1 **Changes in species richness and composition in European acidic grasslands**

2 **over the past 70 years – the contribution of cumulative atmospheric**

3 **nitrogen deposition**

4

5 Running Title: Nitrogen deposition and species richness

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24 **Abstract**

25 Our study investigates the negative impact of nitrogen (N) deposition on species richness in

26 acidic grasslands, based on a temporal comparison of vegetation data spanning a period of

27 almost 70 years.

28   We compiled a large data base of plots assigned to the *Violion caninae* grassland type,

29 composed of managed, but unfertilized semi-natural grasslands on nutrient-poor, acidic soils.

30 In total 1,114 plots, mainly from Great Britain, the Netherlands and Germany, were compiled,

31 dating back to 1939. Environmental site information included geographical and soil (mean

32 Ellenberg values) variables as well as estimates of cumulative N and sulphur (S) deposition

33 since 1939. Statistical analyses were carried out separately for the data sub-sets from the three

34 regions.

35   In all regions, the vegetation differentiation was mainly related to soil acidity and nutrient

36 availability, as well as to the year of sampling and the cumulative amounts of N and S

37 deposition. Plot-species richness of vascular plants and bryophytes (analysed for Great Britain

38 only) decreased with time and analyses suggest these are affected by various factors, notably

39 soil pH, but also latitude and cumulative N deposition. The latter explained more of the

40 variation in species number than the year of sampling and cumulative S deposition, which

41 supports the interpretation that the decline in species richness is mainly caused by increasing

42 N availability and less by altered management and soil acidification. For Great Britain and

43 Germany, cumulative N deposition showed a strong negative relationship with several

44 biodiversity measures, especially the proportion of dicots, whereas it was positively related to

45 the proportion of grass species. In general, our results give temporal evidence for the negative

46 effect of N deposition on species richness in semi-natural vegetation.

Introduction

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

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

72   the validity of such an approach is not known (but see, for example, Clark & Tilman 2008). In

73   addition, the experiments are often simple in the sense that they do not reflect the complexity

74   of different effects acting simultaneously on the ecosystem. Therefore, experiments should be

75   complemented by observations in systems that have not been experimentally manipulated.

76   There are two basic approaches to describe the effects of N deposition on species richness

77   and composition in descriptive field studies: historical analyses and spatial comparisons. The

78   latter can be regarded as a space-for-time substitution and relies on spatial gradients in

79   deposition levels across a region. Comparisons between more- and less-polluted areas have

80   been conducted primarily in forests (Diekmann *et al.* 1999; Diekmann & Falkengren-Grerup

81   2002; Köchy & Bråkenhielm 2007). On a national scale across Great Britain, areas with

82   different pollution levels were compared for various ecosystems (Smart *et al.* 2004). Many of

83   these studies have shown an increase in relatively N-demanding species and a decrease in

84   species indicative of less fertile habitats. Recently, Stevens *et al.* (2004, 2006) provided the

85   first direct evidence on a large spatial scale for the negative impact of N deposition on plant

86   species richness by including modelled rates of deposition, together with many other

87   environmental predictors, into a regression analysis with data from acidic grassland plots

88   across Great Britain.

89   The time series approach is based on a temporal comparison of plot data. By comparing

90   old and new vegetation records in permanent plots, changes in species composition over time

91   may be detected and ecologically interpreted. This approach has proven successful in the

92   study of N-driven changes in forest vegetation (Thimonier *et al.* 1992, 1994; Diekmann *et al.*

93   1999). What limits this method of examining N deposition effects is the lack of permanent

94   plots, which if they exist at all, either have not been established for a long time, or their exact

95   location is not well documented. Furthermore, many habitat types are dominated by long-

96 lived plants where changes occur relatively slowly and therefore are difficult to detect in

97 short-term permanent plot studies.

98   In this study we apply an alternative time series type method to examine the impact of N

99 deposition on species composition and richness: using data from several regions, we compare

100 large numbers of plots from sites collected over a long period of time, including both

101 permanent and non-permanent plots. Here, the disadvantage of not comparing repeated

102 records from permanent plot sites is compensated by the use of a much larger number of plots

103 in the study. An advantage of our approach is that many old records can be used from periods

104 when N deposition levels were still low. This method has rarely been applied to study the

105 effects of atmospheric pollution (but see Diekmann & Duprè 1997), but we believe that the

106 large number of old and new vegetation records and their availability in large national or

107 regional databases make it possible to explore this historical approach further. It must be

108 noted, however, that historical, non-permanent plot data may be biased, if they, for example,

109 are not balanced in the sense that either different regions or different sub-communities are

110 sampled at different time periods, both of which can distort the statistical analysis.

111   In our study we focus on semi-natural acidic grasslands, the same type of vegetation used

112 for the spatial comparison of sites carried out by Stevens *et al.* (2004, 2006). For the first

113 time, estimates of the amount of cumulative N deposition for different sites based on present-

114 day modelling data are included in the species richness models. We addressed the following

115 research questions:

116 - Has there been a systematic change in plant species composition and richness in acidic

117 grasslands over the past seven decades?

118 - If so, can these changes be related to the amount of cumulative N deposition and soil factors,

119 especially soil acidity and fertility?

120 - Are species of different life forms affected differently?

121 **Material and Methods**

122

123 *Vegetation data*

124 We compiled historical and recent vegetation data from acid grasslands located in the oceanic

125 to sub-oceanic area of North-Western and Central Europe, including plots from Great Britain,

126 the Netherlands, Germany, Denmark and Sweden. Plots were only considered if the

127 vegetation conformed to the community type *Violion caninae* (or *Festuca ovina-Agrostis*

128 *capillaris-Galium saxatile* grassland sensu Rodwell 1992; Peppler-Lisbach & Petersen 2001),

129 a semi-natural grassland type on nutrient-poor, unfertilised, acidic soils, managed by

130 extensive grazing or, more rarely, mowing. Typical species include grasses such as *Nardus*

131 *stricta, Festuca ovina* and *Agrostis capillaris*, herbaceous dicots (*Arnica montana*, *Galium*

132 *saxatile, Viola canina*) and bryophytes such as *Pleurozium schreberi* and *Rhytidiadelphus*

133 *squarrosus. Violion* grasslands have a relatively uniform species composition across the entire

134 study region. We did not consider acidic grasslands on wetter soils (assigned to the *Juncion*

135 *squarrosi*) or (sub)alpine communities of the alliance *Nardion strictae*, and we also avoided

136 plots for which abandonment of management was indicated. *Violion* grasslands were formerly

137 of much greater extent, but abandonment of grazing has resulted in a considerable reduction

138 in area (Ellenberg 1996). This is especially true in Central Europe, where unfertilized acidic

139 grasslands nowadays persist almost exclusively in nature reserves. Only in Great Britain do

140 they still cover large areas.

