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

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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 1114 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 subsets 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.

Keywords: bryophytes, soil acidity, sulphur deposition, vascular plants, *Violion* grasslands

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Introduction

Atmospheric deposition of reactive nitrogen (N) 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).

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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. Mountford 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, e.g. 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 nonpermanent 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, nonpermanent plot data may be biased, if they, for example, are not balanced in the sense that either different regions or different subcommunities 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?

Materials and methods

Vegetation data

We compiled historical and recent vegetation data from acid grasslands located in the oceanic to suboceanic 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; Peppler-Lisbach & Petersen, 2001), a semi-natural grassland type on nutrient-poor, unfertilized, acidic soils, managed by extensive grazing or, more rarely, mowing. Typical species include grasses such as *Nardus stricta*, *F. ovina* and *A. capillaris*, herbaceous dicots (*Arnica montana*, *G. 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 supporting information). In total, data from 1114 sample plots were compiled, spanning a time period of almost 70 years from 1939 to 2007 (Table 1).

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

We then constructed a second matrix with environmental data, first entering information on the geogra-

Table 1 Survey of vegetation data given separately for the five countries from where plots were compiled

Country	No. of plots	Years of sampling	Plot size (m²)	Altitude (m a.s.l.)	N_{dep} in 2000 (eq ha⁻¹ yr⁻¹)	S_{dep} in 2000 (eq ha⁻¹ yr⁻¹)	Mean L_{EII}	Mean F_{EII}	Mean R_{EII}	Mean 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
The 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

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}).

phical location (longitude, latitude, and altitude) and size of plots. As only the most recent sources contained data on environmental and soil variables, we used mean Ellenberg species indicator values (not cover-weighted) as surrogates (Ellenberg et al., 1992) for the level of light (L_{EII}), soil moisture (F_{EII}), soil pH (R_{EII}), and soil N (N_{EII}, often interpreted as general soil fertility) for all plots. Ellenberg values, a ranking system of ecological niche preferences based on known plant characteristics, have successfully been applied in many ecological studies across Central and Western Europe (see review by Diekmann, 2003) and have also been used to examine spatial patterns and temporal changes of vegetation in response to acid or N deposition (Thimonier et al., 1992; Diekmann & Duprè, 1997). In our study, mean Ellenberg values were calculated in two ways, using all species (vascular plants and bryophytes), or vascular plants only. For sample plots collected in 2007 from Germany, Denmark and Sweden, the correlation between the mean Ellenberg 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 ($N_{dep}2000$) 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 A). 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: $N_{dep}2000 \times (0.1 \times 11 \text{ years}) + N_{dep}2000 \times (0.5 \times 10 \text{ years}) + N_{dep}2000 \times (0.9 \times 10 \text{ years}) + N_{dep}2000 \times (1.3 \times 4 \text{ years})$.

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 subsets 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 programme 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 subsets, altitude in the subset from the Netherlands). To check for multicollinearity, we correlated all variables to each other. In all country, subsets the mean values for N_{Ell} and R_{Ell} were highly positively correlated ($r = 0.67$–0.77). We decided to use mean R_{Ell} instead of mean N_{Ell}, because (1) the variation in species richness explained by the multiple models including mean R_{Ell} and the R^2_{adj} in simple regressions of species richness on mean R_{Ell} 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_{Ell} or mean N_{Ell} 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 subset, 'altitude' (r with latitude $= -0.81$), and, in the English subset, '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 subset 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 programme R.

Results

Overview

The size of the sampled plots varied considerably, ranging from 0.25 to 750 m^2 (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 standardized 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 Nether-

Table 2 Relationship between sample plot scores of DCA axes 1 and 2 and environmental variables: year of sampling, cumulative N and S deposition (cuN_{dep} and cuS_{dep}) and the mean Ellenberg values for light (L_{Ell}), soil moisture (F_{Ell}), pH (R_{Ell}) and nitrogen (N_{Ell})

Country	DCA axis	Gradient length	Year of sampling	CuN_{dep}	CuS_{dep}	Mean L_{Ell}	Mean F_{Ell}	Mean R_{Ell}	Mean N_{Ell}
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

Pearson 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 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.

DCA, detrended correspondence analysis; N, nitrogen; S, sulphur.

lands. The mean Ellenberg values L_{Ell} and F_{Ell} were mostly in the range of 5.5–8 and 4–7.5, respectively, indicating the relatively exposed and dry to mesic environment of the grasslands. The mean values for R_{Ell} and N_{Ell} were between 2 and 4 in most cases, but lower than 2 in 66 plots for R_{Ell} and in 76 plots for N_{Ell}, 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_{Ell} and F_{Ell} decreased slightly with the year of sampling ($R^2_{adj} = 0.075$ and 0.102, respectively, $P < 0.001$), mean R_{Ell} 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_{Ell} that increased considerably over the years ($R^2_{adj} = 0.223$, $P < 0.001$).

