Nitrogen Deposition causes widespread loss of species richness in British Habitats

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Running title: **Nitrogen deposition and loss of species richness**

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Key-words: Countryside Survey, plant traits, acidification, eutrophication, heathland, acid, calcareous, grassland

Article type: Primary research article

Number of words in abstract: 155

Number of words in main text: 4304

Abstract

We use national scale data to test the hypothesis that N deposition is strongly negatively correlated with plant species richness in a wide range of ecosystem types. Vegetation plots from a national ecological surveillance programme were drawn from heathland, acid, calcareous and mesotrophic grassland habitats. Mean species number and mean plant traits were calculated for each plot and related to atmospheric N deposition. There was a significant reduction in species richness with N deposition in acid grassland and heathland even after fitting covarying factors.

In acid grassland and heathland, evidence from trait changes suggested that acidification rather than increased fertility was responsible for species loss. In contrast, calcareous grassland showed evidence of eutrophication in response to increasing N deposition.

Loss of species richness from chronic N deposition is apparent in infertile grasslands and heathland. Mechanisms associated with loss of species richness differ between habitats so mitigation of N deposition should be targeted to habitat type.

Introduction

In industrialised countries the burning and processing of fossil fuels for energy production and the manufacture and application of agricultural fertilisers and manure have resulted in the release of nitrogen (N) and Sulphur (S) compounds into the environment. There has been a substantial reduction of Sulphur emissions in the UK since 1970 (NEGTAP, 2001), however, atmospheric deposition of anthropogenic N in the form of NOy and NHx remains a significant source of such compounds and has been associated with a variety of environmental problems, most of which have been predicted to increase over the next century (Laurance, 2001, Tilman *et al.*, 2002,). The potential effects of N on natural ecosystems include direct toxicity, eutrophication and acidification (Gordon *et al.*, 1999, Jones and Ashenden, 2000,), all of which can drive changes in biodiversity and species composition (Heil and Diemont, 1983, Aerts *et al.*, 1992, Carroll *et al.*, 2003). Natural and semi-natural ecosystems impacted most by N deposition are typically infertile and have high biodiversity value through the presence of specialist species. Such species often possess resilient traits that confer tolerance to grazing and low nutrient availability but are often unable to respond to additional nutrient inputs and are at risk of competition from more nutrient-demanding species. This results in shifts in species composition and loss of diversity (Wedin and Tilman, 1996, Achermann and Bobbink, 2003). Effects of N deposition on communities are often the result of interactions with other factors such as management regime (e.g. grazing pressure and its interactions with fertilisation significantly affect species composition; (Alonso *et al.*, 2001), pests and disease (e.g. increased foliar N increases palatability to herbivores and increases the likelihood and severity of insect outbreaks; (Aerts and Bobbink, 1999) and plant responses to climatic conditions (e.g. increased impact of drought and susceptibility to frost ; Power *et al.*, 1998).

Numerous experimental additions of N have demonstrated profound ecological effects in a variety of habitats (Morecroft *et al.*, 1994, Power *et al.*, 1995, Power *et al.*, 1998, Roem *et al.*, 2002, Carroll *et al.*, 2003, Clark and Tilman, 2008) . These experiments are invaluable in determining the impacts of N deposition on ecosystems and in elucidating mechanisms, but they often use high nutrient inputs over shorter timescales in an attempt to mimic the high cumulative dose that results from low-level, chronic atmospheric deposition. Experiments are also not exposed to the range of covarying factors that can suppress or enhance responses to atmospheric N inputs, including productivity, soil pH, sulphur deposition, climate and land-use. Analyses that simultaneously incorporate such impacts can potentially result in realistic testing of hypotheses of ecological change in highly managed, temperate ecosystems.

In Britain, a recent large-scale signal attribution study (Stevens *et al.*, 2004) demonstrated a strong negative relationship between atmospheric N deposition and species richness in one type of acid grassland. Potentially confounding factors were well crossed or kept constant along a N deposition gradient. This, combined with rigorous targeting of the same type of plant community across a large geographical area, optimised the chance of isolating a large-scale atmospheric N deposition signal. This study demonstrated a clear correlation between N deposition and biodiversity loss, but was unable to quantify the effect in a wider range of habitat types relative to other drivers. We build on this study, using a larger Great Britain-wide surveillance dataset and also use plant traits to attempt to explain the mechanisms behind observed community changes (Maskell *et al.*, 2006).

