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**Changes in species richness and composition in European acidic grasslands
over the past 70 years – the contribution of cumulative atmospheric
nitrogen deposition**

Running Title: Nitrogen deposition and species richness

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Abstract

Our study investigates the negative impact of nitrogen (N) deposition on species richness in acidic grasslands, based on a temporal comparison of vegetation data spanning a period of almost 70 years.

We compiled a large data base of plots assigned to the *Violion caninae* grassland type, composed of managed, but unfertilized semi-natural grasslands on nutrient-poor, acidic soils. In total 1,114 plots, mainly from Great Britain, the Netherlands and Germany, were compiled, dating back to 1939. Environmental site information included geographical and soil (mean Ellenberg values) variables as well as estimates of cumulative N and sulphur (S) deposition since 1939. Statistical analyses were carried out separately for the data sub-sets from the three regions.

In all regions, the vegetation differentiation was mainly related to soil acidity and nutrient availability, as well as to the year of sampling and the cumulative amounts of N and S deposition. Plot-species richness of vascular plants and bryophytes (analysed for Great Britain only) decreased with time and analyses suggest these are affected by various factors, notably soil pH, but also latitude and cumulative N deposition. The latter explained more of the variation in species number than the year of sampling and cumulative S deposition, which supports the interpretation that the decline in species richness is mainly caused by increasing N availability and less by altered management and soil acidification. For Great Britain and Germany, cumulative N deposition showed a strong negative relationship with several biodiversity measures, especially the proportion of dicots, whereas it was positively related to the proportion of grass species. In general, our results give temporal evidence for the negative effect of N deposition on species richness in semi-natural vegetation.

Introduction

Atmospheric deposition of reactive nitrogen has become one of the largest issues of concern in environmental science and policy (e.g., Millenium Ecosystem Assessment 2005). Unlike sulphur (S), the emissions of N in Europe have been stable or have only marginally declined in recent years (Berge *et al.* 1999), and are projected to increase during the next decades, both in Europe and in other parts of the world (Dentener *et al.* 2006; Gruber & Galloway 2008). Effects of N deposition have been reported from many terrestrial and aquatic ecosystems (Vitousek *et al.* 1997; Bouwman *et al.* 2002) and from many European countries (EMEP 2000). Effects on ecosystems are many and varied (Fangmeier *et al.* 1994): for example, N deposition may influence soil microbial processes (Aber *et al.* 2003), be toxic to plants at high levels of deposition (Sheppard 2002), increase the sensitivity of plants to environmental stress (Carroll *et al.* 1999), and change general resource levels resulting in altered competitive interactions leading to changes in species composition and richness (Bobbink *et al.* 1998; Stevens *et al.* 2004). Eutrophication and acidification are assumed to be major drivers of (plant) diversity loss, together with land use change (Sala *et al.* 2000; Millenium Ecosystem Assessment 2005).

The effects of N deposition have been studied in several vegetation types, mainly by means of experimental N addition in the field (e.g., Mountfort *et al.* 1993; Wedin & Tilman 1996; Gough *et al.* 2000; Phoenix *et al.* 2003; Clark & Tilman 2008). The experimental approach allows an examination of the quantitative effects of the N addition in a highly controlled way. It has, however, some drawbacks: experiments are normally conducted for a limited number of years (with the notable exception of the 150-year Rothamsted experiments; Crawley *et al.* 2005; Silvertown *et al.* 2006), and the amounts of N applied often include unrealistically high doses with the aim of determining long-term impacts in the short term, yet

the validity of such an approach is not known (but see, for example, Clark & Tilman 2008). In addition, the experiments are often simple in the sense that they do not reflect the complexity of different effects acting simultaneously on the ecosystem. Therefore, experiments should be complemented by observations in systems that have not been experimentally manipulated.

There are two basic approaches to describe the effects of N deposition on species richness and composition in descriptive field studies: historical analyses and spatial comparisons. The latter can be regarded as a space-for-time substitution and relies on spatial gradients in deposition levels across a region. Comparisons between more- and less-polluted areas have been conducted primarily in forests (Diekmann *et al.* 1999; Diekmann & Falkengren-Grerup 2002; Köchy & Bråkenhielm 2007). On a national scale across Great Britain, areas with different pollution levels were compared for various ecosystems (Smart *et al.* 2004). Many of these studies have shown an increase in relatively N-demanding species and a decrease in species indicative of less fertile habitats. Recently, Stevens *et al.* (2004, 2006) provided the first direct evidence on a large spatial scale for the negative impact of N deposition on plant species richness by including modelled rates of deposition, together with many other environmental predictors, into a regression analysis with data from acidic grassland plots across Great Britain.

The time series approach is based on a temporal comparison of plot data. By comparing old and new vegetation records in permanent plots, changes in species composition over time may be detected and ecologically interpreted. This approach has proven successful in the study of N-driven changes in forest vegetation (Thimonier *et al.* 1992, 1994; Diekmann *et al.* 1999). What limits this method of examining N deposition effects is the lack of permanent plots, which if they exist at all, either have not been established for a long time, or their exact location is not well documented. Furthermore, many habitat types are dominated by long-

lived plants where changes occur relatively slowly and therefore are difficult to detect in short-term permanent plot studies.

In this study we apply an alternative time series type method to examine the impact of N deposition on species composition and richness: using data from several regions, we compare large numbers of plots from sites collected over a long period of time, including both permanent and non-permanent plots. Here, the disadvantage of not comparing repeated records from permanent plot sites is compensated by the use of a much larger number of plots in the study. An advantage of our approach is that many old records can be used from periods when N deposition levels were still low. This method has rarely been applied to study the effects of atmospheric pollution (but see Diekmann & Duprè 1997), but we believe that the large number of old and new vegetation records and their availability in large national or regional databases make it possible to explore this historical approach further. It must be noted, however, that historical, non-permanent plot data may be biased, if they, for example, are not balanced in the sense that either different regions or different sub-communities are sampled at different time periods, both of which can distort the statistical analysis.

In our study we focus on semi-natural acidic grasslands, the same type of vegetation used for the spatial comparison of sites carried out by Stevens *et al.* (2004, 2006). For the first time, estimates of the amount of cumulative N deposition for different sites based on present-day modelling data are included in the species richness models. We addressed the following research questions:

- Has there been a systematic change in plant species composition and richness in acidic grasslands over the past seven decades?
- If so, can these changes be related to the amount of cumulative N deposition and soil factors, especially soil acidity and fertility?
- Are species of different life forms affected differently?

Material and Methods

Vegetation data

We compiled historical and recent vegetation data from acid grasslands located in the oceanic to sub-oceanic area of North-Western and Central Europe, including plots from Great Britain, the Netherlands, Germany, Denmark and Sweden. Plots were only considered if the vegetation conformed to the community type *Violion caninae* (or *Festuca ovina*-*Agrostis capillaris*-*Galium saxatile* grassland sensu Rodwell 1992; Peppeler-Lisbach & Petersen 2001), a semi-natural grassland type on nutrient-poor, unfertilised, acidic soils, managed by extensive grazing or, more rarely, mowing. Typical species include grasses such as *Nardus stricta*, *Festuca ovina* and *Agrostis capillaris*, herbaceous dicots (*Arnica montana*, *Galium saxatile*, *Viola canina*) and bryophytes such as *Pleurozium schreberi* and *Rhytidiadelphus squarrosus*. *Violion* grasslands have a relatively uniform species composition across the entire study region. We did not consider acidic grasslands on wetter soils (assigned to the *Juncion squarrosi*) or (sub)alpine communities of the alliance *Nardion strictae*, and we also avoided plots for which abandonment of management was indicated. *Violion* grasslands were formerly of much greater extent, but abandonment of grazing has resulted in a considerable reduction in area (Ellenberg 1996). This is especially true in Central Europe, where unfertilized acidic grasslands nowadays persist almost exclusively in nature reserves. Only in Great Britain do they still cover large areas.

The data were obtained from published literature, databases and newly sampled vegetation analyses (see Electronic appendix). In total, data from 1,114 sample plots were compiled, spanning a time period of almost 70 years from 1939 to 2007 (Table 1).

