

Nitrogen saturation in UK moorlands: the critical role of bryophytes and lichens in determining retention of atmospheric N deposition

C. J. CURTIS,* B. A. EMMETT,† H. GRANT,‡ M. KERNAN,*
B. REYNOLDS† and E. SHILLAND*

*ECRC, University College London, 26 Bedford Way, London WC1H 0AP, UK; †CEH Bangor, Orton Building, Deiniol Road, Bangor, Gwynedd LL57 2UP, UK; and ‡CEH Merlewood, Windermere Road, Grange-Over-Sands, Cumbria LA11 6JU, UK

Summary

1. Anthropogenic nitrogen (N) deposition may have several impacts on upland moorland ecosystems, including changes in vegetation composition, eutrophication and surface water acidification through nitrate leaching, but few studies linking N deposition to key biogeochemical processes have been published.
2. A stable isotope tracer (^{15}N) was used to determine the fate of inorganic N inputs to four moorland catchments across gradients of N deposition and leaching, through 2-weekly additions to experimental plots on major soil types over 1 year.
3. An apparent decline in total ^{15}N recovery from soils and vegetation as the proportion of leached N deposition increased was not significant at the $P = 0.05$ level, but a significant relationship was found for recovery in mosses and lichens.
4. Vegetation retained 31–68% of ^{15}N inputs, and ^{15}N recovery increased significantly ($P = 0.01$) with biomass for all compartments except woody shrubs. Mosses and lichens showed far greater ^{15}N recovery per unit biomass than grasses or ericaceous shrubs. There was no significant variation in the proportion of ^{15}N recovered in higher plants across the N deposition gradient (24–29%). In contrast, the proportion recovered in mosses and lichens declined from 44% to 2% as deposition increased, mirroring a decline in their biomass and showing a highly significant inverse relationship ($P = 0.01$) with nitrate leaching.
5. The proportion of ^{15}N recovered in litter plus surface soils (33–39%) was remarkably constant across the deposition gradient for a variety of soil types. However, significantly declining recovery per unit biomass in litter ($P < 0.05$) suggested progressive N saturation of this sink and increasing importance of retention in underlying surface soils as deposition increased.
6. *Synthesis and applications.* Past studies have demonstrated a decline in mosses and lichens in response to increasing N deposition, but we show here for the first time that reduced N retention might result together with increased nitrate leaching into surface waters. The conservation of bryophyte and lichen flora on moorlands is therefore critical to prevent excessive nitrate leaching and associated surface water acidification and eutrophication. Ensuring management practices such as grazing or burning are at an intensity that does not further degrade the bryophyte and lichen communities may help minimize the impact of N deposition on freshwaters, but the only effective means to reduce the risk of N leaching is a reduction in N emissions.

Key-words: acidification, immobilization, nitrate leaching, nitrogen deposition, stable isotope, uplands

Journal of Applied Ecology (2005) **42**, 507–517
doi: 10.1111/j.1365-2664.2005.01029.x

Introduction

In upland areas of the UK, the atmospheric deposition of reduced and oxidized forms of nitrogen (N) derived from anthropogenic sources may have several adverse impacts on semi-natural moorland ecosystems. Terrestrial effects may include vegetation change through increased foliar N concentrations (Pitcairn, Fowler & Grace 1995; Pitcairn *et al.* 2001) and changes in species composition and diversity (Bobbink 1998; Bobbink, Hornung & Roelofs 1998; Leith *et al.* 2001), with bryophytes being particularly sensitive (Woodin, Press & Lee 1985; Lee, Baxter & Emes 1990; Carroll *et al.* 2000). For a recent review see NEG-TAP (2001). Impacts are not restricted to terrestrial ecosystems; aquatic effects may also occur. For example, increased nitrate (NO₃) leaching can contribute to the acidification of upland surface waters (Allott *et al.* 1995; Curtis *et al.* in press), while eutrophication effects have been observed in mountain lakes in the USA (Sickman, Melack & Clow 2003; Burns 2004). Hence the fate of N deposition inputs, whether retained in catchment vegetation and soils, returned to the atmosphere via denitrification, or leached into surface waters, may affect many aspects of the structure and function of upland ecosystems.

Recognition of the potential problems associated with N deposition has led to the development of various static and dynamic models to predict the fate of deposited N compounds within ecosystems and catchments. Such models are often linked to critical loads for vegetation and surface waters (MERLIN, Cosby *et al.* 1997; FAB, Posch *et al.* 1997; INCA, Whitehead, Wilson & Butterfield 1998; MAGIC, Cosby *et al.* 2001). For many of these models, a key uncertainty is associated with the dynamics of N retention through vegetation uptake and immobilization in organic soil components.

The immediate fate of N inputs can be determined from a variety of techniques. These can provide information on the degree to which N retention is biologically mediated in the terrestrial system. This study investigated the short-term fate of inorganic N inputs to four moorland catchments using a stable isotope tracer (¹⁵N).

The purpose of the study was to determine the fate of ¹⁵N-labelled N inputs over a period of 1 year of additions at four sites selected from the UK Acid Waters Monitoring Network (Monteith & Evans 2000). The sites represented gradients of total N deposition and leaching losses of inorganic N, measured as part of a wider N budget study (Curtis & Simpson 2001). The experiment was designed to test the hypothesis that the efficiency of vegetation and soil in retaining deposited N would be reduced in high N deposition areas because of reduced uptake requirements by plants and reduced microbial immobilization in soils. The tracer experiment quantified the proportion of N inputs lost from the soil-plant system in the short term (1 year; Fig. 1). Information on the degree to which net nitrification may change under increasing deposition loads as the

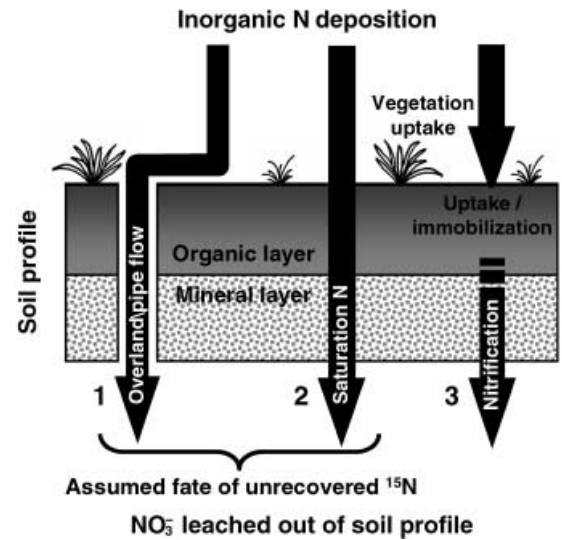


Fig. 1. Schematic diagram of three potential nitrate leaching pathways: (1) 'hydrological' nitrate, which bypasses biological retention mechanisms in the soil-vegetation system via saturation overland flow and/or pipe/macropore flow; (2) 'saturation' nitrate, which is present in excess of biological demand and leaches through the soil profile unchanged; (3) nitrate produced within the soil profile by microbial nitrification, which may have derived originally from anthropogenic N deposition and may be determined by changes in the soil carbon : nitrogen ratio in response to elevated N deposition (Curtis *et al.* 2004). The ¹⁵N addition experiment provides an estimate of the sum of (1) and (2).

carbon : nitrogen ratio of soil organic matter decreases is reported elsewhere (Curtis *et al.* 2004).

