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Contact CEH NORA team at
noraceh@ceh.ac.uk

Title: The role of nitrogen deposition in widespread plant community change across semi-natural habitats

Shortened title: Nitrogen deposition and plant community change

Chris D Field¹, Nancy B Dise¹, Richard J Payne^{1,2}, Andrea J Britton³, Bridget A Emmett⁴, Rachel C Helliwell³, Steve Hughes⁴, M Laurence Jones⁴, Steven Lees⁵, Jonathan R Leake⁵, Ian D Leith⁶, Gareth K Phoenix⁵, Sally A Power⁷, Lucy J Shepperd⁶, Georgina E Southon⁸, Carly J Stevens⁹ Simon J M Caporn¹

¹School of Science and the Environment, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, UK, ² Biological and Environmental Sciences, University of Stirling, Stirling FK94LA, ³James Hutton Institute, Craigiebuckler, Aberdeen, AB15 8QH, UK, ⁴Centre for Ecology and Hydrology Bangor, Environment Centre Wales, Deiniol Road, Bangor, Gwynedd, LL57 2UW, ⁵Sheffield University, The University of Sheffield, Western Bank, Sheffield S10 2TN, UK, ⁶Centre for Ecology and Hydrology Edinburgh, ⁷University of Western Sydney, Locked Bag 1797, Penrith, NSW 2751, ⁸Division of Biology, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, ⁹Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK.

Corresponding author: Chris D Field, 0161 247 2000, 0161 247 6318, c.field@mmu.ac.uk

Author contributions: Field led the survey, carried out some of the heathland and bog surveys, some laboratory analysis, analyzed much of the data, and wrote large sections of the paper; Dise contributed to the design of the study and wrote large sections of the paper; Payne carried out some data analysis and wrote sections of the paper; Britton contributed to the design of the study and carried out some of the survey work; Emmett led the Terrestrial Umbrella research Project under which the survey was funded and contributed to the design of the study; Helliwell contributed to the design of the study, carried out some of the heathland and bog surveys and commented on the paper; Hughes performed a bulk of the soils analysis; Jones contributed to the design of the study, surveyed the sand dune habitat and commented on the paper; Lees carried out some of the grassland surveys and some laboratory analysis; Leake contributed to the design of the study and some of the laboratory methods; Leith contributed to the design of the study, carried out some of the bog surveys and commented on the paper; Phoenix contributed to the design of the study, some of the laboratory methods and commented on the paper; Power contributed to the design of the study and commented on the paper; Shepperd contributed to the design of the study, carried out some of the bog surveys and commented on the paper; Southon carried out some of the heathland surveys and some of the laboratory analysis; Stevens contributed to the design of the study, carried out some of the grassland surveys and commented on the paper; Caporn led the Work Package in the Terrestrial Umbrella Project, conceived of the study and contributed to its design, helped with some of the heathland and bog surveys and commented on the paper.

Abstract

Experimental studies have shown that deposition of reactive nitrogen is an important driver of plant community change, however, most of these experiments are of short duration with unrealistic treatments, and conducted in regions with elevated ambient deposition. Studies of spatial gradients of pollution can complement experimental data and indicate whether the potential impacts demonstrated by experiments are actually occurring in the 'real world'. However targeted surveys exist for only a very few habitats and are not readily comparable. In a coordinated campaign, we determined the species richness and plant community composition of five widespread, semi-natural habitats across Great Britain in sites stratified along gradients of climate and pollution, and related these ecological parameters to major drivers of biodiversity, including climate, pollution deposition, and local edaphic factors. In every habitat we found reduced species richness and changed species composition associated with higher nitrogen deposition, with remarkable consistency in relative species loss across ecosystem types. Whereas the diversity of mosses, lichens, forbs, and graminoids declines with N deposition in different habitats, the cover of graminoids generally increases. Considered alongside previous experimental studies and survey work, our results provide a compelling argument that nitrogen deposition is a widespread and pervasive threat to terrestrial ecosystems.

Keywords: Nitrogen; Sulphur; Climate; Pollution impacts

Introduction

Atmospheric pollution is a major threat to ecosystem structure and function (Bobbink *et al.* 2010; Phoenix *et al.*, 2012), and is recognized as one of the top three threats to global biodiversity (Sala *et al.*, 2000). A large number of field experiments, and some regional-scale gradient studies, have shown that atmospheric nitrogen (N) and sulphur (S) deposition can shift plant species composition and reduce biodiversity in sensitive terrestrial ecosystems (Dise *et al.* 2011). Since at least the eighteenth century and throughout most of the 20th century, atmospheric N (mainly as ammonia [NH₃], and nitrogen oxides [NO_x]), and S pollution (primarily as sulphur dioxide [SO₂]), has increased as a by-product of fossil fuel burning and intensifying agriculture in industrialised countries; and emissions of these pollutants are strongly increasing in many developing regions of the world, particularly Asia (Galloway *et al.*, 2004; Stern, 2005; Erisman *et al.*, 2011).

Since 1970, sulphur emissions have declined dramatically in the developed world following moves to alternative fuels such as natural gas, combined with flue-gas desulphurisation by industry and power stations (Smith *et al.*, 2001): leading to large reductions in S deposition (e.g. in the UK, a 90% reduction was measured between 1970 and 2010 (RoTAP, 2012)). Current atmospheric concentrations of sulphur dioxide probably no longer pose a threat to sensitive plant species in many developed countries, although legacy effects from sulphur pollution may remain for some time to come (Guirriera *et al.* 2011).

On a global scale there has been a six-fold increase in N deposition since 1860, with a further doubling predicted by 2050 (Galloway *et al.*, 2004). Although there has been a modest decline in N deposition in Europe over recent years, this is of a much smaller magnitude than that seen

for S (Erisman *et al.*, 2011). For example, NO_x emissions in the UK fell by 50% between 1986 and 2007 whilst deposition fell by only 22%, largely due to changes in chemical reactions in the atmosphere leading to a more rapid conversion of NO₂ to nitric acid and particulate nitrogen (Fowler *et al.*, 2007). Furthermore, atmospheric concentrations of reduced N as NH₃⁺ have remained essentially unchanged in the UK (RoTAP, 2012).

