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# The interactions between plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition

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**"Capsule":** *Increased nitrogen resulted in loss of plant cover and abundance of individual species.*

**Abstract**

Regular applications of ammonium nitrate (35–140 kg N $ha^{-1}$ $year^{-1}$) and ammonium sulphate (140 kg N $ha^{-1}$ $year^{-1}$) to areas of acidic and calcareous grassland in the Derbyshire Peak District over a period of 6 years, have resulted in significant losses in both overall plant cover, and the abundance of individual species, associated with clear and dose-related increases in shoot nitrogen content. No overall growth response to nitrogen treatment was seen at any stage in the experiment. Phosphorus additions to the calcareous plots did however lead to significant increases in plant cover and total biomass, indicative of phosphorus limitation in this system. Clear and dose-related increases in soil nitrogen mineralization rates were also obtained, consistent with marked effects of the nitrogen additions on soil processes. High nitrification rates were seen on the calcareous plots, and this process was associated with significant acidification of the 140 kg N $ha^{-1}$ $year^{-1}$ treatments.


*Keywords:* Nitrogen; Grassland; Mineralization; Species composition

## 1. Introduction

The atmospheric deposition of reactive nitrogen compounds has increased very significantly over the last 20 years, and now reaches levels of 40 kg N $ha^{-1}$ $year^{-1}$ over large areas of the United Kingdom (NEGTAP, 2001; Goulding et al., 1998). These enhanced nitrogen levels represent a particular threat to semi-natural or unimproved vegetation types such as ombrotrophic mires, moorland, and unimproved grassland, where nutrient inputs have historically been very low.

Increased rates of nitrogen addition have been shown to lead to alterations in root and shoot growth, shifts in overall nutrient limitation (Roem and Berendse, 2000), changes in vegetation structure (Willems et al., 1993), decreased species diversity (Bobbink, 1991; Thurston et al., 1976) and increased susceptibility to a range of environmental stresses, such as frost and drought (Carroll et al., 1999).

Species rich calcareous grasslands growing on shallow nutrient-poor soils provide a habitat for a large number of rare or endangered species (Bobbink and Willems, 1987) and represent a resource of high conservation value. Recent studies on grasslands in the Netherlands exposed to high levels of atmospheric nitrogen have shown a clear expansion in the cover of nitrophilous grasses such as *Brachypodium pinnatum* with subsequent decreases in overall species diversity, and similar results have been obtained as a result of fertilisation experiments

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(Bobbink, 1991; Willems et al., 1993), suggesting that increased rates of nitrogen supply may be responsible for these effects. In many cases calcareous grasslands also show strong phosphorus limitation (Jeffrey and Pigott, 1973; Willems et al., 1993).

Nitrogen addition experiments on other grassland types have shown similar effects, with increased growth and dominance of grasses, associated with accelerated rates of soil acidification, and overall losses in species diversity (Wedin and Tilman, 1996; Mountford et al., 1993; Thurston et al., 1976). Changes in management practices may also represent an important contributory factor, with the loss of grazing further increasing the dominance of the grass species, and also leading to large accumulations of litter (Berendse et al., 1987).

Soil processes have been shown to be altered by nitrogen additions in a number of ways, including effects on mineralization and nitrification rates, soil pH and leaching (Fog, 1988). Studies carried out on permanent grasslands have shown marked effects of both short and long-term nitrogen additions, with clear increases in net nitrogen mineralization and nitrification rates, and evidence of increased rates of leaching of $NO_3^-$ ions in particular (Wedin and Tilman, 1996; Ledgard et al., 1998; Unkovich et al., 1998; Lovell et al., 1995).

In many of the quoted studies however, nitrogen applications have been heavy and infrequent (often once annually). Morecroft et al. (1994), in an experiment designed to more closely mimic natural deposition events, have demonstrated that fortnightly additions of 35–140 kg N $ha^{-1}$ $year^{-1}$ as $NH_4NO_3$ and 140 kg N $ha^{-1}$ $year^{-1}$ as $(NH_4)_2SO_4$ to semi-natural acidic and calcareous grasslands in the Derbyshire Peak District stimulated nitrogen mineralization, and that this stimulation was proportional to the increase in nitrogen deposition. These effects were not however, associated with any clear changes in either vegetation structure, or growth, on the nitrogen treated plots.

Sustained increases in nitrogen mineralization would however be consistent with shifts in other soil processes. Reduced soil pH, high levels of nitrogen availability, and shifts in the balance of nutrients in particular, are strongly associated with losses in species diversity (Roem and Berendse, 2000; Koerselman and Meuleman, 1996) and deleterious changes in overall vegetation structure. The available evidence would therefore suggest, that long-term increases in nitrogen supply would eventually lead to changes in the structure of the vegetation, or the frequency and distribution of individual species on these grasslands.

The present study therefore aims to extend the observations of Morecroft et al. (1994) (made in 1992 following 2 years of nitrogen treatment) over a much longer period and to follow the continued effects of long-term chronic additions of $NH_4NO_3$ and $(NH_4)_2SO_4$ on the interaction between the vegetation structure and soil processes of these grasslands. The floristic composition of the plots has been carefully monitored over the intervening period, and the original mineralization study repeated in 1995–1996. The effects of phosphorus treatment have also been investigated.

## 2. Methods

### 2.1. Site description and nitrogen additions

Experimental additions of nitrogen and glucose were made over a period of 6 years (1990–1996) to areas of grazed acidic grassland (British National Vegetation Classification (NVC) Class U4e, *Festuca–Agrostis–Galium* grassland) and calcareous grassland (NVC class CG2d *Festuca–Helictotrichon* grassland) (Rodwell, 1992), at Wardlow Hay Cop, a small limestone hill (370m national grid reference SK 1773) in the Derbyshire Peak District National Park. The acid grassland (pH 4.1) occurs where glacial loess (nordrach silt loam—average depth 70 cm) has been deposited onto underlying limestone. The calcareous grassland soil is a humic rendzina (pH 6.8) situated on a south facing slope. The site is grazed by cattle between the middle of May and December, with additional sheep in May and June (max. density 20 cattle + 20 ewes and lambs over an area of approximately 20 ha). The total background nitrogen deposition is estimated at 20 kg N $ha^{-1}$ $year^{-1}$ (Morecroft et al., 1994).

Treatments of distilled water, 35, 70 and 140 kg N $ha^{-1}$ $year^{-1}$applied as $NH_4NO_3$ and 140 kg N $ha^{-1}$ $year^{-1}$ applied as $(NH_4)_2SO_4$ were made to contiguous 1 $m^2$ plots replicated in four blocks. One plot in each block was also left untreated. The nitrogen solutions were applied every 14 days over the first 3 years of the study (1990–1993) in 1 l of water using a watering-can with a fine rose, and subsequently once a month in the same volume of water. Drift between plots was minimal. Glucose applications were made at the rate of 720 g glucose $m^{-2}$ $year^{-1,}$ applied bi-annually in the spring and autumn and watered in with distilled water at the rate of 1 l $m^{-2}$. The highest loads applied here are in excess of measured atmospheric deposition, but are not inconsistent with agricultural rates of application. The glucose treatments were intended to reduce nitrogen availability, by stimulating microbial demand. Any dung found in the plots was carefully removed.

In order to obtain more information on possible phosphorus limitation on these grasslands, one previously untreated 1 $m^2$ plot in each block was sub-divided into 4×0.25 $m^2$ plots in April 1992. Treatments were: untreated control, 100 kg N $ha^{-1}$ $year^{-1}$ applied as $NH_4NO_3$, 50 kg phosphorus $ha^{-1}$ $year^{-1}$, applied as solutions of sodium phosphate salts, or 100 kg N $ha^{-1}$ $year^{-1}$ + 50 kg P $ha^{-1}$ $year^{-1}$. These treatments were repeated in May 1995 and subsequently twice annually, in April and October.

### 2.2. Additional plots (1995–1996)

Additional 9 $m^2$ plots were established in 1995 on both the acid and calcareous grasslands. Three blocks were arranged in a randomised block design, with six treatments: water, 35 kg P $ha^{-1}$ $year^{-1}$ applied as sodium phosphate salts, or 35 or 140 kg N $ha^{-1}$ $year^{-1}$ applied as $NH_4NO_3$, ±35 kg P $ha^{-1}$ $year^{-1}$. Treatments were applied quarterly.

### 2.3. Mineralization study

The effect of the nitrogen treatments on soil nitrogen mineralization rates was measured over four periods each of 3-month duration from December 1994 to December 1995. At the start of each time period, duplicate cores of approximately 40 mm diameter were removed from each plot. One of each pair was placed in a polythene bag buried on site at a depth of approximately 50 mm for 3 months while the other was returned to the laboratory for analysis. The cores were weighed and measured in order to allow the calculation of nitrogen release in kg N $ha^{-1}$ $year^{-1}$ and then broken up. Major debris (stones and roots) were removed and duplicate 5g samples of moist soil taken from each core. The samples were added to 25 ml 1M KCl and shaken for 1 h on an end-over-end shaker. The extracts were separated by centrifugation and stored at −20 °C prior to analysis for total $NH_4^+$ and $NO_3^-$ content using a TECATOR FIAStar 5010 flow injection analysis system.

