Work Package 2 Task 6:

The processes controlling uptake and storage of N, and the effects of N deposition on semi-natural vegetation.

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Annual Report
July 2002
Nitrogen Umbrella Project
Department for Environment, Food and Rural Affairs
(TASK 6)

The processes controlling uptake and storage of N and the effects of N deposition on semi-natural vegetation.

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Summary

- *Calluna* growth responded positively for 2 years to all levels of N additions, whereas *Molinia* has only recently increased its growth in the highest wet N treatment, where the spread of *Calluna* has noticeably declined in year 3. This decline in *Calluna* is a direct effect of N and not an interaction with stress. Thus far, all NH_3 treatments continue to stimulate the spread of *Calluna*, at the expense of graminoid proliferation. As these effects were observed in OTCs, they may not be applicable to the field.

- Foliar N concentrations in *Calluna* continue to increase linearly in response to N additions though the absolute values have fallen as the size and structure of the plants have increased. In 3 years *Calluna* has passed through the building and mature phase toward the degenerate phase in the highest N deposition treatment confirming that N deposition accelerates ageing.

- The mechanisms underpinning *Calluna's* growth response to N are unclear. Increases in foliar N have not been associated with increased quantum efficiency of photosystem II.

- N effects on autumn and winter frost hardiness were negligible except in the highest NH_3 treatment where the increased frost sensitivity appears to be concentration, not dose led.

- Measurements of amino acid concentrations in *Calluna* and *Molinia* did not provided any additional information over foliar N data and it is not considered a viable alternative to foliar N for assessing the impact of different N forms on higher moorland plants. Concentrations were much smaller than those found in bryophytes.

- The impacts of NH_3, per unit N deposition continues to exceed those of NH_4^+.

- The risk of *Phytophora* damage to *Calluna* is exacerbated by N deposition, especially as NH_3. Death of the terminal 1 to 2 cm of shoot was observed this summer (2002).

Background

The largest contributor to total N deposition to semi-natural ecosystems is reduced N, emitted mostly from agricultural sources, which deposits either as dry gaseous ammonia (NH_3) or as wet ammonium ions (NH_4^+). Wet N deposition has been reasonably well quantified, however, quantification of N deposition as NH_3 is more complex. NH_3 deposition to vegetation is bi-directional, dependent on foliar N concentration, deposition velocity, canopy resistance, meteorological conditions and the ambient NH_3 concentration. Previous studies (Flechard & Fowler, 1998) have investigated deposition at low ambient NH_3 concentrations to a moorland. In Phase I, NH_3 flux measurements were made to refine NH_3 deposition estimates to moorland vegetation by quantifying the dependence of the surface affinity of the vegetation for NH_3 uptake at a range of ambient NH_3 concentrations and the effects of atmospheric conditions on the NH_3 exchange process. Results showed that calculating NH_3 deposition using a constant deposition velocity overestimates deposition at higher (> 5 $\mu g\ m^{-3}$) ambient NH_3 concentrations by over 100% above 30 $\mu g\ m^{-3}$. During Phase II of this contract we shall continue to refine the relationship between N deposition and NH_3 concentration for a range of monocultures of bryophytes and other moorland species.

Our understanding of the impacts of NH_3 and NH_4^+ on vegetation has been largely derived from pot experiments where it is difficult to simulate competitive effects and also study interactions between N uptake and soil. In the field N driven changes in species composition are often associated with changes in the competitive balance for light as growth is stimulated and a large fraction of the added N is immobilised. The implications of these activities for Critical Loads (CL) needs to be better defined. Using soil-peat and regenerating vegetation from an abandoned peat extraction site, eleven $3m^2$ monoliths were established in the OTC's at CEH Edinburgh to evaluate the differential effects of gaseous NH_3 (0 - 90 $\mu g\ m^3$ NH_3) and solution phase NH_4^+ (0 - 128 kg N ha^{-1} y^{-1}) on a range of moorland plants. Seven plant species were arranged in identical repeating units and spacings in each of 11 chambers (Report phase I). A dose response study was selected, utilising a range of N inputs for the two different reduced N forms, over a replicated study with fewer N additions.

In this second phase the *C. vulgaris* plants in the higher N treatments have progressed beyond the canopy-building phase providing an opportunity to monitor competition effects.

This report describes the results for TASK 6 for the period April 2001 – July 2002.

Objectives Task 6 (2001 – 2004)

- Continue to refine the relationship between ambient NH_3 concentrations and deposition focussing on monocultures of semi-natural species, particularly bryophytes.

- Compare the long-term impacts of wet NH_4^+ and dry NH_3 on stress and competition on the vegetation in the established monoliths.

