for nitrification (see Chapter 5).

The soils at the Etherow are the driest, but most variable, of all sites, especially under the mature Calluna at E2, reflecting the relatively low rainfall at the site. Soil temperatures are slightly higher than at Scoat Tarn, much higher than the Mharcaidh but lower than the Gwy. The soils at 5-10cm depth hardly ever freeze during the monitoring period.

### 3.9 Conclusions and role of budget data

The data presented above show that the study catchments cover a broad gradient in N fluxes for both inputs and outputs, and are very different in terms of internal N cycling, as indicated by the soilwater data. In addition to large differences between sites, major differences in soilwater chemistry are also apparent between soil types within each catchment. All catchments are retaining most of the deposited N, but the leaching of even a small proportion of inputs has a significant impact on surface water
acidity. Terrestrial controls on N leaching are therefore of great importance for modelling the impacts of acid deposition on surface waters.

Grazing losses are not detectable above random variations in biomass and so are assumed to be negligible. Therefore the key terms in FAB, denitrification and immobilisation, must be responsible for the retention of most of the non-leached N. The following chapters aim to determine the fate of retained N deposition, in order to address the question of how well the FAB model represents the major N sinks at present, and how these compare with steady-state predictions of increased NO$_3^-$ leaching.

3.10 References


CHAPTER 4

DENITRIFICATION – FIELD AND LABORATORY FLUX MEASUREMENTS
4. DENITRIFICATION – FIELD AND LABORATORY FLUX MEASUREMENTS

4.1 Introduction: denitrification processes and controls

4.1.1 Aims of the chapter

The denitrification process provides a potential sink for protons associated with N deposition inputs (Klemetsson & Svensson, 1988; Willison & Anderson, 1991), and is therefore included in the FAB model mass balance for N (see Chapter 2). The formulation for calculating the net N sink via denitrification is a source of uncertainty in the model, and results in the prediction of very significant fluxes of N which have not been observed in previous studies of British moorland systems. The primary aims of this chapter are therefore to estimate denitrification rates for typical soils in the four study catchments via both field and laboratory measurements. The major controls on denitrification fluxes are also tested to provide the basis for a critical assessment of the FAB model formulation for denitrification.

4.1.2 Definitions

Denitrification is the reduction of oxidised N species, usually originating as $\text{NO}_3^-$, to gaseous forms which may be lost from the soil to the atmosphere. In this respect, it can act as an important sink for potentially acidifying N deposition. The most important process is generally microbial denitrification, associated with a huge number of different microbial organisms, although abiotic denitrification (chemodenitrification) may also occur under certain conditions (Firestone & Davidson, 1989; Paul & Clark, 1996). Microbial denitrification is carried out mainly by facultative anaerobes which use oxygen as an electron acceptor during respiration when available, but can otherwise use N oxides as electron acceptors, which is then termed denitrification (Parsons et al., 1991; Groffman et al., 1999).

Microbial denitrification follows a pathway which includes several stages, each associated with a particular enzyme, and can produce up to three gaseous N species
Nitrite (NO$_2^-$) is rapidly transformed in soils and rarely occurs at measurable levels. The relative proportions of nitric oxide (NO), nitrous oxide (N$_2$O) and free nitrogen gas (N$_2$) produced vary widely, according to the specific chemical, physical and biological conditions at a site (see below).

Figure 4.1: The microbial pathway of denitrification

For example, the different enzymes may be inhibited to varying degrees by the presence of oxygen or low pH conditions. Nitrous oxide reductase (NOR) is unique in that it is inhibited by acetylene, which provides the basis of the acetylene block method described below (Paul & Clark, 1996). Local conditions also dictate the rates at which the different stages of the process may occur. For example, Dendooven et al. (1994) showed that for a well fertilised agricultural soil, the reduction of NO$_2^-$ to N$_2$O was much more rapid than the reduction of N$_2$O to N$_2$.

The general requirements for microbial denitrification include the following:
1. the absence of O$_2$,
2. the presence of N oxides (usually NO$_3^-$) as an oxidant,
3. the availability of a reductant (usually organic C), and
4. the presence of denitrifying organisms.

Many biotic and abiotic factors interact to determine these controls on the denitrification process. In addition to the availability of oxygen, NO$_3^-$ and organic C, pH and temperature also affect the rate and end products of denitrification, and unfavourable conditions for any one of these factors can completely inhibit it (Davidson & Swank, 1987). High denitrification only occurs where all these factors are optimal, but a complex of microsites in soils often means that there is a continuum of levels for them all. Some of these controls are considered individually below.
4.1.3 N availability

In agricultural soils where NO$_3^-$ is usually present in excess, water availability or the supply of readily decomposable carbon is usually the key control of denitrification, while in non-agricultural soils where competition for available N is high, soil NO$_3^-$ production is usually the key limiting factor (Groffman et al., 1988; Weier et al., 1993).

In unfertilised soils, the supply of NH$_4^+$ from mineralisation is often a dominant control of nitrification and therefore of NO$_3^-$ availability (Groffman et al., 1988). Mineralisation is in turn controlled by several interacting factors including soil type, climate and plant community composition. Nitrification, as an aerobic process, is inhibited by high soil water content.

At concentrations of soilwater NO$_3^-$ lower than circa 20 mgN l$^{-1}$ denitrification appears to follow first-order kinetics, but at greater concentrations denitrification is independent of NO$_3^-$ and follows zero order kinetics, when it may instead be determined by C availability (Paul & Clark, 1996). Ryden (1986) suggested that the greatest potential for denitrification in grassland soils occurs when soil NO$_3^-$-N exceeds 5 $\mu$g g$^{-1}$. Davidson and Swank (1986) found that production of N$_2$O by denitrification in forest soils was positively correlated with ambient NO$_3^-$-N. However, Parsons et al. (1991) suggested that denitrification may occur at soilwater NO$_3^-$ concentrations which are below detection limits, because of the very high affinity of denitrifiers for NO$_3^-$-N. At low levels in wet soils the rate determinant may be the diffusion of NO$_3^-$ to the site of denitrification.

4.1.4 Carbon availability

As most denitrification is carried out by heterotrophic organisms the process is strongly dependent on C availability (Davidson & Swank, 1987; Paul & Clark, 1996). There is a general correlation between total soil organic matter (SOM) content and denitrification potential, but a much better correlation is with the amount of water
soluble carbon measurable in soil, accounting for up to 71% of its denitrification potential (Paul & Clark, 1996). The rhizosphere might therefore be the part of the soil profile expected to be most conducive to denitrification, with easily decomposable C provided by root exudates and exfoliates. Sharp changes in soil water content (drying and re-wetting events), freeze-thaw cycles or other physical disturbances can increase C availability by physical rearrangement of soil particles and by killing microbial cells (Groffman et al., 1988; McCarty & Bremner, 1993).

Carbon affects denitrification more often through indirect effects on O₂ status than through substrate availability, since by stimulating heterotrophic activity C availability promotes oxygen depletion and thus favours denitrifiers over aerobes (Groffman et al., 1988; Christensen et al., 1990). Hence soil respiration (CO₂ production) has been found in some studies to correlate well with denitrification activity (e.g. deCantanzaro & Beauchamp, 1985; Christensen et al., 1990). However, since competition for C is low when oxygen is limiting, C is probably the least common factor directly regulating denitrification in soils.

Not only do NO₃⁻ concentrations and C supply influence denitrification potential; their relative availability has been observed to influence the N₂O:N₂ ratio in the gaseous product (Weier et al., 1993; Paul & Clark, 1996). With high NO₃⁻ and low C supply the ratio is increased, while limited NO₃⁻ decreases the ratio.

4.1.5 Soil moisture

According to Parsons et al. (1991), denitrifying bacteria are chemoheterotrophs that can grow under both aerobic and anaerobic conditions, but only denitrify under anaerobic conditions. Hence those factors which prevent the diffusion of O₂ through soils determine the favourability of conditions for denitrification.

Soil texture influences the diffusion of O₂ through soils, but soil moisture is a more important factor in facilitating denitrification by reducing O₂ movement and availability. The onset of denitrification has been found to occur at c. 60% water-filled pore space (WFPS), while soil moisture is often the biggest single predictor of
variation in denitrification rates in the field (Linn & Doran, 1984; Davidson & Swank, 1986; Parsons et al., 1991; van Kessel et al., 1993; Paul & Clark, 1996). Below 60% WFPS water may limit microbial activity, but at higher levels of soil moisture aerobic activity decreases (Linn & Doran, 1984). The greatest potential for denitrification from grassland soils was associated with WFPS of more than 60-85% by Ryden (1986). de Klein & van Logtestijn (1996) found that for soil moisture, field capacity is the threshold below which denitrification rates are very limited. While N₂O emissions may increase with increasing soil moisture, the diffusion of N₂O out of the soil is also impeded, so that a proportion may not be released until the soil water drains into surface waters (Davidson & Swank, 1986).

Increasing soil moisture was found to enhance overall denitrification in laboratory studies of clay and sandy loam soils, but also changed the ratio of N species liberated (Drury et al., 1992). At low soil moisture values (<20% water content) more NO than N₂O was evolved, and increasing moisture increased the proportion of NO. Above 20-30% soil moisture, the proportion of evolved N₂O approached and then overtook that of NO. At soil moisture values greater than 35% an apparent decrease in denitrification as the evolution of NO + N₂O declined was attributed to further reduction of N₂O to N₂, which was not detected directly in the analysis. According to Davidson et al. (1986), sufficiently reducing conditions for appreciable N₂ production (i.e. further reduction of N₂O) in a study of forest soils occurred only in samples with WFPS greater than 80%.

Factors that control soil water and hence O₂ diffusion are therefore important, including rainfall, activity of plants and soil texture (Groffman et al., 1988). As well as increasing the physical barrier to O₂ movement through soils, soil moisture affects O₂ availability indirectly through its influence on biological activity. For example, a major sink for O₂ in soil is respiration by plant roots and aerobes, which are linked to carbon supply, which is in turn controlled by plant decomposition, root exudates, and microbial cell decomposition (Groffman et al., 1988).

Soil moisture also affects NO₃⁻ supply, both indirectly via the activities of mineralising and nitrifying organisms, and directly, through its effects on the diffusion of NO₃⁻ through the soil matrix (Groffman et al., 1988). In waterlogged
environments, anaerobic conditions inhibit nitrification and thus NO$_3^-$ supply may be restricted. However, anaerobic conditions are necessary for denitrification, so a mechanism for the transport of NO$_3^-$ to waterlogged areas or microsites is required.

Spatial variability in denitrification appears to be greater at low soil moisture values, where heterogeneity in soil moisture restricts denitrification to microsites of low oxygen content. The coefficient of variation between replicated samples is found to be reduced as soil moisture increases, probably due to both increased anaerobic volume and the greater redistribution of soluble C and NO$_3^-$ (Sexstone et al., 1988).

4.1.6 Soil pH

Most denitrifying bacteria grow best in the range pH 6-8 (Paul & Clark, 1996). Denitrification slows down below pH 5 but may still remain significant, while denitrification by organotrophs is negligible or absent below pH 4.

As soil pH decreases, the ratio of N$_2$O to N$_2$ produced by denitrification increases, so that in very acid soils, N$_2$O may be the sole product of denitrification, which removes the need for the C$_2$H$_2$ block technique (Firestone et al., 1980; Hauck, 1986; Tiedje et al., 1989; Christensen et al., 1990; Ineson et al., 1991, 1998; see Section 4.3.3.5 below). Weier and Gilliam (1986) found that in Atlantic Plain coastal soils in the USA, N$_2$O emissions were negligible above pH 5.8, c. 15% of total NO$_3^-$ consumed at pH 5.7 and c. 93% at pH 4.2.

4.1.7 Temperature

Temperature has a direct effect on denitrification rates according to the Arrhenius equation, but it may also have an indirect effect, whereby an increase in soil respiration results in a greater anaerobic volume where denitrification can occur (Smith & Arah, 1990). The latter effect is less pronounced in wet soils where most of the soil volume is anaerobic already.
According to de Klein & van Logtestijn (1996), the highest denitrification rates are found in wet and warm soils, but this combination is unlikely in the field due to the generally negative correlation between soil moisture and soil temperature. They found that a temperature increase from 10 to 20°C led to a ten fold increase in denitrification rates. Other temporal studies have found a negative effect of increased temperature on denitrification, again due to a negative correlation between temperature and soil moisture, for example during summer when soils are warmest but also driest (e.g. Jarvis et al., 1991; Parsons et al., 1991).

Parsons et al. (1991) found that denitrification was not positively correlated with temperature, and occurred at values as low as 2°C, even though most workers cite 2.7 – 10 °C as the lower limit for denitrification. Similarly, Struwe and Kjøller (1991) also found denitrification occurring at 2°C, albeit at a low rate, but rates increased much more rapidly above 10°C. Ryden (1986) found maximum denitrification potentials in soils at temperatures greater than 5°C, while Bailey (1976) found that denitrification was completely inhibited as temperatures were reduced to 5°C.

4.1.8 Nitrification as a source of N\textsubscript{2}O and a sink for N

Nitrification, the oxidation of NH\textsubscript{4}\textsuperscript{+} to NO\textsubscript{3}\textsuperscript{−}, is generally attributed to the autotrophic bacteria Nitrosomonas sp. (oxidising NH\textsubscript{4}\textsuperscript{+} to NO\textsubscript{2}\textsuperscript{−}) and Nitrobacter sp. (oxidising NO\textsubscript{2}\textsuperscript{−} to NO\textsubscript{3}\textsuperscript{−}). Bremner and Blackmer (1978) used NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{−} based fertilisers to show that N\textsubscript{2}O can be released during nitrification of NH\textsubscript{4}\textsuperscript{+} under aerobic conditions as well as by the denitrification of NO\textsubscript{3}\textsuperscript{−}. Hutchinson and Mosier (1979) also found significant nitrification losses of N\textsubscript{2}O after addition of NH\textsubscript{4}\textsuperscript{+} fertilisers. The pathway of N\textsubscript{2}O production during nitrification is now well established (Figure 4.2).

While nitrifying bacteria have been shown to produce both NO and N\textsubscript{2}O, the proportion varies with O\textsubscript{2} concentration and is usually less than 1% of the short-lived NO\textsubscript{2}\textsuperscript{−} intermediate (Paul & Clark, 1996). Goodroad and Keeney (1984) found that only 0.1 to 0.2% of nitrified N was evolved as N\textsubscript{2}O in studies of silt loam soils at different temperature, pH and moisture contents. However, Martikainen (1985) found
that production of N$_2$O in fertilised acid forest soil from nitrification could reach 50% of that from denitrification. Skiba et al. (1992) suggested that N$_2$O produced by nitrification has a better chance of being lost because drier soils allow a better gaseous diffusion.

Figure 4.2: Production of gaseous forms of N during nitrification (redrawn from Paul & Clark, 1996)

Skiba et al. (1993) used the nitrification inhibitor dicyandiamide (DCD) to show that N$_2$O was the product of both nitrification and denitrification in sandy loam soils. In dry soils, DCD additions reduced N$_2$O emissions by more than 40% indicating the importance of nitrification sources, while in wet soils, emissions were not inhibited by DCD additions, indicating that denitrification was the major source.

Although it is important to understand the processes responsible for N$_2$O emissions in order to model them, it is only the net sink for N lost as gaseous emissions from the system which is of interest for mass balance critical load models, so the source of N$_2$O is not in itself important in this regard.
4.1.9 Magnitude of nitrogen loss by denitrification

There is a very large literature on denitrification processes and fluxes, and a selection of published annual fluxes is provided in Table 4.1. While only a few studies of agricultural soils are included for comparison, a wide selection of studies in semi-natural, unfertilised systems is covered.

For agricultural systems the great majority of studies record denitrification fluxes of no more than 10 kgN ha\(^{-1}\) yr\(^{-1}\) and mostly less than 5 kgN ha\(^{-1}\) yr\(^{-1}\). Several studies found negligible fluxes of less than 1 kgN ha\(^{-1}\) yr\(^{-1}\). Only five of the studies cited were carried out on non-forest, semi-natural, unfertilised systems, reflecting the general paucity of information from these areas where denitrification is often assumed to be negligible. Denitrification fluxes are generally found to be at least an order of magnitude greater from fertilised agricultural systems, although the percentage loss of N inputs may be comparable to unfertilised systems.

An earlier literature review of denitrification losses from forest soils by Dutch and Ineson (1990) found a very wide range of published fluxes, from zero to as much as 50 kgN ha\(^{-1}\) yr\(^{-1}\), with higher fluxes often following forest felling.

4.2 Denitrification – field measurements

4.2.1 Procedures for measuring denitrification

Denitrification is difficult to measure for several major reasons (Tiedje et al., 1989):

1. the ultimate product may be N\(_2\) which makes up 80% of the earth’s atmosphere and is therefore difficult to analyse at low concentrations because of the great potential for contamination;
2. measuring substrate depletion is difficult because of the various diverse sources of NO\(_3^-\); and
3. of the biogeochemical processes, denitrification is the most dynamic, with considerable variability over time and space.
Table 4.1: Annual mean N\textsubscript{2}O fluxes from soils published in the literature

<table>
<thead>
<tr>
<th>Soil / system</th>
<th>Denitrification flux kgN ha\textsuperscript{-1} yr\textsuperscript{-1} (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardwood forests, Catskills, USA (whole catchment, soil weighted)</td>
<td>1.1</td>
<td>Ashby et al. (1998)</td>
</tr>
<tr>
<td>Temperate forest, Solling, Germany</td>
<td>0.2 – 7.3</td>
<td>Brumme et al. (1999)</td>
</tr>
<tr>
<td>Spruce – fir forests, USA</td>
<td>&lt;0.1</td>
<td>Castro et al. (1993)</td>
</tr>
<tr>
<td>Unfertilised forests, USA</td>
<td>&lt;0.4</td>
<td>Davidson &amp; Swank (1986)</td>
</tr>
<tr>
<td>Forest soils, Netherlands (review)</td>
<td>0.1 – 2.1</td>
<td>Denier van der Gon (1989)</td>
</tr>
<tr>
<td>Kershope Forest, UK</td>
<td>1.7 – 3.2</td>
<td>Dutch &amp; Ineson (1990)</td>
</tr>
<tr>
<td>Literature review</td>
<td>0 - 50</td>
<td>Dutch &amp; Ineson (1990)</td>
</tr>
<tr>
<td>Forest soils, Michigan</td>
<td>&lt;1 to &gt;40</td>
<td>Groffman &amp; Tiedje (1989)</td>
</tr>
<tr>
<td>Tallgrass prairie, USA</td>
<td>~0 - 10</td>
<td>Groffman et al. (1993)</td>
</tr>
<tr>
<td>Undisturbed forests (review)</td>
<td>&lt;1</td>
<td>Gundersen (1991)</td>
</tr>
<tr>
<td>Clear-cut forests (review)</td>
<td>3-6</td>
<td>Gundersen (1991)</td>
</tr>
<tr>
<td>Riparian/meadow/forest/barren subalpine zones, Sierra Nevada, USA</td>
<td>Max. 6.1 – 8.8</td>
<td>Hixson et al. (1990)</td>
</tr>
<tr>
<td>Irrigated cornfield, California</td>
<td>&lt;4</td>
<td>Hutchinson &amp; Mosier (1979)</td>
</tr>
<tr>
<td>Coniferous forests, UK (deposition c. 100kgN ha\textsuperscript{-1} yr\textsuperscript{-1})</td>
<td>&lt;&lt;max. of 10.5</td>
<td>Ineson et al. (1998)</td>
</tr>
<tr>
<td>Quaking fens, Netherlands</td>
<td>1.1</td>
<td>Koerselman et al. (1989)</td>
</tr>
<tr>
<td>Sagebrush steppe, USA</td>
<td>0.13 – 0.32</td>
<td>Matson et al. (1991)</td>
</tr>
<tr>
<td>Poorly drained agricultural soils with SOM&gt;5% (fertilised)</td>
<td>(25-55)</td>
<td>Meisinger &amp; Randall (1991)</td>
</tr>
<tr>
<td>Wet and dry alpine meadows, Colorado (+250 kgN ha\textsuperscript{-1} yr\textsuperscript{-1})</td>
<td>0.4 (0.08), 0.9 (0.18)</td>
<td>Neff et al. (1994)</td>
</tr>
<tr>
<td>Hardwood forest, USA (soils warmed by 5°C)</td>
<td>&lt;0.1</td>
<td>Peterjohn et al. (1993)</td>
</tr>
<tr>
<td>Agricultural soils (fertilised but non-irrigated, non-manured)</td>
<td>(10)</td>
<td>Von Rheinbaben (1990)</td>
</tr>
<tr>
<td>Sitka spruce / mixed woodland, UK</td>
<td>(3.7, 0.8)</td>
<td>Skiba et al. (1998)</td>
</tr>
<tr>
<td>Grazed fields (fertilised at 360 kgN ha\textsuperscript{-1} yr\textsuperscript{-1}), UK</td>
<td>5</td>
<td>Smith et al. (1995)</td>
</tr>
<tr>
<td>Grazed grassland</td>
<td>8</td>
<td>Smith et al. (1998)</td>
</tr>
<tr>
<td>Wet forest soils (alder, ash)</td>
<td>3.0 – 4.9</td>
<td>Struwe &amp; Kjoller (1991)</td>
</tr>
<tr>
<td>Forest soils, Netherlands</td>
<td>20</td>
<td>Tietema et al. (1991)</td>
</tr>
<tr>
<td>Lowland, cattle-grazed pasture (UK - fertilised)</td>
<td>3.2 (1.3)</td>
<td>Williams et al. (1999)</td>
</tr>
</tbody>
</table>
Denitrification was previously estimated by mass balances, but more recent techniques include tracer N, mass spectrometry, emission spectrometry, gas chromatography and the acetylene blockage technique (Paul & Clark, 1996). The most widely used methods measure the production of N\textsubscript{2}O, easily analysed by gas chromatography and present at low concentrations in the atmosphere, although a disadvantage is that N\textsubscript{2}O is also produced during nitrification as described above.

Field measurements of denitrification were carried out here using a "closed cover" method with static chambers (Hutchinson and Mosier, 1981; Holland et al., 1999). These permit short incubations because N\textsubscript{2}O rapidly accumulates to measurable levels within the small volume of a sealed headspace. One chamber was installed in the central area of each plot (both primary and secondary: see Figure 3.7).

4.2.2 Chamber design and installation

The static denitrification chambers were constructed at UCL during spring 1999 and installed in June/July 1999. The body of each chamber comprised a c. 12 cm long, 15.2 cm diameter screwed access cover with a bevelled bottom edge to facilitate easier cutting into the soil. The body was cut into the soil with a knife to a depth of 3.5-9cm, leaving 8.5-3cm protruding (cf. Hutchinson & Mosier, 1981; Matson et al., 1991; Neff et al., 1994; Smith et al., 1995; Ball et al., 1997; but see also Christensen et al., 1990, who sank 10cm diameter tubes 19cm into a sandy loam soil). If the chambers were cut in too deep there was a risk of water-logging within the chambers, creating a very different micro-environment which could affect denitrification fluxes. Conversely, if the installation depth was too shallow there was a risk of a poor seal, permitting movement of gases into or out of the chamber. Hence the chosen depth represents a compromise between the need for a good gas-tight seal and the need for minimally impeded drainage. The intention was to cause minimal disturbance to the existing soil-vegetation system, and vegetation was left in situ inside the chambers. Since lids had to be fitted to the chambers when sampling for gases, the vegetation had to be confinable within the headspace of the chamber, i.e. grasses could be tucked into the chamber without damage but tall vegetation or heathers had to be avoided when siting the chambers within the plots (cf. Ball et al., 1997).
The screw-fitting lids for the chamber bodies were supplied with rubber O-rings to provide a gas-tight seal. Each lid was drilled with two small holes into which were fitted gas-tight silicone rubber stoppers (subaseal 17mm diameter, Fisher Scientific, Loughborough, UK). The subaseals permitted sampling of the chamber headspace by hypodermic syringe. They are designed to re-seal after piercing with a needle, so they could be re-used many times. Two subaseals were required so that the sample could be taken through one while the other had a needle inserted at the time of sampling to permit equilibration of pressure within the chamber headspace (cf. Neff et al., 1994).

To calculate the volume of the headspace within each chamber a calibration was carried out in the laboratory. Since the chamber with its fitted lid had an irregular shape it was not possible simply to calculate the volume of a cylinder. Upturned, lidded chambers were filled with water in increments of 1cm depth and the volume recorded at each increment to produce a calibration curve. It was therefore possible to calculate the headspace volume for each chamber installed in the field by simply measuring the internal depth, taking an average at several points within each chamber to account for the irregular soil surface. It was assumed that the chamber volume remained constant throughout the sampling period, i.e. the volume of headspace occupied by growing vegetation was assumed to be negligible, and the average depth of installation was not changed. At some plots, internal water-logging necessitated the occasional removal of the chamber bodies to permit drainage; care was taken to reinstall them to the same depth. Although only 3 of 60 chambers were regularly affected (M4D1, S2D1 and G3A3) others were waterlogged on at least one occasion (M2D2, M3D1, M3A1, M3D3, M3A3, M4A1, M4A2, M4D3, M4A3, G1D3, G3A2 and E1D3), with 15 chambers (25% of those installed) affected in total. The problem was by far most widespread at the Allt a’Mharcaidh site, and was presumably due to poor soil drainage properties there, although in some cases chambers may have been dug in too deep.

4.2.3 Sampling methods

After a “settling-in” period (recommended by Matson & Vitousek, 1987) of three months (cf. 2 weeks by Christensen et al., 1990), sampling commenced in September
1999. Chamber bodies remained in situ throughout the sampling period without their lids, to permit normal vegetation growth and microbial activity within them. Gas samples were taken 4-weekly. For reasons of cost, bulk and weight in the field, gas syringes were not used. Instead, the sealed vial method was employed (cf. Matthias et al., 1980; Rolston, 1986; Tiedje et al., 1989; Christensen et al., 1990; Matson et al., 1991). A 60ml plastic syringe fitted with a hypodermic needle was used for the removal of gas samples. Samples were stored in 10ml glass vials fitted with butyl rubber septa (20mm butyl rubber plug, Fisher Scientific, Loughborough, UK). Lids were crimped onto the vials in the laboratory prior to the sampling fieldwork, so the unused vials contained laboratory air.

On arrival at the study area, vials were manually evacuated with the syringe. By fully retracting the syringe with the needle piercing the vial septum, a proportion (60/70) of the laboratory air could be removed. By repeating this process three times the vials were effectively evacuated, with a negligible quantity of laboratory air remaining (in theory, <1%). A baseline, ambient sample from each chamber was taken prior to fitting the lid. With the needle in the chamber headspace, the syringe was pumped three times to ensure that gases in the chamber were well mixed. The ambient sample was injected into a pre-evacuated vial labelled with the plot number and the sample start time was recorded (T=0). The draw-down of the syringe plunger as the needle was inserted into the vial provided an indication of the success of the vial evacuation. If the vial lid was leaking or the vial had not been pre-evacuated, the syringe would not be drawn down and no sample was injected — in these instances the procedures were repeated with another vial (cf. Matson et al., 1991). Draw-down of at least 6mls was considered sufficient for sampling (given the friction of the plunger seal within the syringe) and the remaining sample volume was manually injected into the vial (cf. Groffman et al., 1999). The lid was then fitted tightly onto the chamber body and left in place for 1-2 hours to allow trace gases to accumulate inside the sealed headspace.

After a known interval, the accumulated gas from within the sealed chamber headspace was sampled. The hypodermic needle was inserted into one septum, pumped three times to mix the gases within the chamber, and then a sample was taken after the insertion of a second needle into the other septum to permit pressure
equilibration. The sample was injected into a second, labelled vial and the sampling time recorded (T=sample).

After completion of sampling, chamber lids were removed and the chamber bodies left to freely ventilate until the next sampling 4 weeks later.

4.2.4 Analytical methods

Gas samples were analysed within a week for CO₂, CH₄ and N₂O at CEH Bangor, using a gas chromatograph “Ai Cambridge model 92” fitted with two Porapak QS columns, an Electron Capture Detector (N₂O) and a Flame Ionisation Detector (CO₂, CH₄).

The difference in concentrations between ambient (T=0) and accumulated gas (T=sample) provided the rate of change of concentration. This could then be converted into a flux per unit area of soil using the sample interval, the soil surface area within the chamber and the known headspace volume for each chamber, using the method of Hutchinson and Mosier (1981):

\[ f = \frac{V(C_t - C_0)}{(A \cdot t)} \]

where \( f \) = N₂O flux, \( V \) = internal chamber volume, \( C_0 \) = initial [N₂O], \( C_t \) = [N₂O] at time \( t \) and \( A \) = column cross-sectional area.

4.2.5 Method validation

To check that the static chambers were not leaking and that samples were being collected over a period of linear increase, regular “linearity checks” were carried out on each chamber in the field. A linearity check entailed repeat sampling at 20-30 minute intervals (time recorded) over at least a 2 hour period (cf. Rolston, 1986). Repeat sampling in this way introduced outside air into the chambers via the equilibration needle during sampling, but compared with the chamber headspace

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volume the amount of introduced air was negligible. Plotting of gas concentration results against sample time provided a graphical check on the performance of the method. The chamber lid needed to be left in place long enough to obtain a few data points as a function of time, yet not long enough to result in a flattening of the curve and a decrease in flux; only those data should be used which can be approximated by a straight line (Rolston, 1986). These periodic spot checks indicated that accumulation of both CO₂ and, where denitrifying, N₂O, was adequately linear.

4.2.6 N additions experiment

Since denitrification requires available NO₃⁻ the absence of significant fluxes could be due to a lack of N substrate. Secondary experimental plots (Table 3.5) were therefore used for an N fertiliser addition experiment to test whether denitrification rates would be increased by a greater input of N. Fertiliser was added fortnightly as NH₄NO₃ solution at a rate equivalent to 20 kgN ha⁻¹ yr⁻¹ in 10% of annual estimated runoff (see Table 3.1). The fertiliser was added in identical amounts every two weeks directly into the static chambers after gas sampling had been carried out as above. The interval between fertiliser addition and gas sampling was therefore kept constant, at two weeks. The experiment provided data for comparison with denitrification fluxes from primary plots without N additions. As for the primary plots, soil moisture was measured at the time of gas sampling, using a hand-held Theta-probe.

4.3 Denitrification: laboratory incubation experiments

4.3.1 Rationale: the role of soil core incubation experiments

While the field programme provides measurements for the estimation of actual denitrification fluxes through the study plots and catchments it is constrained by natural conditions (temperature, rainfall, deposition) and the measurement of small fluxes does not necessarily mean that they are always insignificant. The ultimate aim of the study is to model the processes which control N leaching into surface waters so that the potential effects on water quality under different deposition loads can be
assessed. The field sites were selected across a gradient of N deposition and the absence of significant inorganic N in surface waters is a function of deposition inputs as well as retention or removal processes. Hence at the very low deposition site, the Allt a'Mharcaidh, the absence of inorganic N in surface waters is most likely due to the very low inputs; fluxes measured in the field will therefore be small because of the limiting supply of N.

While inputs can be controlled experimentally, e.g. through the addition of NH$_4$NO$_3$ solution as in the denitrification field study (Section 4.2.6), this approach cannot by itself provide a convincing answer to the question of whether denitrification could act as a significant sink for N because of the practical limitations of field measurements. In addition to the uncontrolled effects of temperature etc. mentioned above, there is the problem of temporal variations, which can be very large for denitrification (Tiedje et al., 1989; Dutch & Ineson, 1990). Measurements in the field were taken over 1-2 hours on a monthly basis so it is quite possible that peaks in denitrification fluxes under certain conditions (of N availability, temperature and moisture) could be missed.

In laboratory incubation experiments it is possible to measure the maximum, potential rate of denitrification from a soil by providing the optimal conditions of N supply, moisture and temperature. For soils where denitrification is not detected in the field, the laboratory incubation studies can indicate where denitrification might occur under different conditions, specifically of inorganic N availability. Another advantage of the soil core method is the increased sampling capacity which is particularly important because of the high temporal and spatial variability of denitrification discussed above (Tiedje et al., 1989).

4.3.2 Site selection and sampling

In order to maximise the comparability of results from field and laboratory experiments, soil samples for the laboratory studies were taken from the primary field plots. Since the great spatial variability of soil processes is well known, soil cores were taken from five locations within each plot to increase spatial representativeness
(see Figure 3.7 – labelled A-E). Sample locations were chosen to minimise the likelihood of disturbance from (and to) field installations.

Intact soil cores were obtained in 5cm internal diameter plastic core tubes which were first cut into the soil surface layer with a knife to minimise compaction, prior to being driven into the soil with a mallet (cf. Parkin et al. (1984), who discarded cores compacted more than 5%, rather than "cutting in" the corer). The core tubes were 15cm long and samples were taken at two levels from the same spot to provide a complete core up to a maximum 30cm in length, depending on soil depth. To obtain the lower sample, a small pit had to be dug after taking the first core; again, the small pit size and location was intended to minimise disturbance to field installations.

Sampling was carried out over the period 20-26th July 2000. With three replicated plots per soil type on 13 soil types over 4 catchments, the total number of complete soil cores was 195.

The soil cores obtained for laboratory denitrification experiments were also used for the testing of available soil N, mineralisation and nitrification potentials (Chapter 5), with separate core subsamples used for each (see below). For the main denitrification and mineralisation experiments (phase 2 measurements), soil cores A-D were used (Figure 3.7). Core E was retained for additional denitrification tests (phase 1 experiments). Samples were labelled according to their corresponding plot number (e.g. G12) and location within the plot (i.e. G12A, G12B etc.).

Samples were transported to the laboratory in coolboxes, and then stored at 4°C in a coldroom until required. Given the large number of samples and analyses performed, storage for up to several weeks was necessary, but Parkin et al. (1984) found that storage of cores at this temperature for up to 19 days did not significantly affect the denitrification rate. All phase 2 and some phase 1 measurements were completed within 5 weeks of sampling. The temperature gradient, N additions and C$_2$H$_2$ tests performed within the phase 1 experiments were carried out at 7-9 weeks after sampling.
4.3.2.1 Horizontal splitting of cores A-D (by horizon)

Soil cores were split horizontally into the uppermost, biologically active organic horizons and the deeper organic and mineral soils, so that the most important depths for the different N processes could be assessed. Inspection of the soil cores showed that in general, the surface organic horizons occurred within the uppermost 2-8cm of the core. To ensure the comparability of cores within and between plots, a standard depth of 5cm for the uppermost “top” sample was selected. The depth of the lower “bottom” sample was then constrained by the length of the Mason jars used for incubating the intact cores, to 15cm (Figure 4.3). The remainder of the core was removed and stored.

For the two soil types in the Afon Gwy catchment containing well defined mineral horizons (G2 - peaty gley and G3 - podsol) within the 30cm depth sampled, an extra horizontal subdivision was made. These horizons were found at c. 10-20cm depth and were removed for separate testing. For the G2 and G3 plots the cores were therefore split three ways; surface organic horizons (0 - 5cm), subsurface organic (5 - 10/20cm) and mineral horizons (10/20 - 20/30cm).

4.3.2.2 Vertical splitting of cores A-D for replication

Logistical constraints on the number of incubations for denitrification meant that some soil samples had to be bulked together. In addition, it was necessary to split samples for the separate denitrification and mineralisation experiments, since the available N, mineralisation and nitrification data were required for comparison with the denitrification data. The following strategy was therefore adopted to maximise spatial representativeness of samples while minimising the number of bulked samples for incubation (see Figure 4.3).

1. All A-D samples (tops, bottoms and mineral) were split vertically into two halves. For each sample pair, one half was used in denitrification experiments while the other corresponding half was used in the N mineralisation incubations.

2. For the denitrification samples, tops and bottoms were treated differently. For core top experiments, four half cores from locations A-D were bulked into one sample (e.g. G11 Top). With the longer core bottoms it was not possible to fit four half cores into a Mason jar, so instead diagonally opposite half cores were paired (Figures 3.7 and 4.3), A with C and B with D (e.g. G11 AC and G11 BD...
Bottoms). Each plot therefore generated one top (n = 39) and two bottom (n = 78) samples, plus the extra mineral samples at G2 and G3 (n=12).

3. Sample numbers were less critical for the mineralisation experiments, but a further splitting of samples was required to provide baseline and post-incubation samples (see Chapter 5). Each half core (tops and bottoms) was halved again vertically. Core tops and bottoms were treated identically. The resulting quartered core samples were paired with their diagonal opposites (A with C, B with D) from the plot. Two replicate pairs of quarters (labelled as e.g. 2 × S32 AC tops or 2 × E21 BD bottoms) were therefore produced (see Figure 4.3). Each plot generated four pairs of samples for analysis (AC Tops, AC Bottoms, BD Tops, BD Bottoms – see Figure 4.3). One set was to be used as the baseline sample (available N), and the other was for the corresponding post-incubation analysis.

Figure 4.3: Splitting and amalgamation of soil core samples
4.3.3 Denitrification potential – methods

Denitrification from soil cores was measured in sealed Mason jars with butyl rubber septa (cf. de Klein and van Logtestijn, 1996). Soil samples were generally wetted up with 20ml de-ionised water and placed in a Mason jar with an air-tight lid. Teflon tape around the thread was used to ensure a good seal. Gas samples were removed from the jar via a septum through which a hypodermic needle on a 20ml gas syringe was inserted. The syringe was pumped three times to mix the gases inside the jar, then a needle without an attached syringe was inserted through a second septum while the actual sample was taken, to allow equilibration of air pressure. Both needles were then removed to leave the jar sealed again. Gas syringes were found to retain samples for at least 10-21 days by Jarvis et al. (1991). This procedure could be repeated indefinitely, but the seal would deteriorate if the septa were pierced a large number of times, and the possible feedback effects of gas build up within the jar through time had to be accounted for, i.e. incubation times were kept to a minimum.

The method is comparable with that of Parkin (1987), who incubated soil cores in loosely fitting tubes to facilitate diffusion of \( \text{C}_2\text{H}_2 \) into, and \( \text{N}_2\text{O} \) out of, soil cores. This approach, as with ours, can only be used for soil cores which maintain their structural integrity during transfer out of the core tube. A similar technique was also used by Goulding et al. (1990), although they left intact cores within the coring tubes, which had been drilled with 20 5mm holes to permit diffusion of \( \text{C}_2\text{H}_2 \) into cores. The surface area of the top of the core was, however, still used to derive the areal denitrification rate.

The rate of production of gases was measured simply as the difference in concentrations (ppm) over a known period of time, i.e. a baseline sample was taken at \( t=0 \) and a second sample was taken after a fixed period. Samples were analysed for \( \text{N}_2\text{O} \), \( \text{CO}_2 \) and \( \text{CH}_4 \) by gas chromatograph using identical methods to those for field samples (see Section 4.2.4 above).

Since the volume of the soil sample is known, the total production of \( \text{N}_2\text{O} \) can be calculated. The rate of denitrification can then be expressed in terms of production per unit surface area of soil (cores had a 5cm surface diameter) or per gram dry weight of
soil. Since other studies (e.g. Ineson et al., 1991) found no significant difference between core headspace and soil pore concentrations of N₂O in laboratory incubations, measurement of headspace gases alone was deemed sufficient for estimating fluxes. Furthermore, no account was taken of N₂O dissolved in soilwater (Moraghan & Buresh, 1977), since only the net flux to the atmosphere is required.

The laboratory denitrification experiments were split into two phases. The first phase was designed to test and validate the methods employed through a series of experiments on a small number of surplus test samples. The first phase experiments include checks on the linearity of gas production, effects of wetting, N addition and temperature change and the acetylene test to inhibit gaseous N₂ production. The second phase comprises a full analysis of actual and potential denitrification rates on the bulked soil samples from all experimental plots, for comparison with field rates.

4.3.3.1 Phase 1: Linearity checks

To assess the appropriate time period over which gas production should be measured and to ensure that denitrification is linear over this period, it was necessary to check the method through a series of linearity checks, as recommended by Groffman et al. (1999). For example, Aulakh and Doran (1991) found that in incubations of more than 24 hours, N₂O build up in the headspace could lead to reduced diffusion and entrapment of N₂O in the soil, leading to severe underestimation of the denitrification flux. Davidson et al. (1986) found that rates of N₂O accumulation within incubation bottles were generally linear over the first 24 hours for nonsaturated soils, but non-linear for saturated soils, probably due to NO₃⁻ depletion during incubation.

Eight samples from ‘spare’ soil cores (selected from the ‘E’ samples remaining after the sample bulking procedures above and including all sites) were placed in Mason jars and gas samples taken at intervals over a period of several hours (after 30, 60, 90, 120, 155, 255 and 345 minutes) without removing the lids from the jars. In this way the build-up of gases over a prolonged period can be measured, and an appropriate sample period determined. A graphical plot of the increase in gas concentrations through time reveals whether the rate of production is linear and for how long.
4.3.3.2 Phase 1: Temperature gradient

Since denitrification is a function of temperature (among other variables like moisture) another test of the technique is to measure the change in rates of gas production with temperature. Experimental testing of temperature effects also indicates whether field rates are likely to be temperature limited. This was carried out by repeat measurements on 28 samples from all soils incubated at different temperatures (3.5°, 5.5°, 12° and 15.5°C). Each measurement was carried out over a similar time interval for comparability. The lids were removed for a period between each measurement to ventilate the sample and ensure that each experiment was starting with similar ambient concentrations of gases in the airspace of the sample jar.

4.3.3.3 Phase 1: Wetting effects

Previous work on denitrification has demonstrated that there may be a pulse of N$_2$O production within a very short time after wetting of soils (e.g. Sexstone et al., 1985; de Klein & van Logtestijn, 1996). The potential importance of event-based denitrification fluxes following rainfall in the field can be assessed by laboratory wetting experiments. Furthermore, since the soil samples were to be wetted up in the laboratory to prevent a moisture limitation of denitrification, it was necessary to test the effect of sample wetting prior to incubation. This would prevent the measurement of production rates too soon after wetting.

Gas production rates were measured for a small number of samples (8 – selected across all soil types and depths) at different times after wetting (30, 60, 90, 120 and 150 minutes).

4.3.3.4 Phase 1: N addition effects

In addition to the possibility of event-based denitrification following rainfall, pulses of denitrification activity could also follow high inputs of N deposition independently of wetting effects, for example in a high concentration occult deposition event. The potential effect of adding N in the laboratory (as NH$_4$NO$_3$ solution) could also be the generation of a pulse of denitrification as tested in Section 4.3.3.3 above, but this might be due either to a wetting effect or to an N supply effect. To test the effect of added N independently of wetting, the rate of gas production was measured at different times after the addition of NH$_4$NO$_3$ solution (20ml at 200mg/l$^1$). At this
concentration of inorganic N, denitrification would be expected to follow zero-order dynamics and to be C limited (Paul & Clark, 1996). Comparison of results with those from the post-wetting experiment described above would indicate whether different effects were induced. Unlike similar studies where glucose was added in combination with N to ensure removal of substrate limitation (e.g. Ineson et al., 1991), glucose was not used here because only N limitation is of direct interest.

14 samples were selected from across the range of soil types and depths and N₂O production was measured during incubation at 6°C after 40, 80, 140, 260, 430, 580 and 1575 minutes (26.25 hours). Sample jars were vented between measurements to prevent excessive gas build up, except for the first 3 measurements up to 140 minutes, since it had already been established that denitrification was linear over this period.

4.3.3.5 Phase 1: Acetylene test
An important assumption of this technique is that the major denitrification product is N₂O. However, it is known that under certain conditions denitrification processes can liberate free nitrogen (N₂), the proportion of which is notoriously difficult to measure directly, due to its great abundance in the atmosphere (Payne, 1991). To circumvent this problem and check whether N₂ is a significant denitrification product from a sample, the acetylene block technique is used (e.g. Smith et al., 1978; Parkin et al., 1987; Tiedje et al., 1989). Acetylene inhibits the step in the denitrification process by which N₂O is reduced to N₂ (Paul & Clark, 1996). Comparison of N₂O production rates from a sample with and without acetylene indicates whether N₂ production is important.

The N₂O production rate for a sample was first measured in the standard way (see above). Acetylene gas (C₂H₂), produced by adding water to calcium carbide, was then injected into the sealed sample jar and the procedure was repeated. The volume of C₂H₂ gas injected was sufficient to ensure a 10% concentration (0.1 atm) in the headspace, as recommended by Smith et al. (1978). C₂H₂ generated in this way was used in preference to commercially available compressed C₂H₂ gas because it is free of other contaminants that might interfere with the denitrification assay (Hyman and Arp, 1987). An increase in N₂O production rate after the introduction of C₂H₂
indicates the proportion of the denitrification product which is in the form of N\textsubscript{2} and which is therefore not detected in the GC analysis.

A side effect of this technique is that the soil microbes are adversely affected by C\textsubscript{2}H\textsubscript{2}, so it is effectively a destructive, one-off procedure and samples cannot then be re-used. Nitrification is also inhibited, so N\textsubscript{2}O production by this process will cease.

16 samples selected from all soil types and depths were used in the C\textsubscript{2}H\textsubscript{2} tests.

4.3.3.6 Phase 2: Actual and potential denitrification rates from spatial samples

Having tested the methods in the Phase 1 experiments described above, the full analysis of the pre-prepared spatial survey samples was carried out in Phase 2. Logistical constraints of incubator space and analytical time required the analysis of the soils in three batches; core tops, core bottoms #1 and core bottoms #2 (since there were more than double the number of subsurface “bottom” samples as described in Section 4.3.2.2 above). For each batch, four experiments were carried out before moving onto the next batch:

1) Denitrification at 5°C
2) Denitrification at 15°C
3) Denitrification at 5°C with added N
4) Denitrification at 15°C with added N

The coldroom was used to incubate samples at 5°C, assumed to be a typical lower range value for these sites, while the laboratory incubators were used for 15°C, which was assumed to be within the upper range of soil temperature values and hence to provide an indication of maximum likely rates in the field (cf. Dutch & Ineson, 1990). Each batch was first wetted up with de-ionised water prior to the series of four experiments (i.e. samples were only wetted up once). Gas production was measured after an incubation period of 90-120 minutes. For the two N addition experiments N was added just once, as NH\textsubscript{4}NO\textsubscript{3} solution. The solution was made up to a concentration of 200 mg l\textsuperscript{-1} NH\textsubscript{4}NO\textsubscript{3} (equivalent to 35 mg l\textsuperscript{-1} or 2500 \textmu(eq) l\textsuperscript{-1} of both NH\textsubscript{4}-N and NO\textsubscript{3}-N) and 20ml were added to each soil sample by dripping from a
syringe, to maximise the dispersion of the solution through the sample. These high concentrations should ensure that N limitation does not occur.

4.4 Results: field measurements

4.4.1 Sensitivity of the method

An immediate observation in analysis of baseline samples is the variability in ambient N\textsubscript{2}O levels which determines the sensitivity of the method (Fig. 4.4). On any one sampling occasion, ambient N\textsubscript{2}O may vary by up to 0.05 ppm from the general average of c. 0.4 ppm. For a typical static chamber with a headspace volume of one litre, a change in N\textsubscript{2}O concentration of 0.05 ppm over a 60 minute sampling period is equivalent to an annual flux of 0.3 kgN ha\textsuperscript{-1} yr\textsuperscript{-1}. A flux of ±0.3 kgN ha\textsuperscript{-1} yr\textsuperscript{-1} could therefore be obtained from random variation between two samples in ambient N\textsubscript{2}O alone. It was therefore decided that calculated fluxes in the range ±0.3 kgN ha\textsuperscript{-1} yr\textsuperscript{-1} would be treated conservatively as ‘below detection limit’ or zero for the purposes of calculating annual means. By comparison, the gas-flow core method of Parkin et al. (1984) had a detection limit of c. 0.95 kgN ha\textsuperscript{-1} yr\textsuperscript{-1}.

4.4.2 Annual mean N\textsubscript{2}O fluxes

Mean denitrification fluxes for the 12 samples of the budget year are presented in Tables 4.2a-d. Note that while individual values in the range ±0.3 kgN ha\textsuperscript{-1} yr\textsuperscript{-1} are treated as zeros, annual means from monthly samples may still fall below this threshold value. Fluxes from secondary plots with additions of NH\textsubscript{4}NO\textsubscript{3} are shown in the same tables below the unamended rates for the same soils.

These results show that significant annual mean denitrification rates are observed in only a small number of plots, even at the high deposition sites, indicating large spatial variability in denitrification. While some plots are denitrifying, replicate plots on the same soils often are not, hence mean rates for each soil type without N additions are low, typically <0.5 kgN ha\textsuperscript{-1} yr\textsuperscript{-1} for any given plot and a maximum for a soil type of
0.24 kgN ha\(^{-1}\) yr\(^{-1}\) (burnt *Calluna* at the Etherow; Table 4.2d). Addition of NH\(_4\)NO\(_3\) has little effect on mean denitrification rates at most secondary plots, with only 3 plots at the Mharcaidh showing increased values (Table 4.2a).

**Figure 4.4: Boxplot of variation in ambient N\(_2\)O within the Scoat Tarn catchment (boxes indicate inter-quartile range & median, whiskers indicate data range, *marks outliers > 1.5\times the inter-quartile range outside the box)*

![Boxplot of variation in ambient N\(_2\)O within the Scoat Tarn catchment](image)

**Table 4.2a: Denitrification fluxes at the Allt a’Mharcaidh (kgN ha\(^{-1}\) yr\(^{-1}\))**

<table>
<thead>
<tr>
<th>Plot</th>
<th>Field rate</th>
<th>Plot</th>
<th>Field rate</th>
<th>Plot</th>
<th>Field rate</th>
<th>Plot</th>
<th>Field rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1D1</td>
<td>0.00</td>
<td>M2D1</td>
<td>0.00</td>
<td>M3D1</td>
<td>0.00</td>
<td>M4D1</td>
<td>0.05</td>
</tr>
<tr>
<td>M1D2</td>
<td>0.00</td>
<td>M2D2</td>
<td>0.00</td>
<td>M3D2</td>
<td>0.08</td>
<td>M4D2</td>
<td>0.00</td>
</tr>
<tr>
<td>M1D3</td>
<td>0.00</td>
<td>M2D3</td>
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<td>M4D3</td>
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</tr>
<tr>
<td>Mean</td>
<td>0.00</td>
<td>Mean</td>
<td>0.00</td>
<td>Mean</td>
<td>0.03</td>
<td>Mean</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>+NH(_4)NO(_3)</td>
<td></td>
<td>+NH(_4)NO(_3)</td>
<td></td>
<td>+NH(_4)NO(_3)</td>
<td></td>
<td>+NH(_4)NO(_3)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>Mean</td>
<td>0.49</td>
<td>Mean</td>
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</tbody>
</table>
### Table 4.2b: Denitrification fluxes at the Afon Gwy (kgN ha\(^{-1}\) yr\(^{-1}\))

<table>
<thead>
<tr>
<th>Plot</th>
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<th>Plot</th>
<th>Field rate</th>
<th>Plot</th>
<th>Field rate</th>
<th>Plot</th>
<th>Field rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>GlD1</td>
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<td>G2D1</td>
<td>0.10</td>
<td>G3D1</td>
<td>0.00</td>
<td>G4D1</td>
<td>0.36</td>
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<tr>
<td>GlD2</td>
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<td>G2D2</td>
<td>0.06</td>
<td>G3D2</td>
<td>0.00</td>
<td>G4D2</td>
<td>0.00</td>
</tr>
<tr>
<td>GlD3</td>
<td>0.00</td>
<td>G2D3</td>
<td>0.00</td>
<td>G3D3</td>
<td>0.04</td>
<td>G4D3</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean</td>
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<td>Mean</td>
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<td>Mean</td>
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<td>Mean</td>
<td>0.12</td>
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<table>
<thead>
<tr>
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<th>Plot</th>
<th>+NH(_4)NO(_3) Field rate</th>
<th>Plot</th>
<th>+NH(_4)NO(_3) Field rate</th>
<th>Plot</th>
<th>+NH(_4)NO(_3) Field rate</th>
</tr>
</thead>
<tbody>
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<td>GlA1</td>
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<td>G2A1</td>
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<td>G3A1</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GlA2</td>
<td>0.07</td>
<td>G2A2</td>
<td>0.00</td>
<td>G3A2</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GlA3</td>
<td>0.07</td>
<td>G2A3</td>
<td>0.08</td>
<td>G3A3</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean</td>
<td>0.05</td>
<td>Mean</td>
<td>0.05</td>
<td>Mean</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 4.2c: Denitrification fluxes at Scoat Tarn (kgN ha\(^{-1}\) yr\(^{-1}\))

<table>
<thead>
<tr>
<th>Plot</th>
<th>Field rate</th>
<th>Plot</th>
<th>Field rate</th>
<th>Plot</th>
<th>Field rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SlD1</td>
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<td>S2D1</td>
<td>0.00</td>
<td>S3D1</td>
<td>0.00</td>
</tr>
<tr>
<td>SlD2</td>
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<td>S2D2</td>
<td>0.00</td>
<td>S3D2</td>
<td>0.09</td>
</tr>
<tr>
<td>SlD3</td>
<td>0.00</td>
<td>S2D3</td>
<td>0.03</td>
<td>S3D3</td>
<td>0.05</td>
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<td>Mean</td>
<td>0.01</td>
<td>Mean</td>
<td>0.05</td>
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</table>

### Table 4.2d: Denitrification fluxes at the River Etherow (kgN ha\(^{-1}\) yr\(^{-1}\))

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<th>Plot</th>
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<th>Plot</th>
<th>Field rate</th>
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</thead>
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<td>ElD1</td>
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<td>E2D1</td>
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</tr>
<tr>
<td>ElD2</td>
<td>0.00</td>
<td>E2D2</td>
<td>0.05</td>
</tr>
<tr>
<td>ElD3</td>
<td>0.03</td>
<td>E2D3</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean</td>
<td>0.24</td>
<td>Mean</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plot</th>
<th>+NH(_2)NO(_3) Field rate</th>
<th>Plot</th>
<th>+NH(_2)NO(_3) Field rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ElA1</td>
<td>0.16</td>
<td>E2A1</td>
<td>0.00</td>
</tr>
<tr>
<td>ElA2</td>
<td>0.00</td>
<td>E2A2</td>
<td>0.00</td>
</tr>
<tr>
<td>ElA3</td>
<td>0.07</td>
<td>E2A3</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean</td>
<td>0.08</td>
<td>Mean</td>
<td>0.00</td>
</tr>
</tbody>
</table>
4.4.3 Temporal variability

Inspection of the raw data shows that in addition to the great spatial variability in denitrification, there is also significant temporal variation in the few plots which exhibit measurable denitrification. For example, the plot showing the highest mean rate without N additions at the Etherow (E1D1) only denitrifies from late spring to early autumn (Figure 4.5), while the other two replicated plots show very low values all year round.

In the secondary plots with N additions this temporal variation is even more pronounced, with large denitrification “events” driving the higher figures at the Allt a’Mharcaidh (Figure 4.6). However, inspection of the field records for these plots reveals that these events are associated with waterlogging of the static chambers, leading to elevated denitrification. Hence at the denitrifying Mharcaidh plots it is only the combination of elevated N inputs from NH₄NO₃ additions with waterlogging of the chambers which produces the NO₃⁻ rich, anaerobic conditions necessary for denitrification.

Figure 4.5: Temporal variation in denitrification flux at the River Etherow (burnt Calluna) site (plot E1D1: kgN ha⁻¹ yr⁻¹)
Temporal variations in CO$_2$ flux measured in static chambers are presented in Figure 4.7. While there are too few sites showing denitrification to make a general comparison of temporal variability between sites, CO$_2$ fluxes are easily measurable as indicators of general biological activity, and follow a very similar pattern to soil temperature, peaking in July/August when temperatures are highest. Note that for these field measurements, CO$_2$ fluxes include those from vegetation as well as soil microbes.

Since all four sites are plotted on the same scale, it can be seen that fluxes from Scoat Tarn and particularly the Afon Gwy soils are much greater than at the Mharcaidh or the River Etherow. While the limited activity at the Mharcaidh may be attributed to a combination of low temperatures, high soil moisture and low N availability (see Chapter 3), none of these factors apply at the Etherow, where the soils are much drier, warmer and NO$_3^-$ rich. It is likely that biological activity at the Etherow is restricted more by the impacts of severe atmospheric pollution, as indicated by the very acid soil waters and high acid deposition loads there (Chapter 3). At the Afon Gwy, both soil temperatures and CO$_2$ emissions are much greater than at other sites.
Figure 4.7: Seasonal variation in CO₂ flux in static chambers

Mharcaidh Mean CO₂ Fluxes

Gwy mean CO₂ Fluxes

Scout Tarn mean CO₂ Fluxes

Etherow mean CO₂ Fluxes
4.5 Results: laboratory incubations

4.5.1 Phase 1 results

4.5.1.1 Linearity checks

The accumulation of N\textsubscript{2}O with time in the headspace of the incubation jars is shown in Figure 4.8. For the samples which are producing N\textsubscript{2}O, the increase in concentration is approximately linear, except for S23EB. There is some deviation from linearity within the first hour or so while N\textsubscript{2}O concentrations are still quite low for which the cause is not clear, but the problem of excessive N\textsubscript{2}O concentrations inhibiting the further diffusion of N\textsubscript{2}O from the soil cores does not arise, even for the most active sample plotted on the secondary axis (S31ET: Scoat Tarn deep peat – Top 5cm).

Three samples show no increase in N\textsubscript{2}O (G33ET: Gwy podsol top 5cm, M11ET and M11EB: Mharcaidh peaty ranker top and bottom samples) indicating an absence of denitrification.

The increase in CO\textsubscript{2} within the sample jars during the monitoring period is more linear, but with large variations between samples (Figure 4.9). Part of this variability is due to differences in core volumes and hence headspace volumes, but this can account for only a small proportion of variation because soil volumes are small relative to the headspace and the most active soils are core tops, occupying the smallest volumes. It is noteworthy that of the three samples showing the greatest CO\textsubscript{2} production rates, two (G33ET, M11ET) show no N\textsubscript{2}O production while the third (S31ET) shows by far the highest rate of N\textsubscript{2}O evolution. High rates of soil respiration can increase the anaerobic volume in which denitrification may occur via O\textsubscript{2} consumption, but labile C and N substrates are also required: hence soilwater NO\textsubscript{3}\textsuperscript{-} may be limiting denitrification in the inactive samples with large CO\textsubscript{2} fluxes (see Chapter 3).
Figure 4.8: Increase in headspace N$_2$O concentration with time

![Graph showing increase in headspace N$_2$O concentration with time](image)

Figure 4.9: Increase in headspace CO$_2$ concentration with time

![Graph showing increase in headspace CO$_2$ concentration with time](image)
4.5.1.2 Temperature effects

The rate of increase in the flux of N\textsubscript{2}O in sample jars incubated at different temperatures is shown in Figure 4.10. For the Mharcaidh soils there is no obvious pattern in terms of N\textsubscript{2}O emissions and temperature, as might be expected from soils which are not actively denitrifying (see scale in Fig. 4.10 relative to other sites). Since the jars were vented between each incubation, random differences in ambient N\textsubscript{2}O could account for apparent changes in flux of ±0.15kgN ha\textsuperscript{-1} yr\textsuperscript{-1} (see Section 4.4.1 above – but the headspace volume in the laboratory incubations is always less than 0.5L, hence a greater sensitivity and lower ‘detection limit’), and most Mharcaidh values fall within this range. By contrast, the core tops (0-5cm) from the Gwy show a fairly linear increase in the rate of N\textsubscript{2}O emissions with temperature. In the deeper soil samples from the Gwy, an apparent increase in N\textsubscript{2}O emissions as temperature increases from 4 to 6\textdegree C reverses as the temperature increases further. While this could be due to substrate depletion (since the incubations were performed sequentially with increasing temperature), the very low fluxes fall below the detection limit of the method. Denitrification is therefore much less active in the deeper Gwy soils.

The Scoat Tarn soils show much greater denitrification fluxes than those from other sites (Fig. 4.10) at the highest incubation temperature (15\textdegree C). At temperatures up to 11\textdegree C, fluxes appear to increase in a linear fashion with temperature, as at the Gwy. Unlike the Gwy soils, however, there is a sudden change in slope between the two higher temperatures, indicating that denitrification increases much more rapidly above c. 11\textdegree C. The deeper Scoat soils are similar to those from the Gwy in that there is little evidence of denitrification at any of the incubation temperatures.