Methods

N budget data were summarized for the four experimental catchments (Table 1). Within each site, sample plots were established on the main soil types, giving a total of 13 soils from within the four catchments (Table 1). The Allt a'Mharcaidh site comprised wet and dry heath, with grasses, shrubs, mosses and *Sphagnum* species. The Afon Gwy and Scoat Tarn catchments were dominated by acid grasslands, although shrubs also occurred on the peaty hilltops at the Afon Gwy. The Afon Gwy and Scoat Tarn catchments were subject to low intensity grazing by sheep *Ovis aries* (L.) while red deer *Cervus elaphus* (L.) grazed in the Allt a'Mharcaidh. The study area at the River Etherow was dominated by *Calluna vulgaris* (L.) Hull and was managed as a grouse moor by burning.

Apparent retention of deposited N in catchment soils and vegetation ranged from more than 98% at the Allt a'Mharcaidh to 64% at the River Etherow (Table 1). Nitrate leaching fluxes increased with total inorganic N (TIN) deposition across the four catchments.

On each of the 13 soil study areas within the four N budget catchments, three replicated experimental plots were established. The rectangular plots were 1 m wide and 3 m long, with the long axis along the line of slope of the ground. Following ¹⁵N additions to the whole

Table 1. Experimental catchment details and percentage total inorganic N (TIN) retention according to input–output budgets. Precipitation and deposition fluxes are based on 5-km grid modelled annual mean data for 1998–2001 (CLAG Deposition Fluxes 1997). TIN deposition comprises wet (orographically enhanced) plus dry deposition of oxidized (NO_x) and reduced (NH_y) N. Nitrate leaching fluxes (N_{leach}) are based on mean surface water concentrations and site-specific flow data for the period October 1999–October 2000. Fluxes are expressed in $\text{kg N ha}^{-1} \text{ year}^{-1}$. Total ^{15}N input to each quadrat from tracer additions and deposition (assuming $^{15}\text{N} = 0\%$) is expressed in mg. Experimental soil types each include three replicated sample plots at the specified altitude

Site	Precipitation (mm) and TIN deposition fluxes	^{15}N input, nitrate leaching flux and TIN retention	Experimental soil types ($n = 13$)	Altitude (m a.s.l.)
Allt a'Mharcaidh, Cairngorms, north-east Scotland, NH 881 045				
	Precipitation 1210	^{15}N input 12.68	M1, peaty ranker	700
	NO_x deposition 3.7	N_{leach} flux 0.1	M2, valley peat	570
	NH_y deposition 2.8	% TIN retention 98.4	M3, peaty podsol	495
	TIN deposition 6.4		M4, shallow peat	490
Afon Gwy, Plynlimon, mid-Wales, SN 824 854				
	Precipitation 2258	^{15}N input 13.59	G1, hilltop peat	570
	NO_x deposition 7.3	N_{leach} flux 1.8	G2, peaty gley	550
	NH_y deposition 8.4	% TIN retention 88.5	G3, podsol	480
	TIN deposition 15.7		G4, valley peat	390
Scotat Tarn, Lake District, north-west England, NY 159 104				
	Precipitation 2217	^{15}N input 14.26	S1, podsol	750
	NO_x deposition 10.0	N_{leach} flux 4.9	S2, peaty gley	650
	NH_y deposition 12.5	% TIN retention 78.3	S3, deep peat	590
	TIN deposition 22.5			
River Etherow, S. Pennines, north-west England, SK 116 996				
	Precipitation 1272	^{15}N input 15.06	E1, deep peat (burnt <i>Calluna</i>)	455
	NO_x deposition 15.7	N_{leach} flux 11.0	E2, deep peat (unburnt <i>Calluna</i>)	445
	NH_y deposition 15.0	% TIN retention 64.2		
	TIN deposition 30.7			

plot, a 0.5×0.5 -m quadrat was defined at the centre of each plot for destructive sampling of soils and vegetation. Current $\delta^{15}\text{N}$ values (natural abundance) in soil and vegetation compartments were assessed at three replicated quadrats close to the tracer experiment plots. These were destructively sampled prior to the additions experiment, using the same methods for sampling and analysis as those described for post- ^{15}N additions samples below.

CALCULATION OF ^{15}N TRACER DOSAGE AND APPLICATION METHOD

A very small dosage of labelled $^{15}\text{NH}_4^{15}\text{NO}_3$ solution (30 atom% ^{15}N), equivalent to *c.* $1.5 \text{ kg N ha}^{-1} \text{ year}^{-1}$, was added to minimize the effect of N enrichment. This dosage compares with current deposition inputs of $6.4\text{--}30.7 \text{ kg N ha}^{-1} \text{ year}^{-1}$ for the four study catchments (Table 1). As a significant wetting effect could confound the results, the volume of solution applied was also minimized. The ^{15}N dose was applied fortnightly throughout the year (26 applications). Each dose comprised 1 L of $^{15}\text{NH}_4^{15}\text{NO}_3$ solution at a concentration of 49.81 mg L^{-1} finely sprinkled evenly across the plot, followed by 0.5 L of de-ionized water to help rinse in the labelled N. Rinsing 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 was equivalent to 13 mm of rainfall. The total addition of ^{15}N to the quadrat was

$12.05 \text{ mg } ^{15}\text{N}$. This figure excludes the contribution of 'natural abundance' ^{15}N in deposition, which was estimated assuming a natural abundance value of $\delta^{15}\text{N} = 0\%$ in TIN (Table 1). Published values for $\delta^{15}\text{N}$ of ammonium and NO_3^- in precipitation fall within the range -20 to $+10\%$ (Peterson & Fry 1987). Given the deposition fluxes of TIN at the four sites, the maximum error in calculated recovery of ^{15}N associated with this assumption would be only 0.4% (at the River Etherow) if the actual $\delta^{15}\text{N}$ value for deposition was $\pm 20\%$.

SAMPLING METHODS

At the end of the experimental year, quadrats were destructively sampled for soil and vegetation compartments.

