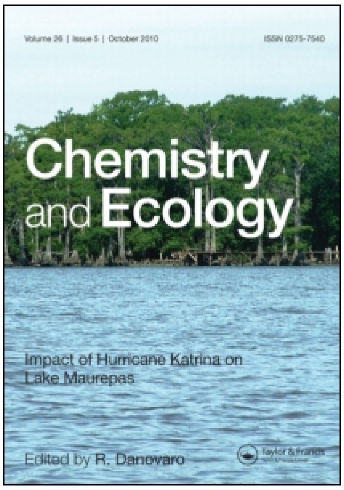

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

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Online publication date: 14 September 2010

To cite this Article Cresser, M. S. , Parveen, Z. , Smart, R. , Deacon, C. , Gammack, S. M. and White, C.(2002) 'The impact of enhanced ammonium sulphate inputs exceeding critical load on calluna vulgaris /peat soil microcosms', Chemistry and Ecology, 18: 3, 195 — 212
To link to this Article: DOI: 10.1080/02757540215058
URL: http://dx.doi.org/10.1080/02757540215058

Chemistry and Ecology, 2002, Vol. 18(3–4), pp. 195–212

THE IMPACT OF ENHANCED AMMONIUM SULPHATE INPUTS EXCEEDING CRITICAL LOAD ON *CALLUNA VULGARIS*/PEAT SOIL MICROCOSMS

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(Received 1 November 2000)

Ammonium sulphate at six concentrations in simulated precipitation has been applied weekly over two years to *Calluna vulgaris* growing in peat soil. The nitrogen deposition treatments were chosen to embrace and exceed critical load. The growth and composition of the *Calluna* and the changes over time in the chemistry of the peat soil and its soil solution were monitored. In spite of significant increases in foliar nitrogen concentration in new shoots, especially in the first year, growth did not increase significantly in response to nitrogen treatment. Several factors could be contributing to the lack of significant growth response. (1) Increasing ammonium input significantly acidified the soil solution, which could adversely effect growth directly. (2) Foliar calcium concentration was reduced significantly in both years by the ammonium sulphate treatments, and more calcium was undoubtedly lost from the rooting zone at higher nitrogen inputs. (3) Foliar phosphate declined significantly between the first and second year, so lack of growth response might also reflect a phosphorus limitation. There was a distinctly visible darkening of the leaves in response to increasing ammonium applications, especially for the first year's growth, and the chlorophyll a and b concentrations in leaves from new growth at the three highest nitrogen treatments were significantly (at $P < 0.05$) higher than those for the control. The pigment concentrations fell markedly by the end of the second season, and treatment effects were much less consistent. It is suggested that pigment analysis therefore probably has little diagnostic value for assessing damage from pollutant nitrogen effects.

Keywords: *Calluna*; Peat; Nitrogen deposition; Acidification; Base cation leaching; Critical load

INTRODUCTION

In Europe, the potential adverse effects of increased nitrogen deposition on sensitive, minimally managed ecosystems have become a cause for considerable concern (UKRGIAN, 1994; Pitcairn and Fowler, 1995). Carroll *et al.* (1999) have recently summarised concisely the problems likely to be encountered when vegetation communities that are adapted natu-

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ISSN 0275-7540 print; ISSN 1029-0370 online
DOI: 10.1080/0275754021000065871

rally to low nitrogen inputs are subjected to elevated nitrogen supply. Possible changes include loss of more sensitive species and a shift in vegetation structure towards more competitive species in response to enhanced nutrient nitrogen. This has stimulated research on the differential response of upland plants in the UK to nitrogen deposition (Leith *et al.*, 1999), and on nitrogen deposition effects on communities likely to be adversely affected. The latter include lowland dry heathlands (Bobbink *et al.*, 1992; Power *et al.*, 1998; Uren *et al.*, 1997) and upland moorland (Lee *et al.*, 1992; Caporn *et al.*, 1995; Carroll *et al.*, 1999).

It is difficult to exploit naturally occurring nitrogen deposition gradients to assess reliably the effects of enhanced nitrogen inputs from the atmosphere on plant communities, because factors other than nitrogen deposition often may also exhibit coincidental gradients. In a recent study, a range of upland plant species was sampled along altitudinal transects in northern Britain because nitrogen deposition was known to increase with altitude along the transects (Hicks *et al.*, 2000). The plant tissue showed marked increases in foliar nitrogen concentration as nitrogen deposition increased with altitude. It was concluded, because foliar phosphate concentration showed no similar significant change, that the nitrogen effect directly reflected increased nitrogen supply. This is, however, debatable in the absence of data on variation in soil phosphate supply along the same transects, because limitation in growth imposed by limited phosphorus supply might indirectly increase nitrogen concentration. It is also difficult to design experiments that unequivocally factor out nitrogen deposition effects *per se* from impacts of changes in intensity of grazing or changes in land use or traditional management practices (Marrs, 1993).

Several researchers have resorted to medium-to long-term simulation experiments in which nitrogen input is as far as possible the only variable. Such experiments may be performed either in the field (Uren *et al.*, 1997; Power *et al.*, 1998; Carroll *et al.*, 1999; Leith *et al.*, 1999) or in more controlled glass house conditions (Yesmin *et al.*, 1995, 1996a, b). A problem even with experiments such as these is that the soil may be well buffered against rapid change. It is not always obvious therefore how long simulated additional nitrogen deposition treatments need to be applied before "damage" symptoms will be observed. Therefore, some such field studies have been continued for several years, on the basis that "for as long as possible" is the best compromise answer to the question "how long for?" (Power *et al.*, 1998; Carroll *et al.*, 1999).

It is sometimes postulated that atmospheric pollutant nitrogen will have a fertiliser effect on plant growth in upland ecosytems, but this is not invariably the case. In a long-running, lowland *Calluna* dry heath study in southern England, ammonium sulphate spray was applied approximately weekly for 7 yr (Power *et al.*, 1998). A final destructive harvest showed significant increases in current year's growth yield and in total dry matter production only for the highest nitrogen treatment applied ($15.4\,\mathrm{kg\,N\,ha^{-1}\,yr^{-1}}$ supplement to the ambient $13–18\,\mathrm{kg\,N\,ha^{-1}\,yr^{-1}}$ at the site). The highest nitrogen treatment also increased current year foliage and litter nitrogen concentrations. Lack of significant growth response at the lower nitrogen treatment suggests a factor other than nitrogen might well have been growth limiting.

