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Changes in species composition of European acid grasslands observed along a gradient of nitrogen deposition.

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Abstract

Question: Which environmental variables affect floristic species composition of acid grasslands in the Atlantic biogeographic region of Europe along a gradient of atmospheric N deposition?

Location: Transect across the Atlantic biogeographic region of Europe including Ireland, Great Britain, Isle of Man, France, Belgium, the Netherlands, Germany, Norway, Denmark, and Sweden.

Materials and Methods: In 153 acid grasslands we assessed plant and bryophyte species composition, soil chemistry (pH, base cations, metal, nitrate and ammonium concentrations, total carbon and nitrogen, and Olsen plant-available phosphorus), climatic variables, N deposition and S deposition. Ordination and variation partitioning were used to determine the relative importance of different drivers on the species composition of the studied grasslands.

Results: Climate, soil and deposition variables explained 24% of the total variation in the species composition. Variance partitioning showed that soil variables explained the most variation in the data set and that climate and geographic variables accounted for slightly less variation. Deposition variables (N and S deposition) explained 9.8% of the variation in the ordination. Species positively associated with N deposition included *Holcus mollis*, and *Leontodon hispidus*. Species negatively associated with N deposition included *Agrostis curtisii*, *Leontodon autumnalis*, *Campanula rotundifolia*, and *Hylocomium splendens*.

Conclusions: Although secondary to climate gradients and soil biogeochemistry, and not as strong as for species richness, the impact of N and S deposition on species composition can be detected in acid grasslands influencing community composition both directly and indirectly, presumably by soil mediated effects.

Introduction

The global nitrogen (N) cycle has been transformed by human activities. The global creation of reactive N increased by a factor of ten from 15 Tg N to 156 Tg N yr^{-1} from 1860 and 1995 and by a further 31 Tg N to 187 Tg N yr^{-1} between 1995 and 2005 (Galloway et al. 2008). With continued growth of the world population and increasing demand for food, pressures on the global nitrogen cycle are set to increase. Excess reactive N in the atmosphere is deposited to terrestrial and aquatic ecosystems as wet and dry deposition. Atmospheric deposition of reactive N is considered a global threat to biodiversity (Sala et al. 2000; Phoenix et al. 2006). Levels of N deposition in Western Europe are among the highest in the world (Galloway et al. 2008) and although there have been small declines in deposition in some regions in recent years (Fagerli and Aas 2008), deposition of N remains high in many areas and critical loads are exceeded in many parts of Europe (Galloway et al. 2008). Sulphur (S) deposition has also increased steadily through the twentieth century, peaking in the 1980's. Between 1880 and 1991 cumulative deposition of S reached 6000 kg S ha^{-1} in high emission areas (Mylona 2002). Since then S deposition has fallen considerably as a result of political initiative in Europe. The 1985 Helsinki Protocol on the Reduction of Sulphur Emissions or their Transboundary Fluxes and the 1994 Oslo Protocol on Further Reduction of Sulphur Emissions have achieved a 60% reduction in S emissions in Europe (1980–1997) (EMEP 1999).

N deposition has the potential to impact on grassland plant community composition in a number of different ways resulting in changes in tissue nutrient stoichiometry and metabolism (e.g. Pitcairn et al. 1998; Gidman et al. 2006; Arroniz-Crespo et al. 2008), changes in species composition (e.g. Mountford et al. 1994; Stevens et al. 2009b), and

changes in species richness (e.g. Stevens et al. 2004; Clark and Tilman 2008; Duprè et al. 2010). There are several ways in which N deposition can bring about these changes.

