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Long-term changes in calcareous grassland vegetation in North-western Germany – No decline in species richness, but a shift in species composition



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ABSTRACT

We aimed to answer the question of whether the species richness and composition of calcareous grasslands in North-western Germany had changed over the last 70 years as a result of atmospheric nitrogen (N) deposition. In total, 1186 plots of *Festuco-Brometea* (alliance *Bromion erecti*) grasslands from the sub-oceanic regions of the country were compiled (1061 plots from literature sources spanning a time period from 1936 to 1996, 125 new plots from 2008). Environmental descriptors recorded for each plot included geographic coordinates, altitude, heat index (combining slope and aspect), mean Ellenberg indicator values for light, soil moisture, soil pH and soil N, and cumulative N deposition (the latter being highly positively correlated with the year of sampling).

In a Detrended Correspondence Analysis, the sample plot scores along axis one were highly correlated with the mean Ellenberg N-values, those along axis two were significantly affected by the year of sampling. In a general linear model, species richness of vascular plants showed a markedly hump-shaped relationship with mean Ellenberg N-value, whereas it was weakly affected by year (cumulative N load). Species with a significant negative trend over time were more often (than expected by chance) habitat specialists of dry grasslands, small, light-demanding and winter-green or evergreen with smaller seeds and scleromorphic leaves. In contrast to what has been found for acidic grasslands, N deposition in calcareous grasslands did not result in a decline in species richness, most likely because calcareous grasslands are water- and phosphorus-limited, and are well-buffered in terms of soil pH. To prevent a further change in species composition towards more mesophytic communities, grassland management by the site managers needs to be intensified.

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1. Introduction

Atmospheric nitrogen (N) deposition and the subsequent eutrophication of terrestrial and aquatic ecosystems have been recognized as global processes where the planetary thresholds have already been overstepped (Rockström et al., 2009). In Central

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Europe the deposition of N exceeds 10–15 kg ha^{−1} year^{−1} in semi-natural vegetation and 20 kg ha^{−1} year^{−1} in coniferous forest, and the values are higher or even much higher in areas of intensive agriculture (Sutton et al., 2011). In Germany, the emission rates for nitrogen oxides have considerably decreased since 1990, whereas those for ammonium have been largely stable over the past years (<http://www.umweltbundesamt.de/>). In general, the emissions of N in Europe and other parts of the world are projected to increase during the next decades (e.g., Gruber and Galloway, 2008). The effects of N deposition on plant-species composition and richness have been documented for several vegetation types, based on three lines of evidence. First, experiments conducted in various habitat

types have shown that N addition is often associated with an increase in biomass and a simultaneous decrease in species richness (Bobbink et al., 2010). In a recent meta-analysis, however, De Schrijver et al. (2011) showed that these effects on plant biomass and richness are vegetation type-dependent and that species of different life forms are differently affected. A second source of evidence relies on descriptive comparisons of areas situated along a geographic gradient in N deposition, for example in North European forests (Diekmann et al., 1999) and in British grasslands and heathlands (Stevens et al., 2004, 2006; Maskell et al., 2010). These spatial studies have largely supported the experimental results, showing a general decline in species richness, a decrease in species indicative of nutrient-poor sites, and a shift in functional types from forbs to grasses. The third way to examine effects of atmospheric pollution is a temporal approach, comparing plots sampled at a time with low N deposition with more recent plots having been exposed to higher deposition levels. Such time-series analyses have mainly been conducted in forests based on the monitoring of permanent plots (e.g., Diekmann et al., 1999), but have also been applied using a non-permanent design (Diekmann and Duprè, 1997; Gaudnik et al., 2011). Provided that the number of plots is large and that the data set is not spatially or temporally biased, this approach has some advantages compared to a permanent plot design in that it can be more representative through covering larger regions and more plots.

Eutrophication-driven changes in European vegetation are particularly well studied in acidic grasslands, which have suffered from a steep decline in species richness mainly caused by an increase in a few competitive grasses at the expense of forbs and dwarf shrubs (Stevens et al., 2006, 2011a; Duprè et al., 2010; Maskell et al., 2010). These temporal trends coincide closely with the results of N-addition experiments in grasslands (see De Schrijver et al., 2011). Surprisingly, the effects of N deposition in calcareous grasslands are less well documented, although these grasslands are among the most species-rich habitat types in Europe and of focal interest to nature conservationists across the continent. In one of the few long-term studies from Britain, Bennie et al. (2006) re-surveyed 92 plots from English chalk grasslands that were first surveyed in 1952–53. They showed a decline in species richness, a decrease in species associated with inherently infertile conditions and an increase in species typical of more mesotrophic grasslands, thereby indicating an effect of nutrient enrichment. In contrast, an increase in α diversity over 70 years was observed in a re-survey of 88 calcareous grassland sites in the English county of Dorset (Newton et al., 2012). In another permanent plot study from southern Germany, revealing similar results, the vegetation changes were attributed mainly to a change in management, namely a decrease in grazing intensity (Hagen, 1996). Again, these changes and their interpretation coincide with results from various N-addition experiments (Bobbink, 1991; Willems et al., 1993; Jacquemyn et al., 2003). Several of the experimental studies suggest that the increasing dominance of *Brachypodium pinnatum* is one of the main proximal reasons for the observed species loss (e.g., Bobbink and Willems, 1987). The evidence for a decrease in species richness and the role of N enrichment in this process, however, are not unequivocal. In an N-addition experiment with chalk grassland species, Wilson et al. (1995) did not observe an increasing cover of grasses (such as *Brachypodium*) and a subsequent loss of species diversity. A recent spatial study from Britain also did not give evidence for a negative impact of N deposition on plant species richness in calcareous grasslands (Maskell et al., 2010).