Within each quadrat, above-ground vegetation was removed at the soil surface using pruning shears. The wet weight of the total bulk sample was measured, and a random subsample of around 25% was taken for sorting and analysis. For all sites, heather (*Calluna* and *Erica* spp.) samples were sorted into green 'year's growth' shoots (removed with scissors) and dead/woody material. Other vegetation was sorted into remaining shrubs (e.g. *Vaccinium* spp.), 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 h, and dry weight was recorded. Large, dried samples of vegetation were coarsely ground using a Fritsch mill fitted with a 2-mm sieve. The vegetation samples were then subsampled

and a further 1–2 g were finely ground using a liquid N-cooled freezer mill (SPEX model 6700, Glen Creston Ltd, Middlesex, UK).

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

Gouge augers (5-cm diameter) were used to obtain soil cores from beneath the turf samples down to the bottom of the upper organic horizon (generally 15–20-cm depth). Soil samples were dried at 40 °C for at least 48 h. Organic soils were coarse- and freezer-milled as for the vegetation and turf samples, but if containing stones they were first coarse milled through a 2-mm sieve (rather than using the Fritsch mill) to remove larger stony fragments. Loss-on-ignition (LOI) at 375 °C was used to estimate bulk density according to the equations of Harrison & Bocoek (1981).

Samples were sent to the NERC Stable Isotope Research Facility at CEH Merlewood (Grange-Over-Sands, UK) 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 $\delta^{15}\text{N}$ were determined using a system comprising a Carlo-Erba NA1500 elemental analyser coupled to a modified Dennis Leigh Technology Isotope Ratio Mass Spectrometer (Provac Services, Crewe, UK).

CALCULATION OF ECOSYSTEM COMPARTMENT N POOLS

In order to determine the total mass of ^{15}N retained per unit area, N pool sizes were quantified within each of the sampled compartments.

Dry weights were multiplied up from each subsampled vegetation compartment 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 $\delta^{15}\text{N}$ figures were used in each plot so that standard deviations in these variables could be calculated for each soil type (Tietema *et al.* 1998).

The turf layer and root samples were treated in the same way as the vegetation samples for the calculation

of N pool sizes. As 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.

Organic horizon thickness, bulk density and %N were used to calculate soil compartment N pool sizes. Mean bulk density (g cm^{-3}) values for the three corresponding replicated soil samples from the natural abundance study (prior to ^{15}N addition) were assumed to be representative for each of the post- ^{15}N addition samples. Horizon thickness for each individual sample was measured when sampling and assumed to be representative for the quadrat.

CALCULATION OF ^{15}N TRACER RECOVERY IN SOIL AND VEGETATION COMPARTMENTS

Atom% ^{15}N values were used to calculate tracer recovery (Hauck & Bremner 1976; Nadelhoffer & Fry 1994):

$$m_{lab} = m_i(A_f - A_i)/(A_{lab} - A_f) \quad \text{eqn 1}$$

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

In some cases, post- ^{15}N addition samples were obtained for compartments for which no natural abundance data were available, but assumptions could be made about the natural abundance of ^{15}N with very small associated errors in relative terms for the calculation of tracer recovery. 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 (see the Appendix). Where natural abundance data were unavailable it was therefore decided to use the mean $\delta^{15}\text{N}$ of other samples of the same compartment from that site or, if none was 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%.

As tracer additions and (estimated) deposition inputs of ^{15}N to the sampled quadrat were known, the mass of ^{15}N retained in each sampled compartment for each plot could be expressed as a percentage of inputs. This was assumed to be the same fate as that of deposition inputs over the experimental year.

STATISTICAL ANALYSES

Individual replicates were combined to create a mean value for each soil type within each individual catchment, resulting in four values for the Allt a'Mharcaidh and Afon Gwy, three for Scoat Tarn and two for the River Etherow. Site differences were then tested using analysis of variance (ANOVA) in a general linear model for ^{15}N recovery in each vegetation compartment and soil horizon. Significance was set at the 5% level and,

where significance was observed, between-site comparisons were carried out using a Tukey's test. All statistical analyses were carried out using MINITAB v.14 (Minitab Inc., State College, PA). Arc sine transformations of percentage data were carried out prior to statistical analyses. A regression approach was used to determine relationships between percentage recovery or biomass and N deposition, and residuals checked for normality.

Results

The biomass data in the Appendix show that there were large differences between sites, both in terms of the dominant vegetation types and in overall biomass per unit area. Recovery of ^{15}N inputs in total vegetation (i.e. the sum of green heather, woody heather, moss plus lichen, live grass, dead grass and root compartments), litter plus surface organic layer and upper organic soil compartments is illustrated in Fig. 2, where mean values (± 1 SD) for each of the 13 soil types across the four catchments are plotted. There is a general decline in overall ^{15}N recovery from left to right, along the gradient of increasing N deposition between sites, which appears to be primarily the result of a decline in recovery in total vegetation. For two sets of samples (M1, M4), the overall mean recovery exceeded 100%, but by less than 1 SD of the mean. For the G2 plots at the Afon Gwy, the mean recovery exceeded 100% by more than 1 SD, but one of the three replicated vegetation samples was lost.

To explore the relationships between recovery in each of the vegetation compartments and the site characteristics at each plot, an exploratory principal components analysis (PCA) was undertaken using CANOCO version 4.5 (ter Braak & Smilauer 2002). The PCA showed that total recovery, represented by the primary axis of variation, was highly correlated with recovery in moss plus lichen but was uncorrelated with any of the

other vegetation compartments, suggesting that recovery in moss plus lichen is the main driver of differences in total recovery. The secondary axis showed an inverse correlation between N retention in the surface organic and litter compartments.

The soil types shown in Table 1 were aggregated into mineral and peat soils and this classification was superimposed onto the PCA biplot (results not shown), revealing that the four plots with the highest recovery were all mineral soils whereas the plots with the lowest recovery were all on peat soils. There was, however, considerable overlap between the two classes in terms of differentiating between high and low recovery levels.

Regression analysis indicated that biomass was a significant and effective predictor of recovery for moss plus lichen, live grass and green shrub components, but for woody shrub components the relationship was much weaker and not significant (Fig. 3). The slopes of the regression lines indicated that the moss plus lichen compartment was the most efficient at retaining ^{15}N per unit biomass.

When retention efficiency per gram dry weight of compartment biomass was plotted for selected compartments averaged by site, between-site differences in retention efficiency independent of biomass were apparent (Fig. 4). In moss plus lichen, live grass and green shrub compartments there was an apparent increase in retention efficiency per unit mass of vegetation along the gradient of increasing deposition, but then a sharp decline in the River Etherow samples; ANOVA indicated that differences between sites were not significant. For litter a rather different pattern was observed, with significant differences between sites and a significant decline in retention efficiency as deposition increased (Fig. 4d). Recovery per unit mass of litter, presumably a result of N immobilization, was more than twice as high at the Allt a'Mharcaidh than at the River Etherow.