[Table 1 here]

146

147 The vegetation data were entered into a database consisting of a sample $\times$ species matrix. As
148 the data were collected at different times and for different purposes, their quality differed as
149 well, and we had to standardise the data which meant that some information was lost. This
150 often involved aggregating taxa: for example, *Luzula campestris* and *L. multiflora* into *L.*
151 *campestris* agg. The nomenclature of vascular plants followed Wisskirchen & Haeupler
152 (1998) and in some cases Tutin *et al.* (1964-1980). For bryophytes we followed a recent
153 reference list (Koperski *et al.* 2000). Species abundances had to be standardised to conform to
154 the simplified Braun-Blanquet scale (Mueller-Dombois & Ellenberg 1974; Dierschke 1994),
155 with '+' (denoting a few individuals) and 1 - 5 (indicating increasing frequency).

156 We then constructed a second matrix with environmental data, first entering information
157 on the geographical location (longitude, latitude, and altitude) and size of plots. As only the
158 most recent sources contained data on environmental and soil variables, we used mean
159 Ellenberg species indicator values (not cover-weighted) as surrogates (Ellenberg *et al.* 1992)
160 for the level of light (L_{EII}), soil moisture (F_{EII}), soil pH (R_{EII}), and soil nitrogen (N_{EII} , often
161 interpreted as general soil fertility) for all plots. Ellenberg values, a ranking system of
162 ecological niche preferences based on known plant characteristics, have successfully been
163 applied in many ecological studies across Central and Western Europe (see review by
164 Diekmann 2003) and have also been used to examine spatial patterns and temporal changes of
165 vegetation in response to acid or nitrogen deposition (Thimonier *et al.* 1992; Diekmann &
166 Duprè 1997). In our study, mean Ellenberg values were calculated in two ways, using all
167 species (vascular plants & bryophytes), or vascular plants only. For sample plots collected in
168 2007 from Germany, Denmark and Sweden, the correlation between the mean Ellenberg
169 values for R and the measured values of soil pH was highly significant ($r = 0.52$, $p < 0.001$),

showing, in accordance with many other studies (Diekmann 2003), that the mean R values can be used as surrogate for soil pH.

Since we were interested in the long-term effects of atmospheric pollution on species richness, we estimated cumulative values of N and S deposition, including both dry and wet components, based on modelled values. The calculation of the variables ‘cumulative nitrogen deposition’ (cuN_{dep}) and ‘cumulative sulphur deposition’ (cuS_{dep}) was based on estimates of N and S deposition for the year 2000 ($\text{N}_{\text{dep}2000}$) obtained from the model FACEM (Pieterse *et al.* 2007). This ‘inferential’ model enabled high-resolution (site-specific) calculations of dry deposition of different compounds. The model combines site characteristics and meteorological information to derive a dry deposition velocity. The latter is then combined with available air concentration data (measured or modelled), resulting in a calculated dry deposition estimate for individual sites. The concentration data used for this study were obtained via the DEHM model (Hertel *et al.* 2006) using EMEP emission data. Available wet deposition data (obtained via EMEP) was then added in order to get estimates of the total deposition (i.e., wet + dry).

To estimate cumulative deposition values over time, we had to (1) consider the different years of sampling, and (2) take into account that the deposition levels have changed considerably during the past decades. In the case of N deposition, it was low until the middle of last century, increasing first slowly and then rapidly until the 1980s, and since then decreasing slightly or remaining more or less constant on a continuously high level (Berge *et al.* 1999; Dentener *et al.* 2006; Gruber & Galloway 2008). S deposition, in contrast, was already relatively high in the 1940s, increased strongly up to the 1970s, and has decreased since then (Berge *et al.* 1999; Lorenz *et al.* 2008). We therefore introduced ‘correction factors’ for the different decades, based on present-day deposition levels (see Appendix 1). First, the N (or S) deposition value of a plot for the year 2000 was multiplied with the

corresponding correction factor to give a deposition estimate for that site for each year. For the calculation, yearly corrected values were added up, starting from the year of the first plot of the data set (1939) until the year in which the plot in question was sampled. For example, for a plot measured in 1973, cuN_{dep} was calculated as: $N_{dep2000} * (0.1 * 11 \text{ years}) + N_{dep2000} * (0.5 * 10 \text{ years}) + N_{dep2000} * (0.9 * 10 \text{ years}) + N_{dep2000} * (1.3 * 4 \text{ years})$.

The final result is an approximation and does not consider changes in land use around the plots that may have taken place between 1939 and present, in which case the current deposition level might not reflect exactly the historical deposition to the sites. It also leaves unconsidered that the deposition curves are not identical between countries. However, on the wide spatial scale at which we are working and the coarse time scale we applied, the calculation accounts both for the major spatial differences in N and the continuous accumulation of N over time. There will already have been some increase in N and S deposition before 1939, but we decided to take this year as a base line and to consider only the deposition that has occurred since then.

Statistical analysis

As we wished to examine whether the effects of N (and S) deposition on species composition were similar in different regions, we subdivided the whole data set into three regional subsets: Germany, Great Britain, and the Netherlands. By this method the differences between countries, not only in environment and species composition, but also in the national environmental and agricultural policies, especially with respect to the intensity and type of management, were taken into account. The relatively few plots from Denmark and Sweden were only used for comparative purposes, as the sampled regions in these countries have lower deposition levels than Central Europe (Table 1).

To examine the changes in species composition over time and the underlying environmental factors responsible for these changes, the three data sub-sets were analysed by means of Detrended Correspondence Analysis (DCA). This method, based on unimodal species responses, was selected because the large floristic gradients resulted in long DCA gradient lengths (see Table 2). The program CANOCO (ter Braak & Smilauer 2002) was used to produce the ordinations. We applied a downweighting of rare species, but otherwise followed the default options. The resulting sample plot scores of the first two DCA axes were then correlated with the following factors: year of sampling, cuN_{dep} , cuS_{dep} , and the mean Ellenberg values for light and soil variables (based on all vascular plant species).

The relationships between environmental factors and species richness were analysed by stepwise multiple linear regression using the programme MINITAB (Version 13.31). If necessary, the descriptors were log-transformed to achieve normal data distribution (plot size in all data sub-sets, altitude in the sub-set from the Netherlands). To check for multicollinearity we correlated all variables to each other. In all country sub-sets the mean values for N_{EII} and R_{EII} were highly positively correlated ($r = 0.67-0.77$). We decided to use mean R_{EII} instead of mean N_{EII} , because (1) the variation in species richness explained by the multiple models including mean R_{EII} and the R^2_{adj} in simple regressions of species richness on mean R_{EII} were in nearly all cases much higher, and (2) it is known that pH has a particularly strong effect on species occurrence and total species richness in low-productive environments on highly acidic soils (e.g., Roem *et al.* 2002). The results of the multiple regression models with either mean R_{EII} or mean N_{EII} were qualitatively very similar. Furthermore, ‘year of sampling’ was omitted from all models (high correlation with cuN_{dep} , $r = 0.74-0.88$) as well as, for the German data sub-set, ‘altitude’ (r with latitude = -0.81), and, in the English sub-set, ‘longitude’ (r with $cuN_{dep} = 0.66$). Finally, because cuN_{dep} and cuS_{dep} were highly positively

correlated ($r = 0.76-0.92$), the models were constructed with only one of the two variables at a time.

For the model, we fitted a stepwise forward method with a threshold of $\alpha = 0.05$. We also applied a backward elimination of variables and a mixed backward and forward method to test the stability of the regressions, by making sure that the results were not affected by the order in which the variables were entered to or eliminated from the models. All methods gave identical results.

Regression models were fitted for the total number of vascular plant species, the number of dicot species, the number of grasses (Gramineae), and the proportions of dicotyledonous species and grasses out of the total number of species. A model for bryophyte species richness was only fitted for the data sub-set from Great Britain, as the plots from Germany and the Netherlands contained too few species (most likely because bryophytes were not always or only incompletely recorded). For the vascular plant models the mean Ellenberg values based on vascular plants were used, for the bryophyte models from Great Britain we used the mean Ellenberg values based on all species. Finally, to visualize the interactions between the explanatory variables, we carried out regression tree analyses using the program R.

