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1 **Loss of forb diversity in relation to nitrogen deposition in the UK:**
2 **regional trends and potential controls**

3

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19

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25 **Running title:** Impacts of nitrogen deposition on acid grasslands.

1 **Abstract**

2 In this study we investigate the impact of nitrogen (N) deposition on the diversity of
3 three different vegetation functional groups – forbs, grasses and mosses – using a field
4 survey of acid grasslands across Great Britain. Our aim is to identify the vegetation
5 types that are most vulnerable to enhanced N deposition, and to shed light on the
6 mechanisms that may be driving N-initiated species changes in the UK. Sixty-eight
7 randomly selected grasslands belonging to the U.K. National Vegetation
8 Classification group U4 (*Festuca ovina*-*Agrostis capillaris*-*Galium saxatile* grassland)
9 were studied along a gradient of atmospheric N deposition ranging from 6 to 36 kg N
10 ha⁻¹ yr⁻¹. At each site, vegetation was surveyed and samples were taken from the
11 topsoil and subsoil. Above-ground plant material was collected from 3 species: a
12 forb, grass and moss.

13 Both the species richness and cover of forbs declined strongly with increasing
14 N deposition, from greater than 8 species/20% cover per m² quadrat at low levels of N
15 to fewer than 2 species/5% cover at the highest N deposition levels. Grasses showed
16 a weak but significant decline in species richness, and a trend toward increasing cover
17 with increasing N input. Mosses showed no trends in either species richness or cover.
18 Most of the decline in plant species richness could be accounted for by the level of
19 ammonium deposition.

20 Soil KCl-extractable ammonium concentration showed a significant positive
21 correlation with N input, but there was no relationship between N deposition and
22 extractable nitrate. In the soil O/A horizon, there was no relationship between N
23 deposition and %N, and only a very weak positive relationship between the level of N
24 deposition and the C:N ratio. Finally, in the vegetation, there was no relationship

1 between N deposition and either shoot tissue N concentration or N:P ratio for any of
2 the three reference species.

3 Combining our regional survey with the results of published N-addition
4 experiments provides compelling evidence that there has been a significant decline in
5 the species richness and cover of forbs across Great Britain, and that the primary
6 cause is competition due to an increase in the cover of grasses in response to enhanced
7 deposition of reactive nitrogen, primarily NH_4^+ .

8

1 **Introduction**

2 Deposition of atmospheric N compounds, predominantly originating from volatilised
3 animal waste, synthetic fertilisers and burning of fossil fuels, has become a worldwide
4 issue of concern during the last few decades, with the effects of acidification and
5 eutrophication observed in Europe, North and South America, the former Soviet
6 Union, large parts of Asia, and areas of Africa (Bouwman *et al.*, 2002). Between 1860
7 and the early 1990s atmospheric deposition of NO_x and NH_y worldwide more than
8 trebled, and it is projected that this will nearly double again to 195 Tg N yr⁻¹ by the
9 year 2050 (Galloway *et al.*, 2004). In Europe, the problems of N deposition are
10 widespread, with over half of terrestrial ecosystems affected by eutrophication in 70%
11 of European countries (EMEP, 2000). In the UK, decadal averages show that between
12 1900 and 2000 total N deposition increased from 0.229 Tg N to 0.402 Tg N.
13 Deposition of both reduced and oxidised N peaked in the 1990s, and is now beginning
14 to decline (Fowler *et al.*, 2004).

15 Nitrogen pollution has a number of potential effects on semi-natural ecosystems. At
16 very high levels of deposition, ammonium (NH₄⁺) is known to be toxic to plants
17 (Sheppard, 2002); however, even at lower levels, serious effects on vegetation can be
18 observed. These include the soil-mediated effects of acidification and eutrophication
19 (in this context, the enrichment of soils by N compounds that are readily available to
20 plants). Eutrophication can lead to increased leaching of N to waterways (Wright *et*
21 *al.*, 2001) and changes in the rates of soil microbial processes (Aber *et al.*, 2003) and
22 has the potential to change the plant species composition of sensitive habitats (Burke
23 *et al.*, 1998). Other observed effects of N deposition include increased susceptibility
24 to pests and disease (*e.g.* increased frequency and severity of heather beetle
25 (*Lochmaea suturalis*) attacks to common heather (*Calluna vulgaris*) (Brunsting and

1 Heil, 1985)) and an increased susceptibility to environmental stressors (*e.g.* increased
2 incidence of late winter injury in *C. vulgaris* (Carroll *et al.*, 1999)).
3 Numerous N-addition experiments in different habitat types have shown the potential
4 for N deposition to impact species richness, soil chemistry and soil microbial
5 processes (*e.g.* Mountford *et al.*, 1993; Phoenix *et al.*, 2003, Wedin and Tilman 1996).
6 Determining that these changes are actually *occurring*, and that they are due to
7 nitrogen pollution, has been more problematic. In the Netherlands, serious effects of
8 N deposition have been inferred, especially the invasion and eventual domination of
9 heathlands by nitrophilous grass species such as purple moor grass (*Molinia caerulea*)
10 (Heil and Diemont, 1983). Supporting the implication of N in these species changes,
11 soil solution N levels in grass-dominated heaths have been shown to be much higher
12 than in heather-dominated ones (Roelofs, 1986). In Great Britain, Smart *et al.* (2004)
13 identified increased cover-weighted Ellenberg fertility scores of semi-natural
14 grasslands and heaths/bogs in areas with high rates of NH_x deposition. Finally,
15 Stevens *et al.* (2004) showed a strong, significant negative relationship between total
16 inorganic N deposition and plant species richness in acid grasslands across Great
17 Britain, and determined that no other likely driver of diversity could explain this
18 pattern.