141   The data were obtained from published literature, databases and newly sampled

142 vegetation analyses (see Electronic appendix). In total, data from 1,114 sample plots were

143 compiled, spanning a time period of almost 70 years from 1939 to 2007 (Table 1).

144

145 [Table 1 here]

6

147   The vegetation data were entered into a database consisting of a sample × species matrix. As

148   the data were collected at different times and for different purposes, their quality differed as

149   well, and we had to standardise the data which meant that some information was lost. This

150   often involved aggregating taxa: for example, *Luzula campestris* and *L. multiflora* into *L.*

151   *campestris* agg. The nomenclature of vascular plants followed Wisskirchen & Haeupler

152   (1998) and in some cases Tutin *et al.* (1964-1980). For bryophytes we followed a recent

153   reference list (Koperski *et al.* 2000). Species abundances had to be standardised to conform to

154   the simplified Braun-Blanquet scale (Mueller-Dombois & Ellenberg 1974; Dierschke 1994),

155   with '+' (denoting a few individuals) and 1 - 5 (indicating increasing frequency).

156   We then constructed a second matrix with environmental data, first entering information

157   on the geographical location (longitude, latitude, and altitude) and size of plots. As only the

158   most recent sources contained data on environmental and soil variables, we used mean

159   Ellenberg species indicator values (not cover-weighted) as surrogates (Ellenberg *et al.* 1992)

160   for the level of light (L_{Ell}), soil moisture (F_{Ell}), soil pH (R_{Ell}), and soil nitrogen (N_{Ell}, often

161   interpreted as general soil fertility) for all plots. Ellenberg values, a ranking system of

162   ecological niche preferences based on known plant characteristics, have successfully been

163   applied in many ecological studies across Central and Western Europe (see review by

164   Diekmann 2003) and have also been used to examine spatial patterns and temporal changes of

165   vegetation in response to acid or nitrogen deposition (Thimonier *et al.* 1992; Diekmann &

166   Duprè 1997). In our study, mean Ellenberg values were calculated in two ways, using all

167   species (vascular plants & bryophytes), or vascular plants only. For sample plots collected in

168   2007 from Germany, Denmark and Sweden, the correlation between the mean Ellenberg

169   values for R and the measured values of soil pH was highly significant ($r = 0.52$, $p < 0.001$),

170   showing, in accordance with many other studies (Diekmann 2003), that the mean R values

171   can be used as surrogate for soil pH.

172   Since we were interested in the long-term effects of atmospheric pollution on species

173   richness, we estimated cumulative values of N and S deposition, including both dry and wet

174   components, based on modelled values. The calculation of the variables 'cumulative nitrogen

175   deposition' (cuN_{dep}) and 'cumulative sulphur deposition' (cuS_{dep}) was based on estimates of N

176   and S deposition for the year 2000 ($N_{dep}2000$) obtained from the model FACEM (Pieterse *et*

177   *al.* 2007). This 'inferential' model enabled high-resolution (site-specific) calculations of dry

178   deposition of different compounds. The model combines site characteristics and

179   meteorological information to derive a dry deposition velocity. The latter is then combined

180   with available air concentration data (measured or modelled), resulting in a calculated dry

181   deposition estimate for individual sites. The concentration data used for this study were

182   obtained via the DEHM model (Hertel *et al.* 2006) using EMEP emission data. Available wet

183   deposition data (obtained via EMEP) was then added in order to get estimates of the total

184   deposition (i.e., wet + dry).

185   To estimate cumulative deposition values over time, we had to (1) consider the different

186   years of sampling, and (2) take into account that the deposition levels have changed

187   considerably during the past decades. In the case of N deposition, it was low until the middle

188   of last century, increasing first slowly and then rapidly until the 1980s, and since then

189   decreasing slightly or remaining more or less constant on a continuously high level (Berge *et*

190   *al.* 1999; Dentener *et al.* 2006; Gruber & Galloway 2008). S deposition, in contrast, was

191   already relatively high in the 1940s, increased strongly up to the 1970s, and has decreased

192   since then (Berge *et al.* 1999; Lorenz *et al.* 2008). We therefore introduced 'correction

193   factors' for the different decades, based on present-day deposition levels (see Appendix 1).

194   First, the N (or S) deposition value of a plot for the year 2000 was multiplied with the

195 corresponding correction factor to give a deposition estimate for that site for each year. For

196 the calculation, yearly corrected values were added up, starting from the year of the first plot

197 of the data set (1939) until the year in which the plot in question was sampled. For example,

198 for a plot measured in 1973, cuN_{dep} was calculated as: $N_{dep}2000 * (0.1* 11 \text{ years}) + N_{dep}2000$

199 $* (0.5 * 10 \text{ years}) + N_{dep}2000 * (0.9 * 10 \text{ years}) + N_{dep}2000 * (1.3 * 4 \text{ years})$.

200   The final result is an approximation and does not consider changes in land use around the

201 plots that may have taken place between 1939 and present, in which case the current

202 deposition level might not reflect exactly the historical deposition to the sites. It also leaves

203 unconsidered that the deposition curves are not identical between countries. However, on the

204 wide spatial scale at which we are working and the coarse time scale we applied, the

205 calculation accounts both for the major spatial differences in N and the continuous

206 accumulation of N over time. There will already have been some increase in N and S

207 deposition before 1939, but we decided to take this year as a base line and to consider only

208 the deposition that has occurred since then.

209

210 *Statistical analysis*

211 As we wished to examine whether the effects of N (and S) deposition on species composition

212 were similar in different regions, we subdivided the whole data set into three regional sub-

213 sets: Germany, Great Britain, and the Netherlands. By this method the differences between

214 countries, not only in environment and species composition, but also in the national

215 environmental and agricultural policies, especially with respect to the intensity and type of

216 management, were taken into account. The relatively few plots from Denmark and Sweden

217 were only used for comparative purposes, as the sampled regions in these countries have

218 lower deposition levels than Central Europe (Table 1).