Numerous field experiments have demonstrated the potential of N to reduce biodiversity, change plant communities, and increase leaching to freshwaters through N saturation (Caporn *et al.*, 1995, Clark and Tilman, 2008, Cunha *et al.*, 2004, Dise *et al.*, 2011, Emmett *et al.*, 1998, Phoenix *et al.* 2012). However, even the longest-established experiments have only been running around 20 years, a fraction of the time to which ecosystems have been exposed to pollution. Other limitations of many existing experimental studies are unrealistically-high and infrequent treatments and high ambient deposition in experimental sites (Cunha *et al.*, 2004; Dise *et al.*, 2011; Phoenix *et al.* 2012).

Whilst experiments are important in determining the causality of change, to demonstrate that experimentally-observed impacts are actually occurring in the landscape, they need to be supported by spatial and temporal surveys. “Space-for-time” surveys assume responses identified over spatial gradients reflect the changes that have occurred over time in response to these variables (Fukami and Wardle, 2005). The principal of space-for-time substitution has been examined by testing results against time-series derived from palaeoecological and historical series and demonstrated to show a high degree of correlation (Blois *et al.*, 2013; Buyantuyez *et al.*, 2012). Similarity is maximised when temporal and spatial variations are similar, site history and management is similar (e.g. Blois *et al.*, 2013) and environmental variables can be separated (Fukami and Wardle, 2005). In ecological studies, spatial and

temporal gradient studies have shown relationships between nitrogen pollution and reduced diversity for acid grassland (Stevens et al., 2010, 2011), sand dune vegetation (Jones et al., 2004), and calcareous grassland (van Den Berg, 2011). However, since the studies used different field and analytical methods, were conducted over different time periods, and did not consider the same suite of alternate drivers, they are not readily comparable. Re-analysis of untargeted national surveys has also revealed relationships between N deposition and species richness for heathland, acid grassland and mesotrophic grassland (Maskell et al., 2010). However, since these surveys were not focused on air pollution impacts and survey locations covered broad habitat classifications, relationships with N deposition were extremely weak against a large background of variability ($r^2 = 0.01-0.17$).

With increasing global change, there is an urgent need to understand the drivers of biodiversity loss such as air pollution, climate change, biotic exchange, and elevated carbon dioxide (Sala *et al.*, 2000). In this paper we report on a unique coordinated study to determine the diversity and vegetation composition of five common semi-natural community types across gradients of climate and air pollution which are representative of those that occur over much of the industrialised world. For each survey, we evaluated all major drivers of vegetation diversity that are measurable at a regional scale. Our aims are to 1) elucidate the main drivers of change in species richness and composition, 2) test whether any changes observed are proportionally consistent across habitats, and 3) evaluate the most likely mechanisms behind any changes. We hypothesise that: H1 N deposition is correlated with lower species richness and changed plant community composition with species changes reflecting those found in experimental studies. H2 N deposition explains more of the variance in the plant data than alternate possible drivers such as climatic gradients. H3 There are significant differences in the sensitivity of different community types with communities most

dominated by bryophytes and most reliant on atmospheric deposition of nutrients most sensitive.

Materials and Methods

In the summer of 2009 vegetation surveys were carried out across Great Britain in five habitats: acid grassland, bog, upland heath, lowland heath, and sand dune. The acid grassland sites were a subset of sites used by Stevens *et al.* (2004); this subset and the sites from the other habitats were selected to control for potentially co-varying gradients of rainfall and temperature, balancing high and low pollutant deposition locations in the generally cooler and wetter northwest and the drier and warmer southeast of the UK. The habitats surveyed have all been identified as being potentially sensitive to air pollution, particularly nitrogen deposition in previous research (Dise *et al.* 2011).

For each habitat a specific Eunis community (European Nature Information System, European Environment agency, 2011) was selected and sites typifying this community chosen following discussion with conservation organisations and land owners. Acid grasslands were Eunis E1.7 with *Agrostis capillaris*, *Festuca ovina*, and *Gallium saxatile* in predominantly upland-acidic grasslands. The bog habitat was represented by Eunis D1; with a combination of *Calluna vulgaris*, *Eriophorum vaginatum* and *Sphagnum capillifolium*. The bogs selected were ombrotrophic in nature, with a combination of lowland raised bogs and a smaller number of upland blanket bogs. Both upland heaths and lowland heaths were covered by Eunis F4.2, although considerable differences exist between the habitats and they were analysed separately. Upland heaths were dominated by *Calluna vulgaris* on acidic, shallow organic soils. Lowland heaths varied from *Calluna*-dominated communities on base-poor,

acidic soils through to floristically-rich *Erica vagans* and *Ulex europaeus* heaths on base-rich brown earth soils (Elkington *et al.*, 2001; Price, 2003). Sand dunes were Eunis B1.4, fixed-dune grasslands dominated by grasses, dicotyledonous herbs and numerous bryophyte species.

For each habitat, 22 to 29 locations were surveyed (135 locations in total, Figure 1). At each site five 2 × 2 m quadrats were located using random numbers within areas representative of the vegetation community studied, and the cover of all plant species including bryophytes and lichens was estimated. Liverworts were excluded to improve consistency between surveyors. In heathlands stratified random sampling was used to capture vegetation in different stages of the *Calluna* growth cycle. Canopy height was recorded at four locations within each quadrat, and two soil cores (top 15 cm, 4 cm diameter) were collected at each site. Soil was refrigerated upon collection and a bulked sample was then analysed for pH, loss on ignition (LOI) and N %. For pH analysis 25 ml deionised water was added to 10 g field-moist soil. The suspension was stirred, stood for 30 minutes and an electrode inserted for 30 seconds before reading pH. For LOI, air dried soil was passed through a 2 mm sieve, 10 g was then weighed and combusted at 375 °C for 16 hours. LOI was used as a proxy for the organic content of the soil, which responds to climatic conditions, particularly in lowland heaths and sand dunes, and can strongly influence species composition. For N %, soil was analysed on a LECO TRUESPEC carbon and Nitrogen Analyzer.

