Work Package 2:

Impacts recovery and controlling processes

Work Package 2 Task 5:

Long-term impacts of enhanced and reduced nitrogen deposition on semi-natural vegetation

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NERC UMBRELLA CONTRACT

SHEFFIELD COMPONENT

REPORT FOR 2002

Introduction

The Sheffield component consists of a small contract supporting a research student to continue studies of the effects of simulated atmospheric N deposition on semi-natural acidic and calcareous grasslands. This represents a continuation of a study initiated in 1990 by the University of Manchester with support from the Department of the Environment. The study is the longest running investigation of the simulated effects of atmospheric nitrogen deposition on grasslands, and has involved at least monthly spraying of the vegetation to achieve a range of nitrogen deposition enhancement of O to 140 kg $ha^{-1}y^{-1}$. The only exception to this was a three month period in the winter of 2001/2002 when spraying was impossible due to the Foot and Mouth outbreak. After spraying was resumed, the annual totals of deposition were achieved by spraying fortnightly for three months, before reverting to the monthly spraying regime. The current contract effectively started on 1 October 2001 with the appointment of Mr Paul Horswill to a PhD studentship.

During the 12 years of the investigation there have been a number of periods when the experiment has been maintained from University resources, both from the Universities of Manchester and Sheffield. The last of these was between May and October 2001. This has only been possible with considerable difficulty.

The Experiment

Plots were established on Wardlow Hay Cop, Derbyshire in 1990 on a shallow calcareous soil supporting typical limestone grassland and on deeper loessic soils supporting an acidic grassland. The sites are c. 100m apart. The plots 1m x 1m were arranged in a randomised block design and each treatment was replicated 4 times. Nitrogen was added as ammonium nitrate to give an enhanced deposition of 3.5, 7 and 14 g N $m^{-2}y^{-1}$. An additional treatment received 14 g N $m^{-2}y^{-1}$ as ammonium sulphate.

Subsequently in 1995 a new series of plots was established with support from the University of Sheffield. These were 3m x 3m and included an enhancement of nitrogen deposition as ammonium nitrate at 3.5 and 14 g $m^{-2}y^{-1}$ with and without phosphorus additions (3.5 g $m^{-2}y^{-1}$). Three blocks were arranged in a randomized block design. These plots were established to allow for more destructive experimentation than was possible in $1m^2$ plots, particularly for the examination of the treatments on soil processes. Again the spraying has been on a monthly basis.

The Umbrella Contract 2001-2004

The plan for this period is to examine the response of the grassland systems to the cessation of experimental treatments, i.e. to examine recovery. This will involve the cessation of treatments on the older plots in August 2002 after 12 years of treatment, allowing 2 years of the contract to study recovery processes. The plots established in 1995 will continue to receive nitrogen treatments for the foreseeable future.

Work in progress is principally to establish base-line measurements at or near the point of cessation of the treatments. This includes measurements of plant and soil chemistry and microbial processes whilst maintaining the current spraying regime.

Results

Some of the most interesting results obtained from the experimental treatments during the last 12 months have been from studies of microbial and decomposition processes. These studies have been particularly aimed at the effects of the treatments on mycorrhizal formation and activity together with initial investigations of decomposition. The latter have demonstrated that, contrary to perceived ecological wisdom, nitrogen enhancement causes a decline in in situ litter decomposition (Fig. 1.) at least at the highest deposition rate. There is no effect at the lower nitrogen treatment. This decline in decomposition appears to be a property of the litter rather than through a direct effect on decomposers because decomposition of untreated litter is not affected by nitrogen treatment when bags containing this litter are added to the experimental plots. Studies of the processes involved are continuing.

Vesicular arbuscular mycorrhizal infection has been measured in several species including Plantago lanceolata on the calcareous plots and Anemone nemorosa on the acidic ones. In both of these species, the nitrogen treatment has reduced mycorrhizal infection. Laboratory studies involving $^{14}CO_2$ experimentation on cores isolated from the plots have shown that plants appear to allocate proportionately more to above ground biomass and less to below ground biomass in enhanced nitrogen-treated plots (Fig. 2.). This may perhaps help to explain the decline in mycorrhizal activity and infection since the mycorrhizas formed may be receiving less carbon from the host. Studies by researchers in Sheffield (Drs D Johnson and J R Leake) as part of the NERC Soil Biodiversity programme have shown that $^{13}CO_2$ labelled field plots lost 5-8% of carbon assimilated through respiration by vesicular arbuscular mycorrhizas within 21 hours. This points to the importance of carbon flow to these organisms in grassland ecosystems. Respiratory loss from soil cores taken from the enhanced nitrogen deposition plots in the present study declined with increasing nitrogen supply (data not shown).

Conclusion

The results continue to suggest that there are major effects of nitrogen deposition on below ground processes. This has been a consistent finding from the early years of the study (see e.g. Morecroft et al 1994). The ease with which such effects are reversible will be a major part of the present contract. Evidence from other studies is that these effects may not be readily reversible, particularly the stimulation of nitrogen mineralisation which may persist for decades (Vinton & Burke, 1995).

References

Morecroft, M D, Sellers, E K & Lee, J A (1994). An experimental investigation into the effects of atmospheric nitrogen deposition on two semi-natural grasslands. Journal of Ecology, 82, 475-483.

Vinton, M A & Burke, I C (1995). Interactions between individual plant species and soil nutrient status in short-grass steppe. Ecology, 76, 1116-1133.

New Publication

The following publication based on work at Wardlow is now in press.

Carroll, J A, Caporn, S J M, Johnson, D, Morecroft, M D & Lee, J A (2002). The interaction between plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition. Environmental Pollution, in press.

Acknowledgements

In the last two years the experiment has been maintained by individuals employed directly on NERC Umbrella funds (P Horswill & C Thorpe), research staff of the University of Sheffield (Dr G Phoenix & I Johnson) and University of Sheffield research students (C MacDonald & T Ames). Without the commitment and enthusiasm of all these individuals this investigation could not be sustained.

J A Lee
J R Leake

July 2002

Figure 1

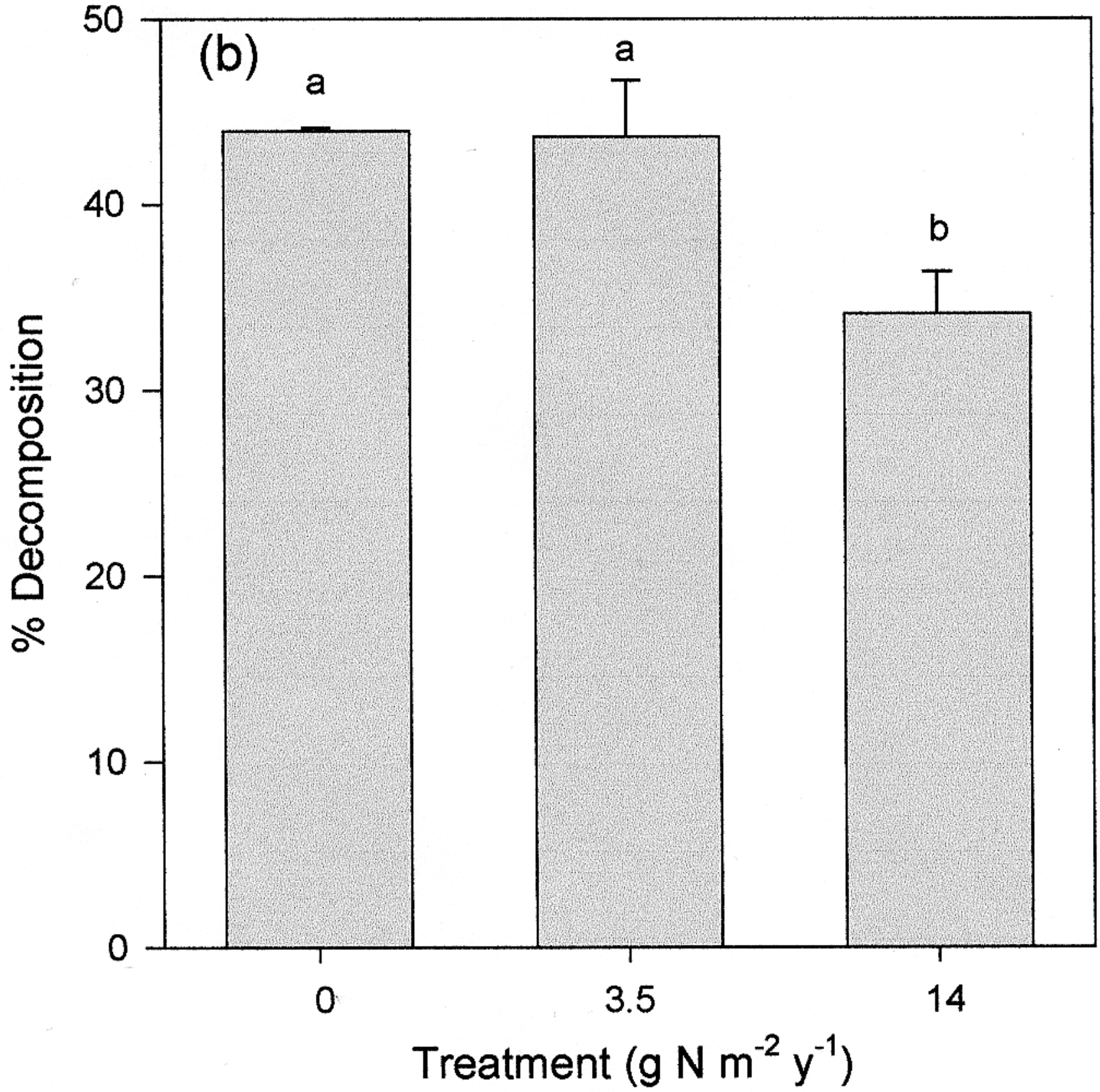
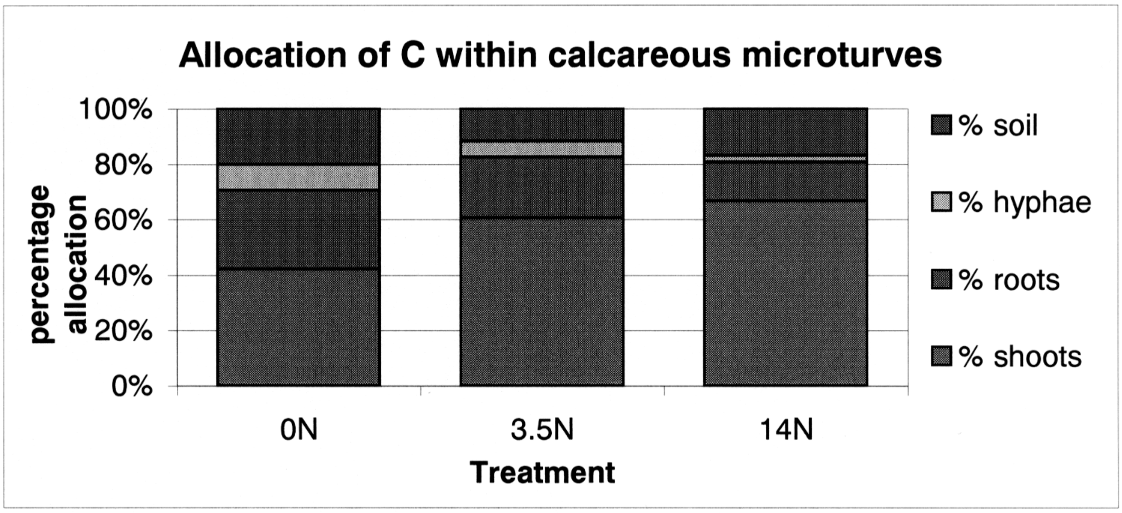
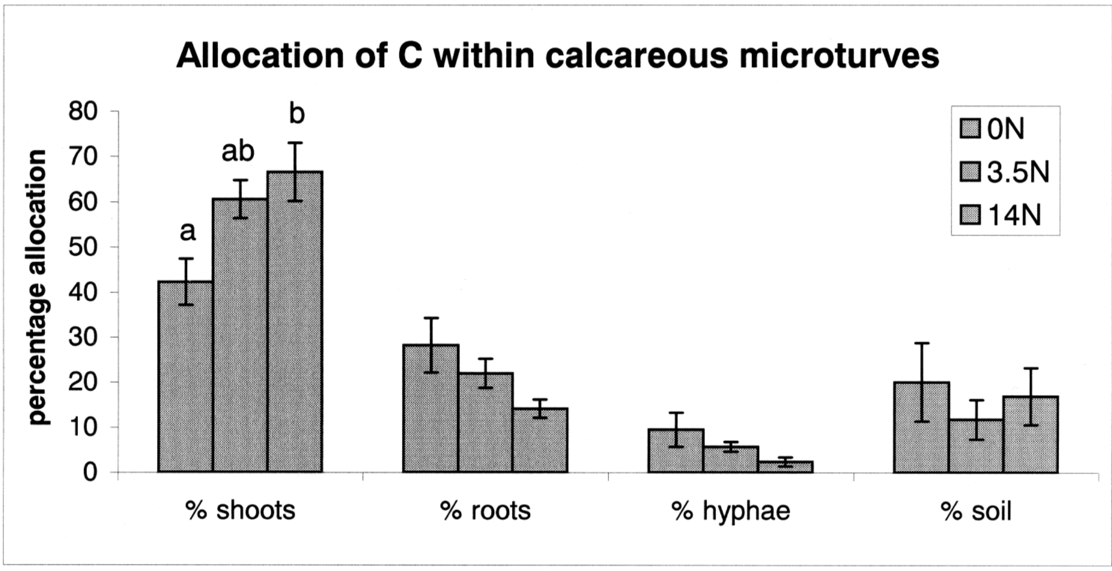


Figure 1. The effect of 7 years of nitrogen additions on percentage decomposition (weight loss) of *in situ* litter after 244 days field incubation in an acid grassland (+/- SE), bars with different letters are significantly different Tukey test ($P < 0.001$).