Results

Overview

The size of the sampled plots varied considerably, ranging from 0.25 to 750 m² (Table 1). The large differences were due to the different objectives of the authors when collecting the data: most of the older plots were sampled in the framework of descriptive vegetation science that did not consider the need of standardised data for a rigorous statistical evaluation. Accordingly, the number of vascular plant species per plot varied strongly, between 3 and 51 in Germany, between 3 and 39 in Great Britain, and between 4 and 35 in the Netherlands. Maximum species richness of bryophytes was 17 in Great Britain, 11 in Germany, and 9 in the Netherlands. The mean Ellenberg values L_{EII} and F_{EII} were mostly in the range of 5.5 to 8 and 4 to 7.5, respectively, indicating the relatively exposed and dry to mesic environment of the grasslands. The mean values for R_{EII} and N_{EII} were between 2 and 4 in most cases, but lower than 2 in 66 plots for R_{EII} and in 76 plots for N_{EII} , reflecting the very acidic and nutrient-poor soils of the sites.

Across all plots there were pronounced temporal changes in mean Ellenberg values: while mean L_{EII} and F_{EII} decreased slightly with the year of sampling ($R^2_{adj} = 0.075$ and 0.102, respectively, $p < 0.001$), mean R_{EII} showed a quadratic relation with time ($R^2_{adj} = 0.098$, $p < 0.001$, as opposed to $R^2_{adj} = 0.066$ for a linear regression), increasing until about 1980 and then stabilizing or even slightly decreasing. Overall the strongest (linear) correlation with time was found for mean N_{EII} that increased considerably over the years ($R^2_{adj} = 0.223$, $p < 0.001$).

[Table 2 here]

Ordination

The DCA ordination analyses for the three countries showed relatively long floristic gradient lengths, varying between 3.3 and 4.3 standard deviation units for axis 1 and between 2.4 and 2.6 units for axis 2 (Table 2). The environmental factors that explained the most variation in species composition were mean R_{EII} and N_{EII} , showing high correlation coefficients with the sample plot scores along the first axis for all countries (absolute values of r between 0.67 and 0.91). Except in one case, the mean F_{EII} and L_{EII} were more closely correlated to the scores along DCA axis 2 than for axis 1, with a maximum $r = 0.56$ for L_{EII} in the German data sub-set. The year of sampling, cuN_{dep} and cuS_{dep} were only weakly correlated with the DCA axes in the Dutch data sub-set. In contrast, for the German plots we found a close relationship between the ordination axes and the three variables, especially for year of sampling ($r = -0.61$). For Great Britain, the correlation with axis 1 was even stronger (for year of sampling $r = -0.70$), that for axis 2 somewhat weaker than for Germany. The strong impact of the year of sampling on the floristic composition of the grasslands is also illustrated in Figure 1, showing DCA ordination diagrams with different symbols representing plots sampled during different decades. The graphs show clear temporal gradients both for Germany and Great Britain, whereas the pattern is less clear for the Netherlands.

[Fig. 1 here]

Stepwise multiple regressions

For all three countries the number of plots for the multiple regressions was reduced due to the lack of information for some of the variables (particularly plot size) in some of the samples. For Germany we used 240 plots, in Great Britain 531 and in the Netherlands 106. Model building started with 8 variables for the data sub-set from the Netherlands and 7 variables for

those from Germany and Great Britain. The final models retained a fairly high number of significant variables, especially for the German and British plots (Table 3). They explained more than half of the variation in total species richness of vascular plants (maximum $R^2_{\text{adj}} = 0.65$) or bryophytes (only Great Britain), and of the number of dicot species. For the number of grasses and for the proportions of dicots or grasses, the models explained much less of the total variation (minimum $R^2_{\text{adj}} = 0.21$).

[Table 3 here]

As expected from the large variation in plot size, the number of species (vascular plants, dicots and grasses) was strongly positively related to the area sampled in all countries. In contrast, there was a weak negative correlation between plot size and the number of bryophytes in Great Britain. With respect to the proportions of dicot and grass species, the effect of plot size was generally less strong and less consistent across countries than for the absolute numbers of dicots and grasses.

Among the geographic variables, latitude was most strongly related to the total and relative numbers of species: except for the proportion of grasses, species richness values increased in Great Britain and decreased in Germany with increasing latitude. In contrast, longitude and altitude showed only weak effects: for Germany, the number of vascular plants and dicots was positively correlated with longitude, and for Great Britain the numbers of dicots and bryophytes increased with increasing altitude.

By far the most important variable in the final models was mean R_{EII} , exhibiting a pronounced positive effect on species numbers except for bryophyte species richness in Great Britain (Table 3). The proportion of dicots was positively related to mean R_{EII} , whereas a negative correlation was observed for the proportion of grasses. In comparison, the effects of

mean L_{EII} and F_{EII} were weaker and non-consistent across the different measures of species richness and countries. In all data sets, however, the proportion of dicots was negatively associated with mean F_{EII} , and, in Great Britain, there was a strong negative effect of mean L_{EII} on the number of bryophytes.

[Fig. 2 here]

Generally the second strongest effect was found for cuN_{dep} , showing a negative relationship to the number of vascular plants and dicots in all countries (Table 3), as well as to the number of bryophytes in Great Britain. This is also displayed in the simple regressions of species richness (corrected for plot size) on cuN_{dep} (Fig. 2). For grass species, cuN_{dep} remained significant only in the final model for the Dutch data sub-set. For the grasslands in Great Britain and Germany, cuN_{dep} also had a strong negative effect on the proportion of dicots, whereas it exhibited a positive impact on the proportion of grass species. It is important to note that the year of sampling, when used instead of cuN_{dep} both in multiple and simple regressions, had a much weaker effect on species numbers, suggesting that the declining species richness is not simply a temporal trend driven by factors other than N deposition.

When using cuS_{dep} instead of cuN_{dep} in the multiple regressions, highly similar results were obtained. The R^2_{adj} values of the final models were mostly slightly lower than for the cuN_{dep} models, but occasionally somewhat higher, and the t values for cuS_{dep} were in most cases smaller than those for cuN_{dep} . The results reflect the strong inter-correlation between the two factors, but also show that cuN_{dep} appears to explain somewhat more of the variation in species richness than cuS_{dep} .

The regression trees for Germany and the Netherlands (figures not shown) confirmed the primary importance of soil acidity for species richness; at given levels of R_{EII} (and partly

latitude), cuN_{dep} also appeared to be important. The regression tree found for Great Britain was highly informative (Fig. 3): here, cuN_{dep} only mattered at lower latitudes where soil acidity was not important, whereas at higher latitudes, cuN_{dep} did not matter at all while R_{EII} was by far the most important factor affecting species richness.

[Fig. 3 here]

When considering the mean proportion of grass species as a function of the total number of vascular plant species in different decades since 1940 for Germany and the Netherlands, the increasing frequency of Gramineae over the years becomes evident (Fig. 4). The mean proportions calculated for the sample plots collected in 2007 from Denmark and Sweden were clearly lower than the corresponding values for Germany and the Netherlands. For Great Britain from which only plot data for three decades was available, the differences in the proportions of grass species between the north and the south support the findings from Fig. 3, whereas there were no clear temporal trends.

[Fig. 4 here]

The changes in the proportions of life forms also become evident from Table 4 showing the relative frequencies of the most common and characteristic species in *Violion* grasslands in plots sampled before 1975 compared to those collected after 1975. Grasses such as *Agrostis capillaris* and *Festuca rubra*, but also ruderals like *Holcus* sp., have clearly increased in all regions, while among the common grasses only *Festuca ovina* has declined. A general decrease in frequency was found for most dwarf shrubs (e.g., *Erica* sp., *Genista* sp. and *Calluna vulgaris*), but also many herbaceous dicots were clearly less common in the more

384 recent than in the older plots, for example *Arnica montana* and *Viola canina*. Among the few
385 dicots that have increased in frequency are *Achillea millefolium* and both *Rumex acetosa* and
386 *R. acetosella*, representing species that are characteristic for more fertile or ruderalized
387 grasslands. On average, species with a decreasing trend had lower N_{EII} values than species
388 with an increasing trend. For bryophytes, we can rely only on the data from Great Britain;
389 here, in accordance with the results given in Table 3, most of the more common species
390 tended to decline over the years (e.g., *Dicranum scoparium*, *Pleurozium schreberi*), whereas
391 only *Rhytidiadelphus squarrosus* and *Scleropodium purum* appeared to increase in frequency.
392 Not reflected in Table 4 is the strong decline in many low-frequent *Violion* species that were
393 already rare before 1975 and have more or less completely disappeared since then.

