New
Phytologist

# Physiological and growth responses of the montane bryophyte *Racomitrium lanuginosum* to atmospheric nitrogen deposition

I. S. K. Pearce[1,2], S. J. Woodin[2] and R. van der Wal[1]

[1]Centre for Ecology and Hydrology, Hill of Brathens, Banchory, Aberdeenshire AB31 4BW, UK; [2]Department of Biological Sciences, University of Aberdeen, AB24 3UU, UK

Author for correspondence:
*I. S. K. Pearce*
*Tel: +44 1330 826 339*
*Fax: +44 1330 823 303*
*Email: iskp@ceh.ac.uk*

Received: *29 April 2003*
Accepted: *13 June 2003*

doi: 10.1046/j.1469-8137.2003.00875.x

## Summary

- The effects of nitrogen (N) deposition on the moss *Racomitrium lanuginosum* within montane heath in Scotland were investigated over 5 yr.
- Permanent field plots were sprayed with $KNO_3$ or $NH_4Cl$ solutions, at doses equivalent to 10 and 40 kg N $ha^{-1}$ $yr^{-1}$, in 3–6 applications each summer.
- *Racomitrium* growth and cover were severely reduced by N addition, whilst the proportion of dead shoots greatly increased. N dose decreased inducibility of shoot nitrate reductase activity (NRA), suggesting that N saturation of *Racomitrium* occurred, and caused an increase in potassium leakage. At high dosage, effects of $NH_4^+$ were more detrimental than $NO_3^-$.
- Physiological responses to N indicate that the habitat's critical load (CL) is exceeded by addition of 10 kg N $ha^{-1}$ $yr^{-1}$. The differential toxicity of the two forms of N suggests that predominant ion type in deposition should be taken into consideration when CLs are set. In contrast to tissue N, NRA correlated well with shoot growth, and may thus be a useful biological indicator of moss condition.

**Key words:** *Racomitrium lanuginosum*, nitrogen deposition, critical load, montane heath, potassium leakage, nitrate reductase activity, ammonium, nitrate.

## Introduction

High latitude montane ecosystems are traditionally nitrogen (N) limited with low rates of elemental cycling and N deposition. However, rates of atmospheric N deposition over western Europe have increased dramatically in recent decades (United Kingdom Review Group on Acid Rain, 1997). Increasing anthropogenic activity, particularly fossil fuel combustion and intensive agriculture, has greatly increased gaseous emissions of N and over large areas of Europe deposition rates now range to an upper level of over 60 kg N $ha^{-1}$ $yr^{-1}$ (Pitcairn *et al.*, 1995). Although areas remote from urban and intensive agricultural pollution sources generally have N deposition rates towards the lower end of the scale (National Expert Group on Transboundary Air Pollution, 2001), montane vegetation can be exposed to higher atmospheric deposition than surrounding lowland sites because of increased precipitation with altitude (United Kingdom Review Group on Acid Rain, 1997). Pollutant concentration of this wet deposition can also be enhanced at high altitude firstly because of the 'seeder-feeder effect' of precipitation, in which cloud water from orographic cloud is washed out by rainfall from higher level cloud, and secondly by long periods of orographic cloud cover causing occult deposition, typically containing 2–5 times the pollutant concentrations of rain (Grace & Unsworth, 1988). Therefore montane areas are potentially at a greater risk of severe and episodic pollution events than surrounding lowlands.

There is a growing body of evidence that increasing levels of atmospheric N deposition have negative impacts on semi-natural habitats (Woodin & Farmer, 1993; Bobbink *et al.*, 1998; Lee, 1998), especially in upland and arctic areas (Carroll *et al.*, 1999; Gordon *et al.*, 2001). These habitats are typically nutrient-poor, with low soil N mineralisation rates, and long-term increases in atmospheric N deposition can result in eutrophication of the ecosystem. This is likely to cause changes in community structure and composition, with the loss of species sensitive to increases in N supply (Lee *et al.*, 1987; Potter *et al.*, 1995; Press *et al.*, 1998) and replacement by more N tolerant or competitive species (Jonasson, 1992; Bobbink *et al.*, 1998; Robinson *et al.*, 1998; Jonasson *et al.*, 1999).

Long-term N addition studies of upland heath have demonstrated increased grass growth (Alonso & Hartley, 1998) coupled with increased sensitivity of *Calluna vulgaris* to winter injury (Lee & Caporn, 1998). However, bryophytes often demonstrate the most rapid negative response to experimental N addition, sometimes being lost completely from communities (Lee & Caporn, 1998; Carroll *et al.*, 1999; Gordon *et al.*, 2001).

Most bryophyte species have no root systems and relatively few species have well-developed rhizoids. They acquire nutrients directly from the atmosphere, making them particularly vulnerable to atmospheric pollution (Bates, 2000). Few experiments have addressed the effect realistic doses of N addition have on physiological responses of montane or upland bryophyte species. Soares & Pearson (1995) found increases in tissue N content, and a reduction in inducible activity of the N assimilating enzyme nitrate reductase, in *Racomitrium lanuginosum* subjected to short-term field misting with 3 mol $NH_4^+$ $m^{-3}$. An 'acid flush' of nitrate and sulphate in snowmelt after prolonged snowlie was observed to cause physiological damage to another montane bryophyte species, *Kiaeria starkei* (Woolgrove & Woodin, 1996c). Whilst indicating high sensitivity to brief pollution events, neither of these studies investigated long-term effects. Habitats such as arctic tundra or montane heath, in which mosses often dominate, are likely to be highly prone to degradation because of the impacts of N pollution. The increase in both the concentration and total deposition of N pollutants with altitude may therefore pose significant harm to montane bryophytes, creating potential for long-term ecological change.

Montane *Racomitrium* heath, dominated by the ectohydric moss *Racomitrium lanuginosum*, is the most extensive near-natural terrestrial community in the UK (Thompson & Baddeley, 1991). However, its cover has been declining in recent decades and in upland areas of England and Wales it has now been replaced by grass dominated communities (Thompson *et al.*, 1987; Ratcliffe & Thompson, 1988). As it cannot directly regulate its nutrient uptake, *Racomitrium* is particularly sensitive to changes in N deposition, and its tissue N content can reflect amounts deposited to it from the atmosphere (Baddeley *et al.*, 1994; Pitcairn *et al.*, 1995). Montane *Racomitrium* heath therefore provides an ideal model system for investigating the effect of N pollution on sensitive, moss-dominated communities.

Studies manipulating doses of N addition on *Racomitrium* have demonstrated a deleterious effect on the moss' survival when exposed to deposition levels equivalent to 20 kg N $ha^{-1}$ $yr^{-1}$ or above (Jones *et al.*, 2002; Pearce & van der Wal, 2002; van der Wal *et al.*, 2003). As deposition estimates for many montane areas in the UK exceed this rate, we have cause for concern that current N pollution is actually damaging sensitive montane vegetation. And indeed, past increases in N deposition have been correlated with deterioration and loss of *Racomitrium* heath across the UK (Thompson & Baddeley, 1991; Bunce *et al.*, 1999).