The soil core top from the burnt Calluna area of the Etherow catchment (E1T) behaves in a similar way to the Scoat soils, in that denitrification increases rapidly above 11\textdegree C. At lower temperatures, and in the lower sample from the same core as well as both top and bottom samples of the core from below the mature Calluna, denitrification fluxes are mostly below the sensitivity range of the method (±0.15 kgN ha\textsuperscript{-1} yr\textsuperscript{-1}).
The equivalent plots for CO₂ fluxes, as indicators of general microbiological activity, are shown in Figure 4.11. Unlike the field measurements, these fluxes are microbial only, since plants are absent from the soil cores (although root fragments may occur in the intact cores).
Patterns of CO₂ emissions are much more consistent between sites and between core tops and bottoms than for N₂O. All samples show either a decrease, or smaller increase in flux between the two lower incubation temperatures, followed by sharp increases in flux at higher temperatures. This initial pattern may be due to differences in the ambient CO₂ level of laboratory air as the samples are vented between each incubation. In most soils, respiration is more responsive to temperature than denitrification, with exponential increases in CO₂ flux as temperature increases.
There is also a consistent pattern of smaller fluxes from the deeper core sections than from the top 5cm sections, despite the smaller volume (generally less than half) of soil in the upper samples. There is a levelling off or even a decrease in fluxes at the highest temperatures, which is more apparent in the lower soils. Hence microbiological activity is much greater in the surface soils, but may become substrate limited by the fourth, highest temperature incubation. With the exception of the surface samples from the Afon Gwy, rates of CO₂ evolution are surprisingly comparable between sites. Fluxes from the Etherow core tops are only slightly lower than the Scoat and Mharcaidh soils.

4.5.1.3 Wetting effects

Concentrations of N₂O and CO₂ after wetting up of soil cores are shown in Figures 4.12 and 4.13. Both figures show that there is no pulse of activity immediately after wetting, and emissions of both trace gases are linear during the 3 hour incubation period. Wetting up of soil cores prior to measuring denitrification is therefore acceptable for ensuring that moisture limitation is minimised. The data also show that wetting up events may not always be an important driver of denitrification pulses in the field, but the antecedent soil moisture conditions are very important in this respect so it cannot be concluded that wetting events are never significant.

![Figure 4.12: Increase in N₂O concentration during incubation after wetting](image)
4.5.1.4 N addition effects

The response of soils incubated at 6°C after N additions is shown in Figure 4.14. Only those soils with a denitrification response are shown; 8 of the 14 samples tested show no net flux of N₂O at any time after N additions. Flux values represent the mean flux since the previous sampling period.

Although the temporal response is complex, most soils follow a broadly similar pattern, at least for the first few hours after N additions. Initial high rates of denitrification within the first 0-2 hours of additions rapidly decline to a minimum value after 1-3 hours. Three of the samples have already passed the first peak in N₂O fluxes when first sampled after 40 minutes, while the other three peak later, at 80 minutes. All samples show a decline after this initial peak, which is subsequently followed by a rapid increase.

Four of the samples show a second, lower peak in emissions after 3-8 hours, after which a second period of decline is followed by varied responses; one shows slow decline, two level off at near-zero rates, and one increases steadily throughout the remaining period of measurement. The other two samples do not show this second
peak; instead they continue to increase in N\textsubscript{2}O emissions, rapidly at first, then more slowly during the period when other samples reach their second peak, then rapidly again.

The CO\textsubscript{2} data show an initial sharp decline followed by a slow increase in emissions (Figure 4.15). The pattern is broadly consistent with that for N\textsubscript{2}O emissions except that the small, secondary peak is generally absent for CO\textsubscript{2}. While the data are lacking to provide an explanation of the observed patterns, these results do confirm that there is an immediate, pulsed microbial response to the addition of NH\textsubscript{4}NO\textsubscript{3} solution, followed by a sharp decline in activity by 3-5 hours, which is then followed by a steady increase in emissions of CO\textsubscript{2} and, for some soils, in denitrification. The initial sharp decline is presumably due to rapid exhaustion of the C substrate, while the longer term response may be governed by changes in microbial populations.

**Figure 4.14: Temporal denitrification response of soils to N additions**

![Figure 4.14: Temporal denitrification response of soils to N additions](image)

It should be noted that the soils are affected by a wetting response as well as the N additions, but Figure 4.12 shows a generally linear response to wetting with distilled water alone. Hence the complex temporal response observed here is mainly due to the...
microbial response to the added N. Whatever the mechanisms responsible for the varied temporal response of N additions, these patterns must be considered in the interpretation of denitrification data following N additions elsewhere, when multiple samples through time may be unavailable.

**Figure 4.15: Temporal response of soil CO₂ emissions to N additions**

4.5.1.5 Inhibition of N₂O reductase with C₂H₂
Fluxes of N₂O from soil cores incubated at 15°C with and without C₂H₂ are shown in Table 4.3, sorted by percentage change after addition of C₂H₂. No figure is provided because fluxes vary by 3 orders of magnitude so the data cannot usefully be plotted on the same axes.

The most notable aspect of the N₂O flux data is the presence of very large baseline values which are an order of magnitude greater than observed in previous core incubations. These highly elevated rates are probably an artefact of the previous experiment from which the samples were obtained, in which N additions were followed by incubation at 6°C for several days. Figure 4.14 above shows that N₂O emissions from certain soils (notable S1T, the top 5cm of the Scoat Tarn podsol)
increased to very high rates within one day of N additions, even at the much lower temperature. It was hypothesised that an expansion of the microbial population could account for this response, and these latest data, following a much longer incubation period and at a higher temperature, support this hypothesis.

Table 4.3: N₂O fluxes (kg N ha⁻¹ yr⁻¹) from soils incubated at 15°C with and without C₂H₂ block

<table>
<thead>
<tr>
<th>Sample</th>
<th>Baseline</th>
<th>+C₂H₂</th>
<th>Net change</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>M4B</td>
<td>1.5</td>
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<td>-1.5</td>
<td>-100</td>
</tr>
<tr>
<td>S2B</td>
<td>117.3</td>
<td>28.6</td>
<td>-88.7</td>
<td>-76</td>
</tr>
<tr>
<td>S3T</td>
<td>134.8</td>
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<td>-72</td>
</tr>
<tr>
<td>M1T</td>
<td>1.2</td>
<td>0.4</td>
<td>-0.9</td>
<td>-71</td>
</tr>
<tr>
<td>G3T</td>
<td>2.0</td>
<td>0.6</td>
<td>-1.3</td>
<td>-67</td>
</tr>
<tr>
<td>E1B</td>
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<td>0.1</td>
<td>-0.1</td>
<td>-61</td>
</tr>
<tr>
<td>M2B</td>
<td>322.6</td>
<td>209.3</td>
<td>-113.2</td>
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<tr>
<td>G4T</td>
<td>87.0</td>
<td>65.4</td>
<td>-21.6</td>
<td>-25</td>
</tr>
<tr>
<td>G1T</td>
<td>8.2</td>
<td>7.0</td>
<td>-1.1</td>
<td>-14</td>
</tr>
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<td>315.9</td>
<td>324.9</td>
<td>9.0</td>
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</tr>
<tr>
<td>S2B</td>
<td>17.9</td>
<td>18.4</td>
<td>0.6</td>
<td>3</td>
</tr>
<tr>
<td>S3T</td>
<td>91.5</td>
<td>100.0</td>
<td>8.5</td>
<td>9</td>
</tr>
<tr>
<td>M3T</td>
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<td>8.4</td>
<td>0.9</td>
<td>13</td>
</tr>
<tr>
<td>E2T</td>
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<td>161.4</td>
<td>19.9</td>
<td>14</td>
</tr>
<tr>
<td>M1T</td>
<td>3.7</td>
<td>5.5</td>
<td>1.8</td>
<td>48</td>
</tr>
<tr>
<td>G2B</td>
<td>1.8</td>
<td>2.8</td>
<td>1.0</td>
<td>55</td>
</tr>
</tbody>
</table>

While it is questionable whether the samples in this condition are in any way representative of the samples used for the phase 2 experiments below, let alone the same soils under natural field conditions, the experimental results are still of interest. There is no systematic change in N₂O emissions following the addition of 10% C₂H₂ to the incubation jars. More samples demonstrate a decrease in N₂O fluxes than an
increase. The only two samples showing a large increase in N\textsubscript{2}O fluxes are also among the slowest denitrifiers. While the reduction of N\textsubscript{2}O to \textsubscript{2}N may not be completely blocked by C\textsubscript{2}H\textsubscript{2}, potentially resulting in an underestimate of N\textsubscript{2}O emissions using this technique (Martin \textit{et al}., 1999), there is no evidence from these data that there is a systematic underestimate obtained from soils incubated without C\textsubscript{2}H\textsubscript{2}.

In absolute terms it is the reductions in N\textsubscript{2}O fluxes which are of greatest significance. Although C\textsubscript{2}H\textsubscript{2} inhibits N\textsubscript{2}O reductase and allows the measurement of gross N\textsubscript{2}O production during denitrification, C\textsubscript{2}H\textsubscript{2} also inhibits nitrification, potentially reducing the supply of NO\textsubscript{3}\textsuperscript{−} and the production of N\textsubscript{2}O which can also occur during nitrification (Robertson & Tiedje, 1987; Ineson \textit{et al}., 1998; Groffman \textit{et al}., 1999; Martin \textit{et al}., 1999), even at partial pressures more than an order of magnitude lower than those required to inhibit N\textsubscript{2}O reductase (Davidson \textit{et al}., 1986; Robertson \textit{et al}., 1986). Therefore, N\textsubscript{2}O production measured using C\textsubscript{2}H\textsubscript{2} may underestimate gross N\textsubscript{2}O production in aerobic incubations. This process would explain the major reductions in N\textsubscript{2}O emissions which are observed here, especially given that the soils were amended with NH\textsubscript{4}NO\textsubscript{3} and should therefore have abundant NH\textsubscript{4}\textsuperscript{+} substrate (35 mg\textsuperscript{−}\textsubscript{1}) for nitrification. In Section 4.1.8 it was suggested that nitrification could account for 50\% of N\textsubscript{2}O emissions from fertilised acid forest soils (Martikainen, 1985); this process could therefore be responsible for a large proportion of N\textsubscript{2}O emissions here. Its contribution is, though, likely to be highly spatially variable and possibly restricted to aerobic microsites, while denitrification is often attributed to anaerobic microsites.

Overall this experiment is inconclusive in assessing the need for the C\textsubscript{2}H\textsubscript{2} block in the soils studied, because of the (suspected) highly modified state of the microbial populations in the samples. However, there is at least little evidence that reduction of N\textsubscript{2}O to \textsubscript{2}N is likely to cause significant underestimation of total denitrification fluxes, particularly if it is assumed that only the absolute rates, and not the nature, of denitrification processes has changed in the soil samples used here.
4.5.2 Phase 2 results

4.5.2.1 Effects of temperature on potential denitrification

Emissions of N$_2$O from soil core tops incubated at 5°C and 15°C are shown in Figure 4.16. At the lower temperature, fluxes of N$_2$O are either absent (Mharcaidh soils and G4 - valley peat at the Gwy) or very low (other Gwy soils, Scoat Tam soils). The Etherow soils show very small negative fluxes at this temperature, suggesting N$_2$O consumption. The effect of warming the soils to 15°C is very marked, with increased, positive fluxes in all soils except M2 (valley peat) and M3 (peaty podsol) at the Mharcaidh. Fluxes are still very low, however, with only G2 (peaty gley, Afon Gwy) and S1 (podsol, Scoat Tarn) exceeding 1 kgN ha$^{-1}$ yr$^{-1}$.

**Figure 4.16: N$_2$O emissions (mean ±1SD) from core tops (0-5cm)**

The response of deeper soils (core bottoms and separate mineral horizons at G2 and G3) is more complex (Fig. 4.17). Even at 5°C, appreciable N$_2$O fluxes (>1 kgN ha$^{-1}$ yr$^{-1}$) occur from the mineral horizons at the Gwy (G2m – peaty gley, G3m – podsol) and all soils from both the Scoat Tarn and Etherow sites.
Elsewhere, N\textsubscript{2}O fluxes are below the detection limit of the method or very small where measurable. Warming the soils to 15°C produces different responses according to the denitrification activity suggested by the lower temperature incubation. In all cases, small increases in N\textsubscript{2}O flux are recorded from those soils which show negligible emissions at 5°C. However, the reverse is true for most of the soils showing appreciable N\textsubscript{2}O emissions (> 1 kgN ha\textsuperscript{-1} yr\textsuperscript{-1}) at the lower temperature; fluxes are much reduced at 15°C. This observation is presumably due to exhaustion of the substrate (either NO\textsubscript{3}\textsuperscript{-} or labile C), which could explain the particularly sharp decline in the mineral horizons from the Gwy soils (see discussion below). The only exception to these two sets of responses is found in the deep peat soil from under burnt Calluna at the Etherow (E1), which shows an increased flux from a relatively high initial value at the lower temperature. This soil is intermediate between the two major groups which are separated by an arbitrary cutoff at 1 kgN ha\textsuperscript{-1} yr\textsuperscript{-1}, and also shows the largest spatial variability (as indicated by standard deviation) at the higher temperature, as observed also for soilwater NO\textsubscript{3}\textsuperscript{-} (see Chapter 3).

The question of NO\textsubscript{3}\textsuperscript{-} limitation is addressed in the N additions experiments below.
4.5.2.2 Effects of N additions on potential denitrification

The effects of adding N to core tops incubated at 5°C are illustrated in Figure 4.18. There is a dramatic increase in the denitrification flux for every soil, including those from the Mharcaidh which show no measurable N₂O flux without N additions at this temperature. These soils are therefore capable of significant rates of denitrification even at this low temperature in the presence of soilwater NO₃⁻.

A similar response is observed for samples incubated at 15°C when N is added (Fig. 4.19). All surface soils show an increase in N₂O emissions when incubated with N at this temperature, relative to soils incubated without the N amendments. The response to increased temperature with N additions is, however, more complex.

Figure 4.20 compares soil core tops incubated at 5°C and 15°C with added N in both cases. The responses to increased temperature are varied and difficult to interpret. Contrary to expectations, many of the soils show a decrease in N₂O flux after warming, although a few show small increases. Only the podsol from Scoat Tarn shows a large increase in N₂O flux after warming, as it also did prior to N additions.

Figure 4.18: N₂O emissions (mean ±1SD) from core tops (0-5cm) incubated at 5°C with and without additions of NH₄NO₃
Figure 4.19: N₂O emissions (mean ±1SD) from core tops (0-5cm) incubated at 15°C with and without additions of NH₄NO₃

![Graph showing N₂O emissions from core tops incubated at 15°C with and without NH₄NO₃ additions.]

Figure 4.20: N₂O emissions (mean ±1SD) from core tops (0-5cm) incubated at 5°C and 15°C with additions of NH₄NO₃

![Graph showing N₂O emissions from core tops incubated at 5°C and 15°C with NH₄NO₃ additions.]

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It might be speculated that the increase in temperature results in a greater proportion of emissions as $N_2$ rather than $N_2O$ (Goodroad & Keeney, 1984; Firestone & Davidson, 1989), although increased $NO_3^-$ and reduced C availability tend to increase the proportion of $N_2O$ relative to $N_2$ (Firestone & Davidson, 1989). However, the phase 1 experiment on the effects of increased temperature showed no such decline in $N_2O$ emissions at c. 15°C.

Instead, because of the order in which the incubations were done, with the N added to soils already at 15°C which were later cooled to 5°C, the greater response at 5°C relative to 15°C is probably due simply to the time lag in response to the N additions. The initial incubations with N at 15°C were sealed within 30 minutes and sampled within 2 hours of N addition, after which the samples were cooled to 5°C and sampled again 4 hours later, around 6 hours after additions. It is apparent from the phase 1 experiment on temporal responses to N additions that $N_2O$ fluxes peak very quickly and are already decreasing after 1-3 hours, after which there is an increase again (Figure 4.14). Hence the fluxes at the higher temperature incubation, sampled after the first peak in $N_2O$ emissions, were dominated by a short term response to N additions and are not comparable with the lower temperature incubation results.

Since problems with sampling too soon after N additions had been noted during the core top experiments, the core bottoms were treated differently. Responses to added N were measured 2 days after additions for both 5°C and 15°C incubations to avoid the early temporal instability and peak in denitrification. The effects of N additions on deeper soils incubated at 5°C are shown in Figure 4.21, and are more complex than for surface soils. Whereas N additions stimulated $N_2O$ emissions from all core tops at 5°C (despite the problems of non steady-state), responses of the deeper soils are mixed. Increased fluxes are found in all the Mharcaidh soils and the organic soils from the Gwy. Decreased fluxes with N additions are found in the two mineral horizons from the Gwy, plus two of the three Scoat soils and both Etherow soils. The size of the decrease in the mineral Gwy soils suggests a depletion of available C, and this must also be true for the other soils which show a decrease with added N.
At 15°C the effects of N additions are more in line with expectations (Fig. 4.22), with most soils showing an increased flux of N\textsubscript{2}O. The only exceptions are soils which show very low emissions both with and without added N, with no significant difference in flux, and the deep peat from the burnt area of the Etherow (E1).

If the N\textsubscript{2}O fluxes from core bottoms with N additions at the different incubation temperatures are plotted together, it is apparent that the increase in temperature does substantially increase N\textsubscript{2}O emissions from all soils (Fig. 4.23).

4.5.2.3 Resampling of N\textsubscript{2}O emissions from core tops

Since the initial experiments to test the response of core tops to N additions were confounded by very short term variations in N\textsubscript{2}O fluxes, a later set of samples was obtained six days after N additions (Figure 4.24). In most samples except those at the Mharcaidh, N\textsubscript{2}O fluxes after 6 days of incubation with N are much higher than those after just 2 hours. In the Mharcaidh samples and the podsol from the Gwy (G3) rates are lower after 6 days than after 2 hours. However, N\textsubscript{2}O emissions are greater 6 days after N additions than they are prior to N additions for all soil core tops.
Figure 4.22: N$_2$O emissions (mean ±1SD) from core bottoms (c. 5-20cm) incubated at 15°C with and without additions of NH$_4$NO$_3$.

Figure 4.23: N$_2$O emissions (mean ±1SD) from core bottoms (c. 5-20cm) incubated at 5°C and 15°C with additions of NH$_4$NO$_3$. 
Again, these results illustrate the problem of measuring the temporal response of soil denitrification to N additions. While all the soils incubated seem to show a similar pattern of an initial rapid pulse of N\textsubscript{2}O emissions, either from denitrification or nitrification, the response thereafter varies between soils. Some soils show a steady increase in N\textsubscript{2}O fluxes over timescales of hours to days following N additions, while others do not, and fluxes may either level off or decrease (Figure 4.14). These different types of response have to be taken into account when attempting to estimate annual mean fluxes of N\textsubscript{2}O in the field, whether from laboratory or direct field measurements.

4.5.2.4 Potential denitrification in whole soil cores

Since soil core tops (0-5cm) and bottoms (c. 5-20cm), plus extra mineral horizons from two of the Gwy soils, were incubated separately, the measured N\textsubscript{2}O fluxes apply only to the particular horizons in the sampled sections. For fluxes to be expressed per unit area of the whole sampled soil profile, net fluxes for each section are summed in Table 4.4. The totals given are maximum possible values for the experimental conditions applied, and are likely to be overestimates of potential field rates because of much more limited diffusion from lower horizons when overlain by surface soils in...
the field. For the 15°C incubations with added N in core tops, the later dataset (6 days after additions) is used, since it is more comparable with the equivalent data for lower soils. The main purpose of the data is to illustrate the differences between catchments, soil types and within vertical soil profiles in terms of potential denitrification activity.

Assessed in this way, the data show that even under near optimal laboratory conditions, the potential for denitrification in these soils is rather low unless N is added, typically 1-3 kg N ha⁻¹ yr⁻¹ at 5°C with the exception of the Mharcaidh soils and the deeper peats at the Gwy. Warming the unamended soils to 15°C creates a mixed response. The uppermost soils all show in increase in mean N₂O fluxes after warming, except for two of the Mharcaidh soils where denitrification is still absent. In the deeper soils, many samples show a reduced N₂O flux which may indicate exhaustion of the available C or NO₃⁻ substrate. The net effect of warming on whole-core rates is that while some soils show an increase, others decrease, and all fluxes are still below 3 kg N ha⁻¹ yr⁻¹.

Addition of N to core tops stimulates denitrification and greatly increases N₂O fluxes at 5°C, suggesting that NO₃⁻ and not C initially limits activity in these samples. However, when the samples are warmed to 15°C with added N, several show a decrease in activity, presumably because of C limitation induced by the high rates of microbial consumption in the previous, lower temperature incubation. This apparent C limitation becomes evident more quickly in the deeper samples which already show a decline in activity when warmed from 5 to 15°C: addition of N results in no increase in activity in several of these samples (e.g. Etherow and most Mharcaidh soils). Hence for the deeper soils, and to a lesser degree the surface soils, denitrification rates cannot increase indefinitely with increased N inputs, because C soon becomes limiting at elevated rates of microbial activity.

4.5.2.5 CO₂ fluxes as indicators of microbial activity

For a general comparison of microbial activity between soils and sites, CO₂ fluxes from unamended cores incubated at 5°C and 15°C are presented in Figures 4.25 and 4.26. Emissions of CO₂ from core tops at both temperatures are much larger for the Gwy soils than elsewhere. Similar low emissions from the Mharcaidh and Etherow soils are found, while slightly higher emissions occur from the Scoat soils.
Table 4.4: Mean N$_2$O fluxes from incubated soil cores (kgN ha$^{-1}$ yr$^{-1}$)

<table>
<thead>
<tr>
<th>Soil</th>
<th>5°C Top</th>
<th>Bottom</th>
<th>Total</th>
<th>15°C Top</th>
<th>Bottom</th>
<th>Total</th>
<th>5°C +N Top</th>
<th>Bottom</th>
<th>Total</th>
<th>15°C +N Top</th>
<th>Bottom</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 Peaty ranker</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
<td>6.0</td>
<td>0.3</td>
<td>6.4</td>
<td>0.2</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
<td>4.7</td>
<td>0.0</td>
<td>4.7</td>
<td>0.7</td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>3.7</td>
<td>0.0</td>
<td>3.8</td>
<td>0.2</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>1.5</td>
<td>0.0</td>
<td>1.5</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.9</td>
<td>1.0</td>
<td>1.1</td>
<td>2.2</td>
<td>3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>0.6</td>
<td>2.5</td>
<td>3.0</td>
<td>1.3</td>
<td>0.5</td>
<td>1.8</td>
<td>2.4</td>
<td>0.9</td>
<td>3.3</td>
<td>3.4</td>
<td>2.0</td>
<td>5.4</td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>0.1</td>
<td>2.3</td>
<td>2.4</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
<td>1.6</td>
<td>0.0</td>
<td>1.6</td>
<td>1.0</td>
<td>0.5</td>
<td>1.4</td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
<td>0.0</td>
<td>0.2</td>
<td>4.4</td>
<td>1.5</td>
<td>6.0</td>
<td>4.4</td>
<td>1.8</td>
<td>6.2</td>
</tr>
<tr>
<td>S1 Podsol</td>
<td>0.4</td>
<td>1.5</td>
<td>2.0</td>
<td>2.0</td>
<td>0.5</td>
<td>2.5</td>
<td>2.2</td>
<td>0.5</td>
<td>2.7</td>
<td>11.8</td>
<td>0.9</td>
<td>12.7</td>
</tr>
<tr>
<td>S2 Peaty gley</td>
<td>0.1</td>
<td>1.2</td>
<td>1.3</td>
<td>0.6</td>
<td>0.3</td>
<td>1.0</td>
<td>8.0</td>
<td>2.6</td>
<td>10.6</td>
<td>4.8</td>
<td>4.2</td>
<td>9.1</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>0.3</td>
<td>2.2</td>
<td>2.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.6</td>
<td>6.1</td>
<td>0.7</td>
<td>6.8</td>
<td>6.3</td>
<td>1.0</td>
<td>7.3</td>
</tr>
<tr>
<td>E1 Peat (burnt)</td>
<td>-0.1</td>
<td>1.1</td>
<td>0.9</td>
<td>0.2</td>
<td>2.3</td>
<td>2.5</td>
<td>0.3</td>
<td>0.4</td>
<td>0.7</td>
<td>0.9</td>
<td>0.9</td>
<td>1.8</td>
</tr>
<tr>
<td>E2 Peat (unburnt)</td>
<td>-0.1</td>
<td>2.1</td>
<td>2.0</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
<td>1.8</td>
<td>-0.1</td>
<td>1.7</td>
<td>0.7</td>
<td>0.1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

For core bottoms the patterns are less consistent. At 5°C the largest emissions occur in the Mharcaidh samples, while the smallest occur in the Scoat and Etherow samples. These differences are obscured to a degree at the higher temperature, but the most notable change is that the greatest emissions are now found in the deep peat samples from under mature Calluna at the Etherow (E2). These differences may be due largely to differences in soil organic matter content, which should be less variable in core tops.

Spatial variability between replicated core samples from each soil, as indicated by the standard deviations in Figures 4.25 and 4.26, appears to be much smaller for CO$_2$ emissions than for N$_2$O emissions, where measurable for the latter (compare Figs. 4.16 to 4.24 above).

4.6 Discussion

4.6.1 Spatial variability in denitrification

There are two important aspects to the spatial variability in denitrification within soils. The first is the vertical variation associated with changes between soil horizons,
which is important at the process level only when laboratory incubations are used to estimate N$_2$O fluxes. The second aspect is the ‘horizontal’ or lateral variation in denitrification rates, which is more widely understood as spatial variation. Since field measurements tend to measure net emissions from the soil surface, only this latter aspect is relevant for quantifying fluxes on an areal basis. Both aspects are discussed separately below.

**Figure 4.25: CO$_2$ fluxes from core tops (0-5cm) incubated at 5°C and 15°C**

![Figure 4.25: CO$_2$ fluxes from core tops (0-5cm) incubated at 5°C and 15°C](image)

**Figure 4.26: CO$_2$ fluxes from core bottoms (c. 5-20cm) incubated at 5°C and 15°C**

![Figure 4.26: CO$_2$ fluxes from core bottoms (c. 5-20cm) incubated at 5°C and 15°C](image)
4.6.1.1 Vertical (within profile) variability

The much higher denitrification rates found here in surface soils than in deeper soils has been observed in several previous studies (e.g. Henrich & Haselwandter, 1991). Jarvis et al. (1991) suggested that most denitrification occurs in the upper 20cm of the soil profile, with a rapid decrease in denitrification potential with depth, following the pattern of organic matter distribution in most grassland soils. This idea was taken further by Parkin (1987), who found that 85% of the denitrification activity in an intact soil core could be attributed not only to the top 1cm of the core, but to a 'hotspot' associated with a single decaying leaf. Similarly, Christensen et al. (1990) found that the highest activity was generally associated with the presence of rotting vegetation, and in one sectioned core was attributed to a layer of grass roots.

Struwe and Kjøller (1991) found that denitrification activity was greatest at a depth of 2-4cm, and decreased down to the maximum sampled depth of 16cm. N₂O production, consumption and transport processes were found to vary markedly over a few cm near the soil surface by Ball et al. (1997). Tietema et al. (1991) showed that the litter layer contributes significantly to annual N₂O emissions from an oak-beech forest. Müller et al. (1997a, 1997b) applied a mechanistic model to predict N₂O emissions from both nitrification and denitrification based only on properties of the top 5cm or 10cm of the soil profile of urine-affected pasture, assuming this to be the most important horizon in this respect. Groffman et al. (1999) point out that although much higher activity may occur in the surface 0-2cm or 0-5cm layer, the lower activity in deeper layers occurs over a greater soil volume and may therefore be significant in terms of overall flux. However, denitrification in the 15-30cm layer was found to equal only 2% of that in the 0-15cm layer by Christensen et al. (1990).

Some authors have suggested that decreased denitrification potentials with increased depth in soils may reflect a lack of energy (carbon) sources to support heterotrophs (e.g. Davidson & Swank, 1987; Ashby et al., 1998). Carbon availability is therefore less likely to be severely limiting in surface horizons of soils where decaying organic matter is more abundant (Parkin, 1987). The earlier onset of apparent C limitation in incubations of deeper soils than of surface samples within the current study (Section 4.5) would seem to support this hypothesis.
In the field, denitrification rates at different depths following N inputs will reflect a balance between the following effects of rainfall (Jarvis et al., 1991):

1. restricting O\textsubscript{2} supply to the surface;
2. moving NO\textsubscript{3}\textsuperscript{-} down the soil profile;
3. influencing the opportunity for competition for NO\textsubscript{3}\textsuperscript{-} between denitrification and microbial or plant uptake; and
4. possible obstruction of the escape of denitrification products and their removal in solution down the profile.

A major conclusion from the results of this study is that for laboratory incubations of soils, the surface organic layer (0-5cm) should never be neglected in assessments of denitrification flux.

4.6.1.2 Horizontal variability

Lateral variations in the capacity of soils to denitrify present one of the biggest problems in attempts to quantify fluxes at the catchment scale. Field measurements of N\textsubscript{2}O fluxes within this study show that measurable, annual mean rates of denitrification occur in only 17% (Mharcaidh) to 50% (Etherow) of the static chambers installed.

Laboratory incubations were more successful in producing measurable fluxes of N\textsubscript{2}O, which provide further evidence of the great spatial variability associated with denitrification (Table 4.5). Coefficients of variation (CVs) are very large for most soils, with many values greater than 100%. Although the number of replicate samples from each soil is very low (n=3), the CVs give some indication of the variability in the data.

For comparison, CVs for CO\textsubscript{2} fluxes from the same samples are provided in Table 4.6. These very low CVs, mostly less than 20% for core tops, though frequently larger for core bottoms, indicate that the processes responsible for CO\textsubscript{2} emissions are much more uniformly distributed in the soil than is denitrification. Similar results have been found by other authors (e.g. Sitaula & Bakken, 1993).
The great spatial variability associated with denitrification, for which field rates vary by up to orders of magnitude, has long been recognised and reported by many authors (e.g. Hutchinson & Mosier, 1981; Rolston, 1986). These variations can occur over widely different spatial scales. Spatial studies by Ball et al. (1997) demonstrated that the highest \( \text{N}_2\text{O} \) emissions could be associated with areas ranging from a few square cm to a few square metres.

### Table 4.5: Coefficients of variation (%) for \( \text{N}_2\text{O} \) fluxes (unamended with N)

<table>
<thead>
<tr>
<th>Soil</th>
<th>Tops (5°C)</th>
<th>Tops (15°C)</th>
<th>Bottoms (5°C)</th>
<th>Bottoms (15°C)</th>
<th>Mineral (5°C)</th>
<th>Mineral (15°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 Peaty ranker</td>
<td>0.0</td>
<td>93.0</td>
<td>0.0</td>
<td>49.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>18.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>155.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>0.0</td>
<td>173.2</td>
<td>244.9</td>
<td>156.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>173.2</td>
<td>136.2</td>
<td>113.7</td>
<td>32.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>44.6</td>
<td>48.5</td>
<td>109.9</td>
<td>57.1</td>
<td>27.1</td>
<td>81.5</td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>173.2</td>
<td>28.8</td>
<td>0.0</td>
<td>162.8</td>
<td>46.9</td>
<td>155.1</td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>0.0</td>
<td>0.4</td>
<td>0.0</td>
<td>244.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1 Podsol</td>
<td>63.6</td>
<td>39.5</td>
<td>19.6</td>
<td>41.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2 Peaty gley</td>
<td>173.2</td>
<td>74.6</td>
<td>37.8</td>
<td>98.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>173.2</td>
<td>71.9</td>
<td>25.0</td>
<td>208.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1 Peat (burnt)</td>
<td>-86.6</td>
<td>48.8</td>
<td>28.8</td>
<td>74.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2 Peat (unburnt)</td>
<td>-86.6</td>
<td>93.3</td>
<td>17.7</td>
<td>90.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Small areas of very high activity, often associated with decaying organic matter (particulate C) or animal excreta, have been termed microsites or hotspots, and result in highly skewed distributions in field measurements of denitrification (Tiedje et al., 1989; Parkin, 1987; Christensen et al., 1990; van Kessel et al., 1993; Williams et al., 1999). While most samples will exhibit low rates, an aggregated dispersion of these microsites results in localised zones of very high activity (Parkin, 1987). The dispersion of hotspots may vary temporally in response to changing conditions in the soil, e.g. moisture (Parkin, 1987).
Table 4.6: Coefficients of variation (\%) for CO\textsubscript{2} fluxes from incubated soils
(unamended with N)

<table>
<thead>
<tr>
<th>Soil</th>
<th>Tops (\textdegree C)</th>
<th>Tops (\textdegree C)</th>
<th>Bottoms (\textdegree C)</th>
<th>Bottoms (\textdegree C)</th>
<th>Mineral (\textdegree C)</th>
<th>Mineral (\textdegree C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 Peaty ranker</td>
<td>13.2</td>
<td>28.4</td>
<td>17.1</td>
<td>31.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>12.7</td>
<td>28.1</td>
<td>17.0</td>
<td>22.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>11.1</td>
<td>11.5</td>
<td>49.7</td>
<td>32.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>16.3</td>
<td>31.3</td>
<td>32.2</td>
<td>69.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>12.0</td>
<td>28.1</td>
<td>29.6</td>
<td>26.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>5.3</td>
<td>11.2</td>
<td>41.5</td>
<td>19.7</td>
<td>27.1</td>
<td>28.3</td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>14.4</td>
<td>61.0</td>
<td>59.7</td>
<td>56.6</td>
<td>46.9</td>
<td>97.2</td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>17.8</td>
<td>21.1</td>
<td>40.5</td>
<td>14.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1 Podsol</td>
<td>20.0</td>
<td>20.0</td>
<td>19.6</td>
<td>22.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2 Peaty gley</td>
<td>13.4</td>
<td>19.0</td>
<td>37.8</td>
<td>10.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>5.0</td>
<td>7.1</td>
<td>25.0</td>
<td>90.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1 Peat (burnt)</td>
<td>15.3</td>
<td>26.8</td>
<td>28.8</td>
<td>32.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2 Peat (unburnt)</td>
<td>16.0</td>
<td>14.3</td>
<td>17.7</td>
<td>13.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

At the other end of the spatial scale, landscape factors are often useful predictors of denitrification. For example, van Kessel \textit{et al.} (1993) found that highest rates occur in footslope and lower-level landforms while the lowest rates are found in divergent shoulder and upper-level landforms. These differences persisted throughout the year regardless of actual denitrification rates, but were smallest after heavy rain. Mohn \textit{et al.} (2000), working on forest soils, found that denitrification rates on drained mounds with raw humus layers were low throughout the year, while in waterlogged depressions rates could peak at 50 kgN ha\textsuperscript{-1} yr\textsuperscript{-1}.

Particularly important components at the landscape scale are the riparian and other seepage zones of catchments (Martin \textit{et al.}, 1999). Cooke and Bryce Cooper (1988) found that the seepage zones of a New Zealand hill pasture had a very high capacity to denitrify inflowing NO\textsubscript{3}\textsuperscript{-}, concluding that under baseflow conditions, most of the influent NO\textsubscript{3}\textsuperscript{-} could be denitrified in these organic rich riparian zones. Ashby \textit{et al.}...
(1998) found significantly higher denitrification in the soils of these areas than elsewhere, with a median rate of 10 kgN ha\(^{-1}\) yr\(^{-1}\) compared with 0-2 kgN ha\(^{-1}\) yr\(^{-1}\) in other catchment soils, but since these soils formed only 0.7% of the catchment area, the catchment weighted flux was only 1.1 kgN ha\(^{-1}\) yr\(^{-1}\). However, high rates of denitrification may not always be measured above riparian soils, because of subsurface flow flushing N\(_2\)O from the soil (Hixson et al., 1990). Hill et al. (2000) suggested that denitrification hotspots in riparian zones only occurred in “redox fronts”, where NO\(_3^-\) rich groundwater interacts with localised supplies of organic matter, so that denitrification may be C-limited in a large proportion of the subsurface riparian zone.

In each of the catchments within the current study, a selection of the major soil types was sampled to provide a representative coverage of the catchment, as far as was logistically possible. Major differences are apparent between soil types in each catchment in the soil incubation study. At the Mharcaidh, denitrification only occurs at measurable rates when N is added. The highest potentials are observed from the surface organic horizons of all soils at 5°C, decreasing with altitude from the peaty ranker (M1) to the shallow peat (M4). The peaty ranker also shows the highest potential at 15°C, this time in the deeper soils, but decreases in potential in surface soils are attributed to exhaustion of available C. At the Gwy, the biggest potentials are found in the mineral soils prior to N additions, especially the peaty gley (G2). Denitrification rates are very low in the hilltop (G1) and valley (G4) peats until N is added, when the highest potentials for all Gwy soils are measured in the valley peat, particularly the surface horizons. All the Scoat Tarn soils show high denitrification potentials prior to N additions, mainly in the deeper soils at 5°C, with a maximum in the lakeside deep peat (S3). At 15°C available C appears to be depleted in the deeper soils and higher potentials occur in the surface horizons, the maximum now being found in the podsol (S1). N additions stimulate denitrification in all soils, but mostly in the core tops. In the Etherow peats, very low denitrification rates are measured in surface soils prior to N additions, but the deeper soils show fairly high potentials. The peat under burnt Calluna (E1) produces the greatest potentials at 15°C, while the peat under mature Calluna (E2) shows greater potentials at 5°C, both with and without N additions.
Although valley peats were sampled at the Mharcaidh and Gwy sites, plus a lakeside peat at Scoat Tarn, these soils are not very representative of the riparian zones in these catchments. It is therefore possible that zones of higher denitrification activity were omitted from the study, leading to an underestimate of mean rates for the whole catchments. Given the conflicting results from other studies in terms of the overall significance of riparian zones to catchment fluxes of N₂O, further attempts to improve estimates of N₂O emissions at this scale should focus more on these areas in future.

4.6.2 Temporal variability in denitrification

As with spatial variations in denitrification, temporal variations are found at very different scales, from rapid responses to wetting or fertilisation within minutes or hours, to seasonal variations linked to climatic factors and interactions with the plant growth cycle. Hutchinson and Mosier (1979) cited the summary of Smith et al. (1978) that “denitrification occurs in periodic bursts, in response to changes in oxygen status, against a background of very slow yet continuous denitrification”. According to Brumme et al. (1999), 3 types of emission patterns can be identified; seasonal, background and event-based.

4.6.2.1 Rapid responses of denitrification to environmental factors

The laboratory incubation experiments indicate that wetting alone has little effect on the soils studied (Fig. 4.12), but wetting in combination with N additions (as NH₄NO₃) causes a rapid response, with at least one initial burst of denitrification within 0-2 hours of additions, sometimes accompanied by a second, smaller pulse of activity within 1-3 hours, followed by a slower response over several hours to days (Fig. 4.14). Similar transient peaks have been found in other studies, although greater availability of N in many cases frequently leads to large responses to wetting alone. The reasons for the occurrence of two pulses in the current study are not known, but it may be speculated that one could be linked to nitrification and the other to denitrification, with these processes having different response times.

On fast-draining soils like sandy loams, the peak in denitrification was found to be very brief, within 1-3 hours after additions (de Klein & van Logtestijn, 1996).
Sexstone et al. (1985) found elevated denitrification occurred in bursts following rainfall of more than 1cm, which peaked at 3-5 hours and returned to normal within 12 hours in a sandy loam, but peaked at 8-12 hours and returned to pre-irrigation levels by 48 hours in a clay loam. Cores from forest soils amended with NO$_3^-$ and glucose showed much higher denitrification rates than unamended cores, which increased to a maximum over several hours then decreased rapidly as the substrate was depleted (Ineson et al., 1991).

Firestone et al. (1980) found a temporal pattern in the denitrification products from 3 soils after the onset of anaerobiosis:

1. from 0 to 1-3 hours N$_2$ was the dominant denitrification product;
2. the NO$_3^-$ reductase activity then increased without a corresponding increase in N$_2$O reductase activity, leading to an extended period of between 1-3 and 16-33 hours during which N$_2$O was an important or dominant product; and
3. after this period, an increase in N$_2$O reductase activity without further increases in NO$_3^-$ reductase occurred, producing generally just N$_2$.

These processes could potentially explain some of the observed responses to N additions in the present study, but are likely to vary in importance with other factors like soil pH and organic matter content.

Other shorter timescale temporal changes may occur, which are not driven by responses to rainfall or fertiliser inputs. Blackmer et al. (1982) found that large diurnal variability in soils (equivalent to fluxes of more than 40 kgN ha$^{-1}$ yr$^{-1}$) could largely be attributed to changes in soil temperature, which affects both microbial production of N$_2$O and solubility of N$_2$O in soil waters.

Some authors have been able to separate responses to fertiliser inputs and wetting over different timescales. Williams et al. (1999) found no immediate effect of fertiliser additions to grazed grassland soils in continuous measurements, but a rainfall event 14 days later caused a burst of emission which lasted for at least 2 weeks. In laboratory incubations, urea additions led to a response within 4 hours, peaking after 6 hours then declining after 24 hours. It was estimated that diurnal variations could cause an underestimate of actual N$_2$O flux by as much as a factor of 5.
In the current study, the potential effects of diurnal variations were explored at the River Etherow during late spring and mid-summer. Sampling of static chambers every 2 hours for a 24 hour period on each visit found no discernible diurnal variation in the very small fluxes observed (Vicky New, unpublished data).

The laboratory studies do, however, suggest that short-term responses to N additions may well have occurred in the field, but the practice of sampling 2 weeks after the previous application means that transient peaks in denitrification would have been missed. In laboratory incubations, N additions increased N$_2$O fluxes by up to an order of magnitude for several days following application. What cannot be quantified here is the effect of the lack of competition for N with plants in the laboratory studies. It seems likely that this process could account for the very low denitrification rates measured in the field, as plant uptake may reduce the availability of both NH$_4^+$ for nitrification and NO$_3^-$ for denitrification.

4.6.2.2 Seasonal patterns of denitrification

Since field rates of denitrification are so low in the current study, temporal variations are barely apparent, with only one plot showing a distinct seasonal pattern (Fig.4.5). Brumme et al. (1999) found that for forests, most sites showed only background emission patterns, ranging from 0.17 to 0.8 kgN ha$^{-1}$ yr$^{-1}$, while two sites with seasonal emission patterns and elevated emissions in summer had annual fluxes of 3 and 7.3 kgN ha$^{-1}$ yr$^{-1}$. By these definitions, all sites within the present study with any measurable denitrification at all show only background emission patterns. Interestingly, Brumme et al. (1999) also suggested that N deposition seems to have no effect on sites with background emissions, but is important at sites with seasonal emissions. This assertion is in agreement with the field measurements here, including plots amended with NH$_4$NO$_3$. In other studies where higher denitrification rates are measured, seasonal patterns are often much more obvious.

In agricultural systems, large seasonal variations in nitrification and denitrification may be associated with cropping practices and the timing of fertiliser additions. Jarvis et al. (1991) recorded a very spiky response in denitrification following fertiliser additions and rainfall on a grazed grassland. In a study by Hutchinson and Mosier (1979), a 3 week period following application of NH$_4^+$ fertiliser accounted for 30% of
the season's N$_2$O flux via vigorous nitrification which evolved N$_2$O directly. Emissions over the growing season totalled c. 2.6 kgN ha$^{-1}$ yr$^{-1}$ with most occurring after just two events, while projected annual emissions were no more than 4 kgN ha$^{-1}$ yr$^{-1}$. Therefore short periods of high emissions do not necessarily lead to large annual mean fluxes.

Seasonal variations are not restricted to agricultural systems. Groffman and Tiedje (1989) found that over 80% of annual denitrification loss from forest soils occurred during 3-6 weeks of high activity in spring and autumn. Tietema et al. (1991) found that N$_2$O flux measurements showed a marked temporal variation, with 3 peak values contributing 65% of the annual emissions from forest soils. Denitrification in tallgrass prairie was found to be highest in spring due to high water and NO$_3^-$ availability because of low plant activity (Groffman et al., 1993).

Dutch and Ineson (1990) suggested that large temporal variation means that measurements are required over a long period of time. Tiedje et al. (1989) went further, stating that consideration of temporal patterns in denitrification can aid in designing sampling strategies. As a minimum guideline they recommended taking 20 cores per site and 12-20 samplings per year, planned to encompass periods of higher activity. The current field study used up to 12 static chambers per site (excluding N additions experiments) which were sampled 12 times during the year, which is slightly lower than the sampling intensity recommended above. It is possible that some peak values may have been missed as a result.

### 4.6.3 Controls on rate and end products of denitrification

The temporal and spatial variability of denitrification is due to the many physical and chemical factors which exert an influence on the presence, rate and end products of the process. When these factors are optimal, variability is reduced and rates increase. For example, Christensen et al. (1990) found that activity in cores with low denitrification was not skewed under conditions where soils were flooded, glucose was added, or during the autumn when plant cover was decaying. The distribution of
rates was always positively skewed in soils at field capacity without a supply of organic matter.

The majors controls on denitrification are discussed below in the context of the experimental results.

4.6.3.1 N availability

Additions of NH$_4$NO$_3$ to static chambers in the field produced no measurable effect after 2 weeks. While rapidly occurring, transient peaks in denitrification may have been missed, there is no ‘conditioning effect’ whereby the background rates of denitrification are increased, at least within the sensitivity of the method (±0.3 kgN ha$^{-1}$ yr$^{-1}$). These results, showing that N inputs have little effect on soils exhibiting background denitrification rates, agree with those of Brumme et al. (1999) if it is assumed that large, short-lived events were not missed.

A similar dose of NH$_4$NO$_3$ (30 kgN ha$^{-1}$ yr$^{-1}$) applied to gley soils under Norway spruce in central Switzerland increased the annual denitrification rate from 1.7 to 2.9 kgN ha$^{-1}$ yr$^{-1}$ (Mohn et al., 2000). The biggest increases in activity followed rainfall events, reaching rates equivalent to almost 50 kgN ha$^{-1}$ yr$^{-1}$. However, denitrification increases following N additions only increased for 24 hours as the soil solution was rapidly depleted in NO$_3$-. A part of the N input is therefore immediately denitrified. This site fits into the “event-based” denitrification class of Brumme et al. (1999).

Laboratory experiments, albeit without the competitive effects of plant uptake, do show that denitrification rates can be greatly increased with a greater supply of NO$_3$-. An initial burst of activity is not sustained for more than a few hours, yet soils sampled after several days of incubation still frequently show elevated denitrification. It would seem that in the short term at least, denitrification may be limited by other factors when NO$_3$- is present in excess. What is not known from the experimental data is whether the elevated rates after several days could be sustained indefinitely with excess NO$_3$-; presumably some other factor like C availability would eventually become limiting.
4.6.3.2 C availability

Although not measured within this study, labile C would appear to limit activity in soils where N is not limiting. Utilisation of readily available C could explain the short term (0-3 hours) pulse of denitrification observed after N additions to soil cores. The gradual increase in denitrification which occurs in some cores over a longer period of days might be due to an expansion of microbial populations providing an increased supply of labile C via decomposition.

The limitations imposed by C supply have been recognised by many authors and were discussed in Section 4.1.4 above. For example, in studies by both Struwe and Kjøller (1991) and Weier et al. (1993) it was found that additions of both C and N were required to generate a large increase in denitrification. Only minor increases were obtained with N alone, supporting the idea that a readily decomposable substrate is required before reduction of added NO$_3^-$ can occur. For this reason, effects of N additions were much greater in surface soils with greater C availability than in subsoils (Weier et al., 1993), which largely matches the findings here.

Other authors have found that C may never be limiting under some circumstances. For example, Koerselman et al. (1989) found that N$_2$O emissions from quaking fens in the Netherlands were limited only by NO$_3^-$ supply, but measured rates in response to ambient N deposition were estimated at just 1.1 kgN ha$^{-1}$ yr$^{-1}$. The possible C limitation on denitrification rates is, however, likely to be of great importance for modelling maximum potential rates under increased N supply for many soils.

4.6.3.3 Soil moisture

Although field rates of denitrification were found to be very low, it is interesting that of the two individual chambers showing the largest N$_2$O emissions, one occurs on the wettest overall soil (Gwy valley peat: G4D1), while the other occurs on the burnt Etherow peat (E1D1) which is one of the driest soils studied. Annual mean soil moisture values are, however, of limited value in predicting potential denitrification, because of temporal variability which may lead to a significant proportion of annual fluxes occurring during short periods when soils may be wettest.
The soil incubation experiments were conducted on soils which had been wetted up with de-ionised water and indicate potential denitrification rates which are much higher than field rates. Denitrification rates from cores without added N are, though, still low (<3 kgN ha\(^{-1}\) yr\(^{-1}\)) compared with N amended cores (up to c. 13 kgN ha\(^{-1}\) yr\(^{-1}\)), and wetting causes no pulse in denitrification like that associated with N additions, showing that substrate limitation is more important in these experiments than moisture limitation.

4.6.3.4 Soilwater pH
In field soils, the largest N\(_2\)O flux was associated with the lowest soil pH (ElD1, pH 3.0). Soil pH does not, therefore, appear to be the primary factor limiting denitrification in the field. In laboratory incubations, the most acid Etherow soils do, however, show much smaller N\(_2\)O fluxes following N additions than less acid soils from other sites. CO\(_2\) emissions are also smallest from the surface soils of the Etherow. This link cannot be attributed directly to pH, since some other factor like C availability may be responsible.

4.6.3.5 Temperature
In laboratory incubation tests along temperature gradients, increased temperature leads to linear increases in N\(_2\)O flux for the Gwy samples, but for Scoat Tarn and Etherow soils the increase is much greater above 11°C (Figure 4.10), which was also observed elsewhere by Struwe and Kjøller (1991). For the spatial samples, incubation at 15°C results in much higher rates of N\(_2\)O emission than at 5°C in core tops, but in core bottoms the effect is confounded to a degree by apparent substrate exhaustion. For samples showing an increase, the proportional effect is not constant between samples.

Despite the effect of increasing temperature, significant emissions are still found even at 5°C in laboratory incubations. Since soil temperatures in study catchments exceed 5°C for periods ranging from 173 to 268 days of the year at the highest Mharcaidh sites and lowest Gwy sites respectively (see Table 3.20), low temperature is unlikely to prevent denitrification for much of the time outside the winter months. The effects of temperature on denitrification are notoriously difficult to predict, given the potential for other confounding factors. Denitrification rates in a hardwood forest in
the USA were found to remain below 0.1 kgN ha\(^{-1}\) yr\(^{-1}\) even during an experiment in which soils were warmed by 5°C over a year (Peterjohn et al., 1993). Temperature is, however, potentially an important factor in governing overall rates, especially given the large differences in temperatures between sites like the Mharcaidh and Gwy catchments.

### 4.6.3.6 End products of denitrification

The results of the C\(_2\)H\(_2\) incubation experiment are ambiguous, but do not, at least, suggest that reduction of N\(_2\)O to N\(_2\) may be a significant problem in the soils studies here. The factors which will increase the proportion of N\(_2\)O relative to N\(_2\) as the end product of denitrification were listed by Firestone & Davidson (1989):

1. increasing oxidant (NO\(_3^+\)),
2. increasing O\(_2\) availability,
3. decreasing C availability,
4. decreasing pH,
5. increasing sulphide,
6. decreasing temperature, and
7. low N\(_2\)O reductase activity.

It seems likely that under field conditions of low soilwater pH and (for some soils) relatively high soilwater NO\(_3^+\), N\(_2\)O should be the major product of denitrification.

The suppression of N\(_2\)O emissions during incubation with C\(_2\)H\(_2\) for more than half of the studied soils could simply indicate exhaustion of the N or C substrate, but since these soils had been amended with N four days prior to this experiment, it seems unlikely that the substrate should be suddenly exhausted in the period of a few hours between baseline and post- C\(_2\)H\(_2\) addition samples. What is perhaps more likely is that inhibition of nitrification is responsible for the reductions in N\(_2\)O emissions from certain cores. While it cannot be established whether the N\(_2\)O originates during the nitrification or ensuing denitrification process, this is of little significance in terms of the actual N sink; the total net flux of gaseous N is the most important figure. Nitrification losses of N\(_2\)O could, therefore, be important following terrestrial N saturation, since NH\(_4^+\) deposition often makes up more than half of total inorganic N deposition.
The significance of NO as a denitrification (or nitrification) product is not known. According to Firestone & Davidson (1989), most studies have shown that very little NO is detected as a denitrification product. While this may have been due to deficiencies of the GC technique used in older studies, more recent mass balance work has confirmed that NO is largely absent in products. It is therefore assumed here that both NO and N$_2$ emissions are negligible.

4.6.4 Soil respiration and denitrification

Peaks in CO$_2$ emissions are often correlated with peaks in N$_2$O emissions, since respiration is a general measure of microbial activity (e.g. Parsons et al., 1991; van Kessel et al., 1993; Ineson et al., 1998). Live plants may confound the measurement of soil respiration, so study soils have to be kept in the dark during incubation (Parsons et al., 1991).

Field measurements of CO$_2$ emissions show clear differences between sites, with much greater values for the Gwy and Scoat Tarn soils than for the Mharcaidh or Etherow sites (Fig. 4.7). These differences hold even for just peat soils (i.e. for G1 and G4 at the Gwy and S3 at Scoat Tarn), and suggest that soil microbial activity is much greater at the Afon Gwy and Scoat Tarn sites. The confounding effects of live vegetation are, however, not known. At the Etherow, where very high N availability relative to other sites might be expected to enhance microbial activity, static chambers are located on bare peats with little (if any) surface vegetation. At the Gwy and Scoat Tarn sites, vegetation (mainly grasses) tends to be present even on the deep peats. Given the short time period over which the chambers were covered and dark (60-90 minutes) during each sampling, residual vegetation sources of CO$_2$ cannot be ruled out.

Laboratory incubations provide CO$_2$ emission rates without the influence of live vegetation (though not necessarily all roots), since surface vegetation was removed. Differences between sites are less apparent for incubated soils (Figs. 4.25-4.26), but for surface soils, activity is much greater at the Gwy than elsewhere, and lowest at the Mharcaidh and Etherow. For deeper soils, CO$_2$ emissions from the Scoat Tarn and
Etherow soils are lowest at 5°C but not at 15°C, though it is very unlikely that these deeper soils will experience such high temperatures in the field. Soil respiration does therefore appear to be lower at the Etherow and higher at the Gwy than elsewhere, even without the effects of vegetation.

4.6.5 Comparison of modelled and measured denitrification rates

The denitrification rates suggested by the FAB model for the soils studied here are shown in Table 4.7 alongside measured rates from field and laboratory studies. Long-term N immobilisation (\(N_{\text{imm}}\)) and fixed denitrification (\(N_{\text{den fixed}}\)) are empirically derived values recommended in Hall et al. (1997) and used for national FAB model applications by Curtis et al. (1999, 2000). FAB denitrification rates (\(N_{\text{den FAB}}\)) are calculated using the standard FAB model formulation (UBA, 1996; Posch et al., 1997), assuming 80% denitrification of net inputs (deposition minus immobilisation) for peat soils and 10% elsewhere (f\(_{\text{de}}\): proportion of net inorganic N denitrified). Estimated total inorganic N deposition is based on mean values for 1995-97 (\(N_{\text{dep}}\): see Table 3.9). All units are kgN ha\(^{-1}\) yr\(^{-1}\) except f\(_{\text{de}}\), which is dimensionless.

It is immediately apparent that denitrification rates estimated using the denitrification fraction method (\(N_{\text{dep FAB}}\), calculated as f\(_{\text{de}}\) \(\times [N_{\text{dep}} - N_{\text{imm}}]\)) are up to an order of magnitude greater than measured rates, while empirical values from the literature (\(N_{\text{den fixed}}\)) are at least the same order of magnitude. The largest values using the denitrification fraction method, at almost 25 kgN ha\(^{-1}\) yr\(^{-1}\) for S3, E1 and E2, are two orders of magnitude greater than field measurements and one order of magnitude greater than estimates from soil cores; for the hilltop peat at the Gwy the difference is three and two orders of magnitude, respectively. These very high rates suggested by the standard FAB model formulation are much greater than any observed rates, with the exception of a core top from the Scoat Tarn podsol (S1T) incubated at 15°C, which was used in the phase 1 temperature gradient experiments (Fig. 4.10) under very artificial conditions.
It can be concluded that the empirical rates suggested in Hall et al. (1997) of 1-4 kgN ha\(^{-1}\) yr\(^{-1}\) are much more representative of current conditions than those calculated using the denitrification fraction method for peat soil. However, for other soil types, denitrification estimated as 10% of net inorganic N inputs (i.e. for non-peat soils) are generally very close to rates measured in laboratory studies. These results suggest that 10% is a realistic figure for all soils.

Table 4.7: Potential denitrification rates for soils in the four study catchments according to the FAB model (see text for details)

<table>
<thead>
<tr>
<th>Soil code</th>
<th>Soil type</th>
<th>N(_{\text{imm}})</th>
<th>f(_{\text{de}})</th>
<th>N(_{\text{dep fixed}})</th>
<th>N(_{\text{den fixed}})</th>
<th>N(_{\text{den FAB}})</th>
<th>N(_{\text{den field}})</th>
<th>N(_{\text{den core (5°C)}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Peaty ranker</td>
<td>1.3</td>
<td>0.1</td>
<td>7.3</td>
<td>1</td>
<td>0.43-0.63</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M2</td>
<td>Valley peat</td>
<td>3</td>
<td>0.8</td>
<td>7.3</td>
<td>1</td>
<td>3.44</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M3</td>
<td>Peaty podsol</td>
<td>3</td>
<td>0.1</td>
<td>7.3</td>
<td>1</td>
<td>0.43</td>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td>M4</td>
<td>Shallow peat</td>
<td>3</td>
<td>0.8</td>
<td>7.3</td>
<td>1</td>
<td>3.44</td>
<td>0.02</td>
<td>0</td>
</tr>
<tr>
<td>G1</td>
<td>Hilltop peat</td>
<td>3</td>
<td>0.8</td>
<td>27.0</td>
<td>1</td>
<td>19.20</td>
<td>0.02</td>
<td>0.3</td>
</tr>
<tr>
<td>G2</td>
<td>Peaty gley</td>
<td>3</td>
<td>0.1</td>
<td>27.0</td>
<td>4</td>
<td>2.40</td>
<td>0.05</td>
<td>3.0</td>
</tr>
<tr>
<td>G3</td>
<td>Podsol</td>
<td>3</td>
<td>0.1</td>
<td>27.0</td>
<td>1</td>
<td>2.40</td>
<td>0.01</td>
<td>2.4</td>
</tr>
<tr>
<td>G4</td>
<td>Valley peat</td>
<td>3</td>
<td>0.8</td>
<td>27.0</td>
<td>1</td>
<td>19.20</td>
<td>0.12</td>
<td>0</td>
</tr>
<tr>
<td>S1</td>
<td>Podsol</td>
<td>3</td>
<td>0.1</td>
<td>33.6</td>
<td>1</td>
<td>3.06</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>S2</td>
<td>Peaty gley</td>
<td>3</td>
<td>0.1</td>
<td>33.6</td>
<td>4</td>
<td>3.06</td>
<td>0.01</td>
<td>1.3</td>
</tr>
<tr>
<td>S3</td>
<td>Deep peat</td>
<td>3</td>
<td>0.8</td>
<td>33.6</td>
<td>1</td>
<td>24.48</td>
<td>0.05</td>
<td>2.5</td>
</tr>
<tr>
<td>E1</td>
<td>Deep peat</td>
<td>3</td>
<td>0.8</td>
<td>33.7</td>
<td>1</td>
<td>24.56</td>
<td>0.24</td>
<td>0.9</td>
</tr>
<tr>
<td>E2</td>
<td>Burnt peat</td>
<td>3</td>
<td>0.8</td>
<td>33.7</td>
<td>1</td>
<td>24.56</td>
<td>0.02</td>
<td>2.0</td>
</tr>
</tbody>
</table>

4.7 Conclusions

4.7.1 The importance of denitrification as a sink for N deposition

The field measurements indicate that N\(_2\)O fluxes in the four study catchments are very low, in the range 0 – 0.5 kgN ha\(^{-1}\) yr\(^{-1}\) throughout the budget year. It is recognised that several factors, both logistical and methodological, may contribute to underestimates of actual denitrification rates in the field, including spatial variability, temporal variability and the proportion of denitrification products that is emitted as N\(_2\)O.
Spatial coverage of samples is poor and riparian zones may be under-represented, so that field rates may be underestimates of actual rates in certain locations within catchments. Trace gases were only sampled monthly, so that shorter periods of high activity in response to optimal conditions may have been missed. It is assumed that the major product of denitrification in these acid upland soils is N$_2$O so the acetylene block technique was not used in the field, but under certain conditions N$_2$ emissions may be significant, leading to underestimates of total gaseous N losses.

The degree to which field rates may be underestimated is revealed by the laboratory incubation experiments. Soil core incubations show that denitrifying organisms are ubiquitous, so that under optimal conditions with adequate substrate availability, highly elevated rates of denitrification can be induced in all samples. Even without N additions, all soils show measurable denitrification in the laboratory (in the case of Mharcaidh samples and valley peats from the Gwy, only after warming to 15°C), but only at low rates of 0.1 – 3.0 kgN ha$^{-1}$ yr$^{-1}$. These are comparable to the maximum rates recorded from spot samples (rather than annual means) in the field.

Maximum rates recorded during incubations with added NH$_4$NO$_3$ are up to an order of magnitude greater than unamended rates, with the highest values of up to 12.7 kgN ha$^{-1}$ yr$^{-1}$ being recorded for the Scoat Tarn soils, although a proportion of the N$_2$O produced may be derived from nitrification. This means that where low field rates of denitrification are recorded, lack of substrate or unsuitable physical conditions (soil moisture or temperature) are limiting, and not the absence of organisms capable of denitrification.