19 Here we analyse the data described in Stevens *et al.* (2004) in more detail to
20 explore trends in grass, forb and moss species richness, tissue chemistry, and soil
21 chemistry in relation to N deposition, and we explicitly examine the separate effects of
22 ammonium and nitrate. Our aims are to (1) follow the signal of nitrogen in deposition
23 as it passes through the soil and vegetation, and ultimately to its impact on the
24 community composition, (2) identify the vegetation types that are most vulnerable to
25 enhanced atmospheric N deposition, (3) evaluate the usefulness of vegetation- or soil-

1 N enrichment as indicators of N deposition and ecosystem N status, and (4) shed light
2 on the mechanisms that may be driving nitrogen-initiated species changes in the UK
3 and other parts of the world.

4

5 **Materials and methods**

6

7 *Field Methods*

8 A database of UK grasslands belonging to the British National Vegetation
9 Classification community U4 (*Festuca ovina*-*Agrostis capillaris*-*Galium saxatile*
10 grassland) (Rodwell, 1992) was compiled using information supplied by English
11 Nature, the Countryside Council for Wales, and Scottish Natural Heritage. U4
12 grasslands are acid grasslands typical of upland areas, with *Agrostis capillaris*,
13 *Anthoxanthum odoratum*, *Festuca ovina*, *Galium saxatile* and *Potentilla erecta* as
14 constant species. They are predominantly used for rough grazing by sheep and are
15 common across much of the UK with the exception of the southeast. These grasslands
16 are akin to the *Violion caninae* grasslands found throughout Europe. The soils of U4
17 grasslands are very variable in their organic matter content and moisture status,
18 ranging from soils with a high peat content through to sands.

19 From this database, sites of insufficient size and those that were not readily
20 accessible were eliminated. A stratified random sample of 68 grasslands was selected
21 to provide a mix of sites covering low (less than 10 kg N ha⁻¹ yr⁻¹, 9 sites), medium
22 (10-25 kg N ha⁻¹ yr⁻¹, 27 sites) and high (greater than 25 kg N ha⁻¹ yr⁻¹, 32 sites) rates
23 of N deposition. N deposition values were modelled by the Centre for Ecology and
24 Hydrology (CEH), Edinburgh using the CEH National Atmospheric Deposition Model
25 (Smith *et al.*, 2000). This provides total inorganic N deposition, wet and dry N

1 deposition, and oxidised and reduced N deposition at a 5 km resolution. A note was
2 made of any point sources in the immediate area of the field locations that could
3 contribute additional N deposition.

4 The sites ranged geographically from Exmoor, southwest England to
5 Altnaharra in northern Scotland and showed a great deal of variability. Mean altitude,
6 for example, ranged from 15 m above sea level to 692 m above sea level, and mean
7 annual temperature ranged from 6.6 °C to 10.6 °C (Appendix 1). Total inorganic N
8 deposition ranged from 6.2 kg N ha⁻¹ yr⁻¹ to 36.3 kg N ha⁻¹ yr⁻¹. Further details on the
9 range of conditions at the sites can be found Appendix 1.

10 The grasslands were visited between May and September of 2002 and 2003.
11 At each site a one-hectare area consisting of more than 50% U4 grassland was
12 delineated. Within the U4 grassland in this area, 5 randomly located 2 × 2 metre
13 quadrats were placed. Within each quadrat, the percentage cover was estimated by eye
14 for each species present and soil samples were collected. Subsoils were sampled using
15 a 5 cm diameter Dutch auger at a depth of 30–40 cm, taken from the centre of the
16 quadrat. Where the soils were not deep enough to permit this, the samples were
17 collected at the maximum possible depth. Topsoil samples were collected from two
18 opposing corners of the quadrat. These were taken from the top 5 cm of the A horizon
19 or, where there was no A horizon apparent, close to the surface, within the O horizon.
20 Samples were air-dried and stored for fewer than three months prior to analysis.

21 Samples of all above-ground plant material (approximately 30g) were
22 collected for 3 species – *Agrostis capillaris*, *Galium saxatile* and *Rhytidiadelphus*
23 *squarrosus*. These species were selected because they represent a common and
24 abundant grass, herb and moss, respectively. All samples were collected from within
25 the one-ha sampling area, avoiding any patches that appeared visually affected by

1 animal latrines or animal feed bought onto the sites. Samples were collected from
2 within or as close as possible to the randomly located quadrats.

3 A full description of each of the sites visited was made, including UK national
4 grid reference and altitude determined using a global positioning system (GPS).
5 Aspect and slope were also estimated. Data on mean annual rainfall, actual
6 evapotranspiration, potential evapotranspiration and mean monthly maximum soil
7 moisture deficit were obtained from the MORECS database (Thompson *et al.*, 1981).
8 Temperature data averaged for the years 1971-2000 were obtained from the UK
9 Meteorological Office (UK Met. Office, 2004). Grazing was estimated on a scale of 1
10 to 3 where 1 was high intensity and 3 low intensity, and assessed based on evidence
11 from vegetation height and amount of animal faecal material. The presence of
12 enclosures (*e.g.* fences, walls or hedges) was also noted. Estimated rates of sulphur
13 deposition were provided by the Centre for Ecology and Hydrology, Edinburgh.

14

15 *Laboratory methods*

16 All soil samples were air dried and ground to <2 mm prior to analysis. For carbon,
17 nitrogen, and sulphur content, the soil samples were ground to a fine powder using a
18 planetary ball mill, with 0.2 g of sample thoroughly mixed with 1 g of Comcat
19 accelerator in a porcelain boat. C and N concentration of the soils was then
20 determined using a LECO CNS-2000 elemental analyser.