This paper reports effects of 5 yr of N addition to *Racomitrium* in the field, demonstrating the longer term outcome of initial observations (2-yr effects are reported in Pearce & van der Wal (2002)), and making the first contribution to an explanation of physiological mechanisms responsible for the observed loss of *Racomitrium* within montane heath. Background rates of wet N deposition at the site were estimated. The influence of both total N load and ion type (oxidised and reduced forms of N) on *Racomitrium* performance are examined using cover and growth measures, as well as underlying physiological changes in the moss. The implications for estimation of the critical load of N for montane heath are discussed. Results also enable us to suggest an effective biological indicator for monitoring occurrence of nitrogenous pollutant damage to *Racomitrium.*

## Materials and Methods

### Site description

The study site, located on the summit (1068 m above sea level (asl) of Glas Maol in the south-eastern part of the Grampian Mountains (56°53′-N, 3°22′-W) in eastern Scotland, was established in 1998. It falls within the Caenlochan Site of Special Scientific Interest (SSSI) that supports a number of rare arctic-alpine communities predominantly found within base-rich areas and scree slopes. Permanent plots (0.6 m × 0.6 m) were located at two sites on the summit, *c.* 200 m apart, in *Carex bigelowii – Racomitrium lanuginosum* (Hedw.) Brid. montane heath (National Vegetation Classification U10a (Rodwell, 1992)). The *Racomitrium* forms extensive mats up to 10 cm thick and, in addition to *C. bigelowii*, can be accompanied by other graminoids, in particular *Agrostis capillaris*, *Deschampsia flexuosa*, *Festuca ovina* and *Festuca vivipara.* The most recently revised estimate for total N deposition for the Glas Maol area, corrected for altitudinal effects and occult deposition, is approximately 18 kg N $ha^{-1}$ $yr^{-1}$ (M. Sutton, pers. comm.).

### Measurement of wet atmospheric N deposition

Wet deposition of N on the Glas Maol summit plateau was measured between June and October 2002. Both rainfall and occult deposition were collected in two separate gauges located at 1060 m asl. Each gauge consisted of a 13-cm diameter funnel supported on a post 1.5 m above ground level. Occult deposition was collected via a plastic covered wire mesh cylinder attached to the rim of one of the funnels. The cylinder was 10 cm high with a diameter of 10 cm and 1.8 $cm^2$ mesh size. Run-off from the funnels was stored in buried containers and collected weekly between June and August and then either weekly or fortnightly until October.

The volume of precipitation was measured and subsamples transported back to the laboratory, where they were frozen until analysis for $NO_3^-$-N and $NH_4^+$-N concentration using a Tectar FIAstar 5010 Analyser (Sweden). The total amount of N deposited by rainfall in the measuring period was calculated and expressed as kg N $ha^{-1}$. The N concentration in occult deposition was calculated from the difference between the concentration measured in rainfall and that in the combined rainfall and occult precipitation sample.

## Experimental design and treatment application

A nitrogen loading experiment, simulating an increase in atmospheric N deposition in the form of wet deposition episodes, was carried out over five growing seasons from 1998 to 2002. Two forms of N, $KNO_3$ and $NH_4Cl$, were applied in solution to separate 0.6 m × 0.6 m plots at each of the two sites at rates equivalent to 10 and 40 kg N $ha^{-1}$ $yr^{-1}$. These rates represent low and high N deposition levels within the UK (United Kingdom Review Group on Acid Rain, 1997). Distilled water was used as a control. There were five replicate blocks at each site, each containing one plot per treatment. The five different treatments (control, low $NO_3^-$, low $NH_4^+$, high $NO_3^-$, high $NH_4^+$) were applied in 0.5 litre amounts as a fine mist, using a knapsack sprayer. Annual additions were divided into a total of seven applications between June and August 1998–99, and an average of five applications, carried out every two to three weeks, each summer from 2000 to 2002. The low N addition was designed to simulate natural occult pollution episodes at sites in the UK receiving high rates of deposition, taking into account that N concentrations in occult deposition can be an order of magnitude higher than in rainfall (Dollard *et al.*, 1983; United Kingdom Review Group on Acid Rain, 1997). Practical constraints on the volume of water that could be carried to the site meant that the concentration of the high N treatment was of necessity higher than occurs in natural occult deposition.

## Vegetation analysis

**Plant Cover** To test for differences in initial plant cover, a pin frame was used to record first intercepts ('hits') at 36 points within each of the plots on 12 June 1998, before the start of the experiment. No significant differences in plant cover between plots designated to the various treatments could be detected ($0.15 < P < 0.61$, GLMM, as described in data analysis section below). At peak biomass in early September 2000–02 (years 3–5), vegetation cover was recorded at 120 pin points, using first (canopy) and second (ground layer) intercepts. Live and dead *Racomitrium* cover was obtained from the second intercept data.

***Racomitrium* shoot growth** Shoot growth (increase in length at the shoot apex) of *Racomitrium* was measured on samples collected from each plot in early June 2000, cut to 4 cm apical lengths, placed into a Netlon cylinder and carefully replaced into the moss mat within their respective plots. Ten shoots were placed into each cylinder with two cylinders per plot. Shoots were retrieved in September 2000 and tissue in excess of the initial 4 cm was measured as an index of *Racomitrium* shoot growth. Results from the two cylinders were combined to give a mean shoot length increase for each plot.

## *Racomitrium* physiology

**Shoot nitrogen content** Tissue N concentration was analysed in *Racomitrium* randomly sampled from each plot in August 2000. Apical 2 cm lengths were removed from each shoot, washed gently in a stream of distilled water and air dried. The dried plant material was milled and 0.36 g analysed for total N content using a continuous flow colorimetric autoanalyser (Segmented Flow Autoanalyser, Burkard Scientific, Uxbridge, UK), following wet acid digestion (Allen, 1989). Nitrogen content was measured as ammonium by a modified Bertholet reaction (Hinds & Lowe, 1980; Rowland, 1983).

**Nitrate reductase activity** N assimilation capacity of the moss was determined by measuring inducibility of the enzyme nitrate reductase. Samples of *Racomitrium* were collected at random from each plot at the end of August 2000. This material was allowed to acclimatise in a growth chamber under continuous lighting for 72 h before assay in order to avoid possible diurnal effects, and kept moist with distilled water. Assays were carried out on current year's growth, identified as the bright green top section of the shoot. Samples of 10 shoots from a plot were used in each assay, and assays were performed in triplicate. Nitrate reductase activity (NRA) was induced by adding 5 ml of 1 mM $KNO_3$ to half the samples before assay. NRA was measured before induction (constitutive activity) and six hours after induction (induced activity), coincident with peak enzyme activity as determined in initial time course assays (data not shown). Inducible NRA was calculated as the difference between the constitutive and induced activities. Assays were performed *in vivo* using the method of Woodin & Lee (1987). All samples were vacuum infiltrated with 5 ml of 100 mM potassium phosphate buffer containing 0.75% propan-1-ol and 75 mM potassium nitrate and placed in a dark water bath at 25°C for 1 h. They were then removed and placed in a water bath at 80°C for 20 min After cooling for 20 min, 1 ml of solution was removed and added to 1 ml of 1% sulphanilic acid and 1 ml of 0.02% n-1 napthyl-ethylene diamine dihydrochloride, and stood in the dark at 25°C for 20 min. This solution was analysed spectrophotometrically (at 540 nm) for nitrite.

**Membrane potassium leakage** In order to investigate physiological damage, membrane potassium ($K^+$) leakage was measured on *Racomitrium* shoots sampled randomly from each plot in August 2001 (fourth year of treatment). Three samples of five shoots were used from each plot. The apical 2 cm of live green material was removed from each shoot, mechanically shaken in 10 ml distilled water at 300 r.p.m. for 30 min and left to stand for 3 h in controlled temperature and light conditions. The $K^+$ content of the water was determined using a flame photometer. Total remaining tissue $K^+$ content was determined by boiling the shoots in fresh distilled water for 1 h, shaking mechanically, and analysing as before.