In a study of nitrogen deposition effects at an upland *Calluna* moorland site in north Wales, much larger nitrogen treatments were applied monthly over 8 yr, as ammonium nitrate, to give supplementary nitrogen loads of 40, 80 and $120\,\mathrm{kg\ N\,ha^{-1}\ yr^{-1}}$ (Carroll *et al.*, 1999). Initially the treatments resulted in consistent and striking increases in annual shoot extension. However, the effect was not sustained, becoming less significant after 4 yr and insignificant after 8 yr. The change in behaviour was attributed to late winter injury, characterised as browning of shoot tips in spring.

Most studies of nitrogen deposition effects on UK non-forest upland ecosystems, such as those discussed above, have been conducted with a view to establishing if, and, if appropriate, at what input flux, nitrogen deposition "damages" the ecosystem. The criterion for damage is invariably some observable adverse effect upon plant growth or vegetation species distribution. If soil chemical change is considered at all, it is generally assumed that an assessment of the effects of sustained nitrogen inputs on nitrogen mass balance in underlying soils is all that is required. However, adverse effects of inorganic nitrogen inputs from the atmosphere could be due to other soil chemical changes, such as fall in pH of soil solution or excessive loss of nutrient base cations such as potassium, calcium or magnesium. It is widely accepted already that ammonia deposition is a potential cause of indirect soil acidification (van Breemen *et al.*, 1982; Rorison, 1986; UKRGIAN, 1994), and that it may cause direct leaching losses of base cations (White and Cresser, 1998; White and Cresser, 1999).

Unfortunately, because long-term field experiments are both time consuming and expensive to maintain, routine monitoring of soil and/or soil solution chemistry is not always feasible, and scant attention is often paid to soil as an ecosystem component. For example, the soil at the Welsh site was described by Carroll *et al.* (1999) as "well drained shallow peat (8–10 cm deep) overlying a silty clay loam (Hiraethog series)." Based upon this description, there is no indication of the extent to which mineral bio-geochemical weathering could replenish base cations leached as a consequence of the treatment effects. This may be especially important, bearing in mind that high doses of ammonium nitrate were being applied monthly. The omission of soil information thus makes it difficult to assess whether or not the results are site specific.

From the above discussion, there is a real need to study direct effects of ammonium inputs on both soil chemistry and on vegetation simultaneously. The soil/plant ecosystems need to be subjected to a realistic outdoor temperature regime. It seemed to the authors that a strong case could be made for studying *Calluna vulgaris*/peat microcosms in this context, because of the widespread occurrence of *Calluna* growing on peat in the British uplands and its ecological importance. The UK upland environment often favours acidic peat accumulation, and also favours the accumulation of ammonium in soils as a direct consequence of cation exchange reactions (Yesmin *et al.*, 1996a). Replenishment of leached base cations by mineral weathering is not possible, so the systems should be very sensitive to leaching and pH effects of ammonium deposition, and any possible direct biological effects of ammonium accumulation. Moreover, the critical loads of acidity for peats so far calculated for the UK are based on the atmospheric deposition load predicted to cause acidification of peat by 0.2 pH units (Hornung *et al.*, 1995; Smith *et al.*, 1992) , taking into account acidification effects of modelled nitrogen biological transformations. No robustly tested system has yet been suggested for quantifying nitrogen critical loads for peat soils. Moreover, what supposedly happens when a critical load of nitrogen is exceeded, is also poorly understood for peat soils. Thus attempts to relate observed growth effects of nitrogen inputs on *Calluna* to quantifiable soil chemical changes also seem very worthwhile.

The experiment described in this paper is the result of an attempt to relate critical loads and their excedance to measurable soil change and to vegetation effects for *Calluna*/peat ecosystems. The authors have set up a series of experiments to examine the relationships between the effects of various deposition parameters on *Calluna* growth and vitality and on the chemistry of peat and its associated soil solutions. This paper reports changes and effects over 24 months of ammonium sulphate deposition enhancement on the composition of peat, peat soil solution, and of *Calluna vulgaris* growing in the peat.

METHODOLOGY

Collection and Preparation of the Peat

As in our previous study of the effects of simulated sulphuric acid deposition on *Calluna vulgaris*/peat microcosms (Parveen *et al.*, 2000), peat was collected from a profile with peat at a depth of >1 m under *Calluna* moorland in a relatively unpolluted area of Great Britain (Shieldaig, in Western Scotland; OS grid reference NG 852485). The top 50 cm of the peat was sampled, and obvious large roots being removed before bagging. On return to the laboratory, the peat was partially air dried by spreading to a ca. 3 cm layer on polythene sheets on benches in a vacant room. The peat was turned frequently, until the moisture content was sufficiently low to allow it to pass readily through a 5 mm sieve. Replicate sub-samples were then subjected to chemical analysis by routine methods. Chemical and physical properties of the peat have been presented elsewhere (Parveen *et al.*, 2001) but are summarised in Table I.

Calluna Pre-treatment and Establishment of the Pot Experiment

Partial drying of the peat facilitated both homogenisation and potting. Complete air-drying was deemed inappropriate, because of the changes that would be induced in the physical, chemical and biological properties of the peat on full drying. In view of possible variation in moisture content, however, pots subsequently were filled by volume rather than by mass of peat. Thus 8.5 dm^3 of sieved peat was added to each of a series of 15 pots. Each pot had an upper diameter of 25.5 cm and was 21.5 cm deep.

Calluna vulgaris plants (of a single cultivar, obtained from a nursery in Northumberland) were used for the experiment, with a view to minimising variability between responses of individual heather plants. The peat-based nursery potting compost was carefully teased from the roots of the 3-yr plants with fingers (in polythene gloves) and a spray of water.

