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

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1. Summary

This experiment has been set up to refine the critical loads of nitrogen (N) for upland acidic and calcareous grasslands in relation to management regimes, using mesocosms from mid-Wales and from Derbyshire installed in a pollution exposure facility at CEH Bangor, in the context of slowly decreasing anthropogenic N emissions. These controlled environment studies aim to complement field-based N addition experiments being conducted by the University of Sheffield and CEH Bangor. The experiment includes a range of N deposition treatments (2, 10, 20 (ambient) and 55 kg N $ha^{-1}yr^{-1}$) both below and above the current working critical load ranges for these systems, and three levels of simulated grazing (zero, light and heavy), incorporating a degree of selectivity to mimic sheep grazing. Treatments commenced in June 1997 and this report describes key results from 7 ½ years of treatment applications, focusing on the last 3 years.

The aims of this study are to: 1. Suggest refinement of the critical load range for acidic and calcareous grasslands. 2. Evaluate prospects and likely timescales for recovery of ecosystem components. 3. Assess how management can influence application of critical loads at the site level, and modify prospects for recovery.

Acidic mesocosms
Vegetation responses show that different species have different optimum N requirements. Many mosses and lichens, in particular have low N tolerances and are already affected by current ambient deposition. This was borne out by nitrate reductase assays on the most sensitive moss species *Racomitrium lanuginosum.* Four common species are likely to increase in cover if N deposition is reduced to half current levels, and to further reduce in cover if N deposition rises. These species are: *R. lanuginosum, Cladonia furcata, Rhytidiadelphus loreus and Polytrichum juniperinum.* Responses are likely within a few years of changes in N deposition.

In the vascular plants, further increases in N deposition are likely to benefit the shrub *Vaccinium myrtillus* and the grass *Deschampsia flexuosa*, although external factors such as disease and drought sensitivity may interact with N to temper growth of *V. myrtillus*. However, although a few nitrophilic species such as *Galium saxatile*, *D. flexuosa* and *V. myrtillus* show some decreases in the lowest reduction treatment, reductions have to be drastic and responses may take some time.

Species richness is reduced in the N-addition treatment, and slightly increased in the lowest reduction treatment. Mean Ellenberg N and Siebel N numbers have successfully shown these vegetation changes and could be used in acidic grasslands to show where eutrophication is occurring.

N deposition had little effect on litter decomposition or bacterial and fungal biomass. There were few changes in soil chemistry, although there was a decrease in base saturation in the N addition treatment and an increase in the pristine treatment. Soil pH did not significantly alter although given the changes in base saturation, acidification in the 55N treamtnt may occur over a longer time period. There appears to be little leaching of N from the system and a short term ^{15}N tracer study showed that most applied N is taken up by the litter layer and the vegetation. This continued accumulation of N with low natural loss rates suggests that recovery of the soil

system, and hence the vascular plants, from this accumulated N deposition is likely to take decades.

There were significant interactions with the management treatments. The lower plants were able to tolerate higher loads of N deposition in the more intensively managed mesocosms. Preliminary experiments have shown that this is related to Nitrate Reductase activity and the ability to process N at different light levels. The implication is that management can be used to mitigate the effects of N deposition on lower plants by maintaining a more open canopy. However, physical disturbance by grazing animals is likely to damage delicate species and a delicate balance needs to be maintained. The management treatments also altered the competitive balance between *V. myrtillus* and graminoids.

These findings suggest that current ambient inputs are above the critical load for lower plants, which make up 30% of the ground cover and are extremely important for N retention and cycling in this system. Reductions in N deposition are likely to result in increases in some of these species within timescales of a few years. Management aimed at opening up the canopy could be used to mitigate the effects of N deposition in the meantime. A critical load range of 10 – 20 is proposed for this system.

Calcareous mesocosms
Vegetation responses showed few changes in this system. There were fewer mosses and they had higher N tolerances than the acidic species. There were no changes in the vascular plants, although in the last year of the study, there were some increases in biomass in the N-addition treatment. There was no effect of N on either species richness or Ellenberg N and Siebel N numbers. Reductions in N deposition are likely to result in decreases of the moss *Rhytidiadelphus squarrosus*, although few other potential changes could be identified.

There was little effect of N deposition on soil pH, litter decomposition or bacterial or fungal biomass. However, changes in soil water chemistry and appreciable quantities of N leaching (2.9 – 3.6 kg N $ha^{-1}yr^{-1}$) in all but the pristine reduction treatment suggest that even in the half-ambient treatment, supply of N is exceeding demand. The relatively high N leaching is probably a result of P limitation preventing the vegetation taking up much of the incoming N.

As in the acidic mesocosms, the lower plants showed an interaction between N treatments and management, with *R. squarrosus* showing higher N tolerance in the more heavily clipped cores.

Conclusion
These findings suggest that current ambient inputs are above the critical load for this system. Leaching losses at 10 kgn suggest that although few changes in vegetation have been recorded, probably due to P limitation, this is exacerbating the problem of N leaching. Thus you could argue for a lower critical load to protect water quality in a P-limited system. Reductions in N deposition are unlikely to benefit many species and could result in decreases in R. squarrosus. The observed soil chemistry, soil N pools, and soil water chemistry suggest that recovery of the soil system is likely to take decades. A critical load range of 10 – 20 is proposed for this system.

2. Policy relevance

The setting of critical loads for acidic and calcareous grassland systems is of crucial policy relevance. Both these systems are under-studied both in Europe and in the UK, particularly by comparison with forests and heathlands, and understanding the effects of N deposition on biodiversity of these systems is a national priority. This work has implications for management of Racomitrium heath, a UKBAP habitat important for breeding of dotterel (*Charadrius morinellus*), which Britain has a particular obligation to protect as it holds the greatest area of this habitat in Europe. Data from these experiments have been presented widely in the UK and in Europe and have been instrumental in modifying critical loads at the UN-ECE workshop on critical loads for nutrient nitrogen held in Berne, November 2002. This and the CEH field experiment are the only critical load studies on a true upland acid grassland, and one of only two critical load studies on calcareous grasslands. Results from these studies have been incorporated in the DEFRA critical loads mapping exercise.

Results from the acid grassland show that many lower plants are at risk from N deposition. Although often disregarded these are an important component of upland systems. They make up a considerable proportion of the ground cover, usually over 30%, and as they immobilise much of the deposited N, they play an important role in chemical transformations and N release to the rest of the soil-plant system. The moss layer also insulates the soil. Thus, a community without moss cover is likely to result in increased leaching losses of N with consequences for stream water chemistry, and also increased decomposition and erosion of the peaty soil layer. This may well be a factor in the widespread erosion of blanket peat in the Pennines.

Results from the calcareous grassland show that, contrary to established opinion, P limitation may exacerbate the effects of N deposition as far as N leaching is concerned. This has serious implications for stream water quality and the Water Framework Directive 2000/60/EC.

This experiment was the first in Europe to investigate reduction treatments in grasslands. This has yielded interesting results showing the potential benefits of reducing N deposition. Also important in this regard is that many of the responses are linear, showing that even small reductions in deposition are of value. Conversely, inaction, or increasing N deposition is likely to cause further damage. The prospects for recovery of a major component of the acid grassland system, the bryophytes, are particularly promising. It has also given us an indication of likely timescales of recovery. Surprisingly, these can be as rapid as a few years in the case of bryophytes and lichens, and provides visible and tangible evidence to vindicate changes in policy. However, recovery of the soil system, and hence changes in most vascular plants, is likely to take decades.

A further important component of this study has been the interactions with management. Of key significance here is that management can be used to mitigate some of the effects of N deposition by creating a more open canopy. As the clipping levels equate to ESA grazing prescriptions this information can be used to help set stocking levels for a site in order to manage for N effects, or to help interpret the local critical load of N given certain grazing pressure.

3. Project description

This 7 year experiment studies the interacting effects of nitrogen deposition and management on the vegetation and soil of an acidic and a calcareous upland grassland. The experimental approach uses mesocosms under controlled conditions and examines both effects of increasing N deposition and reductions in N inputs. The two major strengths of this study were 1) The inclusion of reduction treatments to assess potential recovery of damaged ecosystems under scenarios of declining N inputs; and 2) The modifying effects of management on N deposition impacts.

Key aims of this study were to:

1. Suggest refinement of the critical load range for acidic and calcareous upland grasslands.
2. Evaluate prospects and likely timescales for recovery of ecosystem components.
3. Assessment of how management can influence application of critical loads at the site level, and how management can modify prospects for recovery.

