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**1 Impacts of atmospheric pollution on the plant communities of British acid grasslands**

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**17 ABSTRACT**

18 Air pollutants are recognised as important agents of ecosystem change but few studies consider  
19 the effects of multiple pollutants and their interactions. Here we use ordination, constrained cluster  
20 analysis and indicator value analyses to identify potential environmental controls on species  
21 composition, ecological groupings and indicator species in a gradient study of UK acid grasslands. The  
22 community composition of these grasslands is affected by climate, grazing, ozone exposure and nitrogen  
23 deposition, with evidence for an interaction between the ecological impacts of base cation and nitrogen  
24 deposition. Ozone is a key agent in species compositional change but is not associated with a reduction

25 in species richness or diversity indices, showing the subtly different drivers on these two aspects of  
26 ecosystem degradation. Our results demonstrate the effects of multiple interacting pollutants, which  
27 may collectively have a greater impact than any individual agent.

28 CAPSULE: Ozone exposure and nitrogen deposition have distinct but additive impacts on the plant  
29 communities of British acid grasslands.

30 KEYWORDS: Ozone, Nitrogen deposition, Base cation deposition, Ordination, Critical loads, Biodiversity

## 31 INTRODUCTION

32 Atmospheric pollution is among the most important drivers of global biodiversity loss and  
33 degradation of ecosystem services (Sala et al. 2000; Phoenix et al. 2006). Pollutants such as sulphur  
34 dioxide, ozone, heavy metals, particulates and nitrogen compounds ( $\text{NO}_x$ ,  $\text{NH}_y$ ,  $\text{HNO}_3$ ) are widespread in  
35 industrialised and agricultural regions, and all are capable of ecological effects. Slowing the rate of global  
36 biodiversity decline is a major global policy priority but the aim of the Convention on Biological Diversity  
37 to reduce the rate of biodiversity loss by 2010 has not been met (Butchart et al. 2010). The relative  
38 importance of different pollutants in driving ecosystem change and biodiversity loss is however poorly  
39 quantified.

40 Three general approaches have been used to investigate the impacts of air pollutants on  
41 vegetation: experiments, time-series, and gradient studies, with each approach having its own  
42 advantages and limitations. Experiments allow precise control of environmental conditions to  
43 investigate cause-effect relationships but are inevitably small scale and of relatively short-duration with  
44 uncertain applicability to real-world situations. Time-series allow a wider temporal scope but cannot  
45 demonstrate cause-effect relationships and are limited by the requirement to use old datasets which  
46 are often of poor or uncertain quality. Gradient studies similarly cannot reveal cause-effect, but by  
47 making a space-for-time substitution allow the use of high-quality contemporary data to investigate  
48 widespread environmental processes and the cumulative impact of long-term pollution exposure. In  
49 studies of air-pollution impacts experimental approaches have been by far the most common to date  
50 though gradient and time series studies are now becoming a much more utilised tool.

51 In the United Kingdom the early onset of the agricultural and industrial revolutions has resulted  
52 in many regions experiencing anthropogenic pollution for amongst the longest periods of any area  
53 worldwide (Fowler et al. 2004). However, the UK also experiences a considerable variability in pollution

54 loading with more remote areas, particularly in the north and west, having levels barely exceeding  
55 natural background. This contrast in pollution loading within a region with relatively homogeneous  
56 climate and agricultural management practises provides a highly suitable location for gradient studies. In  
57 2004 Stevens et al. showed a reduction in the species richness of acid (calcifuge) grasslands along a  
58 gradient of inorganic nitrogen deposition clearly demonstrating that nitrogen is an important agent in  
59 the loss of diversity in this UK Biodiversity Action Plan priority habitat (Mattock 2008) and supporting  
60 the results of experimental studies (Morecroft et al. 1994; Lee & Caporn 1998). More recent studies  
61 have employed this dataset to investigate the impact of nitrogen on dominant plant functional groups  
62 (Stevens et al. 2006) and indicator species (Stevens et al. 2009a), plant physiology (Gidman et al. 2006)  
63 and soil exchangeable metal concentrations (Stevens et al. 2009b). However, a number of questions  
64 remain unaddressed in the dataset. While a great deal of study has focussed on the drivers of  
65 biodiversity loss, the sensitivity of individual plant species has been less studied, particularly with  
66 respect to factors other than nitrogen. Our initial aim is therefore to quantify the relative importance of  
67 a range of potential drivers in determining species composition of these sites: is nitrogen the only  
68 important control or do other natural or anthropogenic drivers match or exceed its importance? How do  
69 individual species respond to these drivers? We also address whether modelled cumulative pollutant  
70 loadings are a better measure of pollution impact than current values (Dupre et al. 2010; RoTAP in  
71 press).