9

219   To examine the changes in species composition over time and the underlying

220 environmental factors responsible for these changes, the three data sub-sets were analysed by

221 means of Detrended Correspondence Analysis (DCA). This method, based on unimodal

222 species responses, was selected because the large floristic gradients resulted in long DCA

223 gradient lengths (see Table 2). The program CANOCO (ter Braak & Smilauer 2002) was used

224 to produce the ordinations. We applied a downweighting of rare species, but otherwise

225 followed the default options. The resulting sample plot scores of the first two DCA axes were

226 then correlated with the following factors: year of sampling, cuN_{dep}, cuS_{dep}, and the mean

227 Ellenberg values for light and soil variables (based on all vascular plant species).

228   The relationships between environmental factors and species richness were analysed by

229 stepwise multiple linear regression using the programme MINITAB (Version 13.31). If

230 necessary, the descriptors were log-transformed to achieve normal data distribution (plot size

231 in all data sub-sets, altitude in the sub-set from the Netherlands). To check for

232 multicollinearity we correlated all variables to each other. In all country sub-sets the mean

233 values for N_{Ell} and R_{Ell} were highly positively correlated (r = 0.67-0.77). We decided to use

234 mean R_{Ell} instead of mean N_{Ell}, because (1) the variation in species richness explained by the

235 multiple models including mean R_{Ell} and the R^2_{adj} in simple regressions of species richness on

236 mean R_{Ell} were in nearly all cases much higher, and (2) it is known that pH has a particularly

237 strong effect on species occurrence and total species richness in low-productive environments

238 on highly acidic soils (e.g., Roem *et al.* 2002). The results of the multiple regression models

239 with either mean R_{Ell} or mean N_{Ell} were qualitatively very similar. Furthermore, 'year of

240 sampling' was omitted from all models (high correlation with cuN_{dep}, r = 0.74-0.88) as well

241 as, for the German data sub-set, 'altitude' (r with latitude = -0.81), and, in the English sub-set,

242 'longitude' (r with cuN_{dep} = 0.66). Finally, because cuN_{dep} and cuS_{dep} were highly positively

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

244   time.

245   For the model, we fitted a stepwise forward method with a threshold of $\alpha = 0.05$. We also

246   applied a backward elimination of variables and a mixed backward and forward method to

247   test the stability of the regressions, by making sure that the results were not affected by the

248   order in which the variables were entered to or eliminated from the models. All methods gave

249   identical results.

250   Regression models were fitted for the total number of vascular plant species, the number

251   of dicot species, the number of grasses (Gramineae), and the proportions of dicotyledonous

252   species and grasses out of the total number of species. A model for bryophyte species richness

253   was only fitted for the data sub-set from Great Britain, as the plots from Germany and the

254   Netherlands contained too few species (most likely because bryophytes were not always or

255   only incompletely recorded). For the vascular plant models the mean Ellenberg values based

256   on vascular plants were used, for the bryophyte models from Great Britain we used the mean

257   Ellenberg values based on all species. Finally, to visualize the interactions between the

258   explanatory variables, we carried out regression tree analyses using the program R.

259 **Results**

260

261 *Overview*

262 The size of the sampled plots varied considerably, ranging from 0.25 to 750 m^2 (Table 1). The

263 large differences were due to the different objectives of the authors when collecting the data:

264 most of the older plots were sampled in the framework of descriptive vegetation science that

265 did not consider the need of standardised data for a rigorous statistical evaluation.

266 Accordingly, the number of vascular plant species per plot varied strongly, between 3 and 51

267 in Germany, between 3 and 39 in Great Britain, and between 4 and 35 in the Netherlands.

268 Maximum species richness of bryophytes was 17 in Great Britain, 11 in Germany, and 9 in

269 the Netherlands. The mean Ellenberg values L_{Ell} and F_{Ell} were mostly in the range of 5.5 to 8

270 and 4 to 7.5, respectively, indicating the relatively exposed and dry to mesic environment of

271 the grasslands. The mean values for R_{Ell} and N_{Ell} were between 2 and 4 in most cases, but

272 lower than 2 in 66 plots for R_{Ell} and in 76 plots for N_{Ell}, reflecting the very acidic and nutrient-

273 poor soils of the sites.

274   Across all plots there were pronounced temporal changes in mean Ellenberg values: while

275 mean L_{Ell} and F_{Ell} decreased slightly with the year of sampling (R^2_{adj} = 0.075 and 0.102,

276 respectively, p < 0.001), mean R_{Ell} showed a quadratic relation with time (R^2_{adj} = 0.098, p <

277 0.001, as opposed to R^2_{adj} = 0.066 for a linear regression), increasing until about 1980 and

278 then stabilizing or even slightly decreasing. Overall the strongest (linear) correlation with

279 time was found for mean N_{Ell} that increased considerably over the years (R^2_{adj} = 0.223, p <

280 0.001).

281

282 [Table 2 here]

283

284 *Ordination*

285 The DCA ordination analyses for the three countries showed relatively long floristic gradient

286 lengths, varying between 3.3 and 4.3 standard deviation units for axis 1 and between 2.4 and

287 2.6 units for axis 2 (Table 2). The environmental factors that explained the most variation in

288 species composition were mean R_{Ell} and N_{Ell}, showing high correlation coefficients with the

289 sample plot scores along the first axis for all countries (absolute values of r between 0.67 and

290 0.91). Except in one case, the mean F_{Ell} and L_{Ell} were more closely correlated to the scores

291 along DCA axis 2 than for axis 1, with a maximum r = 0.56 for L_{Ell} in the German data sub-

292 set. The year of sampling, cuN_{dep} and cuS_{dep} were only weakly correlated with the DCA axes

293 in the Dutch data sub-set. In contrast, for the German plots we found a close relationship

294 between the ordination axes and the three variables, especially for year of sampling (r = -

295 0.61). For Great Britain, the correlation with axis 1 was even stronger (for year of sampling r

296 = -0.70), that for axis 2 somewhat weaker than for Germany. The strong impact of the year of

297 sampling on the floristic composition of the grasslands is also illustrated in Figure 1, showing

298 DCA ordination diagrams with different symbols representing plots sampled during different

299 decades. The graphs show clear temporal gradients both for Germany and Great Britain,

300 whereas the pattern is less clear for the Netherlands.