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 and Ashenden, 2001, Jones et al., 1998).

Treatments

The nitrogen treatments are as follows:

kg N $ha^{-1}yr^{-1}$	
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 based on 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. Solute concentrations in the ambient treatment solutions for NH_4^+ (142 μeq l^{-1}) and NO_3^- (143 μeq l^{-1}) were designed to approximate those reported by Reynolds *et al.* (1996), for hill cloud in mid-Wales.

Nitrogen treatments started on the 18th June 1997. Actual sprayed inputs varied from year to year partly due to refurbishment of the facilities in 1998 and more recently in 2001, however the average was within 2.5% of target.

Background N deposition in the experimental facility

Measurements were made of atmospheric nitrogen dioxide and ammonia concentrations both on site and within the misting tunnels in order to calculate the additional input from dry deposition. NO_2 was measured for one month in April 2002 and on the basis of this data (mean 1.25 $\mu g\ m^{-3}$ and assuming a deposition velocity of 1.13 mm s^{-1}), approximate inputs of 0.14 kg N ha^{-1} yr^{-1} were estimated. NH_3 concentrations were measured on site and in several tunnels for 5 months. The air concentrations vary depending on the N treatment, outside concentrations and the weather conditions, but average around 2.00 $\mu g\ m^{-3}$ inside the tunnels, with the cooling fans running. Assuming a deposition velocity of 15 mm s^{-1} this gives an additional 7.8 kg N ha^{-1} yr^{-1} from NH_3, which together with the NO_2 inputs, gives an additional 8 kg N ha^{-1} yr^{-1} to each wet N deposition treatment. Therefore, on average, the total N treatments are 10, 18, 28 and 63 kg N ha^{-1} yr^{-1}.

Clipping treatments

The clipping treatments are as follows:

1. No clipping
2. Light clipping (11 – 14 cm) –ESA prescription – 25%
3. Heavy clipping (6 – 10 cm) –ESA prescription + 30%

This selective clipping technique was designed to reproduce the impact of sheep selectively grazing vegetation of differing palatability and was based on information from Grant *et al.* (1985) and over 8000 canopy height measurements made at a linked experiment in Mid-Wales (Emmett et al., 1996). Full details of the technique can be found in Jones *et al.* (2001). Clipping treatments were applied twice per year – in April/May and August/September, except for the first clipping of the cores which was in early October 1997.

Statistics

Point quadrat data for each year were analysed using the General Linear Model (GLM) procedure in Minitab 14, with cover from the baseline year used as a covariate for all vascular species. All statistical significances are taken at 95% (i.e. $p < 0.05$). All analysis takes into account the nested design of the experiment with clipping treatments nested within the N treatments.

4. Key findings – Acid grassland

General vegetation responses
Table 1 summarises changes in cover in the dominant vascular and lower plant species. In general, responses of the vascular plants to the N treatments have been slow, although competitive interactions are now becoming apparent. On the other hand, the response of some of the mosses and lichens to the nitrogen treatments in this experiment was almost immediate.

-Rapid response of bryophytes and potential for recovery.
The bryophytes and lichens have shown rapid responses to the N treatments, with the species with the lowest N tolerance usually being the quickest to respond. These

Table 1 Summary of N, clipping and N x clipping interaction effects for the dominant acid mesocosm species for each year. Data were analysed by analysis of variance using the GLM procedure in Minitab with %cover in 1997 as a covariate. +,-,? show direction of effect; **n.s.** denotes a non significant trend; *, **, *** denote significance levels of $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

Species	Nitrogen					Clipping					N * Clipping Interaction				
	'98	'99	'00	'01	'03	'98	'99	'00	'01	'03	'98	'99	'00	'01	'03
Deschampsia flexuosa				?+ *	+ n.s.					+ n.s.				**	*
Festuca ovina						- n.s.	- **	- **	- n.s.	+ n.s.					
Nardus stricta	- n.s.			?- *	?- n.s.	+ **	+ **	+ **	+ ***	+ ***					
Vaccinium myrtillus			+ *	+ *	+ n.s.				+ n.s.	+ n.s.					
Vaccinium vitis-idaea					? *	- ***	- ***	- ***	- ***	- ***					
Galium saxatile	+ n.s.	+ n.s.	+ n.s.	+ n.s.	+ n.s.	+ **	+ ***	+ **	+ **	+ n.s.				*	*
Polytrichum juniperinum			- n.s.	- *		+ **	+ **	+ *							
Rhytidiadelphus loreus	- n.s.	- *		- n.s.		+ n.s.	+ **	+ *							
Hypnum jutlandicum			- *	- n.s.		+ ***	+ ***	+ *	+ n.s.		*				
Racomitrium lanuginosum	- *	- n.s.	- *	- **		+ ***	+ ***	+ ***	+ n.s.			*	***	*	
Cladonia furcata	- n.s.	- n.s.	- n.s.	- n.s.		+ **	+ n.s.	+ *	+ n.s.						

species, in order of decreasing sensitivity as measured by their optimum N level for growth (see Fig. 1), are: *Racomitrium lanuginosum, Cladonia furcata, Polytrichum juniperinum, Rhytidiadelphus loreus, Hypnum jutlandicum*. These results compare favourably with those of other studies (Dirkse and Martakis, 1992, Jones et al., 2002, Pearce and van der Wal, 2002). The optimum N loads for many of these species lie at or below current ambient inputs and we are seeing an increase in cover of *R. lanuginosum, C. furcata, R. loreus,* and *P. juniperinum* in treatments below the ambient (20 N), although not all these are significant. This implies that there is a potential for recovery of these species in the field if N concentrations in the atmosphere were to be reduced, provided management conditions are favourable. These changes may be within a timescale of a few years following marked reductions in N inputs. The further decrease in cover at N deposition loads above the current ambient suggests that the relationship with N deposition is not a simple threshold-based one, and that increased inputs will still have further detrimental effects. Conversely, even small reductions in inputs can have worthwhile consequences for N sensitive species.

-Slower response of vascular plants with implications for recovery

The response of the vascular plants to the N treatments has been slower than the bryophytes and lichens. This is largely because much of the nitrogen uptake is mediated by soil processes and soil biological activity. Other studies in the literature have showed delayed responses to N additions in the higher plants. For example, vascular plant responses at Wardlow Haycop took 3-4 years (Morecroft et al., 1994). The delayed response may be due to immobilisation and uptake of N by soil bacteria before the vascular plants are able to utilise it, or possibly due to P limitation in the soil. Both field sites are thought to be P limited (Gordon et al., 2001, Lee et al., 1996). Changes witnessed within the first few years of this experiment, and reported in detail for the first phase of this project (Jones and Ashenden, 2001), include increased tissue N in *Vaccinium myrtillus* and the grasses, altered flowering intensity and timing in *V. myrtillus* and *Festuca ovina*, and a strong advancement of leaf bud burst of *V. myrtillus* in spring with increasing N deposition. These changes all indicate that the vascular plants were seeing some of the N. However, changes in cover did not become apparent until the 3rd (*V. myrtillus*) or 4th (*D. flexuosa, N. stricta*) year of the experiment (Table 1, Fig. 2). These results are reflected in increasing biomass in the N addition treatment which slightly lagged behind changes in cover (Fig. 3). This increase in biomass was largely attributed to *V. myrtillus* (Jones and Ashenden, 2001). The increase of *V. myrtillus* in the high N treatment here differs from results from a field experiment in mid-Wales where no increase was observed (Gordon et al., 2001). This may be because other factors such as fungal/pathogen attack (Nordin et al., 1998), climatic effects, and selective grazing by sheep of N rich material may operate in the field to reduce any growth advantage. Frost damage is unlikely to be a factor in the field, as earlier experiments showed that N did not alter the frost sensitivity of *V. myrtillus* (Jones and Ashenden, 2001). However, drought may be a controlling factor, which was not investigated in this study. *Deschampsia flexuosa* showed a clear increase with increasing N treatments. This response is not surprising as *D. flexuosa* has responded positively to increased N inputs in the Netherlands, and in pot experiments (Truscott, 1997). There was a positive relationship between growth of *D. flexuosa* and *V. mrytillus*, which has also been shown in other studies (Hester et al., 1991).