On a spatial basis, a failure to identify hotspots of activity indicates that such areas are infrequently encountered, so that their contribution in terms of surface area to N$_2$O fluxes at the catchment scale is likely to be minor. For this reason, Parsons et al. (1991) stated that extrapolation of rates expressed per square metre to a per hectare basis may be inappropriate.

A further consideration is that elevated rates of denitrification tend to be very transient following deposition and wetting events, which would explain why the N additions experiments in the field failed to detect an increase in denitrification rates
after a 2-week time lag. The purpose of the field N additions experiment was, however, to raise the general availability of N rather than induce events, and it might reasonably be expected that conditions conducive to denitrification would be encountered at some point during 12 sets of monthly samples, some of which were carried out while raining. However, none of these samples (from 21 amended and 39 unamended chambers) show a flux of N\(_2\)O which exceeds the general laboratory range of 1-3 kgN ha\(^{-1}\) yr\(^{-1}\). It should be remembered that in the laboratory studies, plants were not competing for the available N as they are in the field. While elevated rates above 1-3 kgN ha\(^{-1}\) yr\(^{-1}\) could occur in some areas for short periods, much lower rates are widespread for most of the year, so this range would appear to be appropriate as an upper limit at the catchment averaged scale.

The elevated denitrification rates observed in the laboratory following N additions suggest that under a future scenario of increased NH\(_4^+\) and NO\(_3^-\) availability following N saturation of the terrestrial ecosystem (see Chapter 1), N losses via nitrification and denitrification may be much larger than they are now. The assumption here is that with competition for available inorganic N removed and N no longer limiting, there are no other factors limiting denitrification. However, the short-lived response to N additions in many soil cores suggests that some other factor, probably labile organic C, may soon become limiting. The level of denitrification which can be sustained by C rather than N limited denitrification in the field is not known.

Studies in heavily fertilised systems have shown varied responses to N additions. Hutchinson and Mosier (1979) found that projected annual emissions from a fertilised, irrigated cornfield in California were no more than 4 kgN ha\(^{-1}\) yr\(^{-1}\). Mosier \textit{et al.} (1991) found that N\(_2\)O emissions were increased by a factor of 2-3 by N fertilisation relative to unfertilised soils (0.7 – 1.1 kgN ha\(^{-1}\) yr\(^{-1}\) in the latter) on shortgrass steppe, but fertiliser applications were very large over a period of years. Neff \textit{et al.} (1994) added fertiliser at 250 kgN ha\(^{-1}\) yr\(^{-1}\) to wet and dry alpine meadows at 3500m elevation in Colorado, and while denitrification rates were increased by an order of magnitude, they still only reached 0.4 and 0.9 kgN ha\(^{-1}\) yr\(^{-1}\) for the dry and wet meadow plots, accounting for just 0.08\% and 0.18\%, respectively, of the previous 2 years fertiliser N inputs. Smith \textit{et al.} (1995) found that denitrification emissions over the growing season from grazed fields under fertiliser additions of 360 kgN ha\(^{-1}\)
reached only 5 kgN ha\(^{-1}\). Williams et al. (1999) found mean annual denitrification fluxes from lowland, cattle-grazed pastures to be 3.2 kgN ha\(^{-1}\) yr\(^{-1}\), just 1.3% of fertiliser applications.

Gundersen (1991) found that the few published studies of denitrification in forests suggest rates of <1 kgN ha\(^{-1}\) yr\(^{-1}\) in undisturbed systems and 3-6 kgN ha\(^{-1}\) yr\(^{-1}\) after clear-cutting, and that high rates may not occur even where soil NO\(_3\)\(^-\) is very high and the soil is wet and warm. It is not known what factors limit the appearance of high denitrification in these systems. In their study on denitrification from Dutch forest soils, Tietema et al. (1991) measured a N\(_2\)O emission rate of 20 kgN ha\(^{-1}\) yr\(^{-1}\), but stated that this was very high compared with results from a literature review (in Dutch) of work on similar soils by Denier van der Gon (1989), which found a range of only 0.1 – 2.1 kgN ha\(^{-1}\) yr\(^{-1}\). The high rate was attributed to a combined effect of hydrology and very high NO\(_3\)\(^-\) availability at this site.

Unfertilised forests in the USA were found to show rates below 0.4 kgN ha\(^{-1}\) yr\(^{-1}\) (Davidson & Swank, 1986). A maximum rate in fertilised forests of 15 kgN ha\(^{-1}\) yr\(^{-1}\) was short lived, and mostly 1-2 orders of magnitude lower. Similarly, Ineson et al. (1998), working in coniferous forests, found that denitrification peaked at 10.5 kgN ha\(^{-1}\) yr\(^{-1}\) in July following a period of rainfall in an area near a pig farm with deposition of c. 100 kgN ha\(^{-1}\) yr\(^{-1}\), but the average was much lower.

Hence while there are many studies that show very high denitrification rates in response to large inputs of inorganic N, in some cases the responses are short-lived and in many the annual fluxes are still comparable to those found here (1-3 kgN ha\(^{-1}\) yr\(^{-1}\)). These very varied responses support the idea that many factors other than N limit denitrification in the field.

Of the four catchments studied, Scoat Tarn is the only site with significant concentrations of soilwater NO\(_3\)\(^-\) throughout the winter and spring periods, but the soils still fail to produce high rates of denitrification in the field. However, N additions to the surface organic layer in the laboratory produce the largest fluxes recorded for any site. It is possible that denitrification at this site is therefore “event-based” and occurs only under optimum conditions of rainfall, C and NO\(_3\)\(^-\) supply,
when large fluxes may occur. These events could have been missed by monthly sampling. The site does, however, provide a clear illustration that high soilwater NO\textsubscript{3} does not necessarily lead to consistently high rates of denitrification.

4.7.2 Assessment of the representation of denitrification in mass balance models

Despite the variety of methods available, annual denitrification budgets remain difficult to establish in most environments because of the high spatial and temporal variability inherent in the process (Tiedje et al., 1989; Parsons et al., 1991). Groffman et al. (1988) suggested that a much greater proportion of the variation in denitrification may be explained at the distal (landscape) scale from soil texture and drainage than at the field scale using more proximal factors such as soil water, NO\textsubscript{3} or CO\textsubscript{2} production. Tiedje et al. (1989) concluded that improvements in quantification of denitrification are more likely to come from better approaches to analyse, model and predict the variability than from further work on methodology, but it is doubtful that denitrification budgets will ever approach the accuracy of most other biogeochemical cycle measurements.

The representation of denitrification in the FAB model can be traced back to just two publications in the 'grey' literature for Dutch soils (Steenvoorden, 1984; Breeuwsma et al., 1991), cited in de Vries et al. (1993). In these studies, denitrification from peat soils reached 80% of net inorganic N inputs, while other freely draining soils denitrified only 10% of inputs. These findings form the basis of the interpolation used within the FAB model to predict denitrification sinks for N in systems where N is no longer limiting for plant growth and is therefore present in excess in soils (Chapter 2).

In Section 4.6.5 it was found that the conventional method for calculating potential denitrification using a "denitrification fraction" seems to grossly overestimate current denitrification rates from peat soils, but is much more realistic for non-peat soils. It should be remembered though that for critical loads models it is the long-term steady-state rate of denitrification which is required, and this implies that N saturation may have occurred, potentially leading to much greater availability of inorganic N than under current conditions. Even with this fact in mind, it still appears that 80%
denitrification in peat soils is a gross overestimate, implying denitrification rates of up to c. 25 kgN ha\(^{-1}\) yr\(^{-1}\), which is more than double the maximum rate observed under N additions in the laboratory. It seems probable that other limiting factors would prevent such high denitrification rates from peat soils in the catchments studied here.

According to Sozanska et al. (2002), the IPCC recommends that N\(_2\)O emissions should be estimated as a fraction of inputs, and suggests emission factors ranging from just 1% for atmospheric N deposition, up to 2% for untreated animal waste (IPCC, 2000). These emission factors are an order of magnitude smaller than those suggested for critical loads mass balances for non-peat soils, and almost two orders of magnitude smaller than the figure of 80% for peat soils, but would provide realistic rates according to field measurements here.

More sophisticated models have been used in attempts to quantify N\(_2\)O emissions at the regional or national scale. In an exercise to model N\(_2\)O emissions at the UK national scale in response to the Kyoto Protocol requirement to provide national inventories for 'greenhouse' gases, Sozanska et al. (2002) used multiple regression to produce a model which predicts emissions from N input, soil moisture, soil temperature and land use. However, they noted that the of the 59 studies which provided the regression data used in the model design, only one was based on a semi-natural, unfertilised system representative of UK heathland or moorland. Skiba et al. (1998) derived a multiple regression equation to predict N\(_2\)O flux from N input, soil moisture and soil temperature. A more complex mechanistic model of N\(_2\)O emissions from agriculture, the DeNitrification-DeComposition (DNDC) model, was applied in the UK by Brown et al. (2002), and suggested maximum, county-average N\(_2\)O fluxes of <7.5 kgN ha\(^{-1}\) for 1990.

Estimates of C availability linked to a topographic index have been used to model denitrification rates under "nitrate-non-limited" (NNL) conditions (Whelan & Gandolfi, 2002). High rates of 33-49 kgN ha\(^{-1}\) yr\(^{-1}\) were predicted for the Slapton Wood catchment, under the assumption that 0.1% of soil organic C is available to heterotrophic micro-organisms. These rates are the same order of magnitude as the 80% figure for peat soils in sites experiencing very high N deposition loads, but are based on lowland soils under grassland, forest and arable land.
In conclusion, it seems very unlikely that the magnitude of the denitrification sink for net inorganic N inputs suggested by studies of Dutch peat soils (80%) is transferable to upland peat soils in the UK. While the adoption of a first order sink term which varies as a proportion of net inputs has been recommended elsewhere (IPCC, 2000) and could be appropriate here, the denitrification fraction should to be calibrated to regional conditions of soils, climate and topography. A figure of 10% seems much more defensible as a upper limit for upland soils in the UK, but without more data there is no basis for suggesting that this is an improvement on the use of fixed, empirical rates derived from the literature.

4.8 References


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SECTION III

LABORATORY AND FIELD MEASUREMENTS OF N PROCESSES
CHAPTER 5

AVAILABLE N, MINERALISATION AND NITRIFICATION POTENTIALS FROM LABORATORY INCUBATION EXPERIMENTS
5. AVAILABLE N, MINERALISATION AND NITRIFICATION POTENTIALS FROM LABORATORY INCUBATION EXPERIMENTS

5.1 Introduction and purpose of the Chapter

5.1.1 Mineralisation

Mineralisation is the degradation of organic N (mostly proteins, amino sugars and nucleic acids) to an inorganic, or mineral, form, \( \text{NH}_4^+ \) (Alexander, 1977; Paul & Clark, 1996), hence it is also commonly referred to as ammonification. It is predominantly mediated by soil microbes which simultaneously assimilate some of the \( \text{NH}_4^+ \) released from the organic N forms during decomposition, in a process known as microbial immobilisation, which effectively converts the \( \text{NH}_4^+ \) back into organic microbial constituents (Alexander, 1977; INDITE, 1994). Since mineralisation and immobilisation proceed concurrently, only a proportion of the total \( \text{NH}_4^+ \) produced is liberated. The total release of \( \text{NH}_4^+ \) is referred to as gross mineralisation, while that transferred to the available pool after simultaneous microbial immobilisation is net mineralisation (INDITE, 1994). Net mineralisation is therefore balanced by factors which affect immobilisation and gross mineralisation.

Gross mineralisation rates are largely dictated by the stability of the organic N compounds being degraded, i.e. the relative proportions of labile and recalcitrant soil organic matter (SOM). For example, much of the N in fresh litter occurs in the form of proteins or their decomposition products, which are rapidly utilised by soil microorganisms, while the large organic molecules in soil humus are very resistant to degradation, with half lives varying from decades to millennia (Alexander, 1977; Tamm, 1991). Hence an initial flush of decomposition, during which two thirds of plant residues may be mineralised in one year, is followed by a much slower breakdown of stable, humic substances (Cresser et al., 1993). Under anaerobic conditions, mineralisation is generally retarded, so that accumulation of undecomposed or partially decomposed organic matter can occur. Mineralisation and general microbial activity are therefore greater in surface soil horizons where fresh organic matter and nutrients are more widely available, declining greatly with soil depth (INDITE, 1994).
Other controlling factors include the size of microbial populations, microbial activity and both physical (moisture, temperature) and chemical (pH) conditions in the soil (INDITE, 1994). Mineralisation rates are generally suppressed by factors which decrease microbial activity, such as low pH and O₂ levels, while they are increased by factors which stimulate microbial activity, for example increases in temperature. Overall, though, mineralisation-immobilisation processes are closely linked to C metabolism, and in unamended soils, organic C and N are mineralised at parallel rates (Alexander, 1977).

The degree to which mineralised NH₄⁺ is immobilised by soil microbes depends on their requirement of N for growth (Paul & Clark, 1996). The factors which determine rates of immobilisation and net mineralisation include the C:N ratios of soil organisms and SOM, as well as the stability of the organic compounds which make up SOM. These factors are discussed further in Chapter 8.

The NH₄⁺ produced by mineralisation is generally very short-lived in the soil environment. There are several possible fates which were listed by Paul and Clark (1996):

1. it is a preferred source of inorganic N for plants and therefore readily taken up;
2. it is generally the preferred source of inorganic N for microbes, which would otherwise have to reduce NO₃⁻, and is rapidly immobilised;
3. it may be held on the soil cation exchange complex;
4. it may be fixed in clays because of its similarity in size to the K⁺ ion;
5. it can be immobilised by reaction with SOM to form stable complexes;
6. in areas of large fertiliser or manure inputs or of decaying vegetation, NH₃ volatilisation may be important; and
7. it can be utilised by certain soil microbes as an energy source in the nitrification process.

Note that leaching of NH₄⁺ into surface waters is not included in this list; it is generally assumed that NH₄⁺ leaching is negligible because of the many processes which reduce its mobility through the soil-vegetation system. While this assumption is usually borne out by observations, particularly in N limited, upland catchments, there
are certain conditions which can lead to leaching losses, and indeed these are observed in the River Etherow subcatchments (Chapter 3). This is a somewhat exceptional site compared with the other study catchments, although it may be typical on a regional basis. However, the major importance of mineralisation within the current study is as a control on the supply of NH$_4^+$ for nitrification and subsequent denitrification or leaching of NO$_3^-$.  

5.1.2 Nitrification

The nitrification process was described in Chapter 4 in the context of the trace gases which it may liberate (Fig. 4.2), but also in terms of its potential importance in regulating the supply of NO$_3^-$ for denitrification. In the same way, in systems where a large proportion of external inputs of inorganic N (e.g. as deposition) are rapidly assimilated or immobilised by plants and microbes, nitrification may be the key process regulating leaching losses of NO$_3^-$ to surface waters. Since nitrification requires an NH$_4^+$ substrate, it is very closely linked to the mineralisation process and hence to the controls on mineralisation (Groffman et al., 1988).

The organisms responsible for nitrification are mainly autotrophs which obtain energy from the oxidation of NH$_4^+$ rather than from the oxidation of organic matter, although some heterotrophs may convert organic matter to NO$_3^-$, especially in acid and forest soils (INDITE, 1994; Paul & Clark, 1996). These heterotrophs are primarily fungi and methylotroph bacteria, and are mostly facultative nitrifiers which produce NO$_3^-$ at a much slower rate than autotrophic nitrifiers (Tamm, 1991). Nitrification may be more sensitive to certain controls than either mineralisation or denitrification (Alexander, 1977). These are discussed below.

5.1.2.1 Aeration and soil moisture

All nitrifiers, whether autotrophs or heterotrophs, are obligate aerobes. Factors which control O$_2$ diffusion in soils, such as soil water status and structure, control nitrification in much the same way as for denitrification, albeit working in the opposite direction, since denitrification requires anaerobiosis. Nitrification is easily suppressed by high soil moisture content, but if soils are too dry, it is also suppressed
in parallel with overall microbiological activity. The optimal moisture content varies with soil type.

5.1.2.2 Acidity

Nitrification is one of the most pH sensitive soil reactions. In agricultural soils, pH optima for nitrification vary from 6.6 – 8.0; rates decrease below pH 6.0 and become negligible below pH 4.5 (Paul & Clark, 1996). In acidic forest soils, heterotrophic nitrification, or autotrophic nitrification in less acid microsites, may account for the lower acid sensitivity of the process. Furthermore, in organic soils, the lack of aluminium and its associated toxicity may permit nitrification at lower pH (Paul & Clark, 1996). However, it is often claimed that in acid soils, nitrification is usually negligible (Alexander, 1977; Tamm, 1991; INDITE, 1994).

Work on Dutch forest and moorland soils has revealed that nitrification can be significant even in very acid conditions, through the activity of acid tolerant autotrophs as well as heterotrophic nitrifiers (van Breemen et al., 1987; de Boer et al., 1989, 1990; Tietema et al., 1992). The conclusion that nitrification in the forest floor layer is mainly autotrophic contradicts the findings of other authors working in Scottish forest soils where nitrification is mainly heterotrophic, and this was attributed to the limiting supply of $\text{NH}_4^+$ in the latter (Tietema et al., 1992).

5.1.2.3 Temperature

As with other microbial processes, nitrification rates are sensitive to temperature. Rates tend to be slow below 5°C, but the process can proceed even in snow covered soils (Paul & Clark, 1996). Similarly, the process slows down above 40°C. The optimum range is around 30-35°C (Alexander, 1977).

Interactions between soil moisture, temperature and competition with plants for available $\text{NH}_4^+$ lead to a seasonal pattern in nitrification, with rates generally highest in spring and autumn and lowest in summer and winter (Paul & Clark, 1996). Periods of greatest nitrification rates do not necessarily coincide with periods of greatest $\text{NO}_3^-$ availability in soils, however, because of the interactive effects of other biota (Alexander, 1977).
5.1.3 Aims of the Chapter

The potential importance of mineralisation and nitrification in soils as controls on N leaching has already been discussed, and these are processes which, while very difficult to measure in the field, lend themselves to analysis through laboratory incubation experiments.

A soil’s N mineralisation potential is a measure of its capacity to transform organic soil N into NH$_4^+$, and is often used as an index of available N for plants (Robertson et al., 1999). It is the most widely used measure of N fertility, linked to both the size of the SOM pool and the activity of the microbial population. Note that in order to quantify net mineralisation it is necessary to quantify the production of both NH$_4^+$ and NO$_3^-$, since consideration of increases in NH$_4^+$ alone would fail to account for the proportion that is rapidly converted to the other inorganic form, NO$_3^-$. Hence NO$_3^-$ is considered a product of mineralisation, even though the preliminary step of ammonification is required. Mineralisation potential is a better indicator of soil fertility than inorganic N concentrations alone, because the supply rate is more important than a measure of instantaneous concentration. Similarly, nitrification potential is more useful, as an indicator of NO$_3^-$ supply rate, than a measurement of soilwater NO$_3^-$ alone.

This chapter therefore aims to characterise the soils from the four study catchments in terms of potential supply rates of total inorganic N (NH$_4^+$ + NO$_3^-$) and NO$_3^-$, as key determinants of potential NO$_3^-$ supply for denitrification and/or leaching into surface waters. Mineralisation and nitrification potentials are assessed by the aerobic incubation method (Robertson et al., 1999).

5.2 Sampling methods

5.2.1 Soil sampling

Soil core sampling techniques and splitting of cores for baseline and incubation samples are described in detail in Chapter 4 (Section 4.3.2). All samples were stored
together prior to the initial baseline analysis in the cold room at 3-4°C for 4 weeks while preliminary sample preparation (core splitting and weighing) was completed.

5.2.2 Sample preparation

Each sample comprised a pair of quartered cores from diagonally opposite locations within a study plot (see Fig. 4.3). Samples were also split into top and bottom levels. As close as possible to the start of the incubation period the minimally disturbed baseline samples were prepared for analysis. Roots and stones (>c.2mm) were removed from the soil as far as possible by hand and the soil broken up and homogenised. Corresponding samples for incubation were placed intact into sealed, gas-permeable polythene bags and incubated at 15°C for 33 days. The bags prevented moisture loss from the samples while permitting gaseous exchange, to minimise the build-up of CO₂ and other gases which might affect the analysis (Gordon et al., 1987). After the incubation period the samples were prepared for analysis in the same way as the baseline samples. Since sample preparation was a laborious process taking almost two weeks for the large number of samples analysed, it was ensured that post-incubation samples were treated in the same order as baseline samples so that the intervals between preparation and analysis were the same. During both pre- and post-incubation sample preparation periods, samples were stored at 4°C in the coldroom.

Available NH₄-N and NO₃-N were measured by KCl extraction (Page et al., 1982; Allen, 1989) from a 10g subsample of well mixed soil. A further subsample taken at the same time was used for analysis of dry-weight and loss on ignition (LOI). Soil moisture was determined as the difference between the weight of field-moist soil at ambient temperature and after 16 hours oven drying (overnight) in a porcelain crucible at 105°C. Organic matter content was determined as the percentage loss on ignition of the dried soil after heating in a muffle furnace at 375°C for 16 hours (overnight) (Ball, 1964).
5.2.3 Available nitrogen by potassium chloride extraction

Molar KCl solution was prepared using GPR grade KCl (since Analar grade can contain high concentrations of inorganic N) and de-ionised water. 100mls of molar KCl were added to 10g of field moist soil in a 250ml conical flask and the flask was covered with clingfilm. The samples were shaken for one hour on a flatbed shaker. Meanwhile, Whatman No. 1 filters were placed in funnels standing in the sample bottles and pre-washed with 50ml molar KCl solution, thereby pre-rinsing all vessels prior to filtration of the sample. The KCl rinse solution was discarded, and the shaken soil plus KCl samples were filtered into the sample bottles. Filtration time was determined by the slowest sample, but was generally c. 3 hours. If samples had to be left to filter overnight the air cooling system in the laboratory was left on to minimise warming of the samples.

Random duplicate soil samples (1 per 20) and three blanks were analysed with each batch of 40-70 samples to account for possible contamination of glassware etc. However, all glassware, bottles, funnels etc. and laboratory space used were dedicated for KCl extractions of available N to avoid cross-contamination from other methods. NH$_4^+$-N and NO$_3^-$-N were analysed by Skalar SA-40 autoanalyser using the modified Berthelot (indophenol) reaction (Krom, 1980; Searle, 1984) and sulphanilamide/NEDA/Cd/Cu reduction method (Navone, 1964; APHA, 1980; Walinga et al., 1989), respectively. Ammonia is chlorinated to monochloramine which reacts with salicylate to form 5-aminosalicylate. After oxidation and oxidative coupling a green coloured complex is formed; the resulting indo-phenol blue colour is measured at 660nm. NO$_3^-$ is reduced to nitrite using a redox couple (cadmium/copper column), the nitrite forming an azo dye complex with sulphanilamide and napthylethylene-diamine dihydrochloride; the resulting pink colour is measured at 540nm.

The rate of net mineralisation is given by the difference in total inorganic N concentrations (NH$_4^+$ + NO$_3^-$) in baseline and post-incubation samples, divided by the incubation period. For net nitrification, the difference between pre- and post-incubation NO$_3^-$ concentrations is divided by the incubation period.
5.3 Results

5.3.1 Soil moisture and LOIs

Soil % moisture and LOI (% organic content) for the pre-incubation, baseline samples are shown in Table 5.1. The large differences in soil organic matter content are more obvious in Figures 5.1a-b. For core tops, differences between soil types and between sites are small (Fig. 5.1a). The Gwy podsol at G3 stands out for having the lowest organic content in the surface horizons, followed by the peaty gley from the Mharcaidh (M3) and the Scoat Tarn soils. All except G3 are highly organic, with LOI values in the range 70-90%.

Table 5.1: Soil % moisture and % LOI

<table>
<thead>
<tr>
<th>Soil code</th>
<th>Soil type</th>
<th>Section</th>
<th>Moisture %</th>
<th>Organic %</th>
</tr>
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<td>91.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>114.2</td>
<td>5.7</td>
</tr>
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<td>Peaty ranker</td>
<td>Bottom</td>
<td>472.4</td>
<td>84.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>75.9</td>
<td>5.4</td>
</tr>
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<td>Top</td>
<td>725.9</td>
<td>95.2</td>
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<td></td>
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<td>109.4</td>
<td>2.6</td>
</tr>
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<td>Bottom</td>
<td>765.2</td>
<td>90.5</td>
</tr>
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<td></td>
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<td>3.6</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td>177.0</td>
<td>5.6</td>
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<td>28.1</td>
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<td>94.5</td>
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<td>Peaty gley</td>
<td>Bottom</td>
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<td>10.0</td>
</tr>
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<td>Podsol</td>
<td>Bottom</td>
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<td></td>
<td></td>
<td>11.2</td>
<td>6.5</td>
</tr>
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<td>10.1</td>
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<td></td>
<td></td>
<td>7.4</td>
<td>2.0</td>
</tr>
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<td>594.3</td>
<td>91.9</td>
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<td>71.3</td>
</tr>
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<td></td>
<td></td>
<td>48.6</td>
<td>11.1</td>
</tr>
<tr>
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<td>Podsol</td>
<td>Bottom</td>
<td>116.7</td>
<td>22.9</td>
</tr>
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<td></td>
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<td>17.3</td>
<td>5.3</td>
</tr>
<tr>
<td>S2</td>
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<td>Top</td>
<td>374.6</td>
<td>81.2</td>
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<td>36.6</td>
<td>7.7</td>
</tr>
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<td>262.1</td>
<td>52.0</td>
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<td></td>
<td></td>
<td>49.6</td>
<td>6.6</td>
</tr>
<tr>
<td>S3</td>
<td>Deep peat</td>
<td>Top</td>
<td>465.6</td>
<td>76.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100.1</td>
<td>23.1</td>
</tr>
<tr>
<td>S3</td>
<td>Deep peat</td>
<td>Bottom</td>
<td>280.8</td>
<td>68.2</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>67.9</td>
<td>11.8</td>
</tr>
<tr>
<td>E1</td>
<td>Peat (burnt)</td>
<td>Top</td>
<td>255.1</td>
<td>85.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>33.5</td>
<td>2.3</td>
</tr>
<tr>
<td>E1</td>
<td>Peat (burnt)</td>
<td>Bottom</td>
<td>396.6</td>
<td>77.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>82.5</td>
<td>20.2</td>
</tr>
<tr>
<td>E2</td>
<td>Peat (unburnt)</td>
<td>Top</td>
<td>334.3</td>
<td>92.0</td>
</tr>
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<td></td>
<td></td>
<td></td>
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<td>2.9</td>
</tr>
<tr>
<td>E2</td>
<td>Peat (unburnt)</td>
<td>Bottom</td>
<td>538.4</td>
<td>90.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40.4</td>
<td>2.9</td>
</tr>
</tbody>
</table>
Differences between soil types are much more apparent in the deeper soils, as expected (Fig. 5.1b). The distinct mineral horizons separated out from the peaty gley (G2) and podsol (G3) at the Gwy contain only 10% organic matter. Other soils with high mineral content in the lower sections of soil cores include M3, the remainder of G3 and the podsol from Scoat Tarn (S1). Elsewhere the organic content is still very high, ranging from just over 50% at the Scoat Tarn peaty gley (S2) up to more than 90% in the deeper peat soils.

The same analyses were performed on the post-incubation samples as a check that soil moisture content had not changed and to extend the number of samples for the calculation of bulk density. Comparison of soil moisture in pre- and post-incubation samples shows that it was effectively retained by the polythene bags used (Fig. 5.2), with most samples falling very close to the 1:1 line. The best-fit line through the data suggests that mean soil moisture declined by around 5% during incubation.

Figure 5.1a: LOI (mean ±1SD) for soil core tops (0-5cm)
Figure 5.1b: LOI (mean ±1SD) for soil core bottoms (5-20cm)

Figure 5.2: Soil moisture (% of dry weight) in pre- and post- incubation samples

\[ y = 0.9444x \]

\[ R^2 = 0.9227 \]
Table 5.2: Soil bulk density derived from LOI (g cm\(^{-3}\))

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Top SD</th>
<th>Bottom SD</th>
<th>Mineral SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 Peaty ranker</td>
<td>0.13</td>
<td>0.17</td>
<td>0.05</td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>0.13</td>
<td>0.15</td>
<td>0.07</td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>0.23</td>
<td>0.71</td>
<td>0.20</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>0.13</td>
<td>0.23</td>
<td>0.21</td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>0.12</td>
<td>0.13</td>
<td>0.02</td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>0.14</td>
<td>0.28</td>
<td>0.11</td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>0.44</td>
<td>0.55</td>
<td>0.07</td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>0.13</td>
<td>0.17</td>
<td>0.05</td>
</tr>
<tr>
<td>S1 Podsol</td>
<td>0.23</td>
<td>0.68</td>
<td>0.07</td>
</tr>
<tr>
<td>S2 Peaty gley</td>
<td>0.17</td>
<td>0.41</td>
<td>0.04</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>0.19</td>
<td>0.27</td>
<td>0.10</td>
</tr>
<tr>
<td>E1 Peat (burnt)</td>
<td>0.15</td>
<td>0.20</td>
<td>0.11</td>
</tr>
<tr>
<td>E2 Peat (unburnt)</td>
<td>0.13</td>
<td>0.14</td>
<td>0.01</td>
</tr>
</tbody>
</table>

The LOI data from both pre- and post-incubation samples were combined and used to estimate bulk density for the sampled horizons, using the equations of Harrison and Bocock (1981).

Surface soils: \[ \text{bulk density} = 1.558 - 0.728(\log_{10}\text{LOI}) \]

Subsurface soils: \[ \text{bulk density} = 1.729 - 0.769(\log_{10}\text{LOI}) \]

The equation for surface soils is used where LOI is greater than 70%, otherwise the subsurface soils equation is used. The mean bulk density data are presented in Table 5.2. Bulk density data allow the scaling up of potential mineralisation and nitrification rates to the catchment scale.

5.3.2 Available N

Mean values for KCl extractable NH\(_4\)-N and NO\(_3\)-N are shown in Tables 5.3a and 5.3b.
Table 5.3a: Soil inorganic N (µgN per gram dry weight soil)

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Section</th>
<th>NH₄-N Mean</th>
<th>NH₄-N SD</th>
<th>NO₃-N Mean</th>
<th>NO₃-N SD</th>
<th>TIN Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 Peaty ranker</td>
<td>Top</td>
<td>0.93</td>
<td>1.09</td>
<td>2.32</td>
<td>1.61</td>
<td>3.25</td>
</tr>
<tr>
<td>M1 Peaty ranker</td>
<td>Bottom</td>
<td>7.01</td>
<td>10.31</td>
<td>0.64</td>
<td>0.63</td>
<td>7.65</td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>Top</td>
<td>18.89</td>
<td>20.13</td>
<td>2.21</td>
<td>0.56</td>
<td>21.09</td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>Bottom</td>
<td>27.97</td>
<td>19.00</td>
<td>4.97</td>
<td>5.64</td>
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</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>Top</td>
<td>1.18</td>
<td>1.14</td>
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<td>0.59</td>
<td>2.13</td>
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<td>1.34</td>
<td>0.56</td>
<td>0.22</td>
<td>2.15</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
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<td>3.02</td>
<td>5.72</td>
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<td>9.28</td>
</tr>
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<td>3.81</td>
<td>2.84</td>
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<td>8.11</td>
<td>15.50</td>
<td>8.00</td>
<td>32.40</td>
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<td>2.09</td>
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<td>8.05</td>
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<td>11.99</td>
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<td>28.76</td>
<td>9.65</td>
<td>13.90</td>
<td>53.36</td>
</tr>
<tr>
<td>E1 Peat (burnt)</td>
<td>Bottom</td>
<td>39.81</td>
<td>67.45</td>
<td>10.24</td>
<td>4.21</td>
<td>50.05</td>
</tr>
<tr>
<td>E2 Peat (unburnt)</td>
<td>Top</td>
<td>92.29</td>
<td>30.64</td>
<td>2.27</td>
<td>0.97</td>
<td>94.56</td>
</tr>
<tr>
<td>E2 Peat (unburnt)</td>
<td>Bottom</td>
<td>109.28</td>
<td>14.46</td>
<td>3.64</td>
<td>2.55</td>
<td>112.93</td>
</tr>
</tbody>
</table>

Figures 5.3 and 5.4 show data expressed per gram of organic matter to remove the influence of differences in mineral content between samples.

For core tops, major differences are apparent between sites (Fig. 5.3). In the Mharcaidh soils, values for both NH₄⁺ and NO₃⁻ are very low, but the valley peat (M2) stands out for having much higher levels of NH₄⁺ than other soils at the site. At the Gwy, levels of NH₄⁺ are low, but similar levels of NO₃⁻ occur (higher in the peaty gley at G2) which are greater than at all other sites except Scoat Tarn. The Scoat soils stand out for having high NO₃⁻ concentrations, particularly in the podsol at S1, while
levels of \( \text{NH}_4^+ \) are higher than all sites except the Etherow. The peat soils from the Etherow show very high levels of \( \text{NH}_4^+ \) but very low \( \text{NO}_3^- \) concentrations, which are comparable to those from the Mharcaidh soils in the E2 (mature Calluna) samples and only slightly higher, but very variable, in the soils from burnt areas (E1).

Table 5.3b: Soil inorganic N (\( \mu \text{gN} \) per gram soil organic matter)

<table>
<thead>
<tr>
<th>SOIL type</th>
<th>Section</th>
<th>Mean NH(_4)-N</th>
<th>SD NH(_4)-N</th>
<th>Mean NO(_3)-N</th>
<th>SD NO(_3)-N</th>
<th>Mean TIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 Peaty ranker</td>
<td>Top</td>
<td>1.02</td>
<td>1.19</td>
<td>2.46</td>
<td>1.65</td>
<td>3.48</td>
</tr>
<tr>
<td>M1 Peaty ranker</td>
<td>Bottom</td>
<td>7.89</td>
<td>11.31</td>
<td>0.74</td>
<td>0.71</td>
<td>8.63</td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>Top</td>
<td>19.75</td>
<td>21.10</td>
<td>2.32</td>
<td>0.59</td>
<td>22.07</td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>Bottom</td>
<td>30.63</td>
<td>20.44</td>
<td>5.45</td>
<td>6.13</td>
<td>36.09</td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>Top</td>
<td>2.07</td>
<td>2.18</td>
<td>1.28</td>
<td>0.63</td>
<td>3.35</td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>Bottom</td>
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<td>1.82</td>
<td>2.26</td>
<td>0.92</td>
<td>7.34</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>Top</td>
<td>3.95</td>
<td>3.31</td>
<td>6.09</td>
<td>3.90</td>
<td>10.04</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>Bottom</td>
<td>5.24</td>
<td>3.33</td>
<td>5.23</td>
<td>2.40</td>
<td>10.48</td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>Top</td>
<td>17.70</td>
<td>8.47</td>
<td>16.31</td>
<td>8.58</td>
<td>34.02</td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>Bottom</td>
<td>8.52</td>
<td>3.19</td>
<td>14.23</td>
<td>7.24</td>
<td>22.75</td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>Top</td>
<td>26.38</td>
<td>12.43</td>
<td>47.14</td>
<td>8.77</td>
<td>73.52</td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>Mineral</td>
<td>28.56</td>
<td>24.48</td>
<td>41.10</td>
<td>11.95</td>
<td>69.66</td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>Bottom</td>
<td>7.32</td>
<td>5.30</td>
<td>33.77</td>
<td>7.92</td>
<td>41.09</td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>Top</td>
<td>25.01</td>
<td>22.11</td>
<td>19.30</td>
<td>10.66</td>
<td>44.30</td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>Mineral</td>
<td>29.14</td>
<td>11.59</td>
<td>21.60</td>
<td>11.59</td>
<td>50.74</td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>Bottom</td>
<td>11.11</td>
<td>5.29</td>
<td>18.23</td>
<td>15.92</td>
<td>29.34</td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>Top</td>
<td>13.85</td>
<td>15.43</td>
<td>14.69</td>
<td>2.71</td>
<td>28.54</td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>Bottom</td>
<td>4.55</td>
<td>3.47</td>
<td>9.67</td>
<td>1.46</td>
<td>14.22</td>
</tr>
<tr>
<td>S1 Podsol</td>
<td>Top</td>
<td>32.98</td>
<td>12.00</td>
<td>103.87</td>
<td>19.12</td>
<td>136.85</td>
</tr>
<tr>
<td>S1 Podsol</td>
<td>Bottom</td>
<td>19.14</td>
<td>12.61</td>
<td>51.14</td>
<td>4.58</td>
<td>70.27</td>
</tr>
<tr>
<td>S2 Peaty gley</td>
<td>Top</td>
<td>83.22</td>
<td>26.59</td>
<td>48.93</td>
<td>9.43</td>
<td>132.15</td>
</tr>
<tr>
<td>S2 Peaty gley</td>
<td>Bottom</td>
<td>7.51</td>
<td>4.05</td>
<td>52.48</td>
<td>15.99</td>
<td>59.99</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>Top</td>
<td>33.36</td>
<td>20.35</td>
<td>25.15</td>
<td>19.16</td>
<td>58.50</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>Bottom</td>
<td>6.43</td>
<td>4.27</td>
<td>5.15</td>
<td>2.48</td>
<td>11.58</td>
</tr>
<tr>
<td>E1 Peat (burnt)</td>
<td>Top</td>
<td>51.83</td>
<td>35.48</td>
<td>11.47</td>
<td>16.83</td>
<td>63.30</td>
</tr>
<tr>
<td>E1 Peat (burnt)</td>
<td>Bottom</td>
<td>43.85</td>
<td>70.39</td>
<td>14.19</td>
<td>6.30</td>
<td>58.04</td>
</tr>
<tr>
<td>E2 Peat (unburnt)</td>
<td>Top</td>
<td>99.73</td>
<td>31.24</td>
<td>2.46</td>
<td>1.01</td>
<td>102.18</td>
</tr>
<tr>
<td>E2 Peat (unburnt)</td>
<td>Bottom</td>
<td>120.60</td>
<td>14.80</td>
<td>4.02</td>
<td>2.78</td>
<td>124.62</td>
</tr>
</tbody>
</table>

Some of these differences are less apparent in the deeper soils (Fig. 5.4), while others are more pronounced. Extractable \( \text{NH}_4^+ \) is very variable between soils at each site, but in the same general range for all sites except the Etherow, where it is much higher, particularly for E2 where values are an order of magnitude greater than for some other soils. Soils from the Gwy and Scoat Tarn still show high \( \text{NO}_3^- \) at this depth, but the
very high value found in the core tops from S1 is not found in the lower samples. Again, very low levels of \( \text{NO}_3^- \) are found in both the Mharcaidh and Etherow soils.

**Figure 5.3: Extractable inorganic N from core tops (0-5cm)**

![Graph showing extractable inorganic N from core tops (0-5cm)](image)

**Figure 5.4: Extractable inorganic N from core bottoms (c. 5-20cm) and mineral horizons (G2 and G3 only)**

![Graph showing extractable inorganic N from core bottoms (c. 5-20cm) and mineral horizons (G2 and G3 only)](image)
Differences in total inorganic N for core tops are illustrated in Figure 5.5. While there is variability between soils within each catchment, there is a pattern of increasing TIN from the Mharcaidh to Scoat Tarn along the gradient of estimated TIN deposition, with an apparent decrease in the Etherow peats from the highest values observed in two of the Scoat Tarn soils.

**Figure 5.5: Total inorganic N in core tops (0-5cm)**

In the core bottoms, total inorganic N is much reduced for the two high NO\textsubscript{3}\cdot Scoat Tarn soils; still slightly higher than most Gwy soils but with a very similar range to the mineral horizon of the peaty gley (G2m). The Mharcaidh soils still show the lowest concentrations, but the high deposition Etherow site now has the highest levels of inorganic N. While the soil from the burnt area (E1) is comparable to the Gwy and Scoat samples, the E2 soil from under mature *Calluna* has by far the highest level of inorganic N.

These baseline values provide an indication of the initial pool sizes of inorganic N available for nitrification and denitrification (see Chapter 4), while the mineralisation
and nitrification data indicate potential supply rates under the same moisture conditions, but at an elevated temperature.

![Figure 5.6: Total inorganic N in core bottoms (5-20cm)](image)

5.3.3 Potential mineralisation

Mineralisation potentials for samples incubated at 15°C over 33 days are presented in Table 5.4, with mean and standard deviation for the 6 paired samples from each soil. Potential mineralisation is expressed in terms of mean daily increase in concentration from the baseline values given in Tables 5.3a-b.

For all soils, mineralisation potentials expressed per gram dry weight of soil are greater in the surface organic horizon that lower down the profile, because of the higher organic content in this layer. The same is generally true for values expressed per gram of soil organic matter, which might be expected if the younger organic matter in the surface horizon is more labile than the older, more decomposed humus lower down, except for the Mharcaidh soils. The very low potentials and small within-profile differences at the Mharcaidh suggest either that the labile organic N is
rapidly mineralised and re-utilised in a very tight cycle within the nutrient poor soils there, or that more labile surface material occupies a very thin layer which is effectively "diluted" by very stable, older humus which makes up the bulk of the 5cm sections analysed.

Table 5.4: Potential mineralisation in incubated soil cores (μgN per gram dry weight soil or per gram organic matter per day)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Soil type</th>
<th>Section</th>
<th>Dry weight Mean</th>
<th>SD</th>
<th>Organic Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Peaty ranker</td>
<td>Top</td>
<td>0.54</td>
<td>0.12</td>
<td>0.57</td>
<td>0.13</td>
</tr>
<tr>
<td>M1</td>
<td>Peaty ranker</td>
<td>Bottom</td>
<td>0.40</td>
<td>0.33</td>
<td>0.53</td>
<td>0.35</td>
</tr>
<tr>
<td>M2</td>
<td>Valley peat</td>
<td>Top</td>
<td>0.15</td>
<td>0.76</td>
<td>0.22</td>
<td>0.91</td>
</tr>
<tr>
<td>M2</td>
<td>Valley peat</td>
<td>Bottom</td>
<td>0.12</td>
<td>0.65</td>
<td>0.22</td>
<td>0.99</td>
</tr>
<tr>
<td>M3</td>
<td>Peaty podsol</td>
<td>Top</td>
<td>0.35</td>
<td>0.18</td>
<td>0.46</td>
<td>0.22</td>
</tr>
<tr>
<td>M3</td>
<td>Peaty podsol</td>
<td>Bottom</td>
<td>0.07</td>
<td>0.10</td>
<td>0.70</td>
<td>0.91</td>
</tr>
<tr>
<td>M4</td>
<td>Shallow peat</td>
<td>Top</td>
<td>0.19</td>
<td>0.26</td>
<td>0.21</td>
<td>0.28</td>
</tr>
<tr>
<td>M4</td>
<td>Shallow peat</td>
<td>Bottom</td>
<td>0.08</td>
<td>0.21</td>
<td>0.11</td>
<td>0.24</td>
</tr>
<tr>
<td>G1</td>
<td>Hilltop peat</td>
<td>Top</td>
<td>5.07</td>
<td>5.80</td>
<td>5.43</td>
<td>6.05</td>
</tr>
<tr>
<td>G1</td>
<td>Hilltop peat</td>
<td>Bottom</td>
<td>0.37</td>
<td>0.52</td>
<td>0.43</td>
<td>0.52</td>
</tr>
<tr>
<td>G2</td>
<td>Peaty gley</td>
<td>Top</td>
<td>6.34</td>
<td>2.34</td>
<td>7.03</td>
<td>2.56</td>
</tr>
<tr>
<td>G2</td>
<td>Peaty gley</td>
<td>Mineral</td>
<td>0.14</td>
<td>0.07</td>
<td>0.67</td>
<td>0.53</td>
</tr>
<tr>
<td>G2</td>
<td>Peaty gley</td>
<td>Bottom</td>
<td>2.16</td>
<td>1.15</td>
<td>3.21</td>
<td>1.56</td>
</tr>
<tr>
<td>G3</td>
<td>Podsol</td>
<td>Top</td>
<td>1.16</td>
<td>0.85</td>
<td>2.41</td>
<td>1.79</td>
</tr>
<tr>
<td>G3</td>
<td>Podsol</td>
<td>Mineral</td>
<td>0.10</td>
<td>0.15</td>
<td>0.83</td>
<td>1.46</td>
</tr>
<tr>
<td>G3</td>
<td>Podsol</td>
<td>Bottom</td>
<td>0.63</td>
<td>0.71</td>
<td>1.67</td>
<td>2.17</td>
</tr>
<tr>
<td>G4</td>
<td>Valley peat</td>
<td>Top</td>
<td>2.33</td>
<td>1.13</td>
<td>2.64</td>
<td>1.30</td>
</tr>
<tr>
<td>G4</td>
<td>Valley peat</td>
<td>Bottom</td>
<td>0.67</td>
<td>0.43</td>
<td>0.82</td>
<td>0.49</td>
</tr>
<tr>
<td>S1</td>
<td>Podsol</td>
<td>Top</td>
<td>9.81</td>
<td>2.43</td>
<td>12.37</td>
<td>2.67</td>
</tr>
<tr>
<td>S1</td>
<td>Podsol</td>
<td>Bottom</td>
<td>0.55</td>
<td>0.25</td>
<td>2.10</td>
<td>0.95</td>
</tr>
<tr>
<td>S2</td>
<td>Peaty gley</td>
<td>Top</td>
<td>8.26</td>
<td>2.65</td>
<td>9.47</td>
<td>3.40</td>
</tr>
<tr>
<td>S2</td>
<td>Peaty gley</td>
<td>Bottom</td>
<td>1.08</td>
<td>0.51</td>
<td>1.96</td>
<td>0.84</td>
</tr>
<tr>
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<td>Deep peat</td>
<td>Top</td>
<td>4.31</td>
<td>1.94</td>
<td>4.57</td>
<td>2.79</td>
</tr>
<tr>
<td>S3</td>
<td>Deep peat</td>
<td>Bottom</td>
<td>0.46</td>
<td>0.36</td>
<td>0.62</td>
<td>0.46</td>
</tr>
<tr>
<td>E1</td>
<td>Peat (burnt)</td>
<td>Top</td>
<td>3.80</td>
<td>1.01</td>
<td>4.48</td>
<td>1.06</td>
</tr>
<tr>
<td>E1</td>
<td>Peat (burnt)</td>
<td>Bottom</td>
<td>0.90</td>
<td>0.28</td>
<td>1.10</td>
<td>0.40</td>
</tr>
<tr>
<td>E2</td>
<td>Peat (unburnt)</td>
<td>Top</td>
<td>6.77</td>
<td>2.07</td>
<td>7.54</td>
<td>2.58</td>
</tr>
<tr>
<td>E2</td>
<td>Peat (unburnt)</td>
<td>Bottom</td>
<td>3.23</td>
<td>1.51</td>
<td>3.75</td>
<td>1.79</td>
</tr>
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</table>

For inter-site comparisons and assessment of the importance of mineralisation in controlling nitrification and losses of inorganic N, it is more useful to consider the
activity of soils in terms of overall fluxes per unit area of soil. Mineralisation potentials were converted into fluxes (kgN ha$^{-1}$ yr$^{-1}$) using bulk density (Table 5.2) and assumed horizon thicknesses of 5cm for core tops, 15cm for core bottoms (except G2 and G3, 12cm) and 3cm for the two mineral samples (G2 and G3). The data are presented in Figure 5.7 and Table 5.5.

Figure 5.7: Potential mineralisation flux in soils incubated at 15°C

Potential mineralisation rates are very low at the Allt a’Mharcaidh site compared with the other study catchments, being an order of magnitude smaller than in most of the other soils sampled. The highest rates are generally associated with the peaty gleys (G2, S2) and podsols (G3, S1) at the Gwy and Scoat Tarn, while the peat under mature Calluna at the Etherow (E2) is exceptional among the peats for its high mineralisation potential. The other peats have somewhat lower mineralisation potentials (G1, G4, S3, E1), but values are still much greater than for any of the Mharcaidh soils.
In the Scoat Tarn soils, plus the hilltop peat at the Gwy (G1), the surface organic horizon (core top, 0-5cm) contributes more to the total flux for the whole core than the lower samples, despite being only one third of the thickness (Table 5.5). Elsewhere the contributions from the deeper organic soils are greater in total, despite lower mineralisation potentials per gram dry weight of soil (Table 5.4). The contributions of the mineral horizons at the Gwy are negligible.

Table 5.5: Potential mineralisation fluxes (kgN ha⁻¹ yr⁻¹) at 15°C

<table>
<thead>
<tr>
<th>Soil</th>
<th>Type</th>
<th>Top</th>
<th>SD</th>
<th>Bottom</th>
<th>SD</th>
<th>Mineral</th>
<th>SD</th>
<th>Core total</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
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<td>12.3</td>
<td>2.7</td>
<td>37.4</td>
<td>30.5</td>
<td>49.7</td>
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<td></td>
</tr>
<tr>
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<td>Valley peat</td>
<td>3.4</td>
<td>17.9</td>
<td>9.6</td>
<td>52.2</td>
<td>13.0</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Peaty podsol</td>
<td>14.3</td>
<td>7.6</td>
<td>26.4</td>
<td>37.0</td>
<td>40.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>Shallow peat</td>
<td>4.5</td>
<td>6.2</td>
<td>10.1</td>
<td>26.7</td>
<td>14.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>Hilltop peat</td>
<td>110.7</td>
<td>126.7</td>
<td>25.8</td>
<td>36.6</td>
<td>136.5</td>
<td></td>
<td></td>
</tr>
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<td>Peaty gley</td>
<td>160.4</td>
<td>59.3</td>
<td>332.2</td>
<td>176.5</td>
<td>13.4</td>
<td>6.8</td>
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<td>67.9</td>
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<td>Podsol</td>
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<td>Peaty gley</td>
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<td>81.7</td>
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<td>496.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>Deep peat</td>
<td>146.0</td>
<td>65.8</td>
<td>67.7</td>
<td>52.5</td>
<td>213.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>Peat (burnt)</td>
<td>106.5</td>
<td>28.3</td>
<td>99.1</td>
<td>31.2</td>
<td>205.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>Peat (unburnt)</td>
<td>160.7</td>
<td>49.2</td>
<td>242.2</td>
<td>113.3</td>
<td>402.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.3.4 Potential nitrification

Nitrification potentials for core tops and bottoms are provided in Table 5.6. As with mineralisation potentials, values are generally greater in the surface organic layer than in the lower soils, but only in soils with significant potentials. In soils with very low nitrification potentials, for example from the Mharcaidh and Etherow, there is no consistent pattern in values for upper and lower soils, whether expressed per gram dry weight of soil or per gram organic matter.
These figures were converted into fluxes per unit area in the same way as for mineralisation potentials, using bulk density and assumed horizon thicknesses. Fluxes are shown in Table 5.7 and Figure 5.8. A similar pattern to that for mineralisation is observed, the major difference being that nitrification potentials are low for both Etherow peats. Again, the highest fluxes are found in the peaty gleys and podsols from the Gwy and Scoat Tarn catchments, but the differences between these soils and the peats from these sites are more pronounced than they are for mineralisation. The contributions of the mineral horizons are again negligible. Some of the peat soils show a net consumption of NO$_3^-$ over the period of incubation.

Table 5.6: Potential nitrification in incubated soil cores (µgN per gram dry weight soil and per gram organic matter per day)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Soil type</th>
<th>Section</th>
<th>Dry weight Mean</th>
<th>SD</th>
<th>Organic Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Peaty ranker</td>
<td>Top</td>
<td>0.15</td>
<td>0.10</td>
<td>0.16</td>
<td>0.11</td>
</tr>
<tr>
<td>M1</td>
<td>Peaty ranker</td>
<td>Bottom</td>
<td>0.19</td>
<td>0.09</td>
<td>0.23</td>
<td>0.09</td>
</tr>
<tr>
<td>M2</td>
<td>Valley peat</td>
<td>Top</td>
<td>0.08</td>
<td>0.05</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td>M2</td>
<td>Valley peat</td>
<td>Bottom</td>
<td>-0.02</td>
<td>0.16</td>
<td>-0.02</td>
<td>0.18</td>
</tr>
<tr>
<td>M3</td>
<td>Peaty podsol</td>
<td>Top</td>
<td>0.03</td>
<td>0.02</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>M3</td>
<td>Peaty podsol</td>
<td>Bottom</td>
<td>0.01</td>
<td>0.01</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>M4</td>
<td>Shallow peat</td>
<td>Top</td>
<td>-0.01</td>
<td>0.10</td>
<td>-0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>M4</td>
<td>Shallow peat</td>
<td>Bottom</td>
<td>-0.01</td>
<td>0.09</td>
<td>0.00</td>
<td>0.11</td>
</tr>
<tr>
<td>G1</td>
<td>Hilltop peat</td>
<td>Top</td>
<td>0.49</td>
<td>0.62</td>
<td>0.54</td>
<td>0.69</td>
</tr>
<tr>
<td>G1</td>
<td>Hilltop peat</td>
<td>Bottom</td>
<td>0.20</td>
<td>0.32</td>
<td>0.24</td>
<td>0.32</td>
</tr>
<tr>
<td>G2</td>
<td>Peaty gley</td>
<td>Top</td>
<td>2.75</td>
<td>0.79</td>
<td>3.05</td>
<td>0.89</td>
</tr>
<tr>
<td>G2</td>
<td>Peaty gley</td>
<td>Mineral</td>
<td>0.14</td>
<td>0.10</td>
<td>0.79</td>
<td>0.78</td>
</tr>
<tr>
<td>G2</td>
<td>Peaty gley</td>
<td>Bottom</td>
<td>1.87</td>
<td>0.98</td>
<td>2.76</td>
<td>1.26</td>
</tr>
<tr>
<td>G3</td>
<td>Podsol</td>
<td>Top</td>
<td>0.42</td>
<td>0.52</td>
<td>0.86</td>
<td>1.13</td>
</tr>
<tr>
<td>G3</td>
<td>Podsol</td>
<td>Mineral</td>
<td>0.04</td>
<td>0.08</td>
<td>0.31</td>
<td>0.75</td>
</tr>
<tr>
<td>G3</td>
<td>Podsol</td>
<td>Bottom</td>
<td>0.41</td>
<td>0.61</td>
<td>1.12</td>
<td>1.88</td>
</tr>
<tr>
<td>G4</td>
<td>Valley peat</td>
<td>Top</td>
<td>-0.17</td>
<td>0.10</td>
<td>-0.18</td>
<td>0.13</td>
</tr>
<tr>
<td>G4</td>
<td>Valley peat</td>
<td>Bottom</td>
<td>0.05</td>
<td>0.16</td>
<td>0.04</td>
<td>0.19</td>
</tr>
<tr>
<td>S1</td>
<td>Podsol</td>
<td>Top</td>
<td>4.07</td>
<td>0.64</td>
<td>5.04</td>
<td>0.62</td>
</tr>
<tr>
<td>S1</td>
<td>Podsol</td>
<td>Bottom</td>
<td>0.55</td>
<td>0.22</td>
<td>2.14</td>
<td>0.72</td>
</tr>
<tr>
<td>S2</td>
<td>Peaty gley</td>
<td>Top</td>
<td>3.82</td>
<td>1.84</td>
<td>4.44</td>
<td>2.32</td>
</tr>
<tr>
<td>S2</td>
<td>Peaty gley</td>
<td>Bottom</td>
<td>1.16</td>
<td>0.44</td>
<td>2.13</td>
<td>0.73</td>
</tr>
<tr>
<td>S3</td>
<td>Deep peat</td>
<td>Top</td>
<td>1.68</td>
<td>0.86</td>
<td>1.74</td>
<td>1.13</td>
</tr>
<tr>
<td>S3</td>
<td>Deep peat</td>
<td>Bottom</td>
<td>0.17</td>
<td>0.18</td>
<td>0.22</td>
<td>0.21</td>
</tr>
<tr>
<td>E1</td>
<td>Peat (burnt)</td>
<td>Top</td>
<td>0.29</td>
<td>0.42</td>
<td>0.33</td>
<td>0.50</td>
</tr>
<tr>
<td>E1</td>
<td>Peat (burnt)</td>
<td>Bottom</td>
<td>0.55</td>
<td>0.25</td>
<td>0.63</td>
<td>0.27</td>
</tr>
<tr>
<td>E2</td>
<td>Peat (unburnt)</td>
<td>Top</td>
<td>0.07</td>
<td>0.05</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>E2</td>
<td>Peat (unburnt)</td>
<td>Bottom</td>
<td>-0.03</td>
<td>0.10</td>
<td>-0.03</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Nitrification is expressed as a percentage of mineralisation in Table 5.8. Values vary from more than 100% to less than zero where NO$_3^-$ consumption occurs. In 9 of the 13 soils, a greater percentage of NH$_4^+$ is nitrified in the lower horizons than in the surface organic layer.

Table 5.7: Potential nitrification fluxes (kgN ha$^{-1}$ yr$^{-1}$) at 15°C

<table>
<thead>
<tr>
<th>Soil</th>
<th>Type</th>
<th>Top SD</th>
<th>Bottom SD</th>
<th>Mineral SD</th>
<th>Core total</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Peaty ranker</td>
<td>3.5</td>
<td>2.3</td>
<td>17.3</td>
<td>8.0</td>
</tr>
<tr>
<td>M2</td>
<td>Valley peat</td>
<td>1.9</td>
<td>1.2</td>
<td>-1.7</td>
<td>12.6</td>
</tr>
<tr>
<td>M3</td>
<td>Peaty podsol</td>
<td>1.4</td>
<td>0.7</td>
<td>4.8</td>
<td>2.9</td>
</tr>
<tr>
<td>M4</td>
<td>Shallow peat</td>
<td>-0.2</td>
<td>2.3</td>
<td>-1.7</td>
<td>11.5</td>
</tr>
<tr>
<td>G1</td>
<td>Hilltop peat</td>
<td>10.6</td>
<td>13.5</td>
<td>14.1</td>
<td>22.6</td>
</tr>
<tr>
<td>G2</td>
<td>Peaty gley</td>
<td>69.6</td>
<td>20.1</td>
<td>287.1</td>
<td>151.2</td>
</tr>
<tr>
<td>G3</td>
<td>Podsol</td>
<td>33.1</td>
<td>41.5</td>
<td>124.1</td>
<td>185.1</td>
</tr>
<tr>
<td>G4</td>
<td>Valley peat</td>
<td>-4.2</td>
<td>2.5</td>
<td>4.5</td>
<td>14.4</td>
</tr>
<tr>
<td>S1</td>
<td>Podsol</td>
<td>172.2</td>
<td>27.3</td>
<td>204.8</td>
<td>82.6</td>
</tr>
<tr>
<td>S2</td>
<td>Peaty gley</td>
<td>117.8</td>
<td>56.7</td>
<td>259.3</td>
<td>99.2</td>
</tr>
<tr>
<td>S3</td>
<td>Deep peat</td>
<td>57.0</td>
<td>29.3</td>
<td>24.7</td>
<td>26.4</td>
</tr>
<tr>
<td>E1</td>
<td>Peat (burnt)</td>
<td>8.0</td>
<td>11.7</td>
<td>60.6</td>
<td>28.2</td>
</tr>
<tr>
<td>E2</td>
<td>Peat (unburnt)</td>
<td>1.8</td>
<td>1.1</td>
<td>-2.0</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Figure 5.8: Potential nitrification flux in soils incubated at 15°C
In the Scoat Tarn samples the percentage of nitrification is relatively constant between soil surface samples, at 39-46%. In the lower soils, the percentage is similar for the peat (S3) but close to 100% for the mineral soils. The value of 107% for S2 indicates that the level of extractable NH$_4^+$ must have declined during incubation. Elsewhere values are very variable between soil types.

**Table 5.8: Nitrification potential as a percentage of mineralisation potential at 15°C**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Type</th>
<th>Top</th>
<th>Bottom</th>
<th>Mineral</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Peaty ranker</td>
<td>28.6</td>
<td>46.2</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>Valley peat</td>
<td>56.6</td>
<td>-17.3</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>Peaty podsol</td>
<td>9.9</td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>Shallow peat</td>
<td>-5.0</td>
<td>-16.7</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>Hilltop peat</td>
<td>9.6</td>
<td>54.6</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>Peaty gley</td>
<td>43.4</td>
<td>86.4</td>
<td>97.5</td>
</tr>
<tr>
<td>G3</td>
<td>Podsol</td>
<td>35.9</td>
<td>65.2</td>
<td>39.3</td>
</tr>
<tr>
<td>G4</td>
<td>Valley peat</td>
<td>-7.4</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>Podsol</td>
<td>41.4</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>Peaty gley</td>
<td>46.3</td>
<td>107.1</td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>Deep peat</td>
<td>39.1</td>
<td>36.5</td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>Peat (burnt)</td>
<td>7.5</td>
<td>61.2</td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>Peat (unburnt)</td>
<td>1.1</td>
<td>-0.8</td>
<td></td>
</tr>
</tbody>
</table>

The relationship between mineralisation and nitrification for core tops and bottoms is illustrated in Figures 5.9 and 5.10.