Extractable $NH_4^+$ and $NO_3^-$ concentrations were expressed as kg N $ha^{-1}$ $year^{-1}$, standardized for a core depth of 4 cm for both sites, and the values in the control cores subtracted from those in the buried samples to give the total rate of nitrogen release (mineralization) for each time period. Nitrification rates were calculated from the extractable $NO_3^-$ concentrations, in the same way. The results ($n=4$ for each treatment) were transformed to natural logarithms and analysed by ANOVA with least significant differences range test (statistical package SPSS 6.0).

Annual mineralization rates were obtained by summation of the four time periods for each treatment, adjusting for the different incubation lengths .The data ($n=4$ for each treatment) were transformed to natural logarithms and analysed by ANOVA with least significant differences range test (SPSS 6.0).

### 2.4. Soil total nitrogen measurements

Total nitrogen was measured in oven dried soil samples (80 °C for 24 h) collected from the plots at the start of the mineralization study. Approximately 30 mg samples of dried soil were digested for 4 h at 375 °C, in a concentrated sulphuric/salicylic acid mixture containing $LiSO_4$:$CuSO_4$ catalyst in the ratio 10:1. The nitrogen and phosphorus content of the diluted extracts was then determined using a TECATOR FIAStar 5010 flow injection analysis system. The data was analyzed by ANOVA ($n=4$ for each treatment) (SPSS 6.0) and presented as mg $g^{-1}$ dry weight of soil.

### 2.5. Soil pH measurements

Fresh soil samples (0–50 mm) were taken from the experimental plots in December 1994 and December 1996 ($n=3$ for each plot), shaken for 1 min in distilled water (1:2 w:v) and the pH measured when stable. Data analysed by ANOVA (SPSS 6.0), $n=4$ for each treatment.

### 2.6. Vegetation survey and analysis methods

The vegetation on the original plots was surveyed annually (1990–1996) using a point quadrat technique. Data from a total of 50 regularly arranged points were recorded on each plot, with quadrats placed in the same position every year, and each species touched scored once. The untransformed data were analyzed by ANOVA ($n=4$ for each treatment) and the results presented as % cover values for each species (SPSS 6.0). Contacts with moss were noted, but individual species were not identified. A total of 25 survey points were recorded on each of the subdivided plots. No significant differences ($P<0.05$) were seen between the untreated and watered controls, and only the untreated control values are shown in the figures.

Plant community composition data was analysed using the TWINSPAN and MATCH vegetation analysis packages (Malloch, 1988, 1992).

Wire-mesh grazing exclosures measuring 0.06 $m^2$ were established on the additional calcareous plots in May 1996, with the aim of measuring the total productivity of the grasslands in the absence of grazing. Three exclosures were placed on each plot, and the total biomass inside each was harvested in August 1996 (total area 0.18 $m^2$/plot). The material collected was dried at 80 °C for 24 h and then weighed. The data was analysed by ANOVA (SPSS 6.0) ($n=3$ for each treatment) and presented as g dry wt for each plot.

### 2.7. Shoot total nitrogen measurements

Shoot material from selected species was collected randomly from all the plots in September 1995. Approximately 30 mg samples of oven dried material were digested as described for the soil samples. The nitrogen and phosphorus content of the diluted extracts was then determined using a TECATOR FIAStar 5010 flow injection analysis system. The data was analyzed by

ANOVA (SPSS 6.0) and presented as mg $g^{-1}$ dry weight of plant material.

## 3. Results

The vegetation survey recorded 22 higher plant species on the untreated acidic grassland plots over the total survey period (1990–1996), including six grasses and 13 forb species. The highest cover values were obtained for the grasses *Festuca ovina* and *Agrostis* spp, and the forbs *Potentilla erecta* and *Galium saxatile*. Bryophyte cover values were also high (56%), in the control plots, with the main species being *Rhytidiadelphus squarrosus* and *Pleurozium schreberi* (Carroll et al., 2000).

A higher total of 29 species were recorded on the calcareous grassland plots, including four grasses and two sedges. The highest cover values obtained were for the grasses *Festuca ovina*, and *Helictotrichon pratense*, the sedge *Carex flacca*, and the forbs *Thymus polytrichus*, *Helianthemum nummularia* and *Sanguisorba minor*. Bryophyte cover was low compared with the acid grassland site (Morecroft et al., 1994), and was not clearly affected by the nitrogen treatments.

### 3.1. Vegetation effects

No significant changes were seen in the overall growth or cover of the higher plants on either the acid or calcareous grassland plots in the first 4 years of treatments (Morecroft et al., 1994), although very significant reductions were obtained in bryophyte cover on the acid grassland plots at the higher rates of nitrogen addition (Table 1) (Carroll et al., 2000). TWINSPAN and MATCH analysis of the data similarly showed no change in the overall species composition or National Vegetation Classification (unpublished data). The results of the 1995 vegetation survey however, showed a general trend towards lower cover values for a number of individual species on both sites. Many of these changes have been confirmed by the results of the 1996 survey, presented here, with a progressive reduction in total plant cover in response to increasing nitrogen additions, at both sites.

### 3.2. Acid grassland plots

The 1996 survey results (Fig. 1a and Table 1) showed significant reductions in total grass and higher plant touches, in response to the high nitrogen treatments. No significant changes were found in the cover of individual higher plant species, but there were clear trends towards reduced cover for a number of species, notably *Potentilla erecta* and *Festuca ovina* and *Agrostis* spp. The reductions in cover both for individual species, and for the total touch data were in all cases greater for the $(NH_4)_2SO_4$ treated plots than for the equivalent $NH_4NO_3$ treatment. One exception to this was the cover of *Nardus stricta* which increased by 49% in the 140 kg $(NH_4)_2SO_4$ N $ha^{-1}$ $year^{-1}$ treatment, an effect that was also noted in the 1995 survey.

#### 3.2.1. Calcareous plots

The 1996 vegetation survey results for the original calcareous plots are shown in Fig. 1b and Table 1. The % cover values for the individual species (Table 1) show a number of interesting patterns seen both in 1995

Table 1
The effect of long-term treatment with 35–140 kg $NH_4NO_3$ N $ha^{-1}$ $year^{-1}$ (AN 35–140) or 140 kg $(NH_4)_2$ $SO_4$ N $ha^{-1}$ $year^{-1}$ (AS140) on the % cover (±S.E.M.) of individual species on the acidic and calcareous grassland plots (June 1996)

| Species names | Control | AN 35 | AN 70 | AN 140 | AS 140 | *F* ratio | *P* |
|---|---|---|---|---|---|---|---|
| Calcareous grassland | | | | | | | |
| *Helianthemum nummularia* | 28.5±3.9 | 16.0±4.2 | 29.0±8.2 | 18.0±4.1 | 13.5±4.5 | | >0.05 |
| *Thymus polytrichus* | 32.5±3.2 | 26.0±1.8 | 25.5±3.1 | 18.5±7.2* | 8.00±2.2* | 2.80 | <0.05 |
| *Plantago lanceolata* | 2.66±0.6 | 6.66±0.6 | 2.00±0.0 | 5.00±1.0 | 10.0±0.0 | | >0.05 |
| *Campanula rotundifolia* | 5.00±2.1 | – | 3.33±1.2 | 9.00±4.9 | 7.00±2.5 | | >0.05 |
| *Koelaria macracantha* | 19.0±1.3 | 14.0±1.4 | 19.5±3.1 | 14.6±0.6 | 31.6±4.6* | 4.32 | <0.05 |
| *Carex flacca* | 33.5±2.2 | 46.0±4.1 | 29.0±5.4 | 21.0±6.6 | 7.00±1.7* | 4.71 | <0.05 |
| *Festuca ovina* | 59.5±4.5 | 54.0±3.2 | 50.5±2.7 | 46.0±8.5 | 43.5±6.0 | | >0.05 |
| *Helictotrichon pratense* | 29.0±4.1 | 29.5±3.8 | 32.0±5.9 | 25.5±4.6 | 17.5±3.3 | | >0.05 |
| *Briza media* | 18.0±3.4 | 21.5±2.5 | 16.5±3.9 | 18.0±6.7 | 8.50±2.1 | | >0.05 |
| Acid grassland | | | | | | | |
| *Agrostis* spp | 61.0±4.0 | 67.0±1.8 | 54.0±5.3 | 57.0±5.8 | 37.5±9.8 | | >0.05 |
| *Festuca* spp | 80.5±6.9 | 72.0±1.6 | 72.0±3.4 | 65.0±7.2 | 63.0±6.2 | | >0.05 |
| *Potentilla erecta* | 23.0±10.0 | 23.5±7.9 | 28.5±5.0 | 20.3±10.7 | 14.0±8.2 | | >0.05 |
| *Nardus stricta* | 24.5±8.0 | 35.5±11.1 | 36.0±11.2 | 29.5±10.4 | 48.5±14.3 | | >0.05 |
| *Bryophyte* spp | 56.0±8.7 | 45.5±9.9 | 33.0±6.9 | 27.5±10.5* | 8.00±2.0* | 6.21 | <0.05 |