TABLE I Selected properties of the Sheildaig peat

pH (water)	4.22
pH ($CaCl_2$)	3.56
Moisture (%)	190
C (%)	38.4
N (%)	2.03
C:N ratio	19
K_{Exch} ($mmol_c\ kg^{-1}$)	1.68
Na_{Exch} ($mmol_c\ kg^{-1}$)	1.94
Mg_{Exch} ($mmol_c\ kg^{-1}$)	9.29
Ca_{Exch} ($mmol_c\ kg^{-1}$)	11.8
CEC ($mmol_c\ kg^{-1}$)	1107
Base saturation (%)	0.23
Al_{Exch} ($mmol_c\ kg^{-1}$)	27.31
Ca:Al ratio	0.43

Some loss of fine roots and associated mycorrhizas occurred, but this was small and the plants with cleaned roots still had a very extensive root systems. Each *Calluna* plant was potted immediately after the root treatment in thoroughly mixed peat, and the pots were then randomised. The transplanted plants were kept outdoors in a sheltered position in light dappled shade for a month prior to nitrogen treatment applications. Soil solution sampling (see below) was started 4 weeks later. The microcosms were lightly sprayed when necessary with deionized water to maintain a suitable moisture content in the peat. In total, 84 plants were treated in this way for a series of four experiments. Only two of these plants showed obvious signs of damage following the treatment, and these two were discarded.

The potted plants were then moved to their permanent site in a 30 cm deep trench in the Cruickshank Botanic Garden at Aberdeen University. They were stood in randomised positions on a 10 cm layer of washed coarse granite chips over a layer of overlapping heavy duty black polythene sheets at the bottom of the trench. Slots were cut in the sheets at intervals to allow drainage water to pass into the underlying freely draining sand. The space between the pots was then filled with washed coarse granite chips up to 1 cm below the pot rims. Thus all treatments experienced the same climate and were subjected to realistic prevailing outdoor temperature.

Nitrogen Treatments and Their Application

In addition to the natural Aberdeen rainfall, all microcosms received a twice weekly supplement of artificial rain, sprinkled over the plants and the surface of the peat, to give an annual total rainfall supplement of 1345 mm. The rain supplement contained the treatments to be applied. Since the annual average rainfall for Aberdeen is 655 mm, the artificial rain treatments result in an annual total precipitation of 2000 mm. This is a realistic value for many other parts of Scotland. Assuming the annual evapo-transpiration for Aberdeen for grassland vegetation to be 410 mm, the effective rainfall (precipitation excess or drainage) would be 1590 mm. Bulk deposition (wet + dry) collectors were also installed to monitor annual natural precipitation amount and composition.

The simulated rain supplements for nitrogen deposition contained ammonium sulphate concentrations to give a range of total ammonium loads from 0 to 27 $kmol_c\,ha^{-1}\,yr^{-1}$ (see Tab. II). However the simulated rainfall contained, in addition, background concentrations of base cations and anions appropriate for north east Scotland. This is essential for simulated rain experiments with soil organic horizons, because it is well known that sodium concentra-

TABLE II Summary of the concentrations ($\mu mol\ l^{-1}$) of the solute species in treatments for the enhanced ammonium deposition experiment

Treatment	*Total N load* $kmol\,ha^{-1}\,yr^{-1}$	$(NH_4)_2SO_4$	*NaCl*	$MgSO_4$	$CaCl_2$
N0	0.00	0.0	118	14.4	7.1
N1	2.09	58.2	118	14.4	7.1
N2	3.8	112	118	14.4	7.1
N3	7.1	238	118	14.4	7.1
N4	13.8	489	118	14.4	7.1
N5	27.1	992	118	14.4	7.1

tion of rainfall influences the extent of retention of atmospheric ammonia by such soils (Duckworth and Cresser, 1991). This fact is often overlooked in nitrogen addition experiments.

In addition to the nitrogen received via the treatment applications, all pots received nitrogen in ambient Aberdeen precipitation. The mean annual nitrogen deposition rate in this precipitation over the two years was 12.5 kg $ha^{-1} yr^{-1}$ of which 6.81 kg was as nitrate and 5.69 kg as ammonium. Thus the control treatment is below the critical load of 15–20 kg N ha^{-1} yr^{-1} proposed for dry heathland ecosystems (Uren *et al.*, 1997).

Soil Solution Sampling and Analysis

A rhizon sampler (Meijboom and van Noordwijk, 1992) was fitted to sample soil solution from 0–10 cm depth in each pot, approximately mid-way between the plant and the pot rim. This was to allow soil solution anion and cation composition to be monitored at regular intervals over approximately 24 months (720 days). Samples were taken at 4- and 6-week intervals in the first and second years, respectively. Upon collection, samples were immediately analysed for pH. They were then stored in a cold room at 4 °C prior to determination of ammonium by automated colorimetry, base cations (Ca^{2+} and Mg^{2+} by atomic absorption spectrometry, $Na^+$and K^+ by flame emission spectrometry) and key anions (SO_4^{2-}, Cl^-, NO_3^-, PO_4^{3-} by ion chromatography). Analysis was generally completed within 7 days of sampling.

Soil Sampling and Analysis

Triplicate sub-samples of the peat were analysed fully at the start of the experiment in October, 1997 (Tab. I), and, thereafter, to assess treatment effects on the soil, in October, 1998 and in April and October, 1999. Sample availability was limited for these analyses, so only pH and carbon and nitrogen were determined. A 10-mm stainless steel cork borer was used to sample the peat to a depth of 10 cm. Roots were carefully removed by hand prior to analysis.

Plant Growth Measurements and Plant Sampling and Analysis

Growth parameters monitored non-destructively included number of flowering shoots per plant, and, as a measure of plant bushiness, the % bare peat surface exposed when viewed from directly above. The latter was estimated three times for each plant, and the mean value taken. Both parameters were measured in October in both 1998 and 1999. Plant height was measured in 1999 only (there were no apparent height trends in 1998).

Plant samples were taken for chemical analysis at annual intervals in October, 1998 and 1999. Ten shoots were harvested at random from each plant from the current season's growth, using sharp scissors. Leaves were removed from the stems and analysed for the plant pigments, chlorophyll a and chlorophyll b (Cresser and O'Neill, 1980), carbon, nitrogen and base cations (Cresser and Parsons, 1979). The pigments were extracted by grinding a sub-sample of fresh material with liquid nitrogen and extracting with 80% acetone. The remainder of the leaf sample was oven dried at 80 °C, weighed and ground, and a 100 mg sub-sample was subjected to a Kjeldahl digestion for base cation analysis (Cresser and Parsons, 1979). A further small sub-sample was used for carbon and nitrogen determination using a CNS analyser.

Statistical Analysis

All data were analysed using the package SPSS9. Treatment effects were tested by ANOVA. Where significant effects were found, LSD values were calculated between individual treatment means at $P \leq 0.05$.