Discussion

General discussion of explanatory variables

Plant species richness and composition are simultaneously affected by various factors. By carefully selecting one, well-defined vegetation type with specific habitat conditions we reduced the number of potentially confounding variables. Since *Violion* grasslands in the oceanic and suboceanic, lowland or hilly parts of Central and Western Europe are quite similar in their general species pool and composition (Ellenberg 1996; Peppler-Lisbach & Petersen 2001), this enables spatial comparisons of species richness data both within and between different countries. Nevertheless, to account for geographic effects, we used the latitude and longitude of each site in the models as surrogates for the climate conditions of temperature and precipitation, for which site-specific data over the time span of interest were largely unavailable. Latitude was significant for some species richness measures, especially in Great Britain where the climatic gradients are steeper than in the other two regions of North-Western Germany and the Netherlands.

Overall, the explanatory variables of primary importance for our study were (in approximate order) soil acidity, plot size, cumulative N deposition, and latitude. There is wide evidence for the strong effects of soil N availability and acidity on species composition (e.g., Ellenberg 1996) and richness (Grime 1979; Duprè *et al.* 2002). Due to the lack of measurements of the expected soil chemical drivers of biodiversity (e.g., pH, nutrient status) for the large majority of plots we had to rely on surrogate variables in the form of mean Ellenberg indicator values, which have been shown to perform well in the context of historical comparisons of vegetation data (see Diekmann 2003). Apart from being indispensable for such studies in the absence of soil chemical measurements, indicator values have the advantage that the plants ‘integrate’ the values of environmental variables that

fluctuate in time and space and cannot be determined by single measurements. The only chemical variables at least partly based on measurement data (and thereafter modelled) are cumulative N and S deposition. Although individual deposition values for NO_x^- and NH_4^+ as well as S compounds for the year 2000 were available, we used the total value for the regression models, because the ratio of, for example, deposited nitrate and ammonium has changed during the last decades (the former tends to decrease, the latter to increase), which made the estimation of deposition values for the separate measures in the past too uncertain.

Plot size was included in the regression models as a co-variable. Species richness increases with increasing area, and therefore it is hardly surprising that plot size in general was the second most important factor in the final models. This effect is even likely to be under-estimated, because most vegetation data in Germany and the Netherlands were sampled in the framework of phytosociological studies in which plots tend to be made larger in species-poor vegetation in order to include more taxa of interest (Chytrý 2001; Schuster & Diekmann 2003). This results in a flatter slope of the species richness-area regression line. The negative effect of plot size on bryophyte species richness found in Great Britain is difficult to explain, but one hypothesis could be that the time and attention devoted to bryophytes are relatively smaller in larger plots than in smaller plots that are easier to survey.

Management is another factor that may be driving species richness: it is known to have a strong effect on species composition and richness in grasslands (Bakker 1989). In non-alpine areas *Violion* grasslands are semi-natural, and the abandonment of grazing or mowing may result in a reduced species richness and an increase in the proportion of grasses at the expense of herbs, thereby ‘mimicking’ the effects of nitrogen deposition. Although we considered plots only if continued and uninterrupted management was indicated, the information provided in the literature was not sufficient to determine the intensity of the grazing / mowing for every plot; nevertheless, it is known that management of semi-natural grasslands in

Central Europe in general has declined (Ellenberg 1996). Changes in management, however, cannot be the only explanation for the observed decline of species richness. First, assuming that these changes have taken place in a similar manner in all European countries, species numbers today should also be similar (and lower than previously), which is not the case: in Denmark and especially Sweden with lower or much lower N deposition levels compared to Germany and the Netherlands, species richness and the proportion of forbs were higher or much higher. Second, if management intensity was declining with time, we would expect a closer correlation between species richness and year of sampling than between species richness and cuN_{dep} , which was not the case. Third, the general decrease in dwarf shrubs found in all regions is difficult to explain with the abandonment of management: these species are assumed to increase when grazing or hay-making cease. All this suggests that management may contribute to explaining the changes in species composition and richness, but is likely to be less important than cuN_{dep} .

The close correlation between cuN_{dep} and cuS_{dep} permitted us to use only one of the two variables in the multiple regression models. We chose to keep cuN_{dep} as it explained more of the variation in species richness and was more closely related to the Ellenberg values than cuS_{dep} . For this we suggest the following explanation: The soil pH of the *Violion* grasslands has always been very low, so that S deposition has little acidifying effect on the environment, and thereby has lesser impact on species richness. In contrast, N deposition adds considerably to the nitrogen availability in the soil, as reflected in the effect of cuN_{dep} on species numbers and N_{EII} . An observation suggesting that S deposition is not so important is that the plots from those regions that have experienced rather high doses of S deposition (the Harz mountains in Germany, Western Denmark and Sweden), but where low N deposition occurs, have relatively high species richness. In addition, if cumulative S deposition was the main factor driving the changes in species richness and composition, one would expect decreasing pH

values over time as reflected in declining mean R_{EII} values, for which there is no evidence (see below).

Ordination analysis

Whilst the use of multiple regressions allowed us to investigate changes in species richness through time, the ordination analysis demonstrated changes in the species composition that occurred largely independently of the number of species. Both types of analysis showed the strong impact of soil acidity and cuN_{dep} on the vegetation of the *Violion* grasslands.

The ordinations for all three countries suggested that the main gradient in species composition is linked to a combined gradient in soil acidity and nutrient availability (Table 2). Mean N_{EII} and especially mean R_{EII} were strongly negatively related to the sample plot scores along DCA axis 1; such negative relationships were also found for year of sampling and cuN_{dep} (except in the Netherlands), reflecting the chronic input of nitrogen over time. The results are, however, based on mean indicator values, and the actual pattern is somewhat more complicated. In Germany, the plots sampled in 2007 appear to have slightly lower N and R indicator scores than those collected in the 1980s and 1990s, at first sight suggesting a decreasing N availability during the last two to three decades. Given that the indicator values for N_{EII} and R_{EII} of the more frequent species in the *Violion* grasslands are strongly positively related ($r = 0.655$, $p < 0.001$, $n = 36$), we hypothesize the following scenario. Initially, the deposition of N leads to an addition of N to the soils and an increase in the number of N-demanding species. As the latter also tend to have higher R_{EII} values, both mean N_{EII} and mean R_{EII} increase, even if soil pH values may not have changed or may be changing very slowly. During the first years of N accumulation, the loss of typical stress-tolerant taxa in the grasslands may at least partly have been compensated by the addition of more competitive newcomers. More recently, the relative amount of NH_4^+ in deposition has increased (Kleijn *et*

al. 2008), resulting in increased acidification of the soil. This may have contributed to the decline in acid-sensitive species that at the same time are more N-demanding, leading to a drop in mean R_{EII} and mean N_{EII} values despite continued N deposition.

It is difficult to say why the resulting patterns in the Netherlands are not as clear as in the two other regions. One reason might be that the *Violion* grasslands in the country were already significantly affected by N deposition before 1939, meaning that considerable floristic changes already took place during a period not covered in the data set used here. In accordance with this interpretation, the relative frequencies of species favoured by N in the plots sampled before 1975 in the Netherlands are on average higher than the corresponding values in Germany and Great Britain, and those of 'N-sensitive' species on average lower. The earlier N deposition in the Netherlands may have been caused by the intensive agricultural land use causing relatively high emissions of N, especially ammonium, during the first half of the last century.