We tested the following hypotheses: 1. the relationship between N deposition and plant species richness found in one type of acid grassland (Stevens et al 2004) is apparent in all habitat types. 2. The effects of N deposition on plant species richness and composition are driven by the fertilising effects of added N, i.e. by eutrophication.

Materials and Methods

The data used in these analyses are from the Countryside Survey (CS) of Great Britain, which is a globally-unique project to monitor ecological and land use change in great detail over the whole nation (http://www.countrysidesurvey.org.uk/). The sample design is based on a series of stratified, randomly selected 1 km squares, which numbered 569 in the 1998 survey. Stratification of sample squares was based on predefined groups derived from a classification of all 1 km squares, comprising a grid covering Britain, based on their topographic, climatic and geological attributes (Bunce *et al.*, 1996).

This sampling design means the dataset is representative of common British ecosystems and therefore of the prevalence and severity of driving forces such as N deposition, agricultural conversion and climate. Hence, the degree of crossing and replication of different drivers and their range of intensities realistically reflects the way they have operated across Britain, rather than sampling being designed to maximise any one gradient length. The methods used for vegetation monitoring have been described in detail in Smart *et al* (2003). A series of vegetation plots was located within each 1 km square using a restricted randomisation procedure designed to reduce aggregation. In each vegetation plot a list was made of all vascular plants and a selected range of the more easily identifiable bryophytes and macro-lichens. Nomenclature followed Stace (1991), Watson (1981). Cover estimates were made to the nearest 5% for all species reaching at least an estimated 5% cover. Linear features (road verges, watercourse banks, hedges and field boundaries) and areal features (fields, unenclosed land and small semi-natural biotope patches) were sampled, but in this study we have used only the areal plots with a consistent size of 2m x 2m. The Countryside Survey took place in 1978, 1990, 1998 and most recently 2007 (data not yet available). We used data from 1998 because this year had the largest sample size (6782, 2 m x 2 m plots). The total number of non-native and native taxa per plot were used to measure species richness (includes counting species recorded to genus only or amalgamations of two taxonomically difficult species).

This is a simple measure of plant diversity. Analyses were carried out including bryophytes, however, in addition tests were repeated without bryophytes and bryophytes were analysed separately.

Vegetation plots were classified to the phyto-sociological units of the British National Vegetation Classification (NVC) (Rodwell, 1992) . We used a new assignment of all CS plots to the NVC based on the pseudo-quadrat approach (a simplified version of the technique described in Critchley *et al* (2001)). Plots were selected that were classified into one of four habitat types using their NVC classification; acid grassland (U1to U9), calcareous grassland (CG2, 3, 4, 6, 8, 10, 11), heathland (H1 to H19 except H5, 6 and 17) and mesotrophic grasslands (MG6 and MG7 only) (Appendix 1). The first three habitats are infertile semi-natural communities, likely to be adversely affected by N deposition whilst mesotrophic grasslands are at the other end of the fertility gradient and may be less likely to react to additional input of nutrients. This gave 895 acid grassland plots, 94 calcareous grassland plots, 459 heathland plots and 1342 mesotrophic grassland plots.

The classification into clearly-defined habitat types ensured that similar communities were compared and this reduced variation in management history among co-classified sites: for example, direct agricultural applications of N are unlikely to have occurred in acid or calcareous grassland and heathland, otherwise the vegetation would no longer be referable to these three infertile community types. However, the plot locations were random in each 1 km square, and thus a relatively high noise to signal ratio is likely *a priori* because sampled stands will exhibit greater floristic variation than if homogenous sampling domains were pre-selected. Nitrogen and Sulphur (S) deposition estimates were taken from 5km by 5km maps for the UK (Smith *et al.*, 2000) and comprise wet deposition and dry deposition of oxidised and reduced nitrogen derived from measured concentration fields and a dry deposition model. Average values of total N and S deposition were calculated for each CS 1 km square as kg ha^{-1} yr^{-1}. Despite a substantial decrease in Sulphur emissions between 1970 and the present, it is known to have an acidifying effect so has been included as a potential explanatory variable in this study.