21 For measurement of soil extractable ammonium and nitrate ion concentration,
22 samples were bulked to give one 10 g composite sample per site. Samples were then
23 leached with 100 ml 1 M KCl in leaching tubes. The extracted solution was analysed
24 for ionic concentration using a Dionex DX100 ion chromatograph with a Ion Pac

1 CS16 column for cations and a IonPac AS9-HC column for anions. Ion concentrations
2 were recorded in mg L⁻¹.

3 Plant samples were washed in deionised water after collection and oven dried
4 at 55°C for 3 days. N concentration was analysed using a LECO CNS-2000 elemental
5 analyser as described above for soil samples. Plant tissue phosphorus (P)
6 concentration was determined using a dry ashing extraction method (Chapman and
7 Pratt, 1961; Ryan *et al.*, 2001) followed by a standard Barton colour complex (MAFF,
8 1986). Absorbance was determined using a Heios Thermo Spectropic colorimeter at a
9 wavelength of 410 nm.

10 A correction for seasonal variation was applied to the tissue element
11 concentrations by collecting plant tissue samples from a single field location
12 (Stockgrove Country Park, Bedfordshire) throughout the growing season. The species
13 composition of this site was representative of the surveyed sites, and the same
14 sampling area and methods were used as in the surveyed sites. Levels of N and P in
15 this plant material were used to make adjustments to all the results from the surveyed
16 sites according to day degrees on the date of sampling (ranging between 284.1 and
17 1026.5 day degrees depending on survey date and location). This only resulted in
18 small adjustments to the tissue element concentrations in *Agrostis* and *Galium* (range
19 in the percent difference between the actual and adjusted element concentrations 0.24
20 – 4.09%). There was no significant change in the tissue N or P content of
21 *Rytidiadelphus* across the growing season. Although there may be differences in the
22 range of seasonal variation across the sites, this was not feasible to measure, and the
23 use of a single representative site provides us with a reasonable estimate.

1 *Data analysis*

2 Stepwise multiple regression was used to create empirical models between measures
3 of species richness and all other likely drivers of biodiversity for which data could be
4 collected (Appendix 1). Regression analysis quantifies the amount of variation in a
5 dependent variable that is explained by one or several correlates. Stepwise regression
6 builds a model from those variables that together explain the most variability in the
7 dependent variable, but are themselves statistically independent. The input data
8 (Appendix 1) and resulting models were examined for any significant violations of the
9 assumptions of the technique. Both linear and non-linear functions were evaluated,
10 and significance levels were set at $p < 0.05$.

11

12 **Results**

13

14 *Species richness*

15 Of the 20 variables entered in the stepwise regression, the most significant correlate to
16 species richness is a negative relationship with the total inorganic N deposition
17 ($r^2 = 0.55$), but this relationship is essentially entirely due to NH_x deposition ($r^2 = 0.54$,
18 $p < 0.001$; Figure 1A). Species richness is also correlated to NO_x deposition both
19 linearly ($r^2 = 0.29$, $p < 0.001$) and, somewhat more strongly, in a negative exponential
20 relationship ($r^2 = 0.33$, $p < 0.001$; Figure 1B), reflecting a lower rate of change in species
21 richness at higher levels of NO_x deposition (Figure 1B). After accounting for N
22 deposition, the most significant regional-scale variable correlated to species diversity
23 is soil pH, which contributes an additional 5% toward explaining the variability in
24 species richness (Stevens et al. 2004).

1 The patterns shown in Figure 1 are driven primarily by a loss of forb cover and
2 richness (Figures 2A and 2B), both of which decline linearly with increased N
3 deposition (cover: $r^2 = 0.32$, $p < 0.01$; richness: $r^2 = 0.47$, $p < 0.01$). Forb cover and
4 richness decline from means of ca 20% and 8 species per quadrat, respectively, at the
5 lowest N deposition levels, to less than 5% and 1-2 species at highest N deposition
6 sites. In particular, the forbs *Plantago lanceolata* (ribwort plantain), *Campanula*
7 *rotundifolia* (harebell), and *Euphrasia officinalis* (eyebright), together with the shrub
8 *Calluna vulgaris* (heather) and the moss *Hylocomium splendens* are consistently
9 reduced in the lower-diversity plots. Grass species richness declines slightly with
10 increasing N deposition (Figure 2D) ($r^2 = 0.16$, $p < 0.01$) but grass cover shows an
11 increasing trend, although this is not significant (Figure 2C) ($r^2 = 0.05$, $p < 0.08$).
12 There is no significant relationship between N deposition and bryophyte cover or
13 richness (Figures 2E and F).
14 It should be noted that, by sampling areas that currently conform to the U4
15 specification, we are excluding areas that may have already converted to another
16 vegetation community. Thus, our calculation of the rate of species richness change
17 with N deposition can be considered a minimum estimate for these grasslands.

18

19 *Soil chemistry*

20 There was no significant relationship between total inorganic N deposition and topsoil
21 %N, and only a very weak positive relationship with C:N ($r^2 = 0.08$, $p < 0.05$; Figure
22 3A). Soil exchangeable nitrate did not show any significant relationship with total
23 NO_x deposition or wet deposition of nitrate. There was a significant linear
24 relationship between soil exchangeable NO_3^- and total inorganic N deposition ($r^2 =$

1 0.12, $p < 0.01$); however, this relationship was driven by only 3 points. When these
2 were removed, N deposition and soil solution NO_3^- were uncorrelated.

3 Soil exchangeable ammonium shows a far clearer relationship with N
4 deposition. Soil ammonium is significantly positively related to wet deposition of
5 NH_4^+ ($r^2 = 0.36$, $p < 0.01$) total NH_x deposition ($r^2 = 0.34$, $p < 0.01$), and, most strongly,
6 to total inorganic N deposition ($r^2 = 0.39$, $p < 0.01$) (Figure 3B). The pH of both
7 topsoil and subsoil is also significantly negatively related to N deposition (Figure 3C),
8 although pH is most strongly related to the combined deposition of N and S (acid
9 deposition). Soil pH is also very likely influenced by site factors such as previous
10 land use, and soil parent material and organic matter content.

11

12 *Plant tissue chemistry*

13 There was no significant relationship between total N deposition and tissue N
14 concentration corrected for day degrees for any of the three species tested (Figure 4).
15 There were also no significant relationships if uncorrected data are used. In addition,
16 none of the species showed any significant relationship between the tissue N:P ratio
17 and total inorganic N deposition (Figure 5).