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

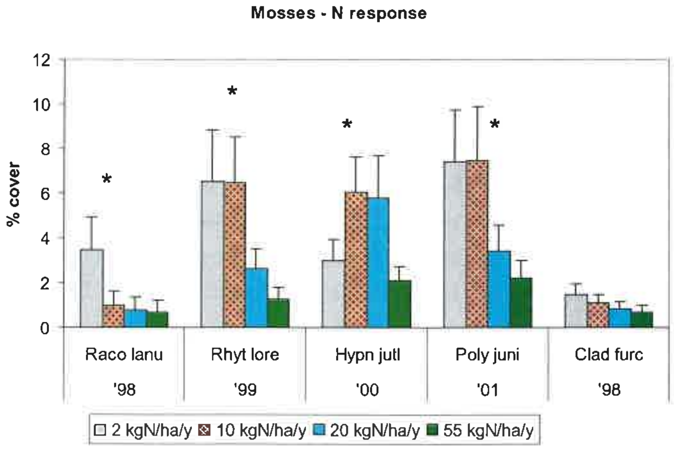
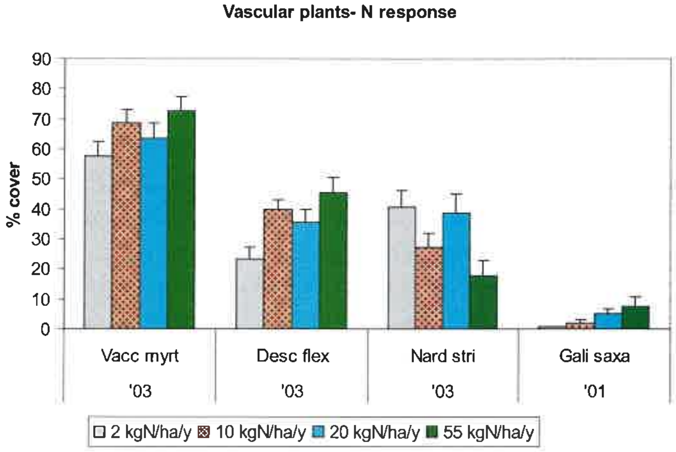


Figure 2 %cover of selected vascular plants in the acid mesocosms under the different N treatments.



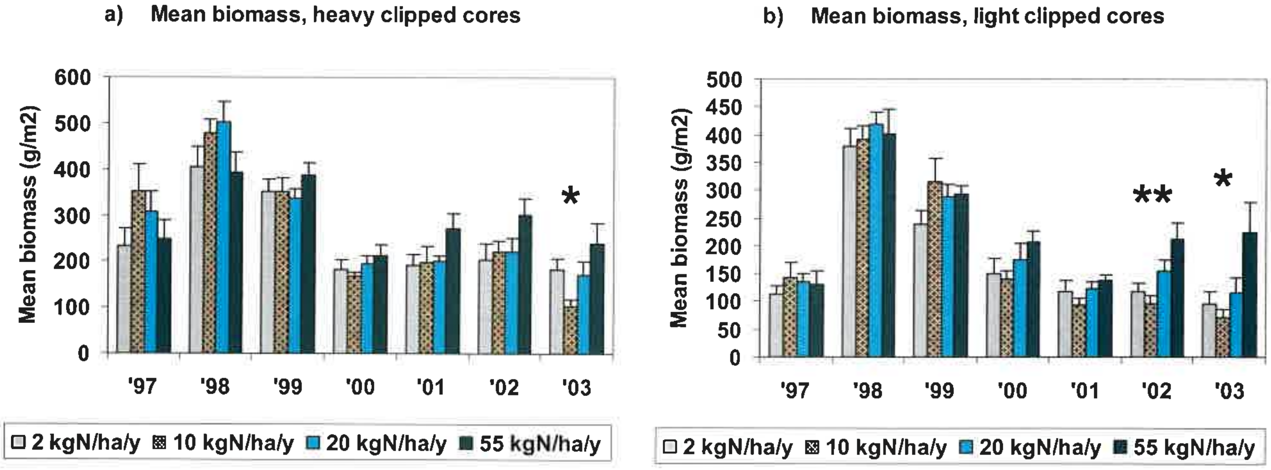


Figure 3 Mean productivity in each N treatment for a) the Heavy clipped cores and b) the Light clipped cores. Acidic grassland.

Results from our study also suggest that there is a strong competitive (ie. negative) interaction between *N. stricta* and *V. myrtillus*, which has also been shown for *N. stricta* and another dwarf shrub, *Calluna vulgaris* (Alonso et al., 2001).

These results indicate that increased N deposition above current ambient will have further consequences such as increases in *V. myrtillus* or *D. flexuosa*. However, these increases may be tempered by other factors such as increased pathogen attack, or drought sensitivity under high N deposition. However, while reductions in N deposition may have some effect on cover of the more nitrophilous species, observed in this experiment in the lowest (2N) treatment. These are only likely following drastic reductions in N inputs, and will take at least 5 years to become apparent.

-Interactions between management and the N treatments

Interactions have been observed in the bryophytes and lichens and, to a much lesser extent in the vascular plants. Although only *R. lanuginosum* showed a significant N x clipping interaction, there appears to be a similar, non significant, effect in the mosses *R. loreus, H. jutlandicum, P. juniperinum* and the lichen *C. furcata,* see for example Fig. 4. All these species show a pattern of increased tolerance to N inputs in the more heavily clipped cores. Therefore, they appear to be able to tolerate higher N inputs in a more open canopy, which has important implications for using management to mitigate effects of N deposition. The precise mechanisms for this increased N tolerance under higher light levels in the clipped cores are unclear, but similar effects have been shown on *R. lanuginosum* by colleagues at CEH Banchory (R. van der Wal, *pers comm*). Nitrate reductase is the rate-limiting enzyme responsible for the first step of assimilation of nitrate by plants, and induction of this enzyme can be suppressed by excess supply of nitrate (Woodin and Lee, 1987). This enzymic reaction has a high energy cost and is controlled by light levels. Therefore, it is possible that, at lower light levels, induction of nitrate reductase is insufficient to assimilate the supply of nitrate, resulting in a more rapid build up of nitrogen toxicity than occurs at higher light levels. Other experiments have shown reduced levels of nitrate reductase activity in experimentally shaded *R. lanuginosum* at the acidic field site, particularly under high ammonium inputs (Jones et al., 2004), and increased levels of nitrate reductase activity in the two reduction treatments in the misting tunnels (Fig. 5), although this was not significant, further suggesting that current ambient deposition exceeds the critical load for this species.

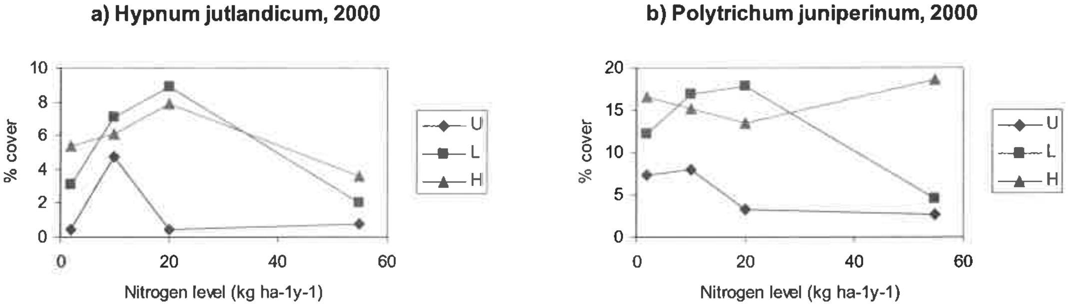


Figure 4 Graphs showing %cover in each N x clipping treatment combination for a) *Hypnum jutlandicum* and b) *Polytrichum juniperinum.*

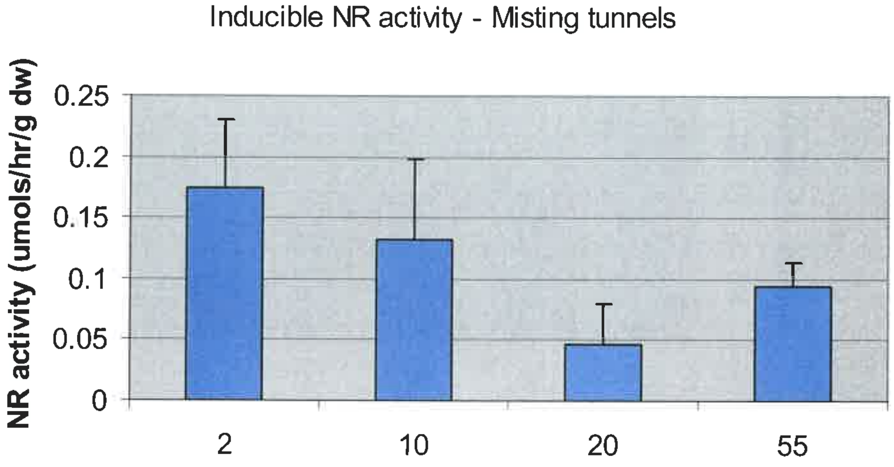


Figure 5. Inducible nitrate reductase activity (NRA; µmol NO_2^- h^{-1} g^{-1} dry weight) in the misting tunnel experiment (Inducible = Induced minus constitutive NR activity), for all four nitrogen treatments.