Regressions were carried out between total N deposition and the number of species in a quadrat as follows. Within each habitat type a mixed model analysis of variance (GLIMMIX procedure in SAS) was used to determine the impact of N deposition on species richness when other potential explanatory variables were taken into account. The number of species was the response variable and then each explanatory variable was tested by entering it last into a sequential model after all other variables (Type 1 tests). The type 1 tests quantify the partial explanatory power of each driver and therefore exclude any overlapping variance between drivers. Explanatory variables included N deposition, S deposition, climatic variables and an indicator representing the degree of grazing as fixed effects. Grazing was represented by the slope coefficient from a linear regression of sheep numbers in each wider 2 km square between 1969 and 2000. Grazing data were taken from the EDINA AgCensus database[1]. Other variables were height of the highest point in the square, average precipitation, mean maximum July and mean minimum January temperatures (long-term averages for the period 1961-1999; www.ukcip.org.uk). The 1 km Countryside Survey square was incorporated as a random effect to account for the non-independence of plots located within the same square. Degrees of freedom were calculated using the approximation of Satterthwaite (1946). A Poisson distribution was specified. Soil data were available for a subset of plots (acid grass N=101, heathland N= 52, calcareous grassland N=22) and thus for these plots we were able to regress soil pH against nitrogen deposition. Further information on the methods used for soil collection and analysis can be found in Emmett *et al* (2009).

To investigate plant traits, each quadrat was assigned a species' cover-weighted mean value for each of a number of attributes. Ellenberg indicator values are integers that range between 1 and 9 and estimate the optimum of each plant species along an environmental gradient e.g.

[1] Downloaded for each 5km sqr containing each CS 1km sqr from the EDINA AgCensus database at www.edina.ac.uk.

light, fertility (Ellenberg et al 1991). Plant species were assigned an Ellenberg indicator value for Fertility (N), Light (L), Moisture (F), and pH (R) using the re-calibrated values for the British Flora, Hill et al (2000). More detailed information on methods used to determine plant traits are available in Smart et al (2003, 2005). Other botanical traits analysed were specific leaf area (SLA), canopy height, leaf nitrogen content, grass/forb ratio and acid preference index. Data on SLA, grass/forb ratio and canopy height were obtained from Grime *et al.*, (2007, 1988), Grime and Hodgson (1969) and Stace (1991). Leaf N data came from Thompson et al (1997). An index of soil acidity preference was calculated using data from Grime and Lloyd (1973). This is similar to the Ellenberg R score, but is a continuous scale representing the frequency with which a species occurs above or below a value of pH 4.5, this being an important threshold below which only species tolerant of aluminium toxicity are found (Grime and Hodgson, 1969, Grime *et al.*, 2007). The index was calculated as the number of sites with soil pH $\leq$4.5 at which a species occurred as a proportion of occupied sites with soil pH > 4.5 (Stevens *et al* 2009 *in press*). Data were not available for all species but sufficient to permit comparisons between sites. Traits were regressed against nitrogen deposition within habitat type.

Results

The distribution of the plots within each habitat type across GB are shown in Figure 1. There were significant negative relationships between total nitrogen deposition (kg N $ha^{-1}yr^{-1}$) and species richness in heathland (r^2=0.17, p<0.001) and acid grassland (r^2=0.09, p<0.001) but not calcareous grassland (r^2=0.005, p=0.05) habitats (Figure 2). There was a very weak negative relationship between species richness and total N deposition in mesotrophic grassland (r^2=0.01, p<0.01).

When other climatic variables were included, N deposition remained a significant explanatory variable for species richness in heathlands, acid grasslands and mesotrophic grasslands, but not for calcareous grassland (Table 1). When tests were repeated excluding bryophytes, results were similar (acid grassland $F=8.2$, $p<0.01$, Calcareous grassland $F=0.01$, $p=0.9$, Heathland $F=6.4$, $p<0.05$ and Mesotrophic grassland $F=3.25$, $p=0.07$). When bryophytes were analysed independently there was only a significant relationship between bryophyte number and N deposition in acid grassland ($F=8.2$, $p<0.001$). Other significant predictors of species richness varied among habitats: sulphur deposition had a significant negative relationship with richness in acid grassland, although not in the other habitats; climate variables were important, but the precise relationships were variable, and estimated grazing intensity had an effect in acid grassland but not the other habitats.