## 72 METHODS

73 Full details of survey methodology are presented in previous publications (Stevens et al. 2004;  
74 2006; 2009a; 2009b); here we provide a brief summary. Sites were selected within a UK National  
75 Vegetation Classification of U4 '*Festuca ovina-Agrostis capillaris-Galium saxatile* grasslands', calcifuge  
76 grasslands which are widely distributed in the British Isles (Rodwell 1992). A series of 64 sites was  
77 selected from southwest England to northern Scotland, stratified to span the gradients of nitrogen  
78 deposition and climate. Sampling areas were selected to avoid areas of significant disturbance and the  
79 cover of all plants estimated to species-level within five randomly positioned 2 x 2 m quadrats. The  
80 mean of the plant species cover estimates in these five quadrats was used as a representation of site  
81 community composition.

82 Data on a wide range of potential environmental drivers of diversity and community  
83 composition were compiled (full details are given in Appendix 1). Climate data were obtained from the  
84 UK Meteorological Office and the MORECS database (Thompson et al. 1981). Data on recent (1996-

85 1998) deposition of total non-marine base cations, sulphur (wet and dry) and nitrogen (wet and dry,  
86 oxidised and reduced), and cumulative nitrogen deposition over a variety of periods were obtained from  
87 the Centre for Ecology and Hydrology CBED-model (Smith et al. 2000) with historical scaling factors  
88 (Fowler et al. 2004). Data on tropospheric ozone exposure were extracted from the UK Air Pollution  
89 Information System (NEGAP 2001), and data on stocking density from the UK Agricultural Census  
90 (DEFRA 2009). Soil samples were collected in the field and pH, nutrient and metal concentrations,  
91 organic content, bulk density and particle size determined in the laboratory following standard methods  
92 (Stevens et al. 2009; Appendix 1). Information on a range of other parameters (aspect, inclination,  
93 presence of enclosures, vegetation height, grazing intensity) was recorded in the field. The total dataset  
94 includes 62 variables that together encompass the most important abiotic controls on community  
95 composition of grasslands that are measureable on a landscape scale (Appendix 1).

96 We use ordination to explore the complex relationships between environmental drivers and the  
97 overall community composition of these grasslands. Our analyses are based on redundancy analysis  
98 (RDA), a constrained ordination technique, with Hellinger-transformed species data (Rao 1995, Legendre  
99 and Gallagher 2001). A forward selection procedure where variables are successively added in order of  
100 their conditional effect with previously selected variables as co-variables was used to identify a minimal  
101 suite of environmental variables. A series of (partial) redundancy analyses were carried out to identify  
102 the proportion of variance explained by each selected variable alone and with other variables  
103 introduced as co-variables (Borcard et al. 1992). The significance of these analyses was tested using  
104 Monte Carlo permutation tests (999 permutations) in CANOCO vers. 4.53 (ter Braak and Šmilauer 1997-  
105 2004). Mean Ellenberg R (acidity) and N (general soil fertility: Hill et al. 1999) and mean C-S-R scores (an  
106 index of plant functional traits: Grime et al., 2008; Hunt et al., 2004), were calculated for the community  
107 composition of each site (Stevens et al. 2010a), and introduced as passive variables in the analysis (i.e.  
108 projected into, but not affecting, the ordination space).

109 To identify ecological groupings of samples along the pollution gradients we trialled a  
110 constrained cluster analysis approach where clusters are constrained to be composed of samples with  
111 similar levels of an environmental variable. We treated our dataset as a transect along a pollution  
112 gradient (nitrogen or ozone) and aimed to identify clusters of samples along this gradient so as to  
113 maximise within-group similarity. Our analysis used constrained incremental sum of squares (CONISS)  
114 clustering (Grimm 1987), an agglomerative method which aims to minimize the increase in total within-  
115 cluster sum of squares when combining adjacent samples (*cf.* Ward 1963) using ZONE vers.1.2 (Juggins

116 1992). We use ANOSIM (Bray-Curtis distance, 999 permutations: Clarke 1993) to determine whether  
117 clusters were significantly different and an indicator species analysis to identify any significant indicator  
118 species. Indicator species were identified using the IndVal technique (999 permutations in INDVAL vers.  
119 2.0; Dufrêne & Legendre (1997) to identify species which had both a high concentration of abundances  
120 (cf. Podani & Csányi 2010) and high fidelity to a single group (Dufrêne and Legendre 1997; McGeoch and  
121 Chown 1998). A taxon with a maximal indicator value of 100% would be found in all samples of a group  
122 and only in that group. Such indicator species characteristic of a group of samples are distinct from  
123 species identified as highly correlated with pollutant variables in ordinations or showing univariate  
124 correlations with pollutants (Stevens et al. 2009), and may be more robust to non-linear species  
125 responses. Separate analyses were conducted using a division derived from constrained cluster analysis,  
126 and a division at the critical load or level.