In the higher plants, the dominant shrub, *V. myrtillus*, did not show a significant interaction. However, its response differed at different N levels. At 55N, it did equally well in all clipping treatments, whereas in the reduction treatments *V. myrtillus* fared worst in the unclipped treatment, where species such as *Juncus squarrosus, Festuca ovina* and *Nardus stricta* appeared to be potential competitors.

Results from both the lower and higher plants suggest that management can alter species responses to N deposition. In the vascular plants, it can alter the competitive interactions between shrubs and grasses. There is a danger that, at higher management intensities, grasses such as *D. flexuosa* which are better able to utilise the N may become competitors with the dwarf shrubs. In the lower plants, opening up the canopy brings benefits by increasing the tolerance of some species to N deposition. However, grazing is the most common management option in upland grasslands and associated impacts such as eutrophication and scorching from dung and urine, and physical disruption by grazers can damage sensitive components of these systems. There is thus a delicate balance to be struck if the aim of managed grazing is to mitigate effects of N deposition.

-Species richness.
There was a significant effect of N on species richness, which decreased in the N addition (55N) treatments and increased in the pristine (2N) treatment, relative to ambient (Fig. 6).

-Ellenberg numbers.
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 (Fig. 7). The shift in mean Ellenberg N number reflects both a reduction in the number of N-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

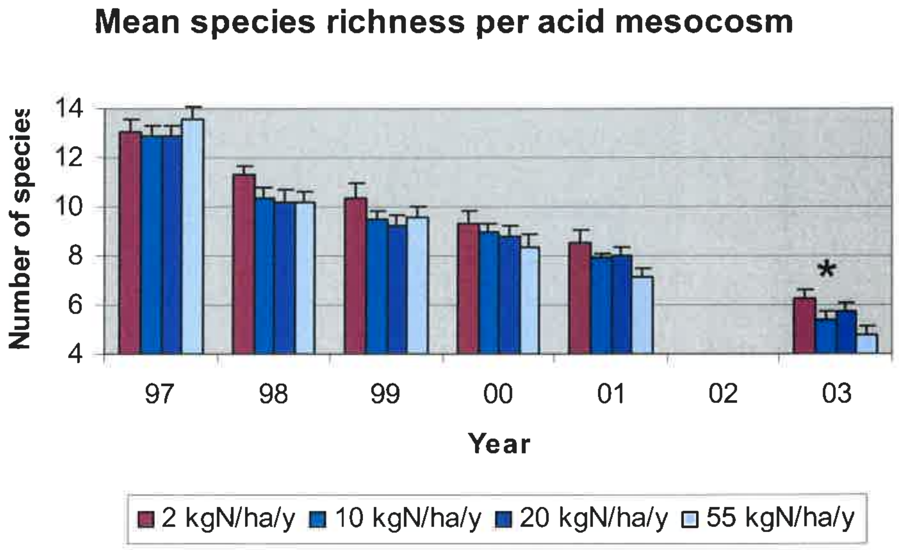


Figure 6. Mean species richness in the acidic mesocosms, across the four N treatments

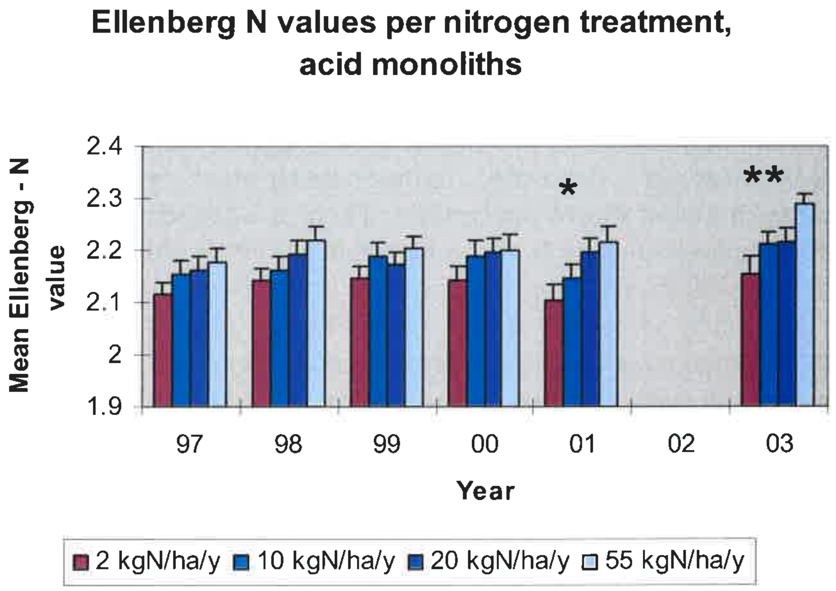


Figure 7. Mean Ellenberg N number in the acidic mesocosms, across the four N treatments

reinforces the benefit of reducing N inputs, and the need to avoid further increases in N deposition. This easy to apply screening technique may prove a useful indicator of eutrophication in upland grasslands.

General soil responses.

Responses in the soil and below ground were less clear than the above ground responses. Many experiments showed no significant results.

-Litter decomposition

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 and there were no effects of N treatment on decomposition rates. However, there is an indication of faster decomposition in the heavy clipped treatment, perhaps because soil temperatures may be higher. The litter quality of the material did not seem to affect the speed of decomposition.

-Bacterial/fungal activity

Soil samples were extracted for analysis of phospho-lipid fatty acids (PLFAs), a component of cell walls. Bardgett *et al.* (1999) studying temperate grasslands have shown that fungal:bacterial ratios are higher in unfertilised grasslands and suggest that this is due to shifts in fungal biomass rather than the bacteria. A study on upland acid grasslands with a range of agricultural improvement (Grayston et al., 2001) showed bacterial PLFAs to be higher in the improved grasslands and fungal PLFAs to be higher in the unimproved grasslands, which were the same NVC community (U4) as the acid cores in this mesocosm experiment. However, in our experiment there were no significant responses to N treatments in the PLFAs of either bacteria or fungi despite changes in biomass and vegetation composition. That we did not find any major shifts in fungal or bacterial populations is not entirely unexpected as most published work has compared soils from different vegetation communities rather than comparing soils from experimental manipulations within one NVC community. What it does suggest is that major shifts in the vegetation and soil communities are necessary over a longer duration of N inputs before we can pick these effects up in the fungal and microbial biomass signatures of PLFAs.

-Root growth

There was an indication of lower root biomass in the mineral layer in the N addition treatment (55N), which was observed both in 2001 and 2003. However, this was not significant.

-Soil chemistry

Soil samples collected at the end of the experiment showed a non-significant increase in pH in the pristine N treatment. There were few changes in soil chemistry. However, there was a non-significant trend towards increased NH4-N in both the litter layer and the combined organic and mineral horizons (Fig. 8). There was also a significant decrease in base saturation in the combined organic and mineral horizons (Fig. 9).

-Soil water chemistry

Soil water samplers installed in the mineral horizon of the cores at the base of the rooting zone showed no leakage of nitrate. Leakage of ammonium-N has been barely detectable, averaging around 0.1 mg l^{-1} which equates to 0.29 kg N ha^{-1} yr^{-1}. Leakage of nitrate-N is also low and equates to 0.23 kg N ha^{-1} yr^{-1}. Leakage is slightly higher in summer than in winter and seems to correspond with times of harvest. The pattern

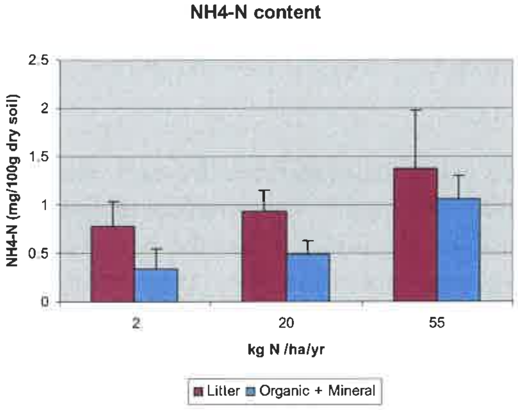


Figure 8. Changes in available NH4-N content of litter layer and the combined organic and mineral layers of the acidic mesocosms, across three N treatments.