The relationship between plant traits and N deposition differed in each habitat type (Table 2). In heathlands increased N deposition was related to decreased Ellenberg R, that is, plants were indicative of more acid soils. There was also a significant negative relationship between soil pH and N deposition in a subset of plots. At the same time potential canopy height increased and Ellenberg light value decreased with higher N. Surprisingly, there was no relationship with Ellenberg N, i.e. plants receiving higher rates of N do not indicate higher soil fertility. N deposition also affected vegetation composition, being related positively to the grass/forb ratio and negatively to the number of forb species. The results of the soil acidity preference index were similar to the Ellenberg R scores, with the species present at high N deposition showing a stronger preference for acid habitats. Acid grassland showed broadly similar patterns: as N deposition increased, Ellenberg R and the number of forb species decreased and the grass/forb ratio and acid preference index increased. Soil pH was negatively related to N deposition in the subset of quadrats for which data were available. Surprisingly, Ellenberg N, SLA and leaf N also declined as N deposition increased.

In calcareous grassland, canopy height, grass/forb ratio, Ellenberg N and leaf N all increased at higher N deposition. In contrast to the situation in heathland and acid grassland, changes in Ellenberg R, soil pH (in a sub-sample of plots) and soil acidity preference index all indicated an increase in plants of more basic soils at high N deposition. In contrast to the other habitat types, there were few relationships between plant traits and N deposition in mesotrophic grasslands. There were slight increases in Ellenberg N (r^2=0.01, p<0.01) and a decrease in Ellenberg L (r^2=0.01, p<0.01) at higher N depositions.

Discussion

We found general support for Hypothesis 1, in that the relationship between N deposition and plant richness previously reported for one type of acid grassland (Stevens et al 2004) was found in other habitat types in Britain. This is an important result because, despite many experimental studies of the impacts of N deposition, determining effects on large-scale semi-natural ecosystems in the presence of other potential drivers has until now not been demonstrated.

The negative relationship between species richness and N deposition held true for heathlands and acid grasslands in general, although the proportion of variation explained was not high (r^2 ranges from 0.09 to 0.17). Stevens et al. (2004) report a higher r^2 value of 0.55 for the relationship between N deposition and species richness; however they specifically chose sites along the N deposition gradient. This would artificially raise their r^2 value so cannot be used to interpret the strength of the relationship for GB as a whole and is not directly comparable with the values reported here. Countryside Survey, in contrast, is a stratified random sample of the vegetation of GB, not designed to optimise signal detection along any specific gradient, nor to control for the species-compositional heterogeneity of the habitats sampled (Smart *et al.*, 2004), and therefore will provide results more representative of the GB countryside in general. There are two main reasons why the r^2 values in this study would be expected to be low. The national estimates of N deposition that are used are modelled values at the 5km scale. Deposition can vary greatly over the small scale and restriction to a 5km scale means that the deposition values are mathematically incapable of explaining variation in vegetation at less than this scale. Vegetation, specifically species richness, also varies greatly at the sub-kilometre scale. Approximately 73% of the variation in species richness of Countryside

Survey plots is at the sub-kilometre square level, leaving a possible maximum of 27% which could be accounted for by variables at higher scales. In this context the observed r^2 values of 0.09 to 0.17 are substantial and to have detected a relationship at all within the limitations of the available deposition data is an important result.
The additional mixed model ANOVA analyses (Table 1) including other explanatory variables are powerful tests, they are designed to take into account the Countryside Survey sampling structure and show significant relationships between species richness and N deposition when other variables are accounted for.

The relationship analysed is spatial, relating geographical patterns of atmospheric deposition to vegetation data in one survey year. A potential problem is that such patterns may coincide with other spatial variables such as rainfall patterns and altitude, which is why it was important to account for other, possibly confounding, variables. Even after accounting for these other variables, N deposition was still the most important explanatory variable for heathlands and acid grassland. This was not the case for calcareous grasslands, where N deposition was not significant when other explanatory variables were included. However, sample size was much lower for calcareous grasslands (94 plots compared to 459 for heathland and 895 for acid grassland), which results in a more restricted distribution across climatic gradients; N deposition was also correlated with mean June temperature and mean January temperature in this habitat type so it was not possible to differentiate N deposition from climatic effects.

Although there was a significant negative relationship between N deposition and species richness in mesotrophic grasslands, this relationship was, as expected, quite weak.
The species composition of this habitat is shaped by deliberate applications of macro-nutrients (P and K in addition to N) necessary to forage production. Hence, the relatively low levels added from atmospheric deposition have little additional effect.