### Data analysis

Data were analysed using Generalised Linear Mixed Models in SAS version 8 with 'treatment' and 'site' as fixed effects and 'plot within site' as a random effect. A specific set of contrasts allowed subsequent testing for the effects of N-application (data from treated plots contrasted with data from control plots), dose (high vs low dose) and ion type ($NO_3^-$ vs $NH_4^+$). Interactions between dose and ion type were investigated by contrasting $NO_3^-$ and $NH_4^+$ at both low and high doses separately. Data on plant cover were arcsin √-transformed to meet assumptions of the statistical models used. Relationships between tissue N, NRA and *Racomitrium* shoot growth were investigated using General Linear Models.

## Results

### Wet atmospheric N deposition

Mean rainfall N concentration between June and October was 0.77 mg $l^{-1}$ whilst the concentration in occult deposition was 2.7 times higher at 2.11 mg $l^{-1}$. Over the whole sampling period, the ratios of $NO_x$-N: $NH_y$-N in rainfall and occult precipitation were 1 : 1.118 ± 0.153 (mean ± 1 SE) and 1 : 1.022 ± 0.166, respectively. Although occult N concentrations appeared more episodic throughout the sample period than rainfall concentrations (Fig. 1), this was largely a result of systematic differences in amplitude as their coefficients of variation were found to be similar (coefficient of variation for rainfall 0.98, occult deposition 0.95). Both forms of wet deposition demonstrated high concentration peaks in June and again in September and October. In the autumnal spike occult N concentration was at its highest at 8.2 mg $l^{-1}$, and this was 4.5 times higher than the rainfall concentration. Total N deposited in rainfall over the 5 months was 5.78 kg N $ha^{-1}$.

### *Racomitrium* cover and condition

Five years of N addition caused progressive loss of live *Racomitrium* cover, and by peak biomass in 2002 this effect was the most pronounced with *Racomitrium* cover for the low and high N treatments being on average 31.6% and 69.7% less than the control plots, respectively (Fig. 2, Table 1). There was also a differential effect of ion type at the high dose. Plots treated with high $NH_4^+$ had a significantly reduced *Racomitrium* cover, occupying only 20.2 ± 3.2% of the ground layer compared to 34.0 ± 4.9% in the high $NO_3^+$ plots (Table 1).

Concomitant with the decrease in live *Racomitrium* cover was an increase in the abundance of dead *Racomitrium* shoots within the N treated plots (Fig. 2). This is listed in Table 1 as a proportion of the total *Racomitrium* cover for each plot. After 5 yr of treatment there was a significant negative effect of N addition on *Racomitrium* shoot survival whereby plots receiving high N doses had a significantly greater proportion of dead shoots than those receiving low doses (Table 1, Fig. 2). There was also a highly significant effect of ion type on the moss at the high but not the low dose, demonstrating that sensitivity to ion type increases with dose. In plots receiving high $NH_4^+$ over half the *Racomitrium* shoots were dead, 54.8 ± 8.7%, compared with only 12.6 ± 3.2% dead in the high $NO_3^-$ treatment.

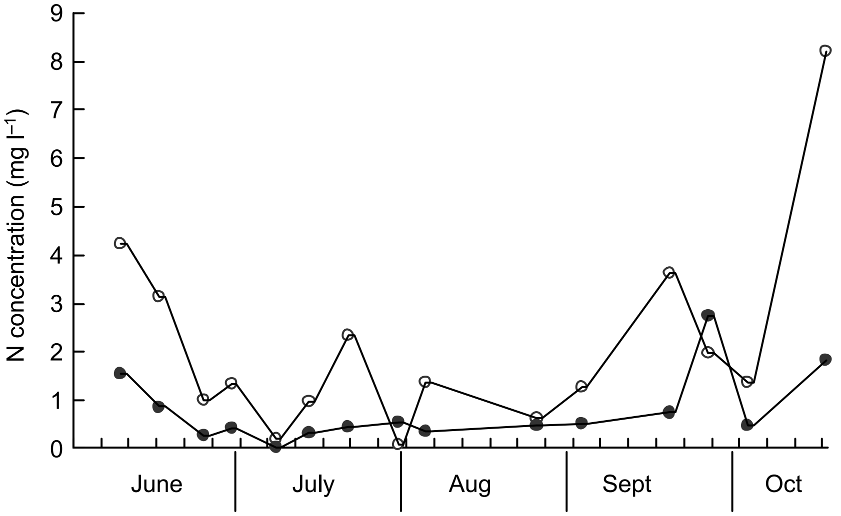

**Fig. 1** Wet deposition of total nitrogen (N) during four months at Glas Maol, 1068 m above sea level. Closed circles, rainfall; open circles, occult deposition.

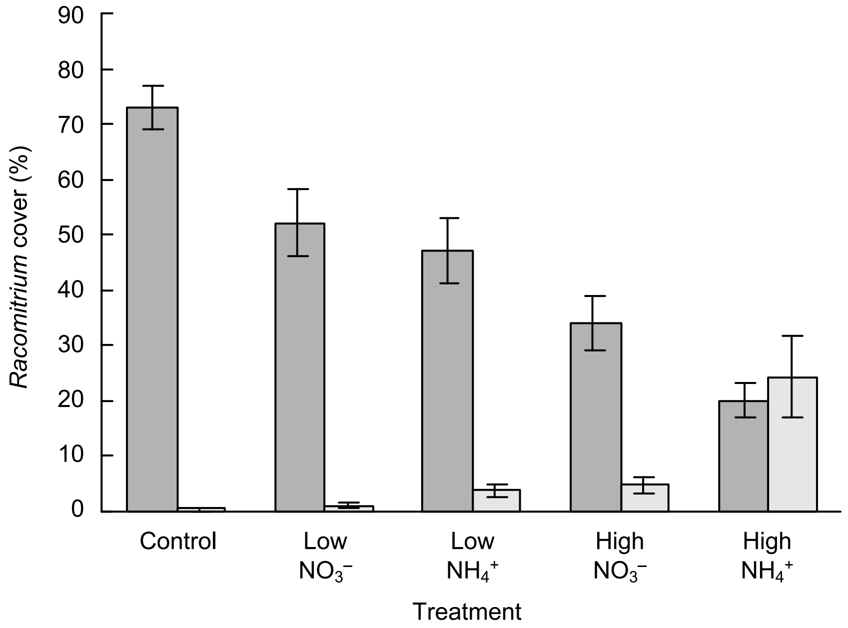

**Fig. 2** Effects of nitrogen treatments on percent ground cover of live (closed columns) and dead (open columns) *Racomitrium* at peak biomass in September after five seasons of treatment. Treatment: low, 10 kg N $ha^{-1}$ $yr^{-1}$; high, 40 kg N $ha^{-1}$ $yr^{-1}$. Bars, ± 1 SE. Significance test results are shown in Table 1.