## 127 RESULTS

128 The results of redundancy analyses are shown in Table 1, Fig. 1 shows bi-plots for an RDA  
129 including all selected variables. Using forward selection, a minimal suite of environmental variables was  
130 identified that collectively explained almost a quarter of the total variance in community composition.  
131 In decreasing order of their marginal effect (i.e. tested without co-variables) these were: modern total  
132 inorganic nitrogen deposition, mean annual potential evapotranspiration, ozone exposure, mean annual  
133 rainfall, non-marine Ca+Mg deposition, cumulative dry deposition of NO<sub>x</sub> since 1900 and a simple field-  
134 estimated index of grazing intensity. Using variance partitioning to examine the conditional effect of  
135 each variable (i.e. with other selected variables as co-variables), ozone exposure emerges as the  
136 strongest independent variable and is the only variable to remain significant if a Bonferroni or Šidak  
137 correction is applied. Current nitrogen deposition loses significance when using variance partitioning  
138 due to strong correlation with cumulative nitrogen, if this is excluded then the result is significant  
139 (P=0.01). The ordination bi-plot (Fig. 1) shows a strong association between the two nitrogen deposition  
140 variables and base cation (Ca+Mg) deposition, as these variables might be expected to have interacting  
141 impacts we also tested an interaction variable, adding 2.6% to the total explained variance and  
142 explaining significant variance independent of both base cation and nitrogen deposition. A similar test of  
143 possible interactions between ozone and nitrogen found no evidence for an interaction.

144 The bi-plots (Fig. 1a&b) suggest various species including *Hylocomium splendens* (bryophyte),  
145 *Euphrasia officinalis* (parasitic forb) and *Plantago lanceolata* (forb) are negatively associated with N  
146 deposition and the bryophyte *Hypnum cupressiforme* positively associated. Projection of C-S-R scores

147 into the ordination space implies that species deleteriously impacted include both competitive (C) and  
148 ruderal (R) species; however univariate correlations with nitrogen are not significant (Stevens et al.  
149 2010a) so this suggestion should be treated with caution. Species negatively associated with nitrogen  
150 are frequently those typical of less acid habitats, as discussed by Stevens et al. (2010a). *Anthoxanthum*  
151 *odoratum* (grass) is negatively associated with grazing intensity and mean annual potential  
152 evapotranspiration, while *Deschampsia flexuosa* (grass) is negatively associated with ozone exposure.  
153 There is a gradient in species richness through the ordination space, clearly showing the reduction in  
154 species richness with increasing N deposition demonstrated previously (Stevens et al. 2004) and also  
155 with grazing intensity and mean annual potential evapotranspiration.

156           Constrained cluster analyses of samples along pollution gradients identifies a sequence of  
157 groupings. For nitrogen, a first division falls between samples with total inorganic N deposition of 14.2  
158 and 14.4 kg ha<sup>-1</sup> yr<sup>-1</sup> and for ozone exposure between samples with ozone exposure of 3148 and 3163  
159 ppb hrs AOT40. In both cases the difference in community composition between these two groups is  
160 highly significant (P<0.003), moderately distinct (R<sub>ANOSIM</sub>>0.2) and it is possible to identify significant  
161 indicator species at the P<0.01 level (Table 2). Groupings identified by subsequent divisions are less  
162 distinct and no indicator species can be identified so we conclude that only the first division is  
163 ecologically useful. IndVal recognizes a number of species as good indicators of low-N sites, but only  
164 one taxon (*Hypnum cupressiforme* agg. , bryophyte) as a good indicator of high-N sites (IV=73, Table 2a).  
165 The best-performing indicators of low-N sites are *Plantago lanceolata* (forb) , *Calluna vulgaris* (dwarf  
166 shrub) and *Lotus corniculatus* (legume forb). Significant indicators of low-ozone sites are *Nardus stricta*  
167 (grass) and *Deschampsia flexuosa* (grass) and indicators of high-ozone sites are *Pseudoscleropodium*  
168 *purum* (bryophyte), *Festuca rubra* (grass) and *Dicranum scoparium* (bryophyte). Very similar results are  
169 obtained using a division based on the results of the constrained cluster analysis or the established  
170 critical load or level (Table 2).