Species associated with low N deposition and thus vulnerable to decline under increased levels are mostly small forbs and bryophytes. In acid grasslands such species include *Hylocomium splendens*, *Plantago lanceolata* and *Campanula rotundifolia*. In heathland *Hylocomium splendens, Campanula rotundifolia, Hypericum pulchrum*, *Viola* sp., *Dactylorhiza maculata* and *Polygala vulgaris*.

The response to N deposition of plant functional traits helped elucidate the mechanism driving changes in communities. Hypothesis 2, that the effects of N deposition on richness and/or composition of plant species are driven by increased fertility, was not supported. Heathlands and acid grasslands showed little or no evidence of eutrophication; there was no increase in Ellenberg N with increasing N deposition, suggesting the communities did not appear to be responding to an increase in fertility. Indeed in acid grasslands the opposite seems to be the case: there were small but significant *reductions* in Ellenberg N, SLA and leaf nitrogen with increased N deposition, all of which are consistent with declines in fertility. This raises the question of what happens to nitrogen deposited on these habitats. Phoenix et al (2003), working in an acid grassland community in the Peak District, northern UK, found that the majority of added nitrogen was immobilised in the soil and so was not freely available to plants, even at high levels of N addition. Further research is required.

Thus in plant communities of acid soils, it seems clear that the observed reduction in richness is not the result of competitive exclusion caused by the response of fast-growing dominants to increased nutrient availability.

The main competing hypothesis is that the reduction in richness is caused by soil acidification associated with increased N deposition. Acid deposition results from the oxidation of NO_2 to NO_3- or of NH_4^+ to NO_3^- which may fall as wet or dry deposition (NEGTAP, 2001). Such deposition may result in acidification of acid-sensitive soils due to direct H+ deposition, nitrification and nitrate leaching, with consequent impacts on both nutrient availability and the presence of toxic metals (Horswill *et al.*, 2008, Bobbink *et al.*, 1998). The bio-availability, mobility and speciation of metals is affected by pH and some such as Al and Pb become more mobile when pH is less than 5 and can interfere with root function or cause chemical stress (Stevens et al 2009). Acid deposition may impact through base cation deficiencies as basic cations (e.g. Na, Mg, Ca) are lost through ion exchange (NEGTAP 2001).

The response to atmospheric N inputs depends on the N status of the site and whether N inputs exceed biotic requirements (NEGTAP, 2001, Crawley *et al.*, 2005). In both acid grasslands and heathland, N deposition was significantly correlated with a decreased mean Ellenberg R score and an increased acid preference index, indicating that plants receiving high rates of atmospheric N are experiencing increased soil acidity. This conclusion is supported by the negative relationship between N deposition and soil pH in the subset of plots for which soil pH was available. In a multifactorial experiment Roem (2002) also found that the influence of nutrient availability was subsidiary to changes in acidity. Few species are able to tolerate a lowering of soil pH and the few that can, such as *Molinia caerulea*, are able to dominate. Soil acidification is not caused only by nitrogen; there was also a significant relationship between sulphate deposition (which also has an acidifying effect) and species richness in acid grasslands, and this effect was additional to that of N deposition.

In heathlands increased N deposition was also linked to an increase in potential canopy height and decrease in Ellenberg L (light) score. Although the simplest interpretation of these changes is successional change, associated with an increase in abundance of taller dominants, such as *Molinia caerulea, Calluna vulgaris, Vaccinium myrtillis,* and a decline in shade-intolerant subordinates, closer inspection reveals a more complex story. N deposition was linked to the decline of a number of small forbs and bryophytes (e.g. *Campanula rotundifolia*, *Hylocomium splendens*), probably through acidification or direct N toxicity. The acid preference index shows that high N deposition is associated with species preferring low pH, suggesting that acidification is the mechanism operating here. Since these species are both small and relatively shade-intolerant, their loss leads inevitably to the apparent successional change observed. An increase in the grass/forb ratio and a decline in the number of forb species with N deposition is consistent with this interpretation. Stevens et al (2006) noted similar patterns.