**Table 1** Summary statistics for the effects of nitrogen addition treatment (with distilled water control), concentration (10 or 40 kg N $ha^{-1}$ $yr^{-1}$) and ion type ($NO_3^-$ or $NH_4^+$) on *Racomitrium* physiological and performance parameters within *Carex bigelowii–Racomitrium lanuginosum* heath

| | Effect of | | | | |
|---|---|---|---|---|---|
| Measured parameters for *Racomitrium* (treatment year in parenthesis) | Effect of Treatment (Treated vs control) NDF = 1 DDF = 32 | N Dose (Low vs high) NDF = 1 DDF = 32 | Ion type ($NO_3^-$ vs $NH_4^+$) NDF = 1 DDF = 32 | Ion type at low dose NDF = 1 DDF = 32 | Ion type at high dose NDF = 1 DDF = 32 |
| Ground cover (5th) | $F = 70.00$*** | $F = 38.22$*** | $F = 6.68$* | $F = 0.91$ ns | $F = 7.13$** |
| Proportion dead shoots (5th) | $F = 27.95$*** | $F = 53.70$*** | $F = 25.14$*** | $F = 0.53$ ns | $F = 58.32$*** |
| Shoot growth (3rd) | $F = 58.05$*** | $F = 37.56$*** | $F = 2.11$ ns | $F = 3.78$* | $F = 0.01$ ns |
| Tissue N content (3rd) | $F = 23.09$*** | $F = 1.42$ ns | $F = 2.10$ ns | $F = 1.59$ ns | $F = 0.62$ ns |
| Inducible NRA (3rd) | $F = 45.27$*** | $F = 9.43$** | $F = 1.67$ ns | $F = 0.00$ ns | $F = 3.24$* |
| Membrane K leakage (4th) | $F = 11.34$** | $F = 9.74$** | $F = 1.99$ ns | $F = 0.47$ ns | $F = 1.70$ ns |

Measurements are made after 3,4 and 5 yr of treatment. NRA, nitrate reductase activity. All tests carried out using Generalised Linear Mixed Models (GLMM) contrast statements. Significance values denoted as *, $P < 0.1$; **, $P < 0.01$; ***, $P < 0.0001$.

### *Racomitrium* shoot growth and physiology

*Racomitrium* growth showed a significant and consistent negative response to N additions. In 2000, the third year of treatment, growth (over 4 months) in the low dose plots averaged at 35% less than the control shoots, whilst in the high dose plots growth was reduced by as much as 74% (Fig. 3a, Table 1). There was a trend ($P = 0.067$) for $NH_4^+$ to have a more detrimental effect than $NO_3^-$ at the low but not the high N dose.

*Racomitrium* shoots taken from N treated plots at the end of the summer season in 2000 had significantly higher tissue N than control shoots (Fig. 3b). The N solutions therefore influenced *Racomitrium* tissue N concentration. However, there were no significant differences between the two N dose rates or ion types (Table 1). Thus although the high N addition was four times greater than the low dose, this was not reflected in moss tissue N concentration.

N addition significantly decreased inducibility of NRA in *Racomitrium* shoots (Fig. 4, Table 1). Inducible NRA in plots receiving the low N dose was 53% less than in the control plots. Activity was even further reduced by the high dose and again there was a differential effect of ion type, with material which had received high $NH_4^+$ addition showing the greatest reduction in enzyme inducibility to only 11% that of the controls.

Total $K^+$ concentration (the sum of both leaked and boiled fractions) in live *Racomitrium* shoots varied across treatments ($F_{4,32} = 14.02$, $P < 0.0001$, Fig. 5a). Shoots from both low dose treatments, and those receiving the high $NO_3^-$ dose,

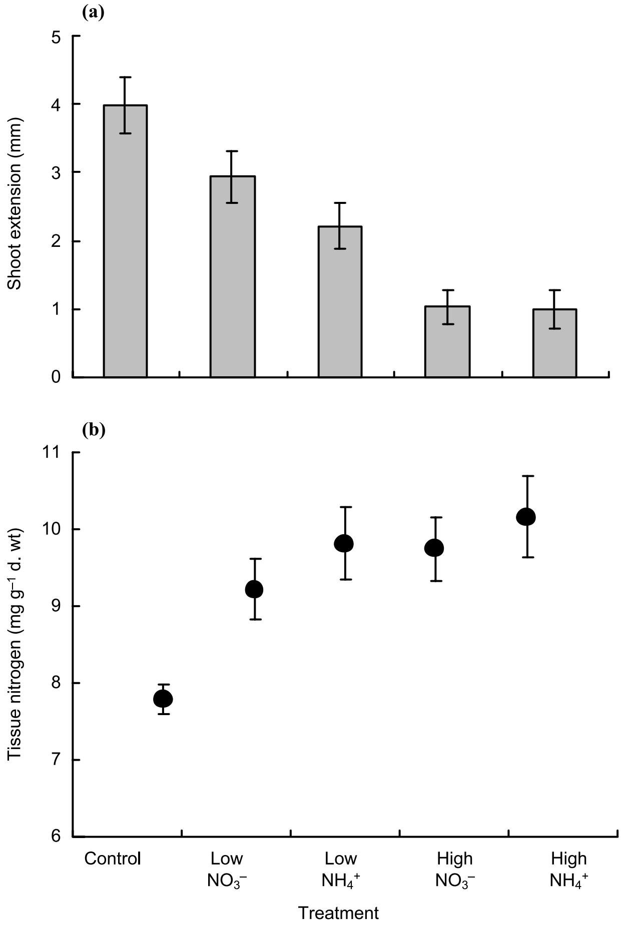

**Fig. 3** Effects of nitrogen treatments on (a) *Racomitrium* shoot extension over 4 months, and (b) tissue N concentration in apical 2-cm segment measured in August after final application in third season of treatment (2000). Treatment: low, 10 kg N ha$^{-1}$ yr$^{-1}$; high, 40 kg N ha$^{-1}$ yr$^{-1}$. Bars, ± 1 SE. Significance test results are shown in Table 1.

contained an average of 0.58 mg g$^{-1}$ more $K^+$ than the control concentration of 1.69 ± 0.08 mg g$^{-1}$. However, moss from plots receiving high $NH_4^+$ had significantly lower $K^+$ concentration than the controls, demonstrating an interaction between dose and ion type ($F_{1,32}$ = 44.88, $P < 0.0001$).

Although oxidised N was applied as $KNO_3$, it did not result in greater tissue $K^+$ concentration in the high compared to the low $KNO_3$ dose treatment. There was also no significant difference in total tissue $K^+$ between moss receiving low doses of $NO_3^-$ and $NH_4^+$-N. It therefore appears that availability of $K^+$ ions in the $KNO_3$ treatment did not result in increased $K^+$ uptake by *Racomitrium.*

All N additions caused significantly increased $K^+$ leakage (expressed as a percentage of total tissue $K^+$ concentration) from the *Racomitrium* tissue ($F_{4,32}$ = 17.27, $P < 0.0001$, Fig. 5b). Material from plots treated with low and high N additions had 2.5 and 3.3 times greater leakage than control material, respectively, indicating that both dose rates caused loss of membrane integrity within the moss tissue. Whereas the dose effect was statistically significant (Table 1), there was no effect of ion type ($P = 0.17$).

### Relationship between *Racomitrium* growth and physiology

Both *Racomitrium* tissue N and inducible NRA were related to shoot growth in order to determine their suitability as indicators of *Racomitrium* condition. Tissue N concentration only showed a weak negative relationship with shoot growth ($F_{1,48}$ = 4.57, $P$ = 0.038, $R^2_{adj}$ = 0.07, Fig. 6a). However, a similar analysis showed moss NRA inducibility to be strongly positively associated with *Racomitrium* shoot growth ($F_{1,48}$ = 21.68; $P < 0.0001$, $R^2_{adj}$ = 0.30, Fig. 6b). It is evident that where enzyme inducibility is lowest, shoot growth is correspondingly reduced.