The pollutant deposition data used were the 5 km² Concentration Based Estimated Deposition (CBED) values for 2004-2006 from the UK Centre for Ecology and Hydrology. Variables for total nitrogen deposition (further divided into wet and dry, and reduced and oxidised forms), total sulphur deposition (split further into wet and dr

y forms) and non-marine base cation deposition (calcium + magnesium) were included in the analysis. Multiple metrics for N deposition are used as deposition patterns are not identical due to differences in sources between reduced and oxidised N and evidence shows that species respond differently to different forms of N (e.g. Sheppard *et al.*, 2008; Dise *et al.*, 2011). However, the total N load and total acid deposition are also important as drivers of long-term N accumulation and acidification. To consider possible legacy effects of pollution, modelled sulphur deposition from a period when sulphur emissions were considerably greater than current (1986-1988) was also included in the analysis. Climate data were based upon UKCP09 5 km² gridded data sets from the UK Met Office. Total annual precipitation and growing degree days (sum of degree days above 5°C) were selected as the most appropriate representations of climatic controls on plant communities, the latter as a stronger control on phenology and plant growth than average temperature alone (Tooke and Battey, 2010). Both precipitation and growing degree data were averaged over the period 1997-2006.

In addition to broad-scale abiotic gradients vegetation may also be affected by a range of local management practices. We attempted to account for this variability in site selection and by including field-assessed indices in data analyses. For acid grasslands and sand dunes we included indices for grazing intensity (absent to intense) and for bogs we included a 'hydrological index' based on evidence for site drainage. For all habitats we attempted to identify sites with consistent habitat management, in the case of heathlands we positioned quadrats to span the *Calluna* growth cycle.

Table 1 provides selected summary information for each habitat.

Statistical analysis

For each site the following metrics were calculated: total species richness (total number of species recorded across the five quadrats), species richness by functional group (moss, lichen, forb, graminoid), and the mean cover of each species and functional group.

Stepwise multiple linear regression was used to model relationships between total species richness, functional group species richness and graminoid cover (identified as an important factor in reduction of grassland forb diversity- Stevens *et al.*, 2006) with respect to the 21 potential driver variables (Table 2). We employed a combination of forward and backward selection: variables were included if they explained significant variation in addition to those already in the model, and excluded if the unexplained variation in a model was not significantly reduced by their removal. Analysis within habitats used absolute species richness but to enable comparison of data between habitats, relative species richness was calculated as the percentage of the maximum number of species found within each habitat. To correct for multiple comparisons, P-values within each habitat were adjusted using sequential Bonferroni corrections in R 2.15 (R Core Team, 2012).

For each habitat, correlations between plant community composition and environmental variables were analysed by ordination using CANOCO for Windows version 4.53 (ter Braak and Smilauer, 2004). A Detrended Correspondence Analysis (DCA; Hill and Gauch, 1980) was first performed to identify the overall community structure. DCA was also used to determine the length of compositional gradients, and in all cases the gradients were short (less than 3 standard deviations). Consequently, we were able to use Redundancy Analysis (RDA, Leps and Smilauer, 2003) to examine significant linear relationships between the cover of individual species and environmental variables. The same environmental variables were used for the

RDAs as for the regressions (Table 2). All data were log-transformed and rare species down-weighted. We used forward selection to identify a minimal suite of significant potential driver variables, first selecting the variable that explained the most variance in community composition (average % cover of each species at each site), then using that as a co-variable to identify the variable that then explained the greatest additional variance. The selection process continued until no further variables explained significant additional variance; a P-value cut-off of $P < 0.05$ was used. Variance partitioning was then carried out using selected variables successively with other selected variables as co-variables. Significance was determined by Monte Carlo permutation tests (999 permutations).

As many of the variables considered were correlated, the selection of some variables over others in both the regression and redundancy analyses should be interpreted with caution: some selected variables may represent a suite of related variables or a broader environmental gradient. For instance, since all N deposition variables are highly correlated, selection of N deposition in a specific form over another should be interpreted with caution. Similarly, relationships with current levels of a driver such as deposition chemistry may reflect the importance of current, past, or cumulative pollutant inputs.

Results

Regression Analyses

Across all habitats combined, 39% of the variability in relative species richness could be explained by a model combining N deposition (dry oxidised) and growing degree days; most of this was due to dry oxidised N ($r^2=0.37$). Within the five habitats, between 39% and 64% of the variability in total species richness could be explained by some combination of the predictor variables (Table 3). Given the many unmeasured environmental variables that

would be expected to impact biodiversity (e.g. historical site management) these are high proportions of total variance. Regression coefficients for acid grassland, bog, lowland heath, and sand dune were all similar, ranging from 0.56 (bog) to 0.64 (lowland heath), upland heath was lower at 0.39.

In acid grassland, total species richness showed the strongest relation to N deposition (dry oxidised N; $r^2 = 0.61$) of all five habitats with no other variable significant in the regression model. The species richness of upland heath was also only related to N deposition in the final regression model (reduced N, $r^2=0.39$, $P=0.002$). For the other three habitats, species richness was most strongly related to other variables: dry S deposition ($r^2=0.56$, $P<0.01$) for bog (dry oxidised N deposition was also correlated, but less strongly than dry S, $r^2=0.52$), growing degree days for lowland heath (followed by altitude, and wet oxidised N deposition), and pH for sand dune (followed by wet oxidised N deposition). The importance of temperature and altitude for lowland heath was primarily driven by two sites: when these are removed, total acid deposition becomes the most important correlate with species richness ($r^2 = 0.20$).

The difference in relative plant species richness along the N deposition gradient was large, with 30-75% lower species richness in the least diverse sites within each habitat, compared with the most diverse (Figure 2A). This curvilinear pattern was broadly similar for acid grasslands, lowland heaths, sand dunes and upland heaths with a consistent rate of % plant species lost with increasing N deposition. In the bog habitat, a shallower, more linear gradient in species loss was observed. Figure 2B-F shows relationships between total plant species richness of the habitats and the four most significant correlates: N deposition, S deposition

(contemporary), growing degree days, and pH, as well as for total acid deposition, 'peak' S deposition (1986-1988) and mean annual precipitation.