$n=4$. ANOVA analysis with LSD range test.
* Significantly different from control, $P<0.05$.

and in the 1996 data presented here, with notably, significant reductions in the cover of *Thymus polytrichus,* and *Carex flacca*, in response to the high nitrogen treatments. Distinct trends towards lower cover values were also seen for *Festuca ovina*, *Helictotrichon pratense, Helianthemum nummularia* and *Briza media*. *Koeleria macracantha*, by contrast, showed a significant increase in cover in response to the $(NH_4)_2SO_4$ treatment and similar patterns were also seen for *Plantago lanceolata* and *Campanula rotundifolia*, although these did not reach significance. Overall the results for total plant touches for the calcareous plots (see Fig. 1b) showed clear trends towards reduced cover, for all the plant groups shown, with significant effects of treatment in the case of the sedges, and higher plants, particularly on the $(NH_4)_2SO_4$ treated plots.

The data for *Thymus polytrichus* are shown in more detail in Fig. 2, presented as the % reduction in cover, in response to treatment, for 1990–1996. Although the data reflects the natural variation in the cover of this species, a clear and significant pattern of increased reductions in response to the 140 kg $NH_4NO_3$ and

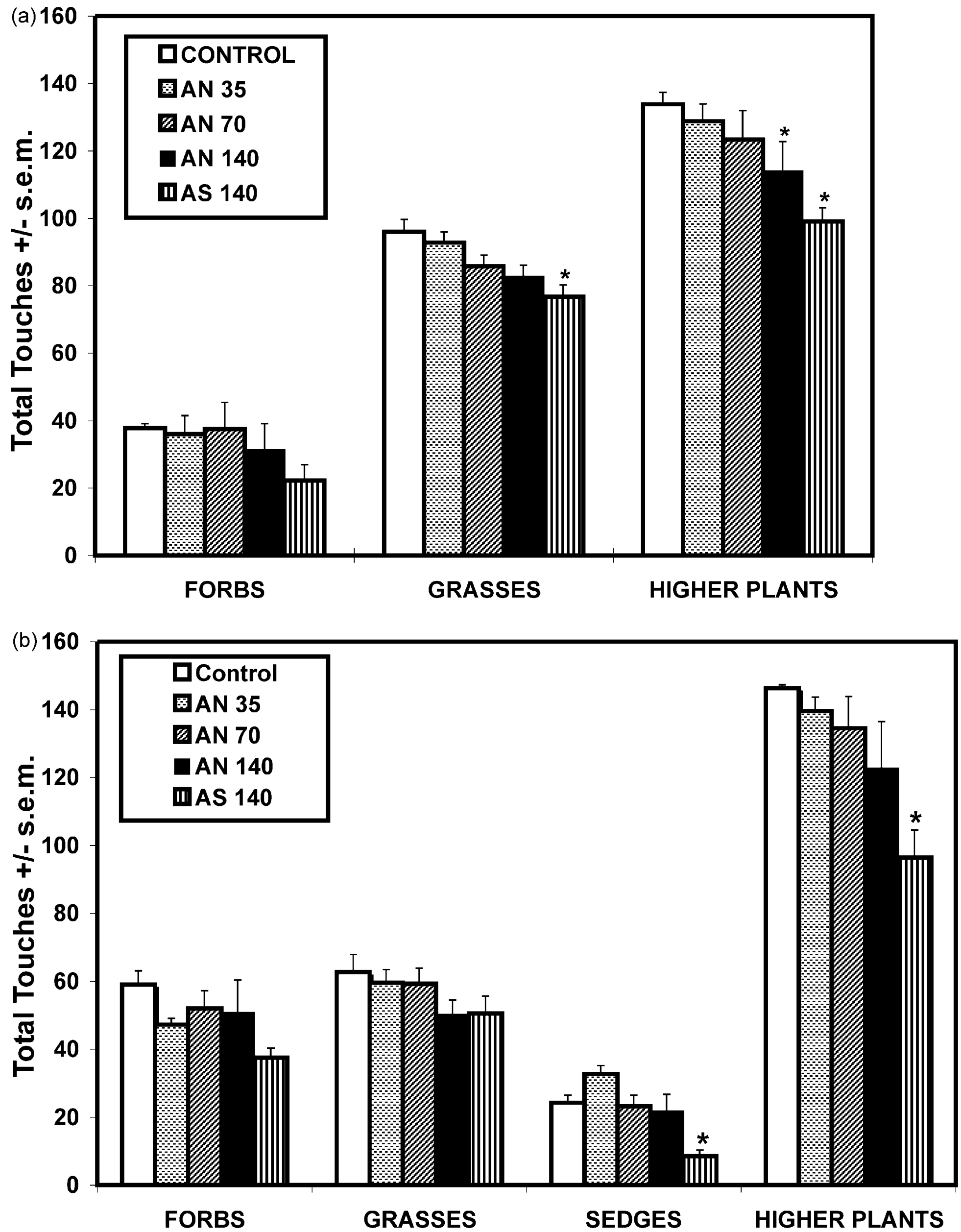


Fig. 1. The effect of long-term treatment with 35–140 kg $NH_4NO_3$ N $ha^{-1}$ $year^{-1}$ (AN 35–140) or 140 kg $(NH_4)_2$ $SO_4$ N $ha^{-1}$ $year^{-1}$ (AS140) on total plant touches. (a) Acid grassland plots. (b) Calcareous grassland plots. June 1996. ANOVA analysis with LSD range test. *Significantly different from control $P<0.05$.

$(NH_4)_2SO_4$ treatments can be seen in the 1994 to 1996 data, with the largest losses in cover in the range of 53–73%, for the $(NH_4)_2SO_4$ treated plots.

### 3.2.2. Effects of phosphorus treatment

The acidic grassland showed no significant changes in cover on the sub-divided N/P treated plots, although there were clear indications of increased sward height in response to the phosphorus treatments (data not shown).

In the case of the calcareous plots, clear changes in cover were obtained on the sub-divided N/P treated plots, and these results are presented in Table 2. Total higher plant, grass and forb touches showed significant increases in cover in response to P and NP treatment, compared with a reduction in the cover of the sedge species, an effect also noted on the 9 m$^2$ plots (data not shown). Sward height was also noticeably higher on the P and NP treated plots. This observation was consistent with the results of the total live biomass harvest from the grazing exclosures established on the additional 9 m$^2$ N/P treated plots in 1996 (Table 3). These also

Table 3
The effect of nitrogen and phosphorus treatments (35 or 140 kg N ha$^{-1}$ year$^{-1}$ ±35 kg P ha$^{-1}$ year$^{-1}$) on the total live biomass of the additional calcareous grassland plots (July 1996)

| Nitrogen and phosphorus additions | Total live biomass (g dry wt ±S.E.M.) |
|---|---|
| Control | 13.9±1.5 a |
| Low N | 15.5±1.0 ab |
| High N | 12.9±1.5 a |
| P | 20.2±1.4 bc |
| Low N+P | 22.7±1.9 bc |
| High N+P | 22.8±1.5 c |

ANOVA analysis with LSD range test. Significant effect of phosphorus treatment, $F$ ratio = 16.3, $P<0.05$. Columns sharing a letter are not significantly different, $P<0.05$.