Another limiting nutrient in many ecosystems is phosphorus (P). The input of P into terrestrial ecosystems from weathering and from the atmosphere is usually low, and if N deposition is high, P may become the limiting nutrient and species with efficient P economies may be favoured (Bobbink et al., 2010; Vitousek et al.,

2010). Both N and P are likely to play an important role for the species richness and variation in species composition, as evidenced for grasslands by, for example, Kleijn et al. (2008) and Ceulemans et al. (2011, 2013). Compared to N, however, P has received only little attention in the study of vegetation changes in semi-natural terrestrial vegetation.

Apart from a recent analysis of the trends in the frequencies of calcareous grassland species in Germany (Jandt et al., 2011), our study is to our knowledge the first large-scale study of temporal changes in the vegetation of calcareous grasslands. It is a follow-up of a similar analysis conducted in acidic grasslands in North-western Europe (Duprè et al., 2010) where the impact of N deposition on species assemblages and diversity were found to be dramatic. Based on an analysis of historical and recent vegetation data from North-western Germany, we aimed to: (1) examine changes in the species composition and richness in calcareous grasslands over the last 70 years in response to N deposition, (2) compare the temporal trends of species with different life-history traits and ecological strategies, (3) assess the role of P for the species composition and richness in calcareous grasslands, and (4) contrast the results with those obtained in acidic grasslands and discuss the underlying causes for the differences between the two grassland types.

2. Material and methods

2.1. Vegetation data

Plot-scale data from calcareous grasslands were compiled from the sub-oceanic areas of North-western Germany (Fig. 1). More southern and continental regions with a much larger pool of calcareous grassland species were avoided to obtain a vegetation data



Fig. 1. Map of the study areas in North-western Germany from where plots of calcareous grasslands were compiled.

set with relatively uniform species composition. We searched exclusively for plots that had been assigned by their authors to the alliance *Bromion erecti*, including grasslands on usually relatively shallow, infertile and high-pH soils, often occurring on south-facing slopes. The decreasing intensity or abandonment of grazing and mowing in semi-natural landscapes has resulted in a strong reduction in the area of traditional calcareous grasslands (Ellenberg and Leuschner, 2010). To minimize confounding management effects, plots were considered for analysis only if active grazing or mowing at the time of sampling was indicated, and if there was no evidence of fertilization. Managed *B. erecti* communities are characterized by a sward of graminoids (the most common species being the grasses *B. pinnatum*, *Festuca ovina* agg., *Koeleria pyramidata*, *Briza media*, *Helictotrichon pratense* and *Bromus erectus*, as well as *Carex flacca* and *C. caryophyllea*) and a large number of forbs such as *Lotus corniculatus*, *Pimpinella saxifraga*, *Sanguisorba minor*, *Thymus pulegioides* and *Cirsium acaule* (Ellenberg and Leuschner, 2010). Calcareous grasslands in the area have a relatively high proportion of threatened and protected plant species, including many orchids like *Gymnadenia conopsea* and *Ophrys insectifera*. Plots were only retained if information on plot size and geographic location were available. The historical data (1061 plots) were obtained mainly from published sources, and to a lesser extent also from unpublished reports. These were supplemented by 125 newly sampled vegetation plots from 2008. The final data sets included 1186 plots, the data sources are listed in Table A1. The oldest plots of calcareous grasslands from the study area available to us were from 1936, the highest number of plots was sampled in the 1980s.

The vegetation data including all vascular plants were entered into a table and standardized. As the precision of species records differed between the studies and also the taxonomic concepts for some species had changed over time, several taxa had to be aggregated. The taxonomic standardization was based on the nomenclature of Wisskirchen and Haeupler (1998). A further standardization concerned the species-abundance measures that were re-coded to conform to the simplified 6-degree scale of Braun-Blanquet (Dierschke, 1994).

2.2. Environmental data

For a causal analysis of the vegetation changes, we compiled a number of environmental descriptors for each plot. These included geographic location (latitude, longitude, altitude) and information about the topography of the site. As slope and aspect are known to have a strong and coupled impact on the plant-species composition of calcareous grasslands (Bennie et al., 2006), we calculated an integrative heat index value = $\cos(\text{aspect} - 225) \cdot \tan(\text{slope})$ according to Parker (1988). As surrogates for measured environmental variables, we calculated mean Ellenberg indicator values (Ellenberg et al., 1992) for light (meanL), soil moisture (meanF), soil pH (meanR), and soil nutrient status (meanN, originally described as reflecting nitrogen availability) for all plots. These values have successfully been applied in other studies examining temporal changes of the vegetation in response to atmospheric pollution (Diekmann and Duprè, 1997; Duprè et al., 2010). As recommended by most researchers (see Diekmann, 2003), the calculation of mean values was based on species presence-absence instead of quantitative data, especially to avoid a bias owing to inter-observer differences in cover estimation. We also did analyses with cover-weighted data, but the results were very similar.

To examine the long-term effects of atmospheric pollution on species composition and richness, we estimated cumulative values of total (wet and dry) N deposition (cumNdep), based on deposition values for the year 2000 obtained from the model FACEM (Pieterse et al., 2007) and the change of deposition values over time. Details of the calculation are described in Duprè et al.

(2010). Cumulative values of sulfur (S) deposition were calculated in a similar manner, but not used in the final analysis for two reasons: first, we assumed that the acidifying effect of S would be well buffered in the calcareous soils, and second, there was a high multi-collinearity between N and S deposition.

No environmental measurements were available for the plots compiled from the literature. For the plots surveyed in 2008, soil samples for the determination of pH, total carbon (C), total N and plant available phosphorus (P) were collected. Soil samples consisted of two mixed sub-samples taken at a depth of 0–10 cm below the litter layer with a core from two opposing corners of the 1 m × 1 m quadrat. In the laboratory, the samples were air dried and passed through a 2 mm sieve prior to analysis. Soil pH was determined in a solution of 10 g of soil and 25 ml of 0.01 M CaCl₂ using a standard glass electrode. Using soil ground to fine powder, C and N were determined with an elemental analyzer. After extraction with ammonium lactate, the content of P was measured by flow-injection analysis. Soil depth was determined as the mean value of five measurements carried out with a marked metal pin.