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_{Ell} and N_{Ell}, 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_{Ell} and L_{Ell} were more closely correlated to the scores along DCA axis 2 than for axis 1, with a maximum $r = 0.56$ for L_{Ell} in the German data subset. The year of sampling, cuN_{dep} and cuS_{dep} were only weakly correlated with the DCA axes in the Dutch data subset. 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 Fig. 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.

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
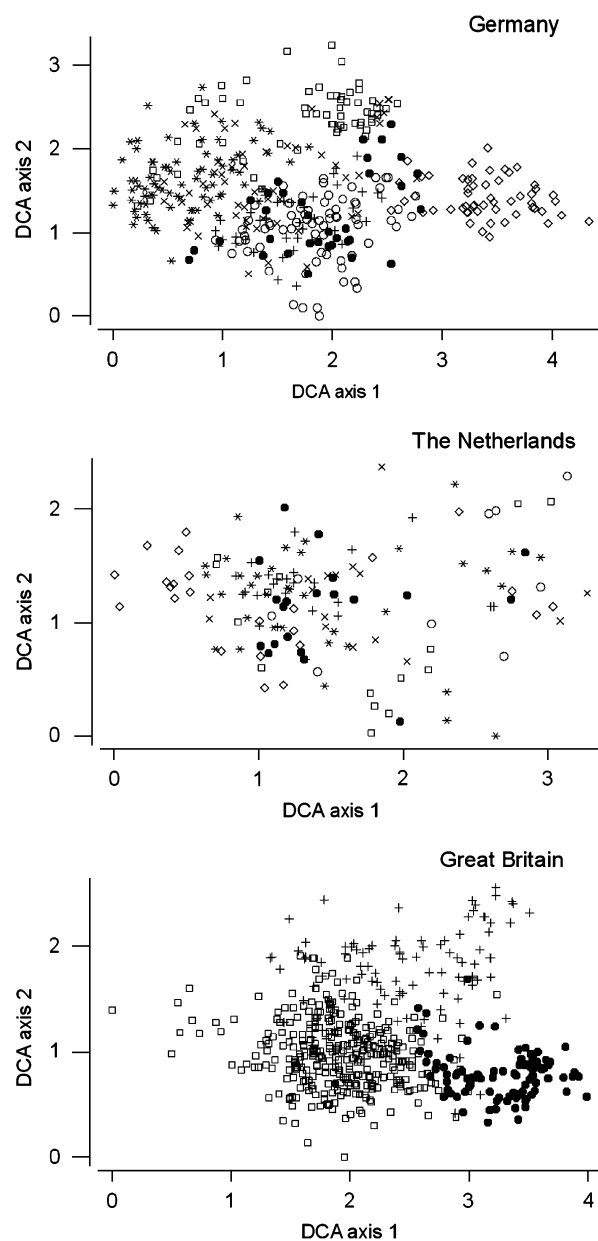

Fig. 1 Detrended correspondence analysis (DCA) ordination diagrams showing the sample plot scores along axes 2 and 1 for the three data subsets from Germany, the Netherlands and Great Britain. The symbols refer to the different decades in which the plots were sampled: ◇,-1949; ○,-1959; ●,-1969; +,-1979; x,-1989; *,-1999; □,-2000.

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 eight variables for the data subset from the Netherlands and seven 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 $R^2_{adj} = 0.65$) 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 $R^2_{adj} = 0.21$).

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_{Ell}, 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_{Ell}, whereas a negative correlation was observed for the proportion of grasses. In comparison, the effects of mean L_{Ell} and F_{Ell} were weaker and nonconsistent across the different measures of species richness and countries. In all data sets, however, the proportion of dicots was negatively associated with mean F_{Ell}, and, in Great Britain, there was a strong negative effect of mean L_{Ell} on the number of bryophytes.

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 subset. 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

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 and altitude), plot size, local environmental descriptors (mean Ellenberg values for light, soil moisture and pH) and cumulative N deposition (cuN_{dep})

Dependent variable	Country	Latitude	Longitude	Altitude	Plot size	Mean L_{Ell}	Mean F_{Ell}	Mean R_{Ell}	CuN_{dep}	R^2_{adj}	CuS_{dep}	R^2_{adj} (CuS_{dep})
No. of vascular plants	D	−4.04	3.09	*	5.79	2.61		12.4	−2.09	65.8	−2.76	66.2
	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