There was no obvious pattern in ^{15}N recovery within the surface organic layer compartment across the

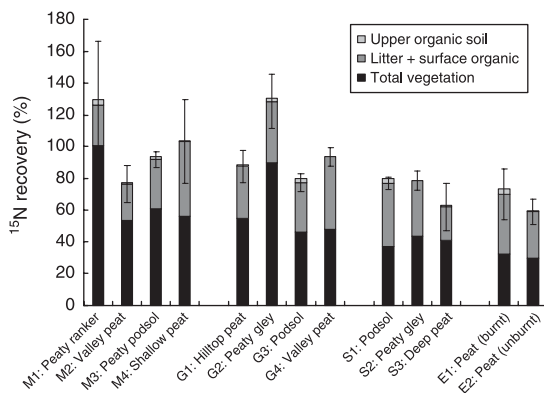


Fig. 2. Relative importance of total vegetation, litter plus surface organic layer and upper organic soil N sinks, measured as percentage retention of ^{15}N inputs (tracer plus deposition). Vertical bars represent ± 1 SD of the mean total value for all summed sinks. Sites are ordered by increasing modelled total N deposition. Soils within sites are ordered by decreasing altitude.

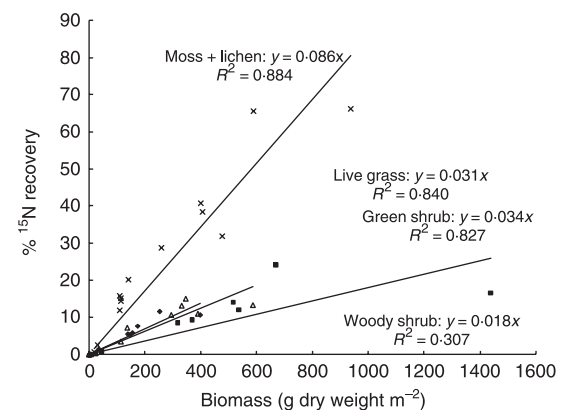


Fig. 3. Recovery of ^{15}N additions within vegetation compartments (%) against compartment biomass. Diamonds, green shrub ($n = 8$); squares, woody shrub ($n = 7$); crosses, moss plus lichen ($n = 13$); triangles, live grass ($n = 12$). All regressions are significant at the $P < 0.01$ level except woody shrub.

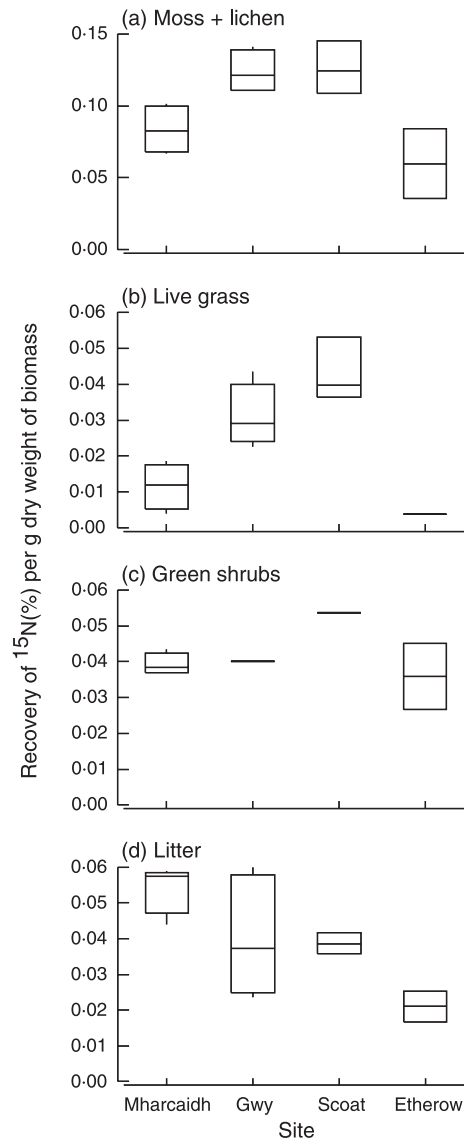


Fig. 4. Retention efficiency of selected vegetation and litter compartments expressed as recovery of ¹⁵N additions (%) per g dry weight compartment biomass. Recovery per unit biomass is plotted against sites ordered by increasing total modelled N deposition. ANOVA indicates no significant difference between sites except for litter ($F = 15.2$, $P = 0.030$). Regression analysis indicates a significant relationship between retention efficiency and deposition (site) only for litter ($R^2 = 90.2$, $P = 0.033$).

deposition gradient represented by the four sites. The variability in mass (see the Appendix) illustrated the variability in soil type. Recovery in the surface organic layer appeared to be more related to recovery in the overlying litter, with a significant inverse linear relationship (Fig. 5). The data suggest that, as retention decreases in the litter layer, there is more scope for leaching of inorganic N into the underlying surface organic layer, where the recovery of ¹⁵N increases accordingly. However, recovery in the combined litter and surface organic layers showed no clear pattern between soils or sites (Fig. 2).

The data suggest that there is a small degree of ¹⁵N recovery in the upper organic soil, generally in the range

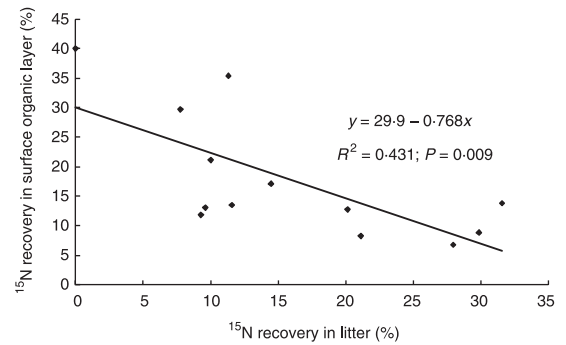


Fig. 5. Relationship between recovery of ¹⁵N inputs in surface organic layer and recovery in litter. Each point represents the mean value of up to three replicated samples per soil type. Regression analysis showed that recovery in the overlying litter layer was a weak but significant predictor of recovery in the surface organic layer.

0–4% of inputs (see the Appendix; Fig. 2), but they must be interpreted with caution. When the $\delta^{15}\text{N}$ values following tracer additions were compared with natural abundance values prior to the experiment (see the Appendix), there was no significant difference in $\delta^{15}\text{N}$ except for soil M1 at the Allt a’Mharcaidh. It must therefore be assumed that while some minor degree of ¹⁵N tracer recovery is likely at these sites, the changes are so small as to be well within the potential measurement errors associated with soil pools. For this reason, the following considerations of total recovery of ¹⁵N exclude the very small proportion in soils below the surface organic and litter layer.

EFFECT OF SOIL TYPE

The combined litter and surface organic layer sinks retain from around a quarter to more than a half of ¹⁵N 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. Comparing recovery rates in the different soils, there were no consistent differences between recovery in surface organic and litter layers overlaying organic and mineral soils or soils in the upper and lower reaches of the catchments.