18 Grazing can affect the relationship between N deposition and tissue N, as re-
19 growth is comprised of young, actively growing shoots. Consequently, we
20 specifically considered grazing as an explanatory variable for regional variation in
21 tissue N. There were no significant relationships between tissue N concentration, P
22 concentration or N:P ratio and grazing intensity, or between these variables and N
23 deposition with grazing intensity forced into the regression equation. However, our
24 estimates of grazing intensity are subjective and it is possible that a stronger regional

1 relationship between N deposition and tissue N or N:P may exist within grasslands
2 that are known to have little grazing pressure.

3

4 **Discussion**

5

6 *Species richness*

7 Both empirical and experimental evidence implicate inorganic N deposition,
8 especially NH_4^+ , in a decline in the species richness of forbs in acid grasslands across
9 Great Britain. Since nitrogen is the limiting nutrient for plant growth in many
10 terrestrial ecosystems, an excess of reactive N to these ecosystems may reduce plant
11 diversity through favouring species adapted to quick exploitation of available
12 nutrients. Nitrogen may also decrease diversity through factors such as nutrient
13 imbalance (e.g. inducing P limitation), soil acidification, or increased susceptibility to
14 diseases or pests (Phoenix et al., 2003; Power et al., 1998; Brunsting and Heil, 1985).

15 In our survey we did not note enhanced pest-related damage in the low-
16 diversity plots, nor could we find any clear indications of nutrient limitation shifts
17 with changing N deposition. Our results showed that soil pH declines in relation to
18 the level of acid deposition, and species richness is higher in higher-pH soils at any
19 level of N deposition, so acidification may play a role in observed species richness
20 differences. This has been clearly shown in several long-term field manipulation
21 experiments using $(\text{NH}_4)_2\text{SO}_4$ (Carroll et al., 2003; Morecroft et al., 1994; Johnson et
22 al., 1986). However, the significant relationship between N deposition and soil
23 exchangeable NH_4^+ , the strong decline in both richness and cover of forbs in relation
24 to other functional groups, and the increased trend in cover of grasses with increasing
25 N deposition, all suggest that the main reason for the decline in overall species

1 richness is competition due to ecosystem N enrichment, resulting in increased
2 dominance of fewer species.

3 Most of the forbs in this study were either low growing, prostrate or rosette in
4 form and therefore not adapted to compete for light. In addition they often do not
5 show the same rate of growth in response to enhanced nutrients that many grasses are
6 capable of. Consequently, at high N deposition, forbs may become light-limited due
7 to shading by plants with a more vigorous growth response. The forbs identified as
8 consistently reduced in the low-diversity plots are, together with heather, either
9 indicators of infertile conditions (*Campanula*, *Euphrasia* and *Calluna*), are recognised
10 as intolerant of competition with vigorous grasses (*Campanula*), or have been shown
11 to decline under experimental N applications (*Plantago* and *Calluna*) (reviewed in
12 Stevens *et al.* 2004).

13 Although grasses also showed a decline in species richness, the positive trend
14 in total grass cover with increasing N deposition suggests increased dominance of a
15 few species. Stevens et al (2004) identified *Nardus stricta* as a species that was more
16 abundant in high-N deposition sites, but the weak overall trend of increasing grass
17 cover with increasing N input is likely due to different species increasing in different
18 locations, and/or several species increasing slightly in cover.

19 Bryophytes as a group show no relationship to N deposition, although this is
20 because some species (*e.g. Hylocomium splendens*) are reduced at high N deposition
21 levels whereas *Hypnum cupressiforme* was positively associated with N deposition
22 (Stevens *et al.*, 2004). Although mosses in general are frequently identified as being
23 sensitive to N inputs (*e.g. Gordon et al.*, 2001), *Hypnum* is relatively pollution-
24 tolerant (Hallinback, 1994), and it is likely that this species has increased at the
25 expense of more sensitive mosses.

1

2 *Soil chemistry*

3 There is essentially no relationship between the N concentration in the topsoil (as %N
4 or C:N) and total inorganic N deposition. One might expect an increase in soil N with
5 increasing N deposition, although along the wide gradient studied the soils varied
6 considerably in their type, structure, and land use history, all of which affect the N
7 content. The soils also have very variable organic matter contents, which in itself
8 causes major differences in the N concentration, since most of the soil N is found in
9 organic matter.

10 These findings are similar to those of Falkengren-Grerup *et al.* (1998) who
11 found no consistent changes in the soil N concentration in woodlands (5 cm depth) at
12 different levels of N deposition, and with Dise *et al.* (1998) who showed no
13 relationship between N deposition and O-horizon C:N in forests across Europe when
14 regional climate differences were accounted for. Several experimental N-addition
15 studies have also failed to show any relationship between soil N concentration or C:N
16 and N application rate (e.g. Morecroft *et al.* 1994, Magill *et al.* 2004, Moldan *et al.* in
17 review). Morecroft *et al.* 1994 suggest increased plant uptake, leaching or
18 denitrification as potential reasons for a lack of response in soil organic matter N
19 concentration to N input, although later work on the same sites has dismissed leaching
20 as a potential cause (Phoenix *et al.*, 2003). It is possible that there is simply
21 insufficient time for N to detectably accumulate in the soil pool as a result of
22 experimental additions, and, in cases where there is no change in C:N, that C may
23 accumulate with N.

24 The poor relationship between soil extractable nitrate and deposition of NO_x,
25 NO₃⁻, or total inorganic N is likely to be due in part to rapid plant uptake combined

1 with the mobility and lability of any excess nitrate (Matson *et al.*, 2002). Nitrate is
2 very mobile in the soil and so may be rapidly leached to lower soil horizons, with
3 microbial immobilisation preventing the nitrate being lost from the system entirely.
4 Conversely, the positive correlation between N deposition and soil extractable
5 ammonium (Figure 3B) may reflect the greater storage of NH_4^+ in the soils
6 (Buchmann *et al.*, 1996). These results, showing retention of NH_4^+ in the topsoil but
7 not of NO_3^- , are consistent with N addition experiments (Emmett *et al.*, 1995;
8 Buchmann *et al.*, 1996; Magill *et al.*, 1997; Stuanes and Kjønaas, 1998).