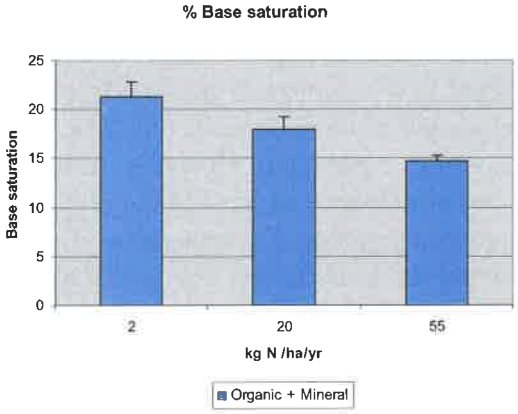


Figure 9. Changes in base saturation of the combined organic and mineral layers of the acidic mesocosms, across three N treatments

of leakage was similar in both clipping treatments and there are no effects of N or clipping treatments. This is slightly surprising as experiments at the acidic field site have shown leakage of nitrate in all N addition treatments including the control, suggesting N saturation. The lack of leakage in this study may be due to increased uptake of N by the plants and incorporation into microbial biomass, or increased losses of N to de-nitrification, although de-nitrification losses in the field were extremely low.

Where is the N going? Results from a 15N tracer study.

A tracer study showed that plants were able to take up some of the applied N very rapidly. Within a week, ^{15}N enrichment in the leaves was higher than any other component of the system (Fig. 10a). Of the three individual species studied, *V. myrtillus* was more efficient at N uptake than the grasses (*D. flexuosa, F. ovina)*. At the highest N treatment, uptake by the bulk vegetation in the mesocosms appeared to tail off, suggesting excess N supply over demand (Fig. 10b). Total recovery of ^{15}N was highest in the 2N treatment and was lower in the 20 and 55N treatments, suggesting that they have a lower demand for N (Fig. 11a). N was preferentially retained in the roots and organic material within the litter layer (Fig. 11b), although approximately 22% of the ^{15}N recovered was taken up by the vegetation and a similar quantity was held in the soil of the litter layer. In the soil compartments, recovery of ^{15}N in the litter layer was highest in the 2N treatment, and in the combined organic and mineral layers, recovery was highest in the 2N and 20N treatments.

These results suggest that demand for N is higher in the lowest reduction treatment and that in the ambient and 55 N treatments, supply is exceeding requirements. They also show that, of the three species studied, *V. myrtillus* is most effective at immediately accessing the applied N, which may explain its strong competitive behaviour in the mesocosms.

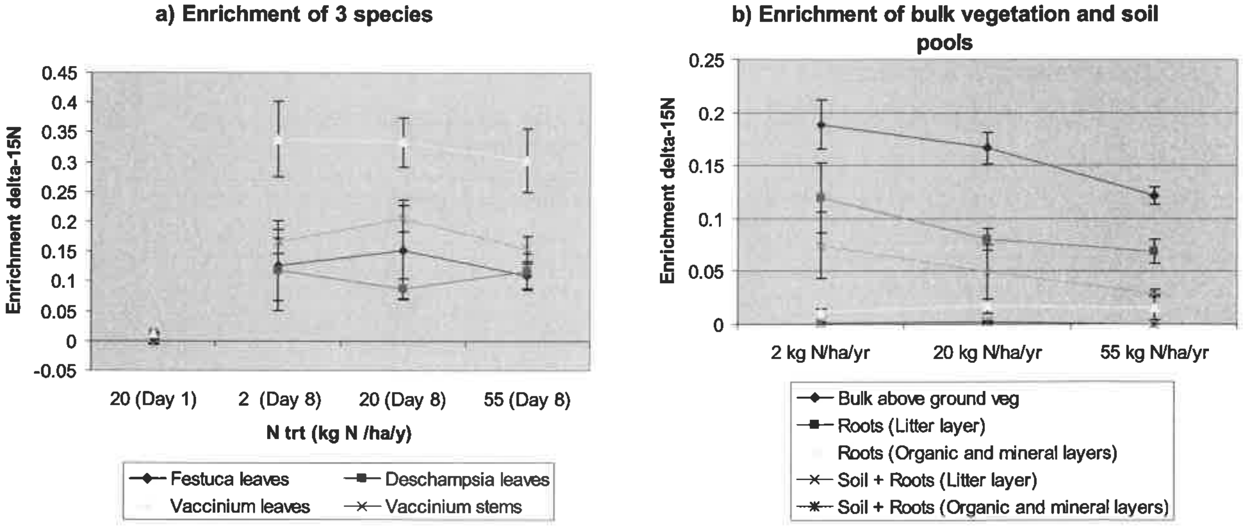


Figure 10. Delta-15N enrichment of the N pool in three of the N treatments (2N, 20N and 55N) for a) 3 competing species and b) bulk vegetation & soil pools.

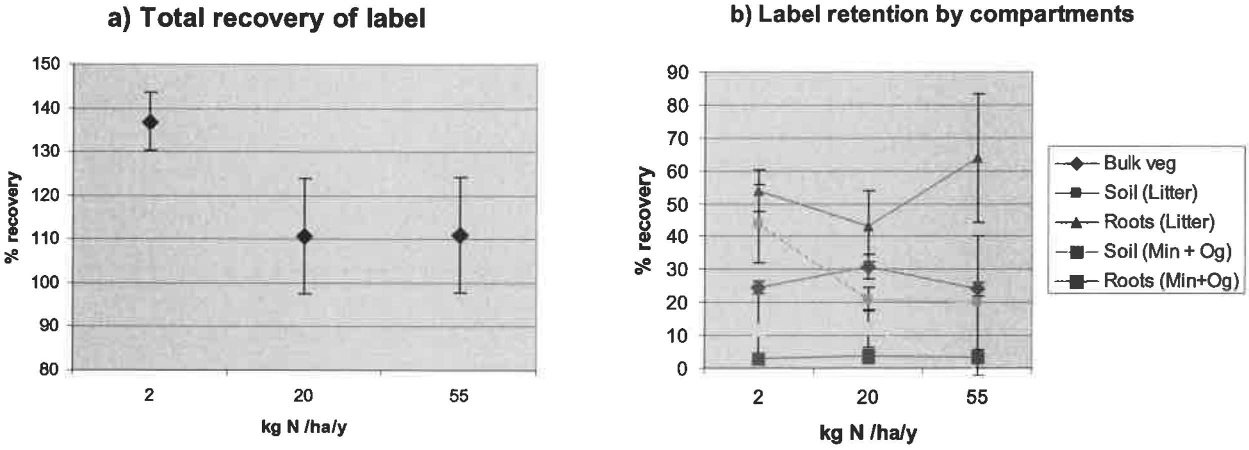


Figure 11. Recovery of labelled ^{15}N showing a) Total recovery in each N treatment and b) Recovery in each vegetation and soil compartment. Only three of the N treatments are used (2N, 20N and 55N).

Nitrogen budget

Table 2 shows the average N pools in the system. These did not differ significantly between N treatments and so are shown as means of all mesocosms. With inputs ranging from 2 – 55 kg N ha^{-1} yr^{-1}, leaching losses remain constant across all treatments at roughly 0.03 kg N ha^{-1} yr^{-1}. Losses from denitrification were measured at the acid field site and were found to be under 1 kg N ha^{-1} yr^{-1}, therefore the majority of the N is being retained in the system. The ^{15}N tracer experiment shows that most of the stored N is taken up by the litter layer, including roots, and in the above ground vegetation. This continued accumulation of N in the system shows that elevated soil N levels will persist for some time as natural loss rates are slow. This has important implications for timescales of recovery and the potential for re-establishment of oligotrophic species which may have been lost already.

Table 2. Average N pools in each compartment of the acid mesocosms, and input and output components

	N pool (kg N ha^{-1} yr^{-1})
Inputs	*2 - 55*
Above ground vegetation	76
Roots	391
Litter layer	988
Soil (organic and mineral)	4383
Denitrification	*< 1*
Leaching	*0.52*

Key findings – Calcareous grassland

General vegetation responses

Table 3 summarises changes in cover of the dominant vascular and lower plant species. In general, very few species have responded to the N treatments, although responses to the clipping treatments occurred relatively quickly and there are some interaction effects.