- Evaluate bio-indicators e.g. amino acid levels and chlorophyll fluorescence as tools for distinguishing between NH_3 and NH_4^+ deposition effects.

- Provide data to refine the setting of Critical Loads to moorland vegetation based on an understanding of underpinning mechanisms.

- Determine how prolonged N deposition affects N uptake and storage by systems that have evolved under low N availability.

Methods

The N dose response study, which began in 1999, was continued for a third year in 2001/02, in order to evaluate the effects of long-term exposure to a range of wet (NH_4^+) and dry (NH_3) N deposition on the established moorland monoliths.

Treatments

Six wet deposition mist treatments (de-ionised water, 8, 16, 32, 64, 128 kg N ha^{-1} y^{-1} as NH_4Cl) were applied over a 25-week period (April –September 2001) at a rate of 12 mm per week (3 applications per week) from individual pressurised vessels via a spray droplet generator. The concentrations of the 6 wet deposition treatments were 0, 0.19, 0.38, 0.76, 1.52 and 2.85 mM NH_4Cl respectively.
There were 5 dry N treatments, ambient NH_3 plus four gaseous NH_3 treatments (6, 20, 50, 90 $\mu g\ m^3$) generated by volatilisation of a pumped 1% aqueous NH_3 solution in the air stream of the OTC manifold. NH_3 treatments were applied continuously throughout the period April – November 2001. NH_3 deposition (kg N ha^{-1} y^{-1}) estimated for 2001 was very similar to that of 2000 (Leith *et al.*, 2001). NH_3 treatment chambers received background ambient NH_3 over winter.
Ambient rainfall was excluded in all treatments (wet & dry) during the treatment period by temporary roofs.

Chemical Analysis
Foliar N, P, K was extracted and converted in an acid digest, then NH_4-N was measured by colorimetry using the indophenol blue method, (Grimshaw *et al.*, 1989) ;Phosphate –P using molybdenum – blue and K by atomic absorption spectrophotometry.

Frost Hardiness
Frost hardiness was measured on detached current years growth using a programmable freezing cabinet. Damage was assessed from ion leakage (Sheppard & Leith, 2002).

Chlorophyll Fluorescence
The responses of photosystem II (PSII) were assessed using a modulated fluorescence meter (HANSATECH Handy Pea). The technique is non-destructive and the cuvettes can accommodate different thicknesses of shoot, making it a useful tool for working with bryophytes. Small cuvettes are placed over 1 cm of current year shoot and left for 20 min to dark-adapt. Illumination is applied and the kinetics of the response analysed. The Fv/Fm ratio is a measure of the maximum quantum efficiency of PSII. Stress can reduce this ratio, although it is not fully understood whether this reduction represents a reversible photo-protective down regulation or an irreversible inactivation of PSII. Measurements were made, 10 weeks into the treatment period on *C. vulgaris* and *M. caerulea* on 10 shoots / tillers per treatment.

Amino acids (aa)
Amino acids were measured by HPLC at the University of Newcastle. These analyses require sophisticated equipment, none standard sample preparation and involve time consuming extractions. However, in conifers the accumulation of specific amino acids has been used as an indicator of excessive N deposition (Rabe, 1990). We measured the aa composition of unreplicated bulked samples of *Calluna* and *Molinia* from the 11 treatments to evaluate their potential use as a bioindicator.

Shoot extension and Biomass
Short extension was monitored in *C. vulgaris* on 15 shoots from April to September 2001. In September the inflorescence and green leaves were removed, separated and weighed for *M. caerulea*. 3 samples of *M. caerulea* per treatment chamber were taken. Sub- samples of these were used for nutrient analysis.

Results and Discussion

Species composition changes.
Currently, (June 2002) *C. vulgaris* is dominating the graminoid species in all wet and dry N treatments except the two controls and the wet 128 kg N ha^{-1} y^{-1} treatment (Fig 2a). In the two control treatments growth has been slow with neither species dominating. However, in the wet 128 kg N ha^{-1} y^{-1} treatment there has been a dramatic change in ground cover with a large decrease in *C. vulgaris*, which has opened the canopy allowing *M. caerulea* cover to increase (figure 2b). This decrease is not due to any secondary stress factors such as frost damage or heather beetle, but appears to be the direct result of applied N deposition over the 3 years *i.e.* t N toxicity. The cause of this loss of vitality in *Calluna* is not clear but may reflect adverse effects on roots and mycorrhiza. This summer, mycorrhiza baiting experiments have been established in the OTC's using young *C. vulgaris* and *Deschampsia flexuosa* plants to assess the impact of the different N deposition and forms on infectivity and root vitality. N_2O emissions will also be assessed to determine if the N supplied has exceeded the demands of the vegetation making more available for transformations by soil microbes.