Given the high recovery of ¹⁵N in vegetation and the strong relationships with biomass, between-site differences in both biomass and ¹⁵N recovery were compared for moss plus lichen, higher plants, litter plus surface organic layer and total (excluding soil) compartments using both ANOVA and regression techniques. Analysis of variance showed that between-site differences in biomass (Fig. 6a) were significant ($P < 0.05$) for all compartments except higher plants ($P = 0.055$). For ¹⁵N recovery (Fig. 6b) the only significant differences were for moss plus lichen ($F = 6.73$, $P = 0.011$), although for total vegetation differences were almost significant ($F = 3.61$, $P = 0.059$, $n = 4$; data not shown).

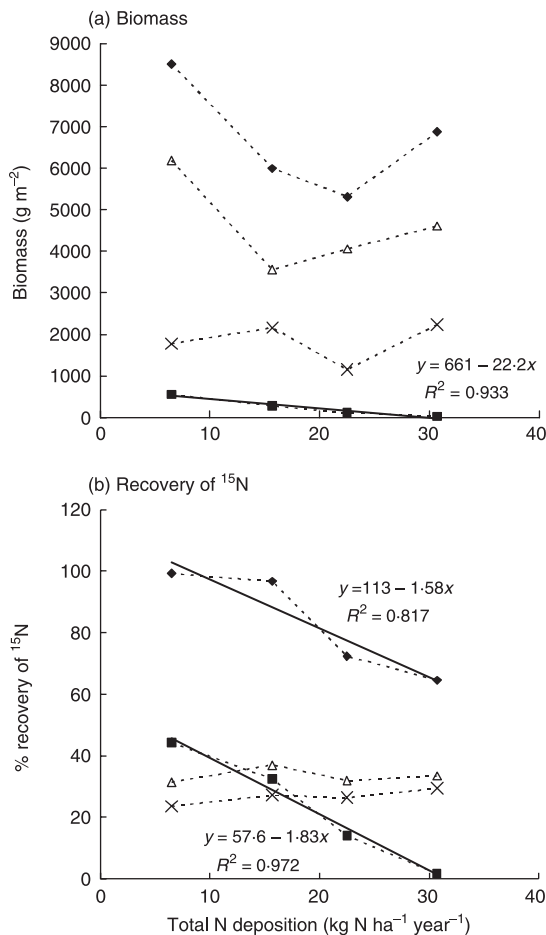


Fig. 6. Relationship between (a) compartment biomass and (b) recovery of ¹⁵N inputs with total N deposition (i.e. by site). Figures for each site are averaged across sampled soil types (arithmetic mean for two to four soils of three replicated samples with no spatial weighting by areal soil coverage; $n = 4$ sites). Diamonds, total for all compartments excluding soil; squares, moss plus lichen; crosses, higher plants; triangles, litter plus surface organic layer.

Regression analysis revealed significant relationships between biomass of moss plus lichen and total N deposition ($R^2 = 0.933$, $P = 0.023$), while the biomass of other compartments was not related to deposition. Regressions of ¹⁵N recovery and total N deposition were significant for moss plus lichen ($R^2 = 0.972$, $P = 0.009$) and total vegetation (data not shown; $R^2 = 0.933$, $P = 0.023$, $n = 4$). While deposition appeared to be a reasonably good predictor of total ¹⁵N recovery (soils excluded; $R^2 = 0.817$), the relationship was not quite significant ($P = 0.063$). The data did, however, support the suggestion in the exploratory PCA that recovery in moss plus lichen was the major driver of differences in total recovery.

Total recovery also appeared to be a good predictor of the proportion of TIN deposition that was lost by leaching into surface waters (Fig. 7) but the regression was not quite significant ($P = 0.051$). However, ¹⁵N recovery in moss plus lichen was a much better predictor of TIN leaching and the regression was significant ($R^2 = 0.971$, $P = 0.010$).

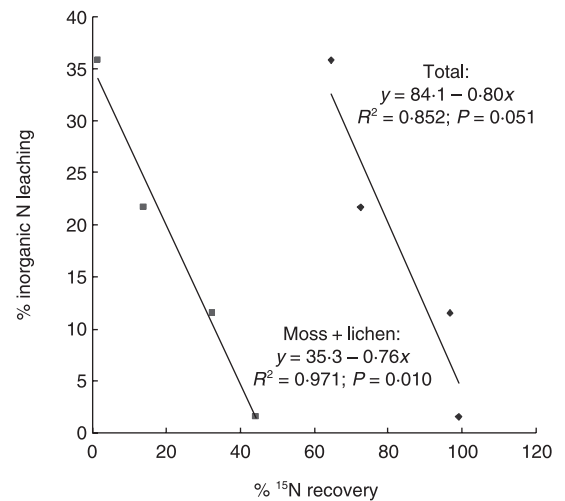


Fig. 7. Relationship between percentage inorganic N leaching (from input–output measurements), total recovery of ¹⁵N inputs (% excluding soils) and recovery in moss plus lichen. Diamonds, total for all compartments excluding soil; squares, moss plus lichen. Each point represents the mean value of two to four soils with up to three replicated samples per soil type.

Discussion

FATE OF ¹⁵N ADDITIONS AND INORGANIC N DEPOSITION

A very large proportion of the ¹⁵N tracer applied to the experimental plots was recovered after 1 year of additions and, by interpolation, most deposition inputs of inorganic N were retained within the soil–plant system over this time scale. Overall, between 65% and 99% of the applied ¹⁵N was recovered from the upper soil–vegetation system, indicating that most N deposition is intercepted in this part of the system and enters the terrestrial N cycle. Thus, up to 35% of applied ¹⁵N was available for immediate leaching as inorganic N (Table 2). Very little of the applied ¹⁵N was recovered in the soils below the surface organic layer (i.e. below 5-cm depth).

VEGETATION UPTAKE OF INORGANIC N

Uptake of ¹⁵N by most vegetation compartments was positively related to vegetation biomass (Fig. 3), while the efficiency of vegetation per unit biomass at retaining N appeared to increase with N deposition up to a threshold, beyond which it declined sharply (e.g. at the River Etherow site; Fig. 4).