Because N is the limiting nutrient in many terrestrial ecosystems the addition of N can increase primary productivity resulting in increased competition for light and other resources. This can lead to an increased dominance of competitive species that are better able to take advantage of the increased nutrients (Bobbink et al. 1998; Hautier et al. 2009). N also has the potential to acidify soils, both through the deposition of nitric acid in precipitation, the oxidation of dry-deposited compounds and an increase in plant uptake and N transformations in the soil. The resultant reductions in soil pH can reduce the available species pool and result in changes in species composition (Schuster and Diekmann 2003; Tyler 2003). N deposition can also result in increased susceptibility to insect herbivory (Brunsting and Heil 1985), increased incidence of drought and frost stress (Caporn et al. 2000; Sheppard and Leith 2002) and, at high air concentrations of NO_2, NH_3 and NH_4^+, can cause leaf damage and growth reduction (Pearson and Stewart 1993) although concentrations this high are generally only found in the immediate vicinity of point sources.

The addition of N to semi-natural vegetation typically results in an increase in competitive species (Wedin and Tilman 1993; Wilson et al. 1995) or a reduction in acid intolerant species (Stevens et al. 2010b). Results from previous studies on acid grasslands have shown that species richness declined in relation to N deposition over both spatial gradients (Stevens et al. 2004; Maskell et al. 2010) and time (Duprè et al. 2010). Changes in species richness and composition in acid grasslands in the UK have been associated with higher KCl-extractable ammonium in the soil, lower pH (Stevens et al. 2006) and higher aluminium and other metal availability in soils (Stevens et al. 2009a).

Changes in species composition in relation to N deposition have previously been examined at local and national scales in a range of habitats (e.g. Smart et al. 2003; Bennie et al. 2006) as well as in experimental manipulations (e.g. Mountford et al. 1993; Carroll et al. 2003). In this investigation we use a survey of 153 acid grasslands belonging to the *Violion caninae* alliance (Schwickerath 1944) in ten countries within the Atlantic biogeographic region of Europe to investigate variation in species composition and their underlying explanatory variables. We examine the variation in species composition in a clearly defined community type along a long N deposition gradient (total atmospheric N deposition ranging from 2.4 - 43.5 kg N ha^{-1} yr^{-1}), and aim to quantify the amount of variation in species composition attributed to different explanatory variables and specifically to deposition variables.

Materials and Methods

One hundred and fifty three *Violion caninae* grasslands were surveyed between 2002 and 2007 within the Atlantic biogeographic zone of Europe (Fig. 1). The acidic grasslands visited were selected in a stratified manner to cover the range of atmospheric N deposition in Europe. Grasslands in the vicinity of point sources of nitrogen (e.g. large pig or poultry farms) were avoided. All of the grasslands were managed by grazing or cutting and none were fertilised. To ensure consistent community selection across the geographic gradient, a list of indicative or dominant species of the community was drawn up and these had to be found on a site before the survey was carried out. Despite the large geographical range over which the community was surveyed, there were no marked differences in the community between countries as shown by the relatively short DCA gradient (Fig. 2). At each site, five randomly located 2 × 2 m quadrats were surveyed within a 1 ha area. Within each quadrat, all vascular plants and bryophytes were identified to a species level and their cover was estimated using the Domin scale (see Rich et al. 2005). Areas within the grassland that belonged to other plant communities (according to the dominant or indicative species) or

were strongly affected by animals, tracks and paths, or were in the rain shadows of trees or hedges were excluded from the survey. A description of the site was made and data collected on latitude, longitude, aspect, slope, extent of grassland, soil depth (to bedrock) and surrounding vegetation.

Soil samples were collected from each quadrat. Topsoil samples were taken at a depth of 0-10 cm below the litter layer. Samples were taken from two opposing corners of the quadrat, bulked to make one sample per quadrat and kept cool during transit.