2.3. Life-history traits

One aim of our study was to examine how far temporal changes in species composition and richness are caused by changing frequencies of species with different life-history traits and ecological strategies. For this, we collected data from the literature on species attributes that we considered to be relevant in the context of the study. We compiled information on the following traits: habitat preference (distinguishing specialists of *Festuco-Brometea* and *Koelerio-Corynephoretea* communities typical of dry and sunny, nutrient-poor habitats, and all other species), general life form, Raunkiaer life form, life span, plant height, ability of vegetative reproduction, leaf anatomy, leaf longevity, seed mass, and Grime strategy (for data sources, trait categories and species values/assignments, see Table A2). The ecological optima of species relative to important soil variables and light were assessed by using the Ellenberg indicator values for soil moisture, soil pH, soil nutrients and light (Ellenberg et al., 1992). Except for seed mass, plant height (interval) and Ellenberg values (ordinal), all variables were nominal.

2.4. Statistical analysis

Differences between plots and changes in species composition over time were analyzed by means of ordination. The data set with the species-plot matrix was examined by Detrended Correspondence Analysis (DCA), using the 'decorana' function of the VEGAN package implemented in R version 2.12.1 (Oksanen et al., 2011). Apart from a down-weighting of rare species (to counteract the influence of outliers), we applied the default options of the program. A second (environmental variable-plot) matrix served to interpret the results. We used the function 'envfit' in VEGAN to fit the environmental variables (year, latitude, longitude, altitude, heat index, meanL, meanF, meanR, meanN, and cumNdep) *post hoc* onto the DCA ordination. The direction of an environmental vector in the resulting diagram shows the direction of the respective gradient, while the length of an arrow reflects the importance of the variable for the variation in species composition. The *P* values for the variables were based on 999 random permutations, meaning that only those variables are displayed in the ordination diagram that had a significant effect on the variation in species composition at *P* < 0.001. The variables 'year' and 'cumNdep' were highly positively correlated (Pearson correlation: *r* = 0.948, *P* < 0.001), therefore only the vector for 'year' is shown.

The relationship between species richness and environmental variables was analyzed by general linear models and generalized

linear models using R version 2.12.1. Plot sizes varied considerably between 1 and 225 m², and the relationship between species richness and area was best described by a regression of log species richness against log plot size ($R^2 = 0.279$). To correct for varying plot sizes we then used the residuals of this regression in the GLM instead of the original species-richness values. A large variation in plot size, however, has been shown to in some cases distort species-richness analyses in vegetation data sets (Dengler and Boch, 2008). Therefore, we also created two sub-sets, one excluding all plots larger than 20 m² (sub-set 1: 822 plots), the second including only plots up to 10 m² (sub-set 2: 389 plots).

To avoid multi-collinearity, separate models were run for 'year' and 'cumNdep'. MeanF and meanN were highly positively correlated. As we assumed that soil moisture is partly captured in the variable 'heat index', we used meanN in the model runs (The results were, however, largely identical to those obtained when using meanF). As species richness often shows unimodal relationships with environmental factors, quadratic terms for all variables were also included in the initial models (after centering of the variables). Each model run started with the full model including all variables, and variables were removed in a stepwise manner until the AIC value was minimized. These models only included variables with a significant or marginally non-significant impact on species richness. After having fitted models for the total number of vascular plant species, we additionally tested the effects of the explanatory variables on the number of typical dry grassland species (habitat specialists according to Table A2) and the percentage of typical dry grassland species. The residuals of total species richness and the number of typical dry grassland species followed a normal distribution, and binomial distribution was applied for the proportion of typical dry grassland species. The main aim of the study was to monitor the change in species richness over time and to examine the roles of cumulative N deposition as well as N and P availability. We therefore do not show all appropriate models based on AIC criteria (Burnham and Anderson, 2002), but only report on one model for each of the dependent variables.

For the statistical analyses for single species, those with fewer than 40 occurrences in the whole data set were omitted in order to reduce the influence of random differences in the abundance of the rarest species between older and more recent plots. To examine whether species had increased or decreased in abundance, the abundance scores were related to the variable 'year' across all sites with linear regression, resulting in three groups of species showing (1) a significant increase over time, (2) a significant decrease over time, or (3) no temporal change in abundance. Using presence-absence values instead of abundance values gave similar results, but fewer species showed significant changes over time. We then compared the life history traits and ecological strategies between increasing and decreasing species using χ^2 -tests (all categorical variables) and Mann-Whitney *U*-test (Ellenberg values, plant height, and seed mass). For the typical dry grassland species with fewer than 40 occurrences in the whole data set, we additionally examined the temporal change in geographic range size, using an analysis by Welk (2002) for whole Germany and a distribution atlas of vascular plants by Garve (2007) for the North-west German region of Niedersachsen from where more than a third of the plots were obtained.

3. Results

The number of vascular plants per plot ranged from 7 to 68. The averages of mean Ellenberg values for soil moisture, pH, nitrogen and light (3.9, 7.1, 2.9 and 7.2, respectively) indicate the dry, base-rich and nutrient-poor soil conditions of the calcareous grasslands and their high light availability. There were pronounced

differences in annual N deposition between sites, with the most polluted grasslands receiving >3 times more N than the least polluted grasslands (840–2882 eq ha⁻¹ year⁻¹; average: 1441).

The DCA analysis based on the species-plot matrix resulted in relatively low eigenvalues and gradient lengths for the first two axes, confirming that the data set included plots with a relatively uniform species composition that can be assigned to the alliance *B. erecti* (i.e., did not comprise plots with a greatly different composition belonging to other alliances of calcareous grasslands, which are widespread under warmer and more continental conditions) (Fig. 2). The site scores in the ordination diagram were well dispersed. Most environmental variables passively fitted to the ordination were significantly correlated to the species composition. The strongest correlation along axis 1 was observed for meanN, its vector pointing to the left. MeanR showed a strong and negative relationship with axis 2. Also year was significantly related to the site scores along axis 2 with its vector pointing down. The perpendicular position of the vectors for meanN and year are in accordance with the weak correlation of the two variables across sites (Pearson correlation: $r = 0.058$). The vector for cumNdep was almost exactly congruent with the one for year (and was, for the sake of legibility, omitted from the graph).