RESULTS

Effect of Enhanced Ammonium Deposition on *Calluna vulgaris*

Mean data for plant parameters (number of flowering shoots per plant and foliar concentrations of chlorophyll a and chlorophyll b (g kg^{-1})) are summarised in Table III. There was no significant treatment effect on visible soil %. Significant treatment effects were evident for the pigments in 1998, with chlorophyll a and b concentrations increasing with nitrogen treatment level. No such trend was seen by 1999, however, and in 1999 the N1 treatment apparently gave spurious results which could not be related to direct visual observation of the plant colour. As expected, the number of flowering shoots per plant was significantly higher in 1999 than in 1998, while % visible soil (data not shown) was significantly lower. There was a dramatic and significant decline in plant pigment concentrations over the second year of growth (Tab. III).

Enhanced ammonium application had a significant ($P < 0.05$) consistent and positive effect on the nitrogen concentration of *Calluna* leaves in 1998, but a rather less consistent effect in 1999 (Tab. IV). After the second year of treatment application, nitrogen concentrations were consistently and significantly lower than those after the first year, except for the control (N0). They were still generally higher than typical literature values of 1.05% for mature *Calluna* plants, however (Batey *et al.*, 1974). Leaf carbon concentration did not show any significant change with treatment, but C:N ratio significantly ($P < 0.05$) decreased with increasing the rate of ammonium application (Tab. IV), especially in 1998 when all treatments from N2 to N5 caused a significant decline in C:N ratio. This reflects primarily the increase in leaf nitrogen % with increasing nitrogen input. In 1999, the change was only significant for the N4 and N5 treatments compared to the N0–N2 treatments.

Leaf calcium concentration in both years, and magnesium concentration in 1999, exhibited significant ($P < 0.05$) effects of ammonia application (Tab. IV). Leaf calcium concentration apparently decreased in response to the higher nitrogen treatments (N4 and N5) in 1998, and

TABLE III Effects of enhanced ammonium sulphate deposition on the number of flowering shoots on the individual *Calluna vulgaris* plants and on foliar pigment concentrations (mg g^{-1}, means of 3 determinations) after 12 and 24 months of treatment application

	Treatment						
Parameter/Year	*N*0	*N*1	*N*2	*N*3	*N*4	*N*5	*LSD* 5%
Flowering shoots/98	107	170	119	113	138	169	ns
Flowering shoots/99	172	274	201	322	240	298	ns
Chlorophyll a/98	1.67	2.32	2.35	2.81	3.46	3.19	0.76
Chlorophyll a/99	2.03	0.88	2.00	1.71	1.65	1.88	0.61
Chlorophyll b/98	0.90	1.11	1.17	1.39	1.71	1.57	0.37
Chlorophyll b/99	1.05	0.59	0.89	0.92	0.90	0.99	0.21

TABLE IV Effects of enhanced ammonium sulphate deposition on base cation and phosphorus ($mg\,g^{-1}$) and carbon and nitrogen (%) concentrations, (all means of 3 determinations) of the *Calluna vulgaris* leaves after 12 and 24 months of treatment application

Parameter/Year	*N*0	*N*1	*N*2	*N*3	*N*4	*N*5	*LSD* 5%
	Treatment						
Calcium/98	4.63	3.85	4.34	4.52	3.64	3.60	0.39
Calcium/99	4.22	4.09	4.41	4.52	3.75	3.35	0.58
Magnesium/98	1.33	1.29	1.41	1.39	1.19	0.99	ns
Magnesium/99	1.23	1.49	1.49	1.47	1.19	1.10	0.19
Potassium/98	3.95	4.04	3.51	3.63	3.20	2.79	0.68
Potassium/99	3.41	4.17	4.13	3.89	4.13	3.15	ns
Phosphorus/98	1.37	1.61	1.24	1.28	1.22	1.43	ns
Phosphorus/99	1.12	1.15	0.97	1.11	0.97	0.96	ns
Nitrogen/98	1.09	1.19	1.36	1.61	1.89	2.40	0.21
Nitrogen/99	1.34	1.02	1.08	1.16	1.32	1.58	1.26
Carbon/98	51.30	53.86	53.54	53.36	53.77	55.08	ns
Carbon/99	52.78	51.21	52.91	51.14	51.95	52.99	ns
C:N ratio/98	47.04	46.05	39.54	33.21	28.43	23.02	5.76
C:N ratio/99	48.82	52.09	49.34	44.27	39.63	33.54	9.04

in response to the N5 treatment in 1999. *Calluna* leaf magnesium concentration in 1999 was significantly increased by the N1, N2 and N3 treatments, but then declined in response to N4 and N5 treatments. In 1998, it also showed a decreasing trend with high nitrogen treatment but statistically the result was not significant. The concentrations in *Calluna* leaves of potassium in 1999, and of sodium and phosphorus in both years did not exhibit any statistically significant change with treatment, but foliar phosphate concentration was significantly lower in 1999 than in 1998. In 1998, however, the N4 and N5 treatments significantly reduced foliar potassium concentration compared to that for the control (N0) and N1 treatments.

Effect of Enhanced Ammonium Deposition on Soil Composition

In October, 1997, at the outset of the experiment, the peat pH value was uniform. In October, 1998, April, 1999, and October, 1999, peat pH values with 0.05 M calcium chloride decreased significantly ($P < 0.05$) with increasing ammonium treatment (Tab. V). Peat pH values with water were rather more variable, as might be expected because variation in the

TABLE V Effects of enhanced ammonium sulphate deposition on peat pH with water and with calcium chloride (means of 3 determinations) at times shown

pH Medium/Time	*N*0	*N*1	*N*2	*N*3	*N*4	*N*5	*LSD* 5%
	Treatment						
Water/10/98	4.30	5.07	4.82	4.70	4.30	4.23	0.13
Water/04/98	4.91	4.77	4.58	4.49	4.12	3.96	0.15
Water/10/99	4.51	4.74	4.40	4.07	3.99	3.74	0.13
Calcium chloride/10/97	3.71	3.63	3.69	3.64	3.69	3.69	ns
Calcium chloride/10/98	3.82	3.83	3.74	3.71	3.65	3.55	0.04
Calcium chloride/04/99	3.85	3.79	3.76	3.69	3.65	3.54	0.05
Calcium chloride/10/99	3.83	3.75	3.65	3.53	3.51	3.32	0.06