Effects of nitrogen addition and soil pH on species richness

The regression results clearly show that species richness in *Violion* grasslands is strongly negatively related to the accumulated N deposition (with the exception of the number of grasses in Germany and Great Britain). This effect is particularly pronounced in Great Britain where bryophyte richness also declines in relation to cuN_{dep} . The results thus support the findings of a spatial comparison across Great Britain (Stevens *et al.* 2004) and from experimental studies (e.g., Mountford *et al.* 1993; Carroll *et al.* 2003; Clark & Tilman 2008), all suggesting the negative effect of N deposition on phytodiversity, at least at higher deposition levels such as in the central and southern parts of Great Britain (see Fig. 3).

The above effect is mainly due to the strong decline in the number of dicot species, whereas the relative number of grasses (except in the Netherlands) has strongly increased.

Therefore, N deposition has favoured grasses such as *Agrostis capillaris* and *Festuca rubra* at the expense of forbs. Again, this is consistent with other observations in *Violion* grasslands made along a geographical deposition gradient in Great Britain (Stevens *et al.* 2006) and with results from N addition experiments (Carroll *et al.* 2003) where grasses were favoured by high N deposition levels. The same response, i.e., a change in vegetation composition in favour of grasses has been reported from experimental sites in other grasslands types (Bobbink 1991; Mountford *et al.* 1993; Wedin & Tilman 1996). One possible reason for this is that grasses are capable of more rapidly exploiting available nitrogen than forbs, resulting in faster growth and a suppression of dicot species of smaller stature (Peppler-Lisbach & Petersen 2001). Accordingly, species that are almost completely lacking from the recent 2007 plots from Germany, such as *Viola canina* and *Veronica officinalis*, are still relatively common in the 2007 plots from the low N-deposition countries Denmark and Sweden.

Soil pH appears to be even more important for species richness than N deposition, having a strong positive effect on both the absolute number of vascular plant species and the proportion of dicots; in contrast, the proportion of grasses decreases with increasing pH (see also Stevens *et al.* 2004). *Violion* grasslands occur on highly acidic soils tolerated by few acid-tolerant species, and any decrease in pH creates an environment that excludes more species, since the majority of species in the European flora have their ecological optima at higher pH values, resulting in hump-backed curves of species richness along the pH gradient (Schuster & Diekmann 2003). The deleterious effect of high soil acidity on most taxa is due to the high H^+ concentration and Al^{3+} toxicity (Peppler 1992; Houdijk *et al.* 1993; Kleijn *et al.* 2008). The pronounced differences in pH (as indicated by the mean R_{EII} values) between plots are mainly caused by original differences between the sites, and partly by temporal changes that have taken place. Although the deposition of acidifying substances has generally decreased during the last two decades, the enhanced NH_4^+ to NO_3^- ratio reported for recent

years may contribute to an acidification that, together with continued N addition, may be responsible for the ongoing decline in plant species richness.

Conclusions

In our paper, based on historical vegetation data from acidic grasslands, we for the first time provide evidence for the strong effects of cumulative N deposition on plant species composition and richness. The findings support the results of spatial gradient analyses and experimental studies in showing that N enrichment of grassland ecosystems is accompanied by a loss of species richness, especially dicots. Future research on the effects of atmospheric deposition on species richness should make use of the different approaches: time series data allows us to track the long-term changes in species composition and reflects the complexity of environmental variation, and therefore complement experimental plot methods that have the advantage of disentangling the effects of single environmental variables, such as soil nitrogen and pH, on the changes in the vegetation.

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References

- Aber JD, Goodale CL, Ollinger SV *et al.* (2003) Is nitrogen deposition altering the nitrogen status of northeastern forests? *Bioscience*, **53**, 375-389.
- Bakker JP (1989) *Nature management by grazing and cutting*. Geobotany 14. Kluwer, Dordrecht, 400 pp.
- Berge E, Bartnicki J, Olendrzynski K, Tsyro, SG (1999) Long-term trends in emissions and transboundary transport of acidifying air pollution in Europe. *Journal of Environmental Management*, **57**, 31-50.
- Bobbink R (1991) Effects of nutrient enrichment in Dutch chalk grasslands. *Journal of Applied Ecology*, **28**, 28-41.
- Bobbink R, Lamers LPM (2002) Effects of increased nitrogen deposition. In: *Air pollution and plant life*, 2nd ed. (eds Bell JND, Treshow M), pp 201-235. JohnWiley & Sons, Chichester.
- Bobbink R, Hornung M, Roelofs JG (1998) The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, **86**, 717-738.
- Bouwman AF, van Vuuren DP, Derwent RG, Posch M (2002) A global analysis of acidification and eutrophication of terrestrial ecosystems. *Water, Air and Soil Pollution*, **141**, 348-382.
- Carroll JA, Caporn SJM, Cawley L, Read DJ, Lee JA (1999) The effect of increased atmospheric nitrogen on *Calluna vulgaris* in upland Britain. *New Phytologist*, **141**, 423-431.
- Carroll JA, Caporn SJM, Johnson D, Morecroft MD, Lee JA (2003) The interaction between plant growth, vegetation structure and soil processes in semi-natural acidic and

593 calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen
594 deposition. *Environmental Pollution*, **121**, 363-376.

595 Chytrý M (2001) Phytosociological data give biased estimates of species richness. *Journal of*
596 *Vegetation Science*, **12**, 439-444.

597 Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition
598 to prairie grasslands. *Nature*, **451**, 712-715.

599 Crawley MJ, Johnston AE, Silvertown J *et al.* (2005) Determinants of species richness in the
600 park grass experiment. *American Naturalist*, **165**, 179-192.

601 Dentener F, Drevet J, Lamarque I *et al.* (2006) Nitrogen and sulphur deposition on regional
602 and global scale: A multimodel evaluation. *Global Biogeochemical Cycles*, **20**,
603 GB4003, doi:10.1029/2005GB002672

604 Diekmann M (2003) Species indicator values as an important tool in applied plant ecology - a
605 review. *Basic and Applied Ecology*, **4**, 493-506.

606 Diekmann M, Brunet J, Rühling Å, Falkengren-Grerup U (1999) Effects of nitrogen
607 deposition: results of a temporal-spatial analysis of deciduous forests in South Sweden.
608 *Plant Biology*, **1**, 471-481.

609 Diekmann M, Duprè C (1997) Acidification and eutrophication of deciduous forests in
610 northwestern Germany demonstrated by indicator species analysis. *Journal of*
611 *Vegetation Science*, **8**, 855-864.

612 Diekmann M, Falkengren-Grerup U (2002) Prediction of species response to atmospheric
613 nitrogen deposition by means of ecological measures and life history traits. *Journal of*
614 *Ecology*, **90**, 108-120.

615 Dierschke H (1994) *Pflanzensoziologie*. Eugen Ulmer, Stuttgart, 683 pp.

616 Duprè C, Wessberg C, Diekmann M (2002) Species richness in deciduous forests: Effects of
617 species pools and environmental variables. *Journal of Vegetation Science*, **13**, 505-516.

618 Ellenberg H (1996) *Vegetation Mitteleuropas mit den Alpen. 5th ed.* Eugen Ulmer, Stuttgart.

619 Ellenberg H, Weber HE, Düll R, Wirth V, Werner W, Paulissen D (1992) Zeigerwerte von
620 Pflanzen in Mitteleuropa. 2nd ed. *Scripta Geobotanica*, **18**, 1-248.

621 EMEP (Co-operative Programme for Monitoring and Evaluation of the Long-Range
622 Transmission of Air pollutants in Europe) (2000) *Transboundary acidification and*
623 *eutrophication in Europe*. Research Report no. **101**, Norwegian Meteorological
624 Institute.

625 Falkengren-Grerup U, Brunet J, Diekmann M (1998) Nitrogen mineralisation in deciduous
626 forest soils in south Sweden in gradients of soil acidity and deposition. *Environmental*
627 *Pollution*, **102 (Suppl. 1)**, 415-420.

628 Fangmeier A, Hadwiger-Fangmeier A, van der Eerden L, Jäger H-J (1994) Effects of
629 atmospheric ammonia on vegetation – a review. *Environmental Pollution*, **86**, 43-82.