Figure 2





The effect of 7 years of simulated pollutant N deposition on the allocation of carbon fixed by grassland turf, 48 h after exposure to 3 MBq of 14 CO_2 for 3 hours in the light.

DEFRA Terrestrial Umbrella – Eutrophication and Acidification of Terrestrial Ecosystems in the UK

Work Package 2, Task 5

Long Term Impacts of Enhanced and Reduced Nitrogen Deposition on Semi-Natural Vegetation

August 2002 (revised) Interim Progress Report

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Executive Summary

1. The long-term upland and lowland heath field manipulation study sites at Ruabon and Budworth (old plots at Ruabon now 13 years old) represent a unique resource for a large number of collaborators. The maintenance and basic monitoring provision for these sites represents a large personnel investment for the MMU group.

Moorland Experiment:

2. The analysis of the full nitrogen budget harvest carried out on the Ruabon "old" plots in March 2000, shows high levels of nitrogen retention (>70% of inputs) in both organic and mineral soil layers, with the standing vegetation accounting for only 5% of the total reservoir. Nitrogen treatment increased the nitrogen content of the fractions, but did not significantly alter this pattern of allocation.

3. The light management burn carried out on these plots in March 2000, removed the vegetation, but left litter and soil layers intact. The impact on the total nitrogen budget has therefore been very limited.

4. The high levels of soil nitrogen immobilisation, and low leaching losses from this site, would be expected to slow recovery from the effects of atmospheric nitrogen deposition.

Lowland heath experiment:

5. The long-term vegetation survey data (1996 – 2001) from the main plots at Budworth, shows the development of an interesting pattern of interaction between the integrity of the *Calluna* and bryophyte canopies, and the rate and success of grass invasion, in response to nitrogen treatment and other environmental stresses.

6. The results of the first year of soil leachate collections from the main plots indicate that overall losses of nitrogen from the organic soil layers are low, consistent with a high level of retention in vegetation and soil pools. The nitrogen budget study planned for this autumn should clarify this.

Questions to be addressed:

7. Work over the next year will concentrate on the effects of management and recovery, and further investigation of the underlying soil processes.

Introduction to Field Experiments

This interim progress report presents a summary of the main new findings in year 2001 – 2002 from long-running nitrogen field manipulation experiments located at an

- Upland heath site near Ruabon in North Wales (commenced 1989)
- Lowland heath site at Little Budworth in Cheshire (commenced 1996)

The full site descriptions, methods and previous results are given in earlier DETR contract reports and elsewhere. These experiments now represent some of the longest-running field manipulation studies still being maintained in the UK, and provide a unique resource for a number of research groups.

Use of site by other research groups

The research groups presently or recently collecting materials or data from our field sites, as an essential part of DEFRA, NERC and other research projects, are shown below:

DEFRA/ previous DETR funded:

- Bryophyte and lichen responses (University of Bradford);
- Soil modelling studies - (CEH Bangor, MLURI);
- ^{15}N soil nitrogen mineralisation studies (CEH Bangor, Merlewood);
- Decomposition studies (Imperial College)

NERC GANE/Other:

- UK *Calluna* Model. (University of Bradford & Imperial College);
- Metabolomics (University of Aberystwyth, CEH Bangor);
- Invertebrate studies (University of Durham, CEH Merlewood);
- Remote sensing of foliar N in *Calluna* (Manchester Metropolitan University).

Manchester Metropolitan University Personnel involved in this research

DEFRA currently funds one student, Nick Ray, in support of the work being done on these field sites. In addition, input to the DEFRA project from the MMU research group includes data generated by Deirdre Wilson (studentship funded by MMU and in collaboration with CEH Merlewood) and Mike Pilkington (studentship funded by NERC *Environmental Diagnostics* and in collaboration with CEH Bangor). Mike Pilkington is currently employed at 40% (*aric*-funded) and is responsible for maintenance, soil leachate and vegetation monitoring work at the Ruabon moorland site. Project support is also provided by Jacky Carroll (Research Associate, MMU-funded) and Neil Cresswell (Microbiology and Molecular Biology Lecturer at MMU).

Upland Heath field manipulation experiment Ruabon – North Wales

Background:

The original "old" plots were first established in 1989, on an area of building-phase *Calluna* with few other species present. The layout consists of 4 blocks of 1m^2 plots receiving control, water, 40, 80 and 120 kg N ha^{-1} y^{-1}.as monthly applications of ammonium nitrate. A light management burn was carried out on the site in April 2000.

Additional larger (2x2 m^2) "new" plots were established in August 1998 on an area close to the original site. The plots are arranged in four randomised blocks receiving control, water, 10, 20, 40 or 120 kg N $ha^{-1}y^{-1}$, 50 kg P ha^{-1} y^{-1}, 20 kg N+50 kg P ha^{-1} y^{-1}, or 120 kg N+50 kg P $ha^{-1}y^{-1.}$ The phosphorus treatment was decreased to 20 kg P ha^{-1} y^{-1}in May 2002.

The results of vegetation and soil studies carried out on the on the "old" plots are presented in previous reports to DETR and publications (e.g. Carroll *et al*, 1999; Lee & Caporn, 1998).

Soil leachate studies:

Collection and analysis of soil leachate from these plots was first established in July 1998. Leachate was collected from below the organic layer using gravity lysimeters and soil solution was sampled below the mineral layer using negative tension lysimeters. Samples were collected monthly from July 1998 to Sept 2000, allowing both the long-term impact of the nitrogen treatments and the immediate effects of the burn treatment to be investigated. The full method details and results are given in reports from 2000 and 2001.

Results from this sampling period showed no significant increase in the flux of total dissolved N from below the mineral layer, in response to nitrogen treatment, although clear upward trends could be seen. Significant increases were seen in the release of NO_3^- from the organic and mineral layer; however, the amounts involved at all times represented a very small percentage (<5%), of the nitrogen added to the plots.

The effects of the burn showed a rapid rise in N flux from both soil layers, with most notably, a 3 fold increase in NH_4^+ release from the organic layer in September 2000, at the highest rate of nitrogen application. Losses from the mineral layer were much lower, but were still increasing at the end of September.

Soil leachate collections were resumed in January 2002, with the aim of monitoring the longer-term effects of the burn treatment on nitrogen and other ionic losses from the soil profile, and this work is ongoing.

Recovery of vegetation on the burnt plots:

Approximately 90% of the above ground *Calluna* was converted to ash, smoke or gases by the burn. The vegetation on the plots was surveyed in August 2000 and a significant negative correlation found between increasing nitrogen additions and both height and cover of *Calluna.*

The survey data for the old plots for 1998 – 2001 are summarised in figs 1 and 2.

The *Calluna* canopy density results for 2001 (fig 1) confirmed the trend seen in 2000, with continued evidence of recovery and a clear negative dose-response to increasing nitrogen inputs. Similar results were obtained for *Calluna* height. Reasons for these trends included phenological effects (*Calluna* on high N plots were developmentally more mature, and therefore regenerated less well), higher burn temperature on high N plots, due to greater biomass, or differences in root structure and other soil processes, resulting from long-term N treatment.

The data for total mean moss pin touches (fig 2) showed a very similar pattern to that seen for *Calluna*, with a clear negative relationship to nitrogen input dose, and in this case, with a return to near 1999 cover levels. This suggested that the effects of nitrogen on moss cover before the burn, may not have been due to shading effects or to the higher accumulation of litter on the high N plots. The effects on moss cover can now be more firmly attributed to direct impacts of nitrogen input or concentration. The lichen cover shows no clear recovery at this stage.

Nitrogen Budget:

A destructive harvest was carried out in March 2000 in order to assess the biomass of nitrogen contained above ground in shoots of *Calluna*. All the vegetation within an area of 15 cm x 100 cm., was harvested from each plot. All the stalks within this quadrat were cut close to the litter surface and air-dried. Sub-samples were taken and divided into current green, previous year's growth and woody material. The original samples and the partitioned sub samples were then oven dried, weighed and ground. The ground samples were bulked to give one sample per plot, digested in sulphuric acid with copper sulphate, sodium sulphate and selenium and assayed colorimetrically for N, P and K content using a Technicon flow injection analyser.

The mass of nitrogen contained in soil was measured using cores taken from inside the plots using a deep-slit borer (2 cm diameter) to penetrate into the clay horizon. Five cores were extracted from each plot and cut into their respective horizons, labelled litter, organic (peat), mineral (gley) and clay. The samples were oven dried, sieved and coarsely ground. After weighing, the soil cores were ball-milled for 90 seconds, and bulked to give one sample per plot, prior to digestion and assay for N, P and K as above.

The total amounts of nitrogen in the various soil and vegetation compartments of the control plots before the management burn are shown in fig 3.The highest nitrogen concentrations (1.2 – 1.4%) were found in the green *Calluna* shoots, and the litter, followed by the peat layers (1%), whereas concentrations in the underlying gley and clay fractions were much lower (0.2%). However, following correction for the differing mass contributions of the various compartments, it can be seen that the largest N pools were those in the peat (50%) and gley (28%) fractions, with the standing vegetation notably contributing only 5% of the total.

The effects of the nitrogen treatments on the total nitrogen content of the soil and vegetation compartments are shown in fig 4. Significant increases were seen (trend analysis, linear response, $p<0.05$) in the nitrogen content in all compartments except for young wood. The most significant linear responses were found in the litter and vegetation, but the organic horizon increased in nitrogen content most steeply.

Fig 6 shows the nitrogen content of the soil and vegetation compartments when compared with the total N inputs over the 11years of treatment. The nitrogen content of the soil, particularly the organic layer, increased in line with total N inputs, and the total N present in the system accounted for between 55% (at 40 kg N ha $^{-1}$ y^{-1}) to 90% (at 120 kg N ha^{-1} y^{-1}) of total inputs to the system. This corresponds to an approximately constant loss of nitrogen from the different treatments, possibly resulting from losses of organic nitrogen through denitrification or lateral flow.

The effects of the burn on the nitrogen content of the various fractions in the control plots are shown in Fig 5. The burn denatured the majority of the standing vegetation whereas the litter and peat layers were unaffected, and the nitrogen content of gley layer increased by 28%. Increasing nitrogen treatment had no significant effect on this pattern.

The increase in the nitrogen content of the gley following the burn could be due to stimulation of litter mineralisation by carbon deposited in the ash from the burnt vegetation. The dense mass of fine mycorrhizal roots found in the litter layer would also be subject to this stimulated mineralisation effects on the rooting zone, as a result of the loss of above-ground vegetation.

Conclusions:

The majority of nitrogen (90%) in both control and treated plots was found in the soil compartments, with >50% in the peat layer.

The biomass and % N content of both green tissue and older woody tissue responded positively to treatment. The nitrogen content of the soil compartments increased in line with additions and accounted for between 50 –90% of inputs over the eleven years of treatment.

The light management burn carried out in April 2000 had only a limited impact on the nitrogen budget of the site, removing in total only about 5% of the total nitrogen in the system, in the form of live biomass, and leaving the major litter and soil nitrogen reservoirs unchanged.

The large amounts of nitrogen sequestered in the soil horizons, and the very limited losses due to leaching, even at high levels of nitrogen input, (see above and report for 2000) were consistent with very high rates of nitrogen immobilisation in this system.

The pulse in leaching losses following the burn, particularly from the high N plots, showed some response of the system to disturbance/management. Overall however, the peat and gley compartments appeared resistant to change, suggesting that reduced nitrogen inputs would not be likely to result in rapid recovery.

Lowland Heath site –Little Budworth Common, Cheshire

***Background*:**

The main plots at Budworth were established in March 1996 with the overall aim of investigating a range of ecological responses to nitrogen, but also with the more specific objective of testing the hypothesis that elevated atmospheric nitrogen deposition increases the sensitivity of *Calluna vulgaris* and associated lowland vegetation to environmental stresses such as drought and heather beetle outbreak.