TABLE VI Effects of enhanced ammonium sulphate deposition on peat carbon and nitrogen concentrations (%) and C:N ratio (means of 3 determinations) at the times shown

Determinant/Time	Treatment						*LSD*
	*N*0	*N*1	*N*2	*N*3	*N*4	*N*5	5%
Carbon %/10/97	36.22	38.82	38.14	34.17	36.56	36.58	ns
Carbon %/10/98	35.10	31.66	34.56	32.24	33.97	32.8	ns
Carbon %/04/99	34.21	33.63	34.01	32.01	36.47	34.31	ns
Carbon %/10/99	37.68	34.02	34.17	36.46	36.88	36.24	2.08
Nitrogen %/10/97	2.20	2.63	2.71	2.87	3.07	3.04	ns
Nitrogen %/10/98	1.75	1.82	2.07	1.92	2.01	1.91	ns
Nitrogen %/04/99	2.04	2.08	2.26	2.11	2.28	1.85	ns
Nitrogen %/10/99	2.18	1.82	1.97	2.05	2.09	2.14	0.15

mobile anion concentration will have a relatively greater influence than when a high dose of sulphate is added. Nevertheless the 1999 data show clear and substantial pH decline with increasing nitrogen input.

As might be expected, peat carbon concentration did not change consistently with treatment on any of the four sampling dates, nor did it change significantly over time. The N1and N2 treatments seemed to give lower % carbon values in October, 1999 compared with the other treatments. Total nitrogen in peat in October, 1998 and in April, 1999 did not exhibit any significant effect in response to enhanced nitrogen treatment. It showed some significant ($P < 0.05$) treatment effects in October, 1999 (Tab. VI), but these were not consistent. No significant effect on peat C:N ratio was found on any of the sampling dates in response to treatment, so this data is not shown.

Effect of Enhanced Ammonium on Change in Soil Solution Composition Over Time

The soil solution pH decreased significantly ($P < 0.05$) with increasing ammonium sulphate application rate, as expected because of the likely high sulphate mobile anion concentrations. In spite of seasonal variations, the trend in treatment effect on hydrogen concentration was similar (N5 > N4 > N3 > N2 > N1 > N0) on almost all sampling dates (Fig. 1). For clarity, LSD values are not shown in the time series plots. However, calculations for individual sampling dates showed that the treatment effect on pH and hydrogen was significant on every occasion. A substantial increase of hydrogen concentration in soil solution was observed over time, especially for the N3, N4 and N5 treatments (Fig. 1). Seasonal trends also were clear, with hydrogen concentration peaks evident in the summers of 1998 and 1999 for all treatments (June to September ran from days 228–349 and 591–712 in 1998 and 1999).

Seasonal trends in the time series plots for chloride concentration in soil solution were very different from those for hydrogen (Fig. 2). There were marked summer minima for chloride especially in 1998. These trends closely reflected the pronounced seasonal variation observed in chloride concentration in precipitation in Aberdeen (data not shown). The latter effect must have been more dominant than any effect of concentration of chloride in soil solution by greater evapotranspiration during the summer. There were no significant or systematic treatment effects upon chloride concentration.

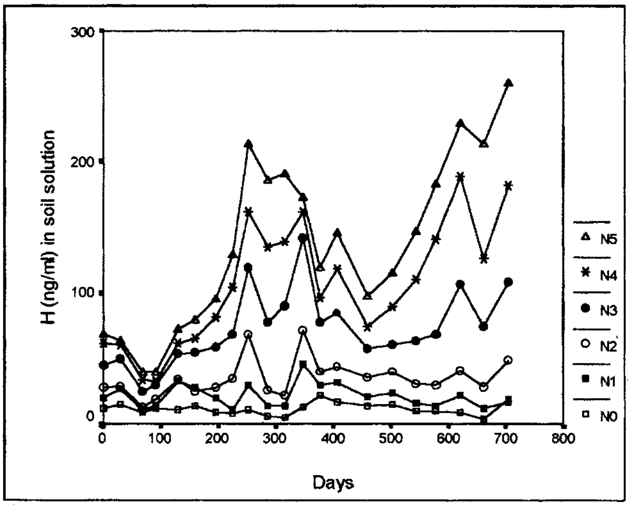

FIGURE 1 Effect of simulated precipitation supplements, formulated to apply 2 to 27 kmol N ha^{-1} yr^{-1} (see Tab. II), on the H^{+} ion concentration of soil solution from the *Calluna*/peat microcosms, over 2 years from October, 1997. In this and all subsequent figures, the microcosms receive, in addition, 0.89 kmol N ha^{-1} yr^{-1} via Aberdeen precipitation.

Sulphate time series plots (Fig. 3) echoed those for hydrogen (Fig. 1), with a consistent and highly significant increase in concentration with increasing ammonium sulphate application rate throughout the experiment, and a pronounced seasonal effect with higher values over the June through September period in both years. However sulphate concentrations were higher in the summer of 1999, than they were in 1998 for all treatments.

Ammonium concentrations in soil solution generally increased with ammonium application rate (Fig. 4), although the effect was not always consistent, especially over the first 130 days. For example, over the period up to 90 days, N1 gave higher ammonium concen-

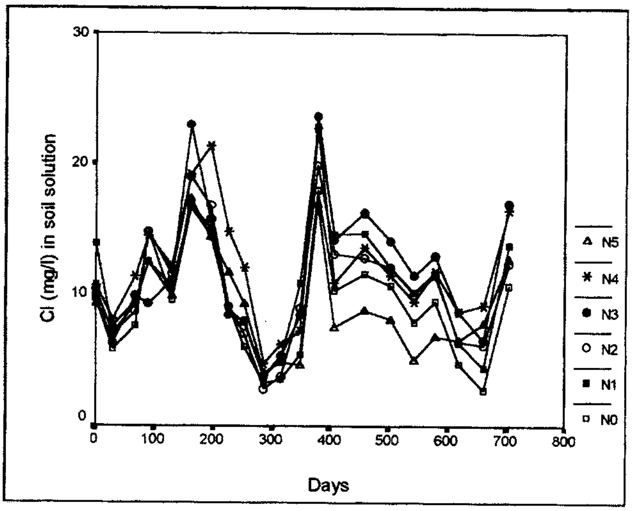

FIGURE 2 Effect of simulated precipitation supplements, formulated to apply 2 to 27 kmol N ha^{-1} yr^{-1} (see Tab. II), on the Cl^{-} concentration of soil solution from the *Calluna*/peat microcosms, over 2 years from October, 1997.