## Discussion

### Wet deposition of N

There is accumulating evidence that the threat of wet N deposition in montane areas has, in the past, been underestimated. Once deposition to vegetation from cloud droplets and dry deposition are added to the 5-month accumulated rainfall of 5.78 kg N ha$^{-1}$ on the Glas Maol summit, the total N deposition figure is likely to fall around the middle (17–20 kg N ha$^{-1}$ yr$^{-1}$) of the UK pollution range (National Expert Group on Transboundary Air Pollution, 2001). The predicted deposition for surrounding lower ground is less than 12 kg N ha$^{-1}$ yr$^{-1}$ (National Expert Group on Transboundary Air Pollution, 2001), demonstrating the effect of increased solute deposition with altitude (Baddeley *et al.*, 1994; National Expert Group on Transboundary Air Pollution, 2001). Past underestimations of N deposition for upland sites that experience prolonged periods of occult deposition, such as the previous figure of 6 kg N ha$^{-1}$ yr$^{-1}$ in north-east Scotland (United Kingdom Review Group on Acid Rain, 1997), have only recently been revised (M. Sutton, pers. comm.).

The frequent occurrence of occult deposition, with average N concentrations 2.7 times higher than in rainfall, makes it an important contributor to N inputs at the site. This contribution can be even higher where montane areas are located in polluted regions. At Great Dun Fell (850 m asl) in Cumbria, N concentration in orographic cloud exceeded that in upwind rain by a factor of between 5 and 10 (Fowler *et al.*, 1988; Fowler *et al.*, 1995; United Kingdom Review Group on Acid Rain, 1997) and occult $NO_3^-$ concentrations reached 2.4 mM (Dollard *et al.*, 1983). This demonstrates the importance of concentration of deposition as well as total amount. The frequency of large peaks in occult N concentration on Glas Maol

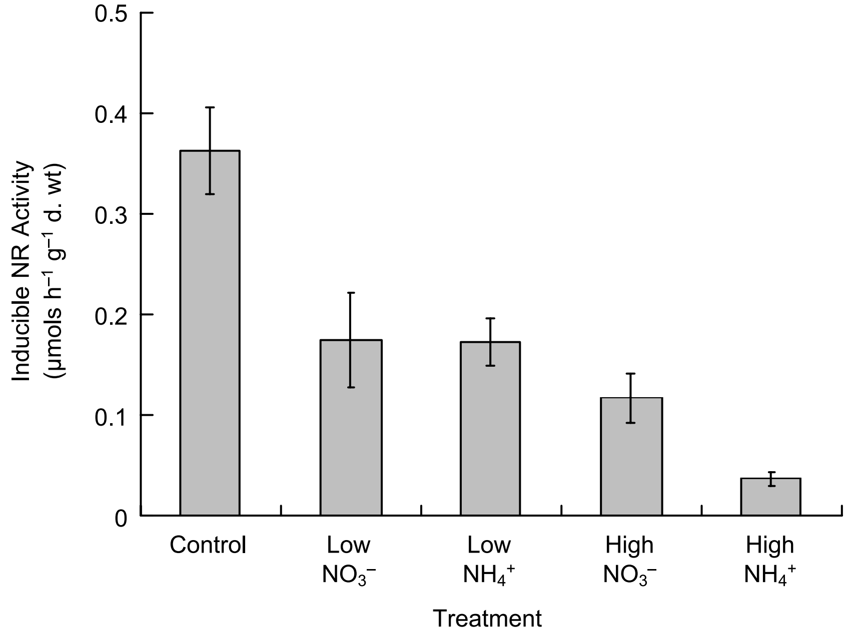

**Fig. 4** The effects of nitrogen treatments on inducible nitrate reductase activity (NRA), calculated as induced – constitutive activity, in current year's growth of *Racomitrium* shoots collected in August 2000 after 3 seasons of treatment. Treatment: low, 10 kg N $ha^{-1}$ $yr^{-1}$; high, 40 kg N $ha^{-1}$ $yr^{-1}$. Bars, ± 1 SE. Significance test results are shown in Table 1.

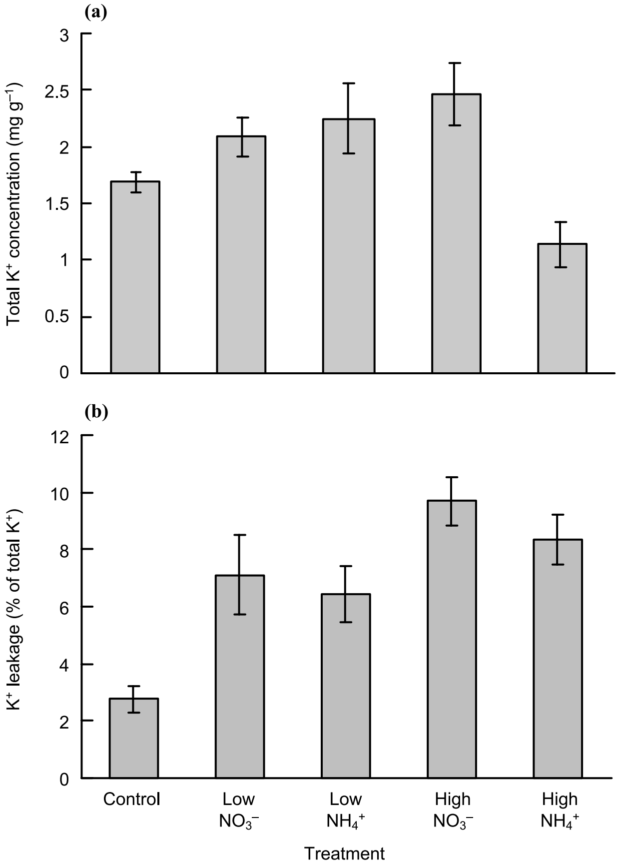

**Fig. 5** Effects of nitrogen treatments on (a) total potassium concentration, and (b) percentage potassium leakage, in current year's growth of *Racomitrium* tissue removed from plots in August 2001 after 4 seasons of treatment. Treatment: low, 10 kg N $ha^{-1}$ $yr^{-1}$; high, 40 kg N $ha^{-1}$ $yr^{-1}$. Bars, ± 1 SE. Significance test results are shown in Table 1.

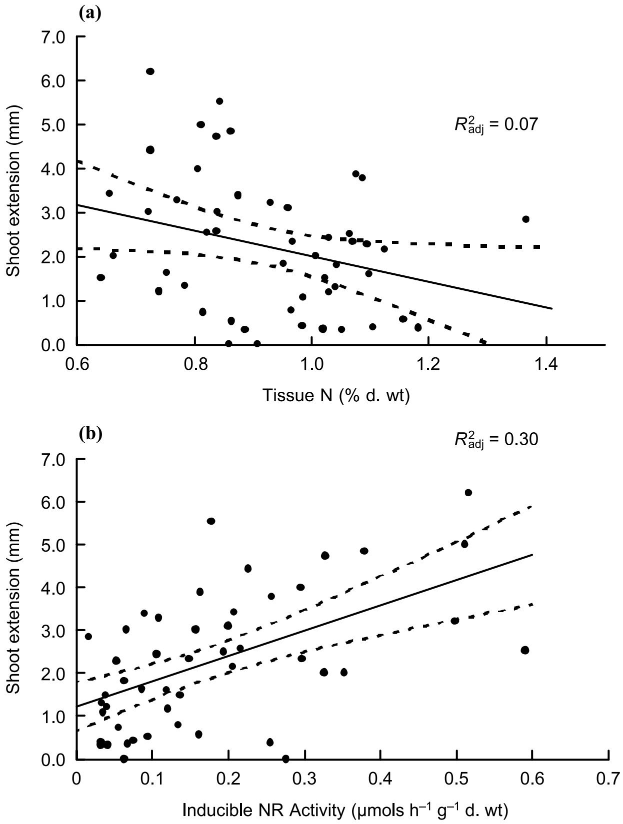

**Fig. 6** Relationship of *Racomitrium* shoot extension measured after 4 months with (a) tissue N content, $y = 4.94 - 2.92x$, $P = 0.03$, $R^2_{adj} = 0.07$, and (b) inducible nitrate reductase activity (NRA) $y = 1.22 + 5.68x$, $P < 0.0001$, $R^2_{adj} = 0.30$, for all plots combined, after three seasons of nitrogen treatment.