301

302 [Fig. 1 here]

303

304 *Stepwise multiple regressions*

305 For all three countries the number of plots for the multiple regressions was reduced due to the

306 lack of information for some of the variables (particularly plot size) in some of the samples.

307 For Germany we used 240 plots, in Great Britain 531 and in the Netherlands 106. Model

308 building started with 8 variables for the data sub-set from the Netherlands and 7 variables for

13

309 those from Germany and Great Britain. The final models retained a fairly high number of

310 significant variables, especially for the German and British plots (Table 3). They explained

311 more than half of the variation in total species richness of vascular plants (maximum R^2_{adj} =

312 0.65) or bryophytes (only Great Britain), and of the number of dicot species. For the number

313 of grasses and for the proportions of dicots or grasses, the models explained much less of the

314 total variation (minimum R^2_{adj} = 0.21).

315

316 [Table 3 here]

317

318 As expected from the large variation in plot size, the number of species (vascular plants,

319 dicots and grasses) was strongly positively related to the area sampled in all countries. In

320 contrast, there was a weak negative correlation between plot size and the number of

321 bryophytes in Great Britain. With respect to the proportions of dicot and grass species, the

322 effect of plot size was generally less strong and less consistent across countries than for the

323 absolute numbers of dicots and grasses.

324 Among the geographic variables, latitude was most strongly related to the total and

325 relative numbers of species: except for the proportion of grasses, species richness values

326 increased in Great Britain and decreased in Germany with increasing latitude. In contrast,

327 longitude and altitude showed only weak effects: for Germany, the number of vascular plants

328 and dicots was positively correlated with longitude, and for Great Britain the numbers of

329 dicots and bryophytes increased with increasing altitude.

330 By far the most important variable in the final models was mean R_{Ell}, exhibiting a

331 pronounced positive effect on species numbers except for bryophyte species richness in Great

332 Britain (Table 3). The proportion of dicots was positively related to mean R_{Ell}, whereas a

333 negative correlation was observed for the proportion of grasses. In comparison, the effects of

334   mean L_{Ell} and F_{Ell} were weaker and non-consistent across the different measures of species

335   richness and countries. In all data sets, however, the proportion of dicots was negatively

336   associated with mean F_{Ell}, and, in Great Britain, there was a strong negative effect of mean

337   L_{Ell} on the number of bryophytes.

338

339   [Fig. 2 here]

340

341   Generally the second strongest effect was found for cuN_{dep}, showing a negative relationship to

342   the number of vascular plants and dicots in all countries (Table 3), as well as to the number of

343   bryophytes in Great Britain. This is also displayed in the simple regressions of species

344   richness (corrected for plot size) on cuN_{dep} (Fig. 2). For grass species, cuN_{dep} remained

345   significant only in the final model for the Dutch data sub-set. For the grasslands in Great

346   Britain and Germany, cuN_{dep} also had a strong negative effect on the proportion of dicots,

347   whereas it exhibited a positive impact on the proportion of grass species. It is important to

348   note that the year of sampling, when used instead of cuN_{dep} both in multiple and simple

349   regressions, had a much weaker effect on species numbers, suggesting that the declining

350   species richness is not simply a temporal trend driven by factors other than N deposition.

351   When using cuS_{dep} instead of cuN_{dep} in the multiple regressions, highly similar results

352   were obtained. The R^2_{adj} values of the final models were mostly slightly lower than for the

353   cuN_{dep} models, but occasionally somewhat higher, and the t values for cuS_{dep} were in most

354   cases smaller than those for cuN_{dep}. The results reflect the strong inter-correlation between the

355   two factors, but also show that cuN_{dep} appears to explain somewhat more of the variation in

356   species richness than cuS_{dep}.

357   The regression trees for Germany and the Netherlands (figures not shown) confirmed the

358   primary importance of soil acidity for species richness; at given levels of R_{Ell} (and partly

15

359   latitude), cuN_{dep} also appeared to be important. The regression tree found for Great Britain

360   was highly informative (Fig. 3): here, cuN_{dep} only mattered at lower latitudes where soil

361   acidity was not important, whereas at higher latitudes, cuN_{dep} did not matter at all while R_{Ell}

362   was by far the most important factor affecting species richness.

363

364   [Fig. 3 here]

365

366   When considering the mean proportion of grass species as a function of the total number of

367   vascular plant species in different decades since 1940 for Germany and the Netherlands, the

368   increasing frequency of Gramineae over the years becomes evident (Fig. 4). The mean

369   proportions calculated for the sample plots collected in 2007 from Denmark and Sweden were

370   clearly lower than the corresponding values for Germany and the Netherlands. For Great

371   Britain from which only plot data for three decades was available, the differences in the

372   proportions of grass species between the north and the south support the findings from Fig. 3,

373   whereas there were no clear temporal trends.

374

375   [Fig. 4 here]

376

377   The changes in the proportions of life forms also become evident from Table 4 showing the

378   relative frequencies of the most common and characteristic species in *Violion* grasslands in

379   plots sampled before 1975 compared to those collected after 1975. Grasses such as *Agrostis*

380   *capillaris* and *Festuca rubra*, but also ruderals like *Holcus* sp., have clearly increased in all

381   regions, while among the common grasses only *Festuca ovina* has declined. A general

382   decrease in frequency was found for most dwarf shrubs (e.g., *Erica* sp., *Genista* sp. and

383   *Calluna vulgaris*), but also many herbaceous dicots were clearly less common in the more

16

384   recent than in the older plots, for example *Arnica montana* and *Viola canina*. Among the few

385   dicots that have increased in frequency are *Achillea millefolium* and both *Rumex acetosa* and

386   *R. acetosella*, representing species that are characteristic for more fertile or ruderalized

387   grasslands. On average, species with a decreasing trend had lower N_{Ell} values than species

388   with an increasing trend. For bryophytes, we can rely only on the data from Great Britain;

389   here, in accordance with the results given in Table 3, most of the more common species

390   tended to decline over the years (e.g*., Dicranum scoparium, Pleurozium schreberi*), whereas

391   only *Rhytidiadelphus squarrosus* and *Scleropodium purum* appeared to increase in frequency.