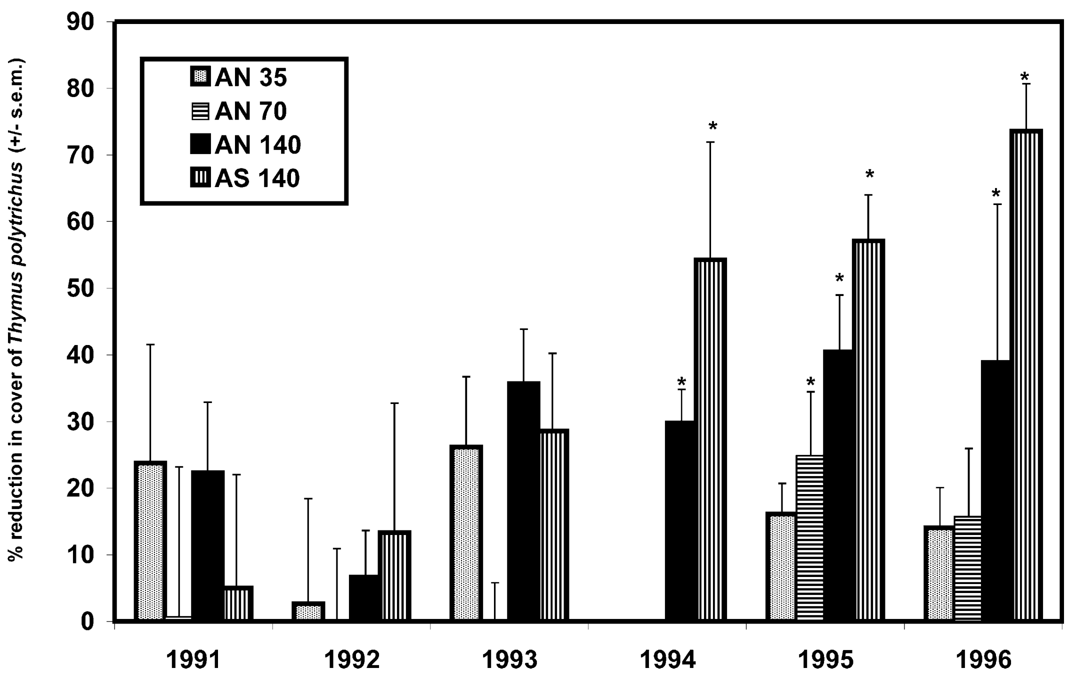


Fig. 2. The effect of long-term treatment with 35–140 kg $NH_4NO_3$ N ha$^{-1}$ year$^{-1}$ (AN 35–140) or 140 kg $(NH_4)_2$ $SO_4$ N ha$^{-1}$ year$^{-1}$ (AS140) on cover of *Thymus polytrichus* (% reduction in cover compared with controls for same year) on calcareous grassland plots. ANOVA analysis with LSD range test. *Significantly different from 35 kg N treatment $P<0.05$.

Table 2
The effect of nitrogen (100 kg N ha$^{-1}$ year$^{-1}$) and phosphorus (50 kg ha$^{-1}$ year$^{-1}$) treatments on total species touches (± S.E.M.) for the calcareous grassland plots (June 1996)

| Vegetation type | Control | N | P | N+P | $F$ ratio | $P$ |
|---|---|---|---|---|---|---|
| Higher plants | 73.7±4.9 | 75.5±6.6 | 92.2±4.1* | 101.2±4.1* | 6.96 | <0.05 |
| Forbs | 30.7±2.5 | 27.8±1.8 | 42.2±3.9* | 44.5±2.2* | 5.97 | <0.05 |
| Sedges | 6.75±2.5 | 13.2±4.1 | 3.50±0.5 | 4.25±1.5 | | >0.05 |
| Grasses | 35.3±1.9 | 34.3±4.3 | 46.0±3.9* | 51.7±3.3* | 5.97 | <0.05 |

$n=4$. ANOVA analysis with LSD range test.
* Significantly different from control, $P<0.05$.

showed the same pattern of increased growth in response to the phosphorus treatments, with no apparent response to nitrogen given alone.

TWINSPAN and MATCH analysis of the 1996 survey data again showed no shift in general species composition for either the acid or the calcareous site as a result of the P and N/P treatments.

### 3.2.3. Shoot nitrogen contents

The shoot nitrogen content of samples collected from the plots in August 1995 (see Fig. 3) showed a very clear dose-response to increasing nitrogen inputs, for the majority of species sampled at both sites. This effect was most pronounced on the acid grassland plots, with increases in the range of 23–76% for the 140 kg $NH_4NO_3$ N $ha^{-1}$ $year^{-1}$ treatment, and 42–92% for shoots collected from the $(NH_4)_2SO_4$ treated plots. The increases in tissue nitrogen on the calcareous plots were generally smaller, with no clear indication of higher levels in samples from the $(NH_4)_2SO_4$ treatments, although some marked effects were seen, notably in the case of *Thymus polytrichus* and the sedge species *Carex flacca* and *Carex caryophyllea*. There was no effect of treatment on shoot phosphorus content (data not shown). Mean N/P ratios of between 19 and 21 were obtained for the control plots at both sites, increasing to

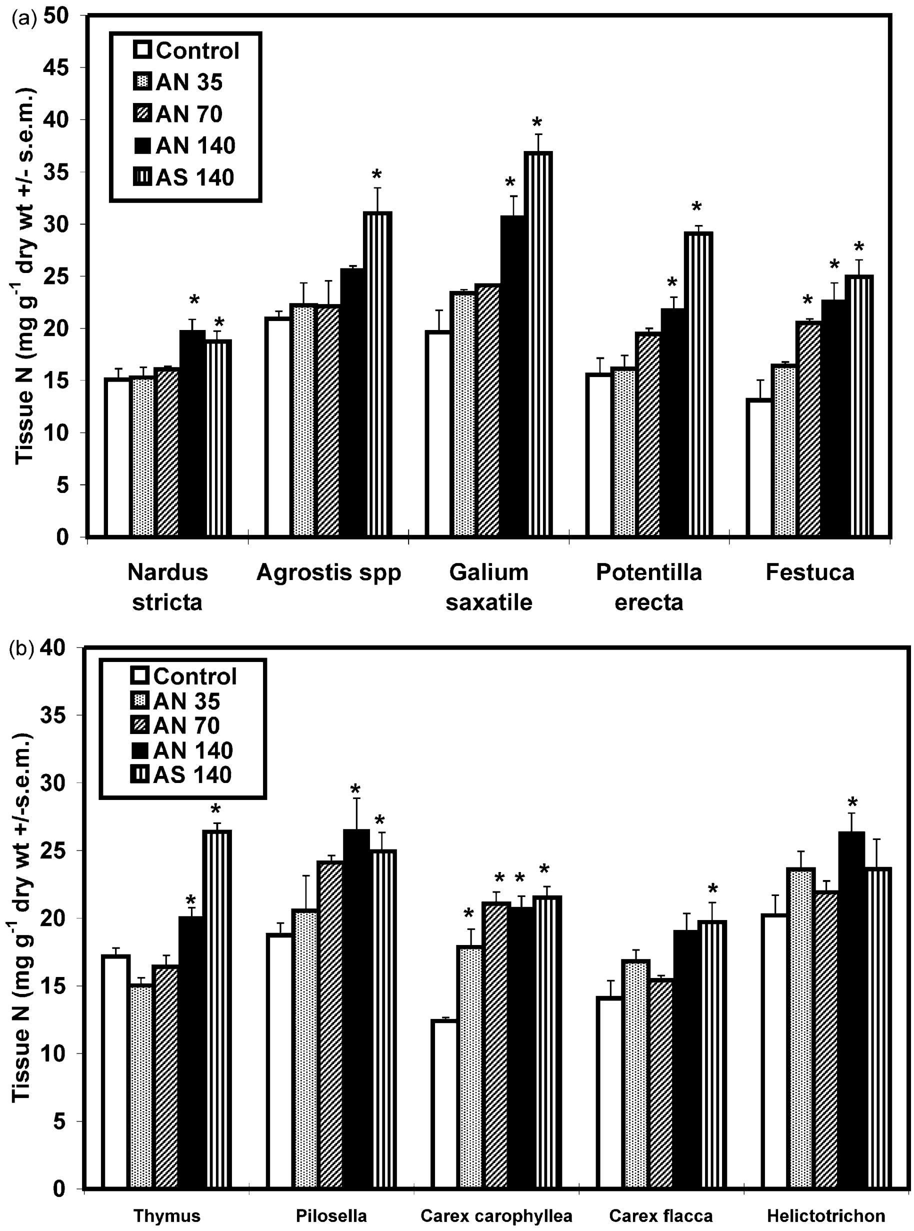


Fig. 3. The effect of long-term treatment with 35–140 kg $NH_4NO_3$ N $ha^{-1}$ $year^{-1}$ (AN 35–140) or 140 kg $(NH_4)_2$ $SO_4$ N $ha^{-1}$ $year^{-1}$ (AS140) on shoot total nitrogen content. (a) Acid grassland plots. (b) Calcareous grassland plots. September 1995. ANOVA analysis with LSD range test. *Significantly different from control $P<0.05$.

maximum values of 25 and 30 on the acid and calcareous plots respectively, in response to the high nitrogen treatments.

### 3.3. Mineralization study

The results for the overall nitrogen mineralization rates for the four quarterly periods of the study are shown in Figs. 4 and 5, for the acid and calcareous grassland plots, respectively. A clear trend towards increased nitrogen mineralization rates in response to increasing nitrogen inputs was seen on both sets of plots, and during all time periods, in addition to a strong seasonal pattern, with maximum rates of nitrogen release during the summer months (June–August).