Foliar nutrient concentrations

Foliar nutrient concentrations are increasingly being used as an indicator of N deposition. Countrywide sampling, comparing present day foliar N concentrations for *C. vulgaris* with samples collected in the 1970's have shown a shift in the upper values from 1.2 to 1.5%, commensurate with the measured increases in N deposition over this period (Val. Kennedy pers. comm.). However, N deposition is not the only variable to affect foliar N concentrations, time of sampling, growth regimes, soil N status and plant age have all been found to influence foliar N concentrations. In this experiment, foliar N has been measured in a range of species to determine how absolute values correspond to N inputs when other factors e.g. soil, timing of sampling, growth regimes and timing of treatment additions are kept constant, and the potential role of interactions with other nutrient availabilities e.g. P and K and stress is minimised.

In all 3 years regression analysis has shown that, independent of species, the linear relationship between foliar N concentration and N deposition is strong and significant ($p<0.05$). In addition, gaseous NH_3 increases foliar N concentrations by more than NH_4^+ per unit N input. It can also be seen (Table 1) that both absolute foliar N concentrations, (Table 2) and the proportional increase in foliar N vary between species e.g. the 128 kg N ha^{-1} y^{-1} treatment almost doubled foliar N in *E. vaginatum* compared with approximately 30% increase in *C. vulgaris.* Despite these large N inputs the mean foliar N concentration has fallen (Table 1, Figure 1 for *Calluna*). Between 1999 and 2001 almost every species, irrespective of treatment has exhibited a 17-34% decline for wet NH_4^+ and 12-19% decline for NH_3, in foliar N (Table 2). During this time foliar P values have not changed nor reflected treatment induced growth effects, which suggest P availability is not a limitation in these monoliths. Comparison of photographs taken at the start of treatment and over successive years indicates a considerable increase in the size of the *Calluna* plants especially in the higher N treatments. The fall in foliar N concentrations may reflect the large increase in plant size, above and probably below ground. This hypothesis can be tested when the chambers are harvested and may also be addressed by the point quadrat analysis, being undertaken later this year. Certainly the additional N has increased the proportion of woody material. It is also possible that plants such as *C. vulgaris,* which have evolved under low N conditions, when transferred to high N deposition, take up more N initially than subsequently once their systems have begun to acclimate. If there is evidence of acclimation to increasing N inputs manifest, via tighter control over N uptake – this will have implications for the interpretation of foliar N data. Interestingly foliar N concentrations in the NH_3 treatments have fallen less than those in the wet deposition treatments where the uptake of NH_3 (via stomata) may be more difficult to control. We shall be installing throughfall collectors in the OTC's to quantify the extent of foliar N uptake.

Effects of N deposition on *Sphagnum capillifolium*

As in 2000, there was a significant linear relationship with both wet and dry N deposition and % N foliar concentration. On an N input basis NH_3 treatments increased foliar N concentration by more than wet deposited N.

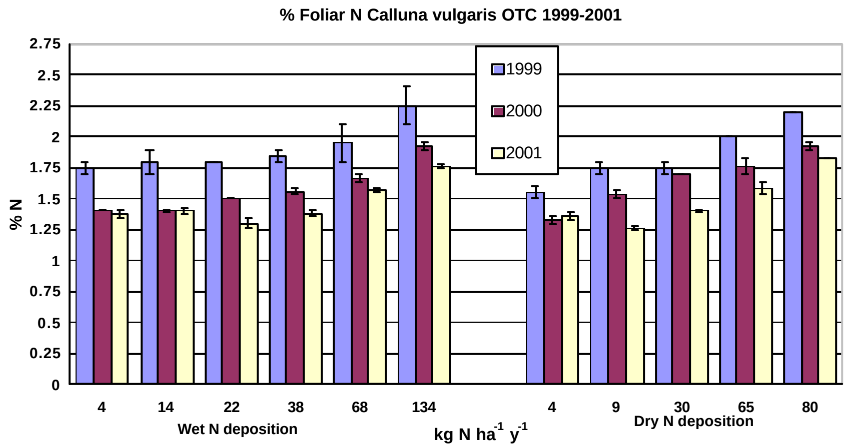

Figure 1. Foliar N concentrations (± 1 s.e.) in current year *C.vulgaris* in wet and dry N deposition treatments for 3 consecutive years (1999 – 2001).