392   Not reflected in Table 4 is the strong decline in many low-frequent *Violion* species that were

393   already rare before 1975 and have more or less completely disappeared since then.

Discussion

General discussion of explanatory variables

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

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

419 fluctuate in time and space and cannot be determined by single measurements. The only

420 chemical variables at least partly based on measurement data (and thereafter modelled) are

421 cumulative N and S deposition. Although individual deposition values for NO_x^- and NH_4^+ as

422 well as S compounds for the year 2000 were available, we used the total value for the

423 regression models, because the ratio of, for example, deposited nitrate and ammonium has

424 changed during the last decades (the former tends to decrease, the latter to increase), which

425 made the estimation of deposition values for the separate measures in the past too uncertain.

426   Plot size was included in the regression models as a co-variable. Species richness

427 increases with increasing area, and therefore it is hardly surprising that plot size in general

428 was the second most important factor in the final models. This effect is even likely to be

429 under-estimated, because most vegetation data in Germany and the Netherlands were sampled

430 in the framework of phytosociological studies in which plots tend to be made larger in

431 species-poor vegetation in order to include more taxa of interest (Chytrý 2001; Schuster &

432 Diekmann 2003). This results in a flatter slope of the species richness-area regression line.

433 The negative effect of plot size on bryophyte species richness found in Great Britain is

434 difficult to explain, but one hypothesis could be that the time and attention devoted to

435 bryophytes are relatively smaller in larger plots than in smaller plots that are easier to survey.

436   Management is another factor that may be driving species richness: it is known to have a

437 strong effect on species composition and richness in grasslands (Bakker 1989). In non-alpine

438 areas *Violion* grasslands are semi-natural, and the abandonment of grazing or mowing may

439 result in a reduced species richness and an increase in the proportion of grasses at the expense

440 of herbs, thereby 'mimicking' the effects of nitrogen deposition. Although we considered

441 plots only if continued and uninterrupted management was indicated, the information

442 provided in the literature was not sufficient to determine the intensity of the grazing / mowing

443 for every plot; nevertheless, it is known that management of semi-natural grasslands in

444   Central Europe in general has declined (Ellenberg 1996). Changes in management, however,

445   cannot be the only explanation for the observed decline of species richness. First, assuming

446   that these changes have taken place in a similar manner in all European countries, species

447   numbers today should also be similar (and lower than previously), which is not the case: in

448   Denmark and especially Sweden with lower or much lower N deposition levels compared to

449   Germany and the Netherlands, species richness and the proportion of forbs were higher or

450   much higher. Second, if management intensity was declining with time, we would expect a

451   closer correlation between species richness and year of sampling than between species

452   richness and cuN_{dep}, which was not the case. Third, the general decrease in dwarf shrubs

453   found in all regions is difficult to explain with the abandonment of management: these species

454   are assumed to increase when grazing or hay-making cease. All this suggests that

455   management may contribute to explaining the changes in species composition and richness,

456   but is likely to be less important than cuN_{dep}.

457   The close correlation between cuN_{dep} and cuS_{dep} permitted us to use only one of the two

458   variables in the multiple regression models. We chose to keep cuN_{dep} as it explained more of

459   the variation in species richness and was more closely related to the Ellenberg values than

460   cuS_{dep}. For this we suggest the following explanation: The soil pH of the *Violion* grasslands

461   has always been very low, so that S deposition has little acidifying effect on the environment,

462   and thereby has lesser impact on species richness. In contrast, N deposition adds considerably

463   to the nitrogen availability in the soil, as reflected in the effect of cuN_{dep} on species numbers

464   and N_{Ell}. An observation suggesting that S deposition is not so important is that the plots from

465   those regions that have experienced rather high doses of S deposition (the Harz mountains in

466   Germany, Western Denmark and Sweden), but where low N deposition occurs, have

467   relatively high species richness. In addition, if cumulative S deposition was the main factor

468   driving the changes in species richness and composition, one would expect decreasing pH

469 values over time as reflected in declining mean R_{Ell} values, for which there is no evidence (see

470 below).

471

472 *Ordination analysis*

473 Whilst the use of multiple regressions allowed us to investigate changes in species richness

474 through time, the ordination analysis demonstrated changes in the species composition that

475 occurred largely independently of the number of species. Both types of analysis showed the

476 strong impact of soil acidity and cuN_{dep} on the vegetation of the *Violion* grasslands.

477   The ordinations for all three countries suggested that the main gradient in species

478 composition is linked to a combined gradient in soil acidity and nutrient availability (Table 2).

479 Mean N_{Ell} and especially mean R_{Ell} were strongly negatively related to the sample plot scores

480 along DCA axis 1; such negative relationships were also found for year of sampling and

481 cuN_{dep} (except in the Netherlands), reflecting the chronic input of nitrogen over time. The

482 results are, however, based on mean indicator values, and the actual pattern is somewhat more

483 complicated. In Germany, the plots sampled in 2007 appear to have slightly lower N and R

484 indicator scores than those collected in the 1980s and 1990s, at first sight suggesting a

485 decreasing N availability during the last two to three decades. Given that the indicator values

486 for N_{Ell} and R_{Ell} of the more frequent species in the *Violion* grasslands are strongly positively

487 related ($r = 0.655$, $p < 0.001$, $n = 36$), we hypothesize the following scenario. Initially, the

488 deposition of N leads to an addition of N to the soils and an increase in the number of N-

489 demanding species. As the latter also tend to have higher R_{Ell} values, both mean N_{Ell} and

490 mean R_{Ell} increase, even if soil pH values may not have changed or may be changing very

491 slowly. During the first years of N accumulation, the loss of typical stress-tolerant taxa in the

492 grasslands may at least partly have been compensated by the addition of more competitive

493 newcomers. More recently, the relative amount of NH_4^+ in deposition has increased (Kleijn *et*

494   *al.* 2008), resulting in increased acidification of the soil. This may have contributed to the

495   decline in acid-sensitive species that at the same time are more N-demanding, leading to a

496   drop in mean R_{Ell} and mean N_{Ell} values despite continued N deposition.