The experiment was organised as four randomised blocks of 2 m^2 plots receiving 0, 20, 60, or 120 kg N ha^{-1} y^{-1} added as ammonium nitrate at fortnightly intervals. In addition to the nitrogen treatments a controlled drought experiment was carried out between May and September 1997. In the summers of 1998 and 1999 there were natural outbreaks of the heather beetle (*Lochmaea suturalis*) on the experimental plots.

The methods and detailed results for the drought experiment and the long-term effect of the treatments and the heather beetle attack on the vegetation were shown in the 2000 - 2001 report to the DETR and in Leigh Cawley's Ph.D thesis (Cawley 2000).

A second set of smaller plots (0.5 x 0.5 m) was established on the same site in March 2000 with the aim of investigating the influence of gap formation in the vegetation canopy, on the competitive interactions between *Calluna*, bryophytes and grasses. Four randomised blocks were laid out, and the plots were treated with either water or 60 kg N ha^{-1} y^{-1} (ammonium nitrate applied fortnightly). Gaps of 20 cm diameter were created and maintained using plastic net to open the *Calluna* canopy. In addition 0.5 g *Deschampsia flexuosa* seed was added to half the plots. This experiment is ongoing, and no results will be presented in this report.

Vegetation Survey Results:

A detailed survey of the vegetation on the main plots has been carried out annually, using a point quadrat technique to record number of touches per pin throughout the period of the experiment. A full summary of all the data for 1996 – 2001 is shown in fig 7.

There was a significant increase in *Calluna* cover on the watered plots from 1996 –1998, with a positive effect of nitrogen treatment. Cover was reduced in 1999, possibly as a result of the heather beetle attack, and this was followed by a recovery in canopy density in 2000 and 2001. The effect of the nitrogen treatment was less clear over the period 1999-2001.

The changes in the *Calluna* canopy density on the droughted plots was similar to that recorded on the watered plots. However, the drought treatment significantly reduced *Calluna* cover in 1997-1998 in the high nitrogen treatments.

The increase in *Deschampsia flexuosa* cover was highly significant in 1997 and 1998 in response to the 120 kg N ha^{-1} yr^{-1} and drought treatments in particular. This increase was probably indicative of a clear stress response to the combined effect of nitrogen, drought and heather beetle on the vegetation structure over this period. Pin touches of this species were reduced again in 1999 in all treatments, when compared to 1998. This trend continued through both 2000 and 2001.

The mean bryophyte cover on the watered plots increased steadily over the experiment. The effect of the nitrogen treatments on the watered plots has been negative overall, with significant reductions in cover at the highest rates of application. Bryophyte cover on the droughted plots was significantly reduced in 1997, but has recovered since, with some indication of a positive response to nitrogen in 1998. Overall the effect of nitrogen on the bryophyte cover on the droughted plots, is less clear than on the watered plots.

The marked increase in grass cover seen on the high nitrogen plots in 1998 has not been sustained. The association between declining grass and increasing moss cover first noted in 1999 (Fig 7) appears to be strengthening, with significant correlations at higher rates of nitrogen application in particular (Fig 8). It is anticipated that the results from the “gap” plots will allow this interaction to be examined in more detail.

Leachate collections

Collection and analysis of soil leachate from the original $2m^2$ plots was first established in December 2000. The methods used for the installation of leachate collectors were similar to those used at the Ruabon field site. At Little Budworth, however, leachate was only collected from below the organic layer (using gravity lysimeters), the sandy nature of the soil being unsuitable for the use of negative tension lysimeters to collect leachate samples from lower in the soil profile. The gravity lysimeters were installed at 10 cm below ground level (the average depth of the main rooting zone for *Calluna vulgaris* at the site). Samples have been collected monthly from December 2000 to present.

The data presented in fig 9 for the period of March 2001 – February 2002 has shown a clear increase in nitrogen flux through the organic layers of the soil, at the highest rate of nitrogen treatment. Mean outputs of 3.79, 2.09 and 5.89 kg N ha^{-1} yr^{-1} were obtained for nitrate, ammonium and total inorganic N respectively at 120 kg N ha^{-1} yr^{-1}, compared with values of less than 1 kg N ha^{-1} y^{-1} for the control treatments. This however still represents a very small proportion of the total nitrogen inputs to these plots (20 – 140 kg N ha^{-1} yr^{-1}), particularly at high treatment levels. These results are consistent with high levels of immobilisation in the soil and vegetation, and may suggest a similar pattern to that seen on the moorland plots, although the soil type (sandy podzol) is very different.

Leachate collections have continued this year (2002), and a full harvest of the plots will take place in the Autumn. This should allow the various soil and vegetation nitrogen pools to be assessed in more detail.

Conclusions:

The long-term vegetation survey data (1996 – 2001) from the main plots at Budworth shows the development of an interesting pattern of interaction between the integrity of the *Calluna* and bryophyte canopies, and the rate and success of grass invasion, in response to nitrogen treatment and other environmental stresses.

The results of the first year of leachate collections from the main plots indicate that overall losses of nitrogen from the organic soil layers are low, consistent with a high level of retention in vegetation and soil pools. The biomass harvest to be carried out this autumn should clarify this.

References

CARROLL, J.A., CAPORN, S.J.M., CAWLEY, L., READ, D.J., LEE, J.A. 1999. The effect of increased atmospheric nitrogen deposition on *Calluna vulgaris* in upland Britain. *New Phytologist* 141 (3) 423-431.

LEE, J.A. & CAPORN, S.J.M. 1998. Ecological effects of atmospheric reactive nitrogen deposition on semi-natural terrestrial ecosystems. *New Phytologist* 139:127-134.

CAWLEY,L.E. (2000) PhD thesis Manchester Metropolitan University

Questions to be addressed in planned work (2002-2004)

Calluna moorland 'old' (1989) plots

- How will *Calluna* and any other higher plant competitors recover from fire management at different levels of nitrogen application?
- Soon after burning nitrogen the leaching increased from all plots but particularly from the highest N treatment. Two years later, have these high rates been sustained?
- Is the increase in nitrogen leaching that is related to raised nitrogen supply explained by faster rates of mineralisation or lower immobilisation (^{15}N study – collaboration with CEH).

The new Calluna moorland (1998) plots (2 x 2 m) will be divided into half receiving no added nitrogen and the other half experiencing the continued inputs.

- Will a step down in nitrogen input result in short term recovery (or change) in various attributes, including the following:
  - *Calluna*, moss and lichen abundance and growth?
  - Plant and soil nitrogen status?
  - Soil biology (microbial, invertebrate) populations and related mineralisation rates?

Lowland heath nitrogen-treated plots. Soil studies will increase to enable the following to be addressed:

- How do the increases in nitrogen leaching relate to changes in soil microbial populations and changes in soil invertebrates?
- Is the increase in nitrogen leaching that is related to raised nitrogen supply explained by faster rates of mineralisation or lower immobilisation (^{15}N study – collaboration with CEH)?

The lowland heath Calluna has matured to the stage where management of some nature would be beneficial. In late 2002 we plan to cut the vegetation in line with local practice.

- How will a management cut of *Calluna* – in the presence of continued nitrogen application - affect the following:
- Competition between grass, heather and moss?
- Nitrogen leaching rates and other soil solution nutrients?
- As part of the cut, soil and plant samples will be taken to estimate the fate of added nitrogen ('the budget') in the system

The Lowland heath Gap x Nitrogen experiment

- The 'gap' experiment will be continued to see if grass develops in the gaps created in the *Calluna* canopy

Fig 1: Vegetation Survey Results for *Calluna vulgaris.* Ruabon "old plots", showing the effects of the management burn in April 2000.

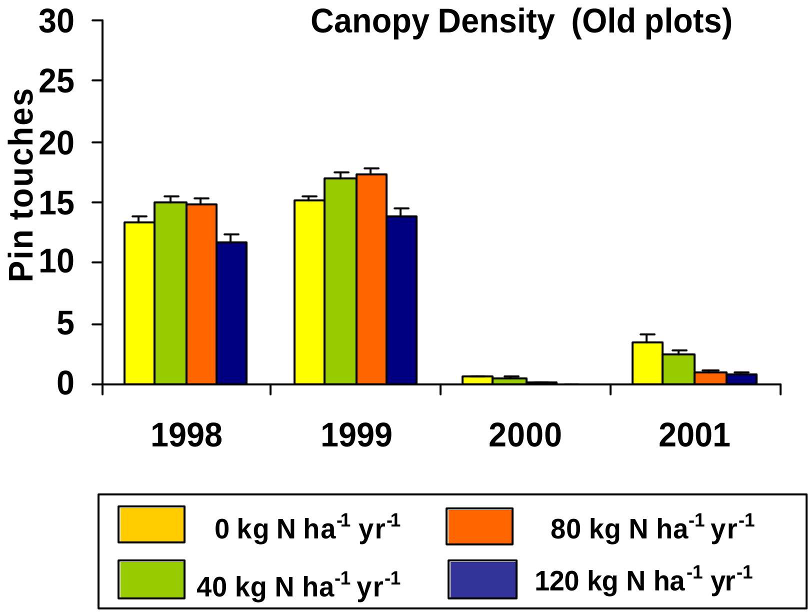


Fig 2: Vegetation Survey Results for *Vaccinium myrtillis* and total moss and lichen cover on the Ruabon "old plots", showing the effects of the management burn in April 2000

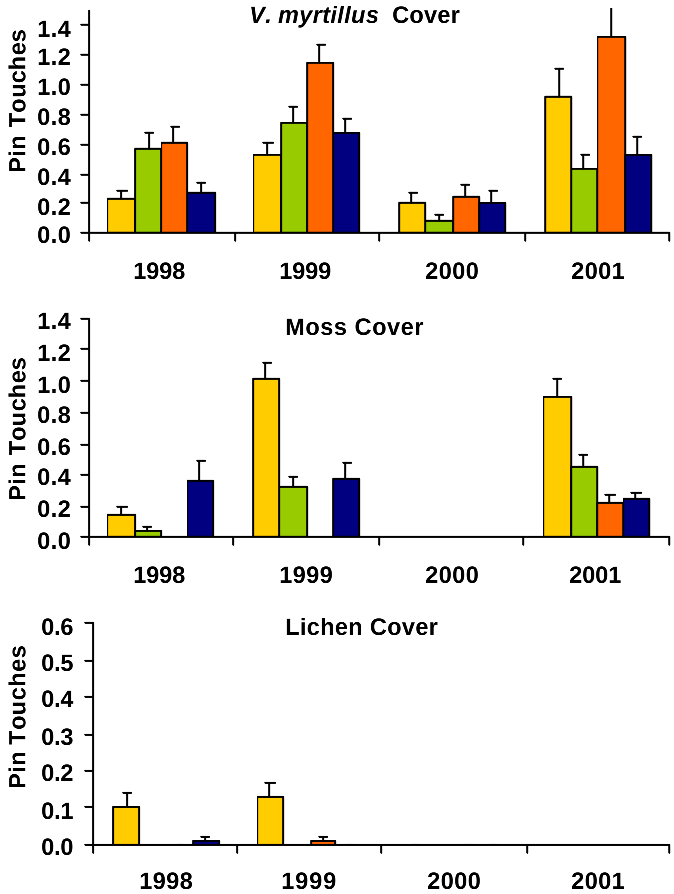
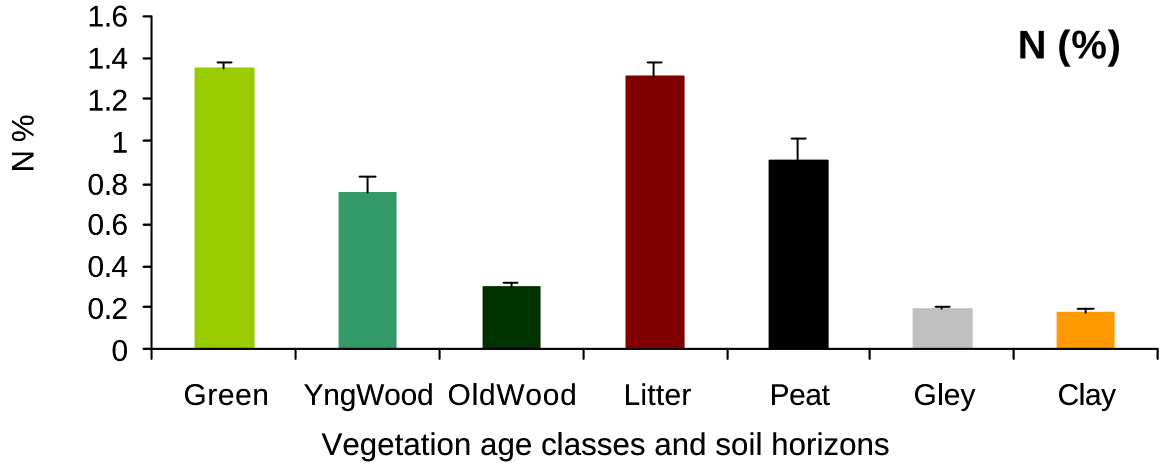
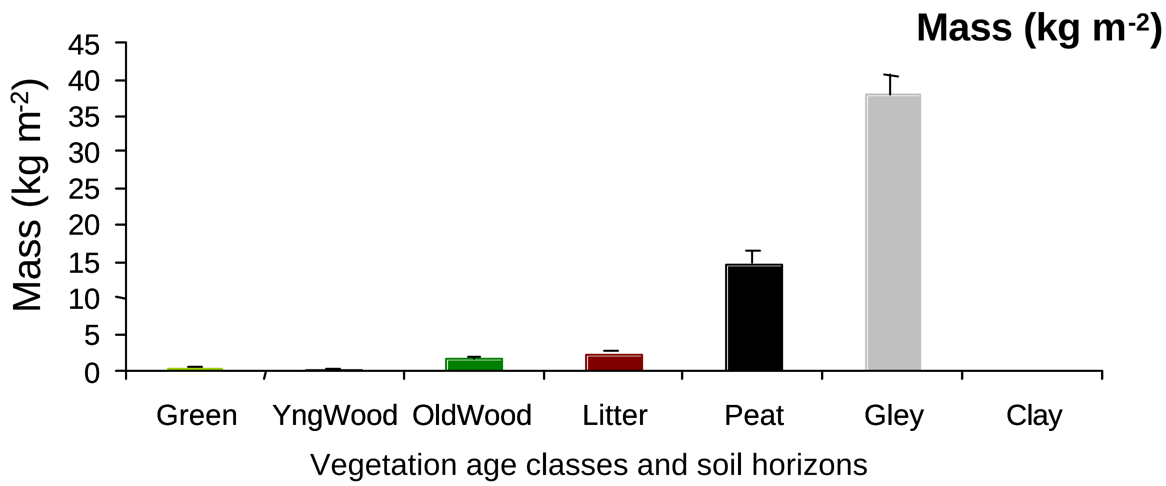