#### 3.3.1. Acid grassland plots

Control mineralization rates on the acid grassland plots were in the range 2.8–14.7 mg N $m^{-2}$ $day^{-1}$ (Fig. 4a) for the December–March, March–May and September–November time periods, increasing to 48–64 mg N $m^{-2}$ $day^{-1}$ during the June–August period. There were no significant differences between the control and water treatments, but lower mineralization rates were

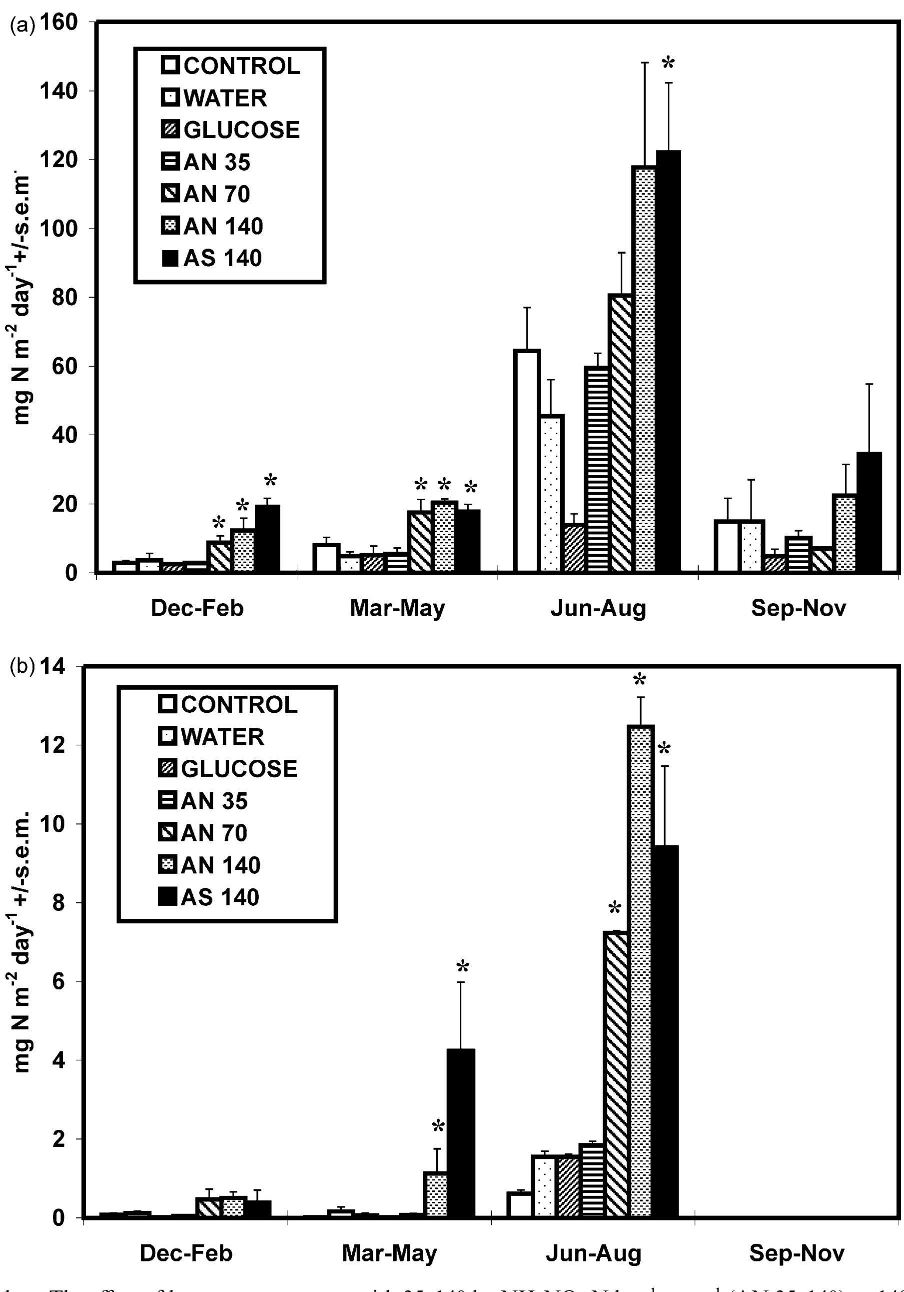


Fig. 4. Acid grassland plots. The effect of long-term treatment with 35–140 kg $NH_4NO_3$ N $ha^{-1}$ $year^{-1}$ (AN 35–140) or 140 kg $(NH_4)_2$ $SO_4$ N $ha^{-1}$ $year^{-1}$ (AS140) on (a) seasonal mineralization rates (b) seasonal nitrification rates (1994–1995). ANOVA analysis with LSD range test: significant effect of treatment in first three time periods $P < 0.05$. *Significantly different from equivalent control at $P < 0.05$.

obtained in the glucose treated plots, during June–August and September–November.

Long-term nitrogen additions markedly increased nitrogen mineralization rates in the first three time periods, with significant differences between control and treated groups for the 70 and 140 kg $NH_4NO_3$ N $ha^{-1}$ $year^{-1}$ and 140 kg $(NH_4)_2SO_4$ N $ha^{-1}$ $year^{-1}$ treatments, during the December–February and March–May periods. Mineralization rates were much higher during June–August, but the effect of the nitrogen additions was less clear, due to increased variability during this period.

Nitrification rates on the acid grassland were very low (Fig. 4b), with control values of less than 0.5 mg N $m^{-2}$ $day^{-1}$. There were however clear indications of increased nitrification rates in response to the 70 and 140 kg N $ha^{-1}$ $year^{-1}$ additions, with significant effects of treatment in the first three time periods. However only 2% of total nitrogen released during the December–May period was in the form of $NO_3^-$, increasing to 7–10% in June–August.

There was no detectable nitrification during September–November. Overall, the effects of the nitrogen treatments on both mineralization and nitrification