confirms that montane areas can be at risk of severe pollution events. The relative contribution of $NO_x$ and $NH_y$ to total deposition at Glas Maol is equal, and the summer wet deposition ratio of 1 $NO_x$-N: 1.07 $NH_y$-N is consistent with the total annual N deposition ratio of 1 : 1.057 predicted by the national deposition database for the 5 km grid square within which Glas Maol occurs (M. Sutton, pers. comm.). The high N concentrations found in both occult deposition and rainfall in early summer may be caused by lowland agricultural activity, such as fertiliser application, and the dramatic peak in autumn occurred following the common and widespread activity of heather burning on surrounding grouse moorland, thus demonstrating a possible seasonal influence on deposition concentrations.

## Effects of N addition on *Racomitrium*

### Cover and growth

After 5 yr of treatment, N additions had a highly detrimental effect on *Racomitrium* cover within the montane heath community. This is a continuation of the pattern observed by Pearce & van der Wal (2002), and is consistent with the findings of other N addition studies within similar habitats. In subarctic Sweden, additions of 100 kg N $ha^{-1}$ $yr^{-1}$ of N and P for 3 yr reduced bryophyte cover within shrub heath by 50% (Potter *et al.*, 1995). Similarly in Alaskan tussock tundra combined nutrients reduced the growth of *Aulocomnium* spp. (Chapin & Shaver, 1985). The results obtained in this study show a clear dose related relationship. Negative effects are significant even with the low N dose applied, and at high N, not only is half the cover of live *Racomitrium* lost, but proportion of dead shoots is dramatically increased. Bryophyte cover can therefore be extremely sensitive to increases in N deposition.

The reduction in *Racomitrium* cover following N treatment reflected its severely reduced shoot growth. *Racomitrium* shoots kept in a mist exposure facility also showed growth reduction with N addition (Jones *et al.*, 2002; van der Wal *et al.*, 2003). As it is an ectohydric species typically found in nutrient-poor environments, *Racomitrium* is more vulnerable to direct toxic effects of excess N than endohydric bryophytes or vascular plants. Differing responses between bryophyte species with contrasting nutrient requirements were seen in arctic heath vegetation following treatment with 10 kg N $ha^{-1}$ $yr^{-1}$ and 5 kg P $ha^{-1}$ $yr^{-1}$ (Woolgrove & Woodin, 1996c; Gordon *et al.*, 2001). After 8 yr nutrophilous species, such as *Polytrichum juniperinum*, were beginning to replace other species such as *Dicranum scoparium*, whose cover was reduced. The exact mechanism by which N treatment directly reduces *Racomitrium* growth is unclear. However, disruption to the moss' physiology was evident. Therefore, death of *Racomitrium* within montane heath may be caused by direct toxic effects of N addition.

### Physiological status

Even at low N additions the physiological response of *Racomitrium* suggests that it is not N limited. This was demonstrated by greatly reduced inducibility of NRA after additions of only 10 kg N $ha^{-1}$ $yr^{-1}$. Nitrate reductase is both substrate inducible and end product inhibited. Therefore if N is being assimilated faster than it can be utilised by the moss, inducibility of the enzyme will eventually be regulated so that its activity is reduced (Woodin *et al.*, 1985). As montane moss heath naturally has a low nutrient availability any N additions either as oxidised or reduced forms would be expected to either not effect, or cause an increase in, NRA inducibility within the moss. However, the observed reduction in inducibility suggests that *Racomitrium* within the heath community is already N saturated.

Nitrogen treatment also resulted in damage to moss cell membranes. The increased $K^+$ leakage observed in *Racomitrium* after 4 yr of N treatment is consistent with other findings that N addition potentially results in loss of membrane integrity and increased solute leakage in bryophytes (Woolgrove & Woodin, 1996c). In higher plants, cell wall damage following exposure to N treatments may occur as a result of faster shoot growth and delayed hardening making plants susceptible to environmental stresses, such as drought or freezing (Robinson *et al.*, 1998; Carroll *et al.*, 1999; Gordon *et al.*, 1999). However in *Racomitrium* increasing N additions caused a dramatic reduction in growth, so this is unlikely to be the mechanism behind the observed damage.

The slower growth of N treated moss may explain the increased total $K^+$ concentrations in apical tissue. The exception to this, of significantly reduced tissue $K^+$ in moss treated with the high $NH_4^+$ dose, might be explained by $NH_4^+$ exchange with $K^+$ ions, although such an effect was not apparent at low $NH_4^+$ additions. It is perhaps more likely that high toxicity of the high $NH_4^+$ dose caused cell solute leakage to occur over a more prolonged period than the other N treatments, resulting in significant loss of $K^+$ from the *Racomitrium* tissue.

Membrane damage and solute leakage in N treated moss may explain the increasingly 'black' appearance of *Racomitrium*, with degraded macro-structure apparent in previous years' growth (*pers. obs.*), as well as the extensive occurrence of dead shoots in high dose plots. This provides evidence that both oxidised and particularly reduced N additions have had a direct toxic effect on *Racomitrium*, causing physiological damage, loss of membrane integrity, and ultimately shoot death at high doses.

The tissue N concentration of *Racomitrium* sampled from control plots, averaged as 7.79 mg N $g^{-1}$ d. wt, readily compares with levels of 6–9 mg N $g^{-1}$ d. wt found in moss collected from similar mountain summits in north-east Scotland (Baddeley *et al.*, 1994). As expected, N content of the moss significantly increased with N addition, demonstrating the ability of *Racomitrium* tissue to reflect surrounding atmospheric N inputs (Pitcairn *et al.*, 1995). However, as has been previously observed in bryophytes (Carroll *et al.*, 2000), *Racomitrium* tissue N concentration does not increase linearly but tends towards saturation with high N inputs. This, combined with the decrease in NRA, suggests that excess N was not being assimilated. It is also possible that, as with $K^+$, assimilated N solutes were being leaked from the damaged moss tissue. Bryophytes can play an important role in scavenging deposited N and so acting as efficient buffers for N inputs to a community (Woodin & Lee, 1987; Gordon *et al.*, 2001). However, in our study the saturation of *Racomitrium* tissue N and reduction in NRA following application of 10 kg N ha$^{-1}$ yr$^{-1}$ demonstrates that the moss is no longer acting as an N sink. Declining N sink strength in plants within a Swedish subarctic montane heath resulted in extra nutrients being absorbed by microorganisms and an increase in soil $NH_4^+$ concentrations (Jonasson *et al.*, 1999). Therefore the loss of live *Racomitrium* cover in N treated plots, and solute leaching from damaged moss tissue, is likely to have implications for N turnover above and below ground within this community.