Across all communities, nitrogen deposition was the most common explanatory variable for changes in species richness or graminoid cover. Out of 22 separate regression analyses on functional groups, some form of N deposition was the strongest correlate to species richness or cover in 11 cases, growing degree days in three cases, pH in two cases, acid [S+N] deposition in one case, and S deposition in one case. In four cases none of the potential correlates was significantly related to species richness (Table 3). Mean annual precipitation, extreme temperature range, grazing, hydrologic index, soil N % and $[Ca^{2+}+Mg^{2+}]$ deposition were not significant in any model when other drivers were included.

In all cases where N deposition is selected in a model, the relationship with species richness is negative. The species richness of acid grassland shows consistently strong negative relationships to N deposition (oxidised N) across all functional groups measured, particularly for forbs and mosses. N deposition (reduced N) is also the dominant correlate for upland heath lichen and graminoid richness. N, S, and total acid deposition appear equally important in bogs. In addition to N deposition, growing degree days (negative relationship) and soil pH (generally positive relationship) are dominant correlates to species richness in lowland heath and sand dune.

Across habitats and within functional groups, N deposition is consistently the dominant correlate to species richness of lichens, although significant regression models could only be developed for bogs and upland heath (in the other three habitats there were either no significant relationships explaining lichen species richness, or lichens were limited to only a

small number of sites). N deposition (reduced N) is also the strongest correlate to graminoid cover in four of five habitats.

In contrast to species richness, in all cases the relationship of nitrogen deposition with graminoid cover is positive: acid grassland, bog, upland heath and lowland heath sites receiving higher levels of N deposition (particularly NH_4^+) have consistently higher cover of graminoids, although graminoid species richness is either lower or unchanged. Forb species richness appears to be negatively related to acidity across habitats: oxidised N deposition (acid grassland), acid deposition (bog) and pH (sand dune) are the strongest correlates; in sand dunes the differences in pH reflect the differences between calcified and decalcified (more acidic) sites. Forb abundance in both lowland and upland heath was limited and not statistically analysed. N deposition, growing degree days, and S deposition are variously the most significant correlates with moss and graminoid species richness except in bogs, where none of the independent predictor variables are significant. The secondary significance of loss on ignition (after oxidised N deposition) for moss diversity in sand dune habitats may reflect gradients in soil type and climate, with more organic-rich soils at wetter sites supporting greater diversity.

Species composition

A form of nitrogen deposition was identified as a significant correlate to species composition in all habitats but sand dunes (Table 3, Figure 3). N deposition was the most important correlate for acid grassland and upland heath species composition and the second most important correlate in bogs (after hydrological index) and lowland heath (after growing degree days). Sand dune species composition was most significantly correlated to local site (pH) and climate factors (precipitation, growing degree days), with peak sulphur deposition

following these in importance (Figure 3E). Dry nitrogen deposition explained only marginally less of the remaining variance than peak S deposition, however (5.1% versus 5.3%).

In addition to N and S deposition, growing degree days was a significant correlate to species richness in all but bog habitats. A hydrology factor played a role for the wettest (bog – hydrological index) and driest (sand dune - precipitation) habitats, and pH was important for lowland heath and sand dune species abundance, perhaps reflecting underlying substrate differences. Moisture and pH may both be reflected in the significance of LOI for lowland heath habitats.

Many of the species showing a negative relationship between abundance and N deposition were forbs, mosses, and lichens. Forb species negatively correlated with nitrogen deposition include *Euphrasia officinalis*, *Plantago lanceolata*, *Lotus corniculatus* (3a. acid grassland – see Stevens *et al.*, (2006) for more details of acid grassland responses), *Narthecium ossifragum* and *Drosera intermedia* (bog). Lichens and mosses negatively associated with nitrogen deposition including the lichen genus *Cladonia*, particularly *C. portentosa* (bog, upland heath and lowland heath), the moss *Pleurozium schreberi* (upland heaths) and the moss *Hylocomium splendens* (acid grassland, upland heath, lowland heath and sand dunes).

Some mosses and lichens also appeared positively associated with nitrogen, such as *Brachythecium rutabulum* and *Cladonia fimbriata* (upland heath and lowland heath), and *Campylopus introflexus* (lowland heath). Graminoid species that increased in cover in relation to N deposition included the grass *Deschampsia flexuosa* in heathlands and the sedge *Eriophorum vaginatum* in bogs.

Discussion

By conducting simultaneous targeted surveys, we have found that many sensitive terrestrial ecosystems have a consistent rate of % plant species loss with increasing N deposition, signifying that relative plant species loss in relation to diffuse pollution may be an emergent property of sensitive ecosystems. The proportional change in plant species richness along the N deposition gradient is very similar for acid grassland, upland heath, lowland heath, and sand dune. Species richness declines by about 40% of maximum species richness from the lowest to the highest N deposition sites in acid grassland and heathland, and by about 20% in sand dune habitats over about half of the N deposition gradient (Figure 2A). Bogs show a more gradual change of about 20% over the same gradient of N as acid grassland and heathland.

There is strong agreement between the results of regression analyses on univariate data (total plant richness, functional group richness, graminoid cover) and the redundancy analyses of community composition. Both sets of results suggest that, of the climate, local site, and pollution drivers investigated (Table 1), the compositions of acid grassland and upland heath communities are most strongly related to nitrogen deposition. Species composition of bog habitats is most strongly related to acid deposition levels and hydrology, and that of lowland heath is related most strongly to growing season temperature, nitrogen deposition, and local site factors (reflected in altitude and soil pH). The species composition of sand dune habitats is most strongly related to soil pH, followed by climate and pollutant deposition. However, because the range in N and S deposition over which the sand dune habitats were sampled is half that of the other habitats (Figure 2A), the potential for detecting a signal of pollutant deposition is not as high.