**Figure 5.9: Relationship between mineralisation and nitrification in core tops**

![Graph A: All samples](image)

![Graph B: Excluding non-nitrifiers](image)

\[ R^2 = 0.8426 \]

\[ R^2 = 0.9925 \]
It is apparent that there is a very strong relationship between nitrification and mineralisation potentials, which is slightly stronger in the surface soils. While some relationship between these processes is expected, since nitrification is facilitated by mineralisation which provides the NH$_4^+$ substrate, the proportional rate of nitrification varies greatly between soils (Table 5.8), so the strength of these correlations is surprising. Some scatter is introduced by the presence of soils in which nitrification is very low or absent (Figs. 5.9a and 5.10a). The relationship is strengthened if these samples showing very low proportional rates of nitrifying activity (<10% of mineralisation) are removed (Figs. 5.9b and 5.10b). Relationships with C:N ratio are explored in Chapter 8.

5.3.5 Mineralisation and nitrification as controls for denitrification

It has been shown above that nitrification is strongly correlated with mineralisation, which provides the required NH$_4^+$ substrate. The importance of nitrification in providing the NO$_3^-$ substrate for subsequent denitrification can be tested in the same way. Figure 5.11 shows the relationships between pre-incubation available NH$_4^+$ or NO$_3^-$ and denitrification fluxes from core tops unamended with NH$_4$NO$_3$, at both 5°C and 15°C.
At 5°C there is a reasonable correlation between denitrification flux and available NO$_3^-$, but no relationship with NH$_4^+$ or total inorganic N. This pattern is expected because denitrifiers utilise NO$_3^-$ only; a relationship with NH$_4^+$ would occur only if NH$_4^+$ were a good indicator of nitrification potential. A much better relationship is found for core tops denitrifying at 15°C, which is due to the larger number of samples.
showing denitrification at this higher temperature. The same plots for core bottoms are shown in Figure 5.12.

Figure 5.12: Denitrification and available inorganic N in core bottoms

No relationships are apparent between available NO$_3^-$ or NH$_4^+$ and denitrification from core bottoms at either incubation temperature. A weak relationship is found with
NO₃⁻ at 15°C if one outlier, from the Etherow peat under burnt *Calluna*, is removed ($R^2 = 0.347$; data not shown). This soil has been found to be an outlier in many other respects in previous chapters, due to the effects of recent burning at the site on local nutrient cycling. The weaker relationship between denitrification and available NO₃⁻ in core bottoms supports the assertion in Chapter 4 that the deeper soils are more likely to be C limited than the surface soils.

The very tight correlation between denitrification at 15°C and available NO₃⁻ in core tops shows that for the trace gas incubation experiments, NO₃⁻ was limiting for denitrification. On an annual basis, the supply of NO₃⁻ throughout the year rather than the instantaneous concentration is therefore likely to limit denitrification. Nitrification potential, as well as the closely linked mineralisation potential, is an indicator of NO₃⁻ supply, and denitrification is plotted against both in Figures 5.13-5.14.

**Figure 5.13: Denitrification, mineralisation and nitrification potentials in core tops**
Relationships can be seen between denitrification and mineralisation or nitrification potentials at both temperatures. For reasons already discussed, the stronger relationships with nitrification relative to mineralisation are expected, but the differences are not great, particularly at the higher temperature. Denitrification at 15°C is much more highly correlated with both mineralisation and nitrification potentials than at 5°C. This is due to the incubation of soils for both potentials at 15°C, so that denitrification is being compared with inorganic N supply at the same temperature. At the lower temperature, denitrification activity is much reduced or absent, so there is more scatter in the plot against inorganic N supply at 15°C.

**Figure 5.14: Denitrification, mineralisation and nitrification potentials in core bottoms**
Again, there is no relationship between denitrification and potential mineralisation or nitrification for the lower soils represented by the core bottoms, providing further evidence for the C limitation of denitrification in these horizons.

5.3.6 Mineralisation and nitrification as controls for inorganic N leaching

The inorganic N released by mineralisation and nitrification is available for plant or microbial uptake which retains the N within the plant-soil system, but a proportion may be lost through the processes of denitrification and leaching. It was shown in Figure 5.13 that denitrification in the upper organic layer is strongly correlated with both mineralisation and nitrification potentials. Inorganic N which is not taken up by the biota or denitrified may be leached down the soil profile and into surface waters, usually as NO$_3^-$ which is much more mobile than NH$_4^+$ in soils. It might therefore be expected that average soilwater inorganic N concentrations would be linked to the supply of NH$_4^+$ and NO$_3^-$ by mineralisation and nitrification throughout the soil profile.

Inspection of the data reveals that there is no correlation between mean concentrations of NH$_4^+$ or NO$_3^-$ in soilwaters (field samples at c. 30cm depth – see Chapter 3: Table 3.15) with whole-core mineralisation or nitrification potentials across the four study catchments. Weak correlations are observed if outliers in the data from the Etherow soils are removed (Figure 5.15), which is strongest for total inorganic N and mineralisation, but also apparent for soilwater NO$_3^-$ and nitrification potential, but these correlations are driven entirely by the Scoat Tarn samples. The occurrence of high soilwater NO$_3^-$ and high nitrification and mineralisation potentials in Scoat Tarn soils gives a misleading picture: NH$_4^+$ levels are very similar across the three sites excluding the Etherow, while NO$_3^-$ is zero for the Mharcaidh and Gwy soils. Hence little can be interpreted from these data, other than to speculate that high soilwater NO$_3^-$ may be driven partly by high rates of nitrification and relatively low rates of immobilisation at Scoat Tarn.

The poor relationships between laboratory potentials and field measurements of soilwater inorganic N reflect important limitations of the incubation experiments, which cannot account for the confounding effects of vegetation uptake and atmospheric deposition on attempts to link mineralisation and nitrification with
leaching. Hence while the relationships between mineralisation, nitrification and denitrification potentials in incubated soils are strong, very low rates of denitrification are observed in the field (Chapter 4). The data are, however, useful in showing where net mineralisation and nitrification fluxes may be important when N is available in excess of plant uptake requirements.

**Figure 5.15: Soilwater total inorganic nitrogen and potential mineralisation and nitrification fluxes (excluding Etherow samples)**

5.4 Discussion

5.4.1 Magnitude of potential fluxes

The mineralisation and nitrification potentials found in these soils (13.0 – 620.4 and -1.9 to 377 kgN ha\(^{-1}\) yr\(^{-1}\)) are very high compared with field rates from other studies. For net mineralisation and nitrification, a very wide range of rates have been published for several ecosystems. Schulze *et al.* (1994) suggested that nitrification rates are negligible in interior Alaskan spruce forests and arctic tundra, though calcareous sites have substantial rates of nitrification in the Arctic. Neff *et al.* (1994) found net mineralisation and nitrification rates of 3.7-65.7 kgN ha\(^{-1}\) yr\(^{-1}\) in fertilised alpine meadow soils from Colorado. A range of 20-60 kgN ha\(^{-1}\) yr\(^{-1}\) was suggested for net mineralisation in nutrient poor, acid, British upland soils by Batey (1982).
In forest soils, published net mineralisation rates vary from 13 and 24 kgN ha\(^{-1}\) yr\(^{-1}\) in the surface organic horizons of control and fertilised forest sites (Gundersen, 1998), and a suggested general range of 30-50 kgN ha\(^{-1}\) yr\(^{-1}\) for forests (Gosz, 1981, cited in Gundersen, 1991), to maximum values of 249-451 kgN ha\(^{-1}\) yr\(^{-1}\) in Dutch forest soils (Tietema et al., 1992). Zak et al. (1990) suggested that 120 kgN ha\(^{-1}\) yr\(^{-1}\) was towards the upper end of the range for northern hardwood forests in North America, while Vitousek and Melillo (1979) found rates from several forest studies ranged from 19 to 139 kgN ha\(^{-1}\) yr\(^{-1}\). Rates varied with the dominant tree species in a study by Hill and Shackleton (1989), from 1.2-113.5 kgN ha\(^{-1}\) yr\(^{-1}\) for mineralisation and 2.7-105.3 kgN ha\(^{-1}\) yr\(^{-1}\) for nitrification, while maximum nitrification rates of 179 kgN ha\(^{-1}\) yr\(^{-1}\) were found in a Dutch oak-beech forest by Tietema et al. (1992). High NO\(_3^-\) leaching was attributed to a high net nitrification potential of 0.84 kgN ha\(^{-1}\) day\(^{-1}\) (307 kgN ha\(^{-1}\) yr\(^{-1}\)) in a central Appalachian forest soil (Christ et al., 2002).

The very high figures in the present study are an estimate of maximum rates at the high incubation temperature of 15°C, when even the warmest soils in the field (G3, Gwy podsol and E1, Etherow peat under burnt Calluna) exceed this temperature for just 8 days during the budget year (see Table 3.19). Most of the other soils studied never reach this temperature at 5-10cm depth, and for those that do it is only for 1 or 2 days of the year. Hence the data are not comparable with annual fluxes derived from field rates which are affected by seasonal variations with changing temperature and moisture. The rates are, however, comparable with measures of net nitrification potential obtained in relatively high temperature incubations elsewhere, for example values in excess of 730 kgN ha\(^{-1}\) yr\(^{-1}\) were obtained for forest soils incubated at 21°C by Christ et al. (2002).

5.4.2 Differences between soils and between sites

The measurements of available total inorganic N show an increase across the gradient of N deposition and leaching from the Allt a’Mharcaidh and Afon Gwy to Scoat Tarn, but in the upper organic soils, N availability decreases again at the River Etherow compared with the highest values found at Scoat Tarn. This pattern is very pronounced for available NO\(_3^-\), for which levels at the Etherow are lower than any site
except the Mharcaidh, but particularly high levels of NH$_4^+$ in the Etherow soils make them second only to the Scoat Tarn surface soils in terms of TIN, and in deeper soils the peat under mature Calluna at E2 has by far the highest availability of TIN.

A very similar pattern is found for mineralisation potentials, with extremely low values in the Mharcaidh soils, very high values in the mineral soils from the Gwy and Scoat Tarn, and then lower values again in the Etherow. In the Scoat soils, the upper organic horizon makes a disproportionate contribution to the total potential N flux. If peat soils alone are considered, there is an increasing gradient in mineralisation potentials across the four catchments (Fig. 5.16), in line with total N deposition and inorganic N leaching.

For nitrification potentials, the pattern is again driven mainly by the mineral soils from the Gwy and Scoat Tarn, with values an order of magnitude greater than in the peat soils. If peat soils are plotted together, it is evident that there is a steep increase in nitrification potential across the four sites with increasing N deposition, but at the Etherow, significant nitrification is associated only with the peat under the burnt Calluna (E1 – Fig. 5.16), and is very low under the unburnt area (E2), so at this site, nitrification is important only following burning. Furthermore, it is the deeper soils at E1 which have the higher nitrification potential, while at Scoat Tarn the surface horizon is most active.

**Figure 5.16: Mineralisation and nitrification potentials in peat soils**
The data suggest that nitrification could be the main control on NO$_3^-$ leaching from the study catchments, except at the Etherow, where NH$_4^+$ supply from mineralisation may supply nitrifiers in riparian zones, or leach into surface waters where it may also be nitrified in-stream. At the Allt a'Mharcaidh, very low mineralisation and nitrification potentials indicate a closed N cycle and low activity associated with refractory soil organic matter, which when coupled with the very low N inputs to the site, explain the lack of inorganic N leaching there.

Several factors may contribute to the difference in NO$_3^-$ leaching observed from the soils at the Gwy and Scoat Tarn under similar N deposition loads. Mineralisation and nitrification potentials are much greater in the surface organic layer for all the Scoat Tarn soils than at the Gwy (Figs. 5.7 - 5.8). Differences in the proportion of runoff leaching directly from this horizon into surface waters could account for higher levels of NO$_3^-$ leaching. However, mean soilwater concentrations at 30cm depth (Chapter 3) indicate that very little NO$_3^-$ occurs in the Gwy soils at this depth, but high levels occur at Scoat Tarn, despite the very similar nitrification potentials in the surface 20cm of the overlying soils in G2, S1 and S2.

Climatic differences may have an influence. The higher elevation soils at Scoat Tarn are cooler, and the podsol there (S1) is much wetter than the mineral soils at the Gwy. Hence actual nitrification rates could be lower on average in the Scoat soils than at the Gwy, but the potential for leaching in cool, wet conditions may be greater. Groffman and Tiedje (1988) suggested that increased mineralisation of C and N follows drying and rewetting, due to increased substrate availability from the death of microbial biomass and from physical disruption of the soil environment, but varies more for N mineralisation because it is a function of the C:N ratio of the substrate being mineralised. The soil C:N ratio, coupled with differences in weather patterns, could therefore be factors in explaining the differences between sites (see Chapter 8).

Finally, the distribution of mineral soils in the two catchments may be very different. While a detailed soils map for the Scoat Tarn catchment is unavailable, the two mineral soils predominate on the steep slopes, with peat only occurring in a small area next to the lake itself. There is also a significant proportion of bare rock on the upper slopes. Hence a large proportion of the catchment is either devoid of soils, or covered
in very active mineral soils. Lamontagne (1998) found that mineralisation and leaching rates of inorganic N could be much greater under lichen and moss mats on bedrock that in forest floors where refractory organic matter favours immobilisation. Immediate runoff during deposition, or rapid mineralisation and leaching in areas of lichen and moss, might therefore be expected from areas without substantial soils. At the Gwy, the proportion of the catchment occupied by the most active peaty gley soil (G2) may be relatively minor, while peat soils are widespread on hilltops and in riparian areas. On the gentler slopes the podsol is widespread, and shows a much lower nitrification potential than the peaty gley there, or the mineral soils at Scoat Tarn.

At the Etherow, nitrification rates in the predominant peat soils are likely to be very low, except for slightly elevated rates in hotspots under burnt areas, despite the relatively high soil temperatures and low soil moisture at this site. Hence the very high leaching rates of NO$_3^-$ must reflect direct runoff from deposition inputs. The presence of NH$_4^+$ in streamwaters there further suggests that nitrification is suppressed, and that direct runoff may be important. The speculation in Chapter 3 that low nitrification rates might be expected, given the high soil acidity at the Etherow, is borne out by the data here. It might be further speculated that the very high deposition load of sulphur, and probably of heavy metals, at this site, will have further contributed to the suppression of nitrification. It was shown in Chapter 4 that fluxes of CO$_2$ at the Etherow are much lower than at the Gwy or Scoat Tarn, indicating low rates of microbial activity.

5.4.3 Changes in mineralisation and nitrification induced by N saturation

The role of nitrification in regulating current NO$_3^-$ leaching is not conclusively demonstrated by this study, although it appears to be important at Scoat Tarn and perhaps the Afon Gwy. For critical loads mass balances, it is the potential rate of nitrification and leaching at steady state that is required, and N saturation of the system affects both of these processes. Nitrification has been described as “the gateway to N leaching and denitrification” (Gundersen, 1991), and is assumed to have a fundamental role in future NO$_3^-$ leaching. Aber et al. (1989) stated that nitrification
is a pivotal process as N saturation progresses, being induced by high \( \text{NH}_4^+ \) inputs, even at low soil pH down to 3.5, potentially leading to changes in ecosystem structure and function, as well as increased \( \text{NO}_3^- \) leaching.

In a comparison of similar soils on either side of an upland stream, one bank of which was subjected to very high N loads from septic tank drainage while the other was covered by unimpacted forest, clear signs of N saturation were found, with higher potential mineralisation and nitrification in enriched soils compared with control soils (Hanson et al., 1994). Much of the increased N load was thought to be stored in “passive” SOM pools, and not susceptible to leaching losses. Furthermore, microbial biomass C and N were not significantly greater in the enriched site, so microbes were just a “conduit” for N rather than an increased pool in themselves. Accelerated nitrification has been linked to prolonged, chronic N deposition in forests in the USA and Sweden (McNulty et al., 1996 and Diekmann et al., 1999, both cited in NEGTAP, 2001).

The temporal pattern of \( \text{NO}_3^- \) leaching, as well as the annual flux, is also likely to be affected by increased rates of mineralisation and nitrification. According to Stoddard (1994), N saturation induced by chronic N deposition may influence the timing and magnitude of inorganic N losses by increasing mineralisation through a “priming effect” induced by fertilisation. The early spring maximum in N loss from temperate forests is attributed to microbial mineralisation of organic matter, and subsequent nitrification, occurring before overstorey uptake becomes an important N sink (Zak et al., 1990; Groffman et al., 1993). In unfertilised soils, the supply of \( \text{NH}_4^+ \) from mineralisation is often a dominant control of nitrification and therefore of \( \text{NO}_3^- \) availability, itself being controlled by interactive effects of soil type, climate and plant community composition (Groffman et al., 1988).

A key mechanism by which N saturation could enhance nitrification and \( \text{NO}_3^- \) leaching is a change in the C:N ratio of the organic matter being mineralised. Soil organic matter with a high C:N ratio generally mineralises more slowly (INDITE, 1994), while soils with a C:N ratio greater than 25-30 appear unable to nitrify, due to outcompetition of chemolithotrophic nitrifiers by heterotrophic microbes which immobilise N (Gundersen & Rasmussen, 1990). Hence an increase in the N content of
SOM, and the associated decrease in C:N ratio, could increase mineralisation and stimulate nitrification in soils where it was previously negligible. \( \text{NH}_4^+ \) released in disturbed systems could be immobilised by decomposers and cycled or retained until the effective C:N ratio was low enough to allow net N release (Vitousek & Melillo, 1979). The mechanisms by which the quality of SOM and its C:N ratio may change are discussed in Chapter 8.

5.5 Conclusions

Nitrification potentials are highly correlated with mineralisation potentials in those soils which show a proportional nitrification of more than 10%. Low nitrification potentials are associated both with very limited inorganic N availability (Allt a’Mharcaidh) and very high N availability in severely impacted soils (River Etherow).

At the Allt a’Mharcaidh, the terrestrial ecosystem is so severely N limited that large increases in N deposition would probably be required to stimulate mineralisation and nitrification rates sufficiently to induce \( \text{NO}_3^- \) leaching. Autotrophic nitrifiers are weak competitors for \( \text{NH}_4^+ \), so immobilisation by plants and heterotrophs can suppress nitrification where availability is low (van Miegrot et al., 1990). Furthermore, since N uptake by heterotrophs depends on C availability high C:N ratios will increase competition and reduce nitrification.

At the other extreme, the soils from the Etherow catchment are so highly acidified that nitrification is negligible despite very high levels of \( \text{NH}_4^+ \) in the soils, except where the N cycle is disrupted by burning. This site is not necessarily representative of a typical N saturated catchment, because of the confounding effects of very high S deposition and possibly heavy metals.

In the Afon Gwy and Scoat Tarn catchments, it seems likely that increases in mineralisation and nitrification rates would lead to enhanced \( \text{NO}_3^- \) leaching from soils. However, at the Gwy, inorganic N is currently removed rapidly from soil waters, so immobilisation would have to decrease for increased leaching to take place.
The effects of changes in mineralisation and nitrification rates cannot be assessed without considering changes in N immobilisation at the same time.

5.6 References


CHAPTER 6

N IMMobilisation – estimating the current rate using stable isotopes
6. N IMMOBILISATION – ESTIMATING THE CURRENT RATE USING STABLE ISOTOPES

6.1 Introduction and rationale

Given the relative unimportance of denitrification as a sink for N deposition inputs (Chapter 4) and the minor role of biomass removal through grazing or burning, the key term in the FAB mass-balance for N is long-term immobilisation in refractory soil organic matter. This process cannot be measured directly in a short-term experiment of one year, but techniques are available for determining the immediate fate of N inputs that can provide information on the degree to which N retention is biologically mediated in the terrestrial system. This chapter describes the fate of inorganic N inputs using a stable isotope tracer ($^{15}$N) for this purpose.

6.1.1 Definitions of N immobilisation

Immobilisation of N in terrestrial systems is a widely discussed but often poorly defined process in the literature. In the broadest sense, any N which is retained within the terrestrial system could be considered to have been immobilised there, but the dynamic cycling of N within soil-plant systems means that a temporal context is required in the definition of immobilisation.

The term ‘immobilisation’ is most frequently encountered in the context of microbial immobilisation, which is the biological incorporation of N into the tissue of soil microbes, generally heterotrophic decomposers (Richards, 1987; van Miegrot & Johnson, 1993; INDITE, 1994; Paul & Clark, 1996; Michelsen et al., 1998). While the life span of the average microbe may be very short, the uptake and release of N by the microbial population is a rapid process which ensures that a large pool of N, usually many times greater than the soil mineral N pool (INDITE, 1994), is effectively locked up in the microbial biomass even though there may be large gross fluxes of N into and out of this pool. Microbial immobilisation in this context may be defined as the difference between gross and net mineralisation processes. While gross mineralisation fluxes of N may be very big, a large proportion is generally
immobilised simultaneously in most soils (Richards, 1987; Paul & Clark, 1996; Tietema et al., 1998). Hence the measurement of net mineralisation rates using standard soil incubation techniques does not include the proportion of mineralised N that is very rapidly immobilised again by soil microbes (see Chapter 5). Although it is a very difficult process to measure directly (INDITE, 1994), $^{15}$N tracer techniques have successfully been used to quantify microbial immobilisation (Högberg, 1997).

According to Richards (1987), the mineralisation-immobilisation cycle is a basic concept of soil microbiology, whereby mineralisation and immobilisation of nutrients proceed concurrently, so that there is continual biological turnover in the soil. Implicit in this concept is the assumption that plants cannot compete successfully with microbes for inorganic nutrients, so that only when there is net mineralisation can they satisfy their nutrient demands. Ågren and Bosatta (1996) summarised a conceptual scheme to determine whether net immobilisation or mineralisation will occur in a soil, sometimes called the carbon-element theory, which evolved in the early Twentieth Century. The theory suggests that it is the balance between the availability of an element (here, N) in the decomposing substrate (i.e. the C:N ratio) and the microbial requirement for it that determines whether mineralisation or immobilisation predominates at a given time (see Chapter 8).

The rapid microbial uptake of mineralised N explains the counter-intuitive increases in immobilisation associated with the increased decomposition rates in forest litter described by some authors (Aber et al., 1989; Murdoch & Stoddard, 1992). Indeed, in a rare definition of immobilisation, Vinten and Smith (1993) suggest that while mineralisation is the process by which organic compounds in the soil break down to release $\text{NH}_4^+$ and carbon (as $\text{CO}_2$), immobilisation is the reverse process, by which inorganic N (usually $\text{NH}_4^+$) is incorporated into organic forms as microbial tissue during the decomposition process. However, it is recognised that this may be a short-term process, followed by the slow release of inorganic N from the soil microbial pool (Stoddard, 1994).

Given the simultaneous nature of the microbial mineralisation/immobilisation cycle, it is necessary to consider timescales when defining microbial immobilisation. Calculations of instantaneous immobilisation fluxes might reveal very large numbers,
but it is the net immobilisation which is of interest for modelling the fate of N deposition. The short-term cycling of N within the microbial system is not relevant at the timescales considered by models which consider periods of, say, years to decades. Over such periods, microbial immobilisation can only be a net sink for N if there is an increase in either the N content of the microbes or the overall microbial biomass, or both. Microbial immobilisation in this context is therefore directly comparable to vegetation uptake as a sink for N, and indeed some authors refer to N immobilisation in vegetation (e.g. Gundersen, 1992; Emmett & Reynolds, 1996; Epstein et al., 2001).

Mass balance models for N assume that over long timescales, the N content and biomass of vegetation and microbes cannot increase indefinitely (unless there is a harvesting or removal of biomass from the system), so there can only be finite sinks for N in living biomass.

According to Paul and Clark (1996), the microbial N requirement for growth determines whether N is immobilised in microbes or accumulates in the soil organic matter, but it is this latter process which some modellers consider as immobilisation. Over the long timescales generally considered by N mass balance models, immobilisation refers to the 'permanent' sinks for N in refractory soil organic matter. Tamm (1991) suggested that an uninterrupted accumulation of N would result in its presence in excess in the pool of recycling N, unless it was either removed from the system (by leaching, denitrification, volatilisation or organic matter removal) or permanently immobilised in, for example, peat or sediments. Peat formation, which occurs where conditions are unfavourable for decomposition, generally due to poor drainage, aeration and/or high acidity, causes rapid accumulation of largely undecomposed plant remains (Cresser et al., 1993), which could potentially immobilise large amounts of N. Similarly, in mature boreal forests in cold and wet areas, N may be immobilised in a thick and relatively inactive mor horizon (Tamm, 1991), although disturbance such as fire can stimulate mineralisation and release some of the immobilised N pool.

A specific link between modelling and the perceived definition of immobilisation was provided by Cresser et al. (1993), who pointed out that the acceptable degree of organic N accumulation (i.e. immobilisation) was a matter of debate in critical loads models for N. Even more specifically, Gundersen (1992) used the term $\Delta N_{\text{humus}}$ (N
accumulation in humus), rather than N immobilisation, in his description of a mass balance model for N critical loads, while Kamäri et al. (1992), in the same workshop proceedings, used the term \( N_{\text{imm}} \) (N immobilisation in the terrestrial catchment) for an equivalent mass balance for waters.

Often no distinction is made when discussing immobilisation between N uptake, short-term microbial immobilisation and longer term biotic or abiotic immobilisation in soil organic matter. For example, Reynolds et al. (1992) suggest that N can be immobilised by incorporation into animal, plant and microbial biomass within soils or sediments, while a later paper (Reynolds et al., 1994) refers to immobilisation in soil humus. Groffman et al. (1993) quantify immobilisation in the microbial pool but also in the soil total N pool, and discuss both microbial and vegetation immobilisation processes. Aber (1992) describes a possible impact of large, pulsed N additions to forests as the swamping of microbial immobilisation (see also Gundersen, 1998) but the favouring of rapid, abiotic incorporation into soil organic material.

This latter process refers to chemical immobilisation which is not biologically mediated. While it is generally assumed that most N immobilisation begins with vegetation or microbial uptake, immobilisation may also occur through abiotic processes, such as \( \text{NH}_4^+ \) adsorption via cation exchange in soils and direct abiotic immobilisation in soil organic matter (Aber, 1992; Gundersen, 1998; Högberg, 1997). The processes responsible for direct abiotic immobilisation of N are poorly understood. Richards (1987) describes the process of ‘ammonium fixation’, whereby some of the \( \text{NH}_4^+ \) released during the decomposition of proteins and amino sugars combines with quinones and polyphenols to form products of greater stability against microbial attack, while also pointing out that the chemical nature of about half the total N in soils remains obscure. Ågren and Bosatta (1996) summarised abiotic immobilisation processes as condensation reactions between decomposition products such as phenols and derivatives with either amino acids or ammonia. Elsewhere, \( \text{NH}_4^+ \) ‘fixation’ is described as the holding of \( \text{NH}_4^+ \) within clay minerals (Nommick & Vahtras, 1982, cited in INDITE, 1994).
6.1.2 Principles of $^{15}\text{N}$ tracer studies

While deposition input and leaching or gaseous output fluxes are relatively straightforward to measure, the fate of the component of atmospheric N inputs that is retained within the terrestrial soil-vegetation system is much more difficult to ascertain directly. N may be taken up by vegetation or microbes to become incorporated in the biomass, resulting either in increased growth and biomass or in increased tissue N content of the biota, or both. While a proportion of N inputs may be retained in biomass, particularly over the course of just one budget year which may be less than the life cycle of some plants, some will also be stored in refractory organic matter through incomplete mineralisation of dead material. In the longer timescale considered by the FAB model, it is assumed that net N uptake is zero, since N cannot accumulate in living biomass over very long timescales greater than the life of the plants; it will ultimately be either recycled internally within the soil-vegetation system or exported from it. The exception occurs when biomass is removed by harvesting (e.g. of forest) or grazing. Over the long term, net N retention can therefore only occur through immobilisation in refractory organic matter (see above).

Although the N content of soils and vegetation can easily be measured, the effective pool sizes of N are very large compared with annual deposition inputs, even at very high deposition sites (Aber, 1992; Reynolds & Edwards, 1995). Hence the detection of a change in the pool size would require the accurate measurement of incremental changes in N content of soils and vegetation, which are known to be spatially heterogeneous. Commonly used methods for measuring total N (e.g. the Kjeldahl method) are too insensitive to accurately quantify single-season changes in the total N content of a soil-plant system (Hauck, 1986). To overcome the problem of detecting small changes against a very large and variable background and to determine the fate of N inputs in the terrestrial ecosystem, a tracer is required, and this is available in the stable isotope $^{15}\text{N}$.

Stable isotope techniques provide a means of tracing the fate of N inputs to ecosystems, whether the source is biological fixation, fertiliser additions or atmospheric deposition. If the natural abundance of a scarce, stable isotope of N is
known in an ecosystem compartment, then highly elevated, artificial inputs of the otherwise rare stable isotope can be used to determine the fate of the added N.

In the atmosphere, by far the most abundant isotope of N is $^{14}\text{N}$, but another stable isotope, $^{15}\text{N}$, also occurs naturally in much lower quantities. The ‘standard’ $^{15}\text{N}:^{14}\text{N}$ ratio of atmospheric N$_2$ is generally taken as 1:272, but several physical and biological processes can result in a fractionation of the two isotopes, leading to changes in the relative proportion of $^{15}\text{N}$ within different compounds and in different parts of an ecosystem. For this reason, prior to the experimental addition of stable isotope tracers it is necessary to quantify the natural abundance of $^{15}\text{N}$ in each component of the ecosystem being studied. Natural abundance data can be ignored if a highly concentrated $^{15}\text{N}$ tracer is used (e.g. 99% enriched) but this was precluded on the basis of cost here. Note that the term ‘natural’ abundance is slightly misleading in that anthropogenic activities such as fertiliser additions or N emissions leading to atmospheric deposition of N species will affect the distribution of $^{15}\text{N}$ in an ecosystem, but measured values are still referred to as natural abundance to distinguish them from values measured after deliberate additions of $^{15}\text{N}$ enriched tracers.

6.1.3 Examples of $^{15}\text{N}$ tracer applications

Studies of $^{15}\text{N}$ natural abundance can provide information about N status and cycling in their own right (see Chapter 7), but they are also a necessary prerequisite to N additions experiments using $^{15}\text{N}$ to trace the fate of N inputs. If the initial concentration, or natural abundance, of $^{15}\text{N}$ is known then increases in abundance (expressed as parts per thousand difference from a standard, $\delta^{15}\text{N}$) following tracer additions can be used to determine the fate of N inputs over the timescale of an experimental study.

For example, Tietema et al. (1998) used a $^{15}\text{N}$ tracer to determine the fate of N deposition in coniferous forests within the NITREX project. They found the organic layer to be one of the most important pools capable of retaining large amounts of
inorganic N, mainly through microbial immobilisation for assimilation of biomass or incorporation into polymerised decomposition products, with 11-47% of inputs retained. Highest retention was found in $^{15}\text{NH}_4^+$ addition experiments, and the absolute retention increased (although the % decreased) with increasing inputs, to a maximum of c. 10 kgN ha$^{-1}$ yr$^{-1}$. Overall the study concluded that the results agree well with the N saturation concept, in which N leaching, indicative of a N saturated system, is attributed to the exceedance of the N demand by biological sinks, and the complementary behaviour of microbial N retention and leaching in response to changed N inputs indicates that the organic layer is the biological sink that is being affected by changes in N input.

Epstein et al. (2001) used 99% enriched $^{15}\text{NH}_4^{15}\text{NO}_3$ to look at the fate of N inputs to grazed shortgrass steppe in Colorado under different plant communities. After 2 years, mean $^{15}$N retention across all plots was 28.3%, most of which (24.9%) was found in soils - a substantially smaller figure than in other reported values for shortgrass steppe. Only 3.4% of added $^{15}$N was found in plants after 2 years, on average. Overall, the plant community that retained the greatest quantities of $^{15}$N over the short-term (1 month) had the least retention after 2 years, probably due to a greater allocation of $^{15}$N to aboveground plant parts susceptible to grazing and with annual turnover. Epstein et al. (2001) concluded that plants with high rates of N sequestration may not therefore be the best for long-term N retention, depending on nutrient allocation patterns.

In other examples of the use of $^{15}$N tracers, Garten et al. (1998) used $^{15}$N enriched, simulated wet deposition to demonstrate that forest canopy N uptake from wet deposition was small compared with dry deposition uptake, while Högberg et al. (1994) used $^{15}$N labelling to investigate the role of mycorrhizae in plant N uptake.

$^{15}$N tracers were used in separate studies of the controls on N losses from Michigan hardwood forests through NO$_3^-$ leaching and denitrification (Zak et al., 1990; Groffman et al., 1993). The earlier study used additions of $^{15}$N labelled NH$_4^+$ and NO$_3^-$ to show that although uptake by spring herbs may influence ecosystem level N fluxes, their regulatory role in the northern forest was less important than that of the microbial community (Zak et al., 1990). Microbial immobilisation was found to be
the most important mechanism retaining N prior to canopy development, while both the pool of N contained in the herbs and their $^{15}\text{N}$ uptake were small compared with microbial biomass and immobilisation. Further studies at the same site linked $^{15}\text{N}$ tracer “snapshot” measurements over 2 day experiments with longer term (8 week) measurements on well-drained and poorly-drained soils with and without herb cover (Groffman et al., 1993). As with the earlier study, microbial biomass and N processing were found to be key regulators of N dynamics. In the well-drained soils, the largest N movement was into microbial biomass and total soil N (i.e. primarily organic) and with this strong immobilisation there was little nitrification or denitrification. Conversely, there was little movement of $^{15}\text{N}$ into the microbial biomass and significantly less accumulation of $^{15}\text{N}$ in the total soil N pool of the poorly drained soils. Availability of $\text{NH}_4^+$ to nitrifiers was therefore high and rates of nitrification very high, leading to the accumulation of $\text{NO}_3^-$ which, when combined with high soil moisture, then led to high denitrification losses and a high potential for leaching losses.

6.1.4 Purpose of the $^{15}\text{N}$ additions study

Given the above discussions on the various interpretations of ‘immobilisation’, it is evident that the long term process of immobilisation in refractory organic matter, while being of most interest for static (equilibrium) mass balance models, is by definition the most difficult to quantify experimentally because of the poor understanding of the processes and the timescales over which it would have to be measured. However, the short term fate of N deposition inputs are still of interest because most N must be biologically cycled before it can be immobilised in unreactive organic compounds within soils.

The purpose of the study which forms the basis of this chapter is to determine the fate of $^{15}\text{N}$ labelled N inputs over a period of one year of additions. As detailed in Section 6.1.1, the processes included under the broad definition of immobilisation are determined by the time period under consideration. While the long term immobilisation of N cannot be determined in a study over just one year, it is possible to quantify both the proportion of the input that is incorporated in the vegetation, and
the proportion that is retained within the soils, presumably via microbial immobilisation. These data should improve our understanding of the initial fate of N deposition and the processes via which long term immobilisation might be controlled. A key assumption is that the $^{15}\text{N}$ tracer inputs will behave in a way which is representative of annual mean deposition inputs of inorganic N.

6.2 Sampling and analytical methods

6.2.1 General approach and baseline data

In order to establish the 'natural' distribution of $^{15}\text{N}$ within catchment soils and vegetation (i.e. prior to the deliberate addition of elevated concentrations of $^{15}\text{N}$), a survey of its natural abundance was required. This survey provides the baseline data against which increases in the abundance of $^{15}\text{N}$ can be assessed, and thereby the proportion of the tracer additions retained within any measured soil or vegetation compartment can be quantified. Techniques for the calculation and application of $^{15}\text{N}$ doses are described in Section 6.2.2 below.

Sampling techniques for the major pools (above-ground vegetation, organic/rooting layer and soils) are described in Section 6.2.3 below. The same techniques were employed for both the $^{15}\text{N}$ natural abundance assessment in Chapter 7 and the post $^{15}\text{N}$ additions sampling described here.

Samples for the natural abundance assessment were taken from within the grazing exclosures and from adjacent areas outside the primary experimental plots, since destructive sampling within the plots was not appropriate at the beginning of the budget year. At the end of the experimental year, post $^{15}\text{N}$ additions samples were taken from the centre of each of the primary experimental plots. The locations of experimental plots are described in Chapter 3.
6.2.2 Calculation of $^{15}$N tracer dosage and application method

While the natural abundance technique provides information on “ambient” values of $^{15}$N and indications of N status from the experimental areas, the fate of atmospheric N inputs was to be established through the addition of labelled $^{15}$NH$_4$$^{15}$NO$_3$. Since it was not the purpose of this particular experiment to examine the effects of additional N enrichment beyond the current levels, the fertilisation effect was minimised by using a very small dosage of labelled solution equivalent to 1.5 kgN ha$^{-1}$ yr$^{-1}$ (assuming the atomic weight of N is 14), compared with a range in current deposition inputs of 7-34 kgN ha$^{-1}$ yr$^{-1}$ for the four study catchments.

Furthermore, since a significant wetting effect could confound the results, the volume of solution applied was also minimised. Since 0.5 x 0.5m quadrats were to be sampled from the centre of primary experimental plots, the $^{15}$N solution was added evenly across each 3 x 1m plot, thereby minimising edge effects (Figure 3.7). The $^{15}$N dose was applied fortnightly in identical amounts throughout the budget year (26 applications). Each dose comprised 1L of solution finely sprinkled evenly across the plot, followed by 0.5L of de-ionised water to help rinse in the labelled N. The 0.5L rinse with de-ionised water was intended to prevent the build up of label on the vegetation surfaces where evaporation could lead to high concentrations of label. The total wetting effect amounted to 39L of water per plot over the course of a year, which is equivalent to 13L m$^{-2}$ or 13mm of rainfall.

Calculation of the required tracer dosage is provided in Appendix 3. The total addition of $^{15}$N to the quadrat (area 0.25m$^2$) was 12.05 mg $^{15}$N. This figure excludes the contribution of “natural abundance” $^{15}$N in deposition.

At the end of the budget year, quadrats were destructively sampled in an identical way to the natural abundance study (see below) to ensure comparability of results.
6.2.3 Sampling methods

6.2.3.1 Vegetation
The vegetation samples collected for the determination of biomass in the grazing exclosure experiment (Chapter 3 above) were used for the measurement of $^{15}$N natural enrichment. At the uppermost Mharcaidh plots (M1) three sets of paired quadrats were sampled specifically for the $^{15}$N study, since none had been sampled for the grazing exclosure work. The wet weight of the total bulk sample was measured, and a representative (random) subsample of around 25% taken for sorting and analysis. For all sites, heather (Calluna or Erica sp.) samples were sorted into ‘year’s growth’ shoots (removed with scissors) and dead/woody material. Other vegetation was sorted into live and dead grass, combined mosses and lichens (with no separation of live and dead), and litter. All samples were weighed wet prior to drying at 40°C for at least 48 hours. The dry weight of all samples was recorded. Large, dried, samples of vegetation were coarsely ground using a Fritsch mill fitted with a 2mm sieve. The vegetation samples were then subsampled and a further 1-2g were finely ground using a liquid N cooled freezer mill (SPEX model 6700, Glen Creston Ltd., Middlesex, UK).

6.2.3.2 Organic/root layer
Two adjacent 10×10cm squares of turf (organic soil and root layer) were removed from the centre of the vegetation plot using a knife. This layer was generally 2-10cm in thickness, and was distinct from the organic and mineral soils below. The paired turf samples were cut in half, and amalgamated into two samples with a half from each original sample. One amalgamated sample was weighed wet prior to drying (“soil organic layer”). The second amalgamated sample was washed and sieved with de-ionised water to extract a “root only” sample. All samples were then dried at 40°C for at least 48 hours. Samples were coarse- and freezer-milled as for the vegetation above.

6.2.3.3 Soils
Gouge augers were used to obtain soil cores from the centre of both 10×10cm turf plots down to either bedrock or 50cm depth, whichever was reached first. Variable
diameter augers were used, starting with a 5cm diameter and moving onto a 3cm diameter auger for the second part of the core. The soil core was separated by horizon (colour change or mineral content) and depths recorded. Soil samples were dried at 40°C for at least 48 hours. Organic soils without stones were coarse- and freezer-milled as for the vegetation and turf samples. Mineral soils and organic soils containing stones were first coarse milled through a 2mm sieve (rather than using the Fritsch mill) to remove larger stony fragments. “Loss-on-ignition” (LOI), measured for each soil sample in the natural abundance survey (Chapter 7), was used in the same way for the corresponding soil samples in this study to estimate bulk density according to the equations of Harrison and Bocock (1981). Soils were bulked into two samples per core as in the natural abundance study, weighted by bulk density (upper organic and lower organic + mineral where present). Bulked samples were then freezer-milled as for other vegetation and organic soils prior to analysis.

Samples were sent to the NERC Stable Isotope Facility at CEH Merlewood for isotopic analysis. Between 5 and 50 mg of sample (depending on approximate N content) were weighed into tin capsules using a Sartorius M3P microbalance before analysis. Total N and δ¹⁵N were determined using a system comprising a Carlo-Erba NA1500 elemental analyser coupled to a modified Dennis Leigh Technology Isotope Ratio Mass Spectrometer.

6.2.4 Calculation of ecosystem compartment N pools

In order to determine the total mass of tracer (and hence deposition) retained per unit area through the measurement of incremental changes in the proportion of total N that is ¹⁵N, it is necessary to quantify the N pool sizes within each of the sampled compartments.

6.2.4.1 Vegetation

Where a compartment had been subsampled within a quadrat, dry weights were multiplied up to give figures for the whole quadrat. Given the great spatial variability in vegetation cover between quadrats and within plots, there was no basis for
assuming that a quadrat was more representative of its associated plot than any other quadrat on the replicate plots. Therefore, for the purposes of calculating component biomass within plots, mean dry weight values were used from the three replicated samples. However, individual sample %N and δ¹⁵N figures were used in each plot so that standard deviations in these variables could be calculated for each soil type (see also Tietema et al., 1998).

6.2.4.2 Organic/root layer
The turf layer and root samples were treated in the same way as the vegetation samples for the calculation of N pool sizes. Mean dry weights from the three replicated plots were used to calculate N pools, while individual sample %N and δ¹⁵N values were used to calculate standard deviations. Since the surface organic material was not analysed separately, the associated N pool was calculated as the difference between that in the whole turf layer sample and that in the roots.

The turf layer and root samples of 100 cm² were assumed to be representative for their associated quadrat (area = 2500 cm²) and plot (area = 30000 cm²).

6.2.4.3 Soils
Soil samples from two horizons were analysed for δ¹⁵N from each plot, with plots replicated three times for a given soil type. Horizon depth, bulk density and %N were required to calculate the compartment N pool size:

\[ \text{mass of N in soil pool} = \text{mass of soil} \times \%N \text{ by mass} \]

and

\[ \frac{\text{soil mass / unit area (g cm}^2\text{)}}{\text{unit area (g cm}^2\text{)}} = \text{horizon thickness (cm)} \times \text{bulk density (g cm}^{-3}\text{)} \].

The analysis for δ¹⁵N provides %N data so it was only necessary to estimate the mass of soil in each sampled horizon per unit area. The bulk densities of the sampled soil horizons were estimated from loss-on-ignition (LOI) data as part of the natural abundance study (see Chapter 7); mean bulk density (g cm⁻³) values for the three
corresponding replicated soil samples from the natural abundance study (prior to $^{15}\text{N}$ addition) were assumed to be representative for each of the post $^{15}\text{N}$ additions samples. Horizon thickness for each individual sample was measured at the time of sampling and assumed to be representative for the quadrat (area = 2500 cm$^2$) and plot (area = 30000 cm$^2$).

Calculation of the N pool for the upper sampled horizon (upper organic = soil depth 1) was straightforward, since a single post $^{15}\text{N}$ additions sample was obtained from each plot. For the lower sampled horizons, an extra stage of calculation was required because natural abundance data were available only for bulked soil samples, generally comprising two separate horizons, but varying from one to three horizons. For comparability, post $^{15}\text{N}$ additions samples, for which separate $\%\text{N}$ and $\delta^{15}\text{N}$ data were available, had to be bulked in the same way. Details of the method for calculating soil N pools and average $\delta^{15}\text{N}$ of bulked soils are provided in Appendix 3.

### 6.2.5 Calculation of $^{15}\text{N}$ tracer retention in soil and vegetation compartments

Atom% $^{15}\text{N}$ values were used to calculate tracer retention (see Appendix 3):

$$m_{lab} = m_i (A_f - A_i) / (A_{lab} - A_f)$$

where $m_{lab} =$ mass of label, $m_i =$ initial mass of N pool, and $A_i$, $A_f$ and $A_{lab}$ are the initial, final and label atom% $^{15}\text{N}$ values.

In some cases, post $^{15}\text{N}$ additions samples were obtained for compartments for which no natural abundance values were available, either due to their absence from the natural abundance plots sampled, or due to methodological differences in the splitting of samples. Examples include mosses at the Afon Gwy, live and dead grass at the Allt a’Mharcaidh and litter from most sites.

While this presented a problem in obtaining a precise measure of $^{15}\text{N}$ tracer retention, the very high values of $\delta^{15}\text{N}$ associated with the post additions samples (generally of
the order of hundreds, and sometimes thousands, per mil: see results below) meant that assumptions could be made about the natural abundance of $^{15}$N with very small associated errors in relative terms. While most natural abundance figures for any compartment were in the range $-10$ to $+10$ $\%$, increases in $^{15}$N abundance following tracer additions were generally hundreds of parts per thousand. Where natural abundance data were unavailable it was therefore decided to use the mean $\delta^{15}$N of other samples of the same compartment from that site, or if none were available, a natural abundance of $0\%$ was assumed. The maximum error introduced by this assumption if the natural abundance figure is out by $10\%$ is almost always less than $5\%$ and often less than $1\%$.

Since tracer additions of $^{15}$N to the sampled quadrat were known ($12.05$ mg $^{15}$N over one year), the mass of tracer retained in each sampled compartment for each plot can be expressed as a percentage of inputs. This is assumed to be the same fate as that of deposition inputs over the experimental year (see discussion).

6.3 Results – fate of $^{15}$N additions

Mean figures for the overall retention of $^{15}$N in all compartments are shown in Table 6.1. Results for vegetation, litter plus surface organic material and soil compartments are presented separately below.

6.3.1 Retention of $^{15}$N in vegetation compartments

Retention of the $^{15}$N tracer in total vegetation (i.e. the sum of green heather, woody heather, lichen + moss, live grass, dead grass and root compartments) is illustrated in Figure 6.1, where mean values $\pm 1$ SD are plotted.

There is an apparent general decline in percentage vegetation retention of $^{15}$N tracer from left to right, along the gradient of increasing N deposition between sites, although the differences between sites are not significant. On the M1 plots from the Allt a'Mharcaidh, the mean value of $105.7\%$ retention, while clearly not possible,
shows a very high standard deviation which demonstrates the particularly great variability on this soil (the peaty ranker). Likewise, the data from the peaty gley plots at the Afon Gwy (G2), which are based on just two samples (one of the three replicate samples was lost), show a mean retention of just over 100% but are very variable. Hence for these two sets of samples the maximum possible retention figure of 100% is easily encompassed within one standard deviation of the mean.

The standard deviations of the mean values for all other plots are generally much smaller, and consideration of the mean retention values in Figure 6.1 and Table 6.1 shows an apparent decline across sites, from c. 56 – 106% at the Mharcaidh and 52 – 102% at the Gwy, to a range of 43 – 52% at Scoat Tarn and 37 – 41% at the Etherow. If these figures are converted into absolute values using deposition estimates (Figure 6.2), the pattern changes. There is a low rate of retention at the Mharcaidh (4-8 kgN ha⁻¹ yr⁻¹), while the highest absolute rates are found at the Gwy (c. 14-28 kgN ha⁻¹ yr⁻¹) and Scoat Tarn (c. 15-17 kgN ha⁻¹ yr⁻¹). In particular, the G2 plots stand out for showing a very high absolute value of mean N retention, while rates on other soil types are mostly comparable at the Gwy and Scoat Tarn. Slightly lower rates are found at the Etherow (c. 13-14 kgN ha⁻¹ yr⁻¹), but the absolute values are still much greater than at the Mharcaidh.

Figure 6.1: Total retention of ¹⁵N tracer in all vegetation compartments (±1SD)
Hence while N retention in vegetation must be linked to N demand, it cannot simply be assumed that decreasing percent retention of N in vegetation as deposition increases reflects a constant N demand which is met by a smaller proportion of inputs. Figure 6.2 shows that absolute values of N retention are much lower at the Mharcaidh than elsewhere, yet there is not the 100% retention of the tracer (except perhaps at M1) that might be expected if N demand alone controlled N retention. Also, at the Gwy the G2 plots show much greater absolute and percentage retention of $^{15}$N than plots on other soil types which are assumed to receive a similar deposition load.

**Figure 6.2: Overall retention of inorganic N deposition in vegetation indicated by $^{15}$N tracers (±1SD)**

The type and density of vegetation cover are also likely to influence the retention of N inputs. The biomass of each of the sampled compartments is listed in Table 6.2, and shows that there are large difference between sites, both in terms of the dominant vegetation types and in overall biomass per unit area. Figure 6.3 shows that there is an apparent relationship, albeit fairly weak, between percentage N retention in above ground vegetation and its total biomass. Inspection of the data in Table 6.2 shows that
the Etherow is an outlier in this regard; in particular, the mature *Calluna* plots at E2 have the highest biomass but the lowest percent retention in vegetation. Hence in this case it is evidently not just biomass which determines N retention. However, if the Etherow data are excluded from the plot of retention against biomass, there is a very strong correlation between percent retention of N in vegetation and total biomass at the other three sites (Figure 6.4).

**Figure 6.3: Tracer retention in above-ground vegetation vs. biomass**

![Graph showing the relationship between biomass and tracer retention in vegetation.](image)

If the same plots are drawn using total plant biomass and retention including roots, the correlations are much weaker, even when the Etherow data are excluded. While the above ground parts of the plants retain tracer in proportion to their biomass, this is not necessarily reflected in the roots. Figure 6.5 shows that while there is a general relationship between root biomass and $^{15}$N retention, there is much scatter. Several factors may be responsible for the weakness of this relationship. Direct uptake of N through foliage could bias retention towards the above ground biomass. The interception of inputs by surface lichens and mosses which are not represented in the root samples could prevent atmospheric N from reaching the roots of other plants; this appears to be the case at the Mharcaidh (see Table 6.1). Another possibility is that
there are differences between species and/or between sites in the proportion of N uptake which is stored in roots directly and that which is translocated into above ground biomass (i.e. new growth). Productivity may be an important factor here but the data are lacking to test this.

Figure 6.4: Tracer retention in above-ground vegetation vs. biomass (excluding Etherow samples)

Since the Etherow plots are much more strongly dominated by *Calluna* than those at other sites, it might be speculated that *Calluna* has a lower N demand than the lichens, mosses and grasses which are more important at other sites.

To compare the effectiveness of a given vegetation compartment as a sink for N at different sites, it is necessary to standardise the data so that percent retention in vegetation is expressed relative to the total vegetation sink. The relative importance of each vegetation component as a proportion of the total vegetation N sink is shown in Table 6.3. Presented in this way, the data show which part of the vegetation is most important in retaining N relative to other vegetation components, and major differences are apparent between sites.
Table 6.1: Mean retention of $^{15}$N tracer (%) in vegetation and soil compartments over one year of additions

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<th>Green heather SD</th>
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<th>Woody heather SD</th>
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<th>Dead grass SD</th>
<th>Moss Mean</th>
<th>Moss SD</th>
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<th>Roots SD</th>
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<th>TOTAL VEGN. SD</th>
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311
Table 6.2: Mean biomass of vegetation compartments on each soil type (g. dry weight m\(^2\))

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<tr>
<td>E1</td>
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<td>0.2</td>
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<td>0.0</td>
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<td>0.0</td>
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<td>294.0</td>
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Table 6.3: Relative importance of vegetation components as proportions of total vegetation N sink (%)


<table>
<thead>
<tr>
<th>SOIL</th>
<th>Green heather</th>
<th>Woody heather</th>
<th>Live grass</th>
<th>Dead grass</th>
<th>Lichen &amp; Moss</th>
<th>Roots</th>
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<tbody>
<tr>
<td>M1 Peaty ranker</td>
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<tr>
<td>M2 Valley peat</td>
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<td>17.2</td>
<td>0.8</td>
<td>9.5</td>
<td>59.5</td>
<td>3.0</td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>9.6</td>
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<td>1.3</td>
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<td>2.5</td>
</tr>
<tr>
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<td>9.8</td>
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<td>0.1</td>
<td>3.1</td>
<td>68.3</td>
<td>3.7</td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>2.5</td>
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<td>8.4</td>
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<tr>
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<tr>
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<td>35.0</td>
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<td>9.2</td>
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<tr>
<td>E1 Peat (burnt)</td>
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</tr>
<tr>
<td>E2 Peat (unburnt)</td>
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<td>0.0</td>
<td>0.0</td>
<td>2.4</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Figure 6.5: Relationship between $^{15}$N tracer retention in roots and root biomass

At the Mharcaidh, shrubs account for only 25-31% of the vegetation N sinks, while lichens and mosses are twice as important, consistently retaining around two thirds of the N which is taken up by the vegetation on all soil types. Grasses and especially roots are only minor components of the total vegetation N sinks at the Mharcaidh.
The Gwy shows the greatest variation between soil types of all four catchments, in terms of which vegetation sinks are most important. Shrubs are insignificant as a sink for N, while grasses provide a significant sink, from 20 to almost 50% of the vegetation N sink. At G4, more of the tracer is retained in the dead grass component than in the standing live grass. Lichens and mosses are the single biggest vegetation N sink on all soils except the podsol at G3, ranging from a third of the total vegetation N sink at G3 up to more than two thirds at G2. Roots also provide a very variable N sink between soil types, from 6 – 29% of the total in vegetation.

Grasses are the single biggest vegetation N sink at Scoat Tarn, accounting for around 50-60% of N retention in vegetation. A greater proportion of the tracer is retained in the dead grass than in the live component at S1 and S3, while the two are very similar at S2. Lichens and mosses are also significant, comprising c. 30-40% of vegetation N sinks over the study period. Plant roots are only a minor N sink at this site.

At the River Etherow, the shrubs are by far the largest vegetation N sink (78-92%), with roots having a greater importance relative to above ground biomass in the burnt Calluna plots at E1, which is consistent with the removal of above ground biomass, but not roots, by burning. Mosses are also more important in the burnt plots than under the mature Calluna, suggesting that they have either been faster to regrow or recolonise than Calluna after burning, or that they were less impacted by the burning itself. Alternatively, direct deposition of tracer onto mosses and lichens is likely to be much more significant when the Calluna “canopy” has been removed by burning.

The relative importance of vegetation N sinks in terms of above-ground biomass (i.e. excluding roots) is shown in Table 6.4. Since roots were shown to be mostly a minor sink for N in Table 6.3 the patterns in the above ground data are very similar. The biggest differences occur at the Gwy, where grasses now comprise around 20-60% of above ground vegetation N sinks, while mosses and lichens increase in importance to make up around 40-80% of the total sink. At the Etherow, the overwhelming importance of shrubs is highlighted still further; they comprise 91-97% of the above ground biomass N sink.
Table 6.4: Relative importance of vegetation components as proportions of total

above-ground vegetation N sink (%)

<table>
<thead>
<tr>
<th>SOIL</th>
<th>Green heather</th>
<th>Woody heather</th>
<th>Live grass</th>
<th>Dead grass</th>
<th>Lichen &amp; Moss</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 Peaty ranker</td>
<td>7.5</td>
<td>24.0</td>
<td>0.3</td>
<td>2.3</td>
<td>65.9</td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>10.4</td>
<td>17.7</td>
<td>0.8</td>
<td>9.7</td>
<td>61.4</td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>9.8</td>
<td>20.2</td>
<td>0.0</td>
<td>1.3</td>
<td>68.7</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>10.2</td>
<td>15.6</td>
<td>0.1</td>
<td>3.2</td>
<td>70.9</td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>3.0</td>
<td>1.7</td>
<td>23.5</td>
<td>9.9</td>
<td>61.9</td>
</tr>
<tr>
<td>G2 Peaty gley</td>
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<tr>
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<tr>
<td>G4 Valley peat</td>
<td>0.0</td>
<td>0.0</td>
<td>10.1</td>
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<td>59.1</td>
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<td>S2 Peaty gley</td>
<td>0.0</td>
<td>0.0</td>
<td>32.2</td>
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<td>38.8</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>0.1</td>
<td>0.0</td>
<td>29.0</td>
<td>38.6</td>
<td>32.3</td>
</tr>
<tr>
<td>E1 Peat (burnt)</td>
<td>40.9</td>
<td>50.0</td>
<td>0.0</td>
<td>0.0</td>
<td>9.1</td>
</tr>
<tr>
<td>E2 Peat (unburnt)</td>
<td>38.3</td>
<td>59.1</td>
<td>0.0</td>
<td>0.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

It is assumed that N retention in vegetation compartments is due to biological uptake of inorganic N, which may have been taken up directly in the \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) forms applied, or, in the case of \( \text{NH}_4^+ \), following rapid nitrification in the surface soil horizons. However, it is also possible that a proportion of the N retained was simply concentrated on vegetation surfaces by evaporation following addition, and it not possible to quantify these processes. It does however, seem unlikely that concentration on foliage by evaporation could have left a significant proportion of the applied tracers in situ over the course of a whole year, given the high rainfall experienced by these sites. The proportion of the \( {}^{15}\text{N} \) tracer which passed through the vegetation pool and was lost via processes such as litterfall during the experimental year is also unknown, but this proportion would inevitably increase if similar analyses were made in successive years following additions. The importance of the dead grass component of vegetation as a sink for N at the Gwy and Scoat Tarn plots illustrates the complexity of following the fate of tracer additions over just one year. It must be assumed that much of the dead grass had grown and then died during the experimental year in order to have retained significant amounts of tracer (assuming that the tracer was incorporated via uptake and had not simply accumulated on the vegetation.
The life cycle of the different plants which retain N must therefore be taken into account.

Since there are many factors which could account for differences in the relative importance of different vegetation types as N sinks between sites, such as differences in distribution, biomass, rooting patterns or in relative ability to intercept deposition inputs directly, it is necessary to consider more standardised measures of their retention efficiency between sites. This can be done by calculating the retention per unit biomass for each vegetation type at each site.

Figure 6.6 shows the relationships between $^{15}$N retention and compartment biomass for green and woody components of shrubs, grasses and mosses/lichens. In all cases there is a strong correlation between retention and biomass. However, when percent retention per unit biomass (g dry weight per square metre) is plotted against sites (i.e. against the deposition gradient) very different patterns are apparent for each vegetation component (Figure 6.7).

Shrubs, which are only widespread at the Mharcaidh and Etherow sites, show no obvious pattern other than a consistently greater retention per unit biomass in the green (year's growth) component than in the woody stems (Figure 6.7a). This is consistent with the uptake of $^{15}$N during the growing season, which is largely incorporated in the new growth, while the older woody material is ‘diluted’ with pre-tracer addition N. Of the green shoot samples, there is little difference in efficiency of tracer retention between the Mharcaidh plots and the solitary sample from the Gwy. The lowest efficiency is found in the solitary sample from Scoat Tarn, which comprised one small shoot, while the biggest range of values occurs at the Etherow. In the new growth of Calluna at E1, the highest tracer retention efficiency for all shrub samples at all sites is found, presumably reflecting the vigorous regrowth of Calluna following burning there. Conversely, the mature Calluna shoots at E2 have the lowest retention efficiency of all sites except the solitary Scoat sample.

Unlike shrubs, similar patterns of retention efficiency are found between live grass and combined lichen/moss samples (Figure 6.7b & d) across the four catchments. The general pattern is of increasing retention efficiency from low values at the Allt
a'Mharcaidh to the highest at Scoat Tarn, with the Afon Gwy intermediate between these two sites, i.e. retention efficiency increases with N deposition inputs for these sites. At the Etherow, however, there is a sharp decline in retention efficiency in both lichens/mosses and grass samples. It should be noted that the same plot using total (live plus dead) grass instead of just live grass, to account for the importance of the tracer retention in the dead grass compartment, does not reveal the same pattern, and this could be attributed to the problems of accounting for vegetation life cycles and separating 'old' (pre-additions) dead samples from 'new' (died during the season of $^{15}$N additions) dead material.

While it might be speculated that these vegetation sinks for N deposition have 'switched off' at the Etherow, other factors must be considered first. In particular, the great dominance of Calluna at the Etherow might suggest that there is simply a greater interception of atmospheric inputs directly by the 'canopy' of Calluna plants. Edwards et al. (1985) suggested that in upland moorlands, a large proportion of NO$_3^-$ deposition may be retained by vegetation foliage which intercepts precipitation. There is, however, no evidence for such a process in the plot for shrubs in Figure 6.7, which shows that the Etherow shrubs are, if anything, less efficient at retaining N than their counterparts at other sites. Furthermore, despite comprising a greater proportion of total and above ground vegetation sinks for $^{15}$N, the Etherow shrubs still only retain less than a third of the $^{15}$N tracer additions, and could not therefore be responsible for the poor efficiency of retention in the grass and moss samples via out-competing them for the inorganic N.