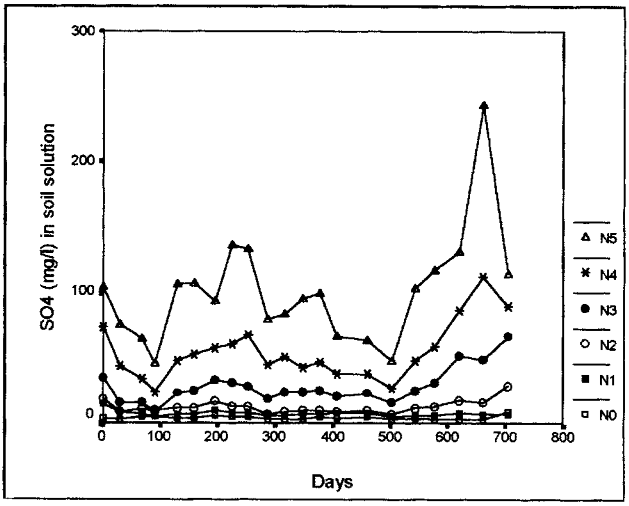

FIGURE 3 Effect of simulated precipitation supplements, formulated to apply 2 to 27 kmol N $ha^{-1}yr^{-1}$ (see Tab. II), on the sulphate concentration of soil solution from the *Calluna*/peat microcosms, over 2 years from October, 1997.

trations than N4. The N1 treatment also resulted in anomalous high nitrate concentrations over the first 170 days (Fig. 5). Ammonium concentrations were generally lower in the May to September period (days 196–348 and 560–712 in 1998 and 1999) than at other times of the year. However, ammonium concentration remained high for the N5 treatment in the second summer (Fig. 4). After cessation of the soil solution monitoring, substantial browning of the *Calluna* foliage was observed in winter for the N5 and N4 treatments.

Apart from the anomalous behaviour for the N1 treatment mentioned in the previous paragraph, the most obvious effect on nitrate concentration was a strong seasonal effect, with pro-

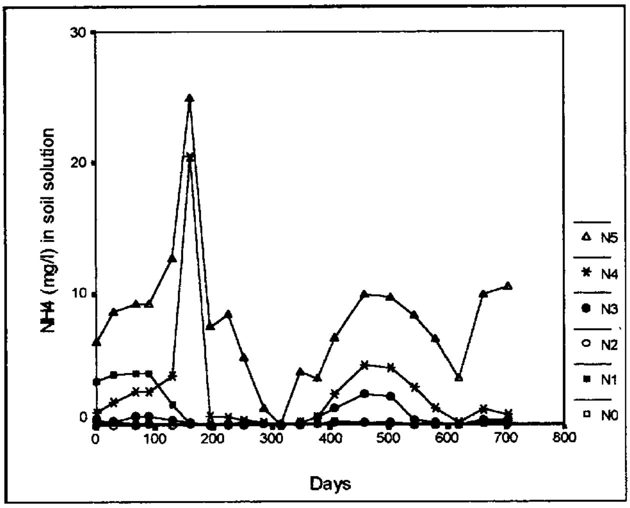

FIGURE 4 Effect of simulated precipitation supplements, formulated to apply 2 to 27 kmol N $ha^{-1}yr^{-1}$ (see Tab. II), on the ammonium ion concentration of soil solution from the *Calluna*/Peat microcosms, over 2 years from October, 1997.

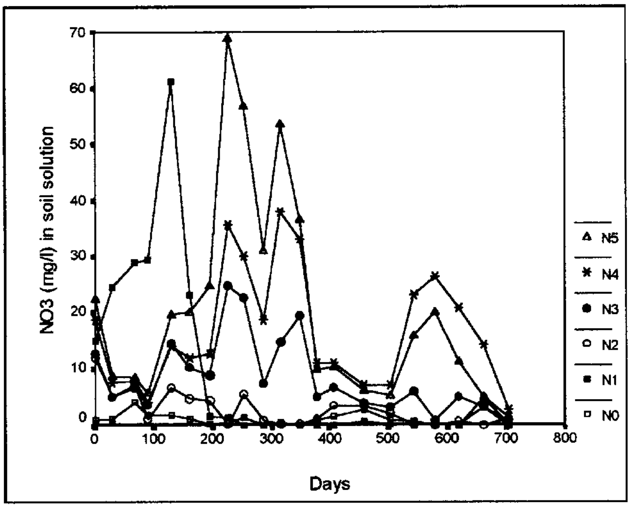

FIGURE 5 Effect of simulated precipitation supplements, formulated to apply 2 to 27 kmol N $ha^{-1} yr^{-1}$ (see Tab. II), on the nitrate ion concentration of soil solution from the *Calluna*/peat microcosms, over 2 years from October, 1997.

nounced spring/early autumn (March–September) peaks (Fig. 5). From days 195–348 (the end of April to the end of September in 1998), there was a clear and highly significant treatment effect. Nitrate concentration in soil solution increased steadily with ammonium concentration of the treatment, indicating a substantial degree of nitrification. The following summer the treatment effect was less clear, with N4 > N5 > N3 > N2 ≅ N1 ≅ N0.

Over the first 300 days, there was a clear and consistent treatment effect upon calcium concentration in the soil solution (Fig. 6). The concentration peaked over the period from March to July, with N5 > N4 > N3> N2 > N1 > N0. By the following summer, the peak was much

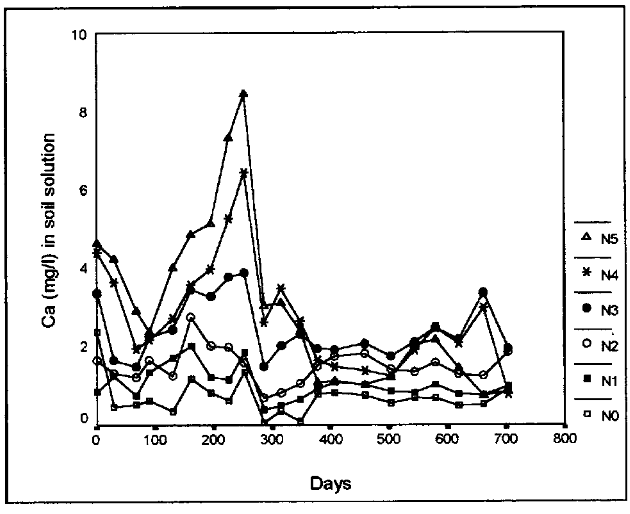

FIGURE 6 Effect of simulated precipitation supplements, formulated to apply 2 to 27 kmol N $ha^{-1} yr^{-1}$ (see Tab. II), on the calcium concentration of soil solution from the *Calluna*/peat microcosms, over 2 years from October, 1997.