497   It is difficult to say why the resulting patterns in the Netherlands are not as clear as in the

498   two other regions. One reason might be that the *Violion* grasslands in the country were

499   already significantly affected by N deposition before 1939, meaning that considerable floristic

500   changes already took place during a period not covered in the data set used here. In

501   accordance with this interpretation, the relative frequencies of species favoured by N in the

502   plots sampled before 1975 in the Netherlands are on average higher than the corresponding

503   values in Germany and Great Britain, and those of 'N-sensitive' species on average lower.

504   The earlier N deposition in the Netherlands may have been caused by the intensive

505   agricultural land use causing relatively high emissions of N, especially ammonium, during the

506   first half of the last century.

507

508   *Effects of nitrogen addition and soil pH on species richness*

509   The regression results clearly show that species richness in *Violion* grasslands is strongly

510   negatively related to the accumulated N deposition (with the exception of the number of

511   grasses in Germany and Great Britain). This effect is particularly pronounced in Great Britain

512   where bryophyte richness also declines in relation to cuN_{dep}. The results thus support the

513   findings of a spatial comparison across Great Britain (Stevens *et al.* 2004) and from

514   experimental studies (e.g., Mountford *et al.* 1993; Carroll *et al.* 2003; Clark & Tilman 2008),

515   all suggesting the negative effect of N deposition on phytodiversity, at least at higher

516   deposition levels such as in the central and southern parts of Great Britain (see Fig. 3).

517   The above effect is mainly due to the strong decline in the number of dicot species,

518   whereas the relative number of grasses (except in the Netherlands) has strongly increased.

519   Therefore, N deposition has favoured grasses such as *Agrostis capillaris* and *Festuca rubra* at

520   the expense of forbs. Again, this is consistent with other observations in *Violion* grasslands

521   made along a geographical deposition gradient in Great Britain (Stevens *et al.* 2006) and with

522   results from N addition experiments (Carroll *et al.* 2003) where grasses were favoured by

523   high N deposition levels. The same response, i.e., a change in vegetation composition in

524   favour of grasses has been reported from experimental sites in other grasslands types

525   (Bobbink 1991; Mountford *et al.* 1993; Wedin & Tilman 1996). One possible reason for this

526   is that grasses are capable of more rapidly exploiting available nitrogen than forbs, resulting

527   in faster growth and a suppression of dicot species of smaller stature (Peppler-Lisbach &

528   Petersen 2001). Accordingly, species that are almost completely lacking from the recent 2007

529   plots from Germany, such as *Viola canina* and *Veronica officinalis,* are still relatively

530   common in the 2007 plots from the low N-deposition countries Denmark and Sweden.

531   Soil pH appears to be even more important for species richness than N deposition, having

532   a strong positive effect on both the absolute number of vascular plant species and the

533   proportion of dicots; in contrast, the proportion of grasses decreases with increasing pH (see

534   also Stevens *et al*. 2004). *Violion* grasslands occur on highly acidic soils tolerated by few

535   acid-tolerant species, and any decrease in pH creates an environment that excludes more

536   species, since the majority of species in the European flora have their ecological optima at

537   higher pH values, resulting in hump-backed curves of species richness along the pH gradient

538   (Schuster & Diekmann 2003). The deleterious effect of high soil acidity on most taxa is due

539   to the high H^+ concentration and Al^{3+} toxicity (Peppler 1992; Houdijk *et al.* 1993; Kleijn *et al.*

540   2008). The pronounced differences in pH (as indicated by the mean R_{Ell} values) between plots

541   are mainly caused by original differences between the sites, and partly by temporal changes

542   that have taken place. Although the deposition of acidifying substances has generally

543   decreased during the last two decades, the enhanced NH_4^+ to NO_3^- ratio reported for recent

544 years may contribute to an acidification that, together with continued N addition, may be

545 responsible for the ongoing decline in plant species richness.

546

547 Conclusions

548 In our paper, based on historical vegetation data from acidic grasslands, we for the first time

549 provide evidence for the strong effects of cumulative N deposition on plant species

550 composition and richness. The findings support the results of spatial gradient analyses and

551 experimental studies in showing that N enrichment of grassland ecosystems is accompanied

552 by a loss of species richness, especially dicots. Future research on the effects of atmospheric

553 deposition on species richness should make use of the different approaches: time series data

554 allows us to track the long-term changes in species composition and reflects the complexity of

555 environmental variation, and therefore complement experimental plot methods that have the

556 advantage of disentangling the effects of single environmental variables, such as soil nitrogen

557 and pH, on the changes in the vegetation.

558

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569

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706 Table 1. Survey of vegetation data given separately for the five countries from where plots were compiled. The number of plots and the ranges of

707 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

708 denote the plot averages of the species' indicator values for light (L_{Ell}), soil moisture (F_{Ell}), pH (R_{Ell}) and nitrogen (N_{Ell}).

709

Country	No. of plots	Years of sampling	Plot size [m^2]	Altitude [m a.s.l.]	N_{dep} in 2000 [eq ha^{-1} y^{-1}]	S_{dep} in 2000 [eq ha^{-1} y^{-1}]	Mean L_{Ell}	Mean F_{Ell}	Mean R_{Ell}	Mean N_{Ell}
Germany	392	1940-2007	1-100	0.5-812	707-2719	361-914	5.7-8.0	3.4-8.5	1.0-5.1	1.5-4.9
Netherlands	144	1939-2006	0.4-750	0.1-59	728-2554	518-972	6.6-8.0	4,3-7.3	1.9-5.7	1.8-4.3
Great Britain	541	1960-2003	0.25-4	9-700	130-868	78-910	3.8-7.7	4.0-8.0	1.6-5.4	1.7-5.7
Denmark	17	2007	4	4-74	1030-1192	437-474	6.5-7.2	4.0-5.5	2.4-3.8	2.2-3.4
Sweden	20	2007	4	64-100	558-563	319-327	6.6-7.1	4.4-5.6	2.3-3.5	2.1-3.4

710

711 Table 2. Relationship between sample plot scores of DCA axes 1 and 2 and environmental variables: year of sampling, cumulative N and S

712 deposition (cuN_{dep} and cuS_{dep}) and the mean Ellenberg values for light (L_{Ell}), soil moisture (F_{Ell}), pH (R_{Ell}) and nitrogen (N_{Ell}). Pearson

713 correlation coefficients are given, with significant values bolded. a = $p < 0.01$, b = $p < 0.05$, all others $p < 0.001$. The eigenvalues for the DCA

714 ordination axes 1 and 2 were 0.45 and 0.22 (Germany), 0.39 and 0.17 (the Netherlands), and 0.33 and 0.21 (Great Britain), respectively.