Table 3 Summary of N, clipping and N x clipping interaction effects for the dominant calcareous mesocosm species for each year. Data were analysed by analysis of variance using the GLM procedure in Minitab with %cover in 1997 as a covariate. +,-,? show direction of effect; **n.s.** denotes a non significant trend; *, **, *** denote significance levels of $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

Species	Nitrogen					Clipping					N * Clipping Interaction				
	'98	'99	'00	'02	'03	'98	'99	'00	'02	'03	'98	'99	'00	'02	'03
Festuca rubra							- ***	- **		+ *					
Agrostis capillaris								+ *	+ n.s.						
Agrostis canina															
Anthoxanthum odoratum							+ *	+ ***	+ **						
Holcus lanatus							+ *	+ **	+ ***	+ ***					
Carex panicea						+ n.s.	+ **	+ ***							
Carex flacca							+ **	- *	+ ***	- ***					
Potentilla erecta							+ *	+ *							
Rhytidiadelphus squarrosus	? *					+ *	+ n.s.	+ ***					*		
Pseudosclerop-odium purum						+ **	+ **	+ ***	+ ***			**			

-Response of bryophytes and potential for recovery.

There were only two bryophyte species with appreciable cover in the calcareous mesocosms. Both *Rhytidiadelphus squarrosus* and *Pseudoscleropodium purum* responded much more slowly to the N treatments than did the acidic grassland species. After 4 years, *R. squarrosus* showed a preference for N levels at 20 N and above, while *P. purum* showed no preference (Fig. 12). This is in line with other studies on calcareous grasslands (Carroll et al., 2000, During and Willems, 1986) and suggests that they are less sensitive to N deposition than their acidic counterparts. These results further suggest that a decrease in N inputs may lead to a reduction in cover of *R. squarrosus*, as its optimum lies at or above current ambient inputs at this site, within a possible timescale of years rather than decades.

-Response of vascular plants and potential for recovery
There do not appear to be any responses of the vascular species to the N treatments, either in the reduction treatments or in the N addition treatment (Fig. 13). At first sight, this might suggest that N has no effect on this system, and that the critical load is above 55N. However, one possible reason for this lack of response is soil P levels. If the plants are P limited, then they are unable to utilise the extra N. P limitation at Wardlow Haycop, a similar site to the calcareous mesocosms source site at Harpurs Hill, has been shown to limit plant responses to N additions (Lee et al., 1996). However, in the last year of this experiment there have been suggestions of a N-addition response, with increases in biomass in the high N treatment, particularly in the light clipped mesocosms (Fig. 14), although this can not be attributed to growth of any particular species. As in the acidic mesocosms, this N response was most pronounced initially in the early summer cut, suggesting P limitation is restricting plant growth as the growing season progresses. Although there have been no changes in the vascular species over the relatively short timescale of this experiment, in the long term, species with efficient P-uptake mechanisms, such as mycorrhizal species and sedges with dauciform roots may gain a competitive advantage. The lack of changes in the reduction treatments suggests that any timescale for recovery is likely to be in the order of decades rather than years.

-Interactions and effects of management
The clipping treatments had numerous significant effects on species abundance (Table 3) with consequences for community composition. However, there were no significant interactions between management and N treatment for the vascular plants.Both of the moss species recorded significant interactions and their response is similar to that of the acidic species, whereby they seem to be able to tolerate higher N loads in the more intensive management treatments (Fig. 15). The implications for management. Evidence from sand dune grasslands in the Netherlands, which are neutral to calcareous, suggests that plant responses are kept in check by grazing (ten Harkel and van der Meulen, 1996).

-Species richness and Ellenberg N numbers
The N treatments had no significant effect on species richness, unlike in the acid mesocosms, nor was there an effect on mean Ellenberg N number per core.

General soil responses
Responses in below ground processes showed some differences between N treatments, although few of these were significant.

-Litter decomposition
Litter decomposition was lower in the N addition (55N) treatment, although this was not significant (Fig. 16), and higher in the clipped treatments. The tissue N content of the source material did not affect decomposition rates, and the slower decomposition may perhaps be due to lower activity of fungal decomposers necessary for the breakdown of lignin in plant tissues.

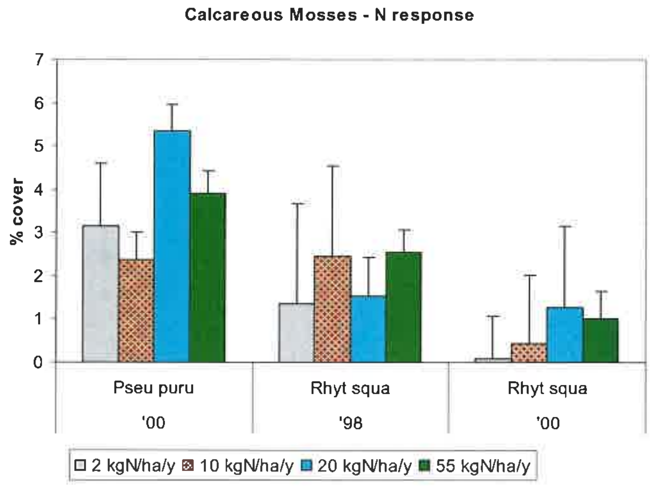


Figure 12. Percentage hits of moss species in calcareous grassland mesocosms. Significant treatment effects for *R. squarrosus* in 1998, carried over from baseline, are included for comparison.

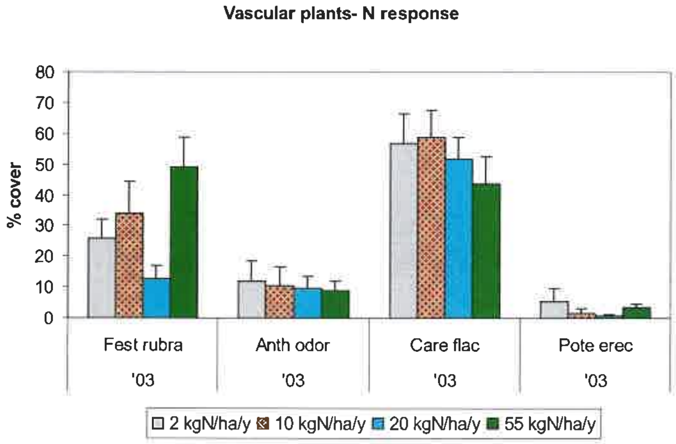


Figure 13. Percentage hits of selected dominant vascular plant species in calcareous grassland mesocosms.

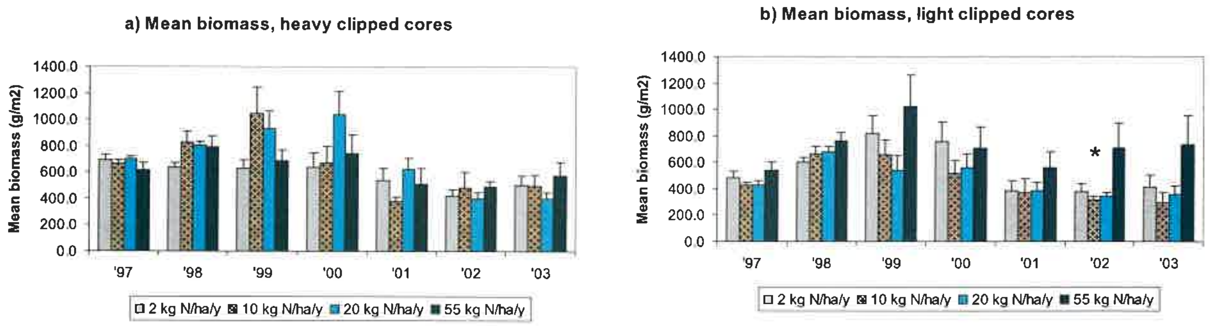


Figure 14. Mean productivity in each N treatment for a) the Heavy clipped cores and b) the Light clipped cores. Calcareous grassland.