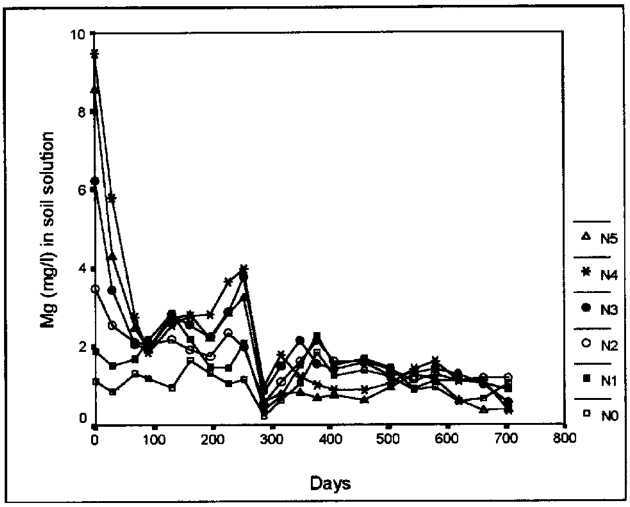

FIGURE 7 Effect of simulated precipitation supplements, formulated to apply 2 to 27 kmol N ha^{-1} yr^{-1} (see Tab. II), on the magnesium ion concentration of soil solution from the *Calluna*/peat microcosms, over 2 years from October, 1997.

less pronounced. The general trend then was N3 > N4 > N2 ≅ N5 > N1 > N0, but there was considerable variation. For magnesium treatment effects were rather more variable than those for calcium, although it was apparent that N5 and N4 resulted in lowest magnesium concentrations over much of the second year (Fig. 7).

DISCUSSION

Effect of Enhanced Ammonium on *Calluna* Growth and Composition

The plant growth data showed no significant treatment effects, in marked contrast to the very pronounced effects on shoot extension reported by Carroll *et al.* (1999) for the first four years of their field experiment with *Calluna* moorland in Wales. The organic horizon depth in their study was 8–10 cm, so the underlying mineral soil could have had a compensatory effect for base cation leaching occurring in response to ammonium nitrate treatments. Furthermore their N application was as ammonium nitrate, rather than ammonium sulphate. Biomass uptake of nitrate by the surface soil (White and Cresser, 1998) and *Calluna* and sphagnum vegetation (Edwards *et al.*, 1985) would at least partially have reduced the extent of base cation leaching. For a substantial period this would delay the acidification of soil solution. As suggested later, in the present experiment, substantial acidification of the soil solution and/or loss of base cations, especially calcium could have been offsetting any beneficial fertiliser effect of high nitrogen inputs. In their nutrient-poor dry heath experiment, Power *et al.* (1998) only observed increased total dry matter yield increase for their highest nitrogen treatment, and that was after 7 yr.

For chlorophyll a and chlorophyll b concentrations, the treatment effects were significant at $P < 0.05$ in 1998, in accordance with the visibly much darker foliage at higher nitrogen inputs after one year's treatment application. However, the significant decline in plant pigment concentrations over the following year of growth (Tab. III) indicates that plant pigments do not provide a useful indicator of nitrogen input effects on *Calluna* ecosystems in the longer term. Table IV shows that foliar nitrogen concentration varied much more in response to nitrogen treatment in 1998 than it did in 1999, so in some respects the lack of a significant treatment effect on plant chlorophyll concentrations in 1999 is not surprising. This, and the lower nitrogen concentrations in 1999 compared to those in 1998, show that for some reason the plants cannot continue to use the excess nitrogen supply, and therefore there is no significant response in pigment concentrations. The pigment concentrations fell substantially over the experiment, even although only new shoots were analysed at the same time in both years. Physiological growth stage is probably better than chronological time for selecting sampling date for pigment analysis. This is difficult to employ in practice, however. Also if, as happened here, ammonium inputs change base cation and pH status of the peat, this is likely to influence leaf pigment concentrations of the *Calluna*.

As in the dry heath study of Power *et al.* (1998) and the altitudinal transects study of Hicks *et al.* (2000), increasing ammonium application significantly increased the nitrogen concentration of *Calluna* leaves. In the second year of the present study, new shoot leaf nitrogen concentrations were consistently and significantly lower than those in the first year. This is in spite of the fact that the extended root system in the second year should have retained more of the nitrogen input from the treatments. As the leaf C:N ratio significantly ($P < 0.05$) decreased with increasing rate of ammonium application, it is reasonable to assume that the decline in nitrogen concentration is simply a plant growth dilution effect. However, the effect is important, because it could be concluded that the suggested use (Hicks *et al.*, 2000) of plant foliar nitrogen data, at least from perennial plants of mixed age, is unlikely to be a useful index of atmospheric nitrogen deposition in regional surveys.

The decreasing leaf calcium concentration at high nitrogen inputs in both years at least in part reflects mobilisation of calcium by cation exchange in response to the elevated ammonium inputs. The treatment effects on calcium concentration in soil solution (Fig. 6) suggest that leaching losses of calcium would have been especially severe in the first year and at the higher nitrogen treatments, before the root system was fully extended. Hence the decline in leaf calcium concentration at N4 and N5 (Tab. IV). The soil solution data for calcium in Figure 6 suggest also that leaching loss of calcium was so great in the first year that treatment effects on soil solution calcium concentration were much reduced and more variable in the second year. However, the leaf calcium concentration of new shoots is not significantly different in the second year from the first, so calcium is unlikely to have become growth limiting.