9

10 *Plant tissue chemistry*

11 None of the three species examined showed a significant correlation between N
12 deposition and tissue N concentration. Since both soil solution chemistry and species
13 richness change with N deposition, it is surprising that plant tissue chemistry does not.
14 This casts doubt on the usefulness of tissue nutrient concentrations as bioindicators of
15 either N deposition or ecosystem N status (*e.g.* Pitcairn *et al.* 2003). Increases in
16 tissue N with experimental applications of N have been shown in *Calluna vulgaris*
17 (Carroll *et al.*, 1999), *Vaccinium vitis-idaea*, *Erica cinerea*, *Eriophorum vaginatum*
18 (Leith *et al.*, 1999), some mosses (Pitcairn *et al.*, 1998, Gordon *et al.* 2001), pine and
19 hardwood tree species (Magill *et al.*, 1997) and some grasses and forbs (Morecroft *et*
20 *al.*, 1994, Carroll *et al.*, 2003). However, many of these responses are to short-term
21 applications using relatively large doses, rather than chronic low-level N deposition.
22 In addition, several experimental additions have failed to show increases in tissue N
23 with N addition. Uren *et al.* (1997) found significant differences in the shoot N
24 concentration of *Calluna vulgaris* at application rates of 7.7 and 15.4 kg N ha⁻¹ yr⁻¹
25 only in the first year of addition. No statistically significant results were found in the

1 subsequent 3 years of the experiment. Pearce and van der Wal (2002) found no
2 increase in tissue N concentration of *Racomitrium lanuginosum* by the end of the
3 growing season in which N was applied.

4 In a paper explicitly linking regional-gradient with experimental N addition
5 studies, Dise and Gundersen (2004) found that the tissue N concentration of conifer
6 needles showed a 'rapid and dynamic' response to N deposition, changing in response
7 to both increasing and decreasing N deposition within 2-3 years. Thus, foliar N may
8 exhibit a plastic response to short-term levels of N deposition, which can vary greatly
9 from year to year. Non-woody plants would likely be even more responsive to short-
10 term fluctuations in N deposition. It is also likely that some plants can rapidly use
11 excess N for increased growth, particularly in N-limited systems. This is supported
12 by optimal partitioning theory which suggests that plants aim to equalise nutrient
13 exchange ratios in order to maximise the efficiency of resource use (Bloom *et al.*,
14 1985). Biomass measurements would provide confirmation of this hypothesis and are
15 a particularly important area of future study. Both possibilities – plasticity of N
16 accumulation, and increased growth – would argue against the use of foliar N as an
17 indicator of long-term N deposition and ecosystem N enrichment.

18 There was also no significant correlation between foliar N:P ratio and N
19 deposition for any of the 3 species examined. The N:P ratio of plant tissues has been
20 suggested as an indicator of nutrient limitation (Koerselman and Meuleman, 1996,
21 Gusewell and Koerselman, 2002). P-limited species would be expected to have a
22 higher N concentration and a lower P concentration, and hence a higher N:P ratio,
23 than N-limited or co-limited species. An N:P mass ratio of greater than 16 has been
24 suggested to indicate P-limited vegetation, one of 13.5–14 co-limitation and a ratio
25 less than 14 N-limitation (Gusewell and Koerselman, 2002).

1 On this basis, *Agrostis* was P-limited at 16% of the sites, 19% of the sites were
2 P-limiting for *Galium*, and 24% were P-limiting for *Rhytidiadelphus*. However, the
3 inferred P limitation occurred across the full range of N deposition (ca 7-35 kg N ha⁻¹
4 yr⁻¹) and was not related to the N deposition flux, and there was little agreement
5 among the three methods on the nutrient that was identified as limiting at any one site.
6 Thus, this study also does not support the use of tissue N to P ratios as an indicator of
7 N deposition, N status, or nutrient limitation on a regional scale. This lack of a
8 relationship between tissue N:P and N deposition could be partially due to the
9 differences in the soils encountered in this study and their capacities to retain N in
10 different forms for potential availability to plants.

11

12 **Conclusions**

13 The primary cause for the decline in the species richness and cover of forbs detected
14 across Great Britain as a function of N deposition is probably competition brought on
15 by an increase in the cover of grasses. We are able to follow the signal of nitrogen
16 enrichment in precipitation into the soil via enhanced extractable NH₄⁺ and reduced
17 pH, although soil solid organic matter chemistry (e.g. %N, C:N) and foliar chemistry
18 were unrelated to input N levels. This may be due to wide variations in soil type and
19 land-use history across the survey sites.

20 Taken together with experimental N manipulations on acid grassland which
21 show declines in the same nutrient-sensitive species, the results of this survey provide
22 compelling evidence that changes in plant community and soil chemistry as a result of
23 elevated N deposition are actually occurring in the UK. In particular, less competitive
24 forbs appear to be suffering at the expense of more vigorous, nitrophilic grasses in
25 areas receiving chronically enhanced NH₄⁺ deposition, which occurs over a

1 widespread area of Great Britain. It is likely that such a reduction in the diversity of
2 forbs, is occurring in other N-impacted areas of the world as well.

3

4

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13

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1 **Figure captions**

2

3 **Figure 1** Declining species richness with increasing A) NH_y deposition ($r^2 = 0.54$) and
4 B) NO_x deposition ($r^2=0.29$).

5

6 **Figure 2** Changes with increasing total inorganic nitrogen deposition in A) forb cover
7 ($r^2 = 0.47$) and B) forb richness ($r^2 = 0.52$), C) grass cover ($r^2 = 0.16$) and D) grass
8 richness ($r^2 = 0.05$; $P < 0.08$) and E) bryophyte cover and F) bryophyte richness.