## 171 DISCUSSION

### 172 *Environmental controls on community composition*

173           Perhaps the most surprising result from our ordinations is the importance of ozone; selected  
174 third in forward selection and with the strongest conditional effect. While previous experimental and  
175 up-scaling studies have suggested the sensitivity of grasslands to ozone (Ashmore et al. 1995; Ashmore  
176 and Ainsworth 1995; Mills et al. 2007; Jones et al. 2007; Bassin et al. 2007a) here we show that this

177 relationship can be detected on a national scale. This is particularly interesting when considering that,  
178 unlike nitrogen and climate, the sampling strategy did not intend to sample the ozone gradient and our  
179 modelled exposure data is based on the AOT40 metric which is increasingly regarded as insensitive  
180 (RoTAP in press). The calculation of AOT40 we use further assumes a three-month May-July sensitivity  
181 period which may be overly conservative and fails to reflect spatial variability in growing season. That  
182 ozone appears so strongly in our analyses despite such possible issues illustrates the strength of the  
183 apparent relationship. To our knowledge this is the first study to show that ozone is an important  
184 control on plant community composition at a national scale.

185 Nitrogen deposition emerges as the strongest correlate when variables are tested  
186 independently. While the importance of nitrogen in determining the species richness of grasslands is  
187 increasingly well demonstrated (Stevens et al. 2004; Maskell et al. 2010; Duprè et al. 2010) the  
188 importance of nitrogen in determining species composition is less well studied on a landscape scale. In  
189 assessing the results of this study it should be remembered that the sampling strategy was deliberately  
190 designed to capture the full nitrogen deposition gradient, it may therefore be that the 'signal' of  
191 nitrogen pollution would be less distinct in a truly random sample (Maskell et al. 2010). The inclusion of  
192 cumulative dry NO<sub>x</sub> deposition in forward selection may suggest some cumulative impact of nitrogen.  
193 Non-marine Ca+Mg deposition is identified in forward selection with evidence for an interaction with  
194 nitrogen deposition. The underlying mechanisms for these relationships are unclear but the results  
195 suggest the need for experimental studies. Given the climatic gradients covered by the sampling area  
196 and the importance of climate to species composition of grasslands (e.g. Suttle 2007; Silvertown et al,  
197 1999) it is inevitable that climatic variables are identified in forward selection: both mean annual  
198 potential evapotranspiration and mean annual rainfall are identified as important here (the latter  
199 particularly correlated with RDA3, not shown in Fig. 1). Similarly it is unsurprising that grazing is also  
200 identified: the impacts of physical disturbance and nutrient input as well as biomass removal are known  
201 to be important (e.g. Crawley 1990; Hulme et al. 2001; Watkinson and Ormerod 2001). That the simple  
202 field-observed index is selected over the census data is probably a reflection on the coarse spatial-  
203 resolution of the gridded census data.

204 Of the variables not identified in forward selection particularly notable is sulphur deposition  
205 which is widely implicated in ecological impacts and from which many British ecosystems carry a legacy  
206 effect even though current deposition levels are much reduced (Lee 1998). Sulphur has been suggested  
207 as a contributor to species loss in acid grasslands (Maskell et al. 2010). In our dataset sulphur deposition



208 is correlated with total nitrogen deposition and particularly with oxidised nitrogen ( $R^2=0.85$ ), reflecting  
209 common origins. It is possible that cumulative  $\text{NO}_x$  deposition, which is included in forward selection,  
210 may be serving as a proxy for the legacy of both cumulative  $\text{NO}_x$  and cumulative sulphur pollution, of  
211 which the latter is not included in our environmental dataset.

#### 212 *Species relationships to nitrogen*

213           There is a high degree of commonality between N-indicating species identified by the ordination  
214 and IndVal, however there are some differences such as the dwarf shrub *Vaccinium vitis-idaea* and the  
215 forb *Lathyrus montanus* (not identified by IndVal) and the forb *Polygala serpyllifolia* (not identified in the  
216 ordination). Such differences are attributable to the different approaches taken with IndVal aiming to  
217 identify species typifying groups rather than to identify correlations. *V. vitis-idaea* and *L. montanus* are  
218 only found in low-N sites but are geographically restricted (only 2 occurrences) and would make poor  
219 indicators while *P. serpyllifolia* is more widespread, present in almost half of the sites with N deposition  
220 below the critical load, and would make a suitable indicator species.

221           The species consistently identified as indicative of low- or high-nitrogen deposition conditions  
222 generally agree with their known ecologies and previous research. The species most strongly positively  
223 associated with N deposition in the ordinations, and the only species identified as an indicator of high-N  
224 sites is the moss *Hypnum cupressiforme* agg.. *Hypnum cupressiforme* is known to be a pollution-tolerant  
225 species (e.g. Adams and Preston 1992; González-Miqueo et al. 2010), characteristic of high-N deposition  
226 sites in many ecosystems (Pitcairn et al. 1998; Mitchell et al. 2005), and resistant to long-term decline  
227 noted in many other N-sensitive bryophytes (Hallingbäck 1992).