In the laboratory, soil samples were air dried and ground to <2 mm prior to analysis. For total carbon (C) and N analysis, soils were ground to a fine powder. Soil pH was determined using a pH probe in a 1:5 slurry of soil and deionised water (Thomas 1996). Nitrate, ammonium, and metal concentrations were analysed using two different methods. Sixty-eight samples from the UK collected in 2002 and 2003 were leached with 1 M KCl (MAFF 1986) and the resulting nitrate and ammonium analysed using an ion chromatograph. Other samples were shaken with 0.4 M NaCl and analysed using an auto-analyser. For all samples metal concentrations were determined using an ICP-MS. A comparison between the two methodologies demonstrated that results were comparable (not shown). Total C and N content of the soil and plant material was analysed using a CN element analyser. Plant-available phosphorus was calculated using an Olsen extract (MAFF 1986). All samples were analysed within three months of collection. Full details of soil analysis are given in Stevens et al. (Stevens et al. 2010a).

Meteorological data for all the sites were obtained from the European Space Agency Monitoring Agriculture with Remote Sensing (MARS) unit (MARS 2009); ten year averages (1996-2006) were calculated for each site for mean annual potential evapotranspiration, mean

minimum daily temperature, mean maximum daily temperature and mean annual rainfall. Radiation index was calculated based on latitude, aspect and slope (Oke 1987).

For each site, total N, reduced N, oxidised N and sulphur (S) deposition data were modelled using the best available deposition model. National models were used for Germany (Gauger et al. 2002), the Netherlands (Van Jaarsveld 1995; Asman and van Jaarsveld 2002; Van Jaarsveld 2004) and the United Kingdom (Smith et al. 2000; NEGTAP 2001). For all other countries the European Monitoring and Evaluation Programme (EMEP) based Integrated Deposition Model (IDEM) (Pieterse et al. 2007) was used. Comparisons between models revealed that results were very similar for many areas where both models were available. The exception was areas with very variable altitude, for these areas national models, which have a smaller resolution than the EMEP model, were used. For all of the models, deposition was calculated as a three-year average (2000-2003).

For the five quadrats at each site, both mean Domin scores (groupings of percentage cover) and constancy values (frequency in the five quadrats) were tested but gave very similar results, so constancy scores were selected for the final analysis. Major gradients were explored using indirect gradient analysis with detrended correspondence analysis (DCA) in CANOCO 4.5 (ter Braak and Smilauer, 2002, Biometris, Wargeningen). Correlation co-efficients between 19 environmental variables (latitude, longitude, radiation index, inclination, management type, mean daily maximum temperature, soil pH, soil aluminium, calcium, magnesium and manganese concentrations, nitrate concentration, ammonium concentration, Olsen phosphorus concentration, total carbon and nitrogen content, C:N, total atmospheric N and S deposition) and site scores of DCA axes were calculated. A log-transformation was applied to some variables to achieve normality. For further analysis, highly inter-correlated variables (r > 0.6) were removed (altitude, radiation index, transpiration, mean daily minimum temperature, rainfall, subsoil pH, iron concentration,

nitrate concentration, ammonium concentration and Olsen extractable phosphate). Latitude and temperature although highly correlated were both retained due to their potential importance as drivers of species composition on such a large geographical scale. A correlation matrix is provided in Appendix S1. To reduce the number of environmental variables, those variables that were significantly correlated with the DCA axes were selected using Minitab 15 (Minitab Inc, 2007, USA). Divalent base cations (calcium, magnesium) and manganese were added together to reduce the number of variables further (Kleinebecker et al. 2008). Sulphur deposition and soil N were retained in the analysis as they were variables of particular interest to this investigation although they were correlated with some other variables. These environmental variables were used in a canonical correspondence analysis (CCA) with forward selection and rare species down-weighted. Variables which did not show a significant relationship in the forward selection were removed. Variance partitioning was conducted by running a series of partial CCAs using three groups of variables: deposition, soil and climate and geographic variables (Table 1) to determine the relative contributions of each group to the overall variance (Borcard et al. 1992). CCA was performed using CANOCO 4.5 (ter Braak and Smilauer, 2002, Biometris, Wargeningen).