9

10 **Figure 3** A) O/A horizon C:N ($r^2=0.08$), B) KCl-extractable ammonium ($r^2 = 0.39$) and
11 C) topsoil pH (A horizon, in H_2O) ($r^2 = 0.39$) in relation to total inorganic nitrogen
12 deposition.

13

14 **Figure 4** Tissue N concentration corrected for day degrees versus total inorganic N
15 deposition for A) *Galium saxatile*, B) *A. capillaris* and C) *R. squarrosus* (no correction
16 for day degrees needed for *R. squarrosus*).

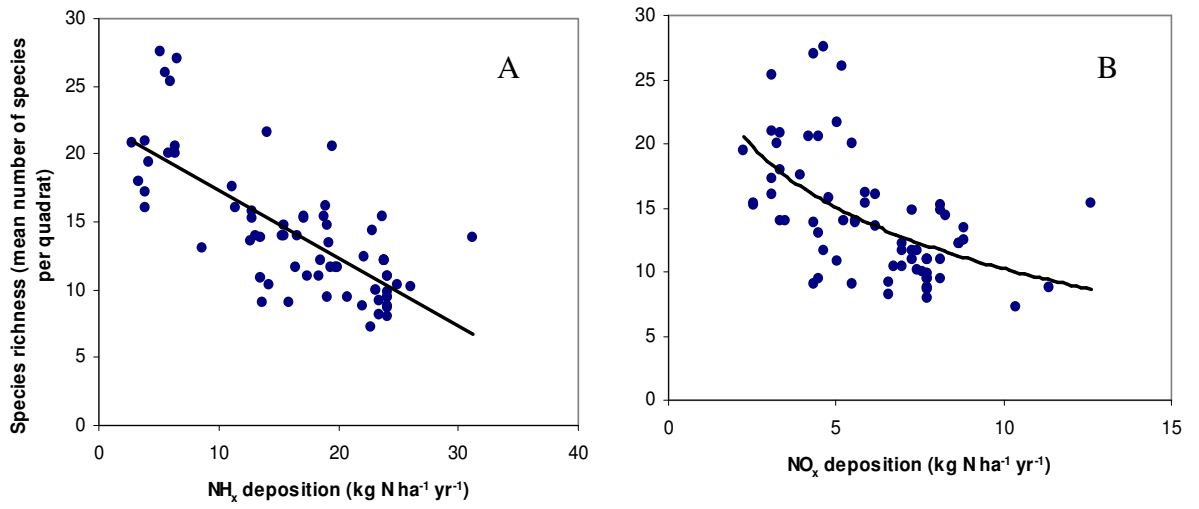
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18 **Figure 5** Tissue N:P ratio versus total inorganic N deposition for A) *Galium saxatile*,
19 B) *A. capillaris* and C) *R. squarrosus*.