228           Species showing apparent deleterious impacts of nitrogen include *Plantago lanceolata*, *Lotus*  
229 *corniculatus*, *Calluna vulgaris*, *Euphrasia officinalis*, *Hylocomium splendens*, and the forb *Campanula*  
230 *rotundifolia*, generally reflecting results from experimental studies. *P. lanceolata* growth and cover has  
231 been shown to be reduced by both N-addition (Mountford et al. 1993; Kirkham et al. 1996) and more  
232 general fertilization in grasslands (Berendse 1983). The legume *Lotus corniculatus* has been shown to be  
233 deleteriously effected by N-enrichment (Jones & Turkington 1986), probably because its ability to fix  
234 nitrogen confers a competitive advantage in low-N environments but an energy cost in high-N  
235 environments. Although experimental N-addition stimulates *C. vulgaris* in the short-term (Carroll et al.  
236 1999), over longer time frames the species may be out-competed by grasses (Terry et al. 2004) leading  
237 many heathlands in high-N regions to convert to grasslands (Heil & Diemont 1983; van Breemen & van

238 Dijk 1988; Bobbink et al. 1998). This response may be mediated by increased sensitivity to frost (Caporn  
 239 et al. 2000), drought (Gordon et al. 2002) and insect attack (Brunsting & Heil 1985; Kerslake et al. 1998;  
 240 Power et al. 1998). A negative correlation of the hemiparasitic annual *Euphrasia officinalis* (a species  
 241 group of complex taxonomy: Silverside 1991) with nitrogen may be linked to a general decline in forb  
 242 cover and a loss of host plants (Stevens et al. 2006). Nitrogen addition has been shown to limit the  
 243 ability of *Campanula rotundifolia* to suppress ruderal invasive species (Hwang & Lauenroth 2008), the  
 244 negative relationship with N deposition here may be attributable to out-competition for resources by  
 245 other species (contrasting with apparent increases in experimental studies of calcareous grasslands:  
 246 Jeffrey & Pigott 1973; Bobbink et al. 1998; Carroll et al. 2003). The negative response of the bryophyte  
 247 *Hylocomium splendens* to N addition is well established (e.g. Dirkse & Martakis 1992; Potter et al. 1995;  
 248 Koranda et al. 2007; Salemaa et al. 2008) and may be reflected in reduced abundance over recent  
 249 decades in many N-impacted areas of Europe (Hallingbäck 1992; Mäkipää & Heikkinen 2003; Dupre et al.  
 250 2010).

#### 251 *Species relationships with ozone*

252 The ordination plots show a positive association of *Festuca rubra*, *Dicranum scoparium* and  
 253 *Pseudoscleropodium purum* with ozone while the indicator species analyses show that these species are  
 254 also significant indicators of high ozone exposure sites. Although reduced biomass (Mortenson 1992,  
 255 Bungener et al. 1999) and increased senescence (Hayes et al. 2006, Williamson et al. 2010) of *F. rubra*  
 256 has been shown under experimental ozone exposure this species appears less sensitive than many of its  
 257 competitors (Brennan & Halisky 1970). Ashmore and Ainsworth (1995) have observed an increase in  
 258 biomass in artificially constructed grassland mesocosms while Ashmore et al. (1995) showed a *F. rubra*-  
 259 dominated sward to be resilient to species compositional change under ozone exposure. There has been  
 260 no experimental study of the response of *P. purum* or *D. scoparium* to ozone, however, the low growth-  
 261 form of these bryophytes may suggest relative tolerance (Hayes et al. 2007).

262 The grasses *Nardus stricta* and *Deschampsia flexuosa* are negatively associated with ozone in  
 263 the ordination plots and identified as indicators of low ozone sites in the IndVal analyses along with the  
 264 rush *Juncus effusus*. In experiments, the above-ground biomass of *N. stricta* has been reduced under  
 265 ozone exposure (Hayes et al. 2006) and visible signs of phytotoxicity have been noted (Bassin et al.  
 266 2007b). The relative growth rate of *D. flexuosa* is reduced by experimental ozone fumigation (Reiling &  
 267 Davison 1992). Hayes et al. (2006) showed that while *Juncus effusus* biomass was not significantly  
 268 reduced by ten weeks of ozone exposure, biomass was reduced following a subsequent over-wintering

269 period without O<sub>3</sub> exposure, perhaps due to increased use of plant resources for defence and repair  
270 mechanisms.