Mosses and lichens were most efficient at retaining incoming N (Carroll *et al.* 2000; Heijmans *et al.* 2002). Lee, Baxter & Emes (1990) described ombrotrophic *Sphagnum* species as an almost perfect sink for atmospheric N inputs under unpolluted conditions. However, the ability of bryophytes to filter out N deposition inputs is impaired at high loads as tissue N content reaches a critical level (Carroll *et al.* 2000). Nordbakken, Ohlson & Högborg (2003) described the failure of the

Table 2. Fraction of unrecovered ^{15}N inputs (% SE) assumed to have been leached into surface waters, and leaching of inorganic N as a percentage of deposition inputs for the four study catchments. Total values excluding soils are shown because of non-significant difference in $\delta^{15}\text{N}$ between pre- and post-addition subsurface soil samples

Site	% unrecovered ^{15}N tracer		
	Total (all sinks)	Total excluding soils	% leaching of N deposition
Allt a'Mharcaidh	0 (14.4)	0.8 (10.4)	1.6
Afon Gwy	1.9 (11.3)	3.3 (11.2)	11.5
Scoat Tarn	26.2 (5.4)	27.6 (5.3)	21.7
River Etherow	33.7 (6.8)	35.5 (5.5)	35.8

'living *Sphagnum* filter' in boreal and ombrotrophic bogs as TIN deposition exceeded 18–20 kg N ha⁻¹ year⁻¹. Work in acid moorland systems suggests that deposition above 10–20 kg N ha⁻¹ year⁻¹ may adversely affect some mosses (Jones, Oxley & Ashenden 2002), with excessive N inputs causing a decline in the cover and stem density of bryophytes (Carroll *et al.* 2000). N deposition may also increase the height and dominance of shrubs, with reduced light penetration excluding plants in the ground layer (Lee & Caporn 1998). Declines in montane *Racomitrium* heaths and lichens have also been attributed to N deposition (Bobbink, Hornung & Roelofs 1998), while Woolgrove & Woodin (1996) suggested that N-induced damage to snowbed bryophyte cover could lead to an increase in the extent of bare ground.

Data from the present study also strongly suggest a decline in cover of moss plus lichen in response to elevated N deposition, which may be largely responsible for an associated increase in the proportion of inorganic N leached (Figs 6b and 7). However, the relationship is not straightforward, as the efficiency of moss plus lichen per unit biomass in retaining N initially increases even though biomass declines (Fig. 4). As deposition increases, moss plus lichen biomass falls more sharply than the initial increase in retention efficiency. As the biomass decline slows, retention efficiency peaks and then declines very steeply (Fig. 8).

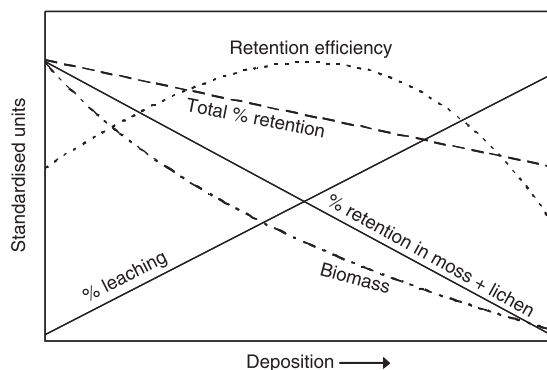


Fig. 8. Change in moss plus lichen properties with increasing deposition. Curves are fitted to actual data points. Units are standardized to percentage of the maximum value. Note that the total percentage retention declines to a much smaller degree than retention in moss plus lichen because of the proportional scale.

The net effect is a large, linear decrease in the proportion of inputs retained by moss plus lichen, in this study from 44% to 2% of inputs. In absolute terms this decrease is very closely matched by a decrease in total retention from 99% to 65% (Fig. 7) but in relative terms the reduction in total retention is only around one-third of that in moss plus lichen (Fig. 8). The increase in the proportion of TIN inputs leached almost exactly mirrors the decrease in retention in moss plus lichen. Even after retention by moss plus lichen has decreased almost to zero, total retention is still around two-thirds of inputs. Other compartments (higher plants, litter, surface organic layer and ultimately soils) are still able to retain a high proportion of TIN inputs even after the near-disappearance of mosses and lichens.

N IMMOBILIZATION IN LITTER AND SOILS

Short-term immobilization of N (corresponding to retention in the upper organic soil plus litter and surface organic compartments in Fig. 2) accounts for the recovery of around 25–50% of N inputs across the four study catchments, with the great majority of this N immobilized in the litter and underlying surface organic layer. Mechanisms may include both biotic and abiotic processes (Aber 1992; Ågren & Bosatta 1996; Högberg 1997; Gundersen 1998).

The proportion of ^{15}N recovered in the combined litter and surface organic layers is relatively constant, suggesting increased absolute retention of deposition, while the relative importance of immobilization increases as moss plus lichen uptake declines (see the Appendix). However, consideration of just the litter layer, when values on all soil types are averaged across each catchment, reveals an apparent decline in immobilization efficiency with increasing deposition (Fig. 4). This may be the result of progressive saturation of the N sinks within the soil layers.

ROLE OF ORGANIC N

This study did not consider the potential role of organic N in total N budgets for these moorland catchments. No data were available for the contribution of organic N to total N deposition, although it may comprise a large proportion of both deposition inputs and leaching outputs in some systems (Michalzik *et al.*

2001; Neff *et al.* 2002; Perakis & Hedin 2002; Phoenix *et al.* 2003). While organic N deposition will contribute to the total N pool from which microbial nitrate may ultimately be generated, there is little published evidence for persistent enhanced organic N leaching in response to N deposition in these soils (Emmett *et al.* 1998). Furthermore, while organic N leaching makes up a significant proportion of total N exports at some of these sites (Curtis 2003), this study focused on inorganic N and its potential contribution to acidification and eutrophication of surface waters.

ROLE OF PHOSPHORUS

This study was based on the assumption that, at least prior to N saturation, terrestrial moorland ecosystems are generally N limited rather than phosphorus (P) limited. However, in some peats and grassland (both acid and calcareous) soils, P limitation has been described and may be enhanced by anthropogenic N deposition, potentially providing a competitive advantage to plants able to use recalcitrant organic forms of P (Turner, Baxter & Whitton 2002; Turner *et al.* 2003).

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 (Fig. 2), as not all possible sinks for N were sampled. Gaseous losses of ^{15}N via denitrification were not measured, but are thought to be relatively insignificant in the four study catchments (Curtis 2003). Recovery in deeper soils could not be quantified; the very low rates of ^{15}N recovery in most soils could be explained by the interception of inputs by vegetation uptake and microbial immobilization in the surface organic layer (cf. Kristensen & McCarty 1999). For those sites where these components of the system retain almost 100% of inputs, it is not known what the potential for immobilization may be lower down the soil profile, as very little of the tracer penetrated this far. However, even at the River Etherow site, where around one-third of ^{15}N inputs were unrecovered from the vegetation, litter and surface organic layer, no changes in $\delta^{15}\text{N}$ in deeper soils were detected. This evidence, taken together with the strong inverse relationship between TIN leaching as a proportion of inputs and total ^{15}N recovery (particularly in moss plus lichen), indicates that the most likely fate of the missing ^{15}N is in drainage waters. Considering the uncertainties in input–output flux measurements and ^{15}N recovery methods, these relationships are surprisingly strong (Fig. 7).