630 Fowler D, Smith R, Muller J, Cape JN, Sutton M, Erisman JW, Fagerli H (2007) Long term
631 trends in sulphur and nitrogen deposition in Europe and the cause of non-linearities.
632 *Water, Air and Soil Pollution: Focus*, **7**, 41-47.

633 Galloway JN, Townsend AR, Erisman JW *et al.* (2008) Transformation of the nitrogen cycle:
634 recent trends, questions and potential solutions. *Science*, **320**, 889-892.

635 Goodall DW (1952) Quantitative aspects of plant distribution. *Biological Review*, **27**, 194-
636 245.

637 Gough L, Osenberg CW, Gross KL, Collins SL (2000) Fertilization effects on species density
638 and primary productivity in herbaceous plant communities. *Oikos*, **89**, 428-439.

639 Grime JP (1979) *Plant strategies and vegetation processes*. John Wiley, Chichester.

640 Gruber N, Galloway JN (2008) An earth-system perspective of the global nitrogen cycle.
641 *Nature*, **451**, 293-296.

642 Houdijk ALFM, Verbeek PJM, van Dijk HFG, Roelofs JGM (1993) Distribution and decline
 643 of endangered herbaceous heathland species in relation to the chemical composition of
 644 the soil. *Plant and Soil*, **148**, 137-143.

645 Koperski M, Sauer M, Braun W, Gradstein SR (2000) Referenzliste der Moose Deutschlands.
 646 *Schriftenreihe für Vegetationskunde*, **34**, 1-519.

647 Köchy M, Bråkenhielm S (2007) Separation of effects of moderate N deposition from natural
 648 change in ground vegetation of forests and bogs. *Forest Ecology and Management*,
 649 **255**, 1654-1663.

650 Lorenz M, Nagel HD, Granke O, Kraft P (2008) Critical loads and their exceedances at
 651 intensive forest monitoring sites in Europe. *Environmental Pollution*, **155**, 426-435.

652 Millenium Ecosystem Assessment (2005) *Ecosystem and human well-being: current state and*
 653 *trends, Vol. 1*. Island Press, Washington, 948 pp.

654 Mountford JO, Lakhani KH, Kirkham FW (1993) Experimental assessment of the effects of
 655 nitrogen addition under hay-cutting and aftermath grazing on the vegetation of
 656 meadows on a Somerset peat moor. *Journal of Applied Ecology*, **30**, 321-332.

657 Mueller-Dombois D, Ellenberg H (1974) *Aims and methods of vegetation ecology*. Wiley,
 658 New York.

659 Peppler C (1992) Die Borstgrasrasen Westdeutschlands. *Dissertationes Botanicae*, **193**, 1-
 660 402.

661 Peppler-Lisbach C, Petersen J (2001) *Synopsis der Pflanzengesellschaften Deutschlands Heft*
 662 *8. Calluno-Ulicetea (G3), Teil 1: Nardetalia strictae. Borstgrasrasen*. Göttingen, 117
 663 pp.

664 Phoenix GK, Booth RE, Leake JR, Read DJ, Grime JP, Lee JA (2003) Effects of enhanced
 665 nitrogen deposition and phosphorus limitation on nitrogen budgets of semi-natural
 666 grasslands. *Global Change Biology*, **9**, 1309-1321.

667 Pieterse G, Bleeker A, Vermeulen AT, Wu Y, Erisman JW (2007) High resolution modelling
 668 of atmosphere-canopy exchange of acidifying and eutrophying components and carbon
 669 dioxide for European forests. *Tellus*, **59B**, 412-424.

670 Rodwell JS (1992) *Grasslands and montane communities*. University Press, Cambridge.

671 Roem WJ, Klees H, Berendse F (2002) Effects of nutrient and acidification on plant species
 672 diversity and seed germination in heathland. *Journal of Applied Ecology*, **39**, 937-948.

673 Sala OE, Chapin FS, Armesto JJ *et al.* (2000) Global biodiversity scenario for the year 2100.
 674 *Science*, **287**, 1770-1774.

675 Schuster B, Diekmann M (2003) Changes in species density along the soil pH gradient –
 676 evidence from German plant communities. *Folia Geobotanica*, **38**, 367-379.

677 Sheppard SC (2002) Three approaches to define the ecotoxicity threshold for atmospheric
 678 ammonia. *Canadian Journal of Soil Science*, **82**, 341-354.

679 Silvertown J, Poulton P, Johnston E, Edwards G, Heard M, Biss PM (2006) The Park Grass
 680 experiment 1856-2006: its contribution to ecology. *Journal of Ecology*, **94**, 801-814.

681 Smart SM, Ashmore MR, Hornung M *et al.* (2004) Detecting the signal of atmospheric N
 682 deposition in recent national-scale vegetation change across Britain. *Water, Air and Soil*
 683 *Pollution: Focus*, **4**, 269-278.

684 Stevens CJ, Dise NB, Mountford JO, Gowing DJG (2004) Impacts of nitrogen deposition on
 685 the species richness of grasslands. *Science*, **303**, 1876-1879.

686 Stevens CJ, Dise NB, Gowing DJG, Mountford JO (2006) Loss of forb diversity in relation to
 687 nitrogen deposition in the UK: regional trends and potential controls. *Global Change*
 688 *Biology*, **12**, 1823-1833.

689 ter Braak CJF, Smilauer P (2002) *CANOCO Reference manual and CanoDraw for Windows*
 690 *User's guide: Software for Canonical Community Ordination (version 4.5)*.
 691 Microcomputer Power Ithaca, NY, 500 pp.

- 692 Thimonier A, Dupouey JL, Timbal J (1992) Floristic changes in the herb-layer vegetation of a
693 deciduous forest in the Lorraine plain under the influence of atmospheric deposition.
694 *Forest Ecology and Management*, **55**, 149-167.
- 695 Thimonier A, Dupouey JL, Bost F, Becker M (1994) Simultaneous eutrophication and
696 acidification of a forest ecosystem in North-East France. *New Phytologist*, **126**, 533-
697 539.
- 698 Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM, Webb DA (1964-1980)
699 *Flora Europaea*. University Press, Cambridge.
- 700 Vitousek PM, Aber JD, Howarth RW *et al.* (1997) Human alteration of the global nitrogen
701 cycle: sources and consequences. *Ecological Applications* **7**, 737-750.
- 702 Wedin DA, Tilman D (1996) Influence of nitrogen loading and species composition on the
703 carbon balance of grasslands. *Science*, **274**, 1720-1723.
- 704 Wisskirchen R, Haeupler H (1998) *Standardliste der Farn- und Blütenpflanzen Deutschlands*.
705 Eugen Ulmer, Stuttgart, 765 pp.

Table 1. Survey of vegetation data given separately for the five countries from where plots were compiled. The number of plots and the ranges of values for the variables are given. The deposition levels of N and S for the year 2000 are abbreviated as N_{dep} and S_{dep}. The mean Ellenberg values denote the plot averages of the species' indicator values for light (L_{EII}), soil moisture (F_{EII}), pH (R_{EII}) and nitrogen (N_{EII}).