The most common and typical dry grassland species are found in the center of the ordination diagram. Species situated at the left-hand side of the graph associated with high meanN values include *Bromus inermis*, *Chaerophyllum temulum*, *Galium aparine*, *Stellaria media*, and *Torilis japonica*, indicative of half-open vegetation on relatively nutrient-rich soils. Species at the right-hand side are, for example, elements of sun-exposed fringes (*Laserpitium latifolium*, *Seseli libanotis*, *Thesium bavarum*) and calcareous rocks (*Gymnocarpium robertianum*, *Gypsophila repens*).

In the GLM, vascular plant species richness was influenced by several environmental variables, often in a unimodal way (Table 1). When year was included in the models, the total R^2_{adj} value amounted to 0.377; with cumNdep instead of year the value (and the whole model) was almost exactly the same. Among the

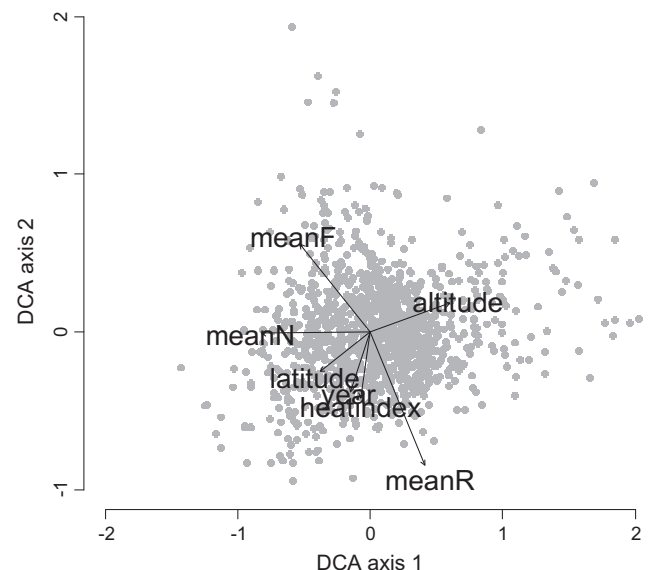


Fig. 2. DCA ordination diagram based on a matrix of 1186 plots and 516 species of calcareous grassland from North-western Germany. The arrows represent an overlay of vectors of the environmental variables fitted *post hoc* to the ordination. Only variables with a significant effect on species composition at $P < 0.001$ are included; the vector for cumulative nitrogen deposition (cumNdep), being almost fully congruent with the one for year, was omitted for better legibility. MeanF = mean Ellenberg value for soil moisture, meanR = mean Ellenberg value for soil reaction (pH), meanN = mean Ellenberg value for soil nitrogen. Eigenvalues and gradient lengths are: axis 1 – 0.165 and 4.245 sd, axis 2 – 0.090 and 2.872 sd.

Table 1

Effects of geographic variables (latitude, longitude, altitude), local environmental descriptors and year on species richness (corrected for plot size, see Section 2) in calcareous grasslands ($n = 1186$) in Germany. The number of vascular plants and the number of typical dry grassland species were analyzed with general linear models, and the proportions of typical dry grassland species with generalized linear models (applying binomial distribution). Each column refers to one model. T values and significance probabilities ($***P < 0.001$, $**P < 0.01$, $*P < 0.05$, $0.05 < P < 0.10$) as well as total R^2_{adj} values are given. The last line shows the R^2_{adj} values of models where year is replaced by cumulative N deposition (cumNdep). Variables that did not remain in the reduced final models are indicated with '-'. MeanL = mean Ellenberg value for light, meanR = mean Ellenberg value for soil reaction (pH), meanN = mean Ellenberg value for soil nitrogen, sub-set 1 includes only plots with an area ranging from 1 to 20 m² ($n = 822$), sub-set 2 plots ranging from 1 to 10 m² ($n = 389$).

	No. of vascular plants			No. of typical dry grassland species			Proportion of typical dry grassland species		
	All plots	Sub-set 1	Sub-set 2	All plots	Sub-set 1	Sub-set 2	All plots	Sub-set 1	Sub-set 2
Latitude	-	-	-	-9.81***	-	-3.79***	-1.74*	-1.76*	-0.75
Longitude	-8.16***	-	-	-	-6.50***	-	-	-	-
Altitude	-	5.40***	-	3.62***	8.68***	0.74	-	-	-
Altitude ²	-	-	-	-2.96**	-5.15***	-1.78*	-	-	-
Heat index	5.31***	-1.84*	-0.30	-	5.06***	0.75	0.53	0.30	0.31
Heat index ²	-7.67***	-6.02***	-5.34***	-	-5.76***	-5.28***	-	-	-
MeanL	1.30	6.83***	4.50***	6.89***	6.72***	5.64***	1.97*	1.42	1.28
MeanL ²	-7.99***	-6.74***	-4.73***	-13.24***	-8.54***	-5.37***	-	-	-
MeanR	-6.51***	4.31***	3.22**	1.95*	1.30	5.75***	2.33*	1.70*	1.10
MeanR ²	-6.15***	-4.09***	-3.39***	-10.90***	-10.65***	-5.41***	-	-	-
MeanN	11.48***	7.24***	6.18***	-3.19**	-3.19***	4.35***	-2.80**	-2.12*	-1.18
MeanN ²	-9.05***	-6.69***	-6.10***	-7.43***	-7.06***	-4.94***	-	-	-
Year	4.15***	2.45*	1.83*	-	-	3.44***	-0.97	-0.61	-0.52
Total R^2_{adj}	0.377	0.324	0.374	0.524	0.542	0.527	(0.699)	(0.715)	(0.636)
Total R^2_{adj} with cumNdep	0.376	0.321	0.387	0.452	0.504	0.531	(0.715)	(0.734)	(0.655)

soil variables, the strongest effect on species richness was found for meanN showing a hump-shaped curve (Fig. 3a). Species richness peaked at intermediate heat index values, with a strong decrease towards the steepest and most sun-exposed slopes. Heat index and meanN were negatively correlated (Pearson correlation: $r = -0.124$, $P < 0.001$). Year and cumNdep had significantly positive effects on the total number of species, which was confirmed by the simple linear model, albeit with a R^2 of only 0.047 (Fig. 3b). The same relationship as described above was obtained for meanN when analyzing sub-sets 1 and 2 (Table 1), while the effect of year on species richness was only marginally or no longer significant.