### Comparative effects of oxidised and reduced N

For several moss parameters an effect of N dose on the response of ion type was strongly evident, with the high dose of reduced N having a more detrimental effect than the high dose of oxidised N. This was demonstrated in high $NH_4^+$ plots by a significantly lower cover of live and higher proportion of dead shoots, greater loss of NRA inducibility, and lower tissue $K^+$ concentration indicating long-term solute leakage. Previous laboratory studies have also demonstrated differential responses of bryophytes to the two forms of N. *Racomitrium* showed preference for $NH_4^+$ uptake when both reduced and oxidised forms of N were available (Soares & Pearson, 1995). When both ions are supplied at the same rate to *Sphagnum* species, $NH_4^+$ causes greater NRA inhibition than $NO_3^-$ (Woodin & Lee, 1987). $NH_4^+$ ions may also increase overall acidity of bryophytes (Pearson & Stewart, 1993) and boreal species such as *Hylocomium splendens* suffered reduced nutrient content and segment length when exposed to solutions of low pH (Bates, 2000). Thus mosses in general appear more sensitive to damage by reduced than by oxidised N.

## Critical load of N for montane heath

### Quantification of the critical load

The very marked changes in NRA, membrane leakage and significant reductions in growth and cover of *Racomitrium* with addition of only 10 kg N ha$^{-1}$ yr$^{-1}$ indicate that its critical load was greatly exceeded by the estimated total received by the moss of *c.* 28 kg N ha$^{-1}$ yr$^{-1}$. Indeed, the effects were so dramatic it is possible that the critical load is already met, or even exceeded, by the background deposition of an estimated 18 kg N ha$^{-1}$ yr$^{-1}$. A recent 8 yr study on an analogous high arctic heath demonstrated negative effects with an N treatment of 10 kg N ha$^{-1}$ yr$^{-1}$ above a background of approximately 1 kg N ha$^{-1}$ yr$^{-1}$ (Gordon *et al.*, 2001). The critical load of N for mountain summit vegetation (EUNIS classification E.4.2), based on effects on bryophytes and lichens, has been set at 5–10 kg N ha$^{-1}$ yr$^{-1}$ (Bobbink *et al.*, 2002). This therefore presents cause for concern as many mountain summits in Britain are likely to exceed this level.

Our study highlights the importance of the deposited N form when considering critical loads for moss heath. The threshold for damage at a particular site may be lower if the predominant form is $NH_y$ rather than $NO_x$ as is the case, for example, in the vicinity of intensive animal stocking (National Expert Group on Transboundary Air Pollution, 2001). The precautionary principal would suggest that the critical load for all sites should be based on the threshold for damage from $NH_y$ dominated deposition, or at least that there should be different critical loads for sites in different pollution climates. The proximity of intensively managed farmland and high livestock densities to montane habitats in the north of England, such as the Pennines, may have been responsible for causing damage to areas of moss heath which have subsequently degraded to grasslands.

### Biological indicators of *Racomitrium* condition and critical load exceedence

It is widely accepted that there is both a general need to be able to monitor biological indicators of critical load exceedence (Bobbink *et al.*, 1996) and a specific need for monitoring the effects of N pollution on the survival of *Racomitrium* within montane moss heath (Thompson & Baddeley, 1991; Bunce *et al.*, 1999). Although *Racomitrium* tissue N may provide a guide to atmospheric N deposition (Pitcairn *et al.*, 1995), the relationship is not linear at high deposition rates and gives no indication of whether vegetation is adversely affected by the deposited N. Whilst biomonitors of deposition are useful, there is an even greater need to identify biological indicators of ecosystem damage and thus of critical load exceedence. Based on our studies, *Racomitrium* shoot growth appears to be an extremely sensitive biological indicator of damage due to atmospheric N deposition, yet it is a time consuming measure. Instead, a closely correlated predictor of growth may provide an alternative monitoring tool. For this purpose, reduction in inducible NRA with increasing N addition proved a much stronger predictor of *Racomitrium* shoot growth than tissue N, therefore highlighting its potential as a performance indicator. Other bryophytes that suffer a decrease in growth following N addition, such as *Sphagnum*, have shown a similar sensitivity of NRA to N loads (Woodin *et al.*, 1985). This close relationship between NRA and *Racomitrium* growth therefore indicates its potential for use as a biological indicator of montane moss heath condition, and of exceedence of the critical load for N deposition.

## Conclusions

This work highlights the importance of occult pollution events in estimating total N deposition at high altitude sites. It provides evidence for the detrimental effects even low doses of reduced or oxidised N have on *Racomitrium* physiology and performance within montane heath. Additions of only 10 kg N $ha^{-1}$ $yr^{-1}$ to a relatively unpolluted site within the UK deposition range were beyond the habitat's critical load, causing toxic effects to *Racomitrium* and loss of cover. This very high sensitivity of the moss to even small increases in atmospheric N deposition demonstrates the potential for loss of ectohydric bryophytes from communities receiving excess N deposition, and supports a low critical load for montane heath. The importance of taking ion type into account when considering the critical load of N for a habitat has also been highlighted. Our findings strongly suggest that atmospheric N deposition may have contributed to loss of *Racomitrium* within montane heath in more polluted areas of Britain. Loss of *Racomitrium* cover will clearly affect ecosystem integrity, leading to changes in N cycling and plant community dynamics. Thus there are longer-term implications for habitat survival, and for potential for recovery once N deposition is reduced through implementation of the Gothenburg protocol (National Expert Group on Transboundary Air Pollution, 2001).

## Acknowledgements

We thank the Invercauld Estate and Scottish Natural Heritage for permission to carry out the work on Glas Maol, and are also indebted to NERC and Aberdeen University for financial support. We would also like to thank Edward Kluen and Louise Newell for their invaluable field assistance, and to Rob Brooker for helpful comments on earlier versions of the text.

## References

**Allen SE. 1989.** *Chemical analysis of ecological materials.* Oxford, UK: Blackwell Scientific Publications.

**Alonso I, Hartley SE. 1998.** Effects of nutrient supply, light availability and herbivory on the growth of heather and three competing grass species. *Plant Ecology* **137**: 203–212.

**Baddeley JA, Thompson DBA, Lee JA. 1994.** Regional and Historical Variation in the Nitrogen Content of *Racomitrium lanuginosum* in Britain in Relation to Atmospheric Nitrogen Deposition. *Environmental Pollution* **84**: 189–196.

**Bates JW. 2000.** Mineral nutrition, substratum ecology, and pollution. In: Shaw J, Goffinet AB, eds. *Bryophyte biology.* Cambridge, UK: University Press, 248–311.

**Bobbink R, Ashmore MR, Braun S, Fluckiger W, van den Wyngaert IJJ. 2002.** *Empirical nitrogen critical loads for natural and semi-natural ecosystems: 2002 update. Background document of the Expert Workshop on Empirical Critical Loads for Nitrogen.* UN ECE Convention on Long–Range Transboundary Air Pollution. Bern, Switzerland: Swiss Agency for the Environment, Forest and Landscape.

**Bobbink R, Hornung M, Roelofs JGM. 1996.** Impirical nitrogen critical loads for natural and semi-natural ecosystems. In: *Manual on Methodologies and Criteria for Mapping Critical Levels and Geographical Areas Where They Are Exceeded.* UN ECE Convention on Long–Range Transboundary Air Pollution. Federal Environmental Agency. Berlin, Germany: Umweltburdensamt.

**Bobbink R, Hornung M, Roelofs JGM. 1998.** The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology* **86**: 717–738.

**Bunce RG, Smart S, Van de Poll H, Watkins J, Scott W. 1999.** *Measuring change in British vegetation.* London, UK: Department of Environment, Transport and Regions.

**Carroll JA, Caporn SJM, Cawley L, Read DJ, Lee JA. 1999.** The effect of increased deposition of atmospheric nitrogen on *Calluna vulgaris* in upland Britain. *New Phytologist* **141**: 423–431.