Fig 3: Nitrogen budget data for Ruabon "old" plots – March 2000 (control plots)





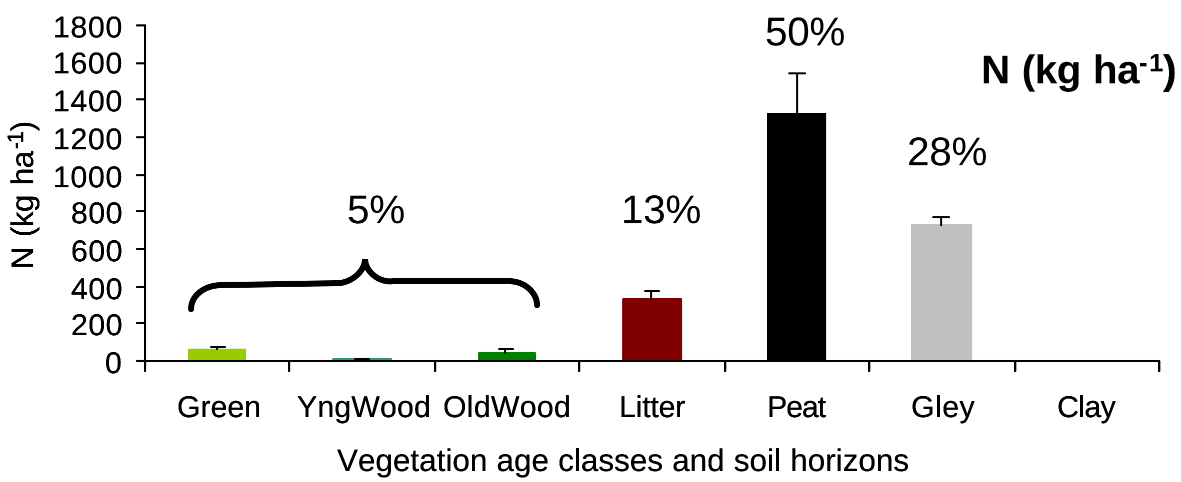
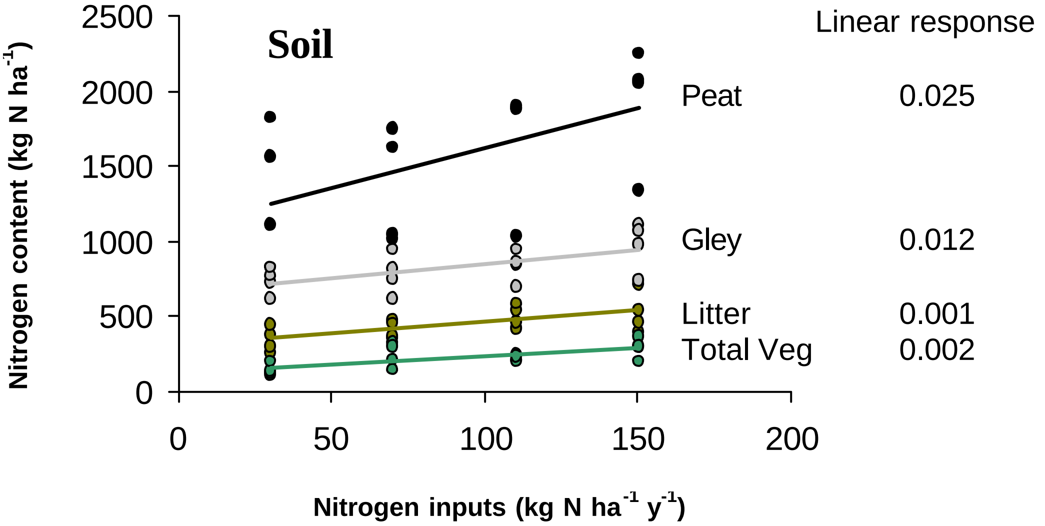


Fig 4: The effect of increasing nitrogen inputs (kg N ha^{-1}yr^{-1}) on nitrogen content of soil and vegetation fractions (kg N ha^{-1}), showing *P* values for linear response analysis. Ruabon "old" plots - March 2000



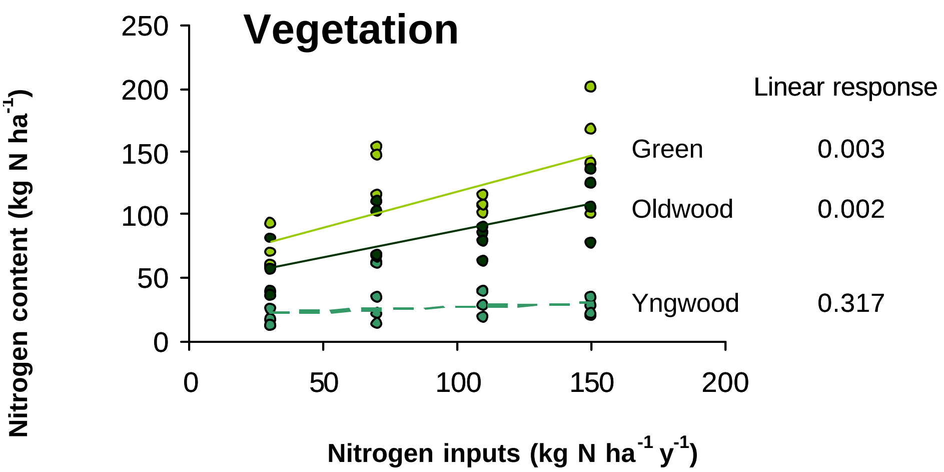


Fig 5: Effect of the management burn (April 2000) on the total nitrogen budget (kg N ha^{-1}) for the Ruabon "old plots"

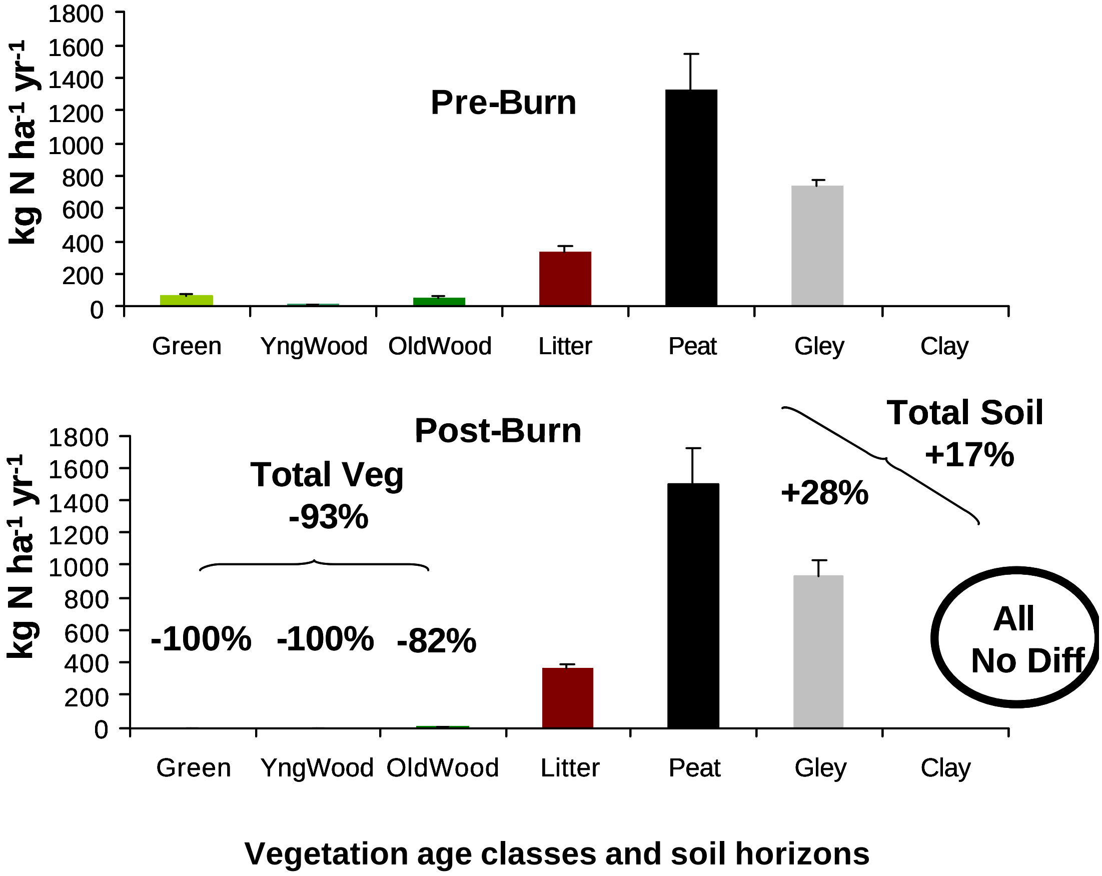


Fig 6: Total nitrogen allocation to soil and vegetation fractions in response to increasing nitrogen inputs (kg N ha^{-1}) to Ruabon "old" plots

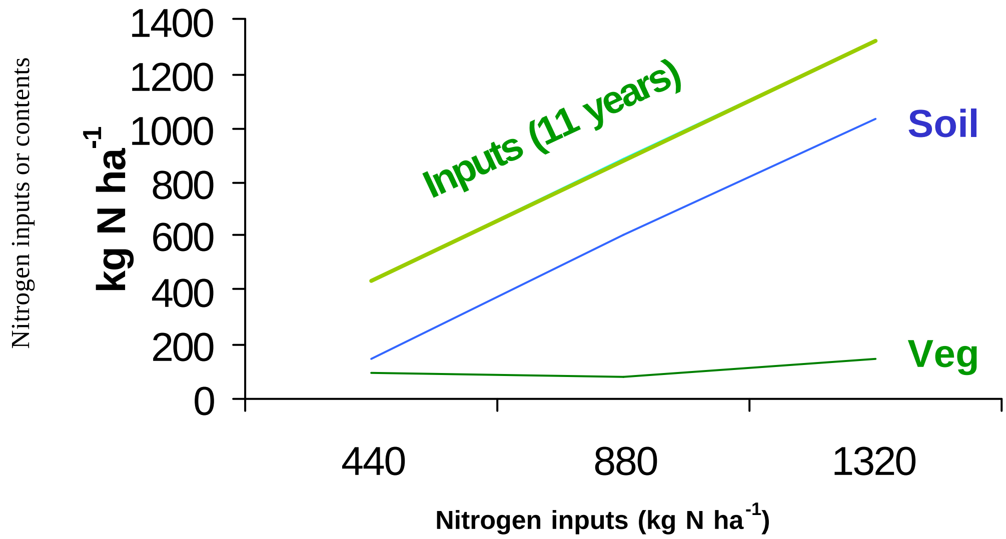


Fig 7: Canopy density data for 1996 –2001 Budworth "main" plots. Effects of nitrogen and drought treatment:

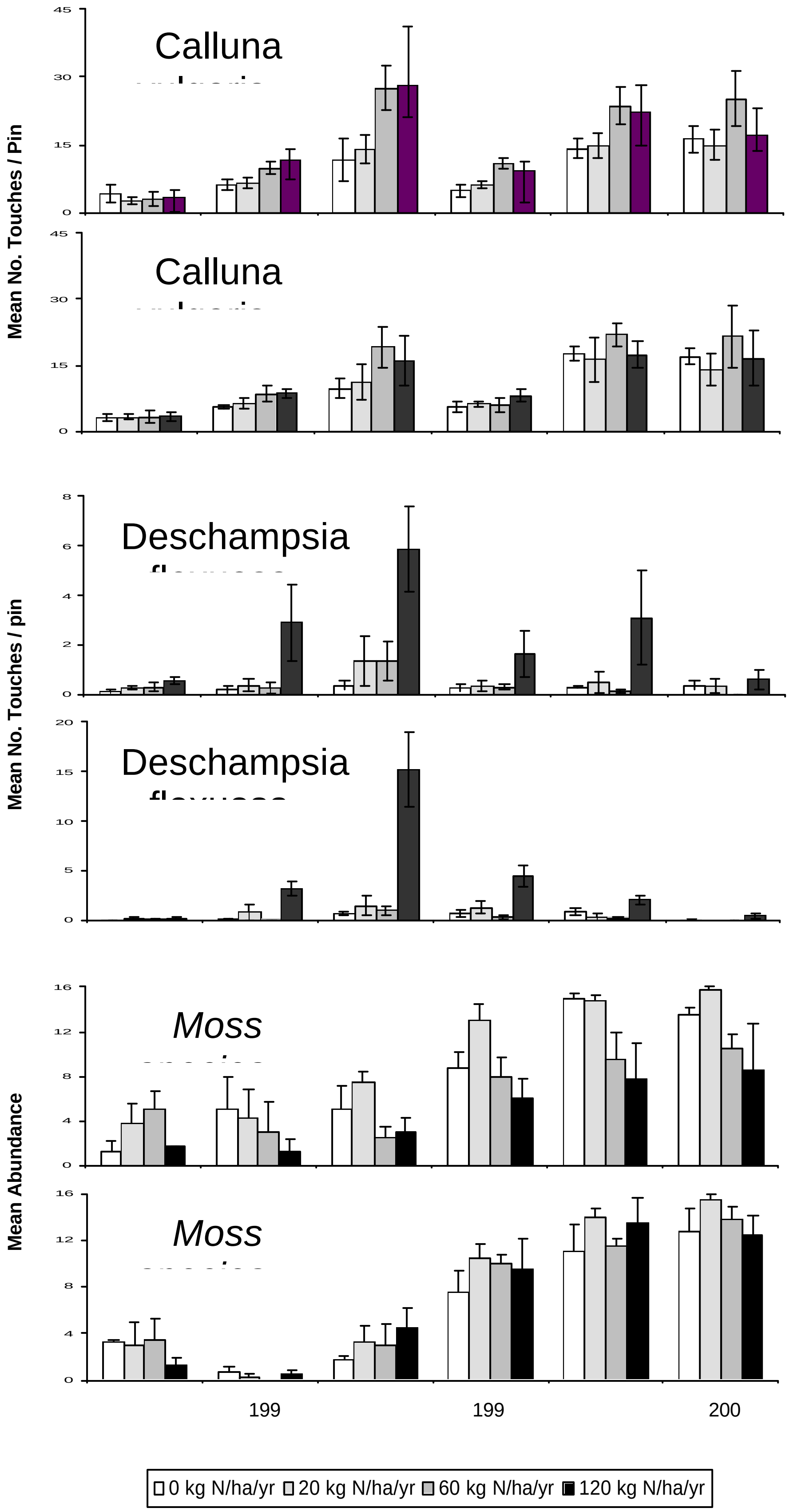


Fig 8: Relationship between total *Deschampsia flexuosa* touches and moss abundance on Budworth "main" plots. Data from 1998-2001.

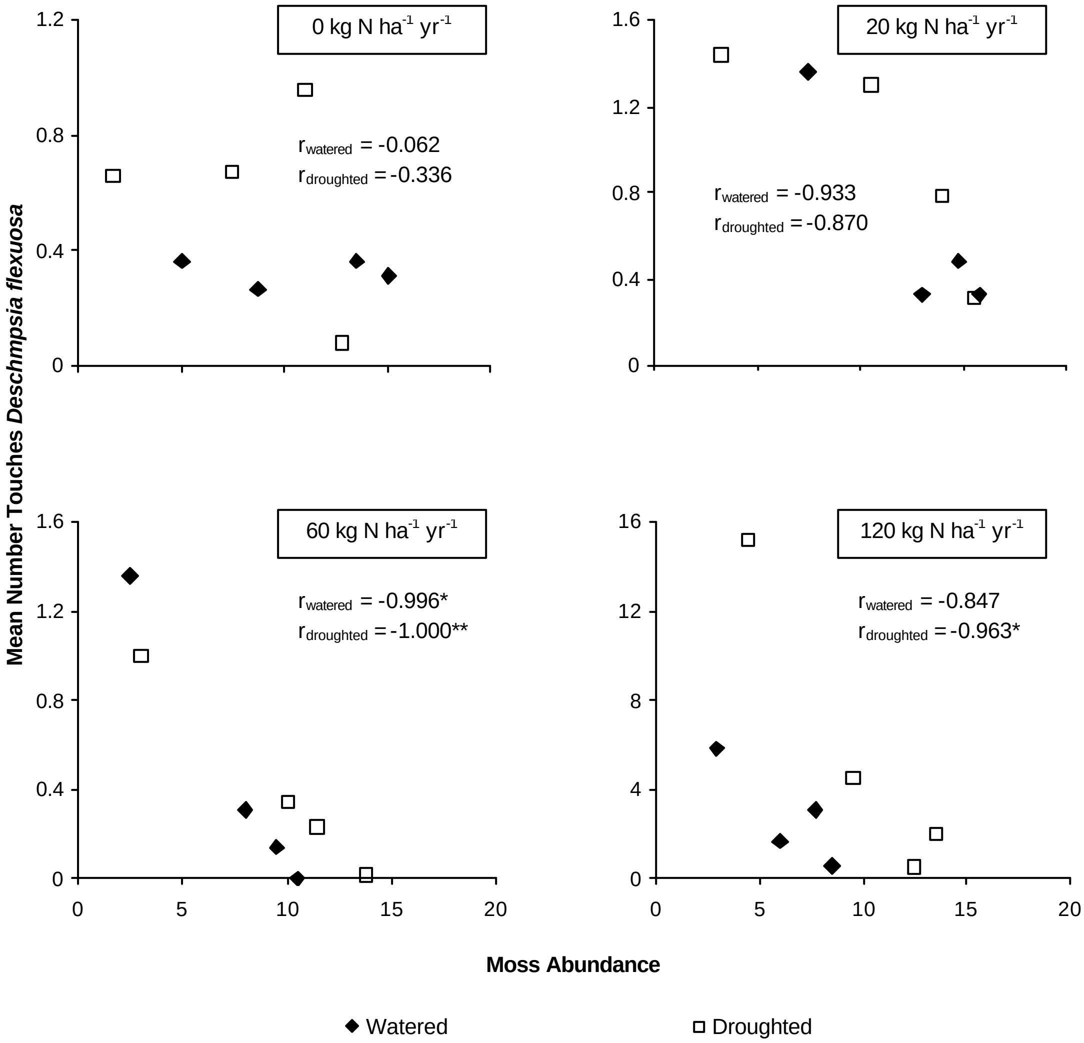
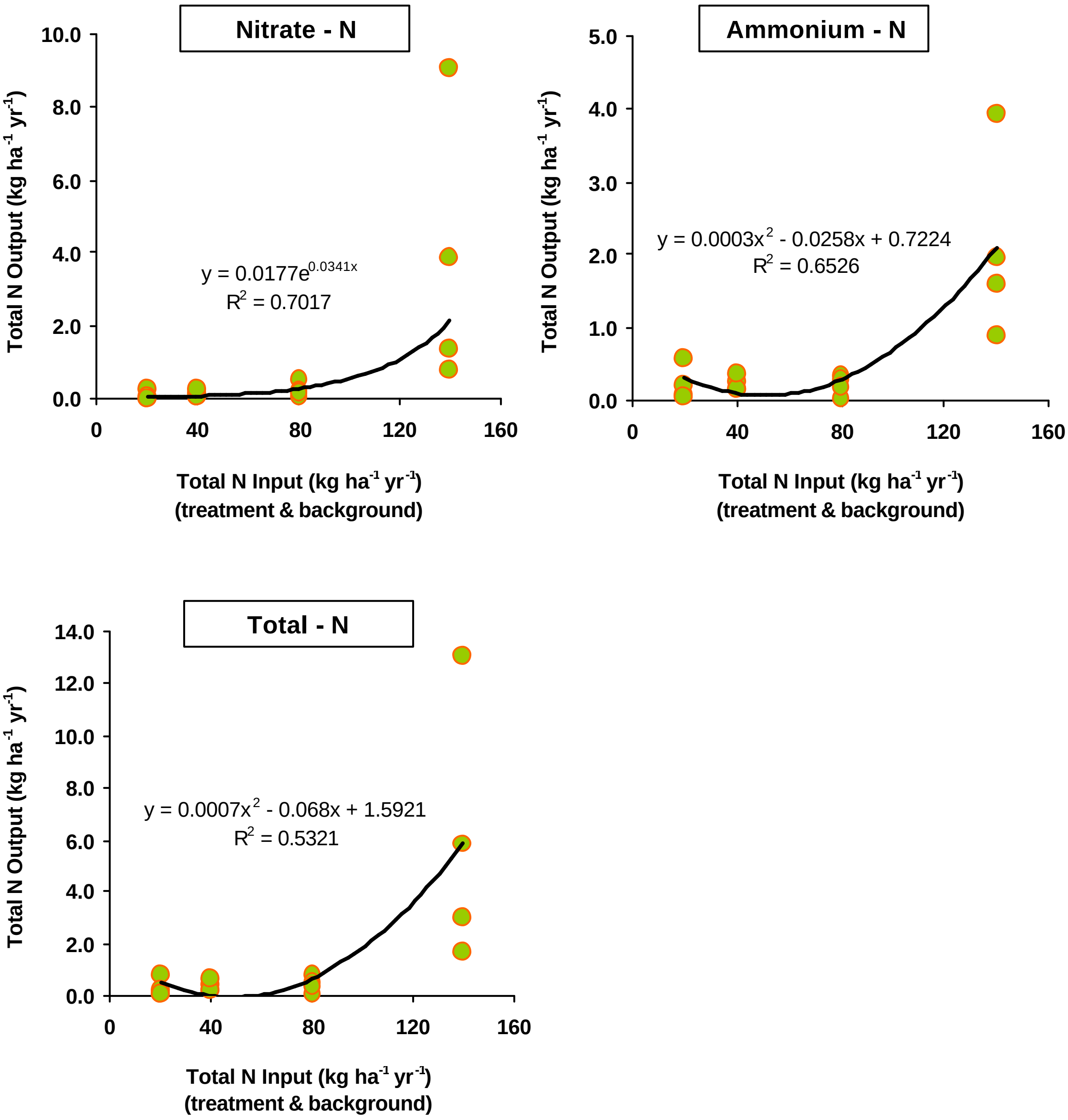


Fig 9: Soil leachate nitrogen fluxes from Budworth “main” plots. 2001 – 2002



Long term impacts of nitrogen deposition on semi-natural vegetation

DEFRA Umbrella Project Annual Report

Sally Power and Emma Green
Imperial College

Introduction

There is now strong evidence that elevated deposition rates of reactive nitrogen has reduced the primary growth limitation by this nutrient in a wide range of semi-natural ecosystems. Many species typical of nutrient-poor environments are unable to compete successfully under more nutrient-rich conditions, with nitrogen deposition thus leading to changes in community composition and the structure and function of ecosystems.

Lowland heathland is one of the fastest declining habitats within Europe; the UK currently has approximately 20% of the remaining lowland heath and its designation as a priority habitat for conservation under UK and European legislation highlights its international importance. Nitrogen inputs can have both direct and indirect effects on plant and soil processes in heathland ecosystems, leading to a disruption of the nitrogen cycle. Whilst high concentrations of NO_2, NO and NH_3 have the ability to directly damage the foliage of plants, such concentrations are rarely seen, except in the vicinity of point sources of emissions. Effects of enhanced nitrogen inputs reported for heathland sites however include changes in plant growth, phenology and chemistry, as well as effects on the soil microbial community and nutrient cycling. Studies have shown that a significant proportion of nitrogen inputs are incorporated into plant material and that, through increases in the quantity and quality of plant litter, this leads to an increase in organic nitrogen stores within the system. Since microbial activity is frequently limited by substrate quality, this may in turn result in an increase in nutrient turnover rates, with long term consequences for community and ecosystem stability.

Aims and objectives

The main aims of the Imperial College study are threefold: 1) to determine the effects and fate of deposited nitrogen on a heathland system, in particular effects on nutrient cycling and the soil microbial community; 2) to investigate the effect of habitat management on ecosystem response to nitrogen and 3) to establish whether management can be used as a tool to promote ecosystem recovery.

The specific objectives of the investigation are as follows:

- To determine plant and microbial responses to enhanced N deposition.
- To determine the fate of deposited N, i.e. the degree to which added N is immobilised in the microbial pool, taken up by plants, leached or denitrified.