NH_4^+	*Calluna vulgaris*	*Molinia caerulea*	*Eriophorum vaginatum*	*Deschampsia flexuosa*	*Narthecium ossifragum*	*Potentilla erecta*
1999	1.90	2.18	1.22	1.92	2.10	2.33
2000	1.57	1.97	1.0	1.48	1.82	1.50
2001	1.46	1.72	0.97	No sample	1.48	1.68
NH_3						
1999	1.85	1.79	1.17	1.62	2.02	2.10
2000*	1.65	1.87	1.10	1.40	1.50	1.50
2001*	1.49	1.49	1.04	No sample	1.62	1.77

Table 1.Mean foliar N concentrations (%N dry wt) for all treatments measured in September in moorland species growing in OTC's from 1998 (pre-treatment) to 2001. Wet deposition treatment inputs range from control to 128 kg N ha^{-1} y^{-1} and dry concentrations up to 90 µg m^{3}. *N deposition in 2000 and 2001 was more than double that in 1999 for NH_3 concentrations > 15 µg m^{3}.

	C. vulgaris	*M. caerulea*	*E. vaginatum*	*D. flexuosa*	*N. ossifragum*	*P. erecta*
Wet						
1999	+29	+46	+96	+100	+19	+39
2000	+38	+32	+92	+137	+70	+73
2001	+23	+40	+73	No sample	+97	+47
Dry						
1999	+42	+35	No sample	+82	+29	+47
2000	+45	+28	+79	+116	+47	+46
2001	+35	+52	+106	No sample	+73	+62

Table 2. Percent change in Foliar N concentrations between control and high N input treatments for a range of species over 3 years of controlled wet and dry N inputs.

Amino acids

Levels of individual amino acids were found to be quite low in both the ericoid and the graminoid species, especially by comparison with bryophyte levels (Carole Pitcairn: pers comm.).

The relationship between N deposition and individual amino acids in foliage was generally poor and there was no obvious difference in response to the different N forms. The tendency for graminoids to accumulate glutamic acid in response to N was observed. *C. vulgaris* which has a relatively low nutrient status (Ellenburg value of 3) showed no signs of accumulating arginine, indicating that N is not being stored as amino acid.

Biomass (2001)

A strong linear relationship was found in *M. caerulea* for tiller biomass, inflorescence (flower and seed production) biomass and wet and dry N deposition.

Frost Hardiness

Frost hardiness measured in the autumn (November 2001) and midwinter (January 2002) indicated no significant effects of N deposition either as NH_3-N or NH_4-N on frost hardiness. However, there has been an increasing tendency over all 3 years of assessments, for increased frost sensitivity in shoots receiving 90 μg NH_3 m^3. This suggests an adverse effect of the high NH_3 concentration rather than the cumulative impact of N dose.

Fluorescence (PEA) measurements (summer 2001)

Measurements of continuous excitation fluorescence (efficiency of photosystem II) in *M. caerulea* indicated some stimulation in response to additions of both N forms. Measurements of *C. vulgaris* showed no effect of N treatments (wet or dry) on Fv/Fm ratio. These observations show that the efficiency of photosystem II was functioning acceptably at approximately 1.25% N in *Calluna* foliage.

Visible foliar injury

Visible foliar injury has been observed in *C. vulgaris,* predominantly in the high dry N treatment and to a lesser extent in the highest wet N treatment in June 2002 (fig. 2c). The symptoms correspond to those caused by *Phytophora* sp. (downy mildew). Previous incidences were recorded 15 years ago coinciding with high rainfall and high water tables (S. Helfer Royal Botanic Gardens, Edinburgh pers. comm..). Brown shoots have also been observed at our field site at Whim Bog, where they are not linked to increased N deposition. However, in the OTC's (Table 3) and also at another N manipulation sites (Simon Caporn pers. Comm.) the incidence of injury is much higher in the higher N treatments.

	NH_3 Concentration (μg m^3)					NH_4^+ Deposition (kg N ha^{-1} y^{-1})					
	Control	6	20	50	90	Control	8	1 6	32	64	128
No. of damaged shoots	0	0	15	20	>400	0	0	0	1	20	>100

Table 3. Number of damaged *Calluna* shoots in each of the N treatments on 30 July 2002.

2a

2b

2c

Figure 2a. A large decrease in *C. vulgaris* and an increase *in M. caerulea* cover the 128 kg N ha $^{-1}$ y^{-1} wet N deposition treatment. 2b. *C. vulgaris* dominating in the 90 μg m^{-3} NH_3 dry treatment with a large decrease in graminiod species cover. 2c. Visible foliar injury to *C. vulgaris*, June 2002.

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