271 Taken overall, the species response clearly shows a decline in ozone-sensitive species and a  
272 relative increase in ozone-tolerant plant species with increasing ozone exposure. In a meta-analysis of  
273 single-species experimental studies (Hayes et al. 2007) *Nardus stricta* was shown to be sensitive to  
274 ozone (relative sensitivity index [RS]= 0.85) and *Festuca rubra* to be comparatively insensitive (RS=0.90);  
275 there was no significant relationship between results of experimental studies for *Deschampsia flexuosa*.  
276 Our results provide good evidence that this conservation priority habitat is being modified by ozone  
277 pollution. These plant community changes are likely to produce impacts on other aspects of the  
278 ecosystem. Effects may be produced below-ground through modification of plant C inputs (Loya et al.  
279 2003, Anderson 2003) and allelopathic chemical production (Kochhar et al 1980) with consequent  
280 impacts on microbial communities (Olszyk et al. 2001), and soil mesofauna (Loranger et al. 2004).

#### 281 *Constrained cluster analysis and ecological groupings*

282 Having established the apparent importance of pollutant variables in determining species  
283 communities of grasslands, we identified groupings of sites along the pollution gradients. The  
284 identification of distinctly differing plant communities solely on the basis of their nitrogen or ozone  
285 status provides independent support for the importance of these variables identified in the ordinations.  
286 It is interesting to note the similarity in the position of these divisions to critical loads and levels: for  
287 nitrogen a division around 14.3 kg ha<sup>-1</sup> yr<sup>-1</sup> lies close to the mid-point of the critical load range of 10-20  
288 kg ha<sup>-1</sup> yr<sup>-1</sup>, (Achermann & Bobbink 2003), and a division at around 3150 ppb hrs AOT40 for ozone  
289 compares to a critical level of 3000 ppb hrs AOT40 (UNECE-CLRTAP 2004).

#### 290 *Differential controls on diversity and community composition*

291 Many landscape-scale studies of pollutant impacts on vegetation have been focussed on  
292 biodiversity, with “biodiversity” in practise often taken narrowly to mean the species richness of a  
293 quadrat sample. The limitations of such an approach are apparent in our dataset with results suggesting  
294 that ozone has the greatest independent impact on community composition of acid grasslands but has  
295 no detectable impact (P>0.05) on diversity *per se*, whether assessed by species richness or a diversity  
296 index incorporating equitability (Shannon’s H or Simpson’s D). It appears that ozone pollution is  
297 changing the composition of acid grasslands with some species increasing and some declining but  
298 without an overall loss of species richness or a detectable shift in their evenness of distribution. Studies

299 focussed solely on species richness could well conclude that ozone exposure is unimportant in this  
300 ecosystem, overlooking what may be an important driver of ecological change. Both diversity and  
301 community composition are important in assessing pollutant impacts, so both should be considered as  
302 they may be affected by different environmental drivers.

### 303 *The importance of multiple drivers*

304 Our study is one of very few to consider the combined impacts of multiple pollutants, providing  
305 good evidence for the impacts of nitrogen and ozone with distinct responses to each and evidence for  
306 possible interactions of nitrogen with base cation deposition. No species are identified as indicators of  
307 both nitrogen and ozone pollution and while nitrogen is clearly correlated with a loss of species richness  
308 there is no significant correlation between species richness and ozone. The importance of both nitrogen  
309 and ozone has implications for the interpretation of individual species responses. For instance *Dicranum*  
310 *scoparium* has been shown to be sensitive to N in some experimental studies (e.g. Mitchell et al. 2004;  
311 Olsson & Kellner 2006; Leith et al. 2008) but here it is shown to be more strongly associated with ozone.  
312 While *D. scoparium* may well be affected by nitrogen, the results here suggest that its distribution in  
313 these acid grasslands is more determined by its insensitivity to ozone than its sensitivity to nitrogen.

314 Our results have important implications for the conservation and management of acid  
315 grasslands supporting much recent work in showing the importance of nitrogen deposition but also  
316 suggesting that ozone exposure is similarly important and that the impacts of nitrogen may be related to  
317 deposition of non-marine base cations. Changes in plant communities are but one part of ecosystem  
318 response to pollution however it is probable that the changes in plant communities we demonstrate  
319 here produce consequent changes in other ecosystem components leading to a more general decline in  
320 ecosystem quality and loss of ecosystem services.

321

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330

## 331 REFERENCES

332 Achermann, B., Bobbink, R., 2003. Empirical critical loads for nitrogen, Environmental  
333 documentation no. 164. Swiss Agency for the Environment, Forests and Landscape, Berne, Switzerland.

334 Adams, K.J., Preston, C.D., 1992. Evidence for the effects of atmospheric pollution on bryophytes  
335 from national and local recording, in: Harding, P.T. (Ed.), Biological recording of changes in British  
336 wildlife. HMSO, London.

337 Andersen, C.P., 2003. Source-sink balance and carbon allocation below ground in plants exposed  
338 to ozone. *New Phytologist* 157, 213-228.

339 Ashmore, M.R., Ainsworth, N., 1995. The effects of ozone and cutting on the species  
340 composition of artificial grassland communities. *Functional Ecology* 9, 708-712.