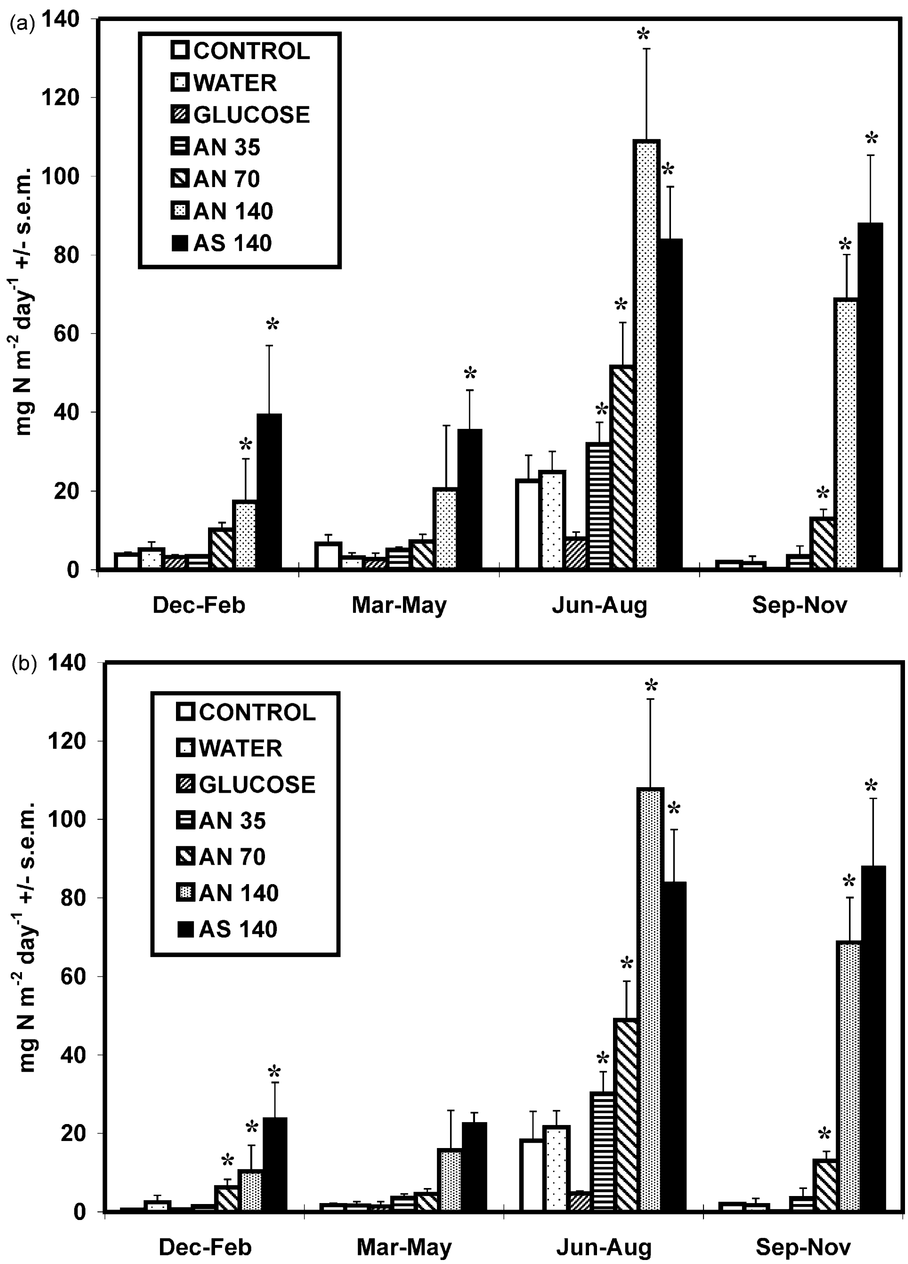


Fig. 5. Calcareous grassland plots. The effect of long-term treatment with 35–140 kg $NH_4NO_3$ N $ha^{-1}$ $year^{-1}$ (AN 35–140) or 140 kg $(NH_4)_2$ $SO_4$ N $ha^{-1}$ $year^{-1}$ (AS14) on (a) seasonal mineralization rates (b) seasonal nitrification rates (1994–1995). ANOVA analysis with LSD range test: significant effect of treatment in first three time periods $P<0.05$. *Significantly different from equivalent control at $P<0.05$.

appeared to be confined to the 70 and 140 kg N $ha^{-1}$ $year^{-1}$ treatments, with no apparent increases in the plots receiving the lower dose of 35 kg N $ha^{-1}$ $year^{-1}$.

### *3.3.2. Calcareous grassland plots*

Mineralization rates on the calcareous grassland plots for the four time periods studied (Fig. 5a), showed a generally similar pattern to that obtained on the acid grassland. Control values were in the range 1.2–5.2 mg N $m^{-2}$ $day^{-1}$ for the December–February, March–May and September–November time periods, but reached much higher levels (22.6–24.8 mg N $m^{-2}$ $day^{-1}$) during the summer months. There was again some indication of lower rates of nitrogen release from the glucose treated plots, particularly in the June–August and September–November time periods, but this did not reach statistical significance.

Nitrogen treatment significantly increased nitrogen mineralization rates on the calcareous plots in all four time periods. This effect was more marked than that obtained in the acid grassland plots. There was again no indication of increased rates at the lowest rate of nitrogen addition.

Nitrification on the calcareous grassland plots (Fig. 5b) accounted for in excess of 90% of the total nitrogen released during the June–August time period. During the December–February period however, this situation was reversed, with between 52 and 86% of total nitrogen in the control plots recovered as $NH_4^+$ ion. Significant increases in nitrification were also seen at the lowest rate of nitrogen addition in June–August.

The mean annual nitrogen mineralization rates for the calcareous and acid grassland plots (Fig. 6) showed a highly significant relationship between nitrogen treatments and mineralization rate, on both sets of plots, with a positive dose–response to increasing nitrogen additions. Annual mineralization rates on the acid grassland plots ranged from 58–67 kg N $ha^{-1}$ $year^{-1}$ for the control and water treated plots, to values of 139 and 169 kg N $ha^{-1}$ $year^{-1}$ for the 140 kg $NH_4NO_3$ N $ha^{-1}$ $year^{-1}$ and 140 kg $(NH_4)_2SO_4$ N $ha^{-1}$ $year^{-1}$ treatments respectively, representing an increase of approximately 2–4 fold in response to the highest treatments.

Control values for the calcareous plots were lower overall (29–30 kg N $ha^{-1}$ $year^{-1}$), but maximum values (170 and 200 kg N $ha^{-1}$ $year^{-1}$ respectively for the 140 kg $NH_4NO_3$ N $ha^{-1}$ $year^{-1}$ and $(NH_4)_2SO_4$ N $ha^{-1}$ $year^{-1}$ treatments) were similar to those on the acid grassland. The annual mineralization figures also confirm the results from the quarterly figures in showing no effect of the lowest 35 kg N $ha^{-1}$ $year^{-1}$ treatment on either the acidic or calcareous plots. Maximum mineralization rates were higher on both sites on the $(NH_4)_2SO_4$ treated plots, although the differences were not significant, and clearly lower mineralization rates were also obtained on the glucose treated plots, when compared with the controls.

Analysis of soil pH values on the calcareous site (Table 4) undertaken in 1996, showed a significant and dose related decrease in pH in response to all the nitrogen treatments with significant differences between control and treated plots at 70 kg N $ha^{-1}$ $year^{-1}$ treatment levels. A larger pH reduction was obtained on the 140 kg $(NH_4)_2SO_4$ N $ha^{-1}$ $year^{-1}$ treatments (significantly different from 140 kg $NH_4NO_3$ N $ha^{-1}$ $year^{-1}$ $P<0.05$), representing a drop of a further pH unit, when compared with data from 1994. On the acidic plots by contrast (mean soil pH $4.4 \pm 0.14$), no significant reduction in soil pH was obtained on any of the treated plots (data not shown).

Total soil nitrogen contents, analysed in December 1994 were in the range 7.6–10.1 and 8.6–10.5 mg N $g^{-1}$ dry weight for the acid and calcareous plots respectively, with no indication of any effect of the nitrogen treatments.

Table 4
Soil pH values for calcareous grassland plots receiving 35–140 kg $NH_4NO_3$ N $ha^{-1}$ $year^{-1}$ (AN 35–140) or 140 kg $(NH_4)_2$ $SO_4$ N $ha^{-1}$ $year^{-1}$ (AS140) (1996)

| Nitrogen additions | pH ±S.E.M. |
|---|---|
| Control | 6.8±0.08 a |
| 35AN | 6.5±0.06 a |
| 70AN | 6.3±0.04 b |
| 140AN | 6.1±0.08 b |
| 140AS | 5.2±0.11 c |

$n=4$. ANOVA analysis with LSD range test. Significant effect of treatment $F$ ratio = 56.58, $P<0.05$. Groups sharing a letter are not significantly different, $P<0.05$.

## 4. Discussion

The results presented here show that long-term application of simulated pollutant nitrogen to semi-natural acidic and calcareous grasslands has lead to marked changes in overall plant cover, nitrogen mineralization and soil pH.

### *4.1. Vegetation effects*

Few changes in species composition were seen over the first four years of the study (1990–1994), with no overall growth response to treatment (Morecroft et al., 1994). The slow onset of change excludes the likelihood of direct effects of the ionic strength of the solutions applied on the higher plants. Significant changes in cover and species composition were however observed on the calcareous plots in 1995 and 1996, in response to the high nitrogen additions. These could be due to a number of factors including direct effects of tissue nitrogen accumulation, or secondary effects resulting from changes in soil processes, most notably the marked acidification of the high nitrogen treated plots on the calcareous grassland.

The rapid acidification of unlimed grasslands treated with $(NH_4)_2SO_4$ fertilisers is a well known phenomenon in commercial farming. In the 130 year old "Park–Grass" experiment at Rothamsted, plots receiving $(NH_4)_2SO_4$ have shown a progressive and dose-related fall in soil pH (5.5–3.7) (Johnston et al., 1986). Studies both at Rothamsted and on other grasslands (Thurston et al., 1976; Johnston et al., 1986; Roem and Berendse, 2000) have also shown a strong negative association between acidification and species diversity. Roem and Berendse (2000) in an extensive study of heathland and grassland sites also found that species numbers and diversity were negatively correlated with increasing N:P and N:K ratios (Koerselman and Meuleman, 1996). The decreases in pH obtained on the calcareous plots in this study, in response to the highest levels of nitrogen addition, would be consistent with decreases in the relative availability of potassium and magnesium (Marschner, 1986) and also with increased mobilisation of aluminium (Blake et al., 1994). Taken together with the high levels of nitrogen this could lead to a pattern of nutrient imbalance and increased soil toxicity that would disadvantage the more calcicolous species in particular. This conclusion would receive some support from the significant increases in shoot tissue nitrogen content seen in *Thymus polytrichus* and *Carex flacca*, the two species showing the highest losses in cover on the calcareous plots.

The decreases in cover seen at high rates of nitrogen application on the acidic grassland plots, cannot however be explained in terms of acidification, since no significant changes in pH occurred. Much larger accumulations in shoot tissue nitrogen were obtained in species from these plots, notably in response to the $(NH_4)_2SO_4$ treatments, but these were not correlated with reductions in the cover of individual species, except in the case of *Agrostis* spp. Reductions of 26% were however obtained in total higher plant touches on the $(NH_4)_2SO_4$ treated acidic grassland plots, indicative of a possible relationship between cover, plant nitrogen uptake and increased levels of $NH_4^+$ ion availability. This would be consistent with the marked spread of *Nardus stricta*, a grass tolerant of highly acidic grassland in which $NH_4^+$ ion uptake would predominate.