715

Country	DCA axis	Gradient length	Year of sampling	CuN_{dep}	CuS_{dep}	Mean L_{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

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

Dependent 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	D	-5.09		#			-4.45	8.70	-5.43	51.1	-4.75	51.1
dicots	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	D			#	-2.76	2.12		-7.08	6.81	47.5	6.43	46.4
grasses	NL					2.89		-6.66		33.1		33.1
	GB		#		-5.66	5.66	2.94	-4.77	12.2	37.4	9.22	36.4
No. of bryophytes	GB	4.46	#	5.29	-2.90	-9.23			-6.83	52.6	-5.46	51.1

Table 4. Relative frequency of species (in %) in the data sub-sets from Germany, the

726 Netherlands and Great Britain, for plots sampled before (old) and after (new) 1975. Only the

727 most common taxa are shown. Within a life form, the species are ordered according to their

728 general patterns of change in frequency over time (increasing; stable, unclear or differing

729 between regions; decreasing). Bryophyte species are shown only for Great Britain. N_{Ell} =

730 Ellenberg value for nitrogen.

731

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

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

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

Figure legends

803   Fig. 1. DCA ordination diagrams showing the sample plot scores along axes 2 and 1 for the

804   three data sub-sets from Germany, the Netherlands and Great Britain. The symbols refer to

805   the different decades in which the plots were sampled: ◊: -1949. ○: -1959.●: -1969. +: -1979.

806   x: -1989. *: -1999. □: 2000-.

807

808   Fig. 2. Effects of cumulative N deposition (in mol ha^{-1} y^{-1} * 1000) on the species number of

809   vascular plants and bryophytes (only Great Britain) in the three regional data sub-sets; species

810   richness values were corrected for plot size (i.e., residuals were used). Germany: $R^2_{adj.}$ =

811   0.098, n = 241; the Netherlands: $R^2_{adj.}$ = 0.125, n = 106; Great Britain - vascular plants: $R^2_{adj.}$

812   = 0.234, n = 532, bryophytes: $R^2_{adj.}$ = 0.159; all p < 0.001.

813

814   Fig. 3. Regression tree of the relationship between species richness of vascular plants

815   (corrected for plot size) and the variables R_{Ell}, latitude and cumulative N deposition (cuN$_{dep}$)

816   for the data sub-set from Great Britain (n = 532).

817

818   Fig. 4. Mean proportion of grass species on the total number of vascular plants in different

819   decades since 1940. The continuous line shows the results for Germany (D), the dashed line

820   are those for the Netherlands (NL). For Great Britain (GB), mean values are given for those

821   decades for which vegetation data was available, separately for plots from the south (○, -s) (<

822   56.1° N, see Fig. 3) and the north (●, -n). For comparison, also the mean values for the 2007

823   sample plots from Sweden (□, S) and Denmark (*, Dk) are given.

824 Appendix 1. Correction factors used for the calculation of values of cumulative nitrogen (N)

825 and sulphur (S) deposition since 1939.

826

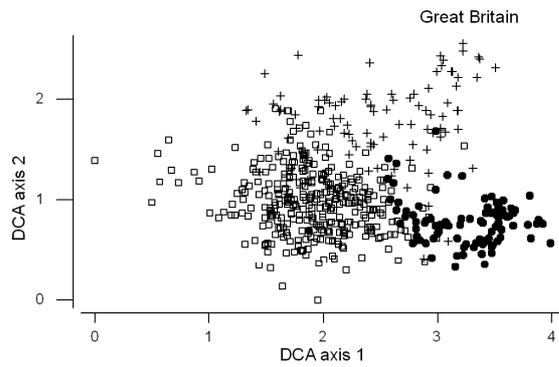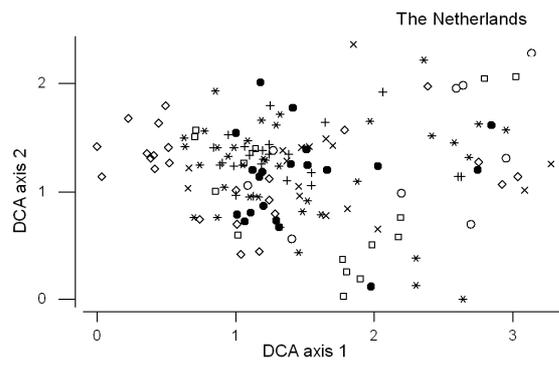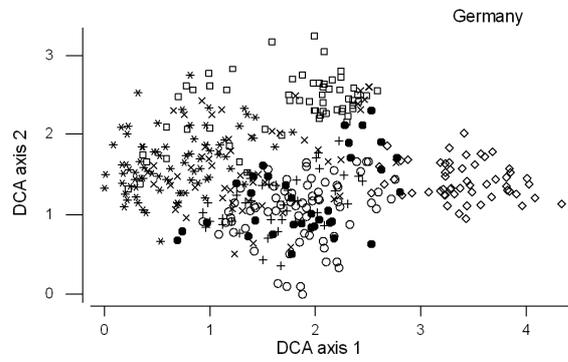|	1939-49	1950-59	1960-69	1970-79	1980-89	1990-99	2000-07
N	0.1	0.5	0.9	1.3	1.1	1	1
S	0.8	1.4	1.7	1.8	1.6	1.4	0.8