341 Ashmore, M.R., Thwaites, R.H., Ainsworth, N., Cousins, D.A., Power, S.A., Morton, A.J., 1995.  
342 Effects of ozone on calcareous grassland communities. *Water, Air and Soil Pollution* 85, 1527-1532.

343 Bassin, S., Volk, M., Fuhrer, J., 2007a. Factors affecting the ozone sensitivity of temperate  
344 European grasslands: An overview. *Environmental Pollution* 146, 678-691.

345 Bassin, S., Volk, M., Suter, M., Buchmann, N., Fuhrer, J. 2007b. Nitrogen but not ozone affects  
346 productivity and community composition of subalpine grassland after three years of treatment. *New*  
347 *Phytologist* 175, 523-534.

348 Berendse, F., 1983. Interspecific competition and niche differentiation between *Plantago*  
349 *lanceolata* and *Anthoxanthum odoratum* in a natural hayfield. *Journal of Ecology* 71, 379-390.

350 Bobbink, R., Hornung, M., Roelofs, J.G.M., 1998. The effects of air-borne nitrogen pollutants on  
351 species diversity in natural and semi-natural European vegetation. *Journal of Ecology* 86, 717-738.

352 Borcard, D., Legendre, P., Drapeau, P., 1992. Partialling out the spatial component of ecological  
353 variation. *Ecology* 73, 1045-1055.

354 Brennan, E., Halisky, P.M., 1970. Response of turfgrass cultivars to ozone and sulfur dioxide in  
355 the atmosphere. *Phytopathology* 60, 1544-1546.

- 356 Brunsting, A.M.H., Heil, G.W., 1985. The role of nutrients in the interactions between a  
357 herbivorous beetle and some competing plant species in heathlands. *Oikos* 44, 23-26.
- 358 Bungener, P., Nussbaum, S., Grub, A., Fuhrer, J., 1999. Growth response of grassland species to  
359 ozone in relation to soil moisture condition and plant strategy. *New Phytologist* 142, 283-293.
- 360 Butchart, S.H.M., 44 others, 2010. Global biodiversity: indicators of recent declines. *Science* 328,  
361 1164-1168.
- 362 Caporn, S.J.M., Ashenden, T.W., Lee, J.A., 2000. The effect of exposure to NO<sub>2</sub> and SO<sub>2</sub> on frost  
363 hardiness in *Calluna vulgaris*. *Environmental and Experimental Botany* 43, 111-119.
- 364 Carroll, J.A., Caporn, S.J.M., Cawley, L., Read, D.J., Lee, J.A., 1999. The effect of increased  
365 deposition of atmospheric nitrogen on *Calluna vulgaris* in upland Britain. *New Phytologist* 141, 423-431.
- 366 Carroll, J.A., Caporn, S.J.M., Johnson, D., Morecroft, C., Lee, J.A. 2003. The interactions between  
367 plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands  
368 receiving long-term inputs of simulated pollutant nitrogen deposition. *Environmental Pollution* 121, 363-  
369 376.
- 370 Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure.  
371 *Australian Journal of Ecology* 18, 117-143.
- 372 Coyle, M., Fowler, D., Smith, R.I., Weston, K., Stedman, J. R. 2002. Quantifying the spatial  
373 distribution of surface ozone concentration in the UK. *Atmospheric Environment* 36, 1013-1024.
- 374 Crawley, M.J., 1990. Rabbit grazing, plant competition and seedling recruitment in acid  
375 grassland. *Journal of Applied Ecology* 27, 803-820.
- 376 DEFRA 2009. June survey of agriculture and horticulture: UK- final results. UK National Statistics,  
377 Newport, UK.
- 378 Dirkse, G.M., Martakis, G.F.P., 1992. Effects of fertilizer on bryophytes in Swedish experiments  
379 on forest fertilization. *Biological Conservation* 59, 155-161.
- 380 Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species: The need for a  
381 flexible asymmetrical approach. *Ecological Monographs* 67, 345-366.