- To quantify rates of N turnover and hence determine the relationship between immobilisation and mineralisation.
- To investigate the importance of nutrient ratios in driving ecosystem response to nitrogen and how responses differ, for example, in a P limited system.
- To ascertain whether the results from this investigation can predict what action is necessary to protect heathland ecosystems also contribute to a refinement of current critical loads for nitrogen.

Experimental background and methodology

The experimental site forms part of Thursley Common National Nature Reserve, in Surrey. The study comprises two separate long term manipulation experiments: 1) a "recovery" experiment and 2) a nitrogen-management interaction experiment. The recovery experiment, begun in 1998, follows on from an earlier study where heathland plots received either 0 (control), 7.7 (low) or 15.4 (high) kg ha^{-1} yr^{-1}, over a seven year period (1989-1996). No further nitrogen additions were made to these plots after September 1996. Plots were managed in February 1998, using four different management techniques:

1. Low intensity mow - above-ground biomass removal only
2. Low temperature (management) burn - above-ground biomass removal
3. High intensity mow - removal of above-ground biomass and litter
4. High temperature (simulated accidental) burn - removal of above-ground biomass, litter and humus

Management treatments are nested within nitrogen treatment plots, giving individual (sub-plot) dimensions of $4m^2$. All treatments are replicated in four blocks.

The nitrogen-management experiment began in April 1998 and involves fortnightly addition of $(NH_4)_2SO_4$, as a fine spray, at rates of either 0 or 30 kg ha^{-1} yr^{-1}. Regular measurements of above and below-ground plant, microbial and soil chemical characteristics have been carried out since 1998, formerly as part of a NERC-funded PhD study (1998-2000). The current phase of research at this site, funded under the DEFRA umbrella programme involves investigation of above- and below-ground ecosystem response to nitrogen, ecosystem recovery from eutrophication, and will also involve the use of stable isotopes to facilitate a more mechanistic understanding of impacts on nitrogen cycling in the field.

Plant growth responses are measured annually, in October, using point quadrats to record the following:

- Presence /absence of *Calluna* and other species
- Cover repetition/canopy density
- Height of the *Calluna* canopy
- Current year's extension growth and whether the shoot is flowering
- Understorey species/bare ground

Foliar (N&P) and soil (N) chemical analyses were carried out following digestion in sulphuric acid, with a selenium catalyst. Litter decomposition rates were calculated by measuring weight loss of *Calluna* litter, collected from the relevant sub-plots, in fine mesh bags over different time intervals. Statistical analysis was carried out using Statistica for

Windows version 5.1. The normality of the data was determined using univariate tests (Cochran C, Hartley and Bartlet). Where necessary, data were either log or, in the case of percentage data, arcsin transformed. Significant differences were tested using a two way ANOVA and post hoc analysis was performed using the Scheffé test.

A review of the literature concerning the effects of nitrogen deposition on heathlands and on nutrient cycling has already been undertaken (and submitted as a separate report in April 2002). The main, experimental focus of the current investigation is now underway and can be divided into five distinct sections:

1. Plant responses to enhanced N deposition, N reduction and the effect of different management regimes; monitoring both biological and chemical changes (2001-2004).
2. Characterisation of the microbial community and how it is affected by enhanced N deposition, N reduction and different management strategies (2001-2003)
3. Fate of the deposited N, determined by investigations into the nitrogen cycle, using biological tracers (namely ^{15}N) (2003)
4. Studies into the role of the soil microbial community in nutrient cycling to provide an insight into the functional implications of N deposition for nutrient cycling and the speed of ecosystem recovery (2003-2004)
5. The influence of other nutrients on heathland response to nitrogen (2003-2004)

The information provided within this annual report highlights the results of the field work completed during the first nine months of this investigation. Additional data collected during the proceeding four year period are also included to draw attention to some of the responses which took place during the first few years following management.

Results and discussion

Nitrogen-management interaction plots

The point quadrat data showed that there was a significant effect of nitrogen on shoot length of *Calluna* from 1999-2001. In 2001, shoot length was increased from 16.4 mm in the control plots to 21.3 mm in those receiving additional nitrogen. These data are illustrated in Figure 1 for a) 1999 and b) 2001. Significant effects of management on shoot growth were apparent in 1998-2000. Although the magnitude of differences between managements was similar in 2001, the effect of management treatments was not statistically significant on this occasion (Table 2.1).

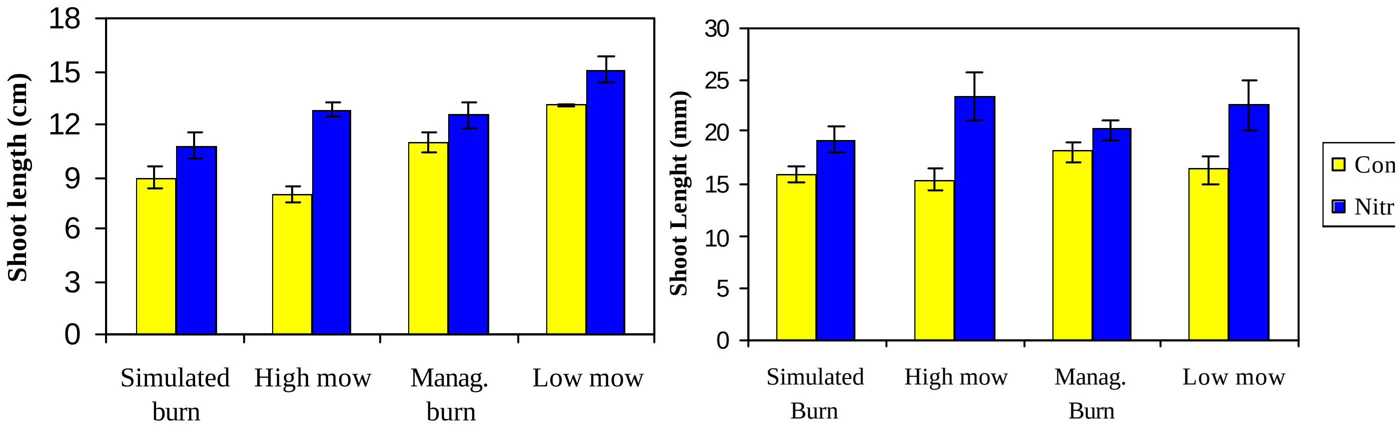


Figure 1. Mean Calluna shoot length following 0 (control) or 30 kg N ha^{-1} yr^{-1} for each management practice for a) 1999 and b) 2001. Error bars represent ± 1 SEM.

The height of the *Calluna* canopy has also been significantly increased by nitrogen additions with values of 14.2 cm in the control plots and 18.9 cm in the nitrogen addition plots (Table 1). Whilst canopy height was consistently increased by nitrogen addition between 1998 and 2000, 2001 was the first time that this effect was statistically significant. Canopy height was not significantly affected by management (Table 1), although the high intensity mow plots had slightly lower canopies than the other managements. A significant effect of nitrogen treatment was also found for canopy density, although there were no significant management or interaction effects for this parameter either.

A significant interaction between nitrogen and management treatments ($F_{3,4}$=3.26, $P<0.05$) was seen for shoot growth in 1999. In the first two years following management, this was indicative of a larger response to nitrogen in plots which had undergone the greatest removal of organic nitrogen stores. For example, in 1999 nitrogen addition increased shoot length by 39.2% in the high intensity managements (high mow and simulated accidental burn), compared to 15.0% in the lower intensity managements (low mow and management burn). Although a similar trend was seen in 2000, a greater shoot response to N in low mow plots in 2001 meant that this interaction is no longer statistically significant. Whilst it is recognised that shoot growth will vary between years, and that factors other than N availability are also differentially altered by habitat management (e.g. soil moisture and availability of other nutrients), there is a suggestion that removal of organic (nitrogen) stores may affect plant response to inorganic nitrogen inputs in the early years following management. As organic material begins to be returned to the soil through litter, these differences may reduce. It will be of particular value to monitor the rate at which differences between managements change with time since this will inform both the choice and interval of management recommendations for sites under different deposition scenarios.

	Shoot length (mm)	Canopy height (cm)	Canopy density	% bare ground	% flowering	% N	% P	
Control	High burn	15.9	14.6	1.49	52	61	0.95	0.079
	High mow	15.4	12.8	1.56	44	58	-	-
	Manag. burn	18.1	14.9	1.52	45	54	-	-
	Low mow	16.4	14.5	1.74	37	55	0.95	0.068
Nitrogen	High burn	19.2	18.6	1.85	35	55	1.05	0.076
	High mow	23.4	18.3	1.96	22	69	-	-
	Manag. burn	20.2	19.0	2.10	23	71	-	-
	Low mow	22.6	19.6	2.08	14	68	1.04	0.067
ANOVA	Nitrogen Management Interaction	19.3 *** 0.8 n.s. 0.0 n.s.	37.5*** 0.8 n.s. 0.2 n.s.	15.4 *** 0.5 n.s. 0.1 n.s.	19.7 *** 2.6 n.s. 0.3 n.s.	2.0 n.s. 0.3 n.s. 0.6 n.s.	20.3*** 0.3 n.s. 0.6 n.s.	0.6 ns 13.2** 0.1 ns

*Table 1. Summary of nitrogen and management effects on above-ground parameters in 2001 **/*** represent significant treatment effects at the P<0.01/P<0.001 level of probability..*

As might be expected, the proportion of bare ground within sub-plots was significantly reduced by nitrogen addition (Figure 2). Whilst the effect of management was not statistically significant, there was nonetheless a trend towards a higher proportion of bare ground in those plots which had been more intensively managed (high intensity mow and simulated accidental burn).

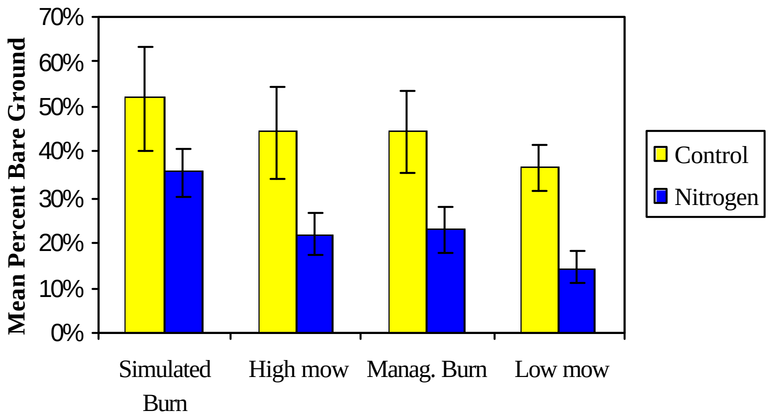


Figure 2. Mean percent bare ground in 2001 for control (0 kg N ha^{-1} yr^{-1}) or nitrogen addition (30 kg N ha^{-1} yr^{-1}) plots for each management practice. Error bars represent ± 1 SEM

Since bare ground provides opportunities for invasion by other species, any benefits arising from the reduction of organic nitrogen stores must be considered in the context of the ability of regenerating *Calluna* to establish a full canopy and thus dominate over other species competing for light.

Foliar nitrogen and phosphorus concentrations were analysed for current year's shoots in October 2001, for the low intensity mow and simulated accidental burn plots. Significant effects of nitrogen treatment were found, with foliar N concentrations increased from 0.95% in control plots to 1.04% in those receiving additional N. A significant effect of management was also found for shoot phosphorus concentration; higher concentrations were found in plots which had previously undergone a high temperature burn (Figure 3). This is indeed interesting since there is currently much interest in the role of phosphorus as a limitation on plant and ecosystem response to nitrogen addition. Whilst phosphorus is not in fact currently limiting at Thursley (as indicated by both plant response to N addition and N:P ratios of 12-16), management by fire appears to affect P availability and may therefore have an influence on the response of P-limited systems to nitrogen deposition. Changes in foliar P concentration over the next few years should reveal information on temporal aspects of this management response and thus longer term implications of this aspect of management by burning.

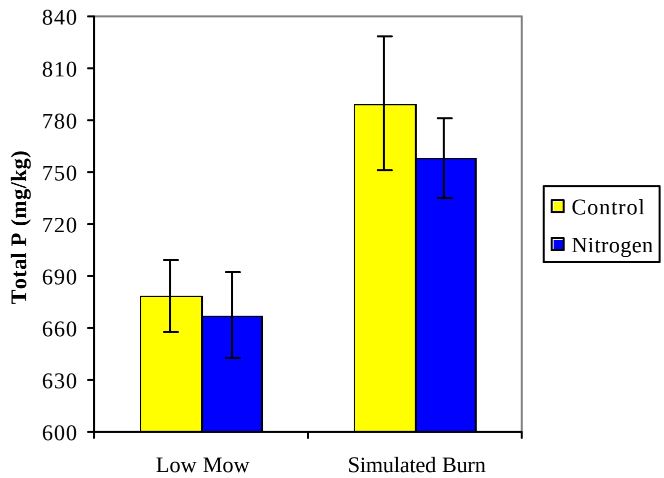


Figure 3. Mean shoot P content (mg kg^{-1}) in 2001 for control (0 kg N ha^{-1} yr^{-1}) or nitrogen addition (30 kg N ha^{-1} yr^{-1}) plots for the low intensity mow and simulated burn. Error bars represent ± 1 SEM

A number of below-ground response to both nitrogen addition and habitat management have also been investigated in these experimental plots since 1998. The most important effect observed to date has been on the rate of litter decomposition. Both nitrogen addition ($F_{1,18\ (36\ weeks)}$=23.7, P<0.001) and habitat management ($F_{3,18\ (36\ weeks)}$=16.9, p<0.001) affected the rate of decomposition, with the fastest weight loss seen in the low intensity mow plots which received additional nitrogen. These data are illustrated in Power *et al.* (2001).