Results

A total of 398 species were found in the 153 sites. The species recorded most frequently in the data set were *Agrostis capillaris* L. (150 sites), *Luzula campestris* (L.) DC. (128 sites), *Rhytidiadelphus squarrosus* (Hedw.) Warnst (124 sites), *Potentilla erecta* (L.) Räuschel (116 sites) and *Galium saxatile* L. (113 sites). Grassland swards were typically grass dominated with variable amounts of forb and bryophyte cover. DCA (Fig. 2) showed good overlap between the sites surveyed in different countries but a latitudinal gradient is apparent on axis 1. The DCA ordination analyses showed relatively short gradient lengths considering the large geographical variance in the grasslands surveyed. The gradient length of axis 1 was

2.73 and that of axis 2 was 2.27. The total inertia in the DCA was 3.006. The sample scores of axis 1 of the DCA analysis were significantly correlated with a number of variables. Significant correlations with an r value of greater than 0.4 were observed for latitude, management type, mean daily maximum temperature, topsoil pH, aluminium concentration, and carbon content. For axis 2 of the DCA analysis sample scores were significantly and strongly correlated with base cation concentration and soil C:N ratio. Sample scores on axis 3 were significantly correlated with total nitrogen deposition and longitude (Table 2).

After excluding highly inter-correlated variables we used eleven variables (Table 1) in the CCA. These variables explained 24% of the total variation in the species composition. Variance partitioning of the explained variation showed that soil variables (topsoil pH, log aluminium concentration, log C content, log N content, C:N ratio) were the group that explained the most variation in the data set, accounting for 38.0% of the constrained total inertia. Climate and geographic variables (latitude, longitude and mean daily maximum temperature) accounted for 30.8% of the variation in the constrained total inertia. A further 13.3% of the variation was accounted for a combination of these variables. Deposition variables (N and S deposition) alone explained 9.8% of the variation in the constrained total inertia with a further 6.2% overlap in explanatory power between deposition and soil variables. The remaining 1.9% of the variation was explained by overlap between the three variable groups (Fig. 3).

CCA was also used to identify species positively and negatively associated with N deposition. For this constrained ordination N and S deposition were used as environmental variables and all other variables were used as co-variables. Fig. 4 shows only those species which occurred in more than 10% of sites. Species most strongly positively associated with N deposition in the ordination diagram were *Holcus mollis* L., *Leontodon hispidus* L., *Festuca ovina sensu lato* L., *Nardus stricta* L., *Cerastium fontanum* Baumg. and *Juncus*

effusus L. Species that were rarer within the dataset but showed a particularly strong association with high N deposition were *Senecio jacobaea* L. and *Cynosurus cristatus* L. Species most strongly negatively associated with N deposition were *Agrostis curtisii* Kerguélen, *Viola riviniana* Reichenb., *Leontodon autumnalis* L., *Campanula rotundifolia* L., and *Hylocomium splendens* (Hedw.) Br. Eur. Species that were rarer within the dataset but showed a particularly strong association with low N deposition were *Vaccinium vitis-idaea* L. and *Hypericum pulchrum* L.

Discussion

Climate and geographic variables explain almost a third of species composition variation in our study. Further influence of climate may have been missed as we did not consider the hydrology and water holding capacity of each of soils at the sites. Given the large spatial gradient over which this study has been conducted the importance of climate in influencing species composition is also of no surprise. The variability in climatic factors across the gradient is large with mean daily minimum temperatures ranging from -0.6 to 10.2 ^{o}C and mean daily maximum temperatures ranging from 6.8 to 18.8 ^{o}C. Rainfall also varies considerably across the gradient from 498 mm per year to 1971 mm per year.