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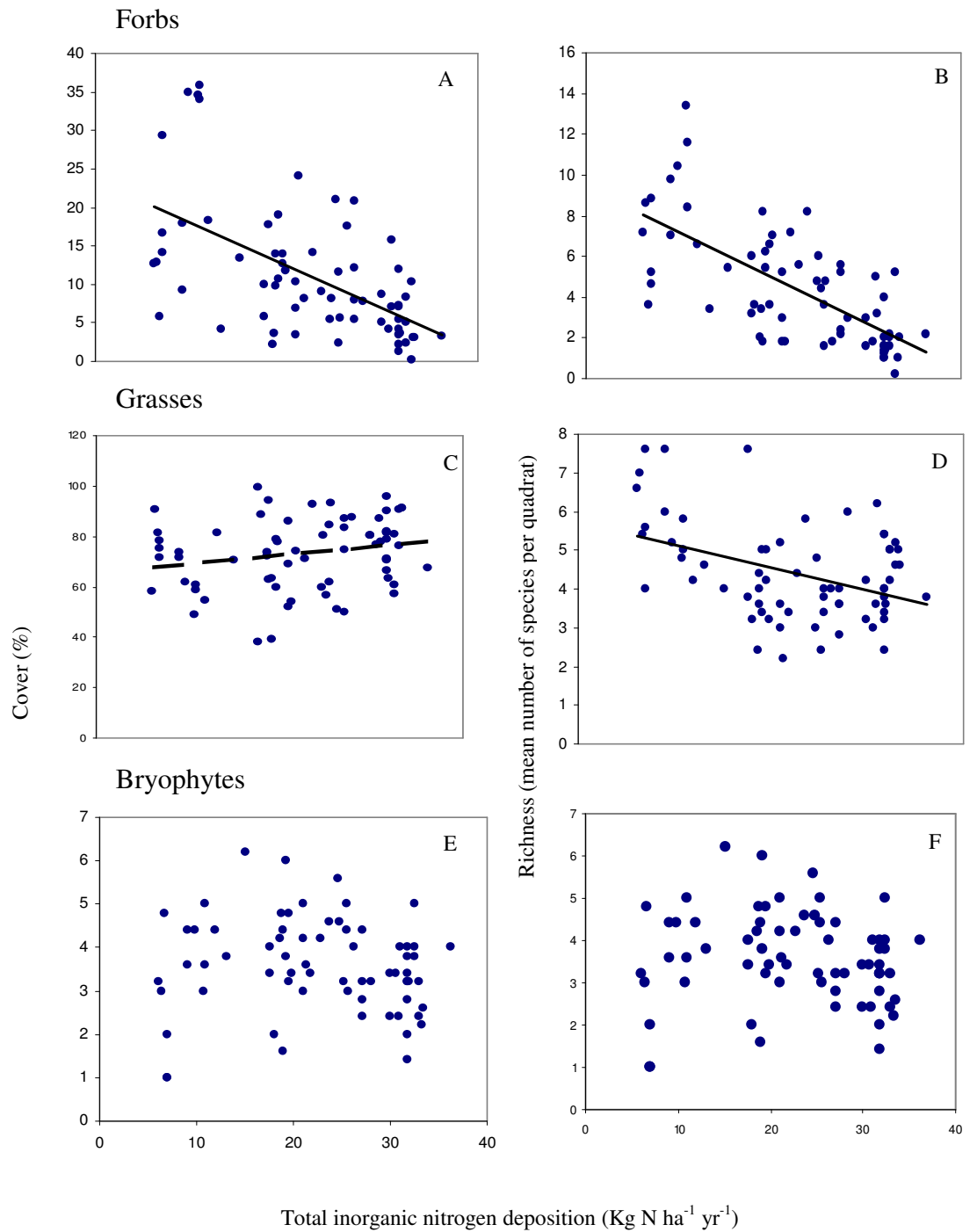
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1 **Figure 1**
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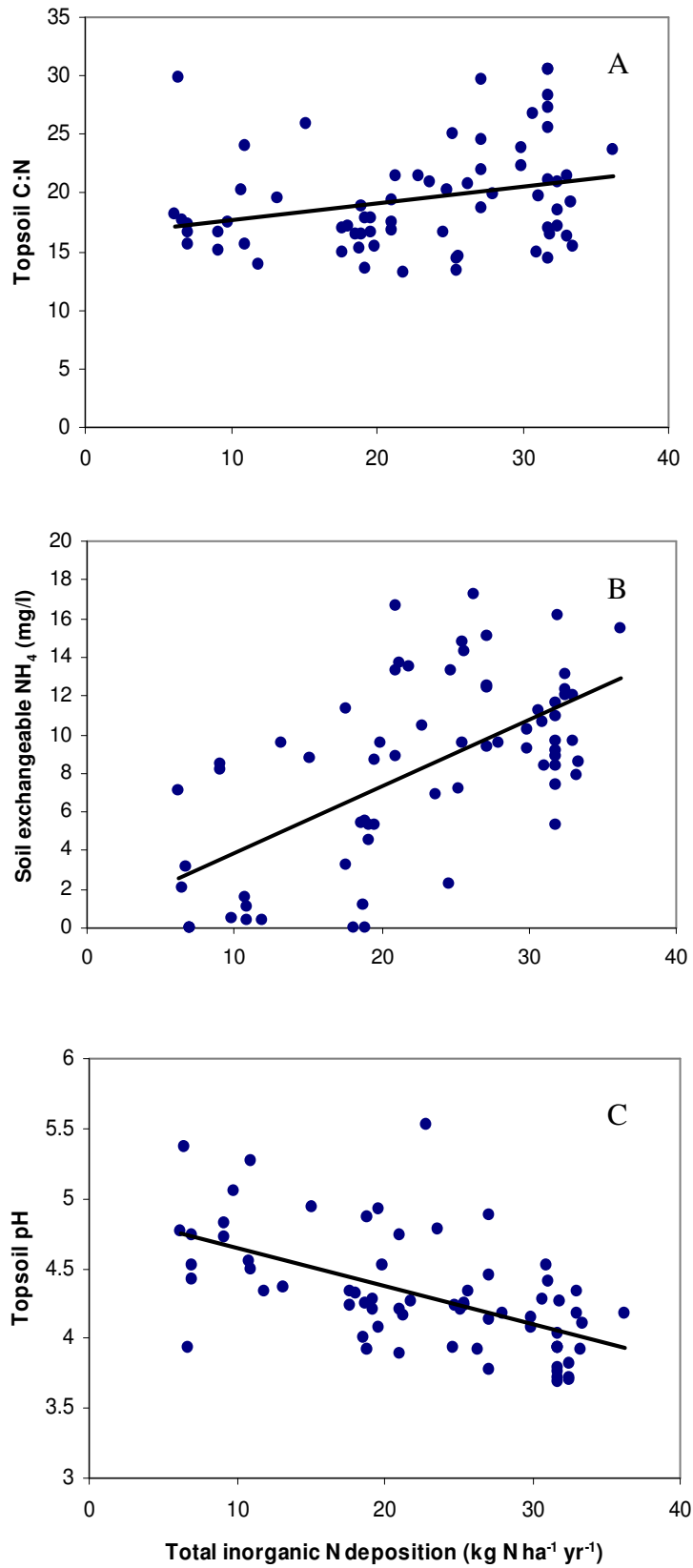
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1 **Figure 2.**