Of the five habitats, the impact of N deposition is clearest for acid grassland and upland heath. In acid grassland, the species richness of each functional group investigated declines with N

deposition, and N deposition is the only significant correlate to species richness for any functional group. Concurrently, the cover of graminoids, particularly *Deschampsia flexuosa*, increases with N deposition. In their analysis of a 68-site dataset of UK acid grassland, Stevens *et al* (2004, 2006) also showed strong negative relationships between N deposition and species richness after accounting for other drivers, as did Duprè *et al* (2010) in their meta-analysis of over 1000 acid grassland survey plots in Europe sampled at various intervals over 60 years. Both studies also reported secondary negative relationships between species richness, soil pH, and climate, and an increase in graminoids with N deposition, all consistent with our findings. Taken together, these studies show that the nutrient enrichment from chronically elevated N deposition enhances the growth of graminoids and suppresses the growth of lower plants and forbs adapted to more nutrient-poor conditions, and that this effect is stronger in grassland on more acid soils and in warmer, drier climates.

The species richness of upland heath shows a similar decline with increasing N deposition as acid grassland (Figure 2A, B), with N deposition the only significant correlate. The relationship is less strong (r^2 of 0.39) than for acid grassland ($r^2=0.61$). Indeed, upland heath is the only habitat in which more than 50% of the variability in species richness remains unexplained by our predictor variables (Table 3). This suggests that an unmeasured factor or factors plays a strong role in vegetation diversity. Of the five habitats, upland heath is the most heavily managed (primarily by fire or cutting, as grouse moors), and it is likely that species richness and composition in this habitat are also strongly related to the frequency and intensity of management. Long-term N addition experiments in upland heath show a clear impact on lower plants such as bryophytes and lichens, probably due to shading from the increased growth of *Calluna vulgaris* (Carroll *et al.*, 1999; Pilkington *et al.*, 2007). Our results reflect this, and provide convincing evidence that nitrogen is a significant driver of change in this habitat, potentially modifying the effects of management.

In bogs, the lower proportional fall in species richness with increasing N deposition (Fig. 2A) may reflect the importance of hydrology which was found to be a stronger driver of change in species composition than N. Therefore, species in waterlogged hollows and lawns may be less sensitive to pollutant deposition, since in these microsites hydrology plays a strong role in plant community responses, and colonization by graminoid or shrub species that could take advantage of enhanced nutrient levels is reduced due to a high water table (Blodau, 2002; Limpens *et al.*, 2003). Supporting this, the relative importance of dry deposition or total acid deposition for explaining species richness in bogs (Table 3) suggests a stronger role of direct pollutant impacts on vegetation than in other habitats.

In drier hummocks, however, a fertilizing effect of N deposition may emerge as hydrological constraints on vegetation response are lifted and faster growing shrubs profit over *Sphagnum*. In an N-addition experiment on a Canadian ombrotrophic bog Bubier *et al.* (2007) found that ericoid cover increased at the expense of *Sphagnum*, and in a UK N-addition experiment, Sheppard *et al.* (2011) found high concentrations of NH₃ had a catastrophic effect on *Calluna vulgaris* and bryophytes including *Sphagnum capillifolium*, whereas the sedge *Eriophorum vaginatum* increased in cover. Some indication that this may be occurring at a landscape scale was found in our study, with a strong positive relationship between cover of the sedge *Eriophorum vaginatum* and N deposition, though we found no relationship with declining *Sphagnum* cover.

In lowland heaths, our analyses suggest that the diversity and composition of vegetation is most strongly driven by growing season temperature, with nitrogen deposition a secondary driver. In all habitats, the relationship between species richness and growing degree days appears negative (Figure 2): that is, species richness is higher in regions with shorter growing seasons. The strong relationship for lowland heath, however, may in part reflect the site distribution, with most sites at the warmer end of the scale and on more sandy soils, and fewer (but species-

rich) sites in cooler, less-polluted locations on the more organic soils of the northern lowland heaths (Figure 2). This is also reflected by species composition, with many species characteristic of wetter habitats ordinated toward lower growing degree days (Figure 4D). Nevertheless, nitrogen deposition is still a significant correlate to species richness and composition in lowland heath (Table 3). This is supported by experimental studies that have found reductions in lichen diversity at low levels of N addition, growth stimulation of *Calluna vulgaris* (Power *et al.*, 2006; Southon *et al.*, 2012) and conversion of heaths to grassland in European N-addition experiments (Heil and Diemont, 1983; Aerts *et al.*, 1990). In our study graminoid cover was similarly correlated with increasing N deposition.

Sand dunes are by far the most species-rich of the five habitats, reaching nearly 80 species per site (totaled across five 2 × 2 m quadrats), almost double that of the next richest habitats. As with the other habitats there is a strong negative correlation between species richness and nitrogen deposition, but soil pH also plays a major role. This may reflect the relatively short pollution gradient sampled for sand dunes (5 - 17 kg N ha⁻¹ yr⁻¹), or the potential for decalcification in response to increased rainfall, the extent of podzolisation, and the formation of a thicker layer of more-acidic organic matter in cooler and wetter areas (Sevink, 1991). Nonetheless, the clear negative relationship of nitrogen with species richness matches that from other surveys (Jones *et al.* 2004; Remke *et al.* 2009).

Whilst contemporary change may be more strongly driven by N deposition, historic sulphur (S) pollution may also have a legacy effect through base cation depletion and lowered pH (Evans *et al.*, in press). Vegetation in some areas of the UK was strongly affected by S deposition in the 1970s and 1980s and in these cases, where populations of plants were severely reduced (e.g. Lee, 1998), a legacy impact of S is likely to influence contemporary species richness with current day or cumulative N hindering recovery. Current S concentrations are low compared to the critical level of 20 µg m⁻³ (e.g. Over the range 1.5-11.2 µg m⁻³ for the bog

habitat), whilst N current deposition to many of the sites is above critical loads (e.g. N deposition range 5.9 to 30.9; Critical load range 5 to 10 depending on water table). These patterns are similar across the habitats and it is suggested that, when considering current pollutant concentrations and deposition, that N is the driver of the changes observed.