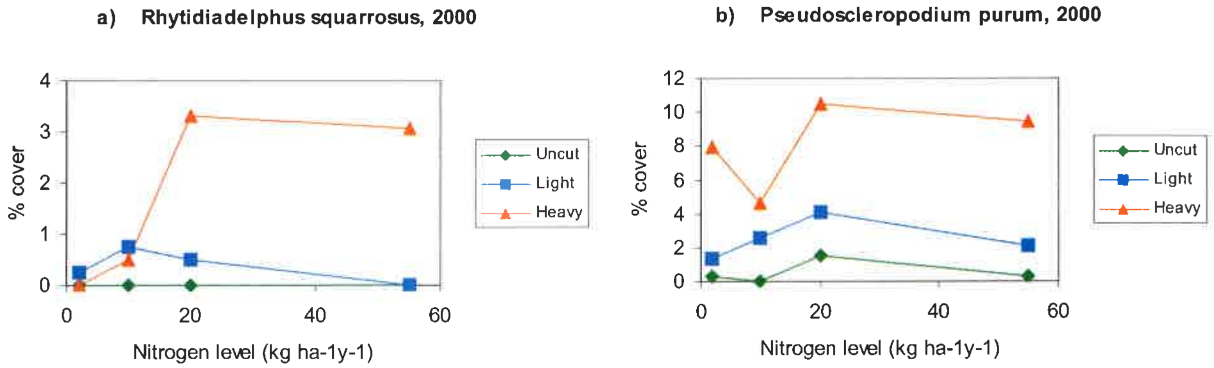


Figure 15. Graphs showing %cover in each N x clipping treatment combination for a) *Rhytidiadelphus squarrosus* and b) *Pseudoscleropodium purum*..

-Bacterial/fungal activity
There was a non-significant trend towards increased bacterial:fungal ratios in the higher N treatments, as revealed by soil PLFA analysis (Fig. 17). The bacterial:fungal ratio also increased in the heavy clipped treatment. These changes were driven by both small increases in bacterial PLFA abundance, and decreased fungal PLFA abundance.

-Root growth
There was no significant effect of N on root biomass, although biomass was highest in the 10N and 20N treatments, and lowest in the 55N treatment.

-Soil chemistry
Soil chemistry showed no change in soil pH between the N treatments. This is not surprising as this is a calcareous system and strongly buffered against changes in soil pH due to nitrification. There were no N effects on thickness of the organic layer.

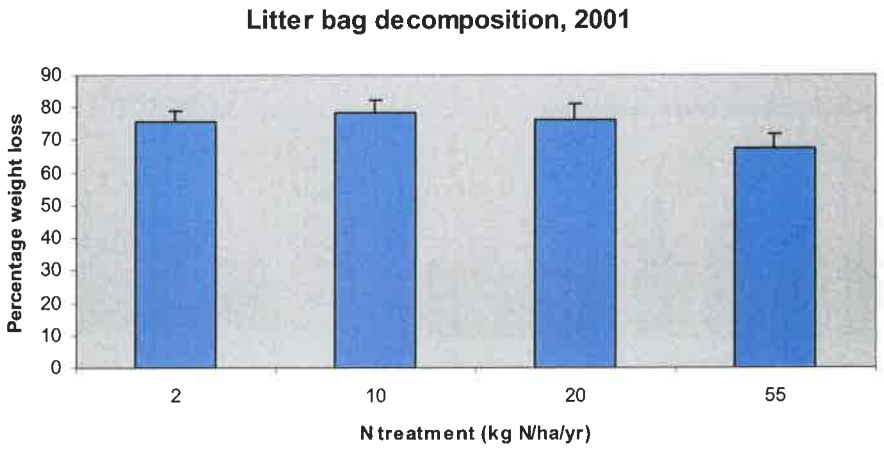


Figure 16. Decomposition of litter in litter bags in each N treatment. Calcareous grassland.

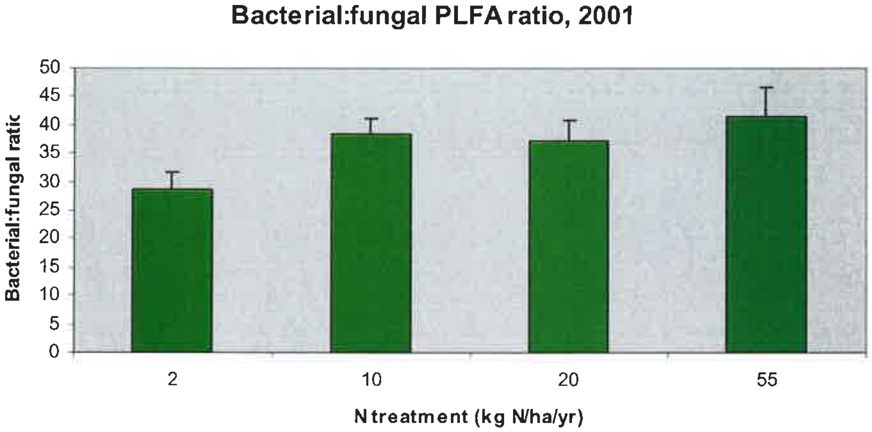


Figure 17. Bacterial:fungal PLFA ratios in each N treatment. Calcareous grassland.

-Soil water chemistry
Suction lysimeters installed within the first few years of the experiment showed a gradual divergence of the ambient and 55N treatments from the two reduction treatments with respect to pH (Fig. 18) and GRAN alkalinity (Fig. 19) of the soil water. Nitrogen leaching occurred in winter when plant growth was not taking it up and showed differences between the N treatments. Ammonium concentrations were low but gave 0.41 kg N ha^{-1} yr^{-1} losses on average. Most N leached was in the form of nitrate (Fig. 20) which averaged losses of 2.01 kg N ha^{-1} yr^{-1}. Both the ambient (20N) and the 10N treatments leached N (roughly 2.9 kg N ha^{-1} yr^{-1} each), while leaching was even higher in the 55N treatment (3.6 kg N ha^{-1} yr^{-1}). Only in the pristine (2N) treatment was N leaching below 1 kg N ha^{-1} yr^{-1} (0.46 kg N ha^{-1} yr^{-1}). This suggests that the system is N saturated at current ambient inputs, and that major reductions in inputs are necessary before plant uptake can compensate for inputs and any reductions in N leaching become apparent. One reason for this appreciable leaching, even in the 10N reduction treatment is that P limitation is preventing the plants from taking up the incoming N. This has important consequences for setting of critical loads. Previously it has been argued that you could set a higher critical load in a P limited system, as few effects are observed (Achermann and Bobbink, 2003). However, this study suggests that P limitation exacerbates problems of N leaching, with serious consequences for stream water chemistry. Thus you could argue for a lower critical load in a P limited system.

In this study, there were some effects of management on N leaching. Other studies comparing ungrazed with grazed systems suggest that leaching decreases with grazing e.g. ten Harkel (1998). However, in this experiment, leaching increased with management intensity, and was higher in the heavy clipped cores than the light clipped (Fig. 21). Lysimeters were not installed in the unclipped cores, so no comparison can be made with an unmanaged system. Although no ^{15}N tracer study was conducted in the calcareous mesocosms, the uptake of N is likely to be in similar pools. The disparity between inputs and leaching losses also shows that most of the applied N is being retained within the system, and inputs exceed losses even in the lowest reduction treatment.

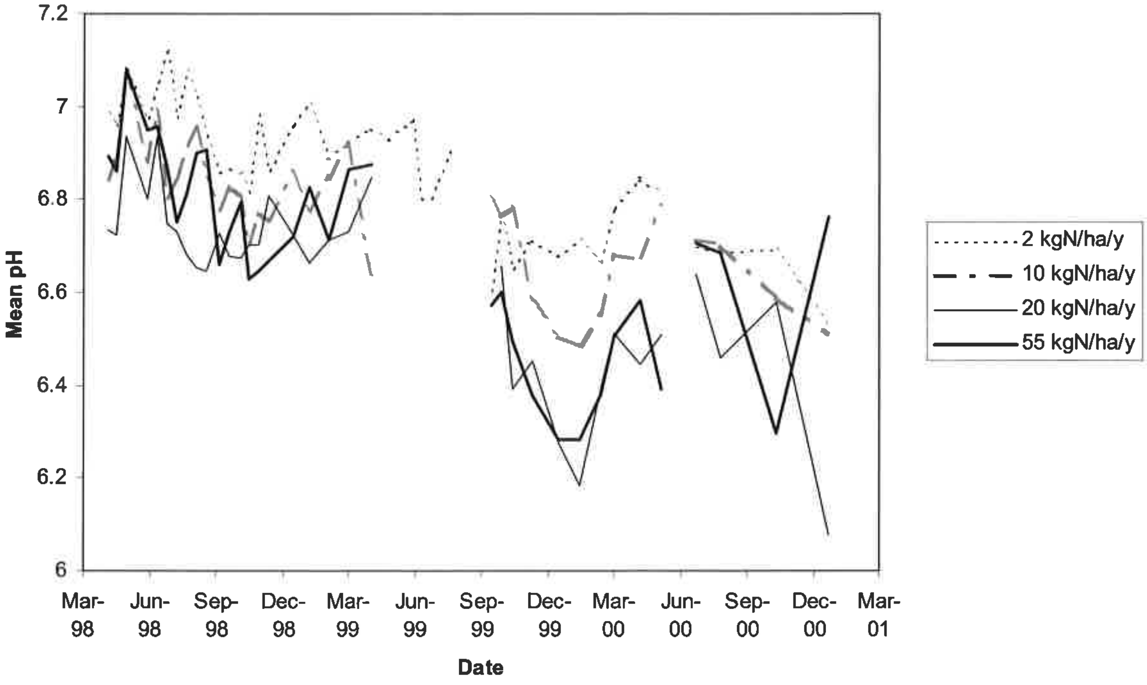


Figure 18. Mean pH of soil water from the mineral horizon in the four nitrogen treatments (pooled means across light and heavy clipping treatments).