For the root samples, the same pattern is observed as for grasses and lichens/mosses with the exception of the burnt Calluna plots at E1 (Figure 6.7c). Retention efficiency is lowest at the Mharcaidh, high (and similar) at Scoat Tarn and the Afon Gwy, and decreases again under the mature Calluna at E2. The burnt Calluna plots at E1 are exceptional because of the recent disturbance by burning (as discussed above), and the data show a very high retention efficiency but great spatial variability.

Overall, several general patterns can be observed in terms of the retention of the $^{15}$N tracer across the deposition gradient represented by the four catchments, despite differences in vegetation types and relative biomass between sites.
Figure 6.6: Relationship between tracer retention (%) and compartment biomass

a: Green shoots of shrubs

b: Woody component of shrubs

c: Live grass

d: Mosses & lichens
Figure 6.7: $^{15}N$ Retention (% per g dry weight compartmental biomass)
1. The proportion of the $^{15}$N tracer retained in total vegetation declines along the gradient of increasing deposition inputs (Figure 6.2).

2. There are strong correlations between percent $^{15}$N retention in a vegetation compartment and its biomass, which is weakest for roots (Figure 6.6).

3. The efficiency of all vegetation compartments in retaining the $^{15}$N tracer, except perhaps for shrubs, increases with increasing deposition from the Mharcaidh and Gwy to Scoat Tarn, but decreases again at the most impacted site, the River Etherow (Figure 6.7).

Therefore, with increasing N deposition it appears that vegetation uptake of inorganic N within the sampled plants increases from the Mharcaidh to the Afon Gwy and Scoat Tarn, leading to a greater retention of N per unit biomass, but that differences in biomass between sites have a greater influence on overall vegetation N retention than these differences in efficiency (see Figure 6.4). With a smaller above ground biomass per unit area at the Gwy and Scoat Tarn plots than at the Mharcaidh, the decline in net vegetation retention of N along this gradient can be attributed simply to these differences in biomass.

The River Etherow plots, however, do not fit into this general pattern. Despite the highest biomass of all sites occurring on the mature Calluna plots at E2, vegetation retention there is the lowest of all sites. The grasses and mosses/lichens at the Etherow also demonstrate much lower N retention per unit biomass than other sites, despite the large deposition load at the Etherow. It can only be concluded that vegetation at the Etherow is severely N saturated, to the extent that there is not just a levelling off of vegetation N uptake with enhanced deposition there, but there is actually a decline in uptake to lower levels than are found at other sites where N deposition inputs are lower. What cannot be ascertained is whether this effect is due solely to N saturation or to other related factors like soil acidification, which is also most pronounced at the Etherow, and caused by very high sulphur as well as N deposition.
6.3.2 Litter and surface organic layer

Since the litter layer and surface organic horizon are intimately linked, to the extent that at some plots it was not possible to obtain a distinct litter sample, the N retention data for these compartments are presented together in Figure 6.8.

There is no obvious pattern in N retention within these compartments across the deposition gradient represented by the four sites. At the Mharcaidh, litter consistently retains around 10-12% of N inputs across the four soil types, while the surface organic layer, although very variable between soils (14-37%), provides a greater N sink than the litter on each soil type.

At the Gwy the situation is reversed, with the surface organic layer retaining around 10-20% of N inputs while litter accounts for 16-36%. Only on the podsol at G3 does the litter retain less N than the surface organic layer.

**Figure 6.8: Retention of $^{15}$N tracer in litter and the surface organic layer (±1SD)**

The relative importance of these two N sinks varies widely between soil types at Scoat Tarn. On the highest podsol plots at S1, very high retention in the surface
organic layer corresponds with zero retention in the litter. Since these plots were the only ones where a separate litter layer could not be defined, it is not surprising that particularly high retention is found in the surface organic layer which must incorporate any litter. On the peaty gley plots at S2 litter is a much more important N sink (33%) than the surface organic layer (8%), while on the lakeside peat plots at S3 the figures are very similar, at 11% and 14% for litter and surface organic layer respectively.

Similarly, at the Etherow there are large differences between burnt and unburnt plots. On the burnt \emph{Calluna} plots (El) retention in the surface organic layer is very high (37%) but variable between plots, while N retention in the litter is much lower (10%) and more consistent between plots. The situation is reversed on the mature \emph{Calluna} plots (E2), where litter is the more important sink (26%) compared with the surface organic layer (10%). This reversal is presumably due to the removal of litter by burning on the El plots while a distinct litter layer is still present under the mature \emph{Calluna}.

Given the variability in the relative importance of these two compartments as N sinks between sites and soil types, their combined values are illustrated for simplicity in Figure 6.9. These combined sinks retain from around a quarter to more than a half of $^{15}$N tracer inputs, and while the high standard deviations indicate great spatial variability within any set of three replicated plots, they undoubtedly provide a very significant potential sink for N deposition.

While the retention of $^{15}$N tracer within vegetation during the period of additions reflects N uptake by the plants, the process or processes responsible for N retention in the litter and surface organic compartments which are composed of dead plant material (thereby ruling out direct plant uptake) are less obvious. The retention of $^{15}$N within the litter and surface organic layer compartments within one year of the commencement of $^{15}$N additions is presumably due mainly to microbial immobilisation processes. It seems unlikely that significant litterfall of enriched material would have occurred within such a short time following additions, and even less likely that physical return of enriched material and mixing could account for the
enrichment of the surface organic layer underlying the litter. Abiotic immobilisation may also have played a part, but this cannot be quantified from existing data.

Figure 6.9: Total mean $^{15}$N tracer retention in litter and surface organic horizon compartments (±1 SD)

A plot of $^{15}$N tracer retention against litter dry weight in Figure 6.10 shows that there is a fairly strong relationship between these variables but a lot of scatter. Removal of the Etherow data from this plot, given the evidence from the vegetation data that N uptake appears to be partially suppressed there, improves the strength of this relationship (Figure 6.11), but only slightly. The litter layer at the Etherow, in particular at E2, is unique in being almost totally dominated by Calluna litter.

These plots simply demonstrate that in sample plots where the mass of litter is greater, a higher proportion of tracer is immobilised in the litter layer. If retention per unit mass of litter is plotted for each site along the gradient of N deposition, no obvious pattern is visible in the data (Figure 6.12). However, if these data are averaged across all soil types within each of the four study catchments (without any spatial weighting of soil coverage), the data appear to show a decline in the efficiency of N immobilisation in the litter layer as deposition increases (Figure 6.13), with more than
twice the retention per unit mass of litter at the Mharcaidh as at the Etherow. In other words, N is much more effectively immobilised in the litter of the very N limited system at the Mharcaidh, compared with the N saturated system at the Etherow. The Gwy and Scoat Tarn sites are intermediate between these two extremes.

**Figure 6.10: Tracer retention against dry weight of litter for all plots**

![Graph showing tracer retention against dry weight of litter for all plots.](image)

**Figure 6.11: Tracer retention against dry weight of litter (excluding Etherow)**

![Graph showing tracer retention against dry weight of litter (excluding Etherow).](image)
Figure 6.12: Tracer retention (%) per unit mass of litter (±1SD)

Figure 6.13: Mean tracer retention (%) per unit dry weight of litter, averaged across soil types for each catchment (no spatial weighting)
6.3.3 Soils

Two sets of soil samples from the upper organic and lower organic plus mineral horizons were analysed for $^{15}$N. Retention of the $^{15}$N tracer in the upper organic soil, generally at a depth of 5 - 15cm below the surface (i.e. around 10cm thick), is shown in Figure 6.14.

![Figure 6.14: Retention of $^{15}$N tracer in upper organic soils (±1SD)](image)

While there is no apparent pattern in $^{15}$N retention between soils or sites, the data indicate that there is a small degree of retention in the upper organic soil, generally in the range 0-4% of inputs. Although the data appear to show that retention, while low, is observed in most soils at all sites, they must be interpreted with caution. Figure 6.15 shows that when the $\delta^{15}$N values following tracer additions are compared with natural abundance values prior to the experiment, there is no significant difference in $\delta^{15}$N except for soil M1 at the Mharcaidh. It must therefore be assumed that while some minor degree of $^{15}$N tracer retention is likely at these sites, the changes are so small as to be well within the potential measurement errors associated with soil pools.
The data are even less conclusive for the deeper soil samples, with very high variability between plots for some soils, and several negative values, some of them large (Figure 6.16). These data are problematic because of the methods employed for bulking which greatly compounded the inherent uncertainties associated with spatial variability in soil properties.

Figure 6.16: Retention of $^{15}$N tracer in bulked soil samples (deeper organic plus mineral where present; $\pm 1$SD)
The bulking of soil horizons to make up the composite "soil depth 2" samples relied upon a series of assumptions, each with large associated uncertainties. A major weakness in the data is the assumption that identical horizons were used to make up composite samples for natural abundance and post $^{15}$N additions analysis. However, since the soil horizons sampled for natural abundance were not described in detail it cannot be known with any certainty that similar horizons were bulked for post additions analysis. The small cross-sectional area of the soil core samples (maximum 2×5cm diameter cores, i.e. 39.3 cm$^2$) means that the spatial representativeness of the samples is likely to be poor. In the very uniform peat soils at the Mharcaidh (M1 and M2) and the Etherow, where this problem seems to have been minimal, only a very small proportion of the tracer is recovered from the deeper horizons. Where spatial variability is much greater the mean retention of $^{15}$N tracer comes out as a negative value in several cases, which is clearly impossible. Given the above uncertainties, the deeper soil samples are excluded from further analysis.

6.4 Discussion

6.4.1 Fate of $^{15}$N additions and inorganic N deposition

The results described above show that a very large proportion of the $^{15}$N tracer applied to the experimental plots was recovered after one year of additions, and by interpolation, the same proportions of deposition inputs of inorganic N are also retained within the soil-plant system over this timescale. The total retention of tracer, summed for total vegetation, litter plus surface organic layer, and upper organic soil (to c. 10-15cm) is shown in Figure 6.17. The number of soils for which recovery of tracer exceeds 100% and the large standard deviation for some soils show the great spatial variability in the data and the uncertainty associated with the method. However, recovery of tracer was very good compared with similar experiments in forest systems.

It is apparent that the added tracer was very tightly retained within the study plots. The only soil plots where the mean retention value is greater than 100% by more than
one standard deviation are those at G2 (peaty gley at the Afon Gwy), but these are also the only plots at which one of the vegetation samples was lost, leaving only two replicate samples which could account for the anomalous value.

Figure 6.17: Overall tracer retention in all compartments (±1SD)

More generally, the highest retention values are found at the Mharcaidh and Afon Gwy sites, where most soil types show retention of close to 100%. The lowest retention at the Mharcaidh is found on the valley peat soils at M2, while at the Gwy it is on the peaty podsol at G3. At Scoat Tarn the mean retention for all three sets of soil plots is less than 100%, with the lowest (although most variable) figure occurring in the deep lakeside peat at S3. Likewise, the soils and vegetation at the Etherow do not retain all the applied $^{15}$N tracer, and the lowest figure for any set of soil plots is found at the mature Calluna plots there (E2). Overall, at least three quarters of the applied $^{15}$N was recovered from the upper soil-vegetation system, indicating that most N deposition must be intercepted in this part of the system and enters the biological N cycle. Only a very small proportion of inputs is potentially available for immediate leaching as inorganic N, but of the maximum 25% of inputs unaccounted for, other
sinks like denitrification or immobilisation deeper in the soil profile could reduce this proportion still further.

The relative importance of the vegetation, litter plus surface organic layer and soil compartments as $^{15}$N tracer sinks is shown in Table 6.5 and Figure 6.18. Vegetation and the surface organic/litter layer retain a very large proportion of the tracer at all plots, while the soils below the surface organic layer (i.e. below 5cm depth) are of very little importance as a sink for $^{15}$N over the course of the experimental year.

A larger proportion is generally retained in the vegetation than in the litter and surface organic layer, except at G4 (Gwy valley peat), S1 (Scoat Tarn podsol) and the Etherow plots, where these two sinks are of almost equal importance. These results are comparable to those of Gundersen (1998) and Tietema et al. (1998), both working on forest plots, in terms of the overall proportion of $^{15}$N tracer recovered.

### Table 6.5: Overall fate of $^{15}$N tracer additions (% retention)

<table>
<thead>
<tr>
<th>SOIL</th>
<th>Total vegetation</th>
<th>Litter/surface organic</th>
<th>Upper organic soil</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>M1 Peaty ranker</td>
<td>105.7</td>
<td>32.0</td>
<td>26.3</td>
<td>11.2</td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>56.3</td>
<td>5.3</td>
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</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>63.8</td>
<td>4.7</td>
<td>32.7</td>
<td>6.9</td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>59.1</td>
<td>9.7</td>
<td>49.1</td>
<td>16.7</td>
</tr>
<tr>
<td>G1 Hilltop peat</td>
<td>61.6</td>
<td>8.5</td>
<td>37.2</td>
<td>5.6</td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>101.9</td>
<td>12.5</td>
<td>43.1</td>
<td>7.0</td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>51.8</td>
<td>4.0</td>
<td>35.6</td>
<td>12.6</td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>54.1</td>
<td>6.0</td>
<td>51.4</td>
<td>10.5</td>
</tr>
<tr>
<td>S1 Podsol</td>
<td>43.4</td>
<td>4.0</td>
<td>47.2</td>
<td>4.7</td>
</tr>
<tr>
<td>S2 Peaty gley</td>
<td>51.6</td>
<td>7.4</td>
<td>40.9</td>
<td>9.5</td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>48.1</td>
<td>7.0</td>
<td>25.0</td>
<td>14.9</td>
</tr>
<tr>
<td>E1 Peat (burnt)</td>
<td>40.6</td>
<td>12.4</td>
<td>46.8</td>
<td>17.9</td>
</tr>
<tr>
<td>E2 Peat (unburnt)</td>
<td>37.0</td>
<td>3.7</td>
<td>36.7</td>
<td>6.3</td>
</tr>
</tbody>
</table>
The dominant processes responsible for the retention of the $^{15}$N tracer, and by interpolation, inorganic N deposition, are discussed separately below.

**6.4.2 N immobilisation in litter and soils**

In the definitions of immobilisation discussed in Section 6.1 above, it is suggested that in the strict sense, N immobilisation is taken to mean the assimilation of inorganic N into the microbial biomass. This immobilised N will then be partly recycled within the microbial N pool and partly stored as refractory organic compounds within the litter-soil system. Since the age of soil organic matter increases with depth, the proportion of labile organic N must also decrease with depth, as the labile fraction will by definition be mineralised more rapidly than the stable soil organic matter. According to Smith (1994), N is concentrated in the upper 10-15cm of the soil profile and generally decreases exponentially with depth, by up to an order of magnitude at 1m depth. This decrease mirrors that of both the soil organic matter and soil microbial biomass, and is the reason why most N cycling studies concentrate on
the 0-15cm zone when measuring processes such as mineralisation and immobilisation of N (see Chapter 5). A small fraction of N immobilisation in this zone may have been abiotic, but it is assumed that most is carried out by soil microbes.

Short term immobilisation of N (corresponding to “soil depth 1” plus “litter and surface organic” compartments in Figure 6.18) accounts for the retention of around 25-50% of N inputs across the four study catchments, with the great majority of this N immobilised in the litter and underlying surface organic material. Only a few percent of N is stored within the organic soils below this horizon to a maximum depth of 15-20cm. It is possible that another similarly small fraction is retained in deeper soils, but the uncertainties in the data within this study are too great to quantify this. Koopmans et al. (1996) found that the mineral soil between 25 and 70cm depth below Dutch forest sites retained only 5-10% of added $^{15}$N.

Microbial immobilisation was found to be a much greater sink for N than vegetation uptake prior to canopy development in hardwood forests in the USA (Zak et al., 1990; Groffman et al., 1993). Immobilisation was found to be much stronger in well-drained soils than in poorly-drained soils, leading to greater nitrification, denitrification and leaching potential in the latter. This relationship is not found within the current study. The wettest soil of all thirteen studied, the valley peat at the Gwy (G4 – see Chapter 3), shows the greatest overall immobilisation of $^{15}$N tracer in the litter and surface organic layer and has the highest immobilisation per unit mass of litter. Conversely, the driest soil, the peat under mature Calluna at the Etherow (E1), shows relatively low overall immobilisation in the litter and surface organic layer, and also shows the lowest value of tracer retention per unit mass of litter.

In their $^{15}$N tracer experiments in European forests, Tietema et al. (1998) found that 11-47% of added $^{15}$N was retained in the organic layer, with the highest values in $^{15}$NH$_4^+$ plots rather than those with $^{15}$NO$_3^-$ additions. The authors attributed these findings to the preferential uptake of NH$_4^+$ by soil microbes and also possible adsorption of NH$_4^+$ by cation exchange. It is not possible to determine which inorganic N species were immobilised in the current study because the tracer used contained $^{15}$N in equal proportions as $^{15}$NH$_4^{15}$NO$_3$ and was applied in equal amounts
on each plot. However, it is possible that the proportion of total deposition immobilised in the litter and surface organic layer is likely to have been underestimated by the use of the $^{15}$NH$_4^{15}$NO$_3$ tracer when the proportion of NH$_4$-N in total deposition may be almost double that of NO$_3$-N (see Chapter 3).

There is no evidence for a decline in the capacity of microbes to immobilise N as the level of inputs increases; with the exception of low values at M1, M2 and S3, the proportion of $^{15}$N recovered in the surface organic/litter layer is relatively constant across all four sites (Table 6.5). Similar results were found in a study of $^{15}$NO$_3^{-}$ retention in hardwood forests in the USA (Nadelhoffer et al., 1995), where it was suggested that retention capacity was increased by the stimulation of biological activity at high N inputs. However, contrasting results were found by Tietema et al. (1998), in which the proportion of throughfall N immobilised in the organic layer decreased as the magnitude of inputs increased, but their study covered a much greater range of deposition inputs up to 80 kgN ha$^{-1}$ yr$^{-1}$ (compared with a maximum of 34 kgN ha$^{-1}$ yr$^{-1}$ here). Consideration of just the litter layer, when values on all soil types are averaged across each catchment, does reveal an apparent decline in immobilisation with increasing deposition (see Figure 6.13), but this pattern disappears when litter and the surface organic layer are considered together.

Aber (1992) suggested that an increasing supply of NH$_4^+$ would reduce NO$_3^{-}$ immobilisation via preferential microbial uptake of NH$_4^+$, and would also enhance NO$_3^{-}$ production via nitrification of the increasingly available NH$_4^+$. Furthermore, NO$_3^{-}$ assimilation and uptake may be actively suppressed by excess NH$_4^+$ availability (Bradley, 2001). Hence it is possible that with a constant proportion of inputs immobilised in the surface organic layer, the absolute supply of NH$_4^+$ available for nitrification increases.

The immobilised N cannot be separated into that which has joined the dynamic, microbial N pool and that which is immobilised in the longer term in refractory soil organic matter. Some exchange of the microbial N pool with the N in standing vegetation will occur, but this is of little interest for modelling the long term fate of N within the system. Ultimately the vegetation, too, will contribute to the stable soil organic matter fraction which locks up a proportion of the N pool, through litterfall,
plant death and root death followed by incomplete decomposition, i.e. through the formation of soil humus.

6.4.3 Vegetation uptake of inorganic N

At the highest deposition Etherow and Scoat Tarn plots, around 40-50% of deposited N is taken up directly by the vegetation over the course of a single growing season. This proportion increases to c. 50-60% for most of the lower deposition plots at the Gwy and Allt a’Mharcaidh, with exceptionally high values at M1 and G2, which, while evidently incorrect at greater than 100%, indicate potentially much greater rates of uptake in the vegetation there.

It was suggested in Section 6.1 that it is generally assumed that plants cannot compete successfully with microbes for inorganic N, so that net mineralisation of N is required for plants to satisfy their nutrient demands (Richards, 1987). For N limited plants, vegetation uptake of N would therefore be related to net mineralisation. While no data are available for net mineralisation, absolute vegetation uptake of inorganic N (assuming uptake of deposition to be the same proportion as retention of $^{15}$N tracer in plants) is plotted against potential net mineralisation in Figure 6.19.

There is no apparent relationship between vegetation N uptake and net mineralisation, except perhaps at very low values of potential mineralisation (i.e. at the Allt a’Mharcaidh). Uptake remains relatively constant above a threshold mineralisation value of around 2 $\mu$gN per gram organic matter per day, which may indicate that vegetation uptake reaches a maximum and does not increase further, i.e. N saturation occurs. Differences in biomass between plots are not taken into account, but the low N uptake at the Mharcaidh is due to limited supply rather than low biomass of vegetation. The above relationship could also be weakened by direct foliar uptake of N, which would enable vegetation to assimilate N without competing with soil microbes. This may be particularly important where the biomass of lichens and mosses is large, but again, this does not explain the apparent relationship between N
uptake and mineralisation at the Mharcaidh, where mosses and lichens are an important component of above ground vegetation (see Table 6.4).

**Figure 6.19: Relationship between total vegetation uptake of inorganic N and potential mineralisation in surface organic layer of soils**

Another possibility is that the microbial preference for NH$_4^+$ allows the vegetation to take up NO$_3^-$ from the soils. Tietema (1998) found that gross NO$_3^-$ immobilisation in the organic layer of acid forest soils was negligible compared with gross NH$_4^+$ immobilisation, so competition for NO$_3^-$ is reduced when NH$_4^+$ is available. However, if NH$_4^+$ is present in excess then NO$_3^-$ assimilation and uptake are inhibited (Bradley, 2001), and NO$_3^-$ uptake by trees in areas of high N deposition has been found to be negligible (Rennenberg & Gessler, 1999). The tracer used in the present study contained equal proportions of $^{15}$NH$_4^+$ and $^{15}$NO$_3^-$, while modelled deposition data suggest that NH$_4$-N comprises almost double the proportion of total inorganic N deposition as that of NO$_3$-N (see Chapter 3). It is therefore possible that most of the plant requirement for N is met by NH$_4^+$ rather than NO$_3^-$, so that uptake of $^{15}$N labelled NO$_3^-$ is reduced under higher deposition loads, whereas all inorganic N is utilised at the N limited Mharcaidh site.
The role of vegetation uptake in determining the ultimate fate of N inputs over long timescales (decades or more) cannot be ascertained from the current study. Although a large proportion of N inputs is retained in vegetation within a given growing season, the fate of the assimilated N in successive seasons is not known. Some may be returned to the litter layer within the same growing season, while a proportion will be retained in longer lived parts of the plant, such as the woody stems of shrubs, for several years. Still more may be incorporated in the litter layer in successive years following the season in which uptake occurred. The rates at which organic N in litter and the surface organic horizon are mineralised will vary between sites and between soil types within each catchment.

This problem of timescales of retention in vegetation was well illustrated by Epstein et al. (2001) in their work on shortgrass steppe in the USA. They found that the plant communities that retained most $^{15}$N within one month of additions showed the least retention two years later (as little as 3.4%), probably due to greater grazing removal or annual turnover. Their study shows the immediate sinks for inorganic N, after which supply is governed by mineralisation rates. In the current study, mineralisation and nitrification potentials have been measured in an attempt to link immediate sinks to longer term release of inorganic N (see Chapter 5).

### 6.4.4 Fate of unrecovered $^{15}$N

Despite the very high recovery of $^{15}$N in soils and vegetation, there is still a significant proportion unaccounted for in some soil plots at all sites. Table 6.5 shows that while three of the Mharcaidh soils potentially show around 100% retention, the valley peat at M2 retained only 81% of added $^{15}$N in the sampled compartments. Likewise at the Gwy, only one set of soil plots did not potentially retain around 100% of inputs, and it is located on the podsol at G3. At Scoat Tarn, overall retention ranges from 75% in the lakeside peat to 94% in the podsol, while at the Etherow, the mature Calluna plots retained only 74% of added $^{15}$N compared with 91% on the recently burned plots at E1. Hence a maximum of 25% of inputs remain unrecovered in two sets of plots (S3 and E2), while at all other plots except the Mharcaidh valley peat (M2), where 20% is
unaccounted for, less than 10% of inputs cannot be found in the sampled compartments.

The fate of the missing $^{15}$N, and the corresponding proportion of the atmospheric deposition flux, cannot be ascertained from the stable isotope work alone, since not all possible sinks for N were sampled. Gaseous losses via denitrification were not measured, but are thought to be relatively insignificant in the four study catchments (see Chapter 4). Retention in deeper soils could not be quantified, but could potentially account for a few percent of inputs, which in most cases could bring retention close to 100%. Furthermore, the very low rates of $^{15}$N retention in most soils can be explained by the interception of inputs by vegetation uptake and microbial immobilisation in the surface organic layer. For those sites where these components of the system retain almost 100% of inputs, it is not known what the potential for immobilisation may be lower down the soil profile, since very little of the tracer penetrated this far. Only in those plots where a significant proportion of the applied tracer was not recovered is it possible that the retention capacity of the soil profile was tested, although it has then to be assumed that denitrification losses were negligible.

The proportion of $^{15}$N not recovered, averaged across all soil types for each catchment (without spatial weighting of soil cover) and the percentage of inorganic N leaching (relative to deposition inputs - see Chapter 3) are very closely matched, given the uncertainties in input-output flux measurements and the $^{15}$N recovery methods (Table 6.6).

<table>
<thead>
<tr>
<th>Site</th>
<th>% unrecovered $^{15}$N tracer</th>
<th>% leaching of N deposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allt a'Mharcaidh</td>
<td>(0)</td>
<td>1.1</td>
</tr>
<tr>
<td>Afon Gwy</td>
<td>(0)</td>
<td>6.7</td>
</tr>
<tr>
<td>Scoat Tarn</td>
<td>12.9</td>
<td>14.5</td>
</tr>
<tr>
<td>River Etherow</td>
<td>17.1</td>
<td>32.5</td>
</tr>
</tbody>
</table>
For all four catchments, percentage retention of the $^{15}\text{N}$ tracer during the experimental year is greater than the percentage retention of deposition inputs. Hence it is not necessary to invoke the processes of denitrification or immobilisation at depth in the soil profile to explain the proportion of the $^{15}\text{N}$ tracer that was not recovered in the vegetation, litter or surface soils; the missing $^{15}\text{N}$ could have been lost through NO$_3^-$ leaching. In fact it is necessary to invoke processes other than simple direct leaching of N inputs through the soil profile to explain the retention of a greater proportion of $^{15}\text{N}$ than (apparently) of inorganic N deposition.

Mineralisation and nitrification of organic N produced during previous seasons may contribute to NO$_3^-$ leaching, since the uptake, return in litter and mineralisation of organic N within a single growing season would not produce the disparity in the figures in Table 6.6. If the cycle of vegetation uptake, litter return and subsequent mineralisation/nitrification takes longer than one year, at least for a proportion of vegetation uptake (e.g. in certain plant species), the $^{15}\text{N}$ tracer technique applied over just one year will underestimate the level of NO$_3^-$ leaching. Alternatively, the problem of weighting up from individual soil types to the catchment scale could easily account for the relatively small differences observed in Table 6.6.

The $^{15}\text{N}$ tracer experiments cannot assess the proportion of NO$_3^-$ leaching that occurs through a hydrological routing of deposition inputs which bypasses the plant-surface soil system. For lakes, direct deposition to the lake surface may be significant, especially where the lake surface area makes up a large proportion of the catchment area. For stream catchments, direct deposition to the stream surface is likely to be negligible, but some proportion of precipitation inputs may have minimal contact with soils or vegetation, for example through rapid drainage into soil macropores. The proportion of NO$_3^-$ leaching which reaches surface waters via such routes is often referred to as “hydrological nitrate” (e.g. A. Henriksen, cited in Moldan et al., 1995). Given the tracer application technique used, with very small volumes added periodically, the movement of inorganic N by rapid drainage is not accounted for.
6.5 Conclusions

The $^{15}$N additions experiments show that despite large uncertainties in the method due to the great spatial variability in the distribution and properties of soils and vegetation, a very large proportion of added $^{15}$N is retained within the plants or litter and surface organic layer of soils during the first year of additions. A maximum of 25% of added inorganic N was unrecovered at the end of the experimental year. Fluxes of inorganic N through these soils are therefore primarily mediated by biological processes, and the scope for direct leaching of inorganic N down the soil profile is limited, except potentially under conditions of very rapid drainage, which were not tested here.

The proportion of inorganic N that is immobilised in both the litter and surface organic layer remains relatively constant across the N deposition gradient represented by the four study catchments, so the absolute amount increases with deposition. Immobilisation efficiency in the litter layer alone appears to decrease with increasing deposition. However, immobilisation may have been underestimated by the use of a tracer containing equal proportions of $^{15}$NH$_4^+$ and $^{15}$NO$_3^-$, since NH$_4^+$ makes up a much larger proportion of total deposition than NO$_3^-$, and is preferentially immobilised by soil microbes.

The proportional vegetation uptake of N, which is presumably mainly of NO$_3^-$ due to rapid microbial immobilisation of NH$_4^+$, does, though variable, appear to decrease with increasing deposition. This implies that NO$_3^-$ is present in excess of vegetation requirements at higher levels of deposition. Whether the excess of NO$_3^-$ is due solely to deposition inputs of NO$_3^-$ or is compounded by the nitrification of NH$_4^+$ which is present in excess of microbial requirements, the result is an increase in the availability of NO$_3^-$ for denitrification or leaching. Since denitrification losses are thought to be very small, leaching losses are the most likely fate of the excess NO$_3^-$. Given that the proportion of unrecovered $^{15}$N tracer is smaller than the proportional leaching loss of NO$_3^-$ from catchment budget calculations, it is likely that nitrification of mineralised NH$_4^+$ is contributing to the leaching of NO$_3^-$.

The recovery of such a large proportion of the $^{15}$N tracer in the vegetation and soil components of the system implies that there is much scope for long term
immobilisation of inorganic N in soil organic matter, both through the microbial and vegetation uptake and decay routes. These experiments have demonstrated that although long term immobilisation cannot be quantified in a short term experiment over just one year, biologically mediated processes are found to account for the short term retention of a very large proportion of inorganic N inputs, and as such, a biological model of N immobilisation, saturation and leaching is required to explain the long term fate of N deposition.

While vegetation uptake provides a temporary sink for N deposition over the life of the plant and increases with the magnitude of inputs, the proportional increases in retention are lower than those in deposition, so that overall retention efficiency decreases as N inputs increase. N immobilisation may decrease in the litter layer as inputs increase, but remains at a relatively constant proportion of inputs across the gradient of N deposition in the combined litter and surface organic layer. If immobilisation is controlled primarily by NH$_4^+$ supply and vegetation uptake is more dependent on NO$_3^-$ supply, then the balance of reduced and oxidised forms of N in deposition may determine the long term immobilisation and leaching of inorganic N in these systems. As NH$_4^+$ supply increases, first NO$_3^-$ immobilisation and then plant uptake of NO$_3^-$ will decrease (Bradley, 2001; Rennenberg & Gessler, 1999), while increased nitrification may add to the NO$_3^-$ pool available for leaching. This pattern may be reflected in the C:N ratio of labile organic matter (Emmett et al., 1998).

Changes in the composition of the litter and surface organic layers in terms of the quality of soil organic matter, in particular C:N ratio, as controls on the balance between immobilisation, mineralisation and leaching of inorganic N, are explored in Chapter 8.

6.6 References


Denitrification. SSSA Special Publication Number 18, Soil Science Society of America, Madison, Wisconsin, USA, pp.59-72.


SECTION IV

THE STATE OF THE SYSTEM:
MEASURES OF N SATURATION
CHAPTER 7

ESTABLISHING N SATURATION STATUS I

- $^{15}$N NATURAL ABUNDANCE
7. ESTABLISHING N SATURATION STATUS I - $^{15}\text{N}$ NATURAL ABUNDANCE

7.1 Introduction

7.1.1 Rationale for the $^{15}\text{N}$ study

While the primary purpose of the $^{15}\text{N}$ natural abundance measurements was to provide the baseline data for the $^{15}\text{N}$ tracer studies and estimates of N immobilisation in Chapter 6, the natural abundance data are of intrinsic value in providing information on terrestrial N cycling and saturation status. The distribution of $^{15}\text{N}$ around the various plant and soil compartments can provide information both on N processes and the sources of N utilised by different parts of the ecosystem.

In the context of the current study, the major value of the $^{15}\text{N}$ natural abundance data lies in the provision of an indirect means of comparing N saturation status between sites, with indications of the predominant processes responsible for the retention or leaching of inorganic N which are not provided by the simple input-output fluxes reported in Chapter 3.

This chapter will therefore provide an assessment of the state of the study catchments, or the degree of N saturation, in terms of the importance of N deposition inputs relative to biological demand. It will also provide an indication of the key processes which determine the sources and fate of N in catchments across a gradient from strong biological N limitation to severe N saturation. While such studies have frequently been reported from forest ecosystems in the literature, this is one of the first to attempt an assessment of N saturation status in moorland systems.

7.1.2 General principles of isotopic fractionation and natural abundance measurement

Processes that either chemically transform or physically transport N may result in isotopic fractionation, whereby the lighter isotope $^{14}\text{N}$ is preferentially lost, leaving a
substrate enriched in $^{15}$N (Nadelhoffer & Fry, 1994). The degree of fractionation varies between different processes, so the flux of N resulting from a given process is not the only factor to consider in N budget calculations. Nadelhoffer and Fry (1994) distinguish between two categories of important processes; those which lead to high N fluxes but result in only slight fractionation, and those associated with smaller N fluxes but resulting in a larger degree of fractionation. The processes which are associated with the greatest degree of isotopic N fractionation include ammonia volatilisation and denitrification, neither of which is likely to result in a very significant N flux in the acid moorland systems considered here (see Chapter 4 on the results of denitrification studies). Two processes which are likely to be much more important in determining the natural abundance of $^{15}$N in the four study catchments are mineralisation and nitrification, which can involve very large fluxes of N albeit with a lesser degree of fractionation than ammonia volatilisation or denitrification.

An important further consideration is that fractionation can only occur where the transformation of N from one form to another is incomplete, since complete transformation would result in a product with the same ratio of $^{15}$N:$^{14}$N as the original substrate (Peterson & Fry, 1987). For example, partial nitrification of NH$_4^+$ produces NO$_3^-$ depleted in $^{15}$N relative to the residual NH$_4^+$, which is simultaneously enriched, but complete nitrification would result in NO$_3^-$ with the same ratio of $^{15}$N:$^{14}$N as the original NH$_4^+$ substrate (Nadelhoffer & Fry, 1994). Furthermore, products along a sequence of reactions should, if fractionation factors were equal (and in the same direction) for each reaction, become progressively depleted, but because fractionation factors vary, products further down the sequence may become more enriched than the original substrate (Högberg, 1997). For example, since nitrification discriminates against $^{15}$N more strongly than the mineralisation process, NH$_4^+$ can become enriched relative to the organic N from which it is derived.

The abundance of $^{15}$N in a sample is normally expressed as $\delta^{15}$N, which is calculated as parts per thousand differences from a standard (Nadelhoffer & Fry, 1994):

$$\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000[\%]$$
where $R_{\text{sample}}$ and $R_{\text{standard}}$ are the $^{15}\text{N}:{ }^{14}\text{N}$ ratios of the samples and standards, respectively. The standard is atmospheric $\text{N}_2$, as described in Chapter 6.

In most terrestrial ecosystems $\delta^{15}\text{N}$ values for plant tissues and bulk soils are generally between $-10$ and $+15\%$, representing a range of only 0.3626 to 0.3718 atom\% $^{15}\text{N}$ (Nadelhoffer & Fry, 1994). The results of a literature survey of $\delta^{15}\text{N}$ values for soils and vegetation are presented in Table 7.1.

Since fractionation occurs during mineralisation, nitrification and denitrification, it can result in $^{15}\text{N}$ enrichment of the total soil $\text{N}$ pool through leaching of $^{15}\text{N}$ depleted $\text{NO}_3^-$ or gaseous loss of $^{15}\text{N}$ depleted $\text{NO}$, $\text{N}_2\text{O}$ or $\text{N}_2$ (Johannisson & Högberg, 1994; Nadelhoffer & Fry, 1994; Emmett et al., 1998; Austin & Vitousek, 1998). Furthermore, since mineralisation results in $\text{N}$ fractionation, residual organic compounds in litter and soil humus tend to be enriched in $^{15}\text{N}$, while the inorganic $\text{N}$ produced by mineralisation is depleted in $^{15}\text{N}$, and strong evidence for this process is provided by consistently observed patterns of lower $\delta^{15}\text{N}$ in vegetation than soils (Nadelhoffer & Fry, 1988; Gebauer & Dietrich, 1993; Nadelhoffer & Fry, 1994). While some authors (e.g. Högberg, 1997; Hobbie et al., 1999) have argued that there is very little evidence of significant fractionation during mineralisation of $\text{N}$ from larger molecules in soils, this does not necessarily contradict the assertion of Nadelhoffer & Fry (1994) that mineralisation does result in significant fractionation, because they invoke the intermediate processes of microbial uptake and subsequent release of inorganic $\text{N}$ through remineralisation. This process is associated with significant fractionation, as microbial decomposition leaves enriched organic material, some of which is labile and some of which may be incorporated in the refractory soil $\text{N}$ pool.

For plants the situation is more complex than for soils. Most soil $\text{N}$ is bound in forms not readily available to plants, with only a few per cent becoming available during the growing season, so the $\delta^{15}\text{N}$ of soil total $\text{N}$ is not a good indicator of the $\delta^{15}\text{N}$ of plant available $\text{N}$ (Högberg, 1997). Incomplete nitrification results in $^{15}\text{N}$ enrichment of residual $\text{NH}_4^+$ which may be taken up preferentially by plants and microbes. $^{15}\text{N}$
enrichment of plant foliage relative to external (deposition) sources of N can therefore result from the uptake of $^{15}$N enriched NH$_4^+$-N from soil solution.

Furthermore, the relative change in the foliage is greater than in the soil total N because of the large, inactive N pool in the soil (Johannisson & Högberg, 1994; Emmett et al., 1998). The $\delta^{15}$N of soil total N is dominated by the isotopic signature of stable organic matter, which is not likely to change significantly over a timescale of decades (Högberg, 1997). However, soil inorganic N may already be depleted relative to soil organic matter because of the fractionation which occurs during mineralisation (see above). In general, tree tissues and fresh litter are slightly depleted in $^{15}$N relative to soils, and $\delta^{15}$N values increase with depth in soil profiles, since the older the soil organic matter, the greater the history of fractionation which has occurred (Nadelhoffer & Fry, 1988, 1994). However, other authors have reported inconsistent patterns in $\delta^{15}$N with soil depth, sometimes finding an increase with depth in the upper horizons, which decreases again in the deeper horizons (Shearer et al., 1978).

Plant $^{15}$N abundance therefore reflects the more active soil N in soil solution or on ion exchange sites and therefore the more recent history of N dynamics at a site (Johannisson & Högberg, 1994; Högberg, 1997; Emmett et al., 1998). The $^{15}$N enrichment of vegetation relative to external inorganic N sources (e.g. atmospheric deposition) will depend on the 'history' of the N assimilated through uptake from soils. While NH$_4^+$ is, in general, preferentially taken up by vegetation relative to NO$_3^-$, the NH$_4^+$ may come directly from deposition (no change in $\delta^{15}$N of biomass relative to source) or from mineralised organic N ($^{15}$N depleted relative to soil organic matter). Furthermore, in soil waters the $\delta^{15}$N of NH$_4^+$ taken up by vegetation depends also on the degree to which nitrification has resulted in differences in $\delta^{15}$N between NH$_4^+$ and NO$_3^-$ through fractionation.

The results of each individual fractionation process are summarised below.

1. Mineralisation produces $^{15}$N depleted NH$_4^+$ (relative to the organic N source) and leaves behind $^{15}$N enriched soil organic matter.
2. Nitrification produces $^{15}\text{N}$ depleted $\text{NO}_3^{-}$ and leaves a $^{15}\text{N}$ enriched $\text{NH}_4^{+}$ substrate which may or may not then be depleted relative to the organic N source.

3. Denitrification leaves a $^{15}\text{N}$ enriched $\text{NO}_3^{-}$ substrate relative to gaseous N losses, but if the $\text{NO}_3^{-}$ source was nitrification it will initially have been $^{15}\text{N}$ depleted (relative to soil water $\text{NH}_4^{+}$).

4. Leaching of $^{15}\text{N}$ depleted $\text{NO}_3^{-}$ (produced by nitrification), or denitrification losses of $^{15}\text{N}$ depleted gases, results in the overall $^{15}\text{N}$ enrichment of the system.

Thus the differences in $\delta^{15}\text{N}$ between soil water $\text{NH}_4^{+}$ and $\text{NO}_3^{-}$, leachate, deposition sources and organic N pools in different ecosystem compartments depends on the routing of N through the ecosystem N cycle and the relative importance of the different fractionation processes. For example, studies by Farrell et al. (1996) investigating the use of $\delta^{15}\text{N}$ of deep-leached soil water $\text{NO}_3^{-}$ as a potential indicator of spatial variability in denitrification (through $^{15}\text{N}$ enrichment of the remaining $\text{NO}_3^{-}$) found that it provided only a semi-quantitative measure because mineralisation of enriched soil organic matter was also an important control on $\text{NO}_3^{-}$ leaching and its $\delta^{15}\text{N}$ value.

While N losses will generally lead to $^{15}\text{N}$ enrichment of a system, the direction of change in $\delta^{15}\text{N}$ of a system retaining N depends on the $\delta^{15}\text{N}$ value of the inputs. For example, when fertiliser N is $^{15}\text{N}$ depleted relative to soil N, fertilisation should lower the $\delta^{15}\text{N}$ of the system, but when N inputs are large enough to lead to leaching and/or denitrification losses (of $^{15}\text{N}$ depleted products), this initial decrease is followed by an enrichment in $^{15}\text{N}$ (Högberg, 1990, 1991; Johannisson & Högberg, 1994). In either case, the overall change in $\delta^{15}\text{N}$ for soils is likely to be small because of the very large N pool relative to inputs. Similarly, Austin and Vitousek (1998) suggested that atmospheric sources tend to add $^{15}\text{N}$ depleted N to a system, since N fixation incorporates N with $\delta^{15}\text{N}$ near 0‰, and deposition, while variable, is generally depleted in $^{15}\text{N}$, especially in unpolluted areas. They cited a study of very young soils in Hawaii whose dominant N input is atmospheric deposition, which were found to have negative $\delta^{15}\text{N}$ values (Vitousek et al., 1989).
<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Soil $\delta^{15}$N range</th>
<th>Type</th>
<th>Vegn. $\delta^{15}$N range</th>
<th>Type</th>
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<td>Peterson &amp; Fry, 1987</td>
<td>(Review)</td>
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<td>Varied</td>
<td>-8 to +3</td>
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<td>-</td>
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<td>LTER sites, USA</td>
<td>-4 to &gt; +8</td>
<td>Organic &amp; mineral</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
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<td>(Review)</td>
<td>-</td>
<td>-</td>
<td>-6 to +13</td>
<td>Forests</td>
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<td>(Review)</td>
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<td>*Bulk soils &amp; vegn.</td>
<td>Down to c.-10</td>
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<td>S.Sweden norway spruce</td>
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<td>Emmett et al., 1998</td>
<td>NITREX forests (Europe)</td>
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<td>Forest floor &amp; soils</td>
<td>-5 to +4</td>
<td>Needles/twigs/wood</td>
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<tr>
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<td>Tundra heath/forest in Sweden, Siberia, Greenland</td>
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<td>-</td>
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<td>Tundra vegetation</td>
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<td>Forest soils</td>
<td>-4.5 to –2.2</td>
<td>Ferns</td>
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<td>Macquarie Island</td>
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<td>Peat / macrofossils</td>
<td>+7 to +16</td>
<td>Tundra-like subantarctic vegetation</td>
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<td>Miller &amp; Bowman, 2002</td>
<td>Alpine meadows, USA</td>
<td>-1 to +7</td>
<td>Alpine soils</td>
<td>c. -2 to +3</td>
<td>Alpine vegetation</td>
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</table>
It is evident that natural abundance measurements can provide two types of information (Peterson & Fry, 1987):

1. Where physical and chemical processes have led to fractionation, the resulting isotopic distribution provides *process information*.

2. Natural abundance may also provide information about the origins of N in a sample, i.e. *source information*.

Examples of these two types of studies and their inter-relationships are given below.

### 7.1.3 Natural abundance of $^{15}N$ in process studies

Several studies have used $^{15}N$ natural abundance as a measure of past and current overall N status and rates of N cycling within forests, often via the calculation of enrichment factors (Austin & Vitousek, 1998; Emmett *et al.*, 1998; Koerner *et al.*, 1997, 1999; Koopmans *et al.*, 1997; Näsholm *et al.*, 1997). Increases in the supply of inorganic N to an ecosystem can lead to increased rates of biological cycling and hence to increased fractionation, which can be reflected in a greater increase in $\delta^{15}N$ of vegetation relative to soil, because of the much greater size of the total soil N pool, which is effectively diluted by old soil organic matter (Johannisson & Högberg, 1994). Hence the change in total soil $\delta^{15}N$ is less evident than that of vegetation or surface organic horizons. The differences in $\delta^{15}N$ between plant and soil may therefore decrease with increasing N supply, and plants may even acquire a higher $\delta^{15}N$ than older soil horizons under high N inputs, due to increasingly higher enrichment of active N pools (Johannisson & Högberg, 1994). However, exchange of N between active and passive pools should, with time in a *closed* system, reduce the magnitude of enrichment of active pools.

Fractionation is most likely when NO$_3^-$ accumulates, such as when net N mineralisation and nitrification rates are high due to elevated inputs, or when nitrification temporarily exceeds NO$_3^-$ consumption (Nadelhoffer & Fry, 1994). When deposition inputs are high, the likelihood of higher rates of N cycling, incomplete but high rates of nitrification and vegetation uptake of $^{15}N$ enriched NH$_4^+$ increases. The
relationship is not straightforward, however. Näsholm et al. (1997) found that arginine in spruce needles increased with N supply, reducing NH$_4^+$ uptake, so that nitrification was stimulated in the soil and the NH$_4^+$ that was taken up was $^{15}$N enriched. Högberg (1990) found that the $\delta^{15}$N of the grass Deschampsia flexuosa under forests treated with urea increased faster than those treated with the same amount of N as NH$_4$NO$_3$, and attributed this to greater uptake of $^{15}$N enriched NH$_4^+$-N resulting from increased volatilisation and nitrification under urea treatments. Similar results were also found for Scots pine needles in these plots (Högberg, 1991), with a correlation between increased $\delta^{15}$N and leaching of (depleted) NO$_3^-$ from soils. Further analysis of data from the same forest N addition plots revealed a strong correlation between increases in needle $\delta^{15}$N and the proportion of N additions lost from the system, by calculating the change in total N stores within the soils and tree biomass (Högberg & Johannisson, 1993).

In oak forest soils, litter $\delta^{15}$N was found to be similar to the $\delta^{15}$N of the upper organic soil horizons through mixing and rapid decomposition processes, while $\delta^{15}$N increased down the soil profile because of the overall isotopic fractionation during decomposition of soil organic matter (Nadelhoffer & Fry, 1988). In a study of terrestrial organic matter contributions to aquatic food webs, Fry (1991) showed an overall pattern of progressive $^{15}$N enrichment of plant, litter, organic soil and mineral soil in forests. A general pattern of decreasing $\delta^{15}$N was suggested by Schulze et al. (1994) as NO$_3^-$ < NH$_4^+$ < old soil organic matter, while fresh litter should be intermediate between NH$_4^+$ and old soil organic matter.

In a study of nutrient dynamics in Hawaiian forests, Austin and Vitousek (1998) used decreasing soil and foliage $\delta^{15}$N values with increasing precipitation to show that N losses (i.e. of $^{15}$N depleted N) relative to pool sizes were greater in the drier sites in spite of more rapid turnover, which they interpreted as an indication of more ‘open’ N cycling. In wetter sites, total N pools were greater but the N cycle was more closed and N losses were smaller relative to the total pool, so enrichment of the remaining N pool via losses of $^{15}$N depleted N was much reduced.
To allow for initial differences in soil and plant $\delta^{15}\text{N}$ values when comparing sites, for example due to previous land management, the enrichment factor ($\epsilon_{ps}$) may be used (Garten, 1993; Garten & van Miegrot, 1994; Koopmans et al., 1997; Näsholm et al., 1997; Emmett et al., 1998). It can be approximated as the difference between $^{15}\text{N}$ abundance in the substrate and product when the substrate is a much larger pool than the product (Garten, 1993), in this case between soil and vegetation:

$$\epsilon_{ps} = \delta^{15}\text{N}_{\text{vegetation}} - \delta^{15}\text{N}_{\text{soil}}$$

The component of the soil measured here is very important, because of the changes in $\delta^{15}\text{N}$ which can occur down the soil profile (see below).

In their natural abundance studies at European forest sites (NITREX), Emmett et al. (1998) found that low $\delta^{15}\text{N}$ values are maintained in the upper soil horizons by a tight cycle between tree uptake and return of $^{15}\text{N}$ depleted foliage and root litter, while soil $\delta^{15}\text{N}$ values generally increased with depth due to transport of $^{15}\text{N}$ enriched soil humus down the soil profile in combination with continued fractionation during the mineralisation and nitrification processes. These findings matched those of Nadelhoffer and Fry (1988) working in oak forests in Wisconsin a decade earlier. Emmett et al. (1998) also found that leaching losses were dominated by $\text{NO}_3^-$ at all sites which reflected the $\delta^{15}\text{N}$ signature of incoming $\text{NO}_3^-\text{N}$ rather than $\text{NH}_4^-\text{N}$ at 2 out of 3 sites, suggesting that retention of incoming $\text{NO}_3^-\text{N}$ may be lower than that of $\text{NH}_4^-\text{N}$, i.e. information about the source of leached $\text{N}$ was also provided.

Overall, the NITREX studies concluded that $^{15}\text{N}$ enrichment of the vegetation relative to soil (mean of the most active soil horizons $\text{Ah}$, $+5\text{cm}$ and $5$-$15\text{cm}$) reflects differences in $\text{N}$ status, fluxes and cycling rates in forests (Emmett et al., 1998). With the exception of the Aber site, $\epsilon_{ps}$ increased (became less negative) with $\text{N}$ deposition, i.e. the $\delta^{15}\text{N}$ value of foliage approached the $\delta^{15}\text{N}$ of the soil more closely. Similarly, Näsholm et al. (1997) had found that $\epsilon_{ps}$ (using the soil $\text{H}$ horizon) was a better predictor of forest $\text{N}$ saturation status and $\text{NO}_3^-$ leaching than foliage $\delta^{15}\text{N}$ alone.
The potential changes in the relationship between vegetation, surface soil and soil profile $\delta^{15}N$ values were nicely summarised by Högberg (1997). In most systems plants are found to have lower $\delta^{15}N$ than soil total N and redeposition of depleted litter onto the soil surface explains why the $\delta^{15}N$ of surface horizons in many forests is lower than further down the profile. Such systems are likely to be N limited, N aggrading forests, where low nitrification rates mean little or no isotopic enrichment of $\text{NH}_4^+$ which is already depleted relative to soil organic matter, so that where $\text{NH}_4^+$ uptake is preferred, progressive depletion at the soil surface may occur through litter return. By contrast, where high N inputs stimulate nitrification and thus $^{15}N$ enriched $\text{NH}_4^+$ production, plants preferentially using the $\text{NH}_4^+$ will progressively enrich the soil surface. Furthermore, depleted $\text{NO}_3^-$ which is readily lost from the upper profile might be partly retained further down, so that an initial increase in $\delta^{15}N$ with depth might turn into a decrease further down the profile. Overall, low $\delta^{15}N$ in the surface organic layer appears to indicate N limitation and low nitrification, while a higher $\delta^{15}N$ in the surface layer than in deeper layers appears to indicate high rates of nitrification, which may also lead to leaching losses of $\text{NO}_3^-$ from the system.

In a rather different application of $\delta^{15}N$ data, decreasing natural abundance values of $^{15}N$ in herbarium samples of vegetation sampled at different times during the last century from the western Mediterranean were used to investigate vegetation response to increased atmospheric CO$_2$ (Peñuelas & Estiarte, 1997). Assuming that the $\delta^{15}N$ of a plant is primarily determined by the $\delta^{15}N$ of the N source, they concluded that decreasing leaf $\delta^{15}N$ this century indicates some combination of the following factors in response to increased carbon availability in an area of low N deposition (c. 3 kgN ha$^{-1}$ yr$^{-1}$):

1. lower N losses in ecosystems, because all N losses (denitrification, ammonia volatilisation or $\text{NO}_3^-$ leaching) should enrich the remaining N;
2. use of increasingly mineralised N (which is isotopically lighter); and/or
3. a larger proportion of N coming from fixation, since atmospheric N has a lower $\delta^{15}N$ than soil.

Measured decreases in tissue N concentrations are consistent with the view that N limitations constrain any positive growth response to elevated CO$_2$, but the $\delta^{15}N$ data
here indicate that these ecosystems may cope with increasing plant N demands by decreasing ecosystem N losses and increasing fixation and mineralisation.

7.1.4 Natural abundance of $^{15}$N as an indicator of N sources

Natural abundance studies have also provided evidence of the different N sources preferred by various plants. These include studies of spatial variations in N sources (atmospheric versus soil, different rooting depths etc.) as well as chemical variations in the N form utilised (N$_2$ fixation, readily available mineral/organic and stable organic forms). Such studies invariably have to consider fractionation and cycling processes as well as direct N sources.

Natural abundance of foliar $^{15}$N was shown by Garten (1993) to indicate spatial differences in soil N availability and foliar N sources in the Walker Branch watershed, USA. Greater values of $\delta^{15}$N (less negative) in valley bottom tree foliage compared with similar samples from a ridge site resulted from higher soil N availability, nitrification potential and overall N status in the valley bottom site. Root uptake of $^{15}$N enriched inorganic N was greater in the valley bottom site, while direct uptake of atmospherically deposited, isotopically light NH$_4^+$ was more important in the ridge site.

The gradient in $\delta^{15}$N down a soil profile has been used to determine the preferred source for plant N uptake. Gebauer and Dietrich (1993) compared the $\delta^{15}$N values of tree species, ericaceous and non-ericaceous shrubs, grass and fungi with the soil $\delta^{15}$N of their rooting zones in a Bavarian forest. They found little difference in the $\delta^{15}$N of different tree species despite their different root distributions, concluding that all the studied species preferentially utilise N from the same soil horizon, the surface organic layer. Shrubs and grass showed similar $\delta^{15}$N values to the trees, except that twigs from ericaceous shrubs had the lowest values of all compartments. Fungi had the highest $\delta^{15}$N values, reflecting their ability to utilise organic N which is $^{15}$N enriched. Fungi therefore seem to prefer the N fraction that is directly available to them but not
to higher plants, which is energetically cheaper for biomass production than inorganic N (Gebauer and Dietrich, 1993).

Similarly, Schulze et al. (1994) measured $\delta^{15}$N in spruce trees (*Picea glauca* and *P. mariana*), an ericoid shrub (*Vaccinium vitis-idaea*) and a grass (*Calamagrostis canadensis*) along a 250km transect at the northern treeline of Alaska, where severe N limitation caused some needle discoloration in the trees. They found that $\delta^{15}$N values were significantly lower in spruce than in *Vaccinium* along the whole transect on various soil types, and both were lower than in *Calamagrostis*, indicating that the ericoid shrub *Vaccinium* and the grass *Calamagrostis* had access to N that was $^{15}$N enriched relative to tree sources. Their conclusion was that in N limited systems, competition for N encouraged the utilisation of N from different sources; the grass used its deep roots to access $^{15}$N enriched inorganic N from deep in the soil profile, while the ericoid shrub had associated mycorrhizae that could utilise $^{15}$N enriched, slowly decomposing, soil organic matter sources. The trees were restricted to using readily available litter organic N or inorganic N sources, which were both depleted in $^{15}$N relative to more slowly decomposing organics or N sources deeper in the profile.

However, when they compared these results with those from a German forest subject to high N deposition loads which caused NO$_3^-$ leaching and denitrification (not found in the Alaskan forest), Schulze et al. (1994) found that the differences between comparable tree, shrub and grass species disappeared. They concluded that there may be an advantage to plants using organic N in a nutrient limited environment, which disappears when N is readily available, i.e. these species do not compete for N when it is present to the extent that NO$_3^-$ leaches. Furthermore, they found that differences in $\delta^{15}$N of different species may increase with N inputs while N is limiting, but these differences disappear when N is present in excess.

In contrast to the results of Schulze et al. (1994), Michelsen et al. (1996) used $^{15}$N natural abundances to show that it was not the rooting depth which allowed different plant species to access different N sources in subarctic Swedish heaths and fellfields, but differences in mycorrhizal species which access either inorganic N or labile organic N from fresh litter. Mosses and lichens depended more on atmospheric
sources, either through $N_2$ fixation or direct uptake from precipitation. In further studies of nutrient poor tundra ecosystems it was found that there were clear differences in $\delta^{15}N$ between non-mycorrhizal plants using inorganic $N$ sources (high $\delta^{15}N$ from enriched $NH_4^+$) and ecto- or ericoid mycorrhizal plants, as well as mosses and lichens, which could access near surface organic $N$ sources and amino acids (low $\delta^{15}N$) directly (Michelsen et al., 1998).

Miller and Bowman (2002) carried out field and greenhouse studies on alpine meadow species in the USA to show that co-occurring alpine species may be differentially utilising soil $NH_4^+$, $NO_3^-$ and organic $N$ (glycine). In these ecosystems, most plants obtained the majority of their $N$ in the first half of the growing season from the pulse following snowmelt. All species could take up all forms in the greenhouse, but were not uniformly flexible in their resource $N$ use. They also found an inverse relationship between foliar $\delta^{15}N$ and $NO_3^-$ reductase activity, suggesting that species with depleted foliar $\delta^{15}N$ might rely on $NO_3^-$ to a greater degree than $NH_4^+$ or organic $N$. Several species appeared to utilise $NO_3^-$ disproportionately well, even though net nitrification rates in the dry meadows are low during the growing season and exchangeable $NO_3^-$ concentrations are frequently an order of magnitude lower than $NH_4^+$ and DON. Overall, they concluded that dry meadow species appear to have the potential to partition soil $N$ on the basis of $N$ form, but at these sites are relatively flexible in their $N$ use. However, species showing the greatest ability to uptake inorganic $N$ would be favoured by increasing fertiliser or atmospheric inputs.

A study of the fractionation of $N$ isotopes during mycorrhizal uptake found that the fungal species became enriched relative to the plant, but the small size of the fungal $N$ pool meant that the $\delta^{15}N$ of $N$ taken up by the plant was not significantly changed during uptake (Högberg et al., 1999). The experiments also implied that plant $\delta^{15}N$ is a good approximation of the signature of the $N$ source, as assumed in the above studies of Gebauer and Dietrich (1993) and Schulze et al. (1994), but only where $N$ is limiting growth. These conclusions were contradicted by later work in which different types of mycorrhizal fungi were found to show species-specific fractionations during uptake and metabolism which shifted the $\delta^{15}N$ of the fungi to different degrees and sometimes in different directions, depending on substrate (Emmerton et al., 2001a).
Thus differences in the δ¹⁵N of mycorrhizal plants could be influenced directly by the mycorrhizae, confounding attempts to compare foliage δ¹⁵N between mycorrhizal and non-mycorrhizal species and to link them to specific N sources (Emmerton et al., 2001b).

The relative abundance of ¹⁵N in individual soil amino acids was found by Ostle et al. (1999) to be influenced by original substrate quality and by the nature of subsequent biological transformations. The authors suggested that heterotroph communities evolved under different soil conditions, land uses and climates will therefore produce characteristic isotopic fractionations during organic matter decomposition, so that natural abundance δ¹⁵N values of specific compounds derived from recalcitrant N fractions may provide a robust time-integrated signature of past soil organic matter transformations. The δ¹⁵N values of individual amino acids could then provide a means to determine subtle differences in soil biological quality.

Lipson and Näsholm (2001) cited the work of Ostle et al. (1999) above, which found positive δ¹⁵N values in certain amino acids, as providing evidence that labile soil organic N was not necessarily ¹⁵N depleted. Therefore great care has to taken with the attribution of organic N sources on the basis of δ¹⁵N values in plants.

In a study of ¹⁵N natural abundance in old and new forests in the Vosges Mountains of France, Koerner et al (1997) found that the δ¹⁵N values of vegetation and soils of new forests were not only greater than old forests, but that differences between areas of new forest were determined by previous land use, which ranged from garden, meadow and pasture to arable. Nitrogen isotopic enrichment of previously cultivated areas were interpreted either as a result of fertilisation by animal manure with much higher δ¹⁵N than forest soils, or of indirect ¹⁵N enrichment by activation of processes leading to losses of ¹⁵N depleted N. Whatever the causes of such a δ¹⁵N increase, Koerner et al (1997) concluded that ¹⁵N natural abundance could be used as an indicator of former agricultural land use where no direct historical data are available. The δ¹⁵N of surface soil (0-5cm) and understorey vegetation (the fern Dryopteris carthusiana) provided particularly good tracers of historic land use almost a century after
afforestation (Koerner et al., 1999), with apparent differences in soil sources of fern N between previously fertilised and unfertilised areas.