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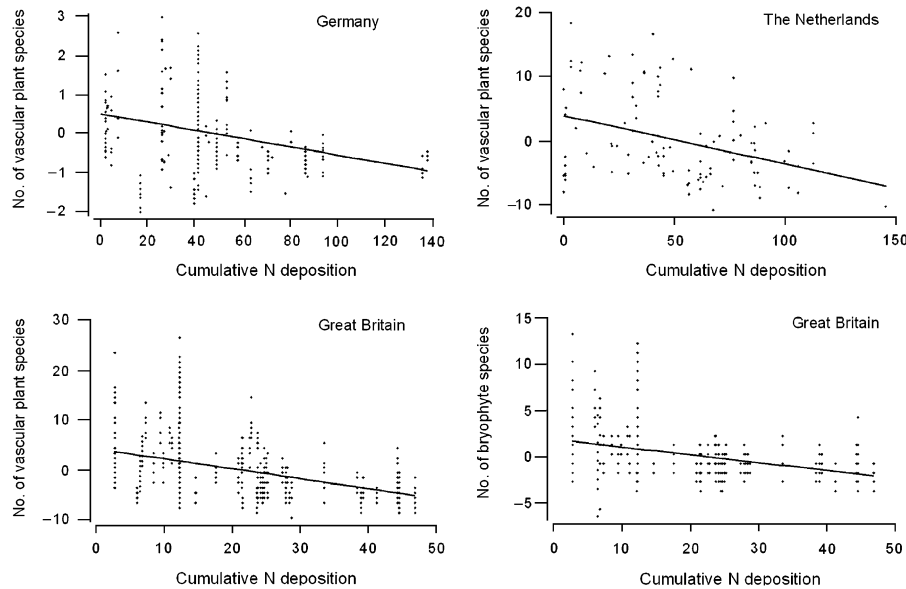
Fig. 2 Effects of cumulative nitrogen (N) deposition (in mol ha^{-1} × 1000) on the species number of vascular plants and bryophytes (only Great Britain) in the three regional data subsets; species richness values were corrected for plot size (i.e. residuals were used). Germany: $R^2_{adj.} = 0.098$,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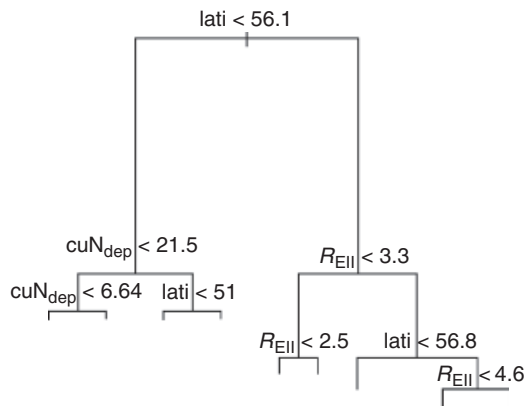

Fig. 3 Regression tree of the relationship between species richness of vascular plants (corrected for plot size) and the variables R_{Ell}, latitude and cumulative nitrogen (N) deposition (cuN$_{dep}$) for the data subset from Great Britain ($n = 532$).

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 intercorrelation 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_{Ell} (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_{Ell} was by far the most important factor affecting species richness.

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.

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 with those collected after 1975. Grasses
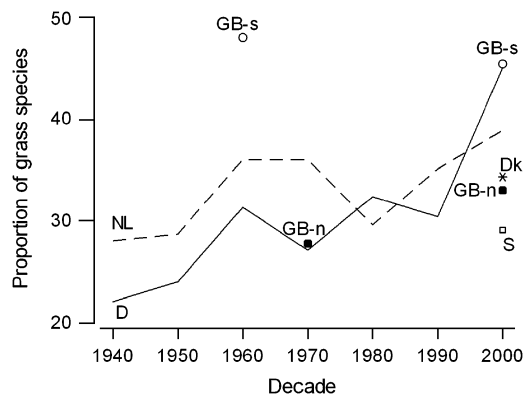
Fig. 4 Mean proportion of grass species on the total number of vascular plants in different decades since 1940. The continuous line shows the results for Germany (D), the dashed line are those for the Netherlands (NL). For Great Britain (GB), mean values are given for those decades for which vegetation data was available, separately for plots from the south (○ , s) (<56.1°N, see Fig. 3) and the north (● , n). For comparison, also the mean values for the 2007 sample plots from Sweden (□, S) and Denmark (*, Dk) are given.

such as *A. capillaris* and *Festuca rubra*, but also ruderals like *Holcus* sp., have clearly increased in all regions, while among the common grasses only *F. 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 recent than in the older plots, for example *A. montana* and *V. canina*. Among the few dicots that have increased in frequency are *Achillea millefolium* and both *Rumex acetosa* and *R. acetosella*, representing species that are characteristic for more fertile or ruderalized grasslands. On average, species with a decreasing trend had lower N_{Ell} values than species with an increasing trend. For bryophytes, we can rely only on the data from Great Britain; here, in accordance with the results given in Table 3, most of the more common species tended to decline over the years (e.g. *Dicranum scoparium*, *P. schreberi*), whereas only *R. squarrosus* and *Scleropodium purum* appeared to increase in frequency. Not reflected in Table 4 is the strong decline in many low-frequent *Violion* species that were 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). Owing 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 covariable. 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 underestimated, because most vegetation data in Germany and the Netherlands were sampled in the framework of phytosociological studies in which plots tend to be