Since litter was collected before nitrogen additions began, the quality of the substrate being decomposed was uniform between treatments in this particular study. The effects of both nitrogen and management must therefore have been via the soil environment, with either the activity or biomass (or both) of the decomposer microorganism community being stimulated by inorganic nitrogen addition. A further study is now underway, to investigate the effect of litter quality as well as soil environment on decomposition rates. Information from these two studies will feed into the ongoing modelling work being undertaken jointly with Bradford University, to quantify the effect of nitrogen deposition on nutrient turnover rates in UK heathlands, which is an integral component of the Heathsol model.

Recovery experiment

Prior to management taking place, there had been very clear, statistically significant effects of former nitrogen addition (1989-1996) in terms of shoot growth, chemistry and litter production. The gradient of increased organic material removal with increasing intensity of management was therefore expected to reduce nitrogen stores to different extents in the four management treatments, with the hypothesis that the simulated high temperature burn and high intensity mow might be more effective than the other managements at reducing differences between former control and high nitrogen plots. Missing data has led to the exclusion of soil nitrogen data for the low intensity mow treatment from statistical analysis. However, the results summarised in Table 2 confirm that soil N stores were reduced to a greater extent following more intensive managements. A small effect of former nitrogen additions was still apparent following management, in particular in the high temperature burn plots, although this was not statistically significant. This suggests that in areas receiving high deposition inputs of nitrogen, availability of this nutrient may remain relatively high, even after removal of almost all plant, litter and humus material.

Management	Former N treatment (1989-1996)	
	Control	High N
Management burn	26.0[ab]	30.6[b]
High intensity mow	22.8[ab]	24.7[ab]
Simulated accidental burn	15.2[a]	21.0[ab]
Nitrogen: $F_{1,18}$=3.79, P>0.05 Management: $F_{2,18}$=7.82, P<0.001 Interaction: $F_{2,18}$=0.31, P>0.05		

Table 2. Effects of management on soil nitrogen content in the recovery plots.

The effect of former nitrogen additions on plant re-growth following management was still apparent several years after additions had ceased. In 2000, plants in the former high nitrogen plots had 26% longer shoots than those in the controls ($F_{1,24}$=9.48, P<0.05), compared to a stimulation of 47% in 1996. However, no significant effect of management was seen at this time (Figure 4).

In 2001, the effect of nitrogen addition on shoot growth was not statistically significant (Table 3). Further monitoring of these plots will reveal whether 2001 represented the point at which shoot growth differences between former nitrogen and control plots were no longer apparent, or whether this was an anomalous year. Either way, above-ground growth is just one of many parameters which will be measured over the next three growth seasons, and does not in itself constitute recovery.

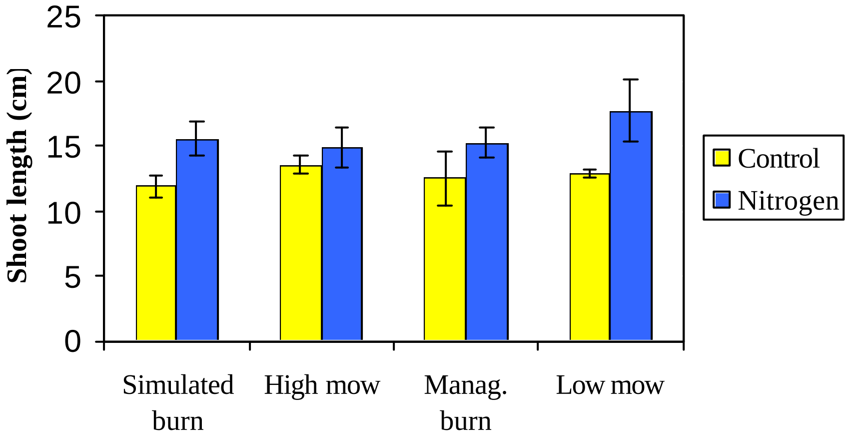


Figure 4. Effects of habitat management and former nitrogen treatment on Calluna shoot length in the recovery plots in 2000.

Foliar chemistry of plants in the recovery plots in 1999 and 2001 has recently been analysed and results indicate that there was no significant effect of nitrogen treatment on October foliar nitrogen concentrations (Table 3). However, since there was rarely an effect of N treatment on foliar concentrations at this time of year (as a result of growth dilution), even when additions were ongoing, this is not surprising.

	Shoot length (mm)	Canopy height (cm)	Canopy density	% bare ground	% flowering	% N	% P
Control	16.85	15.52	1.60	42.5	62.5	0.980	0.066
High nitrogen	17.05	17.25	1.76	37.5	58.0	0.985	0.069
ANOVA: Nitrogen	0.2 n.s.	2.6 n.s.	0.2 n.s.	0.3 n.s.	0.1 n.s.	1.7 n.s	0.3 n.s

Table 3. Effects of former nitrogen treatment on above-ground parameters in recovery plots, 2001.

An effect of management on foliar nitrogen concentrations was apparent in 1999 ($F = 8.34$, $P < 0.05$), although this was no longer significant in 2001. Foliar P concentrations and N:P ratios were determined in 2001, but were not significantly affected by either management or former nitrogen treatments.

Ongoing and future work

Ongoing determination of the effects of nitrogen and habitat management on the soil microbial community is currently underway. Measurements of the soil microbial community have been regularly used in studies of nutrient cycling and provide a measure of the quantity and quality of living microbial biomass present in the soil at a particular point in time (Voroney, 1993). Microbial biomass, nitrogen content, activity and distinction between fungal and bacterial communities will be quantified using selective culture techniques, dehydrogenase activity (INT reductase) assay, denaturing gradient gel electrophoresis

(DGGE) investigation and chloroform – fumigation and extraction. It is anticipated that this will reveal further information on the extent of below-ground disturbance still evident in the "recovery" plots following earlier nitrogen additions.

Detailed analysis of soil moisture content and chemical composition will be undertaken over the next two years, alongside regular measurements of plant performance and chemistry. Biological tracers (^{15}N) will be used to quantify (short term) fluxes and fate of added N at the heathland site.

As with the effects of N on the microbial community, little is known about the role of phosphorus in determining the response of sensitive habitats to N deposition. The importance of P limitation will be investigated using soil cores obtained from sites with low P availability, in a small scale, manipulation experiment in 2003/4.

Conclusions and policy implications

The field experiment at Thursley Common provides clear evidence not only that elevated nitrogen deposition affects plant growth and chemistry, but that there are also effects on ecosystem nitrogen stores and nutrient cycling, mediated via both the plant and microbial communities. The long term implications of an increase in the rate of nitrogen cycling at heathland sites can not yet be determined using data from UK field experiments alone, but an understanding of the mechanisms and processes driving ecosystem response to nitrogen will provide insight into functional changes and inform judgement on the implications of these changes at an ecosystem level. In addition, it will allow accurate parameterisation of dynamic models to predict long term response to changes in deposition inputs of nitrogen in heathland/moorland systems.

Habitat management is an essential feature of semi-natural ecosystems and clearly has an important impact on organic nitrogen stores and heathland response to nitrogen. There is some evidence to suggest that heathland response to inorganic nitrogen inputs is lower (in absolute terms) following more intensive managements which remove a greater proportion of the organic nitrogen stores. This is certainly the case in the first few years following management. However, this must be balanced with greater opportunities for seedling invasion in the early post-management years (which was in fact recorded on site; Barker, 2001), linked to invasion opportunities associated with bare ground. The intensity (and frequency) of management may modify the effect of deposition inputs to sensitive ecosystems, although the timescale for these effects is not yet clear. Management differences may be apparent for several, or very many, years.

Heathland recovery from the effects of elevated nitrogen deposition, following a reduction in inputs, may take many years. Whilst above-ground growth stimulation may be reduced several years after the suspension of nitrogen additions, this is only one of many ecosystem responses. It must also be remembered that the observed responses are following the cessation of inputs of only 15.4 kg N ha^{-1} yr^{-1}, for just a seven year period, and after management had taken place. Many British heathlands have been experiencing elevated inputs over considerably longer timescales, and often at higher deposition rates than those used in this study There is clear evidence that the effects of former nitrogen additions were still apparent in terms of soil nitrogen content, and plant growth four years after additions had ceased. It is therefore likely that effects on the soil microbial community and nutrient cycling are likely to persist for many, possibly very many, years after inputs have been reduced.

Habitat management clearly affects the way in which heathlands respond to management, and may represent a tool to modify the effects of nitrogen deposition to sensitive ecosystems. However, more information is needed on the medium- to long-term effects of both nitrogen deposition and habitat management on nutrient cycling since the availability of nitrogen is an important determinant of plant and microbial performance, and thus of ecosystem function and stability

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Critical loads of nitrogen for acidic and calcareous grasslands in relation to management by grazing

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EXECUTIVE SUMMARY

This experiment aims to: 1). Fine tune the Critical Loads of N for acidic and calcareous upland grasslands, in relation to management by grazing, and 2). Identify the potential for recovery if inputs were to be reduced, and the likely timescales of change. The four N treatments are 2, 10, 20, and 55 kg N /ha/y and the three clipping treatments are Uncut, Light cut and Heavy cut. Treatments started in June 1997 and have now been running for 5 years.

Vegetation responses reveal that different species within these communities have different N optima and take varying lengths of time to adjust to changing inputs. Mosses and lichens responded quickest while vascular plants are now showing significant changes and biomass is increasing in the high N treatment. The mean Ellenberg N number for each treatment reflects N inputs, and may prove a useful indicator of eutrophication.

The below ground parameters show fewer significant responses so far. There is reduced root biomass in the acid cores at high N inputs suggesting less root foraging for nutrients. Litter decomposed faster in the Heavy cut acid cores than the Light cut or Uncut cores. However, the quality of the litter had no effect on decomposition rates.

This valuable experiment is continuing to provide insights into above and below ground responses to the N and clipping treatments and is an important resource for developing and validating predictive models of ecosystem responses to N inputs.

1. BACKGROUND

Since the 1960s, anthropogenic nitrogen in the atmosphere has slowly been increasing in the UK and Europe. While UK emissions stabilised in 1990 and are now starting to decline again (NEGTAP, 2001), the residual soil and vegetation N pools accumulated over time may lead to long term consequences of this enhanced deposition for oligotrophic upland systems. For example, the countryside survey (CS2000) undertaken by CEH (Haines-Young et al., 2000) identified clear evidence of eutrophication in un-improved grasslands, mainly due to increased abundance of more nitrophilous lowland species. Empirical data collected from an acid grassland site at Pwllpeiran, mid-Wales give an input of 20 kg inorganic N ha^{-1} y^{-1} which is compatible with current deposition estimates of 20-25 kg N ha^{-1} y^{-1} for much of upland Wales (UKCLAG, 1997), while deposition to a calcareous grassland at Harpurs Hill, Buxton has been estimated at over 28 kg N ha^{-1} y^{-1} (UKCLAG, 1997). Therefore, inputs at both these sites are already at or above the Critical Loads for acidic and calcareous grasslands of 7-20 kg N ha^{-1} y^{-1} and 14-25 kg N ha^{-1} y^{-1} respectively (Hornung et al., 1995).

The role of additional N deposition in vegetation change in these ecosystems can be assessed using the field experiments at Pwllpeiran (acidic) run by CEH Bangor and Wardlow Hay Cop (calcareous) run by University of Sheffield. However, to test the validity of the critical load ranges or the likely benefits from a decrease in deposition rates, reduction experiments are needed. The cost of having reduction treatments in the field is very high, thus a reduction experiment using mesocosms (cores) from the field sites under more controlled conditions has been established at CEH, Bangor. Therefore, this experiment aimed to investigate the interaction between N deposition and grazing pressure using selective clipping to simulate the effects of sheep grazing. Results from this experiment inform policy decisions on how management practices affect the Critical Loads of these upland grasslands, and on the likelihood and timescales of recovery, if atmospheric N deposition were to reduce further.