	No. of	Years of	Plot size	Altitude	N _{dep} in 2000	S _{dep} in 2000	Mean	Mean	Mean	Mean
Country	plots	sampling	[m ²]	[m a.s.l.]	[eq ha ⁻¹ y ⁻¹]	[eq ha ⁻¹ y ⁻¹]	L _{EII}	F _{EII}	R _{EII}	N _{EII}
Germany	392	1940-2007	1-100	0.5-812	707-2719	361-914	5.7-8.0	3.4-8.5	1.0-5.1	1.5-4.9
Netherlands	144	1939-2006	0.4-750	0.1-59	728-2554	518-972	6.6-8.0	4.3-7.3	1.9-5.7	1.8-4.3
Great Britain	541	1960-2003	0.25-4	9-700	130-868	78-910	3.8-7.7	4.0-8.0	1.6-5.4	1.7-5.7
Denmark	17	2007	4	4-74	1030-1192	437-474	6.5-7.2	4.0-5.5	2.4-3.8	2.2-3.4
Sweden	20	2007	4	64-100	558-563	319-327	6.6-7.1	4.4-5.6	2.3-3.5	2.1-3.4

711 Table 2. Relationship between sample plot scores of DCA axes 1 and 2 and environmental variables: year of sampling, cumulative N and S
712 deposition (cuN_{dep} and cuS_{dep}) and the mean Ellenberg values for light (L_{EII}), soil moisture (F_{EII}), pH (R_{EII}) and nitrogen (N_{EII}). Pearson
713 correlation coefficients are given, with significant values bolded. a = p < 0.01, b = p < 0.05, all others p < 0.001. The eigenvalues for the DCA
714 ordination axes 1 and 2 were 0.45 and 0.22 (Germany), 0.39 and 0.17 (the Netherlands), and 0.33 and 0.21 (Great Britain), respectively.
715

Country	DCA axis	Gradient length	Year of sampling	CuN _{dep}	CuS _{dep}	Mean L _{EII}	Mean F _{EII}	Mean R _{EII}	Mean N _{EII}
Germany	1	4.34	-0.611	-0.329	-0.621	0.283	0.265	-0.762	-0.864
	2	2.35	0.570	0.462	0.529	-0.560	-0.155^a	-0.446	-0.090
The Netherlands	1	3.28	0.139	-0.028	0.152	-0.072	-0.207^a	0.896	0.673
	2	2.37	-0.084	-0.204^b	-0.059	0.110	-0.416	0.138	-0.098
Great Britain	1	4.00	-0.704	-0.365	-0.232	-0.307	0.172	-0.778	-0.718
	2	2.55	0.181	0.323	-0.268	0.508	0.333	-0.161	-0.012

Table 3. Significant variables in stepwise (multiple linear) regressions of species richness in *Violion* grasslands in Germany (D, n = 240), The Netherlands (NL, n = 106) and Great Britain (GB, n = 531), including geographical variables (latitude, longitude, altitude), plot size, local environmental descriptors (mean Ellenberg values for light, soil moisture and pH) and cumulative N deposition (CuN_{dep}). The % measures represent the proportion of the number of species of a particular group out of the total number of species. The analysis was made with forward selection of variables ($\alpha = 0.05$), and the variables remaining in the models are shown with their t-values and sign. Empty cells reflect variables that did not significantly affect the species richness measure in the respective country; # denotes that the variables in question were not entered into the model (to avoid multicollinearity). The last two columns of the table give the results of stepwise multiple regression when using CuS_{dep} instead of CuN_{dep} in the model; here, only the R^2_{adj} of the total model and the t value of CuS_{dep} are shown.

Dependent		Latitude	Longi-	Altitude	Plot	Mean	Mean	Mean	CuN_{dep}	R^2_{adj}	CuS_{dep}	R^2_{adj}
Variable	Country		tude		size	L_{EII}	F_{EII}	R_{EII}				(CuS_{dep})
No. of vascular	D	-4.04	3.09	#	5.79	2.61		12.4	-2.09	65.8	-2.76	66.2
plants	NL	2.37			7.18	-2.07		11.0	-3.90	63.8		57.3
	GB	8.82	#		7.90		2.91	13.2	-7.90	55.8	-5.81	53.5
No. of dicots	D	-4.24	4.47	#	4.69	3.04	-4.82	12.2	-3.40	66.0	-3.37	67.1
	NL				3.97		-2.37	9.70	-2.60	53.6	-5.5	50.8

	GB	8.44	#	2.90	6.77		-2.68	15.3	-7.44	59.7		58.2
No. of grasses	D	-5.87	2.19	#	4.54	4.07		7.49		47.4		47.4
	NL	2.20			5.46				-3.24	24.9		21.3
	GB	6.17	#		4.62	4.08	5.11	4.86		20.7		20.7
% species no. of dicots	D	-5.09		#			-4.45	8.70	-5.43	51.1	-4.75	51.1
	NL						-4.68	4.94		28.7		28.7
	GB	5.19	#		5.15	-2.34	-9.09	8.95	-4.43	49.1	-4.73	49.4
% species no. of grasses	D			#	-2.76	2.12		-7.08	6.81	47.5	6.43	46.4
	NL					2.89		-6.66		33.1		33.1
	GB		#		-5.66	5.66	2.94	-4.77	12.2	37.4	9.22	36.4
No. of bryophytes	GB	4.46	#	5.29	-2.90	-9.23			-6.83	52.6	-5.46	51.1

Table 4. Relative frequency of species (in %) in the data sub-sets from Germany, the Netherlands and Great Britain, for plots sampled before (old) and after (new) 1975. Only the most common taxa are shown. Within a life form, the species are ordered according to their general patterns of change in frequency over time (increasing; stable, unclear or differing between regions; decreasing). Bryophyte species are shown only for Great Britain. N_{EII} = Ellenberg value for nitrogen.

Species		Germany		Great Britain		The Netherlands	
	N_{EII}	old	new	old	new	old	new
No. of plots		177	215	201	340	65	79
Grasses							
<i>Agrostis capillaris</i>	4	60	87	60	98	63	67
<i>Festuca rubra</i>	x	25	65	13	19	74	87
<i>Holcus lanatus</i>	4	19	17	7	28	32	44
<i>Holcus mollis</i>	3	2	11	2	10	5	5
<i>Poa pratensis</i> s.l.	2	15	18	6	7	9	11
<i>Agrostis canina</i>	2	12	3	39	3	39	48
<i>Anthoxanthum odoratum</i>	x	44	45	49	51	35	38
<i>Danthonia decumbens</i>	2	60	31	3	22	91	65
<i>Deschampsia flexuosa</i>	3	28	73	63	34	29	38
<i>Molinia caerulea</i>	1	42	12	5	20	51	61
<i>Nardus stricta</i>	2	59	78	58	34	40	34
<i>Festuca ovina</i>	1	66	53	90	83	9	0

752	Herbaceous dicots and monocots other than grasses							
753	<i>Achillea millefolium</i>	5	14	37	7	14	12	14
754	<i>Rumex acetosa</i>	6	19	46	20	29	5	20
755	<i>Rumex acetosella</i>	2	13	27	6	2	29	54
756								
757	<i>Campanula rotundifolia</i>	2	16	35	13	14	5	1
758	<i>Carex pilulifera</i>	3	43	46	22	23	62	57
759	<i>Galium saxatile</i>	3	36	80	82	79	68	77
760	<i>Hypochaeris radicata</i>	1	31	6	0	10	42	39
761	<i>Lotus corniculatus</i>	3	23	12	6	16	17	13
762	<i>Luzula campestris</i> s.l.	2	56	64	46	50	62	62
763	<i>Plantago lanceolata</i>	x	24	28	8	13	19	8
764	<i>Polygala vulgaris</i>	2	13	13	0	2	8	11
765	<i>Potentilla erecta</i>	2	75	63	50	62	82	58
766	<i>Solidago virgaurea</i>	4	7	14	0	2	11	4
767	<i>Succisa pratensis</i>	2	42	21	4	7	6	5
768	<i>Veronica chamaedrys</i>	x	9	27	1	10	-	-
769	<i>Veronica officinalis</i>	4	22	27	4	4	9	13
770								
771	<i>Arnica montana</i>	2	30	24	-	-	17	10
772	<i>Dactylorhiza maculata</i>	2	11	3	1	1	14	1
773	<i>Hieracium</i> subg. <i>Pilosella</i>	2	37	26	5	4	44	23
774	<i>Juncus squarrosus</i>	1	27	6	54	10	26	14
775	<i>Polygala serpyllifolia</i>	2	2	1	10	7	17	8
776	<i>Prunella vulgaris</i>	7	16	5	6	7	12	9
777	<i>Scorzonera humilis</i>	2	11	1	-	-	2	0
778	<i>Viola canina</i>	2	20	13	1	0	26	14
779								

780	Dwarf shrubs							
781	<i>Empetrum nigrum</i>	2	16	1	1	4	2	6
782	<i>Vaccinium myrtillus</i>	3	12	20	56	27	3	1
783	<i>Vaccinium vitis-idaea</i>	1	1	5	17	1	2	0
784								
785	<i>Calluna vulgaris</i>	1	77	28	21	21	71	62
786	<i>Erica cinerea</i>	1	-	-	8	4	-	-
787	<i>Erica tetralix</i>	2	54	6	4	2	45	27
788	<i>Genista anglica</i>	2	38	5	-	-	32	17
789	<i>Genista pilosa</i>	1	18	2	-	-	8	0
790								
791	Bryophytes							
792	<i>Rhytidiadelphus squarrosus</i>				70	81		
793	<i>Scleropodium purum</i>				12	21		
794								
795	<i>Dicranum scoparium</i>				52	11		
796	<i>Hylocomium splendens</i>				51	22		
797	<i>Hypnum cupressiforme</i> s.l.				61	45		
798	<i>Lophocolea bidentata</i>				49	5		
799	<i>Pleurozium schreberi</i>				62	11		
800	<i>Ptilidium ciliare</i>				42	0		

Figure legends

Fig. 1. DCA ordination diagrams showing the sample plot scores along axes 2 and 1 for the three data sub-sets from Germany, the Netherlands and Great Britain. The symbols refer to the different decades in which the plots were sampled: $\diamond$: -1949. $\circ$: -1959. $\bullet$: -1969. $+$: -1979. $\times$: -1989. $*$: -1999. $\square$: 2000-.