**Carroll JA, Johnson D, Morecroft M, Taylor A, Caporn SJM, Lee JA. 2000.** The effect of long-term nitrogen additions on the bryophyte cover of upland acidic grasslands. *Journal of Bryology* **22**: 83–89.

**Chapin FS, Shaver GR. 1985.** Individualistic growth response of tundra plant species to environmental manipulations in the field. *Ecology* **66**: 564–576.

**Dollard GJ, Unsworth MH, Harve MJ. 1983.** Pollutant transfer in upland regions by occult precipitation. *Nature* **302**: 241–243.

**Fowler D, Cape JN, Leith ID, Choularton TW, Gay MJ, Jones A. 1988.** The influence of altitude on rainfall composition at Great Dun Fell. *Atmospheric Environment* **22**: 1355–1362.

**Fowler D, Leith ID, Binnie J, Crossley A, Inglis DWF, Choularton TW, Gay M, Longhurst JWS, Conland DE. 1995.** Orographic enhancement of wet deposition in the United Kingdom: Continuous monitoring. *Water Air and Soil Pollution* **85**: 2107–2112.

**Gordon C, Woodin SJ, Alexander IJ, Mullins CE. 1999.** Effects of increased temperature, drought and nitrogen supply on two upland perennials of contrasting functional type: *Calluna vulgaris and Pteridium aquilinum. New Phytologist* **142**: 243–258.

**Gordon C, Wynn JM, Woodin SJ. 2001.** Impacts of increased nitrogen supply on high Arctic heath: the importance of bryophytes and phosphorus availability. *New Phytologist* **149**: 461–471.

**Grace J, Unsworth MH. 1988.** Climate and microclimate of the uplands. In: Usher MB, Thompson DBA, eds. *Ecological change in the uplands.* Edinburgh, UK: Blackwell Scientific Publications, 137–150.

**Hinds AA, Lowe LE. 1980.** Application of the Bertholet reasction to the determination of ammonium-N in soil extracts and soil digests. *Communications in Soil Science and Plant Analysis* **11**: 469–475.

**Jonasson S. 1992.** Plant responses to fertilization and species removal in tundra related to community structure and clonality. *Oikos* **63**: 420–429.

**Jonasson S, Michelsen A, Schmidt IK, Nielsen EV. 1999.** Responses in microbes and plants to changed temperature, nutrient, and light regimes in the arctic. *Ecology* **80**: 1828–1843.

**Jones MLM, Oxley ERB, Ashenden TW. 2002.** The influence of nitrogen deposition, competition and desiccation on growth and regeneration of *Racomitrium lanuginosum* (Hedw.) Brid. *Environmental Pollution* **120**: 371–378.

**Lee JA. 1998.** Unintentional experiments with terrestrial ecosystems: ecological effects of sulphur and nitrogen pollutants. *Journal of Ecology* **86**: 1–12.

**Lee JA, Caporn SJM. 1998.** Ecological effects of atmospheric reactive nitrogen deposition on semi-natural terrestrial ecosystems. *New Phytologist* **139**: 127–134.

**Lee JA, Press MC, Woodin S, Ferguson P. 1987.** Responses to acidic deposition of ombrotrophic mires in the UK. In: Hutchinson TC, Meema KM, eds. *Effects of atmospheric pollutants on forests, wetlands and agricultural ecosysytems.* Berlin, Germany: Springer-Verlag, 549–560.

**National Expert Group on Transboundary Air Pollution. 2001.** *Transboundary air pollution: acidification, eutrophication and ground-level ozone in the UK.* London, UK: Department for Environment, Food and Rural Affairs, UK.

**Pearce ISK, van der Wal R. 2002.** Effects of nitrogen deposition on growth and survival of montane *Racomitrium lanuginosum* heath. *Biological Conservation* **104**: 83–89.

**Pearson J, Stewart GR. 1993.** The deposition of atmospheric ammonia and its effects on plants. *New Phytologist* **125**: 283–305.

**Pitcairn CER, Fowler D, Grace J. 1995.** Deposition of fixed atmospheric nitrogen and foliar nitrogen content of bryophytes and *Calluna vulgaris* (L.) Hull. *Environmental Pollution* **88**: 193–205.

**Potter JA, Press MC, Callaghan TV, Lee JA. 1995.** Growth responses of *Polytrichum commune and Hylocomium splendens* to simulated environmental change in the sub-arctic. *New Phytologist* **131**: 533–541.

**Press MC, Potter JA, Burke MJW, Callaghan TV, Lee JA. 1998.** Responses of a subarctic dwarf shrub heath community to simulated environmental change. *Journal of Ecology* **86**: 315–327.

**Ratcliffe DA, Thompson DBA. 1988.** The British uplands: their ecological character and international significance. In: Usher MB, Thompson DBA, eds. Edinburgh, UK: *Ecological change in the uplands.* Blackwell Scientific Publications, 9–36.

**Robinson CH, Wookey PA, Lee JA, Callaghan TV, Press MC. 1998.** Plant community responses to simulated environmental change at a high arctic polar semi-desert. *Ecology* **79**: 856–866.

**Rodwell JS. 1992.** *Grasslands and montane communities.* Cambridge, UK: Cambridge University Press.

**Rowland AP. 1983.** An automated method for the determination of a mmonium-N in ecological materials. *Communications in Soil Science and Plant Analysis* **14**: 49–63.

**Soares A, Pearson J. 1995.** Short-term physiological responses of mosses to atmospheric ammonium and nitrate. *Water Air and Soil Pollution* **93**: 225–242.

**Thompson DBA, Baddeley JA. 1991.** Some effects of acidic deposition on montane *Racomitrium lanuginosum* heaths. In: Woodin SJ, Farmer AM, eds. *The effects of acid deposition on nature conservation in Great Britain, 26.* Peterborough, UK: Nature Conservancy Council, 17–28.

**Thompson DBA, Galbraith H, Horsfield D. 1987.** Ecology and resources of Britain's mountain plateaux: land use issues and conflicts. In: Bell M, Bunce RG, eds. *Agriculture and conservation in the hills and uplands.* Monks Wood, UK: Centre for Ecology and Hydrology, 2–31.

**United Kingdom Review Group on Acid Rain. 1997.** *Acid deposition in the UK 1992–94.* Fourth Report of the United Kingdom Review Group on Acid Rain. London, UK: Department of Environment, Transport and the Regions. AEA Technology plc.

**van der Wal R, Pearce I, Brooker R, Scott D, Welch D, Woodin S. 2003.** Interplay between nitrogen deposition and grazing causes habitat degradation. *Ecology Letters* **6**: 141–146.

**Woodin SJ, Farmer AM. 1993.** Impacts of sulphur and nitrogen deposition on sites and species of nature conservation importance in Great Britain. *Biological Conservation* **63**: 23–30.

**Woodin SJ, Lee JA. 1987.** The effects of nitrate, ammonium and temperature on nitrate reductase-activity in *Sphagnum* species. *New Phytologist* **105**: 103–115.

**Woodin S, Press MC, Lee JA. 1985.** Nitrate reductase activity in *Sphagnum fuscum* in relation to wet deposition of nitrate from the atmosphere. *New Phytologist* **99**: 381–388.

**Woolgrove CE, Woodin SJ. 1996c.** Effects of pollutants in snowmelt on *Kiaeria starkei*, a characteristic species of late snowbed bryophyte dominated vegetation. *New Phytologist* **133**: 519–529.