Fossil peat $^{15}$N natural abundance was compared with current vegetation on the subantarctic Macquarie Island in a palaeoecological reconstruction of the distribution of the island's flora and fauna (Bergstrom et al., 2002). The $\delta^{15}$N of different vegetation samples was found to be linked primarily to the influence of animal excreta and varied according to trophic level of the fauna. They suggested a progressive $^{15}$N enrichment of animal excreta with increasing trophic levels, e.g. vegetation growing next to penguin colonies or small burrowing petrels had an average $\delta^{15}$N of $+7\%o$ while a greater enrichment ($+12.9\%o$) occurred in areas occupied by seals or giant petrels (which are of a higher trophic level). Furthermore, peat $\delta^{15}$N signatures changed throughout fossil peat cores and were associated with changes in fossil composition including plant micro- and macro-fossils, seal hair and skin, providing evidence that vegetation changes have occurred at the cored site over the past 8500 years. Plants using N derived from animals in the upper food chain (seals, giant petrels) have the highest $\delta^{15}$N values of all today, and resemble basal sections of the peat cores. The presence of pollen from the nitrophile *Callitriche antarctic* occurred together with seal fossil remains and isotopically enriched peat. This plant currently occurs mainly in areas disturbed by seals and penguins, forming floating carpets on seal wallows. The peat core fossils map changes (due to tectonic uplift) from eutrophic coastal associations (enriched in $^{15}$N by animal inputs) to relatively depleted inland herb fields or short grasslands today.

The similar N content to current vegetation, the strong changes in peat $\delta^{15}$N and associated fossil evidence suggest that the observed fossil peat isotopic composition on Macquarie Island is predominantly being derived from the N bound to the peat forming constituents, rather than being the result of leached N (Bergstrom et al., 2002). The implication here is that the isotopic composition of the peats reflects the $\delta^{15}$N signature of the vegetation and N sources, and that the peat $\delta^{15}$N values have remained relatively stable with time on Macquarie Island. Peat $\delta^{15}$N values therefore give insight into ecosystem processes that complement the fossil record, providing a potentially valuable palaeoecological tool.
Natural abundance of $^{15}\text{N}$ has also been used in conjunction with that of $^{18}\text{O}$ to determine the proportion of NO$_3^-$ leached from Bavarian forests which comes directly from atmospheric NO$_3^-$ and that which results from nitrification within the soil (Durka et al., 1994). Differences were found between healthy or limed sites, with only 16-30% of spring water NO$_3^-$ derived directly from atmospheric NO$_3^-$, and declining forest stands, where up to 100% of atmospheric NO$_3^-$ inputs were recovered in spring water. In the healthy sites, high levels of atmospheric N inputs still led to significant leaching of NO$_3^-$, but rapid uptake and nitrification processes prevented most atmospheric NO$_3^-$ from leaching directly into surface waters, so that a large proportion of leached NO$_3^-$ had been biologically cycled and produced by nitrification. In the declining forest stands, biological NO$_3^-$ consumption was largely inhibited, leading to a much higher proportion of direct leaching of atmospheric NO$_3^-$ without biological interaction.

In their analysis of natural abundance data from two high N deposition forest sites in the Netherlands (Speuld and Ysselsteyn), Koopmans et al. (1997) used the dynamic N and C cycling and fractionation model NICCCE (van Dam and van Breemen, 1995) to show that $\delta^{15}\text{N}$ of NH$_4^+$ and NO$_3^-$ in deposition was important in influencing $\delta^{15}\text{N}$ of various ecosystem compartments. They concluded that natural abundance studies of forest ecosystem compartments were of limited value in the assessment of the N cycling and saturation status of forests without the concurrent measurement of $\delta^{15}\text{N}$ in deposition inputs and leaching outputs.

Taking the opposite approach but effectively reaching the same conclusion, Marriot et al. (1997) suggested that several factors unrelated to the $\delta^{15}\text{N}$ of a plant’s N source could affect plant $\delta^{15}\text{N}$, including stress, genotype, type/extent of mycorrhizal association and assimilatory and age-dependent changes of signature. These interactions could confound the use of $\delta^{15}\text{N}$ as a tracer of specific N sources in natural ecosystems.

These views were summarised by Högberg (1997), who cited the various factors deemed to affect the $\delta^{15}\text{N}$ of plants by Nadelhoffer et al. (1996):

1. source of plant N (soil, precipitation, gaseous pollutants, fixation);
2. depth of soil from which N is taken up;
3. form of N used (gaseous or aqueous inorganic species, organic sources); or
4. influence of mycorrhizae and associated fractionations.

Thus according to Högberg (1997), and re-iterated by Lipson and Näsholm (2001), data on plant δ^{15}N cannot be used directly in comparisons between ecosystems, but may assist in interpretations of plant N source use in comparisons within ecosystems, especially in experimental settings and with other data or modelling. Multiple stable isotope approaches are especially useful, as they can disentangle source effects from internal fractionations within the system.

An example of a nitrogen stable isotope fractionation model is NIFTE (Nitrogen Isotope Fluxes in Terrestrial Ecosystems), which was developed to improve understanding of the relative importance of different fluxes and fractionations in producing observed patterns of ^{15}N natural abundance (Hobbie et al., 1999). Model runs for spruce and alder forests in Alaska suggested that fractionation during mineralisation must be small (~2%) while fractionation during mycorrhizal transfer of N (but not uptake) could explain many of the observed patterns in δ^{15}N.

7.2 Methods: soil and vegetation sampling

Sampling techniques for ^{15}N natural abundance assessment in the major pools (above-ground vegetation, organic/rooting layer and soils) are identical to those described for the post ^{15}N additions sampling in Chapter 6.

7.3 Results

7.3.1 δ^{15}N in soil and vegetation compartments

The results are summarised below, first looking at differences within each site, and then comparing differences between sites. The summary data on ^{15}N natural
abundance are presented in Table 7.2. Figure 7.1 shows the mean natural abundance \( \delta^{15}N \) values of the sampled vegetation and soil compartments across the various soil types within each of the four catchments, spanning a gradient of N deposition. The four sites are plotted in order of increasing N deposition, but within a site (e.g. M or G plots) soils are plotted in order of decreasing altitude. Lines joining soil plots within each site are purely for illustrative purposes and ease of interpretation.

7.3.1.1 Allt a'Mharcaidh

This catchment is characterised by very pronounced differences between soil and vegetation samples across all soil types. The most striking feature of the Mharcaidh data is the presence of exceptionally low (negative) \( \delta^{15}N \) values in the shrubs, which are among the lowest measured for any plant anywhere (compare data in Table 7.2 with Table 7.1), with mean values down to c. \(-10\%o\) on the valley peat (M2) and shallow peat (M4) and reaching below \(-12\%o\) (one sample was \(-13.6\%o\)) in the year’s growth at M2. The green shoots (year’s growth) generally show lower \( \delta^{15}N \) values than the woody stems; just one of three samples at M4 accounts for the slightly higher mean \( \delta^{15}N \) value in the year’s growth compartment. Large differences in shrub \( \delta^{15}N \) are observed between soil types, with the highest (least depleted) values at the highest altitude plots on the peaty rankers (M1) and the lowest in the valley peat plots (M2).

After the shrubs, the lichen and moss samples (including Sphagnum species) are the most depleted in \( ^{15}N \), with \( \delta^{15}N \) values on all soil types in the range c. \(-4 \) to \(-5\%o\). The grasses and plant root samples are all enriched relative to the shrubs and lichen/moss samples, also exhibiting broadly similar patterns with a very pronounced spatial variation within the catchment, despite a large spatial variability within a given soil type (see high standard deviations in Table 7.2). At the highest altitude plots (M1) one grass sample (of only two analysed from these plots, with \( \delta^{15}N = -9\%o\)) results in a low mean \( \delta^{15}N \) of c. \(-4\%o\), which is lower than the mean value of \(-2.4\%o\) for the plant roots there. In the valley peat plots (M2) the grass in enriched relative to M1, with a mean \( \delta^{15}N \) value of \(-1\%o\). The plant roots at M2 are slightly more depleted (\( \delta^{15}N = -3.6\%o\)) than at M1. However, in the plots at M3 and M4 the root and grass mean \( \delta^{15}N \) values are almost identical, both very enriched relative to all other vegetation compartments at the Mharcaidh (and at the other study sites) and being the only
vegetation samples within the whole study to have positive mean $\delta^{15}N$ values, of c. +2 to +3 (M3) and +1%o (M4).

The $^{15}N$ abundance of the surface organic horizon (which generally includes any litter) is intermediate between that of the shrubs/mosses and the soils ($\delta^{15}N$ in the range c. -2 to -3%o). While the deeper soils are more enriched, the shrubs are much more depleted in $^{15}N$, so the return of $^{15}N$ depleted litter to the surface of otherwise enriched soils might be expected to produce this result. Analysis of a single litter sample from M4 confirmed a low $\delta^{15}N$ value of -6.3%o. The $\delta^{15}N$ value of the surface organic horizon increases with decreasing altitude from M1 to M4.

The most enriched compartment at the Mharcaidh site is the lower organic soil horizon (i.e. >15-20cm depth), with positive mean $\delta^{15}N$ values from c. +3 to almost +10%o at M3. Values of $\delta^{15}N$ in the upper organic horizon (c.5-15cm) are lower than in the deeper soils by c. 1.5 - 4%o but follow the same pattern, and are in turn paralleled by those of the plant roots, but with $\delta^{15}N$ values greater by c. +3.5 to +5.0%o. The increase in $\delta^{15}N$ with soil depth has been frequently reported elsewhere, and suggests that the soils are enriched by mineralisation, which liberates $^{15}N$ depleted NH$_4^+$ relative to soil organic matter N. The observation that root and grass $\delta^{15}N$ values follow the same pattern but are more depleted suggests that the mineralised NH$_4^+$ is taken up by the plant roots.

It is noteworthy that separate root samples were only obtained from the surface organic layer, yet the $\delta^{15}N$ of the roots parallels that of the soils, and not the surface organic material in which they were located.

Overall, roughly parallel patterns between soil types seem to occur in the following compartments, in order of increasing $\delta^{15}N$: shrubs (year's growth) < shrubs (woody) < grasses and roots < upper organic soils < deeper soils. The most enriched soils in terms of these compartments are those at M3, while the most depleted occur at M2. The $\delta^{15}N$ values in the surface organic layer are intermediate between those of the soil and shrub compartments but do not follow the same pattern between soils, perhaps reflecting the different litter contributions of $^{15}N$ from vegetation on each soil.
Lichens and mosses follow an unrelated pattern, possibly suggesting an influence of direct atmospheric sources.

7.3.1.2 Afon Gwy

Patterns of $^{15}$N natural abundance on the four sets of soil plots at the Afon Gwy are much more consistent than at the Mharcaidh, largely due to the more uniform vegetation types at the Gwy (primarily grasses). Here, the predominance of grasses meant that above ground vegetation samples were separated into live and standing dead. Lichen and mosses were not separated out as they formed only minor components of the biomass; instead they were included in the ‘live’ sample. No litter layer was defined; this component was effectively contained within the surface organic layer or ‘turf’ compartment, as at the Mharcaidh.

At each of the four sets of plots at the Gwy, the above ground vegetation samples (both live and dead standing biomass) have the lowest natural abundance values of all compartments, with marginally but consistently lower mean values in the live biomass. The only exceptions are a solitary sample of *Calluna vulgaris* at G1, which has the lowest $\delta^{15}$N value found at the whole site ($-4.6\%o$) and a very marginally lower $\delta^{15}$N value in the roots than in the dead biomass at G3. Very similar $\delta^{15}$N values (range $-1.2$ to $-1.8\%o$) are observed in the above ground biomass on soils G2, G3 and G4, but are significantly lower in the samples from G1 (hilltop peat), the highest altitude plots ($-3.3$ to $-3.5\%o$: see Table 7.2).

Root samples follow the same general pattern, but measured $\delta^{15}$N values are greater than live standing biomass by c. $0.3$ to $1.2\%o$ at all plots. The natural abundance of $^{15}$N in standing dead biomass is intermediate between standing live biomass and roots, except at G3 (podsol), where it is very slightly greater than both.
Table 7.2: $^{15}$N natural abundance summary data (δ$^{15}$N, ‰; n = no. samples)

<table>
<thead>
<tr>
<th>Soil</th>
<th>Shrub - green</th>
<th>Shrub - woody</th>
<th>Grass - live</th>
<th>Grass - dead</th>
<th>Lichen and moss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n  Mean  SD</td>
<td>n  Mean  SD</td>
<td>n  Mean  SD</td>
<td>n  Mean  SD</td>
<td>n  Mean  SD</td>
</tr>
<tr>
<td>M1</td>
<td>3  -7.47  1.16</td>
<td>3  -5.98  0.59</td>
<td>2  -3.99  7.47</td>
<td>0</td>
<td>3  -4.17  0.19</td>
</tr>
<tr>
<td>M2</td>
<td>3  -12.41 1.06</td>
<td>3  -10.17 1.43</td>
<td>3  -1.09  0.73</td>
<td>0</td>
<td>3  -3.92  0.94</td>
</tr>
<tr>
<td>M3</td>
<td>3  -8.51  0.66</td>
<td>3  -6.98  0.27</td>
<td>3  2.24  0.89</td>
<td>0</td>
<td>3  -4.49  0.10</td>
</tr>
<tr>
<td>M4</td>
<td>3  -9.49  0.93</td>
<td>3  -10.07 2.02</td>
<td>3  0.54  0.87</td>
<td>0</td>
<td>3  -4.73  1.59</td>
</tr>
<tr>
<td>G1</td>
<td>1  -4.65 -</td>
<td>1  -4.66 -</td>
<td>3  -3.47  0.18</td>
<td>3  -3.26  0.34</td>
<td>0</td>
</tr>
<tr>
<td>G2</td>
<td>0</td>
<td>0</td>
<td>3  -1.77  0.29</td>
<td>3  -1.42  0.38</td>
<td>0</td>
</tr>
<tr>
<td>G3</td>
<td>0</td>
<td>0</td>
<td>3  -1.72  0.61</td>
<td>3  -1.22  0.47</td>
<td>0</td>
</tr>
<tr>
<td>G4</td>
<td>0</td>
<td>0</td>
<td>3  -1.81  0.15</td>
<td>3  -1.67  1.73</td>
<td>0</td>
</tr>
<tr>
<td>S1</td>
<td>0</td>
<td>0</td>
<td>3  -1.78  0.58</td>
<td>3  -2.00  0.17</td>
<td>1  -2.14 -</td>
</tr>
<tr>
<td>S2</td>
<td>0</td>
<td>0</td>
<td>3  -2.53  0.79</td>
<td>3  -2.58  0.82</td>
<td>0</td>
</tr>
<tr>
<td>S3</td>
<td>0</td>
<td>0</td>
<td>3  -3.32  0.90</td>
<td>3  -3.50  0.17</td>
<td>0</td>
</tr>
<tr>
<td>E1</td>
<td>3  -1.67  2.98</td>
<td>3  -3.51  1.62</td>
<td>0</td>
<td>0</td>
<td>1  -3.78 -</td>
</tr>
<tr>
<td>E2</td>
<td>3  -4.62  0.32</td>
<td>3  -6.13  0.29</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soil</th>
<th>Roots</th>
<th>Surface organic layer</th>
<th>Soil depth 1</th>
<th>Soil depth 2</th>
<th>Other samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n  Mean  SD</td>
<td>n  Mean  SD</td>
<td>n  Mean  SD</td>
<td>n  Mean  SD</td>
<td>n  Mean  SD</td>
</tr>
<tr>
<td>M1</td>
<td>3  -2.37  0.72</td>
<td>3  -3.09  0.40</td>
<td>3  2.19  1.09</td>
<td>3  6.30  0.29</td>
<td>M4 (litter) 1  -6.27 -</td>
</tr>
<tr>
<td>M2</td>
<td>3  -3.59  1.43</td>
<td>3  -2.85  0.41</td>
<td>3  1.32  0.74</td>
<td>3  2.87  0.36</td>
<td>E1 (litter) 2  -4.23  0.21</td>
</tr>
<tr>
<td>M3</td>
<td>3  2.76  8.71</td>
<td>3  -2.06  1.92</td>
<td>3  6.28  3.23</td>
<td>3  9.92  2.45</td>
<td>E1 (woody litter) 3  -5.46  1.58</td>
</tr>
<tr>
<td>M4</td>
<td>2  0.73  2.03</td>
<td>3  -1.74  1.97</td>
<td>3  4.14  0.46</td>
<td>3  5.52  1.18</td>
<td>E2 (litter) 3  -5.68  1.11</td>
</tr>
<tr>
<td>G1</td>
<td>3  -2.25  0.26</td>
<td>3  -2.22  0.89</td>
<td>3  3.09  0.82</td>
<td>3  4.01  1.20</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>3  -1.01  0.20</td>
<td>3  -0.67  0.67</td>
<td>3  4.62  0.53</td>
<td>3  5.44  0.48</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>3  -1.42  0.60</td>
<td>3  -0.96  0.34</td>
<td>3  5.34  0.69</td>
<td>3  6.10  0.41</td>
<td></td>
</tr>
<tr>
<td>G4</td>
<td>3  -1.20  0.67</td>
<td>3  -0.32  0.41</td>
<td>3  2.71  0.85</td>
<td>3  2.72  1.01</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>3  -1.50  0.45</td>
<td>3  -0.05  0.30</td>
<td>3  5.49  1.42</td>
<td>3  7.48  3.16</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>3  -1.19  0.88</td>
<td>3  -0.97  0.78</td>
<td>3  2.80  1.73</td>
<td>3  5.02  0.82</td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>3  -2.24  0.39</td>
<td>3  -1.46  1.18</td>
<td>3  3.20  1.93</td>
<td>3  5.38  2.37</td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>3  -3.02  1.39</td>
<td>3  -3.23  0.19</td>
<td>3  0.89  0.75</td>
<td>3  1.15  0.26</td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>1  -3.93 -</td>
<td>2  -4.23  0.52</td>
<td>3  0.78  0.55</td>
<td>3  1.39  0.67</td>
<td></td>
</tr>
</tbody>
</table>

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Figure 7.1: $\delta^{15}\text{N}$ values for all compartments

Study soil (sites ordered by increasing N deposition)

- Shrubs - green
- Shrubs - woody
- Grass - live
- Grass - dead
- Lichen+Moss
- Roots
- Organic soil
- Soil depth 1
- Soil depth 2
- Litter
- Woody Litter
The surface organic horizon (turf layer) samples are, in general, slightly more enriched in ${}^{15}N$ than all vegetation compartments, with $\delta^{15}N$ values greater by c. 1 to 1.5‰ than the most depleted compartment, the above ground live biomass. As with the vegetation compartments, a lower mean value (-2.2‰) is found at G1 relative to the other sets of plots (-0.3 to -1.0‰). Thus a consistent pattern in $\delta^{15}N$ values is observed for these components, with turf layer > roots > standing dead > live vegetation. The major difference between soil types is the occurrence of consistently lower $\delta^{15}N$ values at G1.

For the deeper soil horizons at the Gwy the general pattern between soils is different, but all soil samples are much more enriched in ${}^{15}N$ than the other compartments, having positive $\delta^{15}N$ values in the range +2.7 to +6.1‰. As at the Mharcaidh, the lower organic horizons have the greatest $\delta^{15}N$ values of all compartments, while values decrease slightly (by c. 1‰ except at G4) in the upper soils. Neither of the soil compartments follows the same pattern between plots as the other compartments, suggesting that the vegetation is more dependent on N sources originating in the surface organic layer.

7.3.1.3 Scoat Tarn
The distribution of ${}^{15}N$ between compartments at Scoat Tarn follows a very similar pattern to that at the Afon Gwy. The lowest natural abundance figures are found in the above ground biomass, which, as in the Gwy samples, is comprised mainly of grasses. There is very little difference in mean $\delta^{15}N$ values for live or dead standing biomass, but the vegetation is slightly more enriched with altitude, ranging from c. -2.0‰ on the podsol at S1 to -3.5‰ lower down the catchment on the peat at S3. The solitary sample of moss/lichen from S1 has an almost identical $\delta^{15}N$ value to the grasses. No shrubs were sampled at Scoat Tarn, although a few plants are scattered around the catchment.

Plant root $\delta^{15}N$ values are again higher at Scoat Tarn than in the above ground biomass, with the smallest difference at S1 (+0.3‰) and more pronounced differences at S2 and S3 (>1.1‰). As at the Gwy, the surface organic horizon (turf layer) is even more enriched relative to the vegetation, with $\delta^{15}N$ values consistently greater than
above ground biomass by c. +1.5 to +1.8‰. At S1 the mean value is 0‰, the highest value for this compartment found at any site.

Like the Gwy soils, the horizons beneath the surface organic layer at Scoat Tarn are significantly enriched relative to the other compartments analysed. The upper organic horizons show high, positive δ^{15}N values, with a maximum of +5.5‰ at S1. Natural abundance values are higher by c.2‰ in the deeper soil horizons, reaching almost +7.5‰ at S1, the highest value found in any soil except the peaty gley (M3) at the Mharcaidh.

For all compartments except plant roots, the highest δ^{15}N values occur at the highest altitude S1 plots. In the above ground biomass and surface organic layer, natural abundance decreases downslope through S2 to S3. This decrease in δ^{15}N with altitude is the opposite to the pattern observed at the Gwy, but at Scoat the highest soil is mineral (podsol) rather than a hilltop peat, as at G1.

7.3.1.4 River Etherow

This site differs from the others in that the vegetation is primarily *Calluna vulgaris*, with only very small proportions of grasses and mosses. Also, the soil is rather uniform deep peat at both sets of plots, the main difference between them being that E1 plots lie on almost bare peat with recently burned *Calluna*, while the E2 plots cover an area of mature *Calluna* plants.

The woody component of the vegetation has the lowest δ^{15}N values, unlike the samples from the only comparable vegetation at the Allt a’Mharcaidh where the green shoots representing the current year’s growth are more depleted than the woody biomass. As might be expected at a much higher deposition site, the shrubs are more enriched at the Etherow, compared with the Mharcaidh, but are still very depleted in \(^{15}\text{N}\), with a minimum mean value of -6.1‰ at E2. The year’s growth samples are enriched relative to the woody component by +1.5 to +1.8‰. A solitary sample of lichen/moss from E1 has a near identical δ^{15}N value to the woody *Calluna* component, at -3.8‰.
The $\delta^{15}N$ values measured in the surface organic horizon (here excluding litter) and plant roots are very similar, although the former are slightly more negative. Unlike the other sites, these compartments are not significantly enriched relative to above ground biomass, except for the woody *Calluna* samples from E2 (almost 2‰ lower). The current year’s growth of *Calluna* at E2 is only very slightly depleted relative to the surface organic horizon (difference of 0.4‰), while at E1 the current year’s growth is enriched by +1.5‰ relative to the surface organic horizon. With the predominance of *Calluna* at this site, obvious litter was separated into extra samples. The litter from under the mature *Calluna* at E2 has a $\delta^{15}N$ value of $-5.7‰$, intermediate between woody and year’s growth components of the *Calluna*. At E1 woody litter was separated from the remainder and is by far the most depleted component, with a $\delta^{15}N$ value (-5.5‰) very close to that of the woody component of above ground biomass and total litter from E2. The remaining litter component is less depleted ($\delta^{15}N = -4.2‰$) with a natural abundance of $^{15}N$ closer to the other compartments. Interpretation of the natural abundance data for E1 is made difficult by the recent burning there, which cleared most of the surface vegetation but left behind some coarser woody material and led to vigorous regrowth of *Calluna* and other plants.

The Etherow has the least enriched soils, with $\delta^{15}N$ values of under +1.4‰ compared with +2 to +6‰ in most soils at the other sites. As at the other sites, there is an enrichment in $^{15}N$ with depth in the soils, but the differences between upper and lower soils are small.

All compartments are more depleted in $^{15}N$ at the E2 (mature *Calluna*) plots than at the recently burned E1 plots, except at depth in the soil where natural abundance values are slightly higher (although more variable). The effect of the recent burning (probably within the 12 month period prior to sampling) in terms of the redistribution of N and in particular $^{15}N$ is not known, but it might be hypothesised that such a redistribution could account for the lesser depletion in vegetation and, to a smaller degree, the upper organic horizons, at E1 compared with E2. A possible redistribution of $^{15}N$ was similarly attributed to the physical mixing and deepening of active horizons following the ploughing of forest soils prior to planting at Aber in Wales, the
only site across a European gradient to show positive $\delta^{15}$N values in trees in the NITREX study (Emmett et al., 1998).

7.3.1.5 Inter-site comparisons

Of the four study catchments, the Allt a’Mharcaidh stands out for having by far the greatest differences between soil and vegetation $\delta^{15}$N values, i.e. the lowest enrichment factor (see below). Furthermore, the most extreme range of $\delta^{15}$N values for vegetation occurred there. As well as having extremely low natural abundance $\delta^{15}$N values for vegetation in the shrub samples, it is also the only site in this study at which vegetation samples with positive $\delta^{15}$N values occurred (grasses, plant roots).

The very low $\delta^{15}$N values from the Mharcaidh shrubs are among the lowest reported in the literature. In most terrestrial ecosystems $\delta^{15}$N values for plants or soils are generally between $-10$ and $+15\%o$ (Nadelhoffer & Fry, 1994). Fry (1991) presented natural abundance data from plants, litter, and soils in a number of non-cultivated ecosystems in the Long Term Ecological Research program (LTER) across the USA, and the lowest $\delta^{15}$N value of c. $-9\%o$ was found in non N-fixing plants. Vitousek et al. (1989) reported values down to $-10\%o$ in Hawaiian forest foliage on very young soils (<200 years) and similarly low values were found in herbarium plant specimens from the Western Mediterranean (Peñuelas & Estiarte, 1997). In more comparable ecosystems to the Mharcaidh site, very low $\delta^{15}$N values down to c. $-10\%o$ have been reported for Arctic and subarctic tundra plants (Schulze et al., 1994; Michelsen et al., 1996, 1998), but in a study of Alpine meadows in the USA, Miller and Bowman (2002) found minimum $\delta^{15}$N values of only c. $-2\%o$.

There is little apparent difference between the Afon Gwy and Scoat Tarn sites; in fact relative to the Mharcaidh and Etherow sites they are remarkable for the similarity of their $^{15}$N natural abundances across all compartments. The Etherow data stand out for revealing the least $^{15}$N enriched soil and most depleted surface organic horizon compartments of all four catchments, despite the very high N deposition at the site.

A consistent pattern across all catchments is observed in the relative increase in $\delta^{15}$N from surface organic horizon to upper organic and then lower organic/mineral soils.
This increase in $^{15}$N natural abundance with depth is generally attributed to a greater history of mineralisation with soil age, which enriches the remaining soil organic matter N pool.

### 7.3.2 Enrichment factors and N deposition

The basis of the enrichment factor ($e$) was described above in Section 7.1.3, where the importance of the soil compartment used in its calculation was highlighted, given the changes in $^{15}$N abundance that are widely found down soil profiles. Emmett et al. (1998) calculated the enrichment factor for tree foliage relative to the most active soil layers (Ah, +5c, and 5-15cm). Garten (1993) used foliage and the surface mineral soil below the Oi horizon. Koopmans et al. (1997) sampled soil horizons at various depths, including LF1 (top 1cm), F2 (5-8cm below), and mineral (0-10, 10-25, 25-50, 50-70), and used the mean $\delta^{15}$N value for the top 5cm of the mineral soil to calculate the enrichment factor. Näsholm et al. (1997) used the organic layer (H horizon). In the current study, $e$ has been calculated relative to the three soil compartments sampled.

If the surface organic horizon (‘turf’ layer) is used to derive $e$ then two patterns are apparent between sites (Figure 7.2a). For shrub samples, $e$ increases from the Mharcaidh and Gwy to the Etherow, although there is large within-site (between soils) variability at the Mharcaidh. However, for grass samples, $e$ decreases from mostly positive (but very variable) values at the Mharcaidh, to values in the range -0.8 to $-1.5\%e$ at the Gwy and further still to c. $-2.0$ at Scoat Tarn.

When the upper organic (generally c. 5-15cm) or lower (> c. 15cm) horizons are used to calculate $e$, all values are negative (Figure 7.2b-c), but the patterns of decreasing $e$ for grasses and increasing $e$ for shrubs is still found along the gradient of increasing N deposition from the Mharcaidh to the Gwy, Scoat Tarn and the Etherow. It might be suggested that the minimum value of $e$ relative to upper organic soils (Figure 7.2b) increases along this gradient, but in fact for the Gwy, Scoat Tarn and Etherow samples the differences are marginal.
It can be seen that similar patterns emerge when enrichment factors are calculated relative to all three soil compartments. It might be argued that for shrubs the change in $\varepsilon$ across sites is more pronounced with soil depth, while for grasses the opposite is true, but overall the patterns are consistent.

For shrubs, the increase in $\varepsilon$ with deposition inputs is consistent with the idea of increased rates of N cycling leading to greater enrichment of (shrub) vegetation relative to soil. However, the within-site variability at the Allt a'Mharcaidh is large compared with the differences observed between sites. Furthermore, the grasses show the opposite pattern (see discussion below).

To clarify the relationships between enrichment factors and deposition, $\varepsilon$ has been plotted against estimated catchment deposition in Figures 7.3-7.5. While there is no apparent relationship between $\delta^{15}N$ of the surface organic layer and N deposition when all sites are considered, removal of the Etherow samples from the plot on the basis of the site's heavily damaged status (see discussion below) shows that for the other three sites, there is a reasonable correlation between these factors (Figure 7.3a). However, since the $\delta^{15}N$ of deposition inputs are not known, and given the different land-use and deposition histories between sites, the enrichment factor should be more strongly correlated with N deposition across all sites. These relationships are shown in Figures 7.3b-c.

While the number of data points is limited by the lack of a universal vegetation compartment across all sites (e.g. shrubs were not sampled at Scoat and grasses were not separately sampled at the Etherow), some strong relationships are suggested by the data. The strongest relationship between $\varepsilon$ and N deposition is found for shrubs (Figure 7.3b), especially for the green shoots representing the year's growth. In this respect the green shoots of shrubs seem to be the most comparable vegetation compartment to the needles of conifers, which have been found to show a similar relationship between $\varepsilon$ and deposition inputs, thus illustrating the use of $\varepsilon$ as an indicator of N saturation status (e.g. Emmett et al., 1998).
Although values of $\varepsilon$ for grasses are also correlated with N deposition, the correlation is weaker and in the opposite direction, i.e. the enrichment factor decreases with increasing N deposition (Figure 7.3c). This finding suggests that the grasses utilise either a different form of N, or one from a different source and generated by a different process to the N utilised by the shrubs (see discussion).

There is no correlation between the $\delta^{15}$N value for the upper organic soils (below 5cm depth) and N deposition (Figure 7.4a), even when the Etherow samples are excluded. However, correlations between $\varepsilon$ and N deposition remain, in the case of shrubs being slightly stronger (Figure 7.4b), while slightly weaker than before for grasses (Figure 7.4c). It might be speculated that the stronger correlation for the enrichment of shrubs relative to the upper organic soils rather than the surface organic material could suggest a deeper source of N being utilised by shrubs, but the differences are very small.

For the deeper organic soils there is still no correlation between $\delta^{15}$N and N deposition, but the correlation between $\varepsilon$ for shrubs and N deposition is stronger than for both of the upper soil compartments (Figure 7.5). However, the relationship between $\varepsilon$ for grasses and N deposition disappears at this depth. The implication is that the shrubs are deeper rooting than the grasses, or at least utilise a source of N which is better reflected in the deeper soils.

7.3.3 Potential mineralisation, $\delta^5$N and $\varepsilon$

The major processes responsible for the fractionation of $^{15}$N and $^{14}$N are generally thought to be nitrification and, to a lesser degree, mineralisation. Denitrification can be a significant fractionating process under certain conditions, but is not thought to be important at these study sites (see Chapter 4). In particular, the enrichment factor may be expected to increase with N inputs and enhanced nitrification rates, which lead to greater plant uptake of $^{15}$N enriched NH$_4^+$, thus reducing the difference between the $\delta^{15}$N values of the soils and vegetation.
Figure 7.2a: Enrichment factors relative to the surface organic (0-5cm) horizon

Figure 7.2b: Enrichment factors relative to the upper organic (c.5-15cm) soil

Figure 7.2c: Enrichment factors relative to the lower organic (c.>15cm) soil
Figure 7.3: $\delta^{15}$N and $\varepsilon$ (relative to surface organic horizon) against total inorganic N deposition

**a: Surface organic layer (Etherow omitted)**

![Graph showing $\delta^{15}$N vs. TIN deposition (kgN/ha/yr) for surface organic layer with $R^2 = 0.562$.](image)

**b: Shrubs**

![Graph showing $\varepsilon$ vs. TIN deposition (kgN/ha/yr) for shrubs with $R^2 = 0.8244$ and $R^2 = 0.6332$.](image)

**c: Grass (live or total)**

![Graph showing $\varepsilon$ vs. TIN deposition (kgN/ha/yr) for grass with $R^2 = 0.6553$.](image)
Figure 7.4: $\delta^{15}N$ and $\varepsilon$ (relative to upper organic soil, below 5cm) against total inorganic N deposition

a: Upper organic soil (Etherow omitted)

TIN deposition (kgN ha$^{-1}$ yr$^{-1}$)

b: Shrubs

TIN deposition (kgN ha$^{-1}$ yr$^{-1}$)

c: Grass (live or total)

TIN deposition (kgN ha$^{-1}$ yr$^{-1}$)
Figure 7.5: $\delta^{15}$N and $\epsilon$ (relative to lower organic soil) against total inorganic N deposition

a: Lower organic soil (Etherow omitted)

b: Shrubs

c: Grass (live or total)
Potential mineralisation and nitrification data are available for the surface organic layer (0-5cm) and organic soils (c. 5-20cm) only, with extra samples from the mineral horizons at the Gwy (see Chapter 5). Potential mineralisation rates in the most similar soil horizons show no apparent relationship with the δ^{15}N values of the surface organic, upper organic and lower organic soils (Fig. 7.6).

When potential mineralisation in the surface organic layer is plotted against vegetation δ^{15}N, the relationships again appear, at first glance, to be rather poor (Figure 7.7). For shrubs, there appears to be two distinct groups of samples, with the most 15N depleted Mharcaidh samples showing an apparent increase in δ^{15}N as potential mineralisation increases, while another group with very high potential mineralisation and higher δ^{15}N values seems to show an increase in δ^{15}N with decreasing potential mineralisation (Figure 7.7a).

For lichens and mosses there are few samples, but the data do suggest a possible increase in δ^{15}N with increasing potential mineralisation in the surface organic layer (Fig. 7.7b). Since lichens and mosses may, like the shrubs, be able to access organic N pools via their associated mycorrhizae, then the same arguments as above will apply.

There is no clear relationship between δ^{15}N of grass or root samples and potential mineralisation in the surface organic layer (Figs. 7.7c-d). Both grasses and roots at the Mharcaidh show a wide range in δ^{15}N while potential mineralisation values are all near zero. The δ^{15}N values of the grass and root samples are very similarly distributed for most sites.

Some rather similar patterns emerge if the organic soils beneath the surface organic layer are considered (Figure 7.8). In the shrub components there is a general increase in δ^{15}N with increasing potential mineralisation in the organic soil (Figure 7.8a), albeit with some scatter, due to the mature Calluna plots from the Etherow (E2). The δ^{15}N of lichen and moss samples still shows an apparent relationship with potential mineralisation in the organic soils (Figure 7.8b) though this is difficult to explain for surface mosses and lichens. It is possible that the inclusion of some Sphagnum samples, which can be deep rooting, may be responsible for this pattern.
Figure 7.6: Potential mineralisation (µgN per g.organic per day) and $\delta^{15}$N in soil compartments

a: Surface organic layer ("turf", 0-5cm)

b: Upper organic soil (c. 5-15cm)

c: Lower organic soil (c. >15cm)
Figure 7.7: Potential mineralisation (µgN per g. organic per day) in surface organic horizon and δ¹⁵N of vegetation compartments

a: Shrubs
- Shrubs - green
- Shrubs - woody

b: Lichens and mosses
- Lichen & moss

с: Grasses
- Grass - live
- Grass - dead

d: Roots
- Roots
For the grasses, there is no apparent relationship between $\delta^{15}$N and potential mineralisation in the organic soil (Figure 7.8c), although a positive correlation might be suggested if the four Mharcaidh samples (with the lowest, near zero mineralisation values) are excluded as outliers. For the root samples (Figure 7.8d) there is no discernible relationship between their $\delta^{15}$N and organic soil mineralisation, but if the E2 (mature Calluna plots at the Etherow) data point is excluded (highest potential mineralisation), the distribution is in fact very close to that of the grasses.

7.3.3.1 Mineralisation and enrichment factors

The enrichment factor relative to the three sampled soil compartments is plotted against the most appropriate data for potential mineralisation in Figure 7.9. The resulting plots are quite different for the surface organic matter and the two soil compartments. When $\varepsilon$ is calculated relative to the surface organic layer, there is an increase in $\varepsilon$ for shrubs which is particularly steep with very low rates of potential mineralisation, but at higher rates is less apparent (Figure 7.9a). The samples showing the sharp increase in $\varepsilon$ are those from the Mharcaidh, which also had $\delta^{15}$N values which were correlated (weakly) with potential mineralisation (see Fig. 7.7). Grasses show the opposite relationship, with $\varepsilon$ decreasing as potential mineralisation increases. Again, it is the Mharcaidh samples which drive this relationship. The most striking feature of this plot is the separation of the Mharcaidh samples, which show a huge range in $\varepsilon$ across shrubs and grasses for a very small range in potential mineralisation values, all near zero, while samples from other sites have a relatively small range in $\varepsilon$ but a very wide range in potential mineralisation.

For $\varepsilon$ relative to the upper organic soil, similar patterns are found but with much more scatter (Fig. 7.9b). Shrub samples show a general increase in $\varepsilon$ with potential mineralisation but the Etherow sample E2 is again an outlier (the highest potential mineralisation value). Very similar patterns are found when $\varepsilon$ is calculated relative to the lower organic soils (Fig. 7.9c).

In general, the above observations are consistent with the relationships found between $\varepsilon$ and total inorganic N deposition in Figures 7.3 - 7.5, as might be expected if potential mineralisation rates increase with N deposition.
Figure 7.8: Potential mineralisation (μgN per g. organic per day) in organic soil (5-20cm) and δ^{15}N of vegetation compartments

- **a: Shrubs**
  - Shrubs - green
  - Shrubs - woody

- **b: Lichens and mosses**
  - Lichen & moss

- **c: Grasses**
  - Grass - live
  - Grass - dead

- **d: Roots**
  - Roots
Figure 7.9: Potential mineralisation (μgN per g, organic per day) and ε

(a) Surface organic layer (0-5cm)

Potential mineralisation (surface organic layer)

(b) Upper organic

Potential mineralisation (organic soil)

(c) Lower organic

Potential mineralisation (organic soil)
7.3.4 Potential nitrification, $\delta^{15}N$ and $\varepsilon$

The above comparisons made between potential mineralisation, N deposition, $^{15}N$ natural abundance and enrichment factors are repeated here for potential nitrification.

In the surface organic layer, while there is no apparent correlation between potential mineralisation rate and $\delta^{15}N$ unless the Etherow data are excluded, the potential nitrification rate increases with $\delta^{15}N$ (Figure 7.10a). Examination of the data reveals that the major difference between the two plots is due to the Etherow samples (see Fig. 7.6), in particular E2, where very high potential mineralisation rates at the lowest values of $\delta^{15}N$ do not correspond with high potential nitrification rates. Otherwise the spread of the data in the two plots is very similar.

Figures 7.10b and 7.10c show a greater scatter in the relationship between potential nitrification and $\delta^{15}N$ of the organic soils, but suggest that there is still a positive correlation between them. The figures show similar distributions to the corresponding plots for potential mineralisation in Figs. 7.6b-c if the Etherow mineralisation data are excluded. While this scatter may be due in part to the possible mismatch in horizons being plotted against each other, it might also be expected, given that the surface organic layer is much more active than the deeper soils, as shown by the much greater range of potential nitrification values. Hence it is no surprise that in the zone of greatest potential nitrification and biological activity, there is also the greatest potential for $^{15}N$ fractionation.

If the potential nitrification rates in the surface organic layer are plotted against the $\delta^{15}N$ values of different vegetation compartments, a consistent pattern emerges (Figs. 7.11a-d). The same pattern of potential nitrification rate increasing with $\delta^{15}N$ observed for the surface organic horizon itself is also observed for $\delta^{15}N$ of shrubs and perhaps lichens/mosses, although the latter have few data points (Figs. 7.11a-b). For grasses (Fig. 7.11c), although samples from the Mharcaidh are strong outliers, even in the remaining data there is no obvious relationship between potential nitrification in the surface organic horizon and grass $\delta^{15}N$. However, the highest $\delta^{15}N$ values are associated with some of the lowest nitrification potentials, suggesting a pattern which
is opposite to that in the shrubs. For roots, a strong relationship holds between potential nitrification and compartment $\delta^{15}\text{N}$ for one group of samples, but a separate group of outliers has elevated $\delta^{15}\text{N}$ values despite near-zero potential nitrification rates (Fig. 7.11d). In the root samples, the outliers are again the Mharcaidh samples, in particular those from M3 and M4, which are the only such samples to have positive $\delta^{15}\text{N}$ values in this study.

In summary, potential nitrification in the surface organic layer only appears to be correlated with compartment $\delta^{15}\text{N}$ for shrubs and roots (except for the root samples from soils M3 and M4 at the Mharcaidh). There are too few data to draw conclusions for lichens and mosses.

If these compartment $\delta^{15}\text{N}$ values are compared with potential nitrification in the organic soils, very similar patterns are found but if anything, the correlations are perhaps stronger (Figure 7.12a-d). The $\delta^{15}\text{N}$ of shrub and, in this case, lichen / moss samples, increases with soil nitrification (Figs. 7.12a-b) as they do for potential mineralisation, while the same outliers are found with the grass and root samples (Figs. 7.12c-d) as in the above comparison. The biggest difference between the mineralisation and nitrification plots is for the root samples, for which there is no apparent correlation with $\delta^{15}\text{N}$ for mineralisation, but there is a strong correlation, at least for a subset of samples, for nitrification.

7.3.5 Nitrification and enrichment factors

When the enrichment factor is plotted against potential nitrification rates, the responses of the different compartments are similar to those found with mineralisation (Figure 7.13). For enrichment factors calculated relative to the surface organic layer, $\varepsilon$ appears to increase with potential nitrification for shrubs, although there is a small range in potential nitrification and much scatter (Figure 7.13a). Conversely, for grasses, while there is a poor relationship between $\varepsilon$ and nitrification, values do, if anything, decrease as potential nitrification increases, largely due to the positive values of $\varepsilon$ at the Mharcaidh where potential nitrification is near zero.
Figure 7.10: Potential nitrification (µgN per g. organic per day) and δ¹⁵N in soil compartments

**a: Surface organic layer ("turf", 0-5cm)**

**b: Upper organic soil (c. 5-15cm)**

**c: Lower organic soil (c. >15cm)**
Figure 7.11: Potential nitrification (μgN per g. organic per day) in surface organic horizon and $\delta^{15}$N of vegetation compartments

a: Shrubs
- Shrub - green
- Shrub - woody

b: Lichens and mosses
- Lichen & moss

c: Grasses
- Grass - live
- Grass - dead

d: Roots
- Roots
Figure 7.12: Potential nitrification (\(\mu g N\) per g. organic per day) in organic soil (5-20cm) and \(\delta^{15}N\) of vegetation compartments

a: Shrubs

- Shrubs - green
- Shrubs - woody

b: Lichens and mosses

- Lichen & moss

c: Grasses

- Grass - live
- Grass - dead

d: Roots

- Roots
When enrichment factors and potential nitrification in the organic soil are considered (Figure 7.13b), the apparent relationship for shrubs breaks down. There is a separation of the Mharcaidh shrub samples, for which potential nitrification values are slightly negative and uncorrelated with $\varepsilon$, from the others, where it is tempting to suggest a correlation with $\varepsilon$ but there are only three pairs of data points. However, for the grasses there is a steady decrease in $\varepsilon$ (which is now negative) at lower values of potential nitrification, which then levels off at very high values of potential nitrification. This relationship could indicate an increasing utilisation of $^{15}$N depleted NO$_3^-$ by grasses as its availability increases. For the lower organic horizons, the same potential nitrification figures are used as for the upper organic soils, since only two levels were analysed (surface 5cm and c.5-20cm), and the patterns in the data are very similar (Figure 7.13c).

7.4 Discussion

There are clear differences in the natural abundance of $^{15}$N both within and between the four study sites, and between soil and vegetation compartments even within a single sample. The results described above may be used in three ways to provide information on the N status of study sites, in terms of supply, availability and rate of cycling, and these are considered separately below.

7.4.1 $\delta^{15}$N as a direct indicator of N status

For a given N limited system it might be expected that, all other things being equal, an increase in N inputs would lead to an increase in the rate of cycling of N within the system. In ecosystem compartments where fractionation leads to enrichment or depletion of $^{15}$N, an increase in N cycling should lead to greater enrichment or depletion, and hence to a change in $\delta^{15}$N. Since nitrification is one of the major fractionating processes, producing $^{15}$N depleted NO$_3^-$ if incomplete and leaving enriched NH$_4^+$, it might therefore be expected that sites or soils with enhanced nitrification would show, for example, higher $\delta^{15}$N values in vegetation which primarily utilises NH$_4^+$.  

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Figure 7.13: Enrichment factor and potential nitrification (µgN per g. organic per day) for soil compartments

a: Surface organic layer (0-5cm)

b: upper organic

c: lower organic

Potential nitrification (surface organic layer)

Potential nitrification (organic soil)
Figure 7.1 shows that there are few apparent trends in $\delta^{15}$N between sites (i.e. with increasing N deposition) for any given compartment unless the Etherow is excluded, when it might be argued that for the other three sites the $\delta^{15}$N of the surface organic horizon increases (see also Figure 7.3). The exclusion of the Etherow site in this context may be justified by consideration of the potential mineralisation and nitrification data, which show that while high rates of potential mineralisation are found, nitrification there is inhibited (see Chapter 5). This is probably due to severe acidification of the peat soils having disrupted the N cycle, to the extent that it is comparable to the N poor Mharcaidh catchment in terms of nitrification potentials.

At all sites the surface organic layer is always the most depleted soil compartment because of the return of depleted litter and standing dead organic matter to the soil surface. Variations in the $\delta^{15}$N of the surface organic layer closely parallel the patterns in the $\delta^{15}$N of vegetation within each catchment. At the Gwy, Scoat Tarn and the mature Calluna plots at the Etherow (E2), the surface organic layer is always more enriched than the sampled vegetation compartments by 1-2‰. At the Mharcaidh and the burnt E1 plot from the River Etherow, the $\delta^{15}$N of the surface material is intermediate between that of the vegetation compartments.

All sites show a steep increase in $\delta^{15}$N from the surface organic layer into the upper and then lower organic soils. This is consistent with the enrichment of soil organic N pools by mineralisation and the depletion of surface material through the return of depleted litter to the soil surface. While this pattern has been reported in some studies (e.g. Nadelhoffer & Fry, 1988, 1994), an initial increase in $\delta^{15}$N with depth in soils, followed by a decrease lower down the profile, has been reported in others (e.g. Shearer et al., 1978). A possible reason suggested for a reversal in $\delta^{15}$N increase at depth is the partial storage of depleted $\text{NO}_3^-$ leached from upper horizons (Högberg, 1997), but there is no evidence for such a process in the data from the current study.

The differences between sites can be further illustrated by plotting potential mineralisation and nitrification against $\delta^{15}$N for the various soil and vegetation compartments. For the surface organic layer of the soil, there is no apparent relationship between potential mineralisation and $\delta^{15}$N, except if the Etherow samples
are excluded (Fig. 7.6). Mineralisation could cause such an increase in $\delta^{15}$N either
directly, by releasing depleted NH$_4^+$ to leave an enriched soil organic matter pool, or
indirectly, by stimulating nitrification and hence the enrichment of the NH$_4^+$ pool
which is then taken up by vegetation and returned to the soil surface in litter. In either
case, it is the removal of depleted inorganic N which results in enrichment at the
surface.

Potential nitrification generally increases with $\delta^{15}$N (Figure 7.10), presumably
reflecting the enrichment of the remaining NH$_4^+$ which, if taken up by vegetation and
then returned to the surface in litter, would enrich the surface organic horizons, as
discussed above. While this observation supports the idea that high rates of
mineralisation can stimulate nitrification, it does not rule out the possibility that
mineralisation may also enrich surface organic material directly. There are weaker
relationships between both potential mineralisation or nitrification and $\delta^{15}$N for the
deeper organic soil compartments, which is probably due to the complex influences of
overlying soil horizons from which different forms of organic and inorganic N may be
transported.

When potential mineralisation in the soil compartments is plotted against the $\delta^{15}$N of
vegetation compartments, several patterns emerge. Most strikingly, the Mharcaidh
samples show different patterns to the rest of the data for all vegetation compartments.
For the shrub samples there is a correlation between potential mineralisation and
$\delta^{15}$N, in particular for the organic soils below the surface layer (Fig. 7.8), despite the
very low values of potential mineralisation at this site. These near zero values mean
that the Mharcaidh samples plot below the other data for all vegetation compartments.
The general pattern for all sites is one of increasing potential mineralisation with $\delta^{15}$N
of shrubs in both surface and lower organic soils. There are very few data points for
lichens and mosses, but there does appear to be an increase in potential mineralisation
with $\delta^{15}$N, again being stronger for the deeper soil horizon. For grasses and roots the
relationships of $\delta^{15}$N with potential mineralisation are very poor, except perhaps for
an increase in grass $\delta^{15}$N with potential mineralisation in the deeper organic soils if
the Mharcaidh samples are excluded.
Overall the relationships between potential nitrification and δ¹⁵N in vegetation compartments are similar to those for mineralisation, but with a few small differences (Figs. 7.11 - 7.12). For shrubs and lichen/moss samples there is a positive relationship between potential nitrification and δ¹⁵N which is stronger for the organic soils than for the surface organic layer. For roots there appear to be two groups of samples. The first group, comprising all the Mharcaidh samples, have very low potential nitrification rates which bear no relationship with compartment δ¹⁵N. The remaining samples seem to show a strong correlation between potential nitrification and δ¹⁵N, especially in the deeper organic soils (Fig. 7.12d). Since nitrification fractionates strongly against ¹⁵N to produce depleted NO₃⁻, the observed increases in δ¹⁵N with potential nitrification suggest that the plants are utilising the remaining NH₄⁺ which is enriched in ¹⁵N by partial nitrification.

One unknown factor here is the δ¹⁵N of N inputs to the study catchments in deposition. It is possible that inputs to the sites have very different δ¹⁵N values and this could potentially cause differences between sites which are not directly linked to the magnitude of N deposition. This problem can be overcome by use of the enrichment factor.

7.4.2 The enrichment factor (ε) as an indicator of N status

While differences in δ¹⁵N for a given compartment between sites may be caused in part by differences in land-use or the δ¹⁵N of deposition inputs, the magnitude of N inputs may still independently cause changes in the relative differences in δ¹⁵N between compartments at a given location through changes in the rate of N cycling. The difference in δ¹⁵N between the N source compartment (usually a soil compartment) and the product (a vegetation compartment) is called the enrichment factor (ε), and should directly reflect the rate of N turnover which is in turn linked to N supply. Hence differences in ε for two given compartments between sites should indicate inter-site differences in N turnover and magnitude of deposition, even if the δ¹⁵N signatures of deposition inputs are different at each site.
Despite the lack of a vegetation compartment present at all four sites, shrubs and grasses both occur at three sites. Figures 7.2 to 7.5 show that there does indeed appear to be a strong correlation between ε and N deposition at these sites, although the presence of only 3 sites in the plots does not allow the calculation of confidence limits. For the woody and especially the green (year’s growth) components of shrubs, a very strong correlation is found between ε and N deposition, with the River Etherow included as one of the three sites (Scoat Tarn is the excluded site). Hence the enrichment factor for shrubs shows a consistent increase with N deposition, which δ¹⁵N alone does not.

For grasses the opposite relationship between ε and N deposition is suggested, with ε declining as N deposition increases. However, this apparent relationship is driven largely by the positive values of ε for the grasses at the Mharcaidh, and as discussed below, resource partitioning of N at this site may explain these anomalous values. While it is possible that this relationship indicates an increasing utilisation of depleted NO₃⁻ as its availability increases with N deposition, it is clear from Figure 7.1 that the δ¹⁵N values of grasses at the Mharcaidh closely parallel those of the soils, whereas those at the Gwy and Scoat Tarn are tightly related to the δ¹⁵N of the surface organic layer. The same pattern might therefore occur if the grasses utilised NH₄⁺ mineralised from these different soil compartments, which could imply that they have to root deeper to find available N.

When the enrichment factor for shrubs and grasses relative to the three soil compartments is plotted against potential mineralisation, there are only weak relationships (Fig. 7.9). There is an increase in ε for shrubs against potential mineralisation, with a more obvious relationship in the deeper soils if outliers are discounted. For grasses there is a slight decrease in ε with increasing potential mineralisation, and the relationship grows weaker down the soil profile. These patterns are repeated when the enrichment factor is plotted against potential nitrification, but the relationship for shrubs is less evident than that found with mineralisation, while that for grasses appears to be stronger.
Since mineralisation provides the necessary substrate for nitrification, there is inevitably some correlation between the two processes; nitrification cannot proceed without the presence of the \( \text{NH}_4^+ \) substrate, although a high rate of mineralisation does not guarantee a high rate of nitrification, because the latter process may be inhibited in acid soils, as at the Etherow. It is therefore not possible to confidently determine from these data whether the observed patterns for shrubs result from the utilisation of labile organic N which has been enriched by mineralisation, or the utilisation of \( \text{NH}_4^+ \) which has been enriched by partial nitrification. The weaker relationship between \( \varepsilon \) for shrubs and potential nitrification, compared with mineralisation, suggests that the former explanation is more likely, although the observed patterns are largely driven by the Mharcaidh data where nitrification is negligible, so the latter process may operate at the other sites.

While the use of the enrichment factor implicitly relies on the assumption that the same source of N is being used by a vegetation compartment at the sites being compared (e.g. shrubs utilising \( \text{NH}_4^+ \) enriched by partial nitrification of the total pool), it has been suggested above that there may be another possible explanation for inter-site differences in \( \delta^{15}N \), which is the utilisation of different N sources between sites. This possibility is in itself linked to differences in N availability between sites.

### 7.4.3 \( \delta^{15}N \) as an indicator of N source in strongly N limited systems

In general, in catchments leaching \( \text{NO}_3^- \) which is produced by nitrification, and hence \( ^{15}N \) depleted, or with significant gaseous loss of \( ^{15}N \) depleted \( \text{NO} \), \( \text{N}_2\text{O} \) or \( \text{N}_2 \) through nitrification or denitrification, the export of depleted forms of N leads to an enrichment of the whole system, relative to the sources of N (Johannisson & Högberg, 1994; Nadelhoffer & Fry, 1994; Emmett et al., 1998; Austin & Vitousek, 1998). It therefore follows that in a catchment like the Allt a'Mharcaidh where leaching and gaseous losses of inorganic N are extremely low, even when expressed as a percentage of the relatively low deposition inputs, this enrichment of the system will not occur, and the average \( \delta^{15}N \) of the system should approach that of the inputs (Högberg, 1990, 1991; Johannisson & Högberg, 1994). Since deposition inputs tend
to be depleted in $^{15}$N, particularly in unpolluted areas (Austin & Vitousek, 1998; Vitousek et al., 1989) this may account at least in part for the extremely low $\delta^{15}$N values observed in the shrub compartment at the Mharcaidh as the whole terrestrial ecosystem is depleted, but unfortunately the $\delta^{15}$N data for deposition inputs are lacking. Furthermore, a closed N cycle at the Mharcaidh would help to explain the high soil $\delta^{15}$N values at depth, where soil organic matter is enriched by mineralisation and the uptake of depleted, mineralised $\text{NH}_4^+$ contributes to the large difference between the shrubs and the deeper soils.

Another possible cause of the extremely low $\delta^{15}$N values in the Mharcaidh shrubs in conjunction with very high values in the soils and grasses there, is the partitioning of N sources between species. In strongly N limited systems, competition for the scarce, available N may force certain components of the system to utilise different sources, either through the uptake of different forms of inorganic or organic N, or through spatial differences in the N pools accessed, for example through different rooting depths (Garten, 1993; Gebauer and Dietrich, 1993; Shulze et al., 1994). It is the removal of this N limitation by anthropogenic N deposition which reduces the need for partitioning of N sources between species and forms the basis for the use of the enrichment factor. Figure 7.1 graphically illustrates the differences which can occur, in this case between the strongly N limited system at the Allt a'Mharcaidh and the other systems where $\text{NO}_3^-$ leaching indicates the presence of inorganic N in excess of biological capacity to utilise it, although independent hydrological effects cannot be ruled out.

The distribution of the $\delta^{15}$N values between the vegetation compartments at the Mharcaidh are comparable to those found by Shulze et al. (1994) at the northern treeline of Alaska, another strongly N limited system. Low nutrient availability there is attributed to temperature limited decomposition which binds N and P in dead soil organic matter (Van Cleve et al., 1991), while deposition inputs are extremely low. Shulze et al. (1994) found that tree foliage was most depleted in $^{15}$N ($\delta^{15}$N = $-7.7\%$o), followed by shrubs ($-4.3\%$o), while grass was enriched in $^{15}$N ($\delta^{15}$N = $+0.9\%$o). These differences were attributed to differences in the pools of N utilised.
The positive values of $\delta^{15}\text{N}$ found in the grasses by Shulze et al. (1994) were linked to their rooting depth, which enabled them to utilise N from deeper soil horizons. Figure 7.1 shows that there is a strong correlation between $\delta^{15}\text{N}$ in grasses, roots and soils below the surface organic layer, which would be consistent with this hypothesis (although roots were sampled only in the surface organic layer, so the implication is that the roots would have to extend into the deeper soils too for this hypothesis to hold). Furthermore, the roots are consistently depleted in $^{15}\text{N}$ by 3-4‰ relative to the upper organic soils below the surface organic horizon, which could suggest that mineralised NH$_4^+$ is the primary source of N for the grasses at the Mharcaidh. While it is unlikely that mineralisation alone could account for such a difference in $\delta^{15}\text{N}$, it must be remembered that there is steep increase in $\delta^{15}\text{N}$ with depth and the average $\delta^{15}\text{N}$ of the upper organic soil may hide a wide range in values, so that if the grasses obtained NH$_4^+$ from the uppermost part of the sampled horizon, the measured difference in $\delta^{15}\text{N}$ would probably be less.

While Shulze et al. (1994) found the tree foliage compartment to be the most depleted in their study, followed by ericoid shrubs, there are no trees within the study areas at the Mharcaidh, where instead the shrubs are the most depleted vegetation compartment. Ericaceous shrubs were similarly found to be the most depleted vegetation compartment in a Bavarian spruce forest by Gebauer and Dietrich (1993), although in their study it was the twigs and not the foliage which were most depleted. The shrubs at the Mharcaidh could, as suggested by Shulze et al. (1994) for the trees at their Alaskan sites, be utilising mineralised NH$_4^+$ from litter and the surface organic layer, themselves already very depleted in $^{15}\text{N}$, thereby contributing to their extremely low $\delta^{15}\text{N}$ values. However, the large difference in $\delta^{15}\text{N}$ between shrubs and the surface organic layer of up to 9‰ seems unlikely to be due solely to this process, since mineralisation is thought to result in only a minor fractionation. The lichen and moss samples have low $\delta^{15}\text{N}$ values in the range $-4$ to $-5$‰ and are thought to obtain much of their N from atmospheric sources. It might therefore be speculated that atmospheric inputs of very depleted N, if taken up directly by shrubs which effectively form the "canopy" at the Mharcaidh, could account for their $\delta^{15}\text{N}$ values, which are lowest in the green shoots. Garten (1993) attributed the lower $\delta^{15}\text{N}$ of tree
foliage on mountain ridge sites compared with their valley counterparts to direct uptake of isotopically light NH\textsubscript{4}\textsuperscript{+} from deposition.

While several studies have suggested that organic N pools may be accessed by fungi and hence by plants with certain mycorrhizal associations, the large difference between soil and shrub $\delta^{15}N$ at the Mharcaidh suggests that this could only be the case here if there is a large difference in the $\delta^{15}N$ of labile organic N and total soil N. Michelsen et al. (1996) found very low $\delta^{15}N$ values down to $-8.8\%$ in shrubs with ericoid or ecto-mycorrhizal associations on N limited subarctic heaths in Sweden. With positive $\delta^{15}N$ values measured in deposition and total soil $\delta^{15}N$ reaching a minimum of around $-1\%$, the very low values in shrubs were attributed to mycorrhizal uptake of a component of labile organic N in fresh litter with very low $\delta^{15}N$. Such a process at the Allt a’Mharcaidh could explain the very low $\delta^{15}N$ values measured in the ericoid shrubs there.