With the number of typical dry grassland species as the response variable, the models based on all plots gave similar results to those found for total species richness with respect to mean Ellenberg values, but had higher total R^2_{adj} values (Table 1). There was, however, a strong negative (instead of a hump-shaped) relationship between the number of dry grassland specialists and meanN, while year had no significant effect. The geographic variables had generally stronger effects on the number of specialist species than on total species richness. Latitude had a pronounced negative effect (also in sub-set 2), indicating the decline in the number of typical dry grassland species towards the north. The species richness of this group peaked at intermediate altitudes. The heat index did not remain in the final model for all plots, whereas it was found to be significant in the models for the two sub-sets. For sub-set 2, year was included in the final model having a positive effect on the number of typical dry grassland species. This effect was, however, no longer visible in a simple linear model (results not shown).

For the proportion of dry grassland species, the final models did not contain any quadratic terms (Table 1). Here, meanN had a negative effect (Fig. 3c) and meanR as well as meanL a positive impact, while year tended to show a negative relationship with the proportion of dry grassland species (Fig. 3d). The results were similar for the models for the two sub-sets, except for much lower significance probabilities. The number and proportion of species typical of mesotrophic conditions increased with time in all data sets (results not shown).

The analysis of temporal trends of species gave several significant differences between different life-history traits. Whereas

generalist species showed an overall increase over time, the number of calcareous grassland specialists has declined ($\chi^2 = 6.206$, $P = 0.012$, $df = 1$; Fig. 4a). Species with a mesomorphic leaf anatomy tended to increase at the expense of those with a scleromorphic or intermediate anatomy ($\chi^2 = 4.721$, $P = 0.094$, $df = 2$; Fig. 4b). For leaf longevity, summer-green and vernal species have increased over time, while winter-green and evergreen species showed the opposite trend ($\chi^2 = 4.773$, $P = 0.029$, $df = 1$; Fig. 4c). No significant differences in temporal trends were observed between species differing in Raunkiaer life form, life span, or general life form (all $P > 0.5$): eight annual and biennial species have decreased over time, five have increased. There were about as many increasing (8) as decreasing (7) grasses (Poaceae), and two out of seven woody species showed a decline. For the Grime strategies we found only weak trends in that competitive species tended to increase at the expense of species with other (intermediate) strategies ($\chi^2 = 4.441$, $P = 0.218$, $df = 3$; Fig. 4d).

Taller plants were over-represented among the increasing species (Mann-Whitney U -test: $W = 502.5$, $P = 0.033$; Fig. 4e), and species with a positive temporal trend tended to have a higher seed mass compared to decreasing species ($W = 468.5$, $P = 0.063$; Fig. 4f). Except in one case (light: $W = 1027$, $P = 0.020$) there were no differences between increasing and decreasing species in their Ellenberg indicator values ($P > 0.15$). *B. pinnatum* did not increase in frequency over the years or in response to cumulative N deposition, and there was no effect of the abundance of the species on total species richness (all $P > 0.05$).

Among the 22 rarest species typical of dry grasslands in the study area for which data were available, 18 have shown a decline or strong decline in Germany over recent decades (Welk, 2002). In the North-west German region of Niedersachsen, 16 out of 27 rare dry grassland species have decreased in their area of occupancy (number of occurrences in grid squares) by 50% or more compared to the period up to 1981 (Garve, 2007). The other species have declined less strongly or remained constant, meaning that none of the species is currently more common than three decades ago.

Across the 125 calcareous grassland plots sampled in Germany in 2008, vascular plant species richness was only weakly, albeit significantly, correlated to the soil P content (linear regression: $R^2 = 0.047$, $P = 0.016$; Fig. 5a). In contrast, P had a strong effect on