827

828 Electronic appendix. Sources of vegetation data used for the analyses, including the number

829 of sample plots, the country of origin, and the year of sampling.
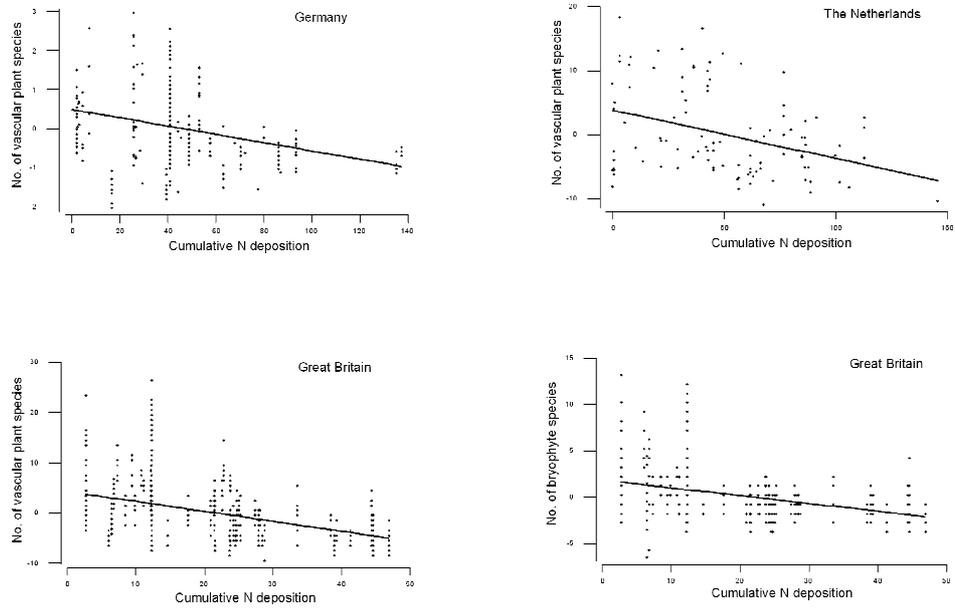
830

Reference	No.	Country	Year
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Bruelheide, H. 1995. Die Grünlandgesellschaften des Harzes und ihre Standortbedingungen. Dissertat. Botan. 244: 1-338.	78	Germany	1991
Buchwald, K. 1940. Die nordwestdeutschen Heiden. Diss. Univers. Heidelberg.	69	Germany	1940
Chadwick, M. J. 1960. *Nardus stricta* L. J. Ecol. 48: 255-267.	10	Great Britain	1960
Dierschke, H. 1979. Die Pflanzengesellschaften des Holtumer Moores und seiner Randgebiete (Nordwest-Deutschland). Mitt. Flor.-soz. Arbeitsgem. N.F. 21: 111-143.	11	Germany	1979
Dierschke, H. & Tüxen, R. 1975. Die Vegetation des Langholter- und Rhauder Meeres und seiner Randgebiete. Mitt. Flor.-soz. Arbeitsgem. N. F. 18: 157-202.	1	Germany	1975
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	60	Germany	2007
	20	Sweden	2007
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Hennekens, S. 2007. SYNBIOSIS-Datenbank, Wageningen, Niederlande.	135	The Netherlands	1936-2006
Horst, K. 1964. Klima und Bodenfaktoren in Zwergstrauch- und Waldgesellschaften des Naturschutzparks Lüneburger Heide. Natursch. Landschaftspfl. Nieders. 2. Hannover.	7	Germany	1964
Huntley, B. 1978. The past and present vegetation of the Caenlochan	103	Great Britain	1978

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Pott, R. & Hüppe, J. 1991. Die Hudelandschaften Nordwestdeutschlands. Westfälisches Museum für Naturkunde, Münster.	8	Germany	1989
Preising, E. 1950. Nordwestdeutsche Borstgras-Gesellschaften. Mitt. Flor.-soz. Arbeitsgem. N. F. 2: 33-41.	21	Germany	1950
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Tüxen, R. 1974. Die Haselünner Kuhweide. Mitt. Flor.-soz. Arbeitsgem. N. F. 17: 69-102.	17	Germany	1974
Walther, K. 1950. Die Pflanzengesellschaften im Grundwasserschadengebiet Lathen-Dörpen am Dortmund-Ems-Seitenkanal. Mitt. Thür. Bot. Ges. Beiheft 2. Weimar.	6	Germany	1950
Walther, K. 1977. Die Vegetation der Gemeindeweide Fuhlkarren bei Meetschow (Kr. Lüchow-Dannenberg). Mitt. Flor.-soz. Arbeitsgem. N. F. 19/20: 253-256.	10	Germany	1977
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    J. Ecol. 54: 535-548.

Welch, D. 1967. Communities containing *Juncus squarrosus* in Upper   67   Great Britain   1967

    Teesdale, England. Vegetatio 14: 229-240.

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    Verden) - aktueller Zustand und langfristige Veränderung. Unveröff.
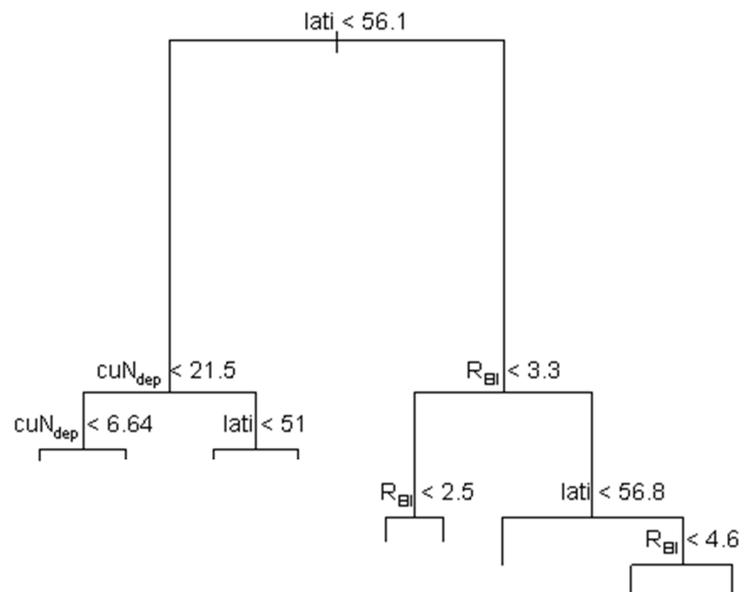
    Diplomarb., Univ. Göttingen.

831

832   Figure 1

833

834   Figure 2

835

836   Figure 3

837

Figure 3 tree diagram. Root: lati < 56.1. Left branch: cuN_dep < 21.5, then cuN_dep < 6.64 and lati < 51. Right branch: R_BI < 3.3, then R_BI < 2.5 and lati < 56.8, then R_BI < 4.6.

838