IMPLICATIONS FOR CONSERVATION AND MANAGEMENT OF UPLAND ECOSYSTEMS

The short-term recovery of such large proportions of inorganic N inputs in vegetation and the active surface

organic layer indicate changes in terrestrial ecosystem structure and function in response to elevated N deposition, in particular the loss of moss and lichen cover. This has implications for the conservation of species that are adapted to low inorganic N availability. Furthermore, effects on aquatic ecosystems must to a large degree be mediated by processes in the terrestrial catchment ecosystems, and direct uptake of atmospheric N by mosses and lichens is particularly important in this regard. Options for the management of vegetation to maintain an efficient sink for incoming N appear to be severely limited, as the mosses and lichens that are the most efficient sink for N are highly sensitive to atmospheric pollutants and may be one of the first ecosystem components to be damaged. Site protection may therefore require action at much larger regional, national or even global scales, with specifically targeted policies for pollutant emission controls. Plot-scale studies such as this one provide process and effect information for the scientists and policymakers responsible for the design and implementation of such controls (Dalton & Brand-Hardy 2003).

Biological models of terrestrial N retention are required if the impacts of N in both terrestrial and freshwater systems are to be predicted. The policy-orientated models used to determine critical loads for N and acidity must continue to take account of terrestrial retention processes, but greater understanding of the links between short-term and long-term (steady-state) rates of vegetation uptake and microbial immobilization are required. The importance of vegetation uptake in mediating nitrate leaching may help to explain why models focusing only on soil profile controls on N leaching cannot reproduce observed leaching patterns. The soil carbon : nitrogen ratio may control leaching through the soil profile over the very long-term (Curtis *et al.* 2004), but effects as a result of vegetation responses to N deposition may be observed much earlier.

CONCLUSIONS

This study provides evidence that the efficiency of retention of incoming N within moorland ecosystems declines across an N deposition gradient, suggesting that N saturation of these ecosystems is in progress. Furthermore, changes in the relative importance of terrestrial sinks from vegetation to soil, apparently related to a loss of the moss and lichen component, and reduced efficiency of N retention by vegetation in polluted sites, indicates a change in the internal N cycle. Ultimately, reductions in N emissions remain the most effective solution to minimizing impacts in the terrestrial ecosystem.

Acknowledgements

This work was jointly funded by National Power and the DETR contract 'Acidification of freshwaters: the role of nitrogen and the prospects for recovery' (EPG1/

3/117). Many colleagues from CEH Bangor, the ECRC at University College London, MLURI, Aberdeen and the Freshwater Fisheries Laboratory, Pitlochry, contributed to the collection of N budget data. Special thanks are due to staff at the Stable Isotope Research Facility at CEH Merlewood, James Shilland at the ECRC, and Jo Porter. Deposition data were provided by CEH Edinburgh. Anonymous referees are thanked for suggested improvements to the original manuscript.

Supplementary material

The following supplementary material is available for this article online.

Appendix S1. Mean biomass, $\delta^{15}\text{N}$ pre- and post-additions, %N (post-addition) and retention of ^{15}N inputs (%) in vegetation and soil compartments over 1 year of additions.

References

- Aber, J.D. (1992) Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. *Trends in Ecology and Evolution*, **7**, 220–224.
- Ågren, G.I. & Bosatta, E. (1996) *Theoretical Ecosystem Ecology: Understanding Element Cycles*. Cambridge University Press, Cambridge, UK.
- Allott, T.E.H., Curtis, C.J., Hall, J., Harriman, R. & Battarbee, R.W. (1995) The impact of nitrogen deposition on upland freshwaters in Great Britain: a regional assessment. *Water, Air and Soil Pollution*, **85**, 297–302.
- Bobbink, R. (1998) Impacts of tropospheric ozone and airborne nitrogenous pollutants on natural and semi-natural ecosystems: a commentary. *New Phytologist*, **139**, 161–168.
- Bobbink, R., Hornung, M. & Roelofs, J.G.M. (1998) The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, **86**, 717–738.
- ter Braak, C.J.F. & Smilauer, P. (2002) *CANOCO Reference Manual and CANODRAW for Windows User's guide: Software for Canonical Community Ordination Version 4.5*. Microcomputer Power, Ithaca, NY.
- Burns, D.A. (2004) The effects of atmospheric nitrogen deposition in the Rocky Mountains of Colorado and Southern Wyoming, USA: a critical review. *Environmental Pollution*, **127**, 257–269.
- Carroll, J.A., Johnson, D., Morecroft, M., Taylor, A., Caporn, S.J.M. & Lee, J.A. (2000) The effect of long-term nitrogen additions on the bryophyte cover of upland acidic grasslands. *Journal of Bryology*, **22**, 83–89.
- CLAG Deposition Fluxes (1997) *Deposition Fluxes of Acidifying Compounds in the United Kingdom*. Critical Loads Advisory Group, Subgroup Report on Deposition Fluxes. ITE, Penicuik, UK.
- Cosby, B.J., Ferrier, R.C., Jenkins, A., Emmett, B.A., Wright, R.F. & Tietema, A. (1997) Modelling the ecosystem effects of nitrogen deposition. Model of ecosystem retention and loss of inorganic nitrogen (MERLIN). *Hydrology and Earth System Sciences*, **1**, 137–158.
- Cosby, B.J., Ferrier, R.C., Jenkins, A. & Wright, R.F. (2001) Modelling the effects of acid deposition: refinements, adjustments and inclusion of nitrogen dynamics in the MAGIC model. *Hydrology and Earth System Sciences*, **5**, 499–517.
- Curtis, C.J. (2003) *An assessment of the representation of moorland nitrogen sinks in static critical load models for freshwater acidity*. PhD Thesis. University College London, London, UK.
- Curtis, C. & Simpson, G. (2001) *Summary of Research under DETR Contract 'Acidification of Freshwaters. The Role of Nitrogen and the Prospects for Recovery'*, EPG113/117. ECRC Research Report No. 79. Environmental Change Research Centre. University College London, London, UK.
- Curtis, C.J., Emmett, B.A., Reynolds, B. & Shilland, J. (2004) Nitrate leaching from moorland soils: can soil C/N ratios indicate N saturation? *Water, Air and Soil Pollution: Focus*, **4**, 359–369.
- Curtis, C.J., Evans, C., Helliwell, R.C. & Monteith, D. (in press) Nitrate leaching as a confounding factor in chemical recovery from acidification in UK upland waters. *Environmental Pollution*, in press.
- Dalton, H. & Brand-Hardy, R. (2003) Nitrogen: the essential public enemy. *Journal of Applied Ecology*, **40**, 771–781.
- Emmett, B.A., Reynolds, B., Silgram, M., Sparks, T.H. & Woods, C. (1998) The consequences of chronic nitrogen additions on N cycling and soil water chemistry in a Sitka spruce stand, North Wales. *Forest Ecology and Management*, **101**, 165–175.
- Gundersen, P. (1998) Effects of enhanced nitrogen deposition in a spruce forest at Klosterhede, Denmark, examined by moderate NH_4NO_3 addition. *Forest Ecology and Management*, **101**, 251–268.
- Harrison, A.F. & Bockock, K.L. (1981) Estimation of soil bulk density from loss-on-ignition values. *Journal of Applied Ecology*, **8**, 919–927.
- Hauck, R.D. & Bremner, J.M. (1976) Use of tracers for soil and fertilizer nitrogen research. *Advances in Agronomy*, **28**, 219–266.
- Heijmans, M.M.P.D., Klees, H., de Visser, W. & Berendse, F. (2002) Effects of increased nitrogen deposition on the distribution of ^{15}N -labeled nitrogen between *Sphagnum* and vascular plants. *Ecosystems*, **5**, 500–508.
- Högberg, P. (1997) Tansley review no. 95: ^{15}N natural abundance in soil–plant systems. *New Phytologist*, **137**, 179–203.
- Jones, M.L.M., Oxley, E.R.B. & Ashenden, T.W. (2002) The influence of nitrogen deposition, competition and desiccation on growth and regeneration of *Racomitrium lanuginosum* (Hedw.) Brid. *Environmental Pollution*, **120**, 371–378.
- Kristensen, H.L. & McCarty, G.W. (1999) Mineralization and immobilisation of nitrogen in heath soil under intact *Calluna*, after heather beetle infestation and nitrogen fertilization. *Applied Soil Ecology*, **13**, 187–198.
- Lee, J.A. & Caporn, S.J.M. (1998) Ecological effects of atmospheric reactive nitrogen deposition on semi-natural terrestrial ecosystems. *New Phytologist*, **139**, 127–134.
- Lee, J.A., Baxter, R. & Emes, M.J. (1990) Responses of *Sphagnum* species to atmospheric nitrogen and sulphur deposition. *Botanical Journal of the Linnean Society*, **104**, 255–265.
- Leith, I.D., Sheppard, L.J., Pitcairn, C.E.R., Cape, J.N., Hill, P.W., Kennedy, V.H., Tang, Y.S., Smith, R.I. & Fowler, D. (2001) Comparison of the effects of wet N deposition (NH_4Cl) and dry N deposition (NH_3) on UK moorland species. *Water, Air and Soil Pollution*, **130**, 1043–1048.
- Michalzik, B., Kalbitz, K., Park, J.-H., Solinger, S. & Matzner, E. (2001) Fluxes and concentrations of dissolved organic carbon and nitrogen: a synthesis for temperate forests. *Biogeochemistry*, **52**, 173–205.
- Monteith, D.T. & Evans, C.D. (2000) *UK Acid Waters Monitoring Network: 10 Year Report. Analysis and Interpretation of Results April 1988–March 1998*. ENSIS Publishing, London, UK.