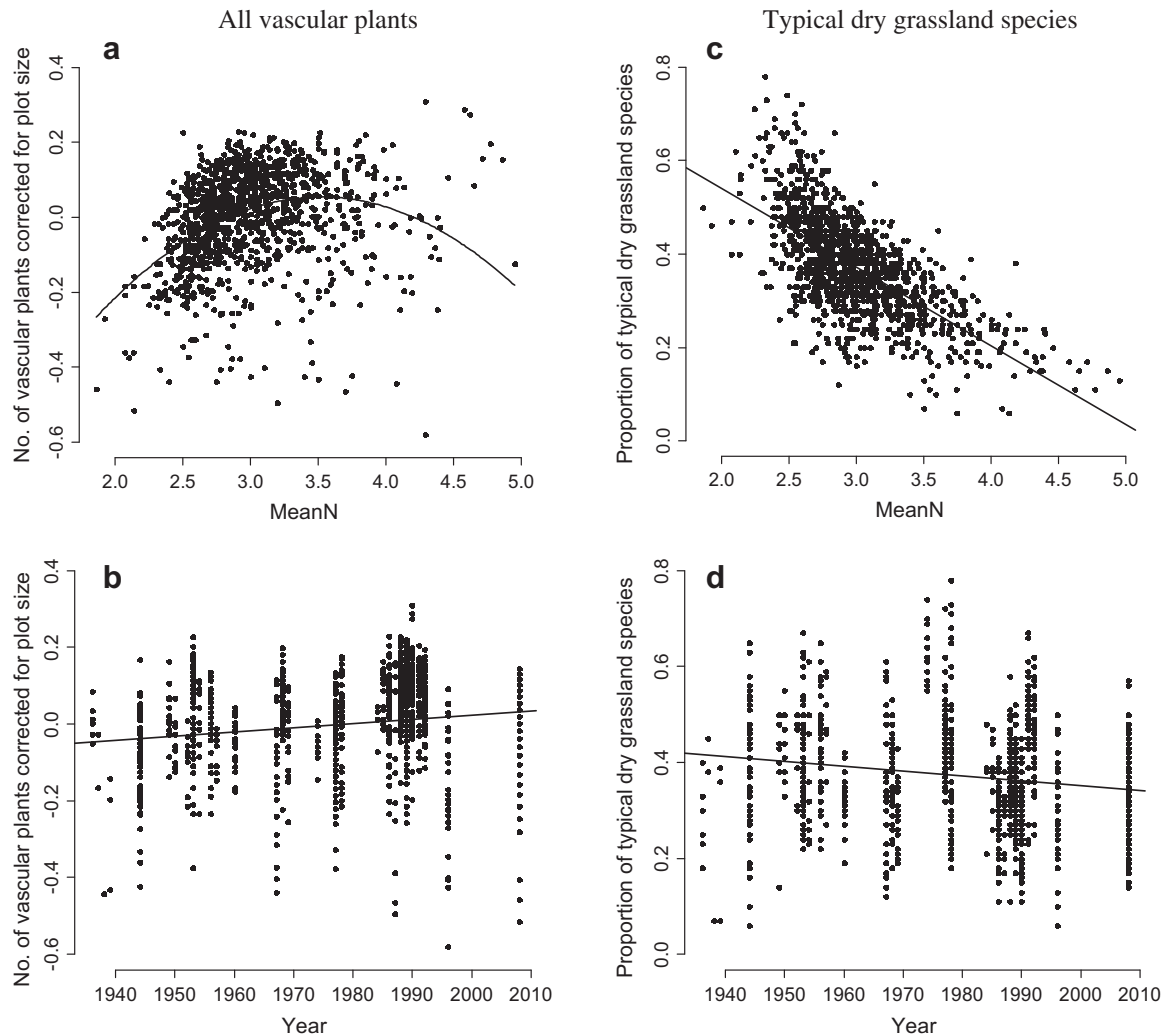


Fig. 3. Relationships between the total number of vascular plants (corrected for plot size) and (a) meanN (mean Ellenberg value for soil nitrogen), and (b) year, and relationships between the proportion of typical dry grassland species and (c) meanN, and (d) year (for all regression analyses $n = 1186$).

the species composition as reflected by the DCA ordination ($R^2 = 0.540$, $P < 0.001$; Fig. 5b). None of the N variables (total N, C/N ratio, N deposition) explained much of the variation in species composition (all $R^2 < 0.09$).

4. Discussion

4.1. Species richness

The analysis of the historical data sets of calcareous grasslands from Germany revealed that there has been no or only a slight positive change in total species richness over the last 70 years since the first vegetation plots from the region were available. The ordination diagram indicated that changes in species composition were more pronounced than those found for species richness, which is also reflected in the partly different temporal trends of species with different life history traits and ecological strategies. These results are in sharp contrast to those observed in acidic grasslands that have undergone a dramatic change both in species richness and composition over the last decades.

During recent decades, calcareous grasslands have suffered from a marked decline in area. While some sites have been fertilized and their agricultural use intensified, most areas, especially on the steeper and drier slopes, have faced an abandonment of

management (mowing and/or grazing), followed by succession to scrub and ultimately forest (Ellenberg and Leuschner, 2010). Since the implementation of the EU Habitats Directive, which recognizes *Festuco-Brometea* communities as a focal habitat type, the remaining grassland sites have largely been protected and preserved by continued management. The results of our study suggest that this management has been successful so far, since plant-species richness has largely remained stable. There was also no evidence of an increase over time of *B. pinnatum*. This species is known to respond positively to experimental N addition (e.g., Bobbink and Willems, 1987) and to a reduction and abandonment of management (Ellenberg and Leuschner, 2010). The lack of a decline in total species richness is unexpected for two reasons. First, the majority of calcareous grasslands in the study area are small in size and often isolated. It has been shown that the fragmentation of these grasslands often has detrimental effects on the fitness of populations of rare species, for example *Gentianella germanica* (Fischer and Matthies, 1998). In addition, the probability of small populations of short-lived plants of going extinct appears to increase with decreasing population size (Matthies et al., 2004). Second, over recent decades, calcareous grasslands have received considerable amounts of airborne N. These are comparable to those in acid grasslands in the same region in which N deposition has had a strong negative impact on species richness (Duprè et al., 2010).