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1 **Figure 3.**
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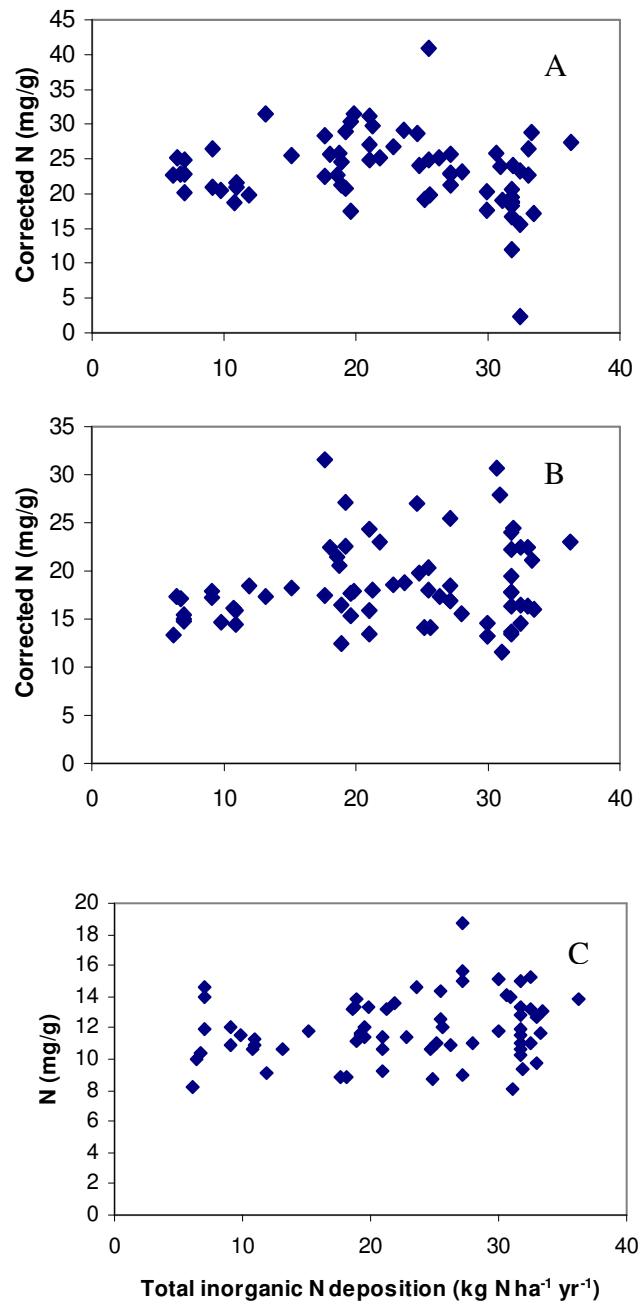
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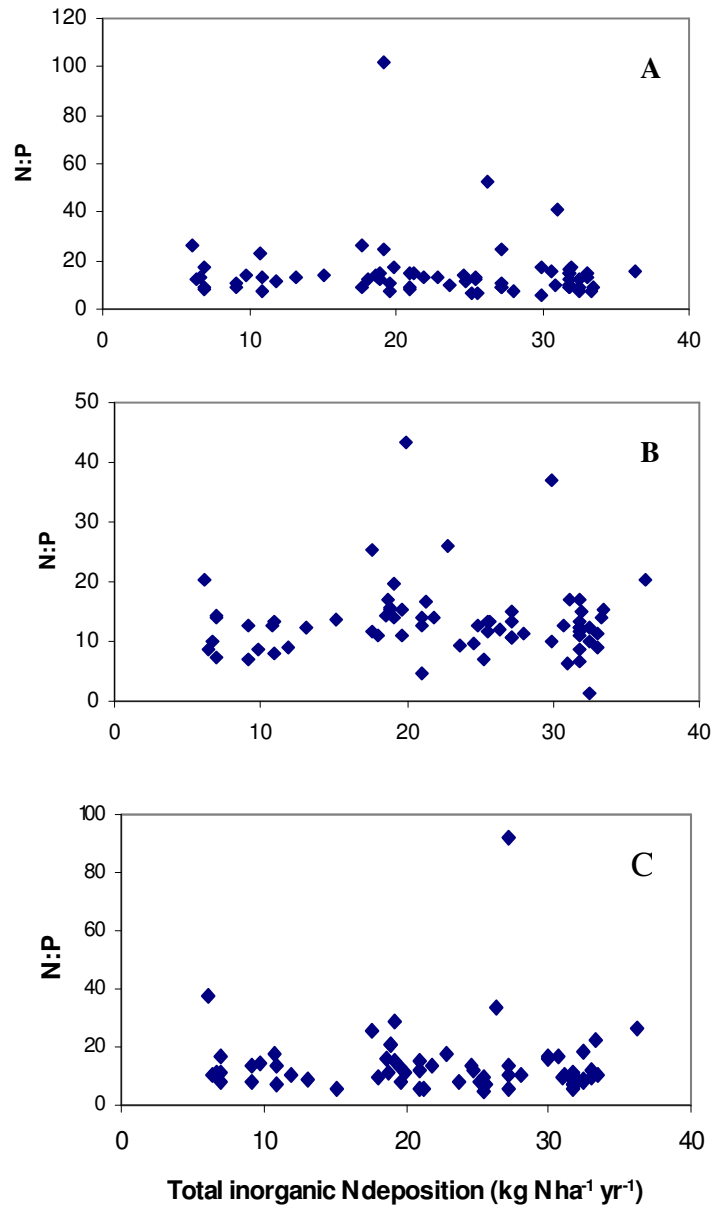
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1 **Figure 5**
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Appendix 1. Variables used in stepwise regressions on plant species richness. Where significant, the direction of simple correlations to species richness are shown (+ or -).

Variable	Range
Total nitrogen deposition (kg N ha ⁻¹ yr ⁻¹) (-)	6.2 – 36.3
Total deposition NH ₃ + NH ₄ ⁺ (kg N ha ⁻¹ yr ⁻¹) (-)	2.8 – 31.2
Total deposition NO + NO ₂ + NO ₃ ⁻ (kg N ha ⁻¹ yr ⁻¹) (-)	2.2 – 12.6
Sulphate deposition (kg SO ₄ ²⁻ S ha ⁻¹ yr ⁻¹) (-)	6.6 – 28.7
Acid deposition (total N + total S, kg ha ⁻¹ yr ⁻¹) (-)	13.0 – 61.4
Topsoil pH (A horizon) (+)	3.7 – 5.5
Subsoil pH (30-40 cm) (+)	3.3 – 5.7
Mean annual temperature (°C) (-)	6.6 – 10.6
Mean annual precipitation (mm) (+)	594 - 3038
Mean annual actual evapotranspiration (mm) (-)	35.7 – 49.3
Mean annual potential evapotranspiration (mm) (-)	35.8 – 54.3
Mean annual soil moisture deficit (mm) (-)	3.4 – 51.4
Altitude (m) (-)	15- 692
Litter cover (%) NS	0 - 24
C:N (by mass) NS	13.3 – 30.2
%N (by mass) NS	0.1 – 1.6
Slope (°) NS	0 - 60
Aspect (°) NS	0 - 315
Grazing intensity (visual inspection, scale 1-3) NS	1 - 3
Enclosure (presence or absence) NS	Presence or absence

5