However, sulphur may accumulate in soils over the long term (Moore *et al.*, 2005). This could mean that recovery from S pollution is delayed in areas where acidifying effect of sulphur persists in the soil (Daniels *et al.*, 2008). Soil pH in the acid grassland, bog, lowland heath and upland heath habitats was strongly correlated with either N or S deposition, suggesting that some extent of acidification by pollutant deposition had occurred. In the bog habitat, forb diversity shows a step decline below around pH 3.8 and in the acid grassland a 'step-down' in species richness was also apparent, in this case below around pH 4.5. This, and the relationship in the bog habitat, could reflect a change in soil acid buffering from base cations to aluminium which occurs around pH 4.5 and which would be more likely in habitats on base-poor soils exposed to long-term acid deposition, such as many in our surveys (Bowman *et al.*, 2008). Therefore it seems that whilst the acidifying potential of contemporary pollution may be limited in comparison to historic levels, plant habitats where pH rests on a buffering threshold may be vulnerable to marginal changes in acidity induced by pollutant deposition over the longer-term (Bowman *et al.*, 2008) and consequential increases in toxicity from aluminium (Stevens *et al.*, 2009).

The reductions in species diversity, shifts in species composition, and consistent response in sensitive species and functional groups across five habitats provide strong evidence that atmospheric nitrogen deposition affects large areas of semi-natural terrestrial habitats. A markedly similar proportional reduction in species richness with increasing N deposition is

observed across environments, and pollutant deposition is identified as a more significant driver of diversity than climate in four habitats. Declines in the species richness of mosses, lichens, forbs, and graminoids with N and/or S deposition, combined with an increase in graminoid cover with increasing NH_4^+ , highlight both the acidifying and eutrophying impacts of these air pollutants. Since it is likely that impacts have developed over many years, the relationships observed reflect a combination of current, cumulative and legacy effects of pollution – both of N and S. We find that lower plants and forbs are particularly sensitive, and habitats dominated by grasses or shrubs may be vulnerable to a shift towards a monoculture. Our study highlights the need for (1) effective management to identify threats and mitigate damage to vulnerable habitats, (2) targeted policy for further abatement of nitrogen pollution, particularly in reduced form, and (3) long-term experimental research to evaluate the interactive responses of plant communities to pollutants, management, and future climate change.

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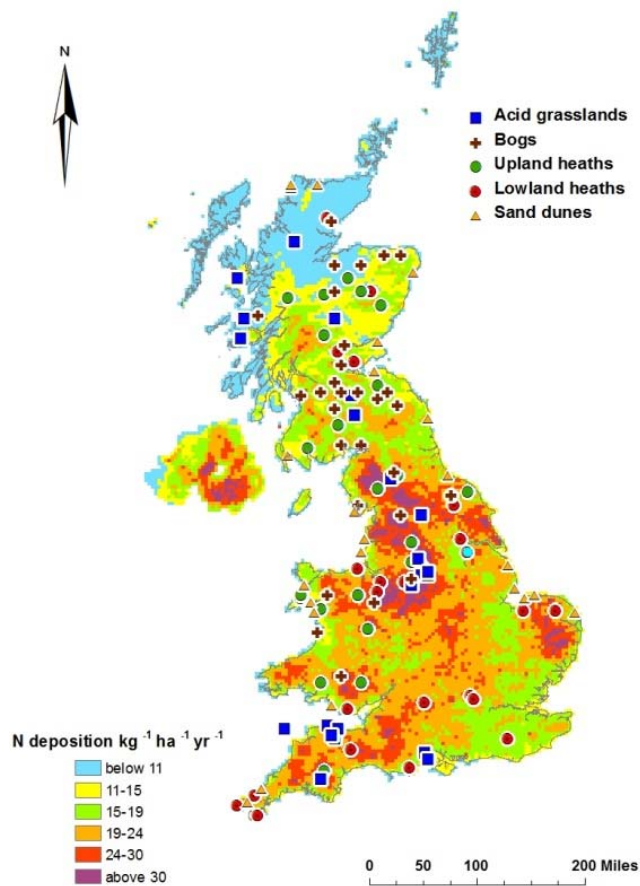


Figure 1. Site survey locations over a map of the UK and a background image of 2004-2006 average N deposition (CBED).