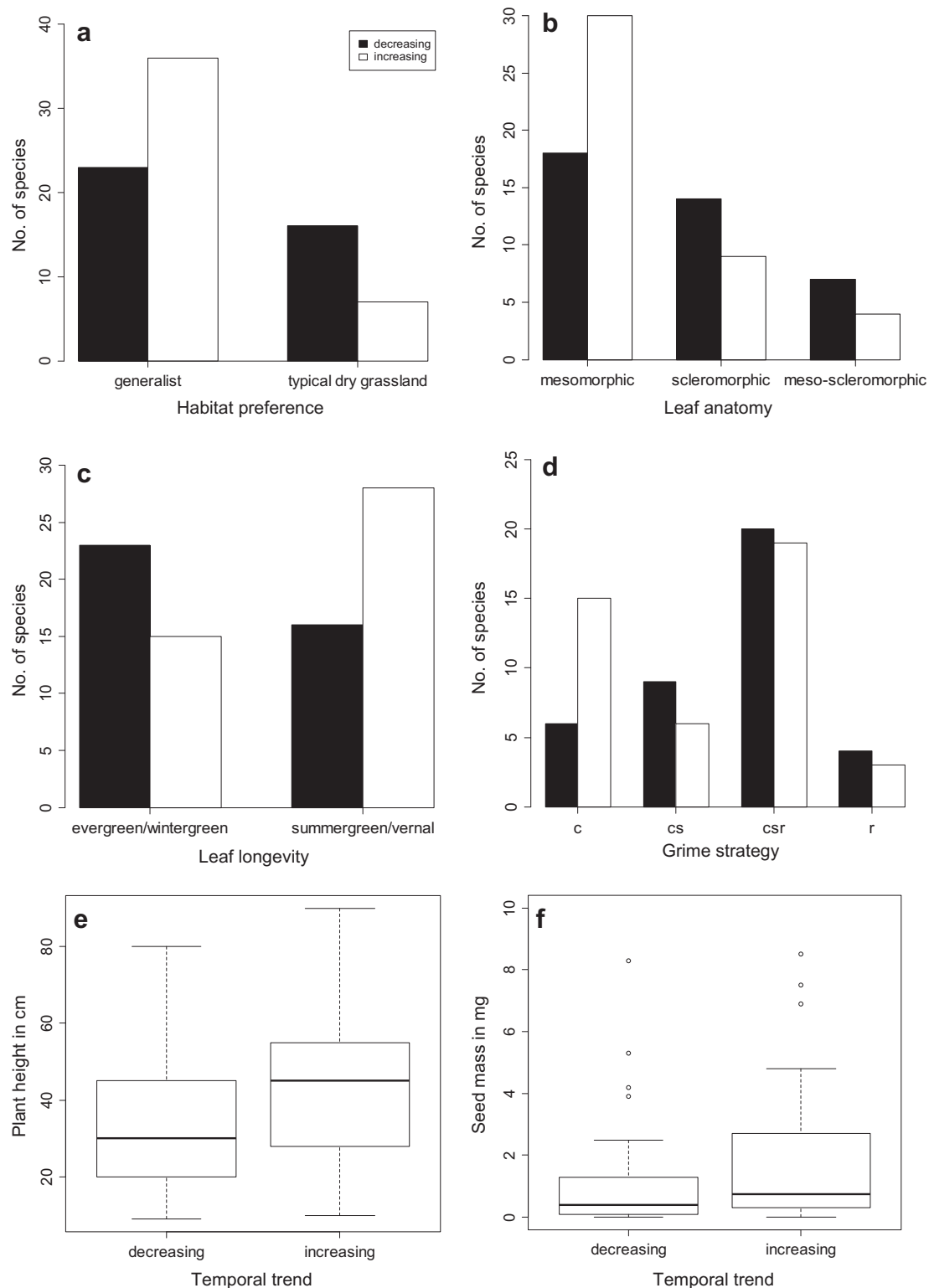


Fig. 4. Temporal trends of species with different life history traits and ecological strategies in German calcareous grasslands. Considered were only those species that showed a significant increase ($n = 43$, gray bars) or decrease ($n = 39$, black bars) over time (see Section 2). For height, the values of seven tree and shrub species were omitted from the analysis; for seed mass, 11 values out of 82 were lacking.

One possible reason for the weak effect of atmospheric N deposition on the number of species in calcareous grasslands emerges from Fig. 3a: total species richness does not generally decline with increasing nutrient availability (as indicated by mean Ellenberg N-values), but shows a hump-shaped relationship with fertility. With respect to total species richness, grasslands with a low

natural N supply (those situated in the left-hand side of the Fig. 3a) may thus even benefit from N addition. Furthermore, on the steepest and most S-exposed sites with shallow dry soils (those with a high heat index), N deposition most likely does not result in an increased N availability for the plants. The reason is that, during rainless periods in the growing season, the soils dry out and water

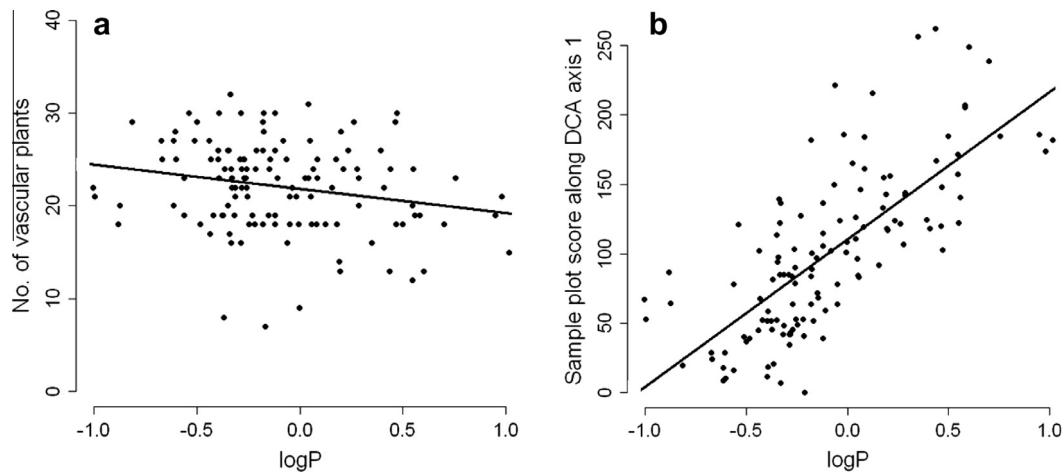


Fig. 5. Effect of soil phosphorus (P) content (log-transformed) on the (a) species richness of vascular plants ($R^2 = 0.047$, $P = 0.016$), and on (b) the main gradient in species composition as reflected by DCA axis 1 ($R^2 = 0.540$, $P < 0.001$) across 125 calcareous grassland plots in Germany sampled in 2008.