In heathland systems, interactions with the management regime are important. In experimental studies interactions between management and N deposition have been shown to be significant (e.g. Alonso et al 2001). *Calluna vulgaris* has been shown to maintain dominance under high N inputs unless the canopy is opened up by stress or disturbance, therefore high N inputs are more likely to result in the loss of *Calluna* in grazed systems (Aerts and Bobbink, 1999) but this could mean an increase in diversity as the canopy is opened up. Rapid growth of *Calluna* under increased N has also been associated with greater prevalence of diseases and frost susceptibility.

In contrast to heathlands and acid grasslands, calcareous grassland showed evidence of eutrophication in response to increasing N deposition. Several changes are indicative of an increase in fertility, such as increased Ellenberg N, SLA, canopy height and leaf N. Nevertheless, the effect of N deposition on richness was not significant, possibly because grazing prevents potential dominants taking advantage of the small increase in fertility. This suggests that calcareous grasslands may be particularly susceptible to N deposition where management becomes less intensive. Experimental studies on calcareous grasslands have found that N deposition can lead to shifts in species composition, with increases in rank grasses and loss of forbs and bryophytes (Morecroft *et al.*, 1994, Lee and Caporn, 1998, Johnson *et al.*, 1999). The increase in the grass/forb ratio with N deposition in calcareous grasslands found in our study is consistent with these experiments. Surprisingly, not only was there no evidence of soil acidification in calcareous grasslands, there was an increase in Ellenberg R, soil pH and species preferring more basic soils with increasing N deposition. We can offer no explanation for this result.

Species richness of mesotrophic grasslands changed only slightly in response to N deposition, and the few small trait changes were consistent with this lack of response. Mesotrophic grasslands are generally found on fertile lowland soils and also tend to receive high inputs of fertiliser, so it is not surprising that extra atmospheric N had little impact on either species richness or plant traits. Other studies have also found that the response of plant functional traits to N deposition varies between sites and habitat types. Suding et al (2005) found that whilst random loss of rare species was a process operating at most sites, particularly at larger spatial scales, functional traits appeared to be better predictors of loss at smaller spatial scales in response to local environmental contingencies (Suding *et al.*, 2005).

Current rates of N deposition to heathland and moorland in Britain range from 5 to 50 kg ha yr (mean 15kg ha yr) (NEGTAP, 2001, Smart *et al.*, 2004). According to the relationships elucidated in this paper these rates have caused and may continue to cause reductions in species richness. These habitats are important for the specialist species that they support (http://www.ukbap.org.uk/habitats). Whilst threats from management activities such as intensification and grazing have been identified the potential impacts of N deposition have not been fully assessed or accounted for. Mechanisms associated with reductions in species richness differ between habitats so mitigation of N deposition and conservation of biodiversity will need to be targeted according to habitat type and management regime.

Acknowledgements

We would like to thank all the staff in the Landuse Research Group at CEH Lancaster who have helped with organization and analysis of CS data. We would like to thank Andy Scott for statistical advice. We would also like to thank the Natural Environment Research Council (NERC) and the Department of the Environment, Food and Rural Affairs (defra) for joint funding the Countryside Survey.

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Table 1: Results from mixed model ANOVA (proc Glimmix procedure) with number of species as response variable and N deposition and climatic variables as explanatory variables. These are type 1 tests with results for tests where the variable quoted is added last. Site was included as a random factor.