In marked contrast to the results obtained from the nitrogen treatments, the addition of phosphorus to the calcareous plots produced clear increases in cover and growth when compared with the control and nitrogen treatments, and these effects were attributable to phosphorus alone. The results from this study confirm the

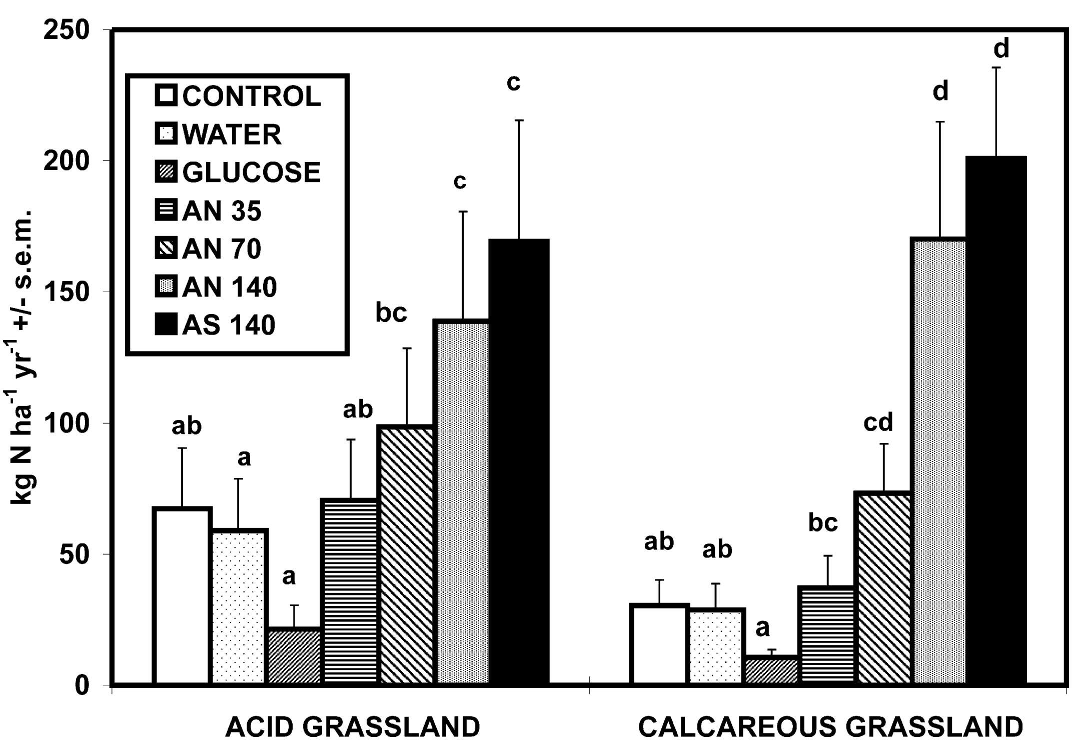


Fig. 6. The effect of long-term treatment with 35–140 kg $NH_4NO_3$ N $ha^{-1}$ $year^{-1}$ (AN 35–140) or 140 kg $(NH_4)_2$ $SO_4$ N $ha^{-1}$ $year^{-1}$ (AS140) on annual mineralization rates (kg N $ha^{-1}$ $year^{-1}$) of acid and calcareous grassland plots (1994–1995). ANOVA analysis with LSD range test. Significant effect of treatment: acid plots *F* ratio = 5.7, $P < 0.05$; calcareous plots *F* ratio = 5.17, $P < 0.05$. Groups sharing a letter are not significantly different $P < 0.05$.

phosphorus limitation of the calcareous plots, a conclusion supported by the results obtained by Johnson et al. (1999) in studies of root surface phosphomonoesterase activity undertaken in 1995. Limitation of this element is a common feature of calcareous grasslands (Jeffrey and Pigott, 1973; Wilson et al., 1995; Willems et al., 1993), and may be largely responsible for the absence of overall growth response, and the very limited effects of the nitrogen additions on species composition. Acidification may also not result in increased phosphorus availability, as calcium phosphates have been shown to remain unavailable for plant uptake in calcareous soils (Faurie and Fardeau, 1990).

Phosphorus additions to the sub-divided 1 $m^2$ plots on the acid grassland did not produce clear changes in cover, possibly because of the higher level of grazing at this site. Acidic grasslands are generally considered to be nitrogen limited (Van der Woude et al., 1994; Mountford et al., 1993), however, the high control N/P ratios in the vegetation at this site suggests that a pattern of phosphorus limitation may well be present (Verhoeven et al., 1996).

### 4.2. Mineralization study

The results obtained in this study show very significant increases in nitrogen mineralization rates in both acid and calcareous grassland soils, in response to six years of nitrogen additions. Annual mineralization rates in the control and water treated plots were in line with those reported by a number of other groups for grassland and heathland habitats (Perkins, 1978; Morecroft et al., 1992; Unkovich et al., 1998; Geens et al., 1991), but higher than those seen by Morecroft et al. (1994) in a similar study on the same plots in 1992. Both the seasonal pattern of mineralization rates and the absolute values obtained in this type of study are likely to be closely related to the detailed climatic conditions and the timing of the samples, with marked effects of soil moisture and temperature (Jamieson et al., 1998; Morecroft et al., 2000; Shaffers, 2000).

The total response to nitrogen treatment obtained by Morecroft et al. (1994) was however of the same order as that seen in this study, with 2–6 fold increases in mineralization rate at the highest rates of nitrogen input on both acid and calcareous grasslands. Wedin and Tilman (1996), in a 12-year study on a series of abandoned fields, obtained very significant effects on net nitrogen mineralization, with control rates of 25 kg N $ha^{-1}$ $year^{-1}$, rising to 100–150 kg N $ha^{-1}$ $year^{-1}$ in response to nitrogen inputs of 50–270 kg N $ha^{-1}$ $year^{-1}$. Large increases in net mineralization were also seen by Gill et al. (1995) on areas of undrained pastureland, following nitrogen inputs of 200 kg N $ha^{-1}$ $year^{-1}$ over 13 years.

Microbial immobilization of nitrogen in unfertilized grasslands has been shown to account for a very high proportion of total mineralized nitrogen and in many cases greatly exceeds plant uptake. A number of studies have shown a clear relationship between increased rates of nitrogen supply and reduced microbial biomass, leading in turn to an increase in the proportion of mineralized nitrogen available for plant uptake (Ledgard et al., 1998; Wedin and Tilman, 1996; Bardgett et al., 1999; Lovell et al., 1995). Similar processes may explain the effects seen in the present study, with the incorporation of plant litter of significantly reduced carbon to nitrogen ratio leading to sustained increases in net mineralization. Measurements of total microbial biomass carbon undertaken at the Wardlow site in 1995 by Johnson et al. (1998) however showed no treatment effects on the calcareous plots, together with a reduction at the highest level of input on the acidic grassland plots. The clear trend towards lower mineralization rates seen in the glucose treated plots would also be consistent with higher levels of nitrogen immobilization by the microbial population in response to increased carbon supply.

The majority of the nitrogen mineralized on the acid grassland plots was recovered in the form of $NH_4^+$ ions, whereas on the calcareous site most of the available $NH_4^+$ was rapidly converted to $NO_3^-$, particularly during the summer months, consistent with the wider tolerance of ammonifying bacteria to conditions of low pH and temperature (Alexander, 1977). Significant nitrification rates were obtained on the acid grassland plots only during the summer months and at the highest levels of nitrogen addition. A similar pattern of response has been seen in a number of studies on both grassland and forest systems (Ledgard et al., 1998; Wedin and Tilman, 1996; Watson and Mills 1998; Aber, 1992) where nitrification rates would normally be expected to be negligible, and have led to the suggestion that $NO_3^-$ leaching from such systems could be used as a marker for nitrogen overload or saturation.

The nitrogen mineralization results obtained in this study showed a strong seasonal pattern, both in the total mineralization rates and also in the balance of net ammonification and nitrification, with maximum nitrification rates during the summer months. A similar pattern was obtained by Morecroft et al. (1994) in the study carried out in 1992, and marked seasonal fluctuations have also been shown in a number of other studies (Davy and Taylor, 1974; Taylor et al.,1982).

The very high rates of nitrification obtained on the calcareous plots during the summer months may be the cause of the decline in the pH of soil samples collected from this site, with very significant acidification of the $(NH_4)_2SO_4$ treatments in particular. Nitrification rates may in some cases be limited by $NH_4^+$ release (Taylor et al., 1982) and higher rates of supply of this ion to the nitrifying bacteria would be consistent with enhanced rates of nitrate production.