- Nadelhoffer, K.J. & Fry, B. (1994) Nitrogen isotope studies in forest ecosystems. *Stable Isotopes in Ecology and Environmental Science* (eds K. Lajtha & R.H. Michener), pp. 22–44. Blackwell Science, Oxford, UK.
- Neff, J.C., Holland, E.A., Dentener, F.J., McDowell, W.H. & Russell, K.M. (2002) The origin, composition and rates of organic nitrogen deposition: a missing piece of the nitrogen cycle? *Biogeochemistry*, **57/58**, 99–136.
- NEG-TAP (2001) *Transboundary Air Pollution: Acidification, Eutrophication and Ground-Level Ozone in the UK*. CEH Edinburgh, Penicuik, UK.
- Nordbakken, J.F., Ohlson, M. & Högberg, P. (2003) Boreal bog plants: nitrogen sources and uptake of recently deposited nitrogen. *Environmental Pollution*, **126**, 191–200.
- Perakis, S.S. & Hedin, L.O. (2002) Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. *Nature*, **415**, 416–419.
- Peterson, B.J. & Fry, B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecological Systems*, **18**, 293–320.
- Phoenix, G.K., Booth, R.E., Leake, J.R., Read, D.J., Grime, J.P. & Lee, J.A. (2003) Effects of enhanced nitrogen deposition and phosphorus limitation on nitrogen budgets of semi-natural grasslands. *Global Change Biology*, **9**, 1309–1321.
- Pitcairn, C.E.R., Fowler, D. & Grace, J. (1995) Deposition of fixed atmospheric nitrogen and foliar nitrogen content of bryophytes and *Calluna vulgaris*. *Environmental Pollution*, **88**, 193–205.
- Pitcairn, C.E.R., Leith, I.D., Fowler, D., Hargreaves, K.J., Moghaddam, M., Kennedy, V.H. & Grannat, L. (2001) Foliar nitrogen as an indicator of nitrogen deposition and critical loads exceedance on a European scale. *Water, Air and Soil Pollution*, **130**, 1037–1042.
- Posch, M., Kämäri, J., Forsius, M., Henriksen, A. & Wilander, A. (1997) Exceedance of critical loads for lakes in Finland, Norway, and Sweden: reduction requirements for acidifying nitrogen and sulphur deposition. *Environmental Management*, **21**, 291–304.
- Sickman, J.O., Melack, J.M. & Clow, D.W. (2003) Evidence for nutrient enrichment of high-elevation lakes in the Sierra Nevada, California. *Limnology and Oceanography*, **48**, 1885–1892.
- Tietema, A., Emmett, B.A., Gundersen, P., Kjønaas, O.J. & Koopmans, C.J. (1998) The fate of ¹⁵N-labelled nitrogen deposition in coniferous forest ecosystems. *Forest Ecology and Management*, **101**, 19–27.
- Turner, B.L., Baxter, R. & Whitton, B.A. (2002) Seasonal phosphatase activity in three characteristic soils of the English uplands polluted by long-term atmospheric nitrogen deposition. *Environmental Pollution*, **120**, 313–317.
- Turner, B.L., Chudek, J.A., Whitton, B.A. & Baxter, R. (2003) Phosphorus composition of upland soils polluted by long-term atmospheric nitrogen deposition. *Biogeochemistry*, **65**, 259–274.
- Whitehead, P.G., Wilson, E.J. & Butterfield, D. (1998) A semi-distributed integrated nitrogen model for multiple source assessment in catchments (INCA). I. Model structure and process equations. *Science of the Total Environment*, **210/211**, 547–558.
- Woodin, S., Press, M.C. & Lee, J.A. (1985) Nitrate reductase activity in *Sphagnum fuscum* in relation to wet deposition of nitrate from the atmosphere. *New Phytologist*, **99**, 381–388.
- Woolgrove, C.E. & Woodin, S.J. (1996) Current and historical relationships between the tissue nitrogen content of a snowbed bryophyte and nitrogenous air pollution. *Environmental Pollution*, **91**, 283–288.

Received 20 June 2004; final copy received 10 February 2005
Editor: Steve Ormerod