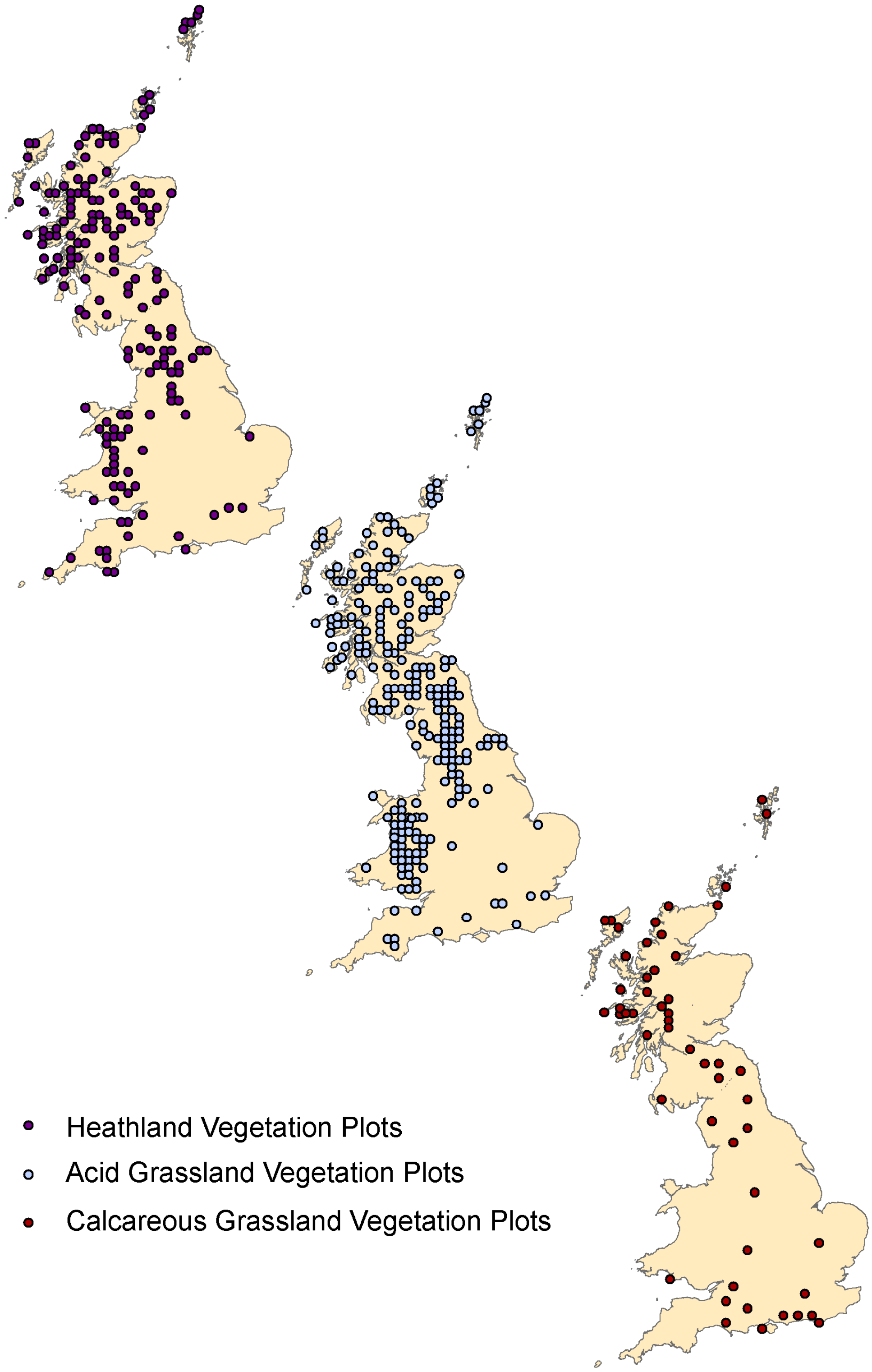
	Heathland		**Acid grassland**		**Calcareous grassland**		**Mesotrophic grassland**	
	F	p	F	p	F	p	F	p
Total N deposition	8.92	<0.01**	12.4	<0.001***	0.03	0.87	4.65	<0.05*
Maximum altitude	0.97	0.33	3.83	0.052	0.56	0.46	0.16	0.69
Mean January temperature	7.49	<0.01**	10.58	<0.01**	0.97	0.33	0.83	0.36
Mean June temperature	1.17	0.28	11.01	<0.01**	0.35	0.56	0.74	0.39
Precipitation	20.58	<0.001***	14.48	<0.001***	3.11	0.08	14.74	<0.001***
Change in sheep numbers	3.24	0.07	6.55	<0.05*	0.13	0.71	0.45	0.5
Sulphate deposition	0.93	0.34	4.18	<0.05*	3.46	0.07	0.49	0.48

Table 2: Results from regressions between N deposition and mean trait values per plot

(***= p<0.001, **=p<0.01, *=p<0.05).