Atmospheric deposition alone explains 9.8% of the variation in species composition in our data set. As shown in Figure 3, there is a strong influence of soils on the species composition found along the gradient of atmospheric deposition used in this study. We need to consider, however, that N and S deposition have the potential to acidify the soils which presents problems in disentangling their impacts on the vegetation community. Soil acidification and consequent mobilisation of metals and reduction in base cation availability have been observed in this grassland community related to N deposition (Stevens et al. 2009a; Stevens et al. 2010b) and changes in soil C:N have also been related to N deposition (Stevens et al.

submitted). As the proportion of variation which is jointly explained by deposition and soils is small it is likely that the influence of deposition on soils is not fully accounted for in the overlap found here. This may be partly due to the large variability in the soil textures and types encountered in this survey leading to differences in how the deposited N is processed in the soil. As a consequence of the influence of N and S deposition on soil chemistry, the variation explained by deposition and the variation explained by soil cannot be considered entirely independent. N and S deposition were considered together in our analysis since they are highly correlated (r=0.45) in our data set which presents problems in disentangling their degree of influence on the community composition.

These results for species composition found in this study contrast with the results obtained when looking at species richness (Stevens et al. 2010a). For species richness, geographic and physical variables (location, climate and site characteristics) explained very little of the variation (less than 1%) whereas here, climate and geographical variables explain almost a third of variation. Species richness was reduced by atmospheric deposition, most likely due to the loss of rare species in the different regions. As a result, in this study the compositional shift is not as evident given that the more dominant species remain the same. The vast majority of the species found in this survey occurred across the whole of the spatial extent of the survey but there were some notable exceptions to this, such as *Agrostis curtisii,* which replaces *A. capillaris* as the dominant grass in some sites in the west of France and the south-west of England. The restricted distribution of *A. curtisii* is thought to be related to climatic and edaphic factors (Ivimey-Cook 1959). There were a number of other species which, although not showing a strongly restricted distribution in our study area, were only found in this community in some geographical areas or were at a much higher abundance in some areas (e.g. *Arnica montana* L.).

Species that were most strongly associated with low N deposition tend to be forbs that are poor competitors and are not tolerant of highly acidic soils. *Viola riviniana* is described by Grime et al. (2007) as intermediate between stress-tolerator and C-S-R strategist but, perhaps more importantly, it is rarely found in the most acid soils. This may also be true of *Campanula rotundifolia,* also intermediate between stress-tolerator and C-S-R but again, rarely found on strongly acid soils (Grime et al. 2007). *C. rotundifolia* is also a poor competitor with vigorous grasses (Sinker et al. 1991) so may not be competing well with grass species that are encouraged by high N deposition. *Leontodon autumnalis* is a species typical of intermediate fertility but is also found commonly on weakly acid soils rather than highly acid soils (Ellenberg et al. 1991; Hill et al. 1999). The moss *Hylocomium splendens* has been shown to decrease with nitrogen additions in several forest experiments. Doses of 30 kg N ha^{-1} yr^{-1} caused a strong decline of *H. splendens* abundance in Sweden (Dirkse and Martakis 1992) and a decline has been identified in coniferous forests in southern Germany over a 20-40 year period. The latter was attributed to sensitivity to acidification (Rodenkirchen 1992). Duprè et al. (2010) also identified a decline in *H. splendens* in Great Britain from the analysis of historic quadrat data collected between 1960 - 75 and 1975 - 2003. The limited distribution of *Agrostis curtisii* means that the strong association with low N deposition for this species should be interpreted with some caution, however, it is a species very typical of infertile habitats (Ellenberg N score of 1 in Hill et al. (1999)).

Species most strongly associated with high N deposition were *Holcus mollis*, *Festuca ovina, Nardus stricta, Cerastium fontanum, Leontodon hispidus* and *Juncus effusus*. None of these species are typical of fertile habitats but given that the vegetation community in which we were working is characterised by extremely poor soils, this is what would be expected. Both *H. mollis* and *J. effusus* tend towards being competitive species and they are both tolerant of very acid soils and *C. fontanum* is a more ruderal species (Grime et al. 2007). An increase in

graminoid species is often associated with increased N deposition (Stevens et al. 2009b; Duprè et al. 2010) and *H. mollis* increased in relative frequency in Germany and Great Britain between two periods, 1939 - 75 and 1975 - 2007. The association of *L. hispidus* with high deposition is more surprising as this species is not typical of highly acidic or nutrient rich habitats (Ellenberg et al. 1991; Hill et al. 1999) and requires further investigation.