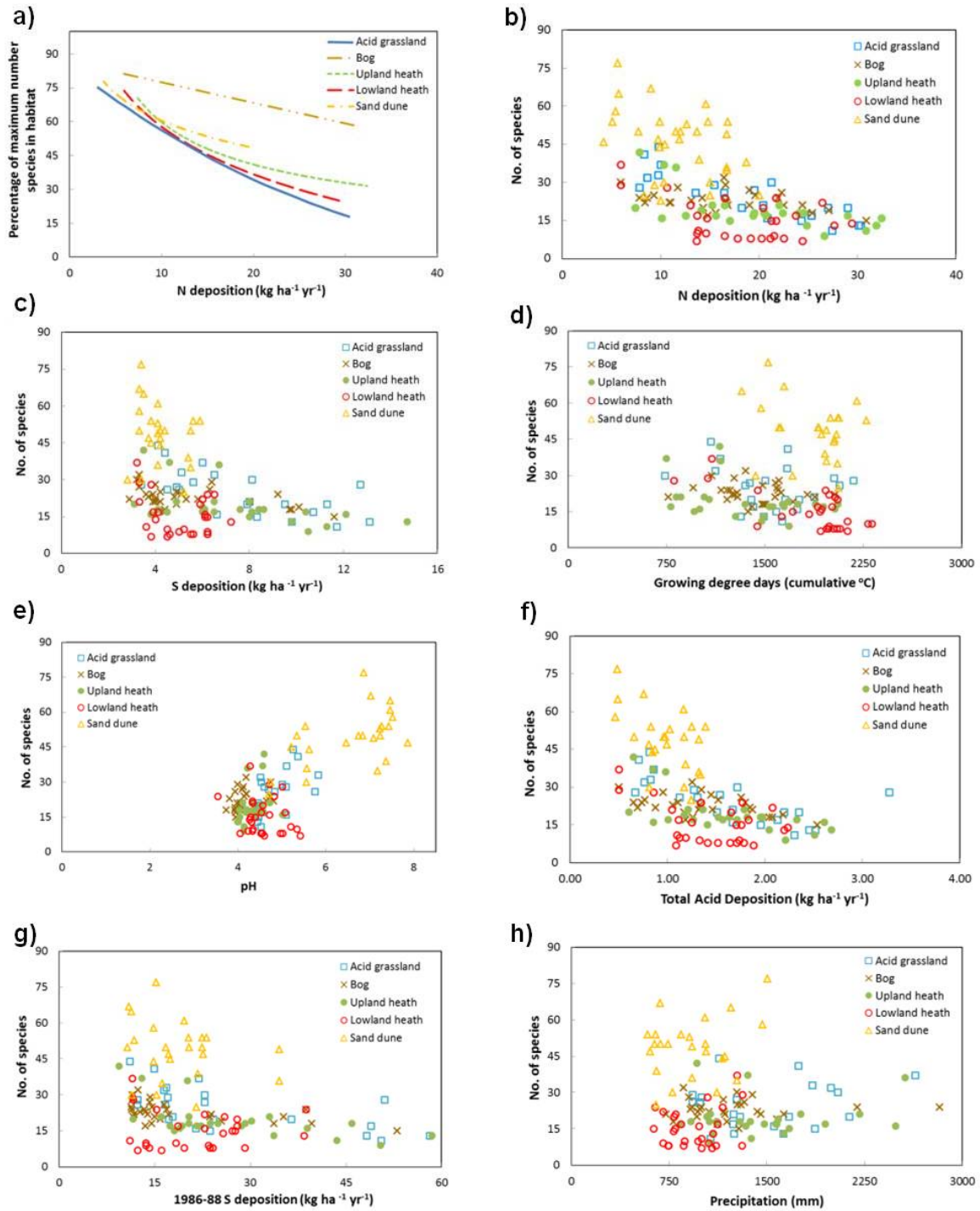


Figure 2. A) Relationship between N deposition and the % of maximum number of species in each habitat. Figures 2B)-2H) Relationships between total species richness of the habitats and the four most significant correlates: N deposition, S deposition, growing degree days (cumulative degrees from days above 5°C), and pH, as well as for total acid deposition, historical S deposition (1986-1988) and mean annual precipitation.

Table 1. Summary of the habitats surveyed and key environmental variables.

Habitat	Eunis code	Number of survey locations	N deposition range (kg N ha⁻¹ yr⁻¹)	Contemporary S deposition range (kg S ha⁻¹ yr⁻¹)	Precipitation range (mm)	Growing degree days range (cumulative degrees C)	pH	Loss on Ignition (LOI) %
Acid grassland	E1.7	22	7.8 - 30.3	3.4 - 13.1	928 - 2639	737 - 2174	4.1 - 6.7	9 - 34
Bog	D1	29	5.9 - 30.9	2.9 - 11.6	727 - 2827	763 - 2034	3.7 - 4.8	86 - 98
Upland heath	F4.2	25	7.4 - 32.4	3.1 - 14.7	836 - 2562	747 - 2055	4.0 - 5.0	14 - 95
Lowland heath	F4.2	27	5.9 - 29.4	3.2 - 7.2	629 - 1313	806 - 2314	3.5 - 5.4	2 - 47
Sand dune	B1.4	24	5.4 - 16.8	2.8 - 5.9	583 - 1502	1323 - 2274	4.7 - 7.9	2 - 9

Table 2. Potential driver variables used in the statistical analysis. Climate and deposition data are annual averages: climate for the years 1997-2006 and current deposition for the years 2004-2006. Local site data were collected or measured during the site visit in 2009.

Variables shown in italics are combinations or subsets of other variables.

Climate	Unit
Growing degree days	cumulative degrees from days above 5 °C
Mean annual precipitation	mm y ⁻¹
Pollutant Deposition	
Total acid deposition (S+N)	kg ha ⁻¹ yr ⁻¹
Total sulphur deposition: non-marine sources	kg S ha ⁻¹ yr ⁻¹
Historical total sulphur deposition: 1986-1988	kg S ha ⁻¹ yr ⁻¹
<i>Wet sulphur deposition</i>	kg S ha ⁻¹ yr ⁻¹
<i>Dry sulphur deposition</i>	kg S ha ⁻¹ yr ⁻¹
Total inorganic nitrogen deposition	kg N ha ⁻¹ yr ⁻¹
<i>Oxidised nitrogen deposition</i>	kg N ha ⁻¹ yr ⁻¹
<i>Reduced nitrogen deposition</i>	kg N ha ⁻¹ yr ⁻¹
<i>Wet oxidised nitrogen deposition</i>	kg N ha ⁻¹ yr ⁻¹
<i>Dry oxidised nitrogen deposition</i>	kg N ha ⁻¹ yr ⁻¹
<i>Wet reduced nitrogen deposition</i>	kg N ha ⁻¹ yr ⁻¹
<i>Dry reduced nitrogen deposition</i>	kg N ha ⁻¹ yr ⁻¹
Calcium + magnesium deposition : non-marine sources	kg [Ca+Mg] ha ⁻¹ yr ⁻¹)
Local Site	
Altitude	(m asl)
Hydrological index (bogs only)	Subjective scale: 1 (very dry, heath like) - 5 (water logged, floating bog).
pH(top 15 cm)	[no unit]
Loss on ignition (top 15 cm)	%
Soil nitrogen (top 15 cm)	%
Grazing intensity (grasslands and sand dunes only)	Subjective scale: 1 (absent) to 3 (intense)

Table 3. Stepwise regression models on species richness (total, and by functional group) and graminoid cover for five terrestrial habitats across Great Britain, using the predictor variables in Table 2. The most significant variable in the model is shown first, followed by the next significant, etc. Arrows indicate the direction of the relationship. R² and P values are for the full model. Cases where any form of N deposition is selected are shown in bold. Also shown are the results of redundancy analyses on species composition, and the proportion of variability in species average % cover explained by each independent variable.