Furthermore, in a more wide-ranging follow-up study in heath and forest tundra ecosystems, Michelsen et al. (1998) found differences in $\delta^{15}N$ of up to almost $8\%$ between ericoid or ecto-mycorrhizal plants and non-mycorrhizal species. They suggested a general pattern in $\delta^{15}N$ of non- or arbuscular mycorrhizal plants $>$ ecto-mycorrhizal plants $>$ ericoid mycorrhizal. Two possible processes were suggested for the $\delta^{15}N$ patterns observed. Since the ecto-mycorrhizal roots were $^{15}N$ enriched relative to leaves, isotopic fractionation could occur at the fungal-plant interface. Alternatively (or additionally) there could be differences in $\delta^{15}N$ between N species, with the microbial N pool becoming $^{15}N$ depleted during NH\textsubscript{4}\textsuperscript{+} immobilisation, with a consequent enrichment of the soil NH\textsubscript{4}\textsuperscript{+} pool. Either of these mechanisms could potentially account for the differences in $\delta^{15}N$ between the shrubs and grasses at the Allt a’Mharcaidh. More recent work by Högberg et al. (1999) suggested that the small size of the fungal N pool meant that the $\delta^{15}N$ of mycorrhizal plants was unlikely to be significantly changed by fractionation at the fungal-plant interface, whereas other work by Emmerton et al. (2001a, b) suggests that the degree of fractionation could indeed sufficiently change the $\delta^{15}N$ of N taken up by the plant that it would no longer be feasible to link it to the original N source.
The very different distribution of $^{15}\text{N}$ in the Mharcaidh soil and vegetation compartments relative to the other sites is due to the severe N limitation at the site. Increased N availability can decrease the dependence of plants on mycorrhizae, affect mycorrhizal fractionation and its relative importance and affect the magnitude of the different N pools available to plants.

7.5 Conclusions

The overall distribution of $^{15}\text{N}$ between compartments in the four study catchments shows that the Allt a’Mharcaidh and Etherow sites are both very different in terms of N cycling from the other two sites and from each other.

At the Allt a’Mharcaidh, where almost all of the low N deposition inputs are retained by the terrestrial ecosystem, the data show an extreme range in $\delta^{15}\text{N}$ between the various soil and vegetation compartments which are comparable with data from studies in strongly N-limited, subarctic environments. While the shrubs exhibit some of the lowest $\delta^{15}\text{N}$ values recorded in the literature, many of the grass samples from the Mharcaidh have much higher $\delta^{15}\text{N}$ values than their counterparts in the other study catchments here. These extreme differences must be due the utilisation of different N sources through the strong competition for scarce available N at this site.

The very low $\delta^{15}\text{N}$ in the shrubs could be due to the uptake of labile forms of organic N, via mycorrhizal associations, which are depleted in $^{15}\text{N}$ relative to the more recalcitrant soil organic N pool. This process could also explain the relative enrichment of the remaining soil organic matter in the organic soils (assuming these to be the rooting zone for shrubs). Mineralisation of this pool would then release $\text{NH}_4^+$ which is slightly depleted in $^{15}\text{N}$ relative to the soil, but still very enriched relative to the organic N utilised by the shrubs. Alternatively, the low shrub $\delta^{15}\text{N}$ values could be due to direct fractionation by active mycorrhizae, again resulting in a relatively enriched $\text{NH}_4^+$ pool. Preferential utilisation of this source of inorganic N would explain the much higher, positive $\delta^{15}\text{N}$ values in the grasses which closely parallel, but are lower than, those in the upper organic soils.
At the other end of the N deposition gradient, the River Etherow site shows strong evidence of an N saturated, severely impacted system. While potential mineralisation rates here are comparable to the other high deposition sites at the Gwy and Scoat Tarn, and much higher than at the Mharcaidh, potential nitrification rates are much lower, being more comparable to those at the N limited Mharcaidh.

In healthy ecosystems with elevated N inputs, losses of inorganic N from the system, either through NO$_3^-$ leaching following nitrification or gaseous losses via denitrification, tend to lead to an enrichment in $^{15}$N of the whole terrestrial ecosystem, since these two processes preferentially remove isotopically light N. Hence there is an increase in the $\delta^{15}$N of surface organic material with N deposition which reflects that of the dominant vegetation from the Mharcaidh to the Gwy and Scoat Tarn, but not at the Etherow.

The lack of nitrification at the Etherow suggests that enrichment of the system via leaching of depleted NO$_3^-$ does not occur. Since denitrification losses are also low (see Chapter 4) the mean $\delta^{15}$N of the whole system is likely to be approaching that of deposition inputs, which, while their $\delta^{15}$N is not known for this site, are generally depleted in $^{15}$N elsewhere. This could explain the low $\delta^{15}$N values in the vegetation and surface organic layer which integrates more recent deposition inputs, particularly at E2 where there has been less recent disturbance by burning. Furthermore, there is little difference in $\delta^{15}$N between surface soil organic matter and plants, because the excess NH$_4^+$ availability removes the need to utilise different N sources and fractionation via nitrification is much reduced. The $\delta^{15}$N of these compartments is, therefore, effectively overwhelmed by that of the deposition inputs.

The absence of nitrification also suggests that the NO$_3^-$ leached from the Etherow soils is probably "hydrologic" NO$_3^-$ which originates directly from deposition with minimal biological interaction in the soil. A similar conclusion was reached for highly impacted Bavarian forests by Durka et al. (1994), where stable isotopes were used to demonstrate that in the most impacted systems, a greater proportion of leached NO$_3^-$ was derived directly from atmospheric inputs, while in healthy systems, a large proportion of leached NO$_3^-$ had been biologically cycled and released by nitrification.
At the Gwy and Scoat Tarn sites, the $\delta^{15}$N of the predominantly grass vegetation closely parallels that of the surface organic horizon but not of the deeper soils sampled. This pattern implies that at these sites a tight N cycle is maintained between the grasses and the surface organic layer in which they root, which nevertheless permits the leaching of inorganic N down the soil profile, where nitrification is enhanced, converting any leached NH$_4^+$ to NO$_3^-$.  

Enrichment factors ($\epsilon$) have also been calculated for the various soil and vegetation components of the ecosystem, but are of limited use here because of the lack of a vegetation component found at all four sites. For the shrubs which were sampled at three sites (excluding Scoat Tarn), $\epsilon$ does appear to increase with N deposition, indicating that the enrichment factor may have potential applications for this type of vegetation. However, care should be taken in the interpretation of these data, since the low $\delta^{15}$N values at the Mharcaidh may to be due to the use of a $^{15}$N depleted organic N pool, and there is only one sample from the Gwy. For the three sites at which grasses were sampled, $\epsilon$ decreased with N deposition, but this pattern is driven largely by the positive values of $\epsilon$ from the Mharcaidh, which are thought to be due to N source partitioning. There is little evidence from these data that the enrichment factor is appropriate for grasses at these sites.  

The surface organic horizon (c. 0-5cm) plays a key role in the determination of $^{15}$N distributions in three of the four study catchments. It is consistently the most $^{15}$N depleted part of the soil profile, having $\delta^{15}$N values lower by 4-6% than the organic soils immediately below it, but is less depleted than the dominant vegetation because litter becomes enriched as mineralisation proceeds. A key observation from this work is that at the Mharcaidh, while different forms of N may be utilised, there is evidence that the whole of the sampled soil profile is contributing to vegetation uptake, whereas at the other three sites with enhanced N deposition, vegetation $\delta^{15}$N is very tightly linked to that of the surface organic layer.  

While there is no direct evidence from the stable isotope work alone to suggest that NO$_3^-$ leaching increases across the N deposition gradient, there is evidence of $^{15}$N enrichment of the vegetation and surface organic layers at the sites where high
potential nitrification rates were found, the Afon Gwy and Scoat Tarn. These findings are consistent with the loss of $^{15}$N depleted NO$_3^-$ from the rooting zone. Furthermore, the tight cycling of N between vegetation and the surface organic material, indicates that any NO$_3^-$ leached below this shallow surface layer should therefore be available for leaching. While it is possible that the $^{15}$N depleted NO$_3^-$ could be immobilised in the deeper soil horizons, there is no evidence for this process from these data; soil $\delta^{15}$N continues to increase through the profile with depth.

Overall, the natural abundance data closely agree with the conclusions of Schulze et al. (1994), whereby low N status sites are characterised by a very variable $\delta^{15}$N signal in vegetation and large enrichment factors, while high N availability leads to reductions in $\delta^{15}$N variability and very low or zero enrichment factors.

7.6 References


CHAPTER 8

ESTABLISHING N SATURATION STATUS
II: C:N RATIOS
8. ESTABLISHING N SATURATION STATUS II: C:N RATIOS

8.1 Introduction: the C:N ratio and N saturation

8.1.1 Historical importance of C:N ratio

The deliberate manipulation of C:N ratios in arable farming has long been practised. It is well known that the addition of crop residues just prior to sowing another crop can cause a temporary N deficiency in the new crop, and that the duration of this deficiency depends on the C:N ratio of the added material (Richards, 1987). The reason for this N deficiency is explained by the relative availability of C and N, and the resulting balance between the tightly coupled processes of mineralisation and immobilisation.

In general, adding material with a C:N ratio of more than 30:1 usually results in net N immobilisation for a period of weeks to months, while incorporating material with a ratio less than 20:1 causes net mineralisation and inorganic N release almost immediately. Addition of crop residues with a C:N ratio of 25:1 results in no net mineralisation or immobilisation, so that N must be added to increase the N content sufficiently to allow decomposition without immobilisation of all the mineralised N (Alexander, 1977; Richards, 1987; Paul & Clark, 1996). The occurrence of net mineralisation at low substrate C:N ratios indicates a relative abundance of N compared to metabolisable C available to heterotrophic decomposers (Alexander, 1977). Conversely, carbon rich material stimulates microbial immobilisation of N (Tamm, 1991).

The critical C:N ratio for crops therefore generally lies in the range 20-30 (Alexander, 1977). At greater C:N ratios, net immobilisation occurs, while at lower values, net mineralisation and nitrification may follow. These observations led to the development of the “nitrogen factor”, defined as the number of units of inorganic N immobilised per 100 units of added residue (Alexander, 1977). This concept, based on the “carbon-element” theory, recognised the fundamental link between the cycling of C and N, and is described in detail by Ågren and Bosatta (1996).
8.1.2 Linking C:N ratio to critical loads

While the relevance of the C:N ratio in regulating the availability of inorganic N for plant uptake in crops was long established, environmental scientists began to realise in the 1970s that it must also have a role in controlling the leaching of inorganic N in non-agricultural systems where it is present in excess of biological requirements. The idea of linking measures of N saturation to critical loads was put forward in the 1986 report of the Nordic Council of Ministers. Nilsson (1986a) suggested that as N accumulates in terrestrial systems, it may reach a state where it is no longer limiting, leading to increased NO₃⁻ leaching. Andersen (1986) stated that a high C:N ratio in litter makes N less easy to extract for micro-organisms, but that with an accumulation of N in the system and greater quantities in annual circulation, organic N may be more easily converted to mineral form. Nilsson (1986b) linked mineralisation and nitrification, as important controls on NO₃⁻ leaching, to substrate quality (litter type). Hence the concepts of C:N ratio and litter quality were inherent in the early development of critical loads models for N.

8.1.3 Effects of the relative availability of C and N on microbial processes

The relative amounts of C and N in both soil microbes and soil organic matter (SOM) are very important in determining the fate of inorganic N within the soil-plant system. For example, whether NH₄⁺ is immobilised by soil microbes or available for nitrification or transfer to the available N pool depends on the microbial N requirement for growth (Paul & Clark, 1996). The C:N ratio of micro-organisms may vary, so that fungi, for example, have a fairly constant C content of approximately 45%, but N contents of 3 to 10%, resulting in C:N ratios from 15:1 to 4.5:1. Thus the amount of inorganic N which can be assimilated and immobilised as organic N by the soil microbial biomass (SMB) will be controlled to an extent by the availability of N relative to C. Furthermore, the rate at which microbially immobilised N is transferred to the available inorganic N pool depends on both the C:N ratio of the substrate and the microbial death rate (Tamm, 1991).
The links between C, N and microbial cycling in forests were summarised by Aber (1992). In strongly N limited systems, most of the inorganic N cycled is in the form of \( \text{NH}_4^+ \), with plant uptake and microbial immobilisation outcompeting nitrifiers for available \( \text{NH}_4^+ \). Gross nitrification rates are therefore low because of a lack of \( \text{NH}_4^+ \), and any \( \text{NO}_3^- \) that is produced is rapidly immobilised to result in zero net nitrification. High rates of gross immobilisation are supported by large pools of labile organic C produced by the decomposition of N poor organic matter, maintaining the tightly closed cycling of N and high C:N ratios in plants, litter, SMB and SOM.

If N supply to an N limited system is increased, for example through atmospheric deposition, more inorganic N is taken up by plants and increased biomass production effectively reduces internal plant C pools (Aber, 1992). Production of labile organic C from N rich litter is also reduced where the N limitation on C metabolism is removed. Biological demand for inorganic N is increasingly met by deposition inputs and gross mineralisation of N enriched organic matter. Excess \( \text{NH}_4^+ \) availability has the dual effect of promoting nitrification (for which it is the substrate) and inhibiting \( \text{NO}_3^- \) immobilisation (since \( \text{NH}_4^+ \) is preferentially immobilised by microbes). Therefore, \( \text{NO}_3^- \) immobilisation is reduced despite increased \( \text{NO}_3^- \) availability from deposition and/or net nitrification. With sufficiently high inputs of inorganic N, \( \text{NO}_3^- \) will accumulate in the soil and the potential for leaching will increase.

Furthermore, N deposition may create a positive feedback in forest N cycling which destabilises the system (Gundersen, 1992). Increased N availability (from deposition) in the tree reduces translocation of N from old to new tissue, so that litterfall has a lower C:N ratio and lignin content. The N rich litter is then more readily mineralised with the reduced N limitation on the process, further increasing N availability to the tree. Hence for plants adapted to low N availability, an important indirect effect of N deposition is the positive feedback of stimulated mineralisation and nitrification, which increases the potential N supply rate within the soil (NEGTAP, 2001).
8.1.4 C:N ratio as an indicator of N saturation and leaching

Since increased N supply from deposition stimulates nitrification and increases the probability of net nitrification occurring, and with the possibility of some inorganic N deposition reaching watercourses after minimal interaction with the soil-vegetation system, it might be expected that some relationship between deposition and leaching of inorganic N might be found.

Dise and Wright (1995) investigated the relationship between throughfall N and NO$_3^-$ leaching in a European forest database. They found that there is negligible NO$_3^-$ leaching from forests with throughfall N below 10 kgN ha$^{-1}$ yr$^{-1}$ and significant leaching from all sites where throughfall N exceeds 25 kgN ha$^{-1}$ yr$^{-1}$. However, in the many forests experiencing throughfall N inputs in the range 10-25 kgN ha$^{-1}$ yr$^{-1}$, some sites leach NO$_3^-$ while others do not.

In a similar later study, Gundersen et al. (1998) found that NO$_3^-$ leaching is very variable from forests receiving deposition loads over a wider range (10-60 kgN ha$^{-1}$ yr$^{-1}$), concluding that deposition alone cannot be used to predict NO$_3^-$ leaching. They suggested that since empirical relationships had been observed between forest floor C:N ratios and NO$_3^-$ leaching, this parameter could provide a useful index. The hypothesis was that forests may differ in the size of the C pool within the active forest floor, so that different levels or cumulative totals of N deposition are required to reduce the C:N ratio to a level where net nitrification is stimulated, leading to NO$_3^-$ leaching. $^{15}$N tracer studies have shown that up to 56% of N in throughfall is retained in the forest floor (Tietema, 1998; Tietema et al., 1998), demonstrating the importance of nitrification as a control on N leaching.

Non- or poorly nitrifying soils are associated with high C:N ratios and very low content of other nutrients like P, K and Ca (Kriebitzsch, 1978; Gundersen, 1992). Vitousek et al. (1982) found fresh litter C:N to be a good indicator of N cycling and availability at a forested site, and therefore a useful predictor of nitrification capacity. Forest studies elsewhere have suggested that nitrification is stimulated when forest floor N exceeds 1.4% (Wilson & Emmett, 1998) or when C:N ratio declines below 27 (Kriebitzsch, 1978). Gundersen and Rasmussen (1988) suggested that forest
ecosystems had the potential to leach NO$_3^-$ if the amount of N in litterfall exceeded 60 kgN ha$^{-1}$ yr$^{-1}$ or if its C:N ratio was below 40. The NITREX project used $^{15}$N tracers to demonstrate that the increase in NO$_3^-$ leaching was due to increased nitrification rates rather than decreased immobilisation (Tietema, 1998, cf. Stark & Hart, 1997).

In European forests, NO$_3^-$ leaching has been linked to the forest floor C:N ratio as an indicator of overall N status (Dise et al., 1998; Emmett et al., 1998a; Gundersen et al., 1998). Gundersen et al. (1998) calculated C:N ratios from the surface organic horizons of forest soils (L, F, H without freshly fallen litter) and compared them with leaching outputs or soilwater NO$_3^-$ concentration. They found that when the forest floor C:N ratio exceeded 30, no NO$_3^-$ leaching was observed, while all sites with a C:N ratio lower than 25 leached some NO$_3^-$. However, in the C:N range 25-30, both high leaching and full N retention were found.

In a similar exercise, Dise et al. (1998) found that the C:N ratio in the organic (OH) horizon can indicate NO$_3^-$ leaching if forest sites are grouped into broad categories of N deposition (measured as throughfall). At sites experiencing deposition of less than 10 kgN ha$^{-1}$ yr$^{-1}$, no NO$_3^-$ leaching occurs regardless of C:N ratio. At intermediate deposition sites (10-20 kgN ha$^{-1}$ yr$^{-1}$) NO$_3^-$ leaching increases with decreasing C:N ratio. At high deposition sites (>20 kgN ha$^{-1}$ yr$^{-1}$), higher leaching of NO$_3^-$ occurs for a given C:N ratio than in intermediate deposition sites, but at very high levels of deposition (>30 kgN ha$^{-1}$ yr$^{-1}$) there is greater scatter in the relationship, suggesting that other factor must be operating, for example luxury NH$_4^+$ uptake, fixation of NH$_4^+$ or shifts in species composition. Hence C:N ratio alone is clearly not the only factor to consider, and though it can provide an indication of the risk of NO$_3^-$ leaching, intrinsic differences in site characteristics also appear to be important.

Assuming that leached NO$_3^-$ is mainly derived from deposition (Durka et al., 1994) prior to the onset of enhanced nitrification, then leaching can be expressed as a proportion of inputs. A plot of this proportion against forest floor C:N ratio shows that a ratio of 24 seems to represent a threshold, below which the proportion of NO$_3^-$ leaching increases, until it exceeds unity (outputs > inputs) suggesting the onset of enhanced nitrification (Emmett et al., 1998b, c).
Andersson et al. (2002) used the C:N ratio of forest soil surface organic layers (L, F, H) as an index of N status and found a correlation with net mineralisation. NO$_3^-$ leaching was poorly correlated except at sites with high N mineralisation, with no leaching where N mineralisation was low. Christ et al. (2002) found that net nitrification potential in central Appalachian forest soils showed a very highly significant correlation with soil C:N ratio and that NO$_3^-$ leaching was correlated with both.

While many studies have found links between C:N ratio and nitrification or NO$_3^-$ leaching, Robertson (1982) found no direct correlation between nitrification and either %N or C:N over a wide range of forest soils. Other studies have found no relationship between NO$_3^-$ concentration in soilwaters and soil C:N ratio, e.g. in 150 Dutch forest soils (Ferrier et al., 1995). Some authors suggested that certain chemical nitrification inhibitors may be produced by litter decomposition, but others found no evidence for this (van Miegrot & Johnson, 1993), and the reasons may instead be linked to litter quality (see below).

8.1.5 Organic matter "quality" as an additional control on mineralisation

The importance of the C:N ratio of organic matter in influencing its decomposition rate has been recognised for many decades, but recent studies have contradicted the long held belief that increased N content in organic residues always leads to accelerated decomposition (INDITE, 1994). N deposition may lead to changes in the quantity and quality of plant litter, which may accelerate the initial stages of decomposition but slow down later stages (Berg et al., 1998; NEGTAP, 2001). Fog (1988) reviewed the literature on the subject and suggested a general pattern linked to both C:N ratio and the "quality" of organic matter. If the C pool is not readily metabolised by the SMB then mineralisation is slow regardless of the C:N ratio of the substrate (Smith, 1994). Decomposition of SOM with a low proportion of recalcitrant organic matter is accelerated by low C:N ratios of less than around 40. The lowest C:N ratio usually occurs in biomass, so it is a relatively labile material (Vinten & Smith, 1993).
In poor quality substrates with high C:N ratios greater than around 50, decomposition is largely controlled by lignin content, and inorganic N inputs may inhibit the enzymes required for lignin degradation, thus slowing the later stages of decomposition and potentially resulting in an increase in N accumulation rates in forest soil organic matter (Berg & Matzner, 1997). A change in the balance of microbial populations from lignin to cellulose decomposers may occur, causing reduced rates of decomposition over a timescale of many years (INDITE, 1994). The major causes of these changes in decomposition rate following inorganic N inputs, often described as “decreased microbial activity”, are increases in decomposer efficiency, faster formation of recalcitrant organic matter and decreased growth rate of decomposers (Ågren et al., 2001). Hence litter quality (defined by the lignin or polyphenol content) as well as C:N ratio is an important control on decomposition rates, so that there is no universal value of C:N ratio at which the switch from immobilisation to net mineralisation and NO$_3^-$ leaching occurs (Richards, 1987; Gundersen, 1992; Vinten & Smith, 1993). The inhibitory effects of N on decomposition were not observed at sites within the NITREX study, suggesting that more work is required in this area (NEGTAP, 2001).

8.1.6 C:N ratios and the N cycle in non-forest systems

Although much research is being carried out in forest systems, there is a general paucity of information on these processes for semi-natural systems typical of the UK uplands (NEGTAP, 2001). In one of the few studies on a heather moorland site (Ruabon, North Wales), prolonged additions of NH$_4$NO$_3$ over 7 years had increased NH$_4^+$ and NO$_3^-$ in the litter layer as well as reducing the C:N ratio, but very little inorganic N had been leached from the soils (Lee et al., 2000, cited in NEGTAP, 2001). In Sphagnum bogs, it has been shown that some N may be assimilated by plants with concomitant C fixation during growth, but excessive N assimilation without growth, perhaps due to P or K limitation, results in decreased plant C:N ratios and a greater tendency to remineralise N or release DON (Aerts et al., 1992; Williams & Anderson, 1999). White et al. (1996) found that at low to moderate levels of N inputs (<9 kgN ha$^{-1}$ yr$^{-1}$) to Calluna moorland on peaty podsol soils, total soil N and C:N ratio increased almost linearly with N deposition, presumably due to the effect of
acidification on organic matter accumulation rate, such that C accumulates faster than N. At higher total N deposition rates (>9 kgN ha\(^{-1}\) yr\(^{-1}\)) the C:N ratio declined with increasing deposition. Hence the few studies on the affects of N additions on moorland soil C:N ratios have produced inconclusive results.

8.1.7 Aims of the chapter

Work in forests has shown that C:N ratio of the forest floor in an important, if not necessarily overriding, control on enhanced mineralisation, net nitrification and NO\(_3^−\) leaching. This parameter therefore has a potential application for modelling the impacts of N deposition on surface water quality and acidification. The potential links between C:N ratio of surface organic material and leaching of NO\(_3^−\) from moorland systems are largely untested. The purpose of this chapter is therefore to explore the links between C:N ratio and other measures of excess N availability in the major soils of the four moorland study catchments.

8.2 Methods: sampling and analysis

Soils analysed for C and N were subsampled from the \(^{15}\)N tracer additions study samples which required the same preparation. Soil sampling, splitting and preparation are described in detail in Chapter 6. However, unlike the \(^{15}\)N analysis, soil samples for CHN analysis were not bulked, so that the vertical distribution of C and N within soils could be quantified. Post \(^{15}\)N additions samples were used: N additions were equivalent to only 1.5 kgN ha\(^{-1}\) yr\(^{-1}\) and should not have measurably changed soil bulk N content. For surface organic horizons, the whole sample including roots was used.

Samples were analysed at the University of Wales, Bangor, using a LECO CHN-2000 C, H and N total element analyser.
8.3 Results: C and N content of soils

8.3.1 N content of soils

The N content of the surface organic horizon samples is plotted for sites along the deposition gradient in Figure 8.1. There is an apparent increase from the Mharcaidh to the Gwy and Scoat Tarn, but then a decrease at the Etherow. Of the mineral soils at Scoat Tarn, only the podsol (S1) has a higher N content than the mineral Gwy soils (G2 and G3), while the peaty gley (S2) shows a similar range to these soils. The peat from Scoat Tarn (S3) has a slightly higher N content than the peats from the Gwy (G1 and G4), but is very variable. The Etherow peats are comparable in N content to those from the Gwy.

Comparisons of N content are confounded to a degree by the mineral content of soils, which is minor in surface samples (although the Gwy podsol G3 has an LOI value of just 48% compared with typical values of 90% elsewhere). For this reason, deeper soils that include mineral horizons in some cases are not plotted here.

8.3.2 C:N ratios of soils

Although mineral content may confound comparisons of per cent N content between soils, the C:N ratio is not affected in this way, since both C and N are derived primarily from the organic component. C:N ratios in the surface organic layer and the two uppermost underlying horizons are shown in Figure 8.2 and listed in Table 8.1. Soil 1 generally represents a horizon from c. 5-15cm depth and Soil 2 represents c. 15-30cm depth, although there is much variation between soils since these samples were defined by horizon.

It can be seen that there is a large within-profile variation in C:N ratio, with the location of the highest values differing between soils. Of the 13 soils sampled, 7 have the highest C:N ratio in the surface organic layer. At the Mharcaidh, the only exception is the peaty gley (M3), while at the Gwy the hilltop peat (G1) has a slightly higher value at depth. At Scoat Tarn only the peaty gley (S2) has the highest C:N ratio.
in the uppermost soil, and at the Etherow, both peats show a much higher value in the deepest sample.

Figure 8.1: Mean %N content (±1SD) of surface organic horizons (0-5cm)

Figure 8.2: Mean C:N ratio for the three uppermost soil horizons (surface organic = 0-5cm, soil depth 1 = c. 5-15cm, soil depth 2 = c.15-30cm)
Since it has been demonstrated in previous chapters that the surface organic horizon is by far the most active in terms of denitrification (Chapter 4) and potential mineralisation and nitrification (Chapter 5), this horizon is deemed the most important control on key soil processes, and hence its C:N ratio is used in subsequent comparisons with other data. Table 8.1 and Figure 8.3 show that although there is spatial variability in C:N ratio (as represented by standard deviations from the mean), even within the same horizon of the same soil, there are major differences between soils and especially between sites.

Maximum C:N ratios of c.43-58 in the Mharcaidh soils compare with a minimum value of 15.7 in the podsol at Scoat Tarn (S1). The decline from the Mharcaidh to the Gwy and Scoat Tarn is very evident, while the higher C:N ratio in the surface of the Etherow peats is very similar to that in the hilltop peat from the Gwy (G1), at around 30. Mean C:N ratios are compared with other aspects of soil biogeochemistry below.

### Table 8.1: C:N ratios in sampled soil horizons

<table>
<thead>
<tr>
<th>SOIL</th>
<th>Surface Mean</th>
<th>SD</th>
<th>Soil1 Mean</th>
<th>SD</th>
<th>Soil2 Mean</th>
<th>SD</th>
<th>Soil3 Mean</th>
<th>SD</th>
<th>Soil4 Mean</th>
<th>SD</th>
<th>Soil5 Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 Peaty ranker</td>
<td>58.4</td>
<td>9.9</td>
<td>41.6</td>
<td>1.6</td>
<td>46.0</td>
<td>1.5</td>
<td>64.8</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2 Valley peat</td>
<td>56.2</td>
<td>4.6</td>
<td>39.3</td>
<td>4.7</td>
<td>29.5</td>
<td>4.9</td>
<td>37.1</td>
<td>3.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3 Peaty podsol</td>
<td>43.2</td>
<td>5.2</td>
<td>47.7</td>
<td>8.3</td>
<td>50.0</td>
<td>6.5</td>
<td>74.1</td>
<td>32.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4 Shallow peat</td>
<td>48.4</td>
<td>6.7</td>
<td>39.0</td>
<td>8.4</td>
<td>36.8</td>
<td>10.6</td>
<td>57.1</td>
<td>17.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gl Hilltop peat</td>
<td>29.8</td>
<td>5.2</td>
<td>21.7</td>
<td>2.3</td>
<td>30.5</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2 Peaty gley</td>
<td>21.2</td>
<td>3.2</td>
<td>18.2</td>
<td>1.7</td>
<td>17.6</td>
<td>1.1</td>
<td>17.3</td>
<td>2.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3 Podsol</td>
<td>23.0</td>
<td>2.8</td>
<td>15.2</td>
<td>0.5</td>
<td>13.7</td>
<td>0.8</td>
<td>15.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G4 Valley peat</td>
<td>25.0</td>
<td>2.7</td>
<td>16.3</td>
<td>2.2</td>
<td>17.9</td>
<td>1.4</td>
<td>19.2</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1 Podsol</td>
<td>15.7</td>
<td>0.5</td>
<td>15.2</td>
<td>0.1</td>
<td>17.6</td>
<td>0.6</td>
<td>15.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2 Peaty gley</td>
<td>21.4</td>
<td>2.0</td>
<td>15.8</td>
<td>0.4</td>
<td>15.7</td>
<td>0.7</td>
<td>17.4</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3 Deep peat</td>
<td>22.2</td>
<td>3.6</td>
<td>19.2</td>
<td>3.8</td>
<td>24.7</td>
<td>2.5</td>
<td>27.2</td>
<td>3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1 Peat (burnt)</td>
<td>28.5</td>
<td>1.0</td>
<td>30.2</td>
<td>2.7</td>
<td>38.3</td>
<td>7.5</td>
<td>44.2</td>
<td>7.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2 Peat (unburnt)</td>
<td>30.4</td>
<td>2.8</td>
<td>29.0</td>
<td>1.0</td>
<td>42.5</td>
<td>2.2</td>
<td>46.9</td>
<td>6.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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8.3.3 C:N ratio and inorganic N in soilwaters

Mean annual concentrations of N species in soilwaters sampled at c. 30cm depth (see Chapter 3) are plotted against the C:N ratio of the surface organic horizon in Figure 8.4. When all sites are included, no clear pattern is apparent, other than very high concentrations of all N species at C:N ratios of around 30 (Fig. 8.4a). Inspection of the data reveals these points to be from the Etherow, and the same plot excluding the Etherow shows that there are some increases in all N species at C:N ratios below 30.

The response to decreases in C:N ratio below the apparent threshold value of 30 varies with N species; NO$_3^-$ increases, NH$_4^+$ decreases and there is no clear pattern for organic N. Since there are no samples with a C:N ratio in the range 30-40 it cannot be assumed that 30 is the true threshold; it is possible that a leaching threshold may occur in this higher range. Furthermore, all the samples with very high C:N ratios come from one site, the Allt a’Mharcaidh, so the data must be interpreted with caution.
Figure 8.4: Surface soil C:N ratio and soilwater N

8.3.4 C:N ratio and mineralisation / nitrification potentials

Another measure of inorganic N supply is the mineralisation potential, and the closely related nitrification potential. These are plotted against C:N ratio of the surface organic horizon in Figures 8.5 and 8.6.

Figure 8.5: Mineralisation potential (μgN per gram organic matter) against C:N ratio in the surface organic horizon (0-5cm)
There is an apparent threshold C:N ratio of around 30 below which significant mineralisation potentials occur (Fig. 8.5), but there is much scatter in the data and no obvious increase in potential with decreasing C:N ratio. For nitrification, the threshold appears to lie between C:N values of around 25 to 30 (Fig. 8.6), i.e. slightly lower than for mineralisation. Furthermore, there is a sharp increase in nitrification potential with decreasing C:N ratio below this threshold. Hence it appears that high rates of nitrification are associated with C:N ratios below a value of around 25.

8.3.5 C:N ratio and trace gas fluxes

In Chapter 4 it was suggested that CO$_2$ fluxes may be a crude indicator of microbial activity, particularly in incubated soil cores where there is no confounding influence of plant respiration. Furthermore, denitrification fluxes of N$_2$O are indicators of NO$_3^-$ availability and therefore potentially of nitrification, although there is again a confounding factor in the direct deposition of NO$_3^-$ onto soils. Both of these trace
gases have been measured in laboratory incubations of soil cores, at 5°C and 15°C, and in 4-weekly measurements of fluxes from static chambers in the field over the period of the budget year. These fluxes are plotted against C:N ratio in Figure 8.7. For laboratory incubations, data from core tops only are used, which are directly comparable with the surface organic layer measurements of C:N ratio.

**Figure 8.7: Trace gas fluxes in laboratory incubations and in the field against C:N ratio**
At 5°C, CO₂ fluxes are very low at high C:N ratios, while they are very variable below an apparent threshold C:N ratio of around 30 (Fig. 8.7a). A more obvious pattern can be seen in the 15°C incubation (Fig. 8.7b), where CO₂ flux generally increases with declining C:N ratio along the whole gradient, i.e. there is no threshold response at this temperature.

For N₂O emissions, there is a threshold in the C:N range 25-30 at 5°C, below which N₂O fluxes increase as C:N declines, albeit with much scatter in the relationship (Fig. 8.7c). Again, a clearer pattern is visible in the data from the 15°C incubation (Fig. 8.7d), with a sharp increase in N₂O flux as C:N decreases below a threshold of around 25.

In field measurements, the mean CO₂ flux data show a rather similar pattern to the laboratory incubations (Fig. 8.7e, cf. 8.7a-b), with no obvious threshold but a general increase in flux as C:N declines. For denitrification fluxes of N₂O, extremely low values from most soils lead to a very poor relationship with C:N ratio, suggesting that factors other than C:N ratio are more important regulators of denitrification in the field, for example soil moisture.

8.3.6 C:N ratio, N deposition and catchment scale leaching of inorganic N

To examine the relationship between C:N ratio and deposition inputs or catchment leaching outputs in streamwaters, it is necessary to aggregate the soil C:N data at the catchment scale. In the absence of data on soil distribution within each catchment to facilitate weighting of the C:N data by soil type, a mean value for all sampled soils is used here, assuming that the soil types are representative for each catchment. Overall mean, minimum and maximum values (using means for each soil type) are plotted against N deposition data in Figure 8.8. Figures on the left show modelled deposition data from the CEH 1995-97 average dataset, while those on the right show measured bulk deposition data, weighted by volume of 2-weekly samples throughout the budget year. Note that the C:N scale is reversed on the right hand side of the charts.
While the number of data points is too small for statistical analysis, there is a clear relationship between the range in C:N ratio observed at each catchment and deposition inputs of inorganic N. The C:N data are best reflected by the bulk deposition figures, which show a smaller flux at the Etherow relative to Scoat Tarn. The modelled data suggest a very similar total N deposition (i.e. including dry deposition) at these two sites. The correlation looks better for NO$_3^-$ than for NH$_4^+$ or TIN in bulk deposition, but the differences are marginal.

The same plots using concentration data rather than bulk deposition fluxes (not shown) reveal a weaker relationship because the highest concentration by far occurs at the Etherow, and it is only the low rainfall at this site that results in a lower flux than Scoat Tarn. Concentrations of both NH$_4^+$ and NO$_3^-$ increase sharply across the assumed N deposition gradient, whereas C:N ratios increase at the top end in the Etherow soils.

Plots of C:N ratio against streamwater leaching fluxes (absolute and percentage of modelled deposition values) are provided in Figure 8.9. There is no apparent correlation between C:N ratio and either NO$_3^-$ or NH$_4^+$ fluxes in the streams. While NO$_3^-$ flux increases sharply along the N deposition gradient, NH$_4^+$ fluxes are only measurable in the Etherow streams, so the pattern in TIN leaching is driven almost entirely by NO$_3^-$. The same plots for inorganic N leaching as a proportion of modelled deposition inputs show very similar patterns to overall leaching fluxes. Again, per cent leaching of NO$_3^-$ (and hence TIN) increases across the deposition gradient for all four catchments, while the C:N ratio is higher in the Etherow soils than at Scoat Tarn.

8.4 Discussion

Clear differences are apparent between sites in terms of the C:N ratio of surface organic horizons, but only some of these are correlated with measured components of the N cycle. There is some variability within each soil type, but differences between soils within each site are still apparent. The most obvious differences between sites are that much higher C:N ratios occur at the Mharcaidh than elsewhere, while the lowest values are generally found in the Scoat Tarn soils. At the Afon Gwy, most
soils have higher C:N ratios than at Scoat Tarn but they are all much lower than at the Mharcaidh, suggesting a gradient of declining C:N ratio with increasing deposition between the three catchments. The Etherow peats do not fit into this gradient. Despite the higher modelled deposition fluxes at the Etherow, its soils have higher C:N ratios than all Gwy and Scoat Tarn soils except for a similar value in the hilltop peat (G1) at the Gwy. If bulk deposition alone is considered, then C:N ratios closely mirror the total input flux of inorganic N.

**Figure 8.8: C:N ratios (points) and N deposition fluxes (bars: kgN ha\(^{-1}\) yr\(^{-1}\))**
For the Mharcaidh, the Gwy and Scoat Tarn, C:N ratios are also inversely related to NO$_3^-$ leaching. As with deposition, the Etherow soils stand out for leaching the greatest flux and greatest proportion of inorganic N relative to inputs, despite their relatively high C:N ratios.

When measurements of inorganic N fluxes and concentrations for each soil are plotted against its C:N ratio, some apparent thresholds are revealed. Soilwater inorganic N concentrations are only high in soils with C:N ratios less than 30. In all the soils with higher C:N ratios, soilwater NH$_4^+$ and NO$_3^-$ are negligible. However, there are no
soils with mean C:N ratios in the range 30-40, so it is not possible to say where the threshold lies. Inspection of the raw data reveals that for the next horizon below the surface organic layer (Soil depth 1, c.5-15cm in Figure 8.2 and Table 8.1), there are samples with C:N ratios in the range 30-40 from all sites except Scoat Tarn, but there is significant overlap between sites and this horizon seems much less useful as an index of N saturation. A further problem in interpretation is that all soils with very high C:N ratios are from the Allt a’Mharcaidh catchment, so other local factors particular to this site, such as severe N limitation and poor organic matter quality cannot be ruled out as reasons for the differences from other sites.

In soils with C:N ratios below the potential threshold of 30, there are some which show negligible soilwater inorganic N and some with very elevated levels. Similar findings have been reported elsewhere (see section 8.1.4). A comparable pattern is seen for mineralisation potentials in laboratory incubations of soil cores at 15°C. While substantial mineralisation potentials are only seen in soils with C:N ratios less than 30, there is little correlation between the two measurements. For nitrification, however, there is a more convincing relationship between increasing nitrification potentials and decreasing C:N ratios below a threshold value of around 25, which is very close to a similar threshold of 27 reported for forest soils by Kriebitzsch (1978). An almost identical pattern is observed in the potential denitrification fluxes observed during incubation at 15°C, indicating that denitrification is tightly coupled to the supply of NO$_3^-$ from nitrification, although field measurements show very low rates in all soils.

Fluxes of CO$_2$ in the field and in laboratory incubations are very low in the high C:N Mharcaidh soils, but there is a wide range in fluxes at lower C:N ratios. If CO$_2$ production is taken as an indicator of microbial activity, then a clue is provided as to the reasons for the divergent behaviour of the Etherow soils. CO$_2$ fluxes in the field are much lower at the Etherow than at the Afon Gwy or Scoat Tarn, being comparable to those at the Mharcaidh (see Chapter 4). Although the presence of live vegetation in the static chambers used for gas flux measurement in the field may confound the interpretation of results in terms of microbial activity, similar, if less dramatic, differences were also observed in laboratory incubations where vegetation had been
removed. Hence low rates of microbial activity appear to be common to both the N poor Mharcaidh soils and the N rich (but relatively high C:N) Etherow soils.

Several hypotheses may be postulated for the high C:N ratios and low microbial activity at the Etherow. Severe soil acidification, and the possible presence of elevated heavy metal concentrations within the catchment due to its proximity to a major road and the Manchester conurbation, may suppress microbial activity. Although the soils there are warmer than at other sites, promoting microbial activity, precipitation is relatively low, being similar to the Mharcaidh and around a half of that at the Gwy and Scoat Tarn, with the lowest soil moisture content of all soils studied under the mature Calluna at E2. Lack of soil moisture in the very uppermost surface layers may therefore stress the soil microbes. Finally, the predominance of Calluna and the management practice of burning may adversely affect the litter quality compared with the other sites where grasses are much more important. Certainly a lack of inorganic N is not the reason for the relatively high C:N ratios in the Etherow peats, since the soilwaters there show the highest concentrations of both NH$_4^+$ and NO$_3^-$.

Furthermore, the very low nitrification potential at this site provides further evidence of suppressed microbial activity (see Chapter 5).

### 8.5 Conclusions

Major differences are demonstrated between the four study catchments, but site specific factors and the small number of sites (4) make it difficult to draw firm conclusions regarding the utility of the C:N ratio as an indicator of N saturation and leaching in moorland catchments.

For 3 of the sites (Mharcaidh, Gwy and Scoat Tarn) there are relationships between inorganic N deposition, NO$_3^-$ leaching and C:N ratio. The flux and proportion of NO$_3^-$ leaching increases with deposition inputs as C:N ratio declines. For soils with a surface organic horizon C:N ratio less than around 25, nitrification may contribute significantly to NO$_3^-$ leaching. Laboratory incubations suggest that the potential for denitrification losses may also increase in the same order, but field data show little evidence of this, perhaps due to problems of spatial and temporal variability. A
threshold for NO$_3^-$ leaching may exist somewhere in the C:N range 30-40, but data in this range were lacking within this study.

At the Allt a’Mharcaidh, the very high C:N ratios may indicate that the low levels of N deposition experienced by the site do not act to reduce C:N ratio. Instead, the accumulation of soil organic matter may increase, effectively providing a permanent store for atmospheric inputs of N (cf. Berg & Matzner, 1997). The C:N ratio may even increase as C sequestration outstrips N assimilation (White et al., 1996). Hence for FAB, a high value of N$_{imm}$ may be required for this type of site.

At the fourth site, the River Etherow catchment, very high rates of NO$_3^-$ leaching and occasional NH$_4^+$ leaching reflect the high deposition inputs to the site, but appear to be decoupled from the C:N ratio of the upper organic horizons. Low nitrification potentials and low rates of CO$_2$ production in the soils, probably reflecting severe acidification stress, indicate that microbial mediation of inorganic N leaching is probably less important at this site. Very high soilwater concentrations of both NH$_4^+$ and NO$_3^-$, due in part to the disturbance of the system by periodic burning, suggest severe N saturation. Plant uptake and microbial immobilisation of NO$_3^-$ are probably very low, allowing the leaching of a large proportion of deposition inputs into surface waters.

Since N saturation is generally associated with low C:N ratios, several possible reasons may explain the discrepancy at this site. The overwhelming dominance of Calluna may result in litter of a different quality, i.e. with different decomposition characteristics, relative to the other sites where other plant species are more important. Early acidification of the peat soils in this most impacted region of Britain may have suppressed microbial activity over many decades, throughout the relatively recent period of enhanced N deposition. Thus the plant-soil system may have had a reduced capacity to assimilate excess inorganic N from the outset, slowing the potential decline in C:N ratio. Finally, limitation by some other nutrient may have led to N saturation earlier than would be expected from C:N ratio alone.

In conclusion, there may be potential in the use of surface organic horizon C:N ratio as an indicator of N saturation status and potential NO$_3^-$ leaching for certain types of
moorland catchments, or in certain regions, but in areas heavily impacted by other pollutants (e.g. S deposition) the relationships may break down. As found in studies of forest systems across Europe, it may be the case that sites have to be grouped according to bands of deposition input, or even of C:N status, in order to determine where C:N ratio may be a useful predictor of NO\textsubscript{3} leaching. Within the current study, the inclusion of only 4 sites, with one at either extreme of the N deposition gradient for the UK, prevents thorough statistical analysis across the whole gradient of N deposition and leaching. Conclusions are therefore only speculative until further data are collected for these systems which are typical of the acid sensitive uplands in this country.

8.6 References

Aber, J.D. (1992) Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. *Tree* 7 (7), 220-224


SECTION V

SYNTHESIS
CHAPTER 9

SYNTHESIS - INTERPRETING THE DIFFERENCES BETWEEN MEASURED AND MODELLED N SINKS
9. SYNTHESIS - INTERPRETING THE DIFFERENCES BETWEEN MEASURED AND MODELLED N SINKS

9.1 Introduction

The critical loads approach for N deposition is firmly based on the assumption that since terrestrial sinks for N must be finite, excess N deposition will ultimately lead to N saturation and subsequent increases in NO$_3^\text{-}$ leaching. The mechanism for increased NO$_3^\text{-}$ leaching could involve either decreased immobilisation of inorganic N or increased mineralisation and nitrification. Some allowance is generally made for "permanent" or sustainable N sinks, for example in the FAB model via the processes of denitrification and long-term immobilisation of N in refractory soil organic matter as it accumulates. For static mass-balance models it is the steady-state rates of these sustainable N sinks that are most important for defining the critical load. Of more immediate concern for dynamic modelling is the degree to which they retard the process of N saturation and delay the onset of increased NO$_3^\text{-}$ leaching, i.e. their effects on the timing of N saturation.

The models further assume that NH$_4^+$ may contribute to acidification via nitrification. This process may act either directly on deposition inputs, or more likely, through indirect effects on net mineralisation and nitrification rates, as assimilated NH$_4^+$ reduces the C:N ratio of organic matter and increases its decomposition rate. A complementary effect may be that the uptake and immobilisation of NO$_3^-$ is reduced through increased NH$_4^+$ availability, which is not only preferentially utilised by many species of plants and microbes, but may also have a direct inhibitory effect on their use of NO$_3^-$. No consideration is given within the models to the possibility that NH$_4^+$ and NO$_3^-$ deposition behave differently in their contributions to a modified N cycle. Uncertainties in these model assumptions formed the basis of this thesis, and the degree to which these issues have been addressed is discussed below.

The importance of NO$_3^-$ in surface water acidification (Chapter 1) and the uncertainties in the static mass-balance modelling approach (Chapter 2) were discussed in Section I. In Chapters 3-8, key aspects of the N cycle in the soils of the four study catchments were described. Major fluxes were quantified in Section II,
including deposition inputs, surface water leaching outputs and denitrification. These measurements provide estimates of major components of the current N budget for the study catchments, but there is no basis for the assumption of a steady-state between N inputs and outputs. Hence static model estimates of output fluxes for inorganic N, which assume a long-term steady-state, cannot be assumed to represent the present state of these systems and cannot be used to test the performance of the model. They can, however, be used to quantify the magnitude of N sinks assumed by the model at steady-state, and these can be compared not only with current measured N sinks, but also with measures of potential N sinks under optimal conditions. Potential denitrification fluxes were quantified in Chapter 4.

Section III provided estimates of potential rates of mineralisation and nitrification in Chapter 5, which are key processes regulating the release of N that has entered the terrestrial N cycle. Chapter 6 described the immediate fate of N deposition inputs using a \(^{15}\)N tracer, and the proportions retained in soils and vegetation after one year. Hence it provided an indication of the proportion of inputs likely to be controlled by the balance between mineralisation and immobilisation, which is determined by the N saturation status of the system.

Two independent measures of N saturation status were described in Section IV. Chapter 7 used the natural abundance of \(^{15}\)N as an indicator of the relative importance of mineralisation and especially nitrification within catchment soils. Chapter 8 considered the C:N ratio as an indicator of N saturation status and its potential for use in dynamic modelling.

This chapter concludes the thesis by bringing together the measurements of N fluxes and cycling within the study catchments at present to provide a characterisation of the catchment soils in terms of current N saturation status. Starting with an assessment of the key limitations of the experimental approach, major conclusions are discussed in relation to the requirements of mass-balance models. Measured fluxes are used to assess how the FAB model representation of steady-state compares with current measurements. The degree to which N saturation status can explain differences between modelled and measured fluxes of N is explored. Key uncertainties for both static (FAB) and dynamic
models of acidification are discussed. Finally, recommendations are made for future research requirements in modelling the impacts of N deposition.

9.2 Limitations of the experimental approach

A fundamental problem with long-term steady-state models such as FAB is the lack of real data against which to test and calibrate them. To test models of N cycling at steady-state with elevated N deposition requires either monitoring data over very long periods of perhaps decades, which are rarely available, or data from sites at all stages of N saturation, including some that have reached a maximum N leaching rate. Within the current study, the best example of such a site is the River Etherow catchment, but the observed N cycle does not match the assumptions of the FAB model. The implications are discussed in detail below.

In more general terms, the study is limited by the number of sites included. This was determined by logistical constraints which are common to this type of research: the measurement of so many parameters and processes is very costly and time consuming. For example, it would have been informative to have measured \(^{15}\)N abundance in deposition and surface waters to determine whether unrecovered tracer additions really were leached into surface waters during the study period. More sample plots in riparian soils might have revealed hotspots of denitrification activity. In general, though, differences between sites are very large for many of the N fluxes measured and the experimental approach provided adequate spatial coverage within catchments, being sufficiently rigorous to characterise them all.

9.3 FAB model representation of catchment scale N sinks

As a first step in comparing measured and modelled aspects of the N cycle it is necessary to calculate steady-state N fluxes according to the FAB mass balance. The soil N sinks employed by the FAB model using national scale (1:250,000) digital soils data and default values for immobilisation \((N_{\text{imm}})\) and denitrification \((N_{\text{den}})\) from Chapter 2 (Table 2.1) are shown in Table 9.1. The denitrification fraction \((F_{\text{de}})\)
calculated according to the recommended method in the UNECE Mapping Manual (UBA, 1996) is also provided. These data are equivalent to those used for national scale FAB mapping applications in the UK (Curtis et al., 2000), and illustrate the difference between national scale and site specific soils information (cf. Chapter 3: Table 3.4).

Table 9.1: Catchment soils data derived from national digital datasets ($N_{imm}$ and $N_{den}$ in kgN ha$^{-1}$ yr$^{-1}$, from Hall et al., 1997: see Table 2.1)

<table>
<thead>
<tr>
<th>Site</th>
<th>Soil Code</th>
<th>Description</th>
<th>% cover</th>
<th>Mapping Code</th>
<th>$N_{imm}$</th>
<th>$N_{den}$</th>
<th>$F_{de}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mharcaidh</td>
<td>335</td>
<td>Subalpine soils</td>
<td>61</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Mharcaidh</td>
<td>336</td>
<td>Alpine soils</td>
<td>31</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Mharcaidh</td>
<td>14</td>
<td>Rankers</td>
<td>8</td>
<td>3.1</td>
<td>1</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Gwy</td>
<td>0654a</td>
<td>Stagnopodsols</td>
<td>53</td>
<td>6.5</td>
<td>3</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Gwy</td>
<td>0611c</td>
<td>Brown podsolic</td>
<td>41</td>
<td>6.1</td>
<td>3</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Gwy</td>
<td>1013a</td>
<td>Raw peat soils</td>
<td>6</td>
<td>10.1</td>
<td>3</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Scoat Tarn</td>
<td>0311e</td>
<td>Rankers</td>
<td>100</td>
<td>3.1</td>
<td>1</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Etherow</td>
<td>1011b</td>
<td>Raw peat soils</td>
<td>100</td>
<td>10.1</td>
<td>3</td>
<td>1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Table 9.2 shows the catchment scale N sinks weighted by soil coverage using the denitrification fraction method. The FAB mass balance is employed to calculate fluxes for immobilisation ($N_{imm}$), denitrification ($N_{den}$), in-lake retention ($N_{ret}$: applies to Scoat Tarn only) and predicted catchment leaching of NO$_3^-$ for the given levels of total inorganic N deposition ($N_{dep}$: CEH 1995-97 mean values, catchment weighted – see Chapter 3). Measured leaching fluxes are provided for comparison.

It can be seen that modelled long-term immobilisation rates are very small, in the range 1-3 kgN ha$^{-1}$ yr$^{-1}$ as in Table 2.1, but denitrification fluxes have a much wider range, since they are calculated as a fraction of net inputs (deposition minus immobilisation). For the Mharcaidh, Gwy and Scoat Tarn catchments the figures are...
comparable to the soil specific values in Table 2.1 (1-4 kgN ha\(^{-1}\) yr\(^{-1}\)), but for the Etherow, which is dominated by peat soils, a very large denitrification flux of almost 24.6 kgN ha\(^{-1}\) yr\(^{-1}\) is predicted. In-lake retention at Scoat Tarn is of the same order of magnitude as terrestrial N sinks.

### Table 9.2: Catchment scale soil sinks and leaching outputs (kgN ha\(^{-1}\) yr\(^{-1}\)) predicted by FAB using the denitrification fraction (F\(_{de}\)) method

<table>
<thead>
<tr>
<th>Site</th>
<th>(N_{dep})</th>
<th>(N_{imm})</th>
<th>(F_{de})</th>
<th>(N_{den})</th>
<th>(N_{ret})</th>
<th>Predicted leaching</th>
<th>Measured leaching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mharcaidh</td>
<td>7.3</td>
<td>2.84</td>
<td>0.10</td>
<td>0.4</td>
<td>4.0</td>
<td>0.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Gwy</td>
<td>27</td>
<td>3</td>
<td>0.14</td>
<td>3.4</td>
<td>20.6</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Scoat Tarn</td>
<td>33.6</td>
<td>1</td>
<td>0.10</td>
<td>3.3</td>
<td>27.0</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Etherow</td>
<td>33.7</td>
<td>3</td>
<td>0.80</td>
<td>24.6</td>
<td>6.1</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

At all sites except the Etherow, predicted leaching fluxes of NO\(_3^-\) are much greater than measured values, by around an order of magnitude. As discussed in Chapter 2, these differences are due to very small immobilisation sinks assumed at steady state, along with low rates of denitrification assumed for non-peat soils. At the Etherow, the removal of 80% of net N inputs remaining after immobilisation leads to a predicted leaching flux which is actually smaller than the observed value. However, there is no current evidence for significant denitrification at this site (see below).

If fixed, soil specific denitrification rates are employed in the FAB model, very similar leaching outputs to those calculated above are predicted for all sites except the Etherow, where an assumed denitrification flux of only 1 kgN ha\(^{-1}\) yr\(^{-1}\) leads to a much greater prediction of NO\(_3^-\) leaching, this time larger than the measured rate (Table 9.3). At the Mharcaidh, the soil-weighted denitrification rate of 1 kgN ha\(^{-1}\) yr\(^{-1}\) is actually greater than that estimated as a percentage (10%) of net inputs in Table 9.2, because of the very low deposition at this site. The choice of method therefore has very large implications for predictions of NO\(_3^-\) leaching at steady-state, and hence for
critical load exceedance. Furthermore, the low values of N immobilisation in conjunction with low rates of denitrification are also responsible for the prediction of much greater leaching than is currently observed. These issues are discussed in detail below.

Table 9.3: Catchment scale soil sinks and leaching outputs (kgN ha\(^{-1}\) yr\(^{-1}\)) predicted by FAB using fixed denitrification

<table>
<thead>
<tr>
<th>Site</th>
<th>(N_{\text{dep}})</th>
<th>(N_{\text{imm}})</th>
<th>(N_{\text{den}})</th>
<th>(N_{\text{ret}})</th>
<th>Predicted leaching</th>
<th>Measured leaching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mharcaidh</td>
<td>7.3</td>
<td>2.84</td>
<td>1</td>
<td>0</td>
<td>3.46</td>
<td>0.1</td>
</tr>
<tr>
<td>Gwy</td>
<td>27</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>23.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Scoat Tarn</td>
<td>33.6</td>
<td>1</td>
<td>1</td>
<td>2.7</td>
<td>29.0</td>
<td>4.9</td>
</tr>
<tr>
<td>Etherow</td>
<td>33.7</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>29.7</td>
<td>11</td>
</tr>
</tbody>
</table>

9.4 Denitrification in mass balance models

In Chapter 4 it is demonstrated that current rates of denitrification are much lower than those suggested by the FAB model, whether using soil specific empirical rates or input dependent denitrification. Experimental N additions show that excess NO\(_3^{-}\) soon leads to C limitation, so denitrification is unlikely to be a significant N sink even following N saturation at some future steady-state. The field and experimental work also show that peat soils do not have the greatest potential for denitrification; the surface organic layer of grassland soils at the Gwy and Scoat Tarn provides by far the highest potentials. The denitrification fraction of 80% for peat soils is deemed much too high for the UK uplands, and a universal figure of 10% is suggested to be more appropriate for all soils including peats.

The recommendation that higher denitrification rates be included in modelling applications for peat soils is based on the assumption that these soils most frequently provide the optimal physical conditions of high soil moisture and labile organic C
supply, so that high rates of denitrification will be less transient than in other soil types where soil moisture declines more rapidly following rain as the soils drain more freely. This assumption may be misguided, at least for UK peats, where high soil moisture at depth does not facilitate denitrification even with elevated NO$_3$ concentrations, probably because of labile C limitation. The much greater activity in the surface organic horizons compared with deeper soils suggests that the main controls on non-NO$_3$ limited denitrification may be soil moisture at the surface and labile C production. At the Etherow, soil moisture in the peats is lower than in any of the other soils studied, so it cannot be assumed that peats are necessarily associated with high surface soil moisture. The valley peat (M2) and shallow peat (M4) at the Allt a’Mharcaidh have very high surface soil moisture compared to the Etherow peats, largely due to the widespread presence of Sphagnum, but denitrification is negligible due to N limitation.

The relative importance of the surface organic horizon for denitrification is further demonstrated by the lack of significant fluxes in the field despite the presence of high soilwater NO$_3$ in deeper soils at both Scoat Tam and the River Etherow. Even with the combination of high soil moisture and high levels of NO$_3$ at a depth of c. 30cm, denitrification is negligible in the field, again presumably due to lack of labile C. In the surface horizons, competition for available N and soil moisture are probably limiting.

In the UK, a proportional denitrification sink of 10% would produce values very close to the range of empirical rates suggested in Hall et al. (1997). With immobilisation rates assumed to be very low following N saturation (values of 1-3 kgN ha$^{-1}$ yr$^{-1}$ are suggested by the previous authors) deposition inputs in the range 10-40 kgN ha$^{-1}$ yr$^{-1}$, which would encompass much of the UK uplands, would result in predicted denitrification fluxes in the range 0.7 – 3.9 kgN ha$^{-1}$ yr$^{-1}$, compared with default values of 1-4 kgN ha$^{-1}$ yr$^{-1}$. These rates are also comparable to those observed for most soils in the soil core incubation experiments, but higher rates of up to 12.7 kgN ha$^{-1}$ yr$^{-1}$ were measured in some soils when NH$_4$NO$_3$ was added at 15°C (Chapter 4). These very high potentials are attributed to the high incubation temperature, the lack of competition with plants and the very high soilwater NH$_4^+$ and NO$_3^-$ concentrations used, with nitrification apparently contributing to the N$_2$O losses, and are not representative of real conditions in the field. Furthermore, they are considerably lower than those suggested by the $F_{de}$ method for peats (80% of net inputs) described above.
The use of an input dependent method (i.e. a first-order term in the model) is preferable to the use of fixed values because the latter method might result in negative fluxes being calculated in very low deposition areas. While negative fluxes of N\textsubscript{2}O may occur through its consumption in soils via further reduction to N\textsubscript{2} by denitrifiers, there is still an overall positive flux of N out of the soil and modelled negative fluxes of N would be incorrect.

Given the great temporal and spatial variability of the denitrification process, it is very difficult to either measure or model at the catchment scale. Several modelling studies are reported in Chapter 4, and all generally require information on factors such as soil moisture, land use and soil temperature. Alternative methods have been suggested for critical loads modelling elsewhere, using kinetic equations (e.g. Grennfelt & Thörmelöf, 1992), but again these are data intensive. Since the current study suggests that on an annual basis, denitrification fluxes are likely to provide only a minor sink for N deposition, the extra work and sampling effort required to parameterise these models may not be justified for critical loads work. The large uncertainties associated with other sinks for N, in particular long-term immobilisation, mean that improvements in the predictive power of models by reducing the uncertainty in denitrification estimates are likely to be relatively trivial. The use of a first-order term for all soils is therefore recommended for critical loads modelling work, with a default denitrification factor of 10% likely to be appropriate in the absence of site specific data or unusual circumstances (such as the presence of wetlands in a major proportion of the catchment). This approach is also appropriate for the dynamic modelling of denitrification flux where changes in the net supply of NO\textsubscript{3}\textsuperscript{-}, for example as a result of increased nitrification, occur.