It is clear from this analysis that N deposition has the potential to influence vegetation community composition in acid grasslands, both directly and indirectly by soil mediated effects. Although secondary to climate gradients and soil biogeochemistry the impact of N and S deposition on species composition can be detected, even at a large spatial scale. These results have important implications for conservation management and suggest that in order to maintain acid grasslands in good condition we need to reduce N deposition or manage grasslands in a way that mitigates its effects.

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Table 1: Grouping of variables used in variance partitioning.

Group	Variables
Deposition	Total inorganic N deposition and S deposition
Soil	Topsoil pH, exchangeable aluminium concentration, exchangeable base cation concentration, % C, %N and C:N ratio
Climate and geographic	Latitude, longitude and mean daily maximum temperature

Table 2. Correlation co-efficients between DCA axis scores and environmental variables.

Significant correlation coefficients are marked as: *= $P<0.05$, **=$P<0.01$, ***= $P<0.001$.

Environmental Variable	Axis 1		Axis 2		Axis 3	
Latitude	-0.649	***	0.070		0.073	
Longitude	0.294	***	-0.255	***	0.627	***
Altitude (m a.s.l.)	-0.360	***	0.155		-0.108	
Log inclination (^{o})	-0.398	***	0.165	*	-0.252	**
Radiation index	0.328	***	-0.042		0.118	
Mean monthly maximum temperature (^{o}C)	0.663	***	-0.131		-0.078	
Mean monthly minimum temperature (^{o}C)	0.468	***	-0.080		-0.290	***
Mean annual rainfall (mm)	-0.029		0.095		-0.258	**
Mean annual evapotranspiration (mm)	-0.604	***	-0.019		-0.078	
Topsoil pH	0.629	***	0.308	***	-0.190	*
Log aluminium concentration (mg kg^{-1} dry soil)	-0.446	***	-0.139		-0.102	
Log base cation concentration (mg kg^{-1} dry soil)	-0.351	***	0.474	***	-0.381	***
Log C (%)	-0.458	***	0.151		0.151	
Log N (%)	-0.203	*	-0.114		-0.113	
C:N	-0.301		-0.447	***	0.166	*
KCl extractable nitrate concentration (mg kg^{-1} dry soil)	-0.368	***	0.397	***	0.179	*
KCl extractable ammonium concentration (mg kg^{-1} dry soil)	-0.196	*	-0.129		-0.011	
Olsen extractable P concentration (mg kg^{-1} dry soil)	0.013		0.073		0.122	
Management type (grazing or mowing)	0.551	***	-0.196	*	-0.018	
Total inorganic N deposition (kg N ha^{-1} yr^{-1})	-0.301	***	-0.293	***	0.464	***
Total inorganic S deposition (kg S ha^{-1} yr^{-1})	-0.355	***	-0.104	***	0.006	

Fig. 1. Distribution of the 153 *Violion caninae* grasslands surveyed in the Altantic biogeographic region of Europe.

Fig. 2. DCA ordination of sites surveyed coded according to country. The gradient lengths for axes 1 and 2 are 2.73 and 2.27 respectively, eigen values are 0.236 and 0.190.

Fig. 3. Amount of variation in species composition described by CCA analysis which is explained by three groups of explanatory variables: deposition (N deposition and S deposition), soil (topsoil pH, aluminium concentration, base cation concentration, carbon content, nitrogen content and C:N ratio) and climate and geographic (latitude, longitude and mean daily maximum temperature). Areas of circles in the Venn diagram show approximately the percent of variation explained relative to the total variation explained by the full CCA model (24%).

Fig. 4. CCA ordination diagram (axes 1 and 2) for all species with N and S deposition as environmental variables and climate and soil variables used as co-variables, rare species are downweighted. Species plotted occurred in more than 10% of sites and species positively or negatively associated with N deposition (assessed by their positions in the ordination diagram) are named.