Table 4 Relative frequency of species (in %) in the data subsets from Germany, the Netherlands and Great Britain, for plots sampled before (old) and after (new) 1975

Species	N_{Ell}	Germany		Great Britain		the Netherlands	
		Old	New	Old	New	Old	New
No. of plots		177	215	201	340	65	79
Grasses							
Agrostis capillaris	4	60	87	60	98	63	67
Festuca rubra	x	25	65	13	19	74	87
Holcus lanatus	4	19	17	7	28	32	44
Holcus mollis	3	2	11	2	10	5	5
Poa pratensis s.l.	2	15	18	6	7	9	11
Agrostis canina	2	12	3	39	3	39	48
Anthoxanthum odoratum	x	44	45	49	51	35	38
Danthonia decumbens	2	60	31	3	22	91	65
Deschampsia flexuosa	3	28	73	63	34	29	38
Molinia caerulea	1	42	12	5	20	51	61
Nardus stricta	2	59	78	58	34	40	34
Festuca ovina	1	66	53	90	83	9	0
Herbaceous dicots and monocots other than grasses							
Achillea millefolium	5	14	37	7	14	12	14
Rumex acetosa	6	19	46	20	29	5	20
Rumex acetosella	2	13	27	6	2	29	54
Campanula rotundifolia	2	16	35	13	14	5	1
Carex pilulifera	3	43	46	22	23	62	57
Galium saxatile	3	36	80	82	79	68	77
Hypochaeris radicata	1	31	6	0	10	42	39
Lotus corniculatus	3	23	12	6	16	17	13
Luzula campestris s.l.	2	56	64	46	50	62	62
Plantago lanceolata	x	24	28	8	13	19	8
Polygala vulgaris	2	13	13	0	2	8	11
Potentilla erecta	2	75	63	50	62	82	58
Solidago virgaurea	4	7	14	0	2	11	4
Succisa pratensis	2	42	21	4	7	6	5
Veronica chamaedrys	x	9	27	1	10	–	–
Veronica officinalis	4	22	27	4	4	9	13
Arnica montana	2	30	24	–	–	17	10
Dactylorhiza maculata	2	11	3	1	1	14	1
Hieracium subg. *Pilosella*	2	37	26	5	4	44	23
Juncus squarrosus	1	27	6	54	10	26	14
Polygala serpyllifolia	2	2	1	10	7	17	8
PruN_{Ell}a vulgaris	7	16	5	6	7	12	9
Scorzonera humilis	2	11	1	–	–	2	0
Viola canina	2	20	13	1	0	26	14
Dwarf shrubs							
Empetrum nigrum	2	16	1	1	4	2	6
Vaccinium myrtillus	3	12	20	56	27	3	1
Vaccinium vitis-idaea	1	1	5	17	1	2	0
Calluna vulgaris	1	77	28	21	21	71	62
Erica cinerea	1	–	–	8	4	–	–
Erica tetralix	2	54	6	4	2	45	27
Genista anglica	2	38	5	–	–	32	17
Genista pilosa	1	18	2	–	–	8	0
Bryophytes							
Rhytidiadelphus squarrosus				70	81		
Scleropodium purum				12	21		

Continued

Table 4. (*Contd.*)

Species	N_{Ell}	Germany		Great Britain		the Netherlands	
		Old	New	Old	New	Old	New
No. of plots		177	215	201	340	65	79
Dicranum scoparium				52	11		
Hylocomium splendens				51	22		
Hypnum cupressiforme s.l.				61	45		
Lophocolea bidentata				49	5		
Pleurozium schreberi				62	11		
Ptilidium ciliare				42	0		

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_{Ell} = Ellenberg value for nitrogen.

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 nonalpine 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 N 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 with 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 N availability in the soil, as reflected in the effect of cuN_{dep} on species numbers and N_{Ell}. 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_{Ell} values, for which there is no evidence.

Ordination analysis

While 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_{Ell} and especially mean R_{Ell} 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 N 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_{Ell} and R_{Ell} 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_{Ell} values, both mean N_{Ell} and mean R_{Ell} 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_{Ell} and mean N_{Ell} 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 N 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 *A. capillaris* and *F. 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 N 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 *V. 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_{Ell} 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 allow 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 N and pH, on the changes in the vegetation.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Sources of vegetation data used for the analyses, including the number of sample plots, the country of origin, and the year of sampling.

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Appendix A

Correction factors used for the calculation of values of cumulative nitrogen (N) and sulphur (S) deposition since 1939

Table A1

	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