The relative unimportance of denitrification as a sink for N in the study catchments implies that other sinks are responsible for the large rates of retention observed. At Scoat Tarn where the absolute rate of retention is greatest, the difference between modelled deposition inputs and leaching outputs is 28.7 kgN ha\textsuperscript{-1} yr\textsuperscript{-1}, of which less than 0.1 kgN ha\textsuperscript{-1} yr\textsuperscript{-1} appears to be lost through denitrification at present. Even assuming 10% denitrification of all the retained N, around 26 kgN ha\textsuperscript{-1} yr\textsuperscript{-1} is unaccounted for. Biological assimilation and biotic or abiotic immobilisation must be responsible for the remainder if it is assumed that grazing losses are negligible. Hence mineralisation and nitrification are likely to be key controls of future NO\textsubscript{3}\textsuperscript{-} leaching.
9.5 Measured and modelled rates of N immobilisation

9.5.1 Modelled rates of N retention and input-output budgets

To estimate the proportion of inputs that is physically retained within the study catchments, it is necessary to make assumptions about the non-measured N losses from the system. To provide a conservative estimate of the retained N that is unaccounted for, estimates at the upper limit of guideline values are used.

Grazing losses are thought to be no more than 2 kgN ha\(^{-1}\) yr\(^{-1}\) from upland catchments with a relatively high stocking density of sheep and <1 kgN ha\(^{-1}\) yr\(^{-1}\) where stocking density is low (INDITE, 1994). The upper value is therefore assumed for the Gwy, Scoat Tarn and River Etherow catchments, while a low value of 0.5 kgN ha\(^{-1}\) yr\(^{-1}\) is assumed for the Mharcaidh catchment where sheep are absent and deer are rarely present. Losses of N during burning are a major unknown, but are typically 4-6 kgN ha\(^{-1}\) yr\(^{-1}\) for British uplands (Batey, 1982). The Etherow is the only site where burning is practised, and an upper limit value of 6 kgN ha\(^{-1}\) yr\(^{-1}\) is assumed here. For denitrification a maximum rate is estimated as 10\% of inputs, which is likely to be an overestimate since the figure should be applied to net inputs after plant uptake and immobilisation. At Scoat Tarn one inflow stream has a slightly higher mean NO\(_3^-\) concentration than the lake while the other stream has a slightly lower concentration (Chapter 3: Table 3.10), so it is assumed that net retention of N in the lake is negligible.

The magnitude of the missing N sinks (i.e. N retained in plants and soils) resulting from these assumptions is shown in Table 9.4. There is a wide range in both absolute and percentage values. The “missing” N is either assimilated by plants and the soil microbial biomass or immobilised in soil organic matter.

It is possible that some of the retained N is lost from the catchment through organic N leaching. Dissolved organic N (DON) is the major form of dissolved N in surface waters at the Allt a’ Mharcaidh, making up over 90\% of total dissolved N leaching. DON was not analysed for the Gwy streamwaters but is generally less important than total inorganic N (TIN) at Scoat Tarn and the Etherow, varying between sampled streams
from 31-44% and 37-55% respectively. Concentrations (rather than proportions of total N) are very similar at the Mharcaidh and Scoat Tarn (see Table 3.10), but much greater in the Etherow streams. All these figures are based on mean concentrations rather than fluxes, but illustrate the potential importance of organic N in catchment scale N budgets.

Table 9.4: Unknown N sinks during the budget year at the catchment scale (kgN ha\(^{-1}\) yr\(^{-1}\))

<table>
<thead>
<tr>
<th>Site</th>
<th>N(_{dep})</th>
<th>Max (N_{den})</th>
<th>N(_{grazed})</th>
<th>N(_{burnt})</th>
<th>Measured leaching</th>
<th>“Missing” N</th>
<th>“Missing” %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mharcaidh</td>
<td>7.3</td>
<td>0.7</td>
<td>0.5</td>
<td>0</td>
<td>0.1</td>
<td>6.0</td>
<td>82</td>
</tr>
<tr>
<td>Gwy</td>
<td>27.0</td>
<td>2.7</td>
<td>2.0</td>
<td>0</td>
<td>1.8</td>
<td>20.5</td>
<td>76</td>
</tr>
<tr>
<td>Scoat Tarn</td>
<td>33.6</td>
<td>3.4</td>
<td>2.0</td>
<td>0</td>
<td>4.9</td>
<td>23.3</td>
<td>69</td>
</tr>
<tr>
<td>Etherow</td>
<td>33.7</td>
<td>3.4</td>
<td>2.0</td>
<td>6.0</td>
<td>11</td>
<td>11.3</td>
<td>34</td>
</tr>
</tbody>
</table>

The soilwater chemistry data (Table 3.15), which are also available for the Gwy soils, show similar DON concentrations in the Mharcaidh and Scoat Tarn soils, but much greater values in the Gwy peats and especially the Etherow soils. DON is proportionally much more important in soilwaters than surface waters at Scoat Tarn and the Etherow, ranging from 65-83% of total dissolved N, but makes up very similar proportions at the Mharcaidh (88-95% in soils compared with 90% for streams).

DON is frequently found to be an important, and often dominant, form of N export from forest and moorland catchments (e.g. INDITE, 1994; Campbell et al., 2000; Hagedorn et al., 2000; McHale et al., 2000), particularly those dominated by organic soils (Chapman & Edwards, 1999). The response of DON production in soils to increased N deposition is poorly understood, as are the mechanisms for its transport into surface waters and its potential impact on the functioning of aquatic ecosystems (INDITE, 1994). Seasonal and annual variations in the ratio of DOC to DON indicate different mechanisms of production and release (Solinger et al., 2001). Some authors have found that DON fluxes are correlated with precipitation or discharge (e.g. Hagedorn et al., 2000; Solinger et al.,
2001) but not with N deposition (Michalzik et al., 2001). Other studies have found that DON release from peat soils increases in response to inputs of S and N (Yesmin et al., 1995) but have not established clear links with leaching into streams (Williams & Anderson, 1999). Hence there is little conclusive evidence that organic N leaching fluxes have changed as a result of increased anthropogenic N deposition. Nevertheless, organic N leaching provides an export flux of N out of catchments which may be missed by the use of chronosequence data alone to estimate long-term rates of N immobilisation.

9.5.2 N retention measured using $^{15}$N tracers

In Chapter 6, stable isotope ($^{15}$N) tracer experiments are used to show that a very large proportion of N inputs are retained within catchment soils and vegetation over the course of a single year, ranging from around 100% retention to a minimum value of c. 75%. The fate of the N inputs varies between catchments.

The biggest proportion of $^{15}$N inputs is generally found in vegetation, with average values of 50-60% at the Allt a’Mharcaidh and Afon Gwy, declining slightly to 40-50% at Scoat Tarn and the River Etherow. Exceptionally high retention of 100% (though evidently subject to some experimental error) is found at M1 (peaty ranker at the Mharcaidh) and G2 (peaty gley at the Gwy). An apparent reduction in total vegetation uptake of N as a proportion of increasing deposition is largely due to differences in biomass between sites, with which retention is highly correlated. These may reflect differences in productivity, but there are no supporting data. Hence the large uptake figures at M1 and G2 are due mainly to the very high above-ground biomass recorded on these soils. At G2, a particularly high biomass of both lichens/mosses and grasses is found, compared with similar mineral soils at Scoat Tarn. Lichens and mosses are responsible for the retention of most of the added $^{15}$N at G2. The high retention is driven by the high biomass; tracer retention per unit mass of lichens/mosses is comparable at both sites, and in some cases larger at Scoat Tarn. Root biomass is also much greater in all the Gwy soils than at Scoat Tarn.

The Etherow is an exception to the pattern linking biomass to N retention. Consideration of the retention efficiency of biomass (% retention per unit mass) reveals that while there is perhaps a slight increase with increasing deposition at the
other three sites, values at the Etherow are much lower. The shrubs at E1 are an exception: vigorous regrowth following burning has resulted in a relatively high uptake efficiency for added $^{15}$N. The reduction in uptake efficiency in the mature Calluna could be due to severe N saturation and excess NH$_4^+$ availability in the soils.

The proportion immobilised in the litter and surface organic layer is relatively constant along the deposition gradient and generally lower than or comparable to that retained in vegetation, varying between soils in the range 25-50%. The proportion immobilised lower down the soil profile is only a few percent, and is negligible compared with retention in biomass and surface soils. If litter alone is considered, the retention efficiency (% per unit mass) appears to decline as deposition increases, but this pattern is obscured when immobilisation in the surface soils is included.

The proportion of $^{15}$N tracer retained in soils (litter + surface organic horizon and underlying organic horizon) from Chapter 6 is converted into current rates of N immobilisation using best available modelled deposition inputs (see Chapter 3: Table 3.9) in Table 9.5. While these figures are only for short-term immobilisation during the monitoring period of one year, they provide an indication of current rates for comparison with the literature-based long-term rates in Tables 9.3-9.4 above.

At the Allt a’Mharcaidh, current N immobilisation rates in the surface organic layer are remarkably similar to the literature based values of 1-3 kgN ha$^{-1}$ yr$^{-1}$ recommended in Hall et al., (1997). Since this site experiences very low N deposition inputs compared with much of the UK, these rates might reasonably be regarded as being close to the “ambient” rates of N immobilisation prior to the onset of anthropogenic N deposition. Furthermore, since the pre-anthropogenic rates are used as the basis of the long-term figures in static mass-balance models, which are derived from chronosequence studies, the data appear to support the recommended range of default values.

At the other sites where the anthropogenic N deposition load is much greater, current rates of N immobilisation are much higher than the proposed long-term rates. The range in values is quite similar for each site, but the differences between soil types do not follow the pattern of recommended values, which suggest a high value of 3 kgN
ha\textsuperscript{-1} yr\textsuperscript{-1} in peats, podsols and stagnohumic gleys, and 1 kgN ha\textsuperscript{-1} yr\textsuperscript{-1} elsewhere. At the Gwy, the lowest current rate is found in the podsol, while the Scoat Tarn podsol shows the highest rate for that site. If these immobilisation rates declined to the suggested long-term values, inorganic N leaching could increase by up to 13 kgN ha\textsuperscript{-1} yr\textsuperscript{-1}, which would have a major impact on surface water ANC.

Table 9.5: Measured rates of short-term N immobilisation (N_{imm}) using \textsuperscript{15}N tracers in surface organic horizon and upper organic soils

<table>
<thead>
<tr>
<th>Site</th>
<th>Soil Type</th>
<th>% of inputs retained</th>
<th>N_{imm} (kgN ha\textsuperscript{-1} yr\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Surface</td>
<td>Organic soil</td>
</tr>
<tr>
<td>Mharcaidh</td>
<td>Peaty ranker</td>
<td>26.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Mharcaidh</td>
<td>Valley peat</td>
<td>23.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Mharcaidh</td>
<td>Peaty podsol</td>
<td>32.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Mharcaidh</td>
<td>Shallow peat</td>
<td>49.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Afon Gwy</td>
<td>Hilltop peat</td>
<td>37.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Afon Gwy</td>
<td>Peaty gley</td>
<td>43.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Afon Gwy</td>
<td>Podsol</td>
<td>35.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Afon Gwy</td>
<td>Valley peat</td>
<td>51.4</td>
<td>-0.3</td>
</tr>
<tr>
<td>Scoat Tarn</td>
<td>Podsol</td>
<td>47.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Scoat Tarn</td>
<td>Peaty gley</td>
<td>40.9</td>
<td>-0.2</td>
</tr>
<tr>
<td>Scoat Tarn</td>
<td>Deep peat</td>
<td>25.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Etherow E1</td>
<td>Peat (burnt)</td>
<td>46.8</td>
<td>3.9</td>
</tr>
<tr>
<td>Etherow E2</td>
<td>Peat (unburnt)</td>
<td>36.7</td>
<td>0.8</td>
</tr>
</tbody>
</table>

At all sites, immobilisation rates in the deeper soils are an order of magnitude less than in the surface soils, and the results of the tracer retention experiment in these samples are not significant. It is not possible to say from the experimental results here whether the deeper soils could become a more important sink in the longer term, following potential increases in inorganic N leaching down the soil profile. The presence of very high concentrations of NH\textsubscript{4}\textsuperscript{+} in the Etherow soilwaters at 30cm depth does suggest that
abiotic immobilisation may be important at this depth, and this process may not have been measured during the short timescale of the tracer experiment because of the "residence time" of N inputs in the living vegetation and upper organic layer.

The current measured rates of N immobilisation do not provide any indication of what the steady-state rates may be with elevated N inputs. It is assumed that the high current rates of up to almost 16 kgN ha\(^{-1}\) yr\(^{-1}\) are not sustainable and will decrease as the large immobilisation flux reduces C:N ratios relatively quickly. However, the Etherow data show that N saturation does not necessarily induce high rates of nitrification, and that NH\(_4^+\) may accumulate in soils. If C:N ratios are not declining in the surface horizons of the Etherow peats, the current immobilisation figures may indicate a sustainable rate, perhaps maintained by abiotic processes. However, abiotic sinks must also have a finite capacity to retain N. The implications of NH\(_4^+\) accumulation are not known, and future research in systems like the Etherow catchment may need to focus on this issue.

The proportion of the \(^{15}\)N tracer unrecovered at the four sites is not detectable in most soils at the Mharcaidh and Gwy catchments, but averages 12.9\% at Scoat Tarn and 17.1\% at the Etherow (see Table 6.6). This pattern is highly consistent with soilwater concentrations of inorganic N, particularly NO\(_3^-\), measured throughout the budget year, which are extremely low at the Mharcaidh and Gwy, high at Scoat Tarn and very high at the Etherow. It is also consistent with the proportion of inputs leached into surface waters (Table 6.6), given the potential errors associated with the tracer methods. The implication is that some of the added \(^{15}\)N may have been leached directly, but since the leached proportion of N deposition inferred from input-output budgets is greater than the proportion of tracer retained, the data suggest that some of the leached NO\(_3^-\) must result from mineralisation and nitrification of older (i.e. pre-experiment) N.

The lack of \(^{15}\)N data for leached NO\(_3^-\) means that it is not possible to say with any certainty what proportion is accounted for by the unrecovered \(^{15}\)N, i.e. by direct leaching of inputs. It is possible that since some of the leached NO\(_3^-\) is derived from older catchment sources of N, there may be other sinks, such as grazing or denitrification, for the unrecovered \(^{15}\)N. These data do not, however, suggest that over
the one year period of tracer additions, a significant proportion has been lost through these processes, since NO$_3^-$ leaching alone is more than sufficient to account for the amount of $^{15}$N unrecovered at the end of the year. Ideally, $^{15}$N natural abundance and post-experimental levels would have been measured in soilwater, streamwater and deposition inputs, but resources were not available for analysis within this study.

When compared with the estimates of “missing” N in Table 9.4, the $^{15}$N data show that plant uptake and microbial immobilisation can easily account for the difference between current and modelled N retention (if estimates of denitrification, grazing and burning losses are realistic), but these are probably short-term processes and not sustainable at these rates over the long term. Possible changes in mineralisation and nitrification could become very important as controls of NO$_3^-$ leaching.

9.6 Mineralisation, nitrification and N saturation status as controls on N leaching: can these factors account for differences between modelled and measured fluxes?

Although nitrification is often assumed to be a key control on NO$_3^-$ leaching under N saturation (Chapter 5), nitrification potentials in this study are not necessarily increased by excess NH$_4^+$ availability. Low nitrification potentials occur at the Allt a'Mharcaidh, which is severely N limited, but also at the River Etherow, where availability of both NH$_4^+$ and NO$_3^-$ is high.

At the Mharcaidh the low nitrification potentials are associated with very low mineralisation potentials, so that NH$_4^+$ supply is limiting. The $^{15}$N tracer experiment (Chapter 6) shows that within the sensitivity of the method, 100% of N inputs are retained in the vegetation or by immobilisation in the surface organic horizons. Plant uptake and microbial immobilisation would therefore outcompete nitrifiers for mineralised N. Very high C:N ratios in the Mharcaidh soils (43.2 – 58.4) demonstrate the scarcity of N in the system and the relative abundance of C strongly favours N immobilisation (Chapter 8).
Natural abundance data for $^{15}$N show an extreme range in $\delta^{15}$N values at the Mharcaidh, with both the highest and lowest figures for vegetation at any site. These data indicate that the plants there utilise N in different forms from throughout the soil profile because of severe N limitation. The low nitrification potentials found in the soils may explain the extremely low $\delta^{15}$N values in shrubs by preventing the enrichment of NH$_4^+$ utilised by plants. Alternatively, $^{15}$N depleted organic sources of N may be accessed by mycorrhizal associations, but it is not possible to determine from existing data which of these mechanisms is responsible for the extremely low $\delta^{15}$N values in shrubs at this site. For grasses, the use of N sources from the enriched deeper soils could explain the very high $\delta^{15}$N values obtained.

Conversely, the Etherow peats show relatively high mineralisation potentials, with the highest values for peat soils at any site. The combination of high mineralisation potentials and high soilwater NH$_4^+$ show that nitrification is not limited by substrate availability at the Etherow, but is instead probably suppressed by very low soil pH (c. 3.0) and possibly other pollutants like heavy metals. The relatively low proportion of $^{15}$N retained by vegetation in the tracer experiment confirms that plant demand for N is much lower than at the other study sites. Relatively high C:N ratios in the surface soils (c. 29-30), despite the very high N deposition load to the site, demonstrate that either N immobilisation is low, or C assimilation keeps pace with immobilisation.

Total retention of N inputs is only slightly lower than that at Scoat Tarn, but a much greater flux is leached at the Etherow. The difference in leaching flux given the relative similarity in deposition and $^{15}$N retention cannot be due to a greater nitrification source at the Etherow, since nitrification potentials there are very low. The lack of nitrification indicates that streamwater NO$_3^-$ is derived either from direct leaching of atmospheric deposition, or from in-stream nitrification of leached NH$_4^+$ which is present in excess in the soils and recorded regularly in one of the minor study streams. Adamson et al. (1998) attributed the presence of NO$_3^-$ in streamwaters draining Pennine peats with little soilwater NO$_3^-$ to riparian or in-stream nitrification, although inorganic N fluxes were much smaller than organic N in this system. The $^{15}$N natural abundance data from the Etherow plots support this hypothesis, since the $\delta^{15}$N values of both the surface organic layer and the deeper organic soils are very low.
compared with the other sites. Leaching or denitrification losses of $^{15}$N-depleted NO$_3^-$ produced by nitrification tends to enrich the total N pool relative to atmospheric sources, whereas direct leaching of atmospherically deposited N is neutral in terms of $^{15}$N and retention of N deposition will generally reduce the average $\delta^{15}$N value of the system. Similarly, the leaching of a large proportion of N deposition may account for the relatively high C:N ratio in the peat soils.

Very high mineralisation and nitrification potentials are found in the mineral soils at both the Gwy and Scoat Tarn. At the Gwy the highest rates for both processes are found in the peaty gley (G2), with the deeper soils having the higher potentials, particularly for nitrification. Despite the high nitrification potentials in the deeper soils, soilwater NO$_3^-$ concentrations are near zero for most of the year. In the podsol (G3), potentials are higher than in peat soils both at the Gwy and elsewhere, but are not as high as in the peaty gley or the Scoat Tarn mineral soils.

Both of the mineral soils which dominate the catchment at Scoat Tarn have mineralisation and nitrification potentials comparable to the highest values in the Gwy, but the surface organic horizons contribute disproportionately to the total for the whole profile. Contrary to the situation in the Gwy soils, the very high nitrification potentials in the surface horizons at Scoat Tarn are associated with high soilwater NO$_3^-$ concentrations at depth. These differences between the two sites must be due to some combination of hydrological factors related to soil drainage properties, or to differences in vegetation uptake of NO$_3^-$. The analytical method used to derive nitrification potentials provides net nitrification values, so net microbial immobilisation of NO$_3^-$ at the Gwy compared with net nitrification at Scoat Tarn cannot be responsible.

The $^{15}$N experiment provides a possible explanation for the observed differences in soilwater N between the Gwy and Scoat Tarn soils. The proportion of $^{15}$N inputs immobilised in the litter and organic layer is comparable at both sites. The proportion retained in vegetation is, however, larger for all soils at the Gwy, especially for the most active Gwy soil, the peaty gley (G2). In this soil, very high mineralisation and nitrification potentials occur at depth, but almost all $^{15}$N tracer added is retained in the
vegetation or combined litter and surface organic layer. The high biomass of lichen and moss peculiar to this soil, combined with a much greater density and biomass of roots in all Gwy soils compared to Scoat Tarn soils, is largely responsible for the greater uptake of added N. These factors may be indicators of greater productivity, but there are no data available within this study to support this. Hence deposition inputs are almost all intercepted by the biota which prevents vertical leaching into deeper soils, and the high biomass of roots and above ground vegetation facilitates the assimilation of all inorganic N released by net mineralisation and nitrification. High potentials for these processes in the deeper soils are therefore not realised in the field because of a limiting supply of N at depth.

Unlike the Gwy soils, where only the podsol (G3) does not retain all added $^{15}$N in the soil surface or vegetation, all the Scoat Tarn soils show a small but significant proportion of unrecovered $^{15}$N. Furthermore, it is a proportion of a larger deposition input. It would appear that the smaller biomass (i.e. density) of roots and above-ground vegetation in the Scoat Tarn soils results in less assimilation of the deposited or nitrified N, permitting some leaching into lower soil horizons and leading to the high observed soilwater NO$_3^-$ concentrations. Absolute rates of retention at the two sites are actually very similar for all soils except G2 (see Chapter 6: Fig. 6.2), with more efficient retention in the vegetation at Scoat Tarn per unit biomass partly balancing the greater total biomass of vegetation at the Gwy.

The importance of the C:N ratio in controlling nitrification and NO$_3^-$ leaching is difficult to ascertain from so few sites, but it is interesting that the lowest C:N ratios are associated with the greatest activity at both the Gwy and Scoat Tarn. A steep increase in nitrification potential is found with declining C:N ratio for all sites, but only below a threshold value of around 25. All the Scoat Tarn soils fall into this category, but the C:N ratio in the highly active peaty podsol (S1: 15.7) is the lowest found within this study, while the other soils have surface horizon C:N ratios of c. 21-22. Likewise at the Gwy, the most active peaty gley soil (G2) has the lowest C:N ratio there (21.2), but the podsol (G3) is the only other soil with a surface C:N ratio (23.0) below the threshold value of 25. The valley peat (G4: 25.0) and hilltop peat (G1: 29.8) have higher C:N ratios and low nitrification potentials. Therefore the C:N ratios may be linked to nitrification potentials and be a potential indicator of NO$_3^-$ leaching at the
catchment scale, but they do not in themselves explain the lack of deeper soilwater 
\( \text{NO}_3^- \) at the Gwy compared with Scoat Tarn.

Hence the key difference between the two sites appears to be related to net uptake by 
vegetation. With similar mineralisation and nitrification potentials in the most active 
soils from the Gwy and Scoat Tarn, but higher temperatures at the Gwy, it is therefore 
possible that more rapid cycling of N at the Gwy, probably through greater 
productivity, results in a greater immobilisation of N in refractory soil organic matter 
over a longer timescale than the one year period covered in this study. However, 
Epstein et al. (2001) found that plants with the greatest short-term uptake rates may 
not show the biggest long-term N retention, possibly due to more rapid turnover or 
susceptibility to grazing losses.

The \( ^{15}\text{N} \) natural abundance study shows that the \( ^{15}\text{N} \) of vegetation at all sites except 
the Mharcaidh is closely associated with that of the surface organic horizon and very 
different from that in deeper soils. The data strengthen the argument that there is a 
tight cycling of inorganic N between plants, litter and the soil surface horizons at 
these sites, but fail to demonstrate the relative importance of nitrification as a source 
of \( \text{NO}_3^- \) leaching. If a significant proportion of \( \text{NO}_3^- \) leached from the soils was 
derived from nitrification, the \( ^{15}\text{N} \) values of plants using the remaining enriched 
NH\(_4^+\) would increase. In forest systems, this process results in an increase in the 
enrichment factor (\( \varepsilon \)) as the \( ^{15}\text{N} \) of foliage approaches that of the surface soils, which 
are generally more enriched in \( ^{15}\text{N} \) than vegetation. For the grasses which dominate 
the vegetation at the Gwy and Scoat Tarn, \( \varepsilon \) is very similar and if anything lower at 
Scoat Tarn, despite the higher deposition load and greater leaching of \( \text{NO}_3^- \). It seems 
likely that very tight cycling of N between the vegetation and surface soil organic 
matter may maintain a consistent difference between the \( ^{15}\text{N} \) value of the two 
compartments. Leaching of depleted \( ^{15}\text{N} \) produced by nitrification may enrich both 
compartments equally, preventing the use of the enrichment factor as an indicator of 
N saturation and \( \text{NO}_3^- \) leaching for grassland systems. The \( ^{15}\text{N} \) value of both grass 
and soil organic matter would increase, but \( \varepsilon \) would change little.
It is tempting to speculate that the $\delta^{15}\text{N}$ of the surface organic layer seems to increase from the Mharcaidh to the Gwy and then Scoat Tarn, but there is some overlap between soil types and the differences are marginal. The highest $\delta^{15}\text{N}$ value is, however, found in the Scoat Tarn podsol which is notable also for its very high mineralisation and nitrification potentials and low C:N ratio. Furthermore, the natural abundance data do show elevated $\delta^{15}\text{N}$ values in the plants and surface organic material of the two catchments with the highest mineralisation and nitrification potentials, the Afon Gwy and Scoat Tarn, suggesting that some losses of isotopically light N do occur.

9.7 N saturation status of catchment soils: comparison with the Stoddard scheme

The scheme proposed by Stoddard (1994) for identifying stages of N saturation is based on changes in the seasonal pattern of $\text{NO}_3^-$ leaching induced by excess N deposition. It is assumed that these changes are biologically mediated, and that seasonality of leaching is not driven directly by deposition. While the scheme itself is not explicitly incorporated in mass-balance models for N, it does form the basis for the mechanisms assumed to control leaching in the long term.

Although some seasonality is apparent in the bulk deposition data within the current study, the seasonal pattern of $\text{NO}_3^-$ leaching does not appear to be driven directly by bulk deposition (although no data are available for seasonal variations in total deposition at the study catchments). Surface water $\text{NO}_3^-$ concentrations follow a much smoother pattern than the very variable and higher concentrations found in bulk deposition, even in the sites leaching a relatively large proportion of inputs (Chapter 3). While hydrological retention time in catchments soils may be a factor, biotic retention and cycling has a major role in controlling leaching patterns, as demonstrated by the retention of very large proportions of N inputs at the Mharcaidh and Gwy sites. Hence the smooth seasonal pattern in $\text{NO}_3^-$ leaching provides indirect evidence of biological mediation and implies that nitrification must be the source of at least some of the leached $\text{NO}_3^-$. 

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The four study catchments were linked to the Stoddard classes of N saturation using existing data in Chapter 3, and the new data collected within the present study provide further confirmation of this classification. It is, however, possible to provide further interpretation of the mechanisms for observed stages of N saturation, and there are some notable departures from the schemes of Stoddard (1994) and Aber et al. (1989).

At the Allt a'Mharcaidh (Stage 0), plant and microbial uptake of deposited N clearly dominate the N budget, retaining 99% of inputs. Very low concentrations of NO$_3^-$ (<3 μeq l$^{-1}$) are observed on a seasonal basis during high flow episodes, but are below detection limits for most of the year due to negligible nitrification and are always much lower than concentrations in bulk deposition (frequently >40 μeq l$^{-1}$).

The Afon Gwy (Stage 1) shows a very strong seasonal pattern in NO$_3^-$ concentrations, with negligible leaching during the summer but elevated concentrations persisting from autumn to late spring. Concentrations during the period of leaching are sometimes higher than those in deposition, indicating that nitrification may be contributing to leaching losses, which is further demonstrated by the retention of almost all N deposition by the vegetation and surface soils. Assimilation of the elevated N inputs increases the N content of vegetation and litter, reducing the C:N ratio in the surface organic layer and increasing the nitrification potential, although nitrifiers face strong competition for available NH$_4^+$ from plant uptake and microbial immobilisation.

Elevated leaching of NO$_3^-$ throughout the year at Scoat Tarn indicates that N saturation has occurred, but a strong seasonal pattern shows that biological control of leaching is still dominant (Stage 2). Annual mean concentrations of NO$_3^-$ are similar in streams and in bulk deposition, although NH$_4^+$ is still strongly retained in the terrestrial system. Low C:N ratios occur in surface organic soils due to the assimilation of a large proportion of the high N deposition load. Biomass is significantly lower than at the Afon Gwy, but this cannot be linked causally to the excess N deposition load. Unlike the Stage 2 class defined for forests by Aber et al.
(1989), the system is still functioning as an important N sink, but N retention is relatively low compared with less N saturated catchments.

Severe N saturation is apparent at the River Etherow, with elevated $\text{NO}_3^-$ leaching all year round and no seasonal pattern, indicating that biological mediation of the leaching process is weak (Stage 3). Occasional periods of $\text{NH}_4^+$ leaching are also observed in some streams. The annual mean concentration of $\text{NO}_3^-$ in streams is greater than that in bulk deposition, suggesting that the catchment is a net source for $\text{NO}_3^-$ through nitrification, although a large proportion of total inorganic N inputs is still retained because $\text{NH}_4^+$ dominates the inputs. Unlike the schemes of Aber et al. (1989) and Stoddard (1994), nitrification does not occur in the dominant soils of the catchment, but may occur in riparian zones or in-stream. However, consideration of the total modelled deposition of oxidised N suggests that despite the relatively small flux in bulk deposition, cloud water and dry deposition are very significant, so that catchment soils are still a net sink for $\text{NO}_3^-$. The data indicate that $\text{NO}_3^-$ leaching in the Etherow streams is equivalent to 87% of total oxidised N deposition (Chapter 3: Table 3.12), providing further evidence that $\text{NO}_3^-$ may be largely decoupled from biological cycling within the plant-soil system. Despite the severity of N saturation the catchment still functions as a net sink for total deposited N, largely through the accumulation of $\text{NH}_4^+$ in soils, which is relatively immobile due to the lack of nitrification. Abiotic processes may be key regulators of $\text{NO}_3^-$ leaching.

The experimental data show a close correspondence with the stages of N saturation proposed by Stoddard (1994) but a key departure from his scheme is that nitrification is not necessarily an important control on $\text{NO}_3^-$ leaching in the most damaged catchment soils. Deposition history of both N and S, along with other possible pollutants, may account for reductions in microbial activity, particularly in the relatively sensitive nitrification process. As a result, another difference is that catchments are not a net source of total N, although $\text{NO}_3^-$ leaching at the Etherow exceeds bulk deposition inputs and is a comparable flux to total modelled deposition of oxidised N. If it can be shown that organic N exports have increased as a result of N deposition, estimates of catchment retention will decrease substantially but there is still net retention at present. The scheme is, however, broadly applicable to the moorland catchments studied, despite being devised for forest systems.
9.8 What components are missing from FAB, and how can it be improved?

Recommendations are made above for a modification to the denitrification component of FAB, to 10% of net inputs. N uptake and export rates are well established for managed coniferous forests which make up the majority of forest in the acid-sensitive UK uplands, but are less well known for moorlands. The key biomass removal processes are grazing and burning. It is well established that in the less intensively grazed uplands, N removal in biomass is relatively trivial compared to input fluxes, and likely to be no more than 1-2 kgN ha\(^{-1}\) yr\(^{-1}\). N losses in burning are more difficult to quantify, largely because of the major disturbance to the N cycle and the stimulation of mineralisation. This process could be important in heather moorland sites like the Etherow catchment which are intensively managed for grouse.

The major uncertainty in the N mass balance is the long-term immobilisation rate that can be sustained under excess N deposition loads. Data from tracer experiments at the low input Mharcaidh site suggest that literature based values for N immobilisation based on chronosequence studies are appropriate there, but would not necessarily reflect the maximum sustainable rate under a higher deposition load. Current rates of short-term immobilisation at the other higher input sites are an order of magnitude greater, but again it cannot be ascertained from this short duration study whether these elevated rates are sustainable. The suggested relationship between C:N ratio and NO\(_3^-\) leaching at 3 of the sites (excluding the Etherow) implies that net mineralisation could increase as C:N ratio declines, accelerating below some threshold value. At the Etherow the C:N ratio may not be a key control of inorganic N leaching because NH\(_4^+\) is present in excess and nitrification is inhibited. Hence it may be appropriate to separate sites into different “types”, for example acid grasslands and heather moorlands, where the key terms of the N mass balance may differ.

Denitrification is relatively unimportant at the Etherow, but NO\(_3^-\) leaching is, despite being extremely high, much lower than predicted by FAB, which assumes that excess NH\(_4^+\) will be nitrified and subsequently leached. One reason for this discrepancy is the very low long-term immobilisation rate allowed by FAB, while immobilisation is still much greater at the Etherow. Another reason is that the FAB assumption of induced nitrification does not apply to the Etherow peats. Extremely high levels of
NH$_4^+$ are present in the soilwaters, but nitrification potentials are very low. This accumulation of NH$_4^+$ in acid peats under heathland has been observed elsewhere in response to elevated N deposition (van Breemen & van Dijk, 1988), often associated with damaged *Calluna*, for example following heather beetle attack (e.g. Kristensen & McCarty, 1999). Nitrification may be inhibited under very high NH$_4^+$ deposition and in very poor condition soils (van Breemen & van Dijk, 1988). Hence it cannot be assumed that with a decline in biological N assimilation and C:N ratio, increased mineralisation will result in elevated nitrification rates and NO$_3^-$ leaching: NH$_4^+$ storage may become important and is not factored into current models. Processes which may suppress nitrification should therefore be taken into account.

Organic N may be the dominant form of N leached from upland soils, particularly in areas experiencing low anthropogenic deposition loads. In acid peat soils like those found at the Etherow, which are common in some of the most acidified regions of the UK, organic N fluxes may decline in relative importance as inorganic N leaching increases, but can be very important in absolute magnitude. One of the greatest uncertainties in the steady-state mass balance for N is whether organic N leaching increases in response to inorganic N deposition and net immobilisation. The importance of this potential sink for acidity is much debated in the literature but given the magnitude of leaching fluxes currently observed, much of the difference between current and chronosequence based N immobilisation rates could be accounted for. Since organic N retention or leaching has no net effect on the acidity balance, the potential sink for N associated acidity is very large.

Several studies have found that there may be a minimum threshold of N deposition below which inorganic N leaching is negligible. For example, Dise and Wright (1995) suggested that inorganic N outputs from forest soils were negligible at deposition loads of less than 9 kgN ha$^{-1}$ yr$^{-1}$. The same threshold was suggested for the accumulation of both C and N in Scottish moorland peaty podsols, with C:N ratios increasing in response to deposition below this threshold (White *et al*., 1999). Such sinks for N may therefore prove to be permanent, i.e. long-term immobilisation. However, Wilson and Emmett (1999) noted that other thresholds have been found elsewhere. If it can be demonstrated that such thresholds are universally applicable for
given soil types, the FAB model could account for them by adjusting the $N_{imm}$ term on a soil specific basis.

A key control on the leaching of all N species is the water flux through soils. Catchment hydrology may therefore be an important factor in the N mass balance, but is difficult to incorporate into national scale models for which only coarse resolution data are generally available. Slope and proportional cover of bare ground may be important regulators of N leaching, while differences in soil drainage properties are likely to be significant. The importance of hydrological NO$_3^-$ has not been quantified, but large observed differences in water chemistry between adjacent stream subcatchments at Scoat Tarn and the River Etherow are likely to be due at least in part to hydrological effects.

A process not accounted for in static models, but with relevance for dynamic models, is that of vegetation change. For example, N deposition has been associated with the failure of *Sphagnum* mosses to recover in much of the Pennines from which they were lost due to S deposition (see INDITE, 1994), and has been linked to changes from *Calluna* heath to grassland in the Netherlands (Aerts & Bobbink, 1999). If long-term rates for the key N fluxes are not changed as a result of such effects, then there is no implication for static critical loads.

**9.9 What are the key considerations for dynamic modelling?**

N retention was initially incorporated into the dynamic acidification model MAGIC as a zero-order term which simply calibrated simulated N leaching to observed values in streamwater for a given N deposition, without taking any account of the processes responsible (Evans et al., 2001). This approach is equivalent to that taken by the SSWC model for total acidity (see Chapter 1). The first version of the model to incorporate an extension to calculate the major N fluxes and their changes through time was called MAGIC-WAND (MAGIC With Additional N Dynamics; Ferrier et al., 1995; Jenkins et al., 1997). Mass balances for inorganic N were incorporated separately for NO$_3^-$ and NH$_4^+$ to include deposition, fixation, fertiliser additions, net mineralisation and nitrification, denitrification and forest uptake. Values derived from the literature were used for rates of mineralisation, denitrification and fixation. Nitrification was included as
a first-order reaction responding to changes in \(\text{NH}_4^+\) concentration, but the plant uptake function, using a Michaelis-Menten equation, was the most important process governing \(\text{NO}_3^-\) leaching (Ferrier et al., 1995).

A problem with the formulation of MAGIC-WAND was the lack of an internal state variable to represent the process of N saturation, so that rate constants for key processes were fixed (Evans et al., 2001). The latest version of the model, MAGIC7, has addressed this issue by incorporating a soil organic matter compartment, with immobilisation controlled by C:N ratio (Cosby et al., 2001). The model first calculates the N supply rate as the sum of deposition and gross mineralisation. Nitrification and plant uptake are then calculated, and N is removed from the soil organic pool if inorganic N is insufficient to meet plant demands. As with MAGIC-WAND, these processes are all first-order with fixed rate coefficients.

The key feature of MAGIC7 is therefore the final, sequential N sink, immobilisation, which is calculated as a function of C:N ratio. Above an upper limit C:N ratio, all available inorganic N after calculation of the above mass balance is immobilised in soil organic matter, i.e. there is net immobilisation. Below a lower limit of C:N ratio, gross immobilisation ceases and 100% of available inorganic N is leached. Between these limits, gross immobilisation in interpolated as a linear function of C:N ratio, so that leaching varies from 0 to 100% across this C:N range. The N saturation process is implicit in that immobilised N reduces the C:N ratio of the soil organic matter pool, and so regulates the rate of immobilisation through a direct feedback mechanism as C:N declines. Conceptually, MAGIC7 therefore assumes that it is a decrease in immobilisation rather than an increase in mineralisation that occurs during N saturation, but the net effect is the same either way.

Other dynamic models have incorporated processes to describe N saturation and release, but they are generally not linked to freshwater critical loads. For example, an identical approach to that used in MAGIC7 was adopted in the SMART soil acidification model to determine immobilisation rates (Posch & de Vries, 1999). The dynamic model MERLIN (Model of Ecosystem Retention and Loss of Inorganic N) was developed specifically to predict N saturation and inorganic N leaching (Cosby et al., 1997; Emmett et al., 1997). The model requires temporal sequences of C fluxes and pools,
hydrological data and external N inputs as input data, and is therefore very data
intensive. Furthermore, it is not linked directly to acidification models like MAGIC and
so has very limited applications in critical loads work. The model does, however,
embrace all the processes discussed for FAB and MAGIC7 and utilise compartment
C:N ratios to determine key process rates, showing a consistency in approach between
all the above dynamic models.

There are subtle differences between the key uncertainties associated with static and
dynamic mass balance models for N. All the problems associated with the static models
should apply equally to the dynamic models over very long timescales of, say, decades
to centuries, depending on the size of the active C pool and the time taken for N
deposition to reduce C:N ratios to critical values, inducing N saturation. Over shorter
periods of decades or less, net N immobilisation may continue and will have to be
included as a sink for N at these timescales. However, it has already been shown that the
C:N ratio is not likely to be a dominant control of N leaching in all systems, and it may
be inapplicable to damaged peat soils like those at the Etherow. Furthermore, other
processes like vegetational change could have major implications for dynamic modelling
over shorter timescales, depending on their effects on the major N fluxes like
immobilisation and mineralisation. Changes in productivity and biomass may occur over
a period of decades and while they may not provide sustainable sinks for N deposition,
net accumulation of N in biomass must be accounted for in the short term.

For example, Lee et al. (1990) described Sphagnum moss as an almost perfect sink for N
deposition, whereby the moss carpet intercepts deposited ions and controls their
availability for the growth of higher plants. This may reflect the situation at the Allt
a’Mharcaidh. However, in polluted areas, the Sphagnum sink may be saturated and its
decomposition accelerated by the higher N content, possibly changing competitive
relationships and causing an increase in the growth of higher plants, while the Sphagnum
debottlenecks. This process, along with very high S deposition, may have contributed to the
loss of Sphagnum from large areas of the Pennines, and may have occurred at the Etherow. If the plants which replace the Sphagnum are a less effective sink for N, then a
feedback mechanism may be induced for N saturation and leaching. The representation
of such processes presents a major challenge for both static and dynamic models.
The ability of the dynamic models to separate the processes regulating NH$_4^+$ and NO$_3^-$ fluxes could prove useful if it can be demonstrated that direct leaching of NO$_3^-$ occurs because its participation in the terrestrial N cycle is inhibited by excess NH$_4^+$, as appears to be the case at the Etherow. For example, denitrification could be incorporated as a first-order term at 10% of net NO$_3^-$ supply to provide consistency with the FAB model.

9.10 Recommendations for future work

The large differences in catchment behaviour that are highlighted, from the N limited Mharcaidh to the severely impacted Etherow, and between heather moor and grassland sites, suggest that the study of a greater number of sites within each of these categories to provide better coverage of the key environmental gradients could provide very useful data for model development and improvement. Future work might focus on the measurement of C:N ratios and soil nitrification potentials as promising indicators of inorganic N leaching behaviour. Factors which might inhibit nitrification need to be better understood and described for incorporation into models.

Finally, the nature of the problems associated with modelling N saturation and leaching demonstrates the vital importance of longer term datasets from environmental monitoring programmes. There are currently very few data with which to test hypotheses relating to the key question of N immobilisation and whether changes in soil C:N ratios or organic N leaching have occurred in response to N deposition. Short-term experimental programmes like this one are useful in identifying the most important processes and parameters for modelling work, but only long time series of the relevant data can demonstrate the degree to which these processes are being successfully modelled.

9.11 References


nitrogen saturation in a Sitka spruce forest, Aber, Wales, UK. *Biogeochemistry* 38, 129-148.


APPENDIX 1: FORMULATION OF THE SSWC MODEL

Acid neutralising capacity (ANC): the critical chemical parameter

ANC is operationally defined as the sum of base cations minus the sum of acid anions (Henriksen et al., 1992), but since critical loads relate to acid deposition inputs it is first necessary to quantify and remove the proportion of ions deriving from neutral sea-spray inputs so that the definition of ANC becomes:

\[ \text{ANC} = [BC_t^*] - [AA_t^*] \]  \hspace{1cm} (Equation 1)

where \([BC_t^*]\) is the current, measured sum of non-marine base cations (\(= \text{Ca}^* + \text{Mg}^* + \text{K}^* + \text{Na}^*\)) and \([AA_t^*]\) is the sum of non-marine acid anions (\(= \text{SO}_4^* + \text{NO}_3\)). It is assumed that all chloride is derived from marine sources; \(^*\) denotes the non-marine component whereby the marine contribution of each ion is subtracted as a proportion of measured chloride concentration from the known ratios of these ions in seawater.

The SSWC model formulation

The equation defining ANC forms the basis of the freshwater modelling approach: empirical relationships are invoked to determine the pre-industrial concentration of base cations (\([BC_0^*]\)), and this effectively sets the long-term critical load because it represents the only source of base cations over the long term. Given a pre-selected critical ANC value, then the freshwater critical load is simply the input flux of acid anions from atmospheric deposition which gives the critical ANC when subtracted from the pre-industrial flux of base cations (Henriksen et al., 1992):

\[ \text{Critical load} = ([BC_0^*] - \text{[ANC}_{\text{crit}}].Q) \]  \hspace{1cm} (Equation 2)

Concentrations are multiplied by runoff \((Q)\) from the site to convert them into fluxes. The critical load is therefore a critical flux of acid anions.
The steady-state water chemistry (SSWC) model employs certain assumptions and empirical relationships in order to determine the “permanent” buffering provided by the pre-industrial base cation concentration ([BC]₀*) which is the sum of weathering ([BC_w]) supply plus base cation deposition ([BC_{dep}]*) if it is assumed that base cation deposition has not significantly changed since pre-industrial times. The first step is to quantify the proportion of measured base cation leaching which is derived from transient ion-exchange processes (BC_{ex}) and is proportional to the load of acid anions. This proportion is represented in the SSWC model by the term “F”, calculated according to the methodology of Brakke et al. (1990):

\[ F = \sin \left( \frac{\Pi [BC]^*}{2} \right) \]  

(Equation 3)

where [BC]ₜ* is measured non-marine base cation concentration and S is a constant which varies regionally according to geology, but from empirical studies is taken as 400 µeq l⁻¹ (Harriman and Christie, 1995). This constant determines the measured non-marine base cation concentration which represents a catchment likely to be unaffected by acid deposition; when [BC]ₜ* = S, F=1 and base cation leaching is increased by exactly the value of the acid anion load, resulting in no change in ANC in runoff. For values of [BC]ₜ* greater than S (400 µeq l⁻¹) F is set to 1 (it would otherwise decrease again with [BC]ₜ* according to the sine function).

F is then used to calculate the pre-industrial base cation concentration according to the following equation (Henriksen et al., 1992):

\[ [BC]₀^* = [BC]ₜ^* - F([AA]ₜ^* - [AA]₀^*) \]  

(Equation 4)

where [AA]₀^* is the pre-acidification concentration of non-marine acid anions from weathering and natural atmospheric sources and the measured leaching rate of non-marine base cations ([BC]ₜ*) represents the sum of weathering, non-marine deposition and ion-exchange sources (BC_{leach}). Data from near-pristine lakes in northern Scotland indicate that “background” concentrations of NO₃⁻ are close to zero, while
“background” concentrations of $SO_4^{2-}$ are determined from empirical relationships between base cations and sulphate in near-pristine lakes (see Henriksen et al., 1990, 1992).

Since $[BC]_0^*$ is now known, the critical load can be defined by Equation 2. If the critical load of acid deposition is exceeded, then when $BC_{\text{leach}}$ has declined to the concentration $[BC]_0^*$ the ANC of the water body will cross the threshold concentration $[\text{ANC}_{\text{crit}}]$. The magnitude of critical load exceedance, expressed as a flux of acid anions, provides the theoretical ANC of the water body when $BC_{\text{leach}}$ has declined to $[BC]_0^*$.

**Critical load exceedance for total acidity with the SSWC model**

Critical load exceedance for total acidity is calculated from sulphur deposition and $NO_3^-$ leaching (Kämäri et al., 1992):

$$\text{Exceedance} = S^*_{\text{dep}} + [NO_3^-] \cdot Q - \text{Critical Load} \quad \text{(Equation 5)}$$

where $S^*_{\text{dep}}$ is non-marine sulphur deposition. $NO_3^-$ deposition cannot be treated in the same way because in general, only a small proportion of it is leached into surface waters; most is retained within the terrestrial part of the catchment. Measured $NO_3^-$ concentration is therefore converted into an exceedance flux (using runoff) to represent the quantity of N deposition which is contributing to exceedance (Kämäri et al., 1992).

**References**


APPENDIX 2: ANALYTICAL METHODS FOR SURFACE AND SOIL WATER CHEMISTRY

Surface waters

1. Mharcaidh, Scoat Tarn and Etherow samples (see Harriman et al., 1990)
Water samples were filtered (0.45μM) prior to analysis. Base cations (Na⁺, K⁺, Ca²⁺, Mg²⁺) were determined by atomic absorption spectroscopy (Perkin Elmer 2380). Acid anions (NO₃⁻, SO₄²⁻, Cl⁻) were determined by ion chromatography using a DIONEX-QIC system. NH₄⁺ was determined using an Orion 901 Ionalyser ion-selective electrode. TOC on the filtered fraction (i.e. DOC) was measured with a TOCSIN carbon analyser (Phase Separation Ltd.). Alkalinity and pH were measured with a Radiometer TTT81 digital titration system with pHM84 research pH meter. Equivalence alkalinity was determined using the dual end-point titration method (to pH 4.5 and 4.2 for positive alkalinities and pH 4.2 and 3.9 for negative alkalinities).

2. Gwy samples (see Patrick et al., 1991)
Base cations and acid anions (SO₄²⁻ and Cl⁻) were measured by ICP/OES at CEH Wallingford. Other determinands were measured at the EA Llanelli Laboratory. Total oxidised N was determined colourimetrically at 520nm. Alkalinity was determined by Mettler DL 40 Memotitrator and pH by E.E.L. pH Meter. DOC was measured with a Technician Air Segmented Autoanalyser II.

Soil waters – suction samplers

NO₃⁻ was measured by ion chromatography using a DIONEX DX500 dual channel system with DIONEX Anion self regenerating suppressor (ASRS-1, 4mm) and CD20 conductivity detector (range 0.003 – 4 mgN l⁻¹).

NH₄⁺ was determined colourimetrically using segmented flow analysis (TRAACS analyser, Bran & Luebbe, Northampton, UK: range 0.003 – 1.0 ppm NH₄-N).
Total dissolved N was determined by a persulphate oxidation procedure. Dissolved organic N was calculated as the difference between total soluble N and \((\text{NO}_3^- + \text{NH}_4^+)^{\prime}\).

References


APPENDIX 3: CALCULATION OF \textsuperscript{15}N TRACER DOSAGE, N POOL SIZES
AND TRACER RETENTION

Tracer dosage

Fortnightly dosage of tracer

The dosage of labelled $\textsuperscript{15}\text{NH}_4\textsuperscript{15}\text{NO}_3$ was calculated as follows.

$$1.5 \text{ kgN ha}^{-1} = 0.15 \text{ gN m}^{-2}$$

For a $3\text{m}^2$ plot with 26 fortnightly additions the required mass of N per dose is:

$$\frac{(3 \times 0.15)}{26} = 0.0173 \text{ gN} = 17.3 \text{ mgN}$$

Assuming the atomic weight of N = 14, then the addition of 17.3 mgN as $\textsuperscript{14}\text{NH}_4\textsuperscript{14}\text{NO}_3$ would require:

$$17.3 \times \frac{(14+4+14+48)}{(14+14)} = 49.43 \text{ mg} \textsuperscript{14}\text{NH}_4\textsuperscript{14}\text{NO}_3$$

However, since the label used was 30 atom\% $\textsuperscript{15}N$ the dosage had to be modified to account for the extra mass of the $\textsuperscript{15}N$.

Following the methodology of Jones \textit{et al.} (1991), if the number of atoms in a sample of N are $\textsuperscript{14}N = p$ and $\textsuperscript{15}N = q$ then the average atomic mass of N ($^{\text{av}}N$) in the sample is given by:

$$^{\text{av}}N = \frac{(14p + 15q)}{(p+q)}$$

Given the atom\% of $\textsuperscript{15}N$ “A” then:

$$q / (p+q) = A/100 \text{ and } p / (p+q) = 1 - (A/100)$$
Therefore the average atomic mass of N can also be expressed as:

\[
{^{av}N} = \frac{14p}{(p+q)} + \frac{15q}{(p+q)} \\
= 14 \left[1-(A/100)\right] + 15 \left[A/100\right] \\
= 14 + \left(A/100\right)
\]

From this equation, the average atomic mass of the \( ^{15}N \) labelled tracer is 14.3. Therefore the \( ^{15}NH_4^{15}NO_3 \) tracer has a molecular weight of 80.6 rather than 80, and the number of moles of tracer N equivalent to 49.43 mg \( ^{14}NH_4^{14}NO_3 \) requires a fortnightly one litre dosage of:

\[
49.43 \times (80.6/80) = 49.81 \text{ mg l}^{-1} \text{ labelled } ^{15}NH_4^{15}NO_3.
\]

**Total annual additions of tracer and \( ^{15}N \)**

With 39 plots and 26 additions, the total mass of label used was 50.5 g. The total dosage of N to each plot of 3 m\(^2\) over the course of one year of additions expressed as a mass flux is:

\[
(49.81 \times 26) \times (28.6/80.6) = 459.54 \text{ mg N yr}^{-1} \\
= 153.18 \text{ mg N m}^{-2} \text{ yr}^{-1} \\
= 1.53 \text{ kg N ha}^{-1} \text{ yr}^{-1}
\]

of which 30 atom\% was \( ^{15}N \). For a sampled quadrat of 0.25 m\(^2\) the total addition of tracer N was therefore \( 153.18 \times 0.25 = 38.295 \) mg.

Using the same notation as above, the percentage mass of \( ^{15}N \) can be expressed as:

\[
\frac{(15q \times 100)}{[(p+q) \times \text{^{av}N}]} \text{ i.e. (mass of } ^{15}N \times 100 \) / (total mass of N)
\]

\[
\frac{(15A/100) \times 100}{\text{^{av}N}} = 15A / \left[14 + (A/100)\right]
\]
\[
= 1500A / (1400 + A)
\]

Given that 30 atom% $^{15}$N was used, the percent by mass of N applied which was $^{15}$N was:

\[
\frac{1500 \times 30}{1400+30} = 31.46\% \text{ by mass}
\]

The total mass dosage of $^{15}$N over the additions year was therefore:

\[
459.54 \text{ mg} \times 0.3146 = 144.57 \text{ mg} \quad ^{15}\text{N per plot}
\]

The total addition of $^{15}$N to the quadrat (area 0.25m$^2$) was:

\[
144.57 \times (0.25 / 3) = 12.05 \text{ mg} \quad ^{15}\text{N}
\]

This figure excludes the contribution of “natural abundance” $^{15}$N in deposition.

**Calculation of soil N pools and $\delta^{15}$N of bulked samples**

If the thickness of the soil horizon is T and the bulk density is BD, then sampled horizons 2 and 3 (counting from the surface, where the upper organic horizon 1 was analysed separately) were bulked in proportion to their respective bulk densities and thickness. For example, to bulk soil horizons 2 and 3, the bulking factor, or weighting, of sample 2 ($W_2$) was calculated as:

\[
W_2 = \frac{BD_2 \times T_2}{(BD_2 \times T_2) + (BD_3 \times T_3)}
\]

where $W_3$ (and $W_4$ where required) was calculated in the same way, such that the sum of all weightings was unity.

To quantify the N pool in the bulked sample, the overall bulked density ($BD_{bulk}$) and \%N ($\%N_{bulk}$) were required. These were calculated from the weightings and individual values from the separate bulked soils:
\[ BD_{\text{bulk}} = (BD_2 \times W_2) + (BD_3 \times W_3) \]

Similarly, for the overall %N in bulked samples 2 and 3:

\[ %N_{\text{bulk}} = (%N_2 \times W_2) + (%N_3 \times W_3) \]

In order to calculate the new $\delta^{15}\text{N}$ value of the bulked sample, it was also necessary to weight by proportional %N, such that:

\[ \delta^{15}\text{N}_{\text{bulk}} = (\delta^{15}\text{N}_2 \times W_2 \times %N_2 / %N_{\text{bulk}}) + (\delta^{15}\text{N}_3 \times W_3 \times %N_3 / %N_{\text{bulk}}) \]

These derived values for the bulked samples were used to estimate the overall N pools in the bulked horizons within each quadrat and plot.

**Calculation of $^{15}\text{N}$ tracer retention in soil and vegetation compartments**

In order to determine the most appropriate equation to use of those published by Nadelhoffer and Fry (1994), the mass balance was reworked from first principles.

The starting point is consideration of the mass of the initial N pool \( m_i \), the mass of labelled N incorporated into the N pool \( m_{lab} \) and the final mass of the N pool \( m_f \), i.e. the total N mass balance:

\[ m_f = m_i + m_{lab} \]

This can be expressed as a mass balance for $^{15}\text{N}$ by using atom% $^{15}\text{N}$ \((A)\) values:

\[ (m_i \times A_i) + (m_{lab} \times A_{lab}) = (m_f \times A_f) \]

If $\delta^{15}\text{N}$ is assumed to be a linear function of atom% $^{15}\text{N}$ then a mass balance for $^{15}\text{N}$ can be expressed by using the $\delta^{15}\text{N}$ values (Nadelhoffer & Fry, 1994), so that:
\[(m_i \times \delta^{15}N_i) + (m_{lab} \times \delta^{15}N_{lab}) = (m_f \times \delta^{15}N_f)\]

But since \(m_f = m_i + m_{lab}\) and hence \(m_i = m_f - m_{lab}\) the above equation can be re-expressed as:

\[
(m_{lab} \times \delta^{15}N_{lab}) = (m_f \times \delta^{15}N_f) - (m_i \times \delta^{15}N_i) = (m_f \times \delta^{15}N_f) - (m_f \times \delta^{15}N_i) + (m_{lab} \times \delta^{15}N_i)
\]

\[
\Rightarrow (m_{lab} \times \delta^{15}N_{lab}) - (m_{lab} \times \delta^{15}N_i) = (m_f \times \delta^{15}N_f) - (m_f \times \delta^{15}N_i)
\]

\[
\Rightarrow m_{lab} = m_f (\delta^{15}N_f - \delta^{15}N_i) / (\delta^{15}N_{lab} - \delta^{15}N_i)
\]

This equation is recommended by Nadelhoffer and Fry (1994) to estimate fluxes into relatively small N pools where the incorporation of the labelled N can be expected to measurably increase the pool size, e.g. decomposing litter, and was used in the study of tracer retention in coniferous forests within NITREX by Tietema et al. (1998).

Alternatively, according to Nadelhoffer and Fry (1994), if the pool is large and the change is too small to measure over the timescale of the experiment, an alternative formulation can be used, assuming \(m_f = m_i\):

\[
(m_{lab} \times \delta^{15}N_{lab}) = (m_i + m_{lab})\delta^{15}N_f - (m_i \times \delta^{15}N_i)
\]

\[
\Rightarrow (m_{lab} \times \delta^{15}N_{lab}) - (m_{lab} \times \delta^{15}N_i) = (m_i \times \delta^{15}N_f) - (m_i \times \delta^{15}N_i)
\]

\[
\Rightarrow m_{lab} = m_i (\delta^{15}N_f - \delta^{15}N_i) / (\delta^{15}N_{lab} - \delta^{15}N_i).
\]

However, all the above mass balance equations assume that \(\delta^{15}N\) is a linear function of atom\% ^{15}N, and this assumption only holds approximately true when sample atom\% ^{15}N (\(A_s\)) is small, i.e. close to the standard value (\(A_0\)).

This can be seen by plotting \(\delta^{15}N\) against A for different ranges of A, using the equation:
\[ \delta^{15}\text{N} = \left[ \frac{(272 \, A_s)}{(100 - A_s)} \right] - 1 \times 1000\% \]

At low values of A the relationship is almost exactly linear (Figure A3.1).

**Figure A3.1: Relationship between \( \delta^{15}\text{N} \) and \( A_s \) for low abundances of \( ^{15}\text{N} \)**

\[ y = 2788x - 1032.3 \]
\[ R^2 = 1 \]

Hence for low abundances of \( ^{15}\text{N} \) the relationship between \( \delta^{15}\text{N} \) and \( A_s \) is almost linear. At such low abundances of \( ^{15}\text{N} \) it is therefore possible to use the simplified assumption made by Johannisson (1996):

\[ \delta^{15}\text{N} = \left( \text{atom}\% \, ^{15}\text{N}_{\text{samp}} - \text{atom}\% \, ^{15}\text{N}_{\text{standard}} \right) / \text{atom}\% \, ^{15}\text{N}_{\text{standard}} \times 1000\% \]

Using this notation, the \( \delta^{15}\text{N} \) of the tracer used in this study (30.02 atom\% \( ^{15}\text{N} \)) is:

\[ \delta^{15}\text{N} = \left[ (30.02 - 0.0003663) / 0.0003663 \right] \times 1000\% = 81954 \% \]
However, when a tracer with a very high $^{15}$N abundance is used, the linear relationship between $\delta^{15}$N and $A_s$ does not hold (Figure A3.2). These differences in $\delta^{15}$N when calculated for the $^{15}$N tracer using the correct rather than the above "linear" method are obvious:

\[
\delta^{15}\text{N} = \left[\frac{(272 \times A_s)}{(100 - A_s)}\right] - 1 \times 1000\% \\
= \left[\frac{(272 \times 30.02)}{(100 - 30.02)}\right] - 1 \times 1000\% \\
= 115682\% 
\]

Therefore when using mass balance techniques to determine the fate of tracers with a very high $^{15}$N abundance, the approximation of Nadelhoffer and Fry (1994) that $\delta^{15}$N can be used in place of atom% $^{15}$N in the mass balance does not hold. Instead, atom% $^{15}$N values should be used directly:

\[
m_{lab} = m_i \frac{(A_f - A_i)}{(A_{lab} - A_f)}
\]

Figure A3.2: Relationship between $\delta^{15}$N and $A_s$ for very high $^{15}$N abundances in tracers
This is the formulation used in the current study, under the assumption that \( m_f = m_i \)
i.e. equivalent to the equation recommended by Nadelhoffer and Fry (1994) for cases
where tracer additions do not measurably increase the overall N pool size, and
approximates that used by Hauck & Bremner (1976), cited in Miller and Bowman
(2002):

\[
F = \frac{[T(A_s - A_b)]}{A_f}
\]

F = weight of N derived from tracer
T = total weight of sample N
\( A_s \) = atom% excess \(^{15}\)N in labelled sample
\( A_b \) = atom% excess \(^{15}\)N in control (background) sample
\( A_f \) = atom% excess \(^{15}\)N in tracer (here = 30.02\%) 

References