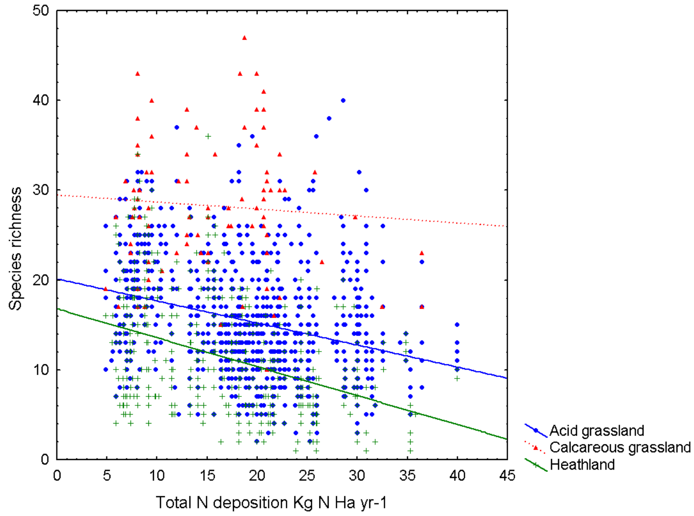
Heathlands N=895			**Acid grassland** N=459		**Calcareous grassland** N=94	
	Direction	r^2	Direction	r^2	Direction	r^2
Ellenberg R	-	0.06***	-	0.09***	+	0.16***
Ellenberg N	n.s.		-	0.01**	+	0.06**
Soil pH	-	0.16**	-	0.05*	+	0.23*
Potential canopy height	+	0.11***	+	0.01*	+	0.1**
Ellenberg Light	-	0.08***	-	0.02***	n.s.	
Grass forb ratio	+	0.07***	+	0.03***	+	0.03*
Number of forbs	-	0.12***	-	0.05***	n.s.	
SLA	n.s.		-	0.04***	+	0.05*
Leaf N	n.s.		-	0.01**	+	0.13***
Acid preference index	+	0.11***	+	0.06***	-	0.12***

Figure 1



Heathland Vegetation Plots
Acid Grassland Vegetation Plots
Calcareous Grassland Vegetation Plots

Figure 2



Species richness
Total N deposition Kg N Ha yr-1
Acid grassland
Calcareous grassland
Heathland
0
10
20
30
40
50
0
5
10
15
20
25
30
35
40
45

Appendix 1

	Acid grassland
U1	*Festuca ovina-Agrostis capillaris-Rumex acetosella* grassland
U2	*Deschampsia flexuosa* grassland
U3	*Agrostis curtisii* grassland
U4	*Festuca ovina-Agrostis capillaris-Galium saxatile* grassland
U5	*Nardus stricta-Galium saxatile* grassland
U6	*Juncus squarrosus-Festuca ovina* grassland
U7	*Nardus stricta-Carex bigelowii* grass-heath
U8	*Carex bigelowii-Polytrcium alpinum* sedge-heath
U9	*Juncus trifidus-Racomitrium lanuginosum* rush-heath
	Heath
H1	*Calluna vulgaris-Festuca ovina* heath
H2	*Calluna vulgaris-Ulex minor* heath
H3	*Ulex minor-Agrostis curtisii* heath
H4	*Ulex gallii-Agrostis curtisii* heath
H7	*Calluna vulgaris-Scilla verna* heath
H8	*Calluna vulgaris-Ulex gallii* heath
H9	*Calluna vulgaris-Deschampsia flexuosa* heath
H10	*Calluna vulgaris- Erica cinerea* heath
H11	*Calluna vulgaris Carex arenaria* heath
H12	*Calluna vulgaris-Vaccinium myrtillus* heath
H13	*Calluna vulgaris-Cladonia arbuscula* heath
H14	*Calluna vulgaris-Racomitrium lanuginosum* heath
H15	*Calluna vulgaris-Juniperus communis* ssp. *nana* heath
H16	*Calluna vulgaris-Arctostaphylos uva-ursi* heath
H18	*Vaccinium myrtillus-Deschampsia flexuosa* heath
H19	*Vaccinium myrtillus-Cladonia arbuscula* heath
	Calcareous grassland
CG2	*Festuca ovina-Avenula pratensis* grassland
CG3	*Bromus erectus* grassland
CG4	*Brachypodium pinnatum* grassland
CG6	*Avenula pubescens* grassland
CG8	*Sesleria albicans-Scabiosa columbaria* grassland
CG10	*Festuca ovina-Agrostis capillaris-Thymus praecox* grassland
CG11	*Festuca ovina-Agrostis capillaris-Alchemilla alpina* grass-heath
	Mesotrophic grassland
MG6	*Lolium perenne-Cynosurus cristatus* grassland, *Lolium Cynosuretum cristati*
MG7	*Lolium perenne* leys and related grasslands