## 5. Conclusions

The results of the vegetation study show that the species composition of the acidic and calcareous grasslands studied in this experiment is slowly changing in response to long-term nitrogen additions, with significant changes in the cover of individual species and reduced overall plant cover at the highest rates of nitrogen addition. The primary phosphorus limitation of the site is likely to be the major factor responsible for the relative stability of the vegetation structure, but continued grazing may also play a part in preventing the shift to more nitrophilous grass species.

Soil processes have been markedly affected by the nitrogen inputs. The very high rates of nitrogen mineralization and associated soil acidification, resulting from high rates of nitrogen supply, are likely to lead to changes in vegetation structure in the longer term.

More immediate considerations may be the effects on soil leaching and drainage processes, with the high rates of nitrification on the calcareous plots in particular likely to be associated with major losses of both $NO_3^-$ and cations to the drainage water from this site. $NH_4^+$ ions are more tightly bound within the soil structure, and leaching from the acid grassland is therefore not likely to be such a problem. Long-term accumulation of $NH_4^+$ ions in the soil could however eventually lead to acidification and possible toxic effects on plant growth on the acid grassland plots.

## References


Aber, J.D., 1992. Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. TREE 7, 220–224.

Alexander, M., 1977. Introduction to Soil Microbiology. Wiley, London.

Bardgett, R.D., Lovell, R.D., Hobbs, P.J., Jarvis, S.C., 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biology and Biochemistry 31, 1021–1030.

Berendse, F., Oudhof, H., Bol, J., 1987. A comparative study on nutrient cycling in wet heathland ecosystems. I. Litter production and nutrient losses from the plant. Oecologia 74, 174–184.

Blake, L., Johnston, A.E., Goulding, K.W.T., 1994. Mobilization of aluminium in soil by acid deposition and its uptake by grass cut for hay. A chemical time bomb. Soil Use and Management 10, 51–55.

Bobbink, R., 1991. Effects of nutrient enrichment in Dutch chalk grassland. Journal of Applied Ecology 28, 28–41.

Bobbink, R., Willems, J.K., 1987. Increasing dominance of *Brachypodium pinnatum* (L.) Beauv. in chalk grassland: a threat to a species rich-ecosystem. Biological Conservation 40, 301–314.

Carroll, J.A., Caporn, S.J.M., Cawley, L., Read, D.J., Lee, J.A., 1999. The effect of increased deposition of atmospheric nitrogen on *Calluna vulgaris* in upland Britain. New Phytologist 141, 423–431.

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

Davy, A.J., Taylor, K., 1974. Seasonal patterns of nitrogen availability in contrasting soils in the Chiltern hills. Journal of Ecology 62, 793–807.

Faurie, G., Fardeau, J.C., 1990. Can acidification associated with nitrification increase available soil phosphate or reduce the rate of phosphate fixation? Biology and Fertility of Soils 10, 145–151.

Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews 63, 433–462.

Geens, E.L., Davies, G.P., Maggs, J.M., Barraclough, D., 1991. The use of mean pool abundances to interpret $^{15}N$ tracer experiments. II. Application. Plant and Soil 131, 97–105.

Gill, K., Jarvis, S.C., Hatch, D.J., 1995. Mineralization of nitrogen in long-term pasture soils: effects of management. Plant and Soil 172, 153–162.

Goulding, K.W.T., Bailey, N.J., Bradbury, N.J., Hargreaves, P., Howe, M., Murphy, D.V., Poulton, P.R., Willison, T.W., 1998. Nitrogen deposition and its contribution to nitrogen cycling and associated soil processes. New Phytologist 139, 49–58.

Jamieson, N., Barraclough, D., Unkovich, M., Monaghan, R., 1998. Soil N dynamics in a natural calcareous grassland under a changing climate. Biol. Fertil. Soils 27, 267–273.

Jeffrey, D.W., Pigott, C.D., 1973. The response of grasslands on sugar limestone in Teesdale to the application of phosphorus and nitrogen. Journal of Ecology 61, 85–92.

Johnson, D., Leake, J.R., Lee, J.A., Campbell, C.D., 1998. Changes in soil microbial biomass and microbial activities in response to 7 years simulated pollutant nitrogen deposition on a heathland and two grasslands. Environmental Pollution 103, 239–250.

Johnson, D., Leake, J.R., Lee, J.A., 1999. The effects of quantity and duration of simulated pollutant nitrogen deposition on root-surface phosphatase activities in calcareous and acid grasslands: a bioassay approach. New Phytologist 141, 433–442.

Johnston, E.E., Goulding, K.W.T., Poulton, P.R., 1986. Soil acidification during more than 100 years under permanent grassland and woodland at Rothamsted. Soil Use and Management 2, 3–10.

Koerselman, W., Meuleman, A.F.M., 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. Journal of Applied Ecology 33, 1441–1450.

Ledgard, S.F., Jarvis, S.C., Hatch, D.J., 1998. Short-term nitrogen fluxes in grassland soils under different long-term nitrogen management regimes. Soil Biol. Biochem. 30, 1233–1241.

Lovell, R.D., Jarvis, S.C., Bardgett, R.D., 1995. Soil microbial biomass and activity in long-term grassland: effects of management changes. Soil. Biol. Biochem. 7, 969–975.

Malloch, A.J.C., 1988. VESPAN II. University of Lancaster, Lancaster.

Malloch, A.J.C., 1992. MATCH. University of Lancaster, Lancaster.

Marschner, H., 1986. Mineral Nutrition of Higher Plants. Academic Press, London.

Morecroft, M.D., Marrs, R.H., Woodward, F.I., 1992. Altitudinal and seasonal trends in soil nitrogen mineralization rate in the Scottish highlands. Journal of Ecology 80, 49–56.

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.

Morecroft, M.D., Burt, T.P., Taylor, M.E., Rowland, A.P., 2000. Effects of the 1995–1997 drought on nitrate leaching in lowland England. Soil Use and Management 16, 117–123.

Mountford, J.O., Lakhani, K.H., Kirkham, F.W., 1993. Experimental assessment of the effects of nitrogen addition under hay-cutting and aftermath grazing on the vegetation of meadows on a Somerset peat moor. Journal of Applied Ecology 30, 321–332.

NEGTAP: National Expert Group on Transboundary Air Pollution, 2001. Acidification, Eutrophication and Ground-level Ozone in the UK. Prepared at CEH Edinburgh on behalf of DEFRA and the devolved administrations.

Perkins, D.F., 1978. The distribution and transfer of energy and nutrients in the *Agostis–Festuca* grassland ecosystem. In: Heal, O.W., Perkins, D.F. (Eds.), Production Ecology of British Moors and montane Grasslands. Springer-Verlag, Berlin, pp. 325–395.

Rodwell, J.S. (Ed.), 1992. British Plant Communities, Vol III: Grasslands and Montane Communities. Cambridge University Press, Cambridge.

Roem, W.J., Berendse, F., 2000. Soil acidity and nutrient supply ratio as possible factors determining changes in plant species diversity in grassland and heathland communities. Biological Conservation 92, 151–161.

Schaffers, A.P., 2000. In situ annual nitrogen mineralization predicted by simple soil properties and short-period field incubation. Plant and Soil 221, 205–219.

Taylor, A.A., De-Felice, J., Havill, D.C., 1982. Seasonal variation in nitrogen availability and utilisation in an acidic and calcareous soil. New Phytologist 92, 141–152.

Thurston, J.M., Williams, E.D., Johnston, A.E., 1976. Modern developments in an experiment on permanent grassland started in 1856: effects of fertilisers and lime on botanical composition and crop and soil analyses. Ann. Agron. 27, 1043–1082.

Unkovich, M., Jamieson, N., Monaghan, R., Barraclough, D., 1998. Nitrogen mineralization and plant nitrogen acquisition in a nitrogen-limited calcareous grassland. Environmental and Experimental Botany 40, 209–219.

Van der Woude, B.J., Pegtel, D.M., Bakker, J.P., 1994. Nutrient limitation after long-term nitrogen fertiliser application in cut grasslands. Journal of Applied Ecology 31, 405–412.

Verhoeven, J.T.A., Koerselman, W., Meuleman, A.F.M., 1996. Nitrogen or phosphorus limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. TREE 11, 494–497.

Watson, C.J., Mills, C.L., 1998. Gross nitrogen transformations in grassland soils as affected by previous management intensity. Soil Biol. Biochem. 6, 743–753.

Wedin, D.A., Tilman, D., 1996. Influence of nitrogen loading and species composition on the carbon balance of grasslands. Science 274, 1720–1723.

Willems, J.H., Peet, R.K., Bik, L., 1993. Changes in chalk grassland structure and species richness resulting from selective nutrient additions. Journal of Vegetation Science 4, 203–212.

Wilson, E.J., Wells, T.C.E., Sparks, T.H., 1995. Are calcareous grasslands in the UK under threat from nitrogen deposition? An experimental determination of a critical load. Journal of Ecology 83, 823–832.