instead of N becomes the main limiting factor for most species. Dryness also results in a low biological activity of soil organisms and a reduced N mineralization (Ellenberg and Leuschner, 2010). The scarce literature on long-term changes in unimproved calcareous grasslands supports the observation that the species richness of these grasslands is *not* strongly affected by N deposition. This was, for example, noted by Partzsch (2000) in a study of porphyry outcrop vegetation in Eastern Germany. This interpretation also coincides with observations made by Bennie et al. (2006) in British chalk grasslands where the total number of species had generally declined over recent decades, most likely as an effect of nutrient enrichment, but these changes were less pronounced on the steepest and most sunny slopes. Another resurvey of calcareous grasslands in the UK also showed a significant, but weak decline in species richness (from on average 41 species in 1990–93 to on average 39 species in 2006–09 in 144 m² plots) (van den Berg et al., 2011). These results coincide with the lack of significant changes in species richness observed in long-term field experiments in the UK (Phoenix et al., 2012). Similar to the results of our study, an increase in local species richness over the last decades was observed in calcareous grassland sites in the county of Dorset in England, owing to a pronounced increase in species typical of mesotrophic conditions (Newton et al., 2012).

Another limiting factor in calcareous grasslands that is not directly associated with nor affected by N deposition is phosphorus. The availability of P to plants is generally low on high-pH soils (Carroll et al., 2003; Phoenix et al., 2003; Olsson and Tyler, 2004; Ellenberg and Leuschner, 2010), and the atmospheric input of P is generally low. P may thus be more limiting for species richness than N. The large importance of P for the differentiation of calcareous grassland vegetation becomes evident in the spatial comparison of plots from 2008 in Germany (Fig. 5). This is in clear contrast to acidic grasslands for which there is much evidence (Roem and Berendse, 2000) that P probably plays a subordinate role for the historical changes in species richness and composition compared to N (but see Ceulemans et al., 2011 for a different view).

Finally, another reason for the relatively weak effect of atmospheric N input on the species richness of calcareous grasslands is related to soil pH. N deposition in acidic grasslands results not only in direct N addition, but also indirectly in acidification and increased ammonium contents or higher ammonium/nitrate ratios, and these changes together cause the deleterious effects on species richness on soils with already low pH (Stevens et al., 2011a,b). The rendzina soils of calcareous grasslands, in contrast, have a large buffering capacity against acidification, and N is predominantly

mineralized as nitrate. In general, the effects of N deposition on soil chemistry and mineralization have been shown to depend on soil and habitat type in extensively managed habitats (Rowe et al., 2012).

4.2. Species composition

Species composition showed a directional change, for which there are three lines of evidence: (1) the variable 'year' had a significant effect on the differentiation of the grassland vegetation as reflected by the 2nd DCA axis (Fig. 2), (2) the proportion of dry grassland species responded differently to the explanatory variables than did the total species richness, with 'year' having a negative effect on the frequency of specialist species in the German grasslands (Fig. 3d), and (3) species with different attributes showed different temporal trends (Fig. 4). Over-represented among the taxa with a significant negative trend over time were species that were smaller and more light-demanding, had smaller seeds and winter-green or evergreen, more scleromorphic leaves, i.e., features typical for dry grassland specialists. Examples include *Acinos arvensis*, *Arenaria serpyllifolia*, *Gentianella ciliata* and *Trifolium campestre*. This coincides well with the results found by Jandt et al. (2011). The slow, but significant change towards a community with a higher share of taller species typical of more mesotrophic grasslands or thermophilic fringes is unlikely to be caused by N deposition and increasing N availability to the plants. First, the mean Ellenberg N scores of plots are unrelated to 'year' and 'cumNdep'. Second, there were no differences in N indicator values between increasing and decreasing species. A re-survey of UK grasslands also revealed no effect of N deposition on the change in mean Ellenberg N values (van den Berg et al., 2011).

We believe that the main reason for the shift in species composition is related to land-use history and management that has a strong long-term effect on species richness and composition (Alard et al., 2005; Köhler et al., 2005; Ellenberg and Leuschner, 2010). Several species with a positive temporal trend are typical elements of thermophilic fringe communities that are known to increase in calcareous grasslands after abandonment (at the expense of typical dry grassland species), such as *Clinopodium vulgare*, *Helictotrichon pubescens*, *Ranunculus polyanthemus* and *Viola hirta* (see also Jandt et al., 2011). This does not mean that the grasslands are no longer managed, but that the management is less intensive than in previous times (see also Hagen, 1996). Apart from management intensity, the form of management may also have changed, with a trend towards a transition from grazing to mowing. The effects

of these two processes are difficult to separate: the marked increase in *B. erectus*, a tall-growing grass, may be caused by lower grazing intensity, but also by the abandonment of management. The same applies to the decline of several thorny or unpalatable species that are indirectly favored by grazing and suffer from canopy closure and shrub encroachment, for example *Carlina vulgaris*, *C. acaule*, *Euphorbia cyparissias* and *G. ciliata* (Ellenberg and Leuschner, 2010).

We are aware that any historical survey of vegetation data not based on truly permanent plots faces the risk of bias, which is introduced if plots from different time periods are sampled with different methods. This may concern differences in plot size (Dengler et al., 2009) or the range of community types, season and phenology (Vymazalová et al., 2012). Estimates of species richness derived from phytosociological data – the majority of plots considered here – also can be associated with a certain bias (Chytrý, 2001; Diekmann et al., 2007). However, there is no indication of any systematic difference between different time periods in how, when and which data were sampled.

In conclusion, unlike acidic grasslands and forests, calcareous grasslands have not suffered from a general decline in vascular plant species richness. However, the different temporal trends of species with different attributes in the German data set reflect a change in species composition. Most notable is the loss of typical dry grassland species, which are often threatened and of focal interest to nature conservation. This loss is alarming as many of these species are long-lived, but have a transient seedbank. Although this trend appears to be slow, it indicates that management not only needs to be maintained in general, but also on an appropriate and sufficiently intensive level. The dry grasslands least susceptible to species loss and change are those on steep and sun-exposed slopes, where water and phosphorus limitation are most pronounced.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocon.2014.02.038>.

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