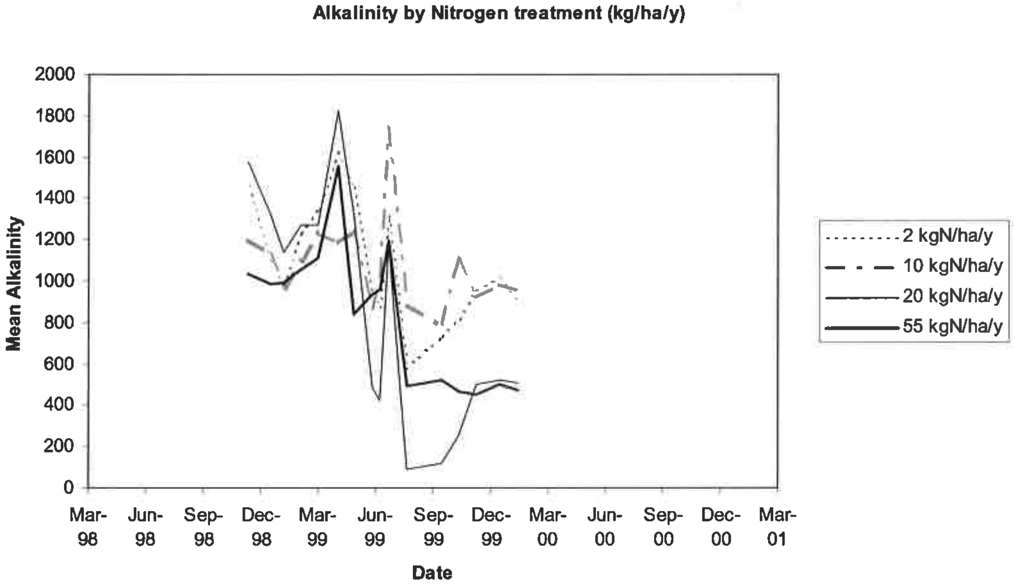


Figure 19. Mean GRAN alkalinity of soil water from the mineral horizon in the four nitrogen treatments (pooled means across light and heavy clipping treatments).

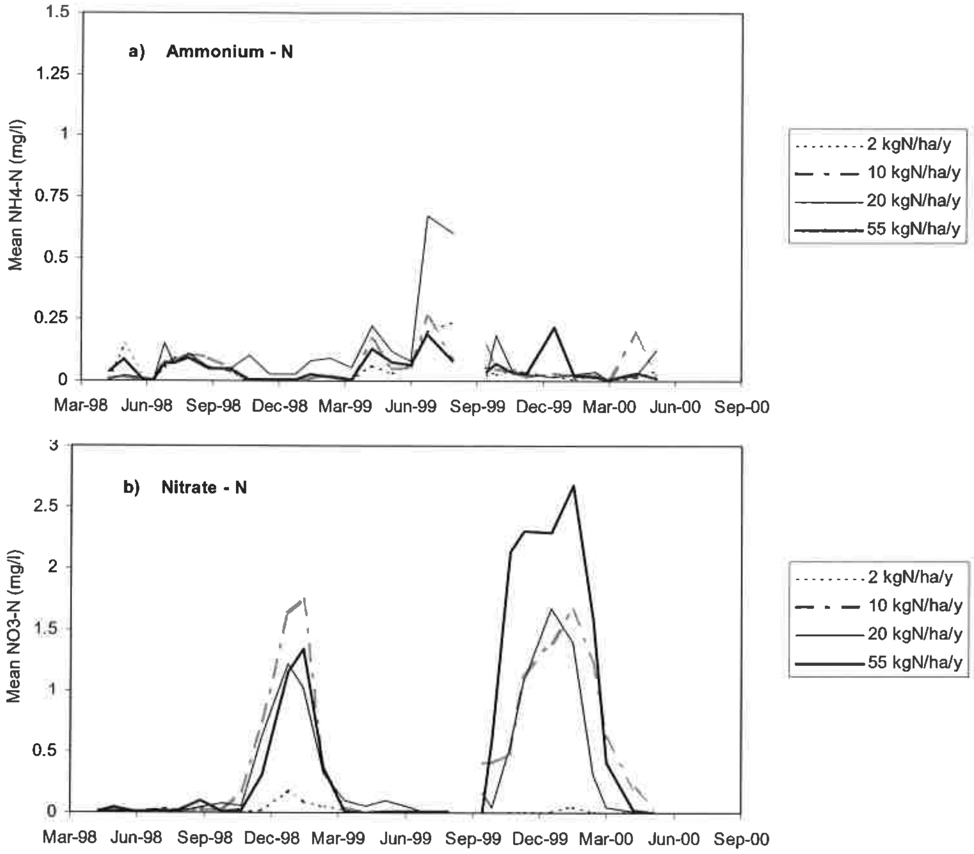


Figure 20. Mean concentrations of N (mg/l) from soil water in the mineral horizon for each nitrogen treatment (pooled across nitrogen treatments) Graphs show concentrations of a) Ammonium – N and b) Nitrate – N.

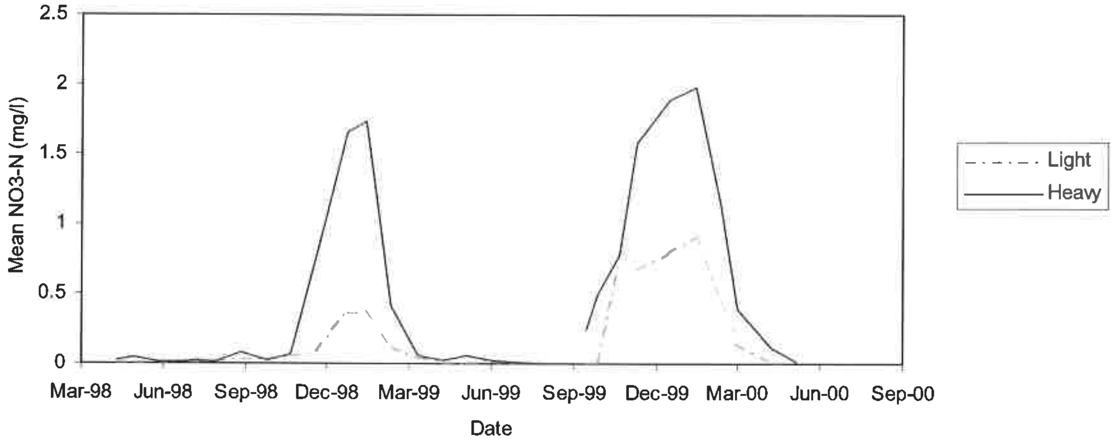


Figure 21. Mean concentrations of N (mg/l) from soil water in the mineral horizon for each nitrogen treatment (pooled across clipping treatments) Graphs show concentrations of Nitrate – N.

Conclusion

The critical loads for both acidic and calcareous systems appear to be exceeded at current ambient deposition. In the acidic grassland this largely impacts on the lower plants whereas in the calcareous system, the impact is largely on water quality. In the latter case, this appears to be exacerbated by P-limitation of plant growth. However, there is potential for recovery of the sensitive moss and lichen species if N deposition is to be reduced. This is likely to occur within a timescale of years. Recovery of the soil system or changes in vascular plants on the other hand are likely to take place over decades. Management does alter the responses to N in both systems, but more markedly in the acidic system. A more intensively managed system allows us to set critical loads at the higher end of the range in the acidic system, but in the calcareous system, the opposite may be the case with regard to water chemistry effects. A critical load range of 10 – 20 kg N ha^{-1} yr^{-1} is appropriate for both these systems.

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