The decline in leaf magnesium concentration at high ammonium inputs, seen in the second year, probably reflects the enhanced leaching loss of magnesium at elevated ammonium inputs seen in Figure 7 over the early stages of the experiment. However, the trend in soil solution concentration with ammonium input is not simple. The leaf magnesium concentrations were, if anything, higher in the second year, so magnesium cannot be growth limiting.

New shoot leaf phosphate concentration was significantly lower in 1999 than in 1998. The possibility that phosphate availability is limiting the *Calluna* growth response to nitrogen cannot therefore be ruled out, although the foliar phosphate concentrations are not low compared with the values reported for upland plants (Leith *et al.*, 1999).

The Effects of Enhanced Ammonium on Soil Composition

The significant decreases in peat pH values in both water and calcium chloride at high ammonium treatments (Tab. V) show both short-term and long-term effects. The long-term effects are clear from the changes over time between October, 1998 and October, 1999. The pH values in water are most indicative of likely effects on, for example, biological activity in the peat. By October, 1999, the pH difference between N0 and N5 treatments was 0.77 pH unit, which could exert a significant effect. Cresser *et al.* (1991) have shown that slightly increasing pH of peat from a moderately polluted region of north eastern Scotland could enhance growth of *Calluna vulgaris* by 40% in a single season. Conversely, it follows that even a quite modest reduction in the pH of an already naturally very acidic ombrotrophic peat could significantly reduce *Calluna* growth. Thus reduction in peat pH in the present experiment could be preventing a fertiliser response from the *Calluna*. However, without further research, this hypothesis must remain speculative.

The rapid growth of the *Calluna*, and the resultant canopy nitrogen storage, resulted in only a small increase in peat nitrogen concentration after two years of treatment application, and no significant increase in the peat C:N ratio. This is in marked contrast with the results of Yesmin *et al.* (1996a), who observed a highly significant fall in peat C:N ratio after only 1 year of ammonium sulphate application to intact *Calluna* moorland microcosms. The *Calluna* growth was more rapid in the current experiment, so that canopy nitrogen storage could readily explain the much slower rate of change in peat nitrogen concentration and C:N ratio. In the absence of a final plant yield measurement, total plant nitrogen uptake cannot be quantified. It is difficult therefore to assess whether the amount of nitrogen added would be likely to cause a detectable change in C:N ratio.

Effect of Enhanced Ammonium Inputs on Change in Soil Solution Composition

The decrease in soil solution pH with increasing ammonium sulphate application rate on all sampling dates shows that the root environment is potentially seriously adversely effected at high ammonium inputs. This sustained effect is probably more relevant to soil biota and *Calluna* growth than the soil solid phase pH results. The clear seasonal trends, with hydrogen concentration peaks evident in summers (Fig. 1), primarily reflect the seasonal trends in sulphate (Fig. 3) and nitrate (Fig. 5) concentration. Nitrification thus must be viewed as having a direct adverse effect upon soil solution pH. The combined effects of these two dominant anions will lower pH via the mobile anion effect. The treatment effect on nitrate is most pronounced in the first summer, and this will compensate for the fact that the sulphate peak was higher in the second summer than in the first. Thus the peaks in hydrogen concentration are similar in both years. The chloride troughs around sampling days 287 and 662 (Fig. 2) almost certainly contribute to the secondary minima in the hydrogen time series plots on the same days (Fig. 1).

The higher concentrations of nitrate and sulphate during the seasons of active growth are almost certainly due to a temperature effect. This suggests that sulphate and nitrate are present in excess of plant requirements, or are being produced by mineralization in zones of the soil where root uptake of the ions is limited. The effect is not just due to increased evapotranspiration in summer, which could exert a concentration effect. If it was, chloride concentration might be expected to increase during the summer, rather than in winter. It is important to note that the soil solution pH is most acidic during the period of greatest potential growth. This possibly is the stage when the greatest adverse effect of excess acidity may occur.

The observation that sulphate concentrations were higher in the summer of 1999 than they were in 1998 for all treatments probably reflects greater sulphur storage in the rapidly expanding canopy in the first year.

Ammonium concentrations in soil solution initially increased rapidly with ammonium application rate, especially for the N4 and N5 treatments (Fig. 4), but after sampling day 160 suddenly fell sharply. The time series plot for nitrate concentration (Fig. 5) suggests that this sharp decline is due to the onset of significant nitrification. Other authors (van Vuren *et al.*, 1992) have reported that ammonium availability limits the production of nitrate in Dutch acid heathland soils. The N1 treatment resulted in earlier peaks in both ammonium and nitrate concentrations, but why this should have happened is not clear.

The consistent treatment effect upon calcium concentration in the soil solution over the first year (Fig. 6) clearly demonstrates that ammonium inputs can result in direct proportional leaching losses of base cations. The less pronounced peak the following summer demonstrates the sensitivity of peat soils to damage by base cation leaching. Basically, once the calcium has been heavily depleted by leaching, it cannot be replaced by mineral weathering. A similar difference between years was seen for magnesium.

CONCLUSIONS

Exposure to ammonium sulphate inputs at levels in excess of suggested nitrogen critical loads for heathland did not perceptibly damage the *Calluna* plants over the two years of the study. However, it adversely effected peat properties directly, via short-term cation mobilisation and acidification, both associated with enhanced mobile anion concentrations, and via longer- term acidification. This acidification could be offsetting any nitrogen fertilisation effect on growth. Enhanced mineralization in the summer means that the sulphate and nitrate mobile anion concentrations in soil solution, and hence the hydrogen concentration, show marked seasonal trends with peaks during active growth. Initial beneficial growth effects for *Calluna* observed on more mineral soils may not be seen on peats, because mineral weathering cannot replenish leached base cations. Further work is still needed to establish whether any damage which might yet be observed if the experiment is continued is a consequence of increased leaf nitrogen concentration or soil acidification.

An unexpected consequence of very high ammonium inputs was a surge in nitrification in the peat, especially during the period of active growth of the *Calluna*. However this effect was much reduced in the second year.

Visible effects on leaf colour were associated with significant treatment effects on measured chlorophyll concentrations in the first year of treatment, but chlorophyll concentrations varied markedly between seasons, reflecting plant nitrogen status. This suggests that plant age would need to be taken into account in any attempt to use plant leaf colour to diagnose atmospheric nitrogen pollution effects.

Acknowledgements

The authors are indebted to NERC and to the UK Department of the Environment, Transport and the Regions for financial support for this research.

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