Fig. 2. Effects of cumulative N deposition (in $\text{mol ha}^{-1} \text{y}^{-1} * 1000$) on the species number of vascular plants and bryophytes (only Great Britain) in the three regional data sub-sets; species richness values were corrected for plot size (i.e., residuals were used). Germany: $R^2_{\text{adj.}} = 0.098$, $n = 241$; the Netherlands: $R^2_{\text{adj.}} = 0.125$, $n = 106$; Great Britain - vascular plants: $R^2_{\text{adj.}} = 0.234$, $n = 532$, bryophytes: $R^2_{\text{adj.}} = 0.159$; all $p < 0.001$.

Fig. 3. Regression tree of the relationship between species richness of vascular plants (corrected for plot size) and the variables R_{ElI} , latitude and cumulative N deposition (cuN_{dep}) for the data sub-set from Great Britain ($n = 532$).

Fig. 4. Mean proportion of grass species on the total number of vascular plants in different decades since 1940. The continuous line shows the results for Germany (D), the dashed line are those for the Netherlands (NL). For Great Britain (GB), mean values are given for those decades for which vegetation data was available, separately for plots from the south ($\circ$, -s) ($< 56.1^\circ \text{N}$, see Fig. 3) and the north ($\bullet$, -n). For comparison, also the mean values for the 2007 sample plots from Sweden ($\square$, S) and Denmark ($*$, Dk) are given.

824 Appendix 1. Correction factors used for the calculation of values of cumulative nitrogen (N)
 825 and sulphur (S) deposition since 1939.

826

	1939-49	1950-59	1960-69	1970-79	1980-89	1990-99	2000-07
N	0.1	0.5	0.9	1.3	1.1	1	1
S	0.8	1.4	1.7	1.8	1.6	1.4	0.8

827

828 Electronic appendix. Sources of vegetation data used for the analyses, including the number
 829 of sample plots, the country of origin, and the year of sampling.
 830

Reference	No.	Country	Year
Birks, H.J.B. 1973. Past and present vegetation of the Isle of Skye. A paleoecological study. Cambridge University Press, Cambridge.	31	Great Britain	1973
Bruehlheide, H. 1995. Die Grünlandgesellschaften des Harzes und ihre Standortbedingungen. Dissertat. Botan. 244: 1-338.	78	Germany	1991
Buchwald, K. 1940. Die nordwestdeutschen Heiden. Diss. Univers. Heidelberg.	69	Germany	1940
Chadwick, M. J. 1960. <i>Nardus stricta</i> L. J. Ecol. 48: 255-267.	10	Great Britain	1960
Dierschke, H. 1979. Die Pflanzengesellschaften des Holtumer Moores und seiner Randgebiete (Nordwest-Deutschland). Mitt. Flor.-soz. Arbeitsgem. N.F. 21: 111-143.	11	Germany	1979
Dierschke, H. & Tüxen, R. 1975. Die Vegetation des Langholter- und Rhader Meeres und seiner Randgebiete. Mitt. Flor.-soz. Arbeitsgem. N. F. 18: 157-202.	1	Germany	1975
Duprè, C. & Diekmann, M. Unpublished data.	17	Denmark	2007
	60	Germany	2007
	20	Sweden	2007
Eddy, A., Welch, D. & Raves, M. 1968. The vegetation of the Moor Hus National Nature Reserve in the Northern Pennines, England. Vegetatio 16: 239-284.	25	Great Britain	1968
Hennekens, S. 2007. SYNBIOSIS-Datenbank, Wageningen, Niederlande.	135	The Netherlands	1936-2006
Horst, K. 1964. Klima und Bodenfaktoren in Zwergstrauch- und Waldgesellschaften des Naturschutzparks Lüneburger Heide. Natursch. Landschaftspfl. Nieders. 2. Hannover.	7	Germany	1964
Huntley, B. 1978. The past and present vegetation of the Caenlochan	103	Great Britain	1978

National Nature Reserve, Scotland. New Phytol. 83: 213-283.			
Jahns, W. 1969. Erläuterungen zu den Kartierungen der realen Vegetation verschiedener Talquerschnitte von Hamme und Wümme. Mskr. Bundesanstalt f. Vegetationskunde, Naturschutz u. Landschaftspfl. Bonn - Bad Godesberg.	23	Germany	1969
Klement, O. 1953. Die Vegetation der Nordseeinsel Wangerooge. Veröff. Inst. Meereskd. Bremerhaven 11: 279-379.	5	Germany	1953
Peppler, C. 1992. Die Borstgrasrasen (Nardetalia) Westdeutschlands. Dissertat. Botan. 193: 1-402	41	Germany	86-89
Pott, R. & Hüppe, J. 1991. Die Hudelandschaften Nordwestdeutschlands. Westfälisches Museum für Naturkunde, Münster.	8	Germany	1989
Preisling, E. 1950. Nordwestdeutsche Borstgras-Gesellschaften. Mitt. Flor.- soz. Arbeitsgem. N. F. 2: 33-41.	21	Germany	1950
Raabe, E. & Saxen, W. 1955. Über <i>Arnica montana</i> und den <i>Nardus</i> - Rasen. Mitt. Arbeitsgem. Flor. Schlesw.-Holst. Hamburg 5: 185-210.	36	Germany	1955
Stevens, C.J., Dise, N.B., Mountford, J.O. & Gowing, D.J. 2004. Impacts of nitro-gen deposition on the species richness of grasslands. Science 303: 1876-1879.	340	Great Britain	2003
Tüxen, R. 1962. Der Maujahn. Veröff. Geobot. Inst. ETH, Stiftung Rübel 37: 267-302.	4	Germany	1962
Tüxen, R. 1974. Die Haselünner Kuhweide. Mitt. Flor.-soz. Arbeitsgem. N. F. 17: 69-102.	17	Germany	1974
Walther, K. 1950. Die Pflanzengesellschaften im Grundwasserschadengebiet Lathen-Dörpen am Dortmund-Ems- Seitenkanal. Mitt. Thür. Bot. Ges. Beiheft 2. Weimar.	6	Germany	1950
Walther, K. 1977. Die Vegetation der Gemeindeweide Fuhlkarren bei Meetschow (Kr. Lüchow-Dannenberg). Mitt. Flor.-soz. Arbeitsgem. N. F. 19/20: 253-256.	10	Germany	1977
Walther, K. 1986. Die Vegetation des Maujahn 1984. Tuexenia 6: 145- 193.	2	Germany	1984

Welch, D. 1966. Biological flora of the British Isles, <i>Juncus squarrosus</i> L. J. Ecol. 54: 535-548.	7	Great Britain	1966
Welch, D. 1967. Communities containing <i>Juncus squarrosus</i> in Upper Teesdale, England. Vegetatio 14: 229-240.	67	Great Britain	1967
Wittig, B. 1990. Flora und Vegetation des Holtumer Moores (Landkreis Verden) - aktueller Zustand und langfristige Veränderung. Unveröff. Diplomarb., Univ. Göttingen.	6	Germany	1988

831

836 Figure 3

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