2. MATERIALS AND METHODS

Collection of material and experimental design

In early 1997, mesocosms (henceforth referred to as cores) were collected from ungrazed areas on an acidic (Pwllpeiran, mid-Wales, Grid Ref 2798,2771) and a calcareous grassland (Harpurs Hill, Buxton, Grid Ref 4054,3705). Intact cores, 30 cm diameter, were removed down to the full soil depth and allocated to exposure tunnels using their source area and results from an initial vegetation survey to aim for even representation of dominant species in each treatment. The experiment is housed in a misting facility consisting of eight polythene tunnels with overhead spray nozzles. This provides two replicate blocks, each comprising four nitrogen treatments in separate tunnels. Within each tunnel there are three clipping treatments, with four replicate cores of each soil type. Thus, for each soil type there are 96 cores in the whole experiment. Full details of the collection methods, experimental design, and treatment applications are given in earlier reports (Jones et. al. 1998, 2001)

Treatments

The nitrogen treatments are as follows:

kg N $ha^{-1}y^{-1}$	
2	Pristine
10	Ambient - 10
20	Ambient
55	Ambient + 35

Nitrogen, in the form of NH_4NO_3 is added to a maritime rain solution (Reynolds et al., 1990) and delivered as a mist with a droplet range of 5-30 μm, similar to that of hill cloud. Mist is applied for 3.5 hours, 3 times per week. Supplementary water is applied by hand once per week to make up the annual rainfall-equivalent of 2000 mm for the acid cores and 1300 mm for the calcareous cores. Nitrogen treatments started on the 18th June 1997.

Clipping treatments

The clipping treatments are as follows:

1. No clipping
2. Light clipping
3. Heavy clipping

The acid cores are cut using a selective clipping method developed for this experiment to simulate the effects of sheep grazing (Jones et al., 2001), while the calcareous cores are cut at the same level as the lowest heights used for the acid cores. Clipping treatments are applied twice per year – in early and late summer. The first clipping of the cores was in early October 1997.

3. PROGRESS TOWARDS OBJECTIVES AND DISCUSSION OF RESULTS

Vegetation change

Annual assessment of %cover in the N and clipping treatments is continuing. Early results from the experiment showed significant changes in %cover of the mosses and lichens both in response to the clipping and, more slowly, to the N treatments. Figure 1 summarises these early responses to N and shows the year in which the optimum N level for each species became apparent. This reinforces the fact that different species have different optima for N, and this will control the direction, the magnitude and the likely timescale of change under future scenarios involving reduced atmospheric N inputs. The consensus from this experiment is that ambient inputs of 20 kg N /ha/y exceed, or are at the upper limit of, the CL threshold for all the dominant acidic moss species. However, the calcareous moss species (*Pseudoscleropodium purum* and *Rhytidiadelphus squarrosus*) have an optimum at or above 20 kg N /ha/y.

In the last year, changes in the vascular plants which were becoming apparent in previous years, have become more marked. Figure 2 summarises these results, and shows that

Vaccinium myrtillus is clearly increasing in the N addition treatment, as are *Galium saxatile* and the fine grasses, to a lesser extent. However, *Nardus stricta* is decreasing in the same treatment and this potential competitive interaction may develop over time.

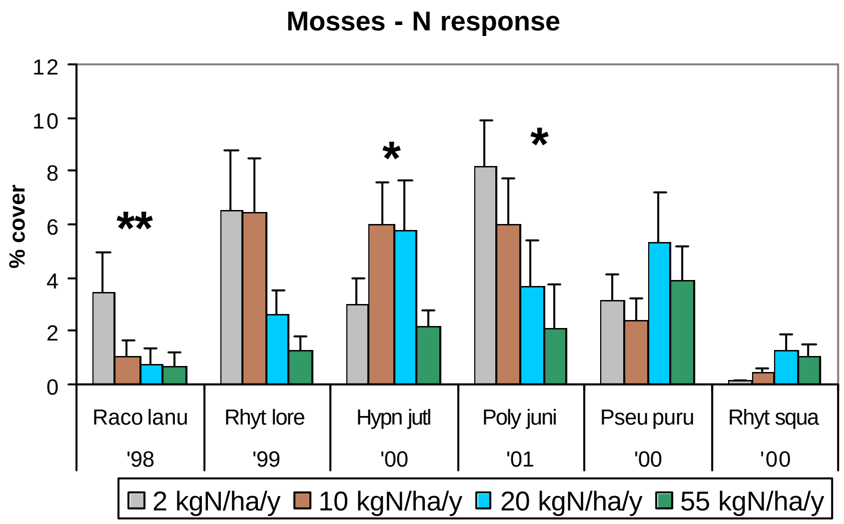

Figure 1. %cover of mosses in the acid and calcareous cores under the different N treatments. Year of data shows when optimum N level became apparent.

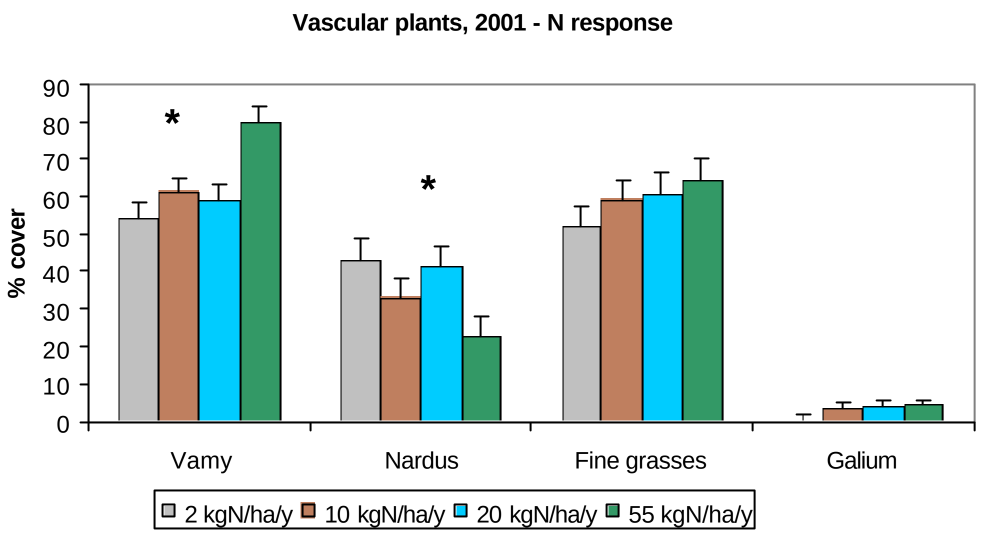

Figure 2. %cover of some of the dominant vascular plants in the acid cores, under the four N treatments, 2001.

The changes in cover of *V. myrtillus* have caused a significant increase in biomass in the acid cores in the high N treatment. However, there is a similar significant increase in biomass in the calcareous cores caused by smaller and, on their own non-significant, increases in cover of grasses, sedges and herbs. These increases are only apparent in the early spring cut and are not apparent in late summer. One possible reason is that the cores are borderline Phosphorus limited and that P deficiency develops during the course of the growing season as plants exhaust the available soil stores. The increased biomass in both soil types at 55N suggests that this level of inputs exceeds the Critical Load for both grassland systems.

Analysis of the mean Ellenberg N numbers for vascular plants, combined with Siebel N numbers for the bryophytes shows a clear divergence between the N treatments in the acid cores. However, there is no clear trend in the calcareous cores. The shift in mean N number reflects both a reduction in the number of sensitive species occurring in the high N treatment, and an increase in the number of species with low N preference in the reduction treatments. This easy to apply screening technique may prove a useful indicator of eutrophication in upland grasslands.

Soil processes

Root biomass

Although there have been exciting changes in the vegetation cover recently, this phase of the experiment has concentrated more on below-ground processes and soil cores taken in spring 2001 showed varying root biomass under the different N and clipping treatments. In the calcareous cores there was significantly lower root biomass in the clipped treatments compared with the unclipped (Figure 3) while in the acid cores there was a non-significant trend towards higher biomass in the heavy clipped treatments (not illustrated). This may reflect differences in response to clipping between the dwarf shrub dominated acid grassland cores and the sedge and herb dominated calcareous grassland cores. Meanwhile, under the N treatments, the acid cores showed a strong but non-significant decrease in root biomass in the high N treatment (Figure 4), which would be expected if roots had to forage less for nutrients. The calcareous cores showed no clear trends in response to N.

New root growth will be assessed when the ingrowth cores are removed and a N budget will be prepared once the soil total N pool has been measured.

PLFA analysis

Soil samples removed for PLFA analysis are currently being analysed. The lipids have been extracted and saponification and derivatisation of the samples prior to analysis on the GC at CEH Merlewood is currently in progress. This will allow us to determine fungal and microbial biomass in the cores.

Decomposition rates

Litter bags were prepared using material from the early summer cut of 2001. A 'transplant' experiment was designed to test whether the quality of the litter, or whether the decomposition environment under the N and clipping treatments would have the greatest effect on decomposition rates. Inter-sample variability was high. However, there is an

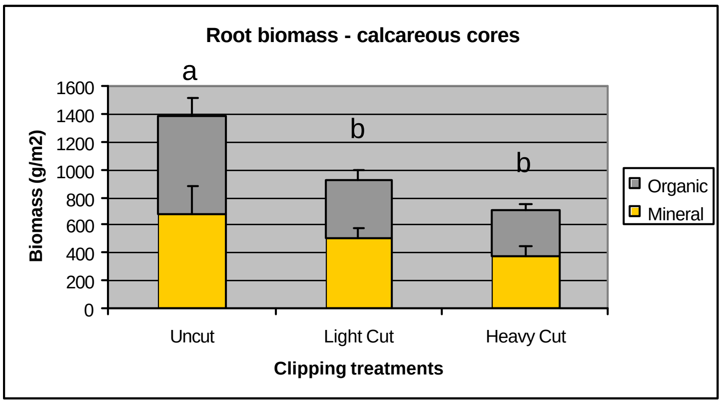

Figure 3. Root biomass in the calcareous cores under the three clipping treatments, 2001

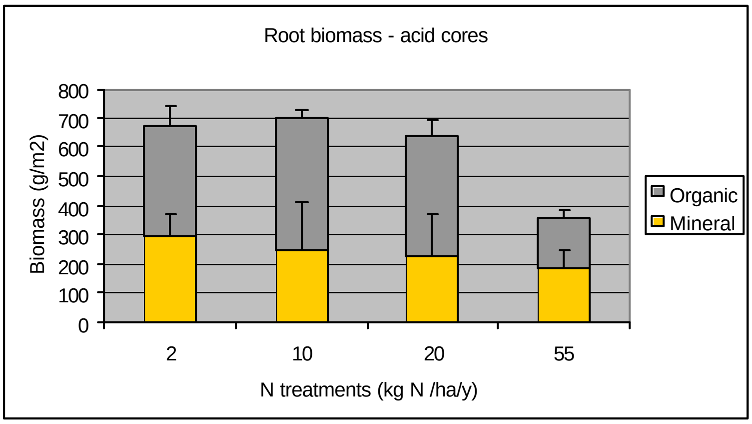

Figure 4. Root biomass in the acid cores under the four N treatments, spring 2001

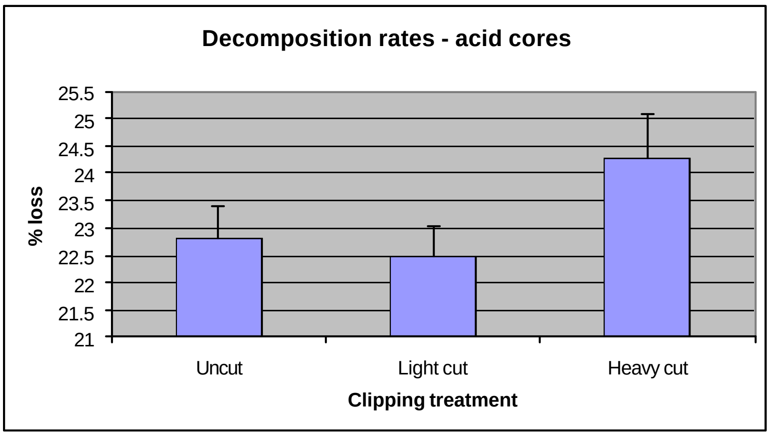

Figure 5. Decomposition of litter (pooled data of litter from different N treatments) in the different clipping treatments in the acid cores.

indication of faster decomposition in the heavy clipped treatment (Figure 5), perhaps because soil temperatures may be higher, and slightly faster decomposition in the high N treatment of the calcareous cores (not illustrated). The litter quality of the material did not seem to affect the speed of decomposition.

4. FURTHER WORK

The N and clipping treatment applications are being maintained. Monitoring of the ongoing changes in the vegetation will continue, with particular emphasis on the vascular plants, and the potential for recovery of the more N-sensitive species will be carefully followed. Further work will try and improve the link between mean N (Ellenberg and Siebel) numbers and Critical Loads of N for these two grasslands. The considerable data set we have built up will be used as part of the adaptation and parameterisation of the SMART-SUMO models for UK vegetation.

Analysis of the PLFA samples will be used to assess whether there is a critical threshold of microbial:fungal biomass ratio in relation to N deposition. A small scale, exploratory, tracer study is planned to identify the sinks for the applied N inputs.

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