Response variable	Acid grassland	Bog	Upland heath	Lowland heath	Sand dune
Species richness (%)	All habitats: Dry-oxidised N deposition (↓) + Growing degree days (↓) (R ² =0.39, P=0.001)				
Species richness (Actual)	Dry-oxidised N deposition (↓) R ² =0.61, P=0.004	Dry-S deposition (↓) R ² =0.56, P=0.02	Reduced N deposition (↓) R ² =0.39, P=0.006	Growing degree days (↓) Altitude (↓) Wet-oxidised N deposition (↓) R ² =0.64, P<0.003	pH (↑) Wet-oxidised N deposition (↓) R ² =0.57, P<0.01
Moss species richness (Actual)	Dry-oxidised N deposition (↓) Reduced N deposition (↓) R ² =0.45, P=0.004	no combination of variables explain significant variation in the data	S deposition (↓) R ² =0.25, P=0.02	Growing degree days (↓) pH (↓) R ² =0.42, P=0.005	oxidised N deposition (↓) LOI (↑) R ² =0.67, P<0.005
Lichen species richness (Actual)	-	Dry-oxidised N deposition (↓) R ² =0.37, P<0.02	Reduced N deposition (↓) R ² =0.26, P<0.02	no combination of variables explain significant variation in the data	no combination of variables explain significant variation in the data

Forb species richness (Actual)	Dry-oxidised N deposition (↓) R ² =0.65, P=0.004	Total acid deposition (↓) R ² =0.39, P=0.006	-	-	pH (↑) Wet-oxidised N deposition (↓) Wet-S deposition (↓) R ² =0.53, P<0.005
Graminoid species richness (Actual)	Dry-oxidised N deposition (↓) R ² =0.28, P=0.004	no combination of variables explain significant variation in the data	Dry-reduced N deposition (↓) Altitude (↓) R ² =0.46, P<0.004	Growing degree days (↓) R ² =0.46, P<0.003	Growing degree days (↓) R ² =0.46, P<0.005
Graminoid cover	Wet-reduced N deposition (↑) LOI (↑) R ² =0.38, P=0.001	Wet-reduced N deposition (↑) Growing degree days (↑) R ² =0.68, P<0.001	Dry-reduced N deposition (↑) R ² =0.24, P=0.014	Dry-reduced N deposition (↑) R ² =0.35, P=0.001	pH (↓) soil C/N (↓) R ² =0.48, P<0.001
Statistically significant drivers of change in species composition and variance partitioning by driver					
Species composition	N deposition 12.8%, P=0.001 Growing degree days 9.1%, P=0.005	Hydrological index 8.3%, P=0.001 Dry-reduced N deposition 7%, P=0.004 Historical S deposition 5.9%, P=0.011	Reduced N deposition 15.8%, P=0.001 Growing degree days 7.9%, P=0.006 Loss on ignition 7.4%, P=0.01	Growing degree days 13.3%, P=0.001 Dry-oxidised N deposition 8.2%, P=0.005 Soil pH 6.5%, P=0.001	pH 14.6%, P=0.001 Precipitation 10.6%, P=0.001 Growing degree days 5.6%, P=0.013 Historical S deposition 5.3%, P=0.035

Table 4 for online appendix. Species showing a strong response to Nitrogen (N) with good distribution across dataset.

Habitat	Specific species showing a strong response to Nitrogen (N) with good distribution across dataset (direction ↑↓)	Relationship equation between mean % cover (y) and N deposition (x)	Comment
Acid grassland	<i>Euphrasia officianlis</i> (↓)	$y = -0.0296x + 0.8399$	
	<i>Hylocomium splendens</i> (↓)	$y = 0.0061x^2 - 0.3975x + 6.699$	
	<i>Lotus corniculatus</i> (↓)	$y = -1.034\ln(x) + 3.5753$	Many sites with little or no cover; sites with cover > 2% exist only where N < 25 kg
	<i>Carex panicea</i> (↑)	$y = 0.0724x - 0.8844$	
	<i>Hypnum cupressiforme</i> (agg.) (↑)	$y = 0.0104x^2 - 0.2508x + 1.8153$	% cover increases markedly above 30 kg N
	<i>Nardus stricta</i> (↑)	$y = 0.298x - 2.3768$	
Bog	<i>Cladonia portentosa</i> (↓)	$y = -0.49x + 15.46$	<i>Cladonia</i> spp. in general decline with N
	<i>Eriophorum vaginatum</i> (↑)	$y = 1.47x + 6.06$	
	<i>Sphagnum fimbriatum</i> (↑)	$y = 0.0028x^2 - 0.0782x + 0.491$	Generally only found at most polluted sites where N > 24 kg
Lowland heathland	<i>Cladonia portentosa</i> (↓)	$y = -3.601\ln(x) + 11.077$	Frequency presence in quadrats declines above 16 kg N
	<i>Hylocomium splendens</i> (↓)	$y = -8.901\ln(x) + 26.711$	Frequency presence in quadrats declines above 16 kg N; at wetter sites only
	<i>Brachythecium rutabulum</i> (↑)	-	Frequency presence in quadrats increases above 18 kg N
	<i>Campylopus introflexus</i> (↑)	-	Little cover below 22 kg N
	<i>Cladonia fimbriata</i> (↑)	$y = 0.1139\ln(x) - 0.231$	Increase in cover and frequency at mid-N levels: 20-25 kg N
Sand dunes	<i>Ammophila arenaria</i> (↓)	$y = -3.63\ln(x) + 11.19$	
	<i>Hylocomium splendens</i> (↓)	$y = -12.08\ln(x) + 32.99$	Frequency presence in quadrats declines above 11 kg N
Upland heathland	<i>Cladonia portentosa</i> (↓)	-	
	<i>Hylocomium splendens</i> (↓)	$y = -15.6\ln(x) + 51.697$	Frequency presence in quadrats declines above 17 kg N; at wetter sites only
	<i>Brachythecium rutabulum</i> (↑)	-	Frequency presence in quadrats increases above 20 kg N
	<i>Cladonia fimbriata</i> (↑)	-	
	<i>Deschampsia flexuosa</i> (↑)	$y = 0.04x^2 - 0.75x + 6.52$	