- 382 Duprè, C., Stevens, C.J., Ranke, T., Bleeker, A., Pepler-Lisbach, C., Gowing, D.J., Dise, N.B.,  
383 Dorland, E., Bobbink, R., Diekmann, M. 2010. Changes in species richness and composition in European  
384 acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen  
385 deposition. *Global Change Biology* 16, 344-357.
- 386 Fowler, D., O'Donoghue, M., Muller, J.B.A., Smith, R.I., Dragosits, U., Skiba, U., Sutton, M.A.  
387 Brimblecombe, P., 2004, A chronology of nitrogen deposition in the UK between 1900 and 2000. *Water,*  
388 *Air and Soil Pollution: Focus* 4, 9-23.
- 389 Gidman, E.A., Stevens, C.J., Goodacre, R., Broadhurst, D., Emmett, B., Gwynn-Jones, D., 2006.  
390 Using metabolic fingerprinting of plants for evaluating nitrogen deposition impacts on a landscape level.  
391 *Global Change Biology* 12, 1460-1465.
- 392 González-Miqueo, L., Elustondo, D., Lasheras, E. & Santamaría, J.M., 2010. Use of native mosses  
393 as biomonitors of heavy metals and nitrogen deposition in the surroundings of two steel works.  
394 *Chemosphere* 78, 965-971.
- 395 Gordon, C., Woodin, S.J., Alexander, I.J., Mullins, C.E., 2002. Effects of increased temperature,  
396 drought and nitrogen supply on two upland perennials of contrasting functional type: *Calluna vulgaris*  
397 and *Pteridium aquilinum*. *New Phytologist* 142, 243-258.
- 398 Grime, J.P., Fridley, J.D., Askew, A.P., Thompson, K., Hodgson, J.G., Bennett, C.R., 2008. Long-  
399 term resistance to simulated climate change in an infertile grassland. *Proceedings of the National*  
400 *Academy of Sciences of the United States of America* 150, 10028-10032.
- 401 Grimm, E.C., 1987. CONISS: A Fortran 77 program for stratigraphically constrained cluster  
402 analysis by the method of incremental sum of squares. *Computers and Geosciences* 13, 13-35.
- 403 Hallingbäck, T., 1992. The effect of air pollution on mosses in southern Sweden. *Biological*  
404 *Conservation* 59, 163-170.
- 405 Hayes, F., Mills, G., Williams, P., Harmens, H., Büker, P., 2006. Impacts of summer ozone on the  
406 growth and overwintering of UK upland vegetation. *Atmospheric Environment* 40, 4088-4097.
- 407 Hayes, F., Jones, M.L.M., Mills, G., Ashmore, M., 2007. Meta-analysis of the relative sensitivity of  
408 semi-natural vegetation species to ozone. *Environmental Pollution* 146, 754-762.



- 409 Heil, G.W., Diemont, W.H., 1983. Raised nutrient levels change heathland into grassland.  
410 *Vegetatio* 53, 113-120.
- 411 Hulme, P.D., Pakeman, R.J., Torvell, L., Fisher, J.M., Gordon, I.J., 2001. The effects of controlled  
412 sheep grazing on the dynamics of upland *Agrostis-Festuca* grassland. *Journal of Applied Ecology* 36, 886-  
413 900.
- 414 Hunt, R., Hodgson, J.G., Thompson, K., Bungener, P., Dunnett, N.P., Askew, A.P., 2004. A new  
415 practical tool for deriving a functional signature for herbaceous vegetation. *Applied Vegetation Science*  
416 7, 163-170.
- 417 Hwang, B.C., Lauenroth, W.K., 2008. Effect of nitrogen, water and neighbour density on the  
418 growth of *Hesperis matronalis* and two native perennials. *Biological Invasions* 10, 771-779.
- 419 Jeffrey, D.W., Pigott, C.D., 1973. The response of grasslands on sugar-limestone in Teesdale to  
420 application of phosphorous and nitrogen. *Journal of Ecology* 61, 85-92.
- 421 Jones, D.A., Turkington, R., 1986. *Lotus corniculatus* L. *Journal of Ecology* 74, 1185-1212.
- 422 Jones, M.L.M., Hayes, F., Mills, G., Sparks, T.H., Fuhrer, J., 2007. Predicting community sensitivity  
423 to ozone using Ellenberg Indicator values. *Environmental Pollution* 146, 744-753.
- 424 Juggins, S., 1992. *The ZONE program, version 1.2*, Unpublished program, University of  
425 Newcastle.
- 426 Kerslake, J.E., Woodin, S.J., Hartley, S.E., 1998. Effects of carbon dioxide and nitrogen  
427 enrichment on a plant-insect interaction: the quality of *Calluna vulgaris* as a host for *Operopheta*  
428 *brumata*. *New Phytologist* 140, 43-53.
- 429 Kirkham, F.W., Mountford, J.O., Wilkins, R.J., 1996. The effects of nitrogen, potassium and  
430 phosphorus addition on the vegetation of a Somerset peat moor under cutting management. *Journal of*  
431 *Applied Ecology* 33, 1013-1029.
- 432 Kochhar, M., Blum, U. Reinert, R.A., 1980. Effects of O<sub>3</sub> and/or fescue on ladino clover:  
433 interactions. *Canadian Journal of Botany* 48, 241-249.

- 434 Koranda, M., Keschbaum, S., Wanek, W., Zechmeister, H., Richter, A., 2007. Physiological  
435 responses of bryophytes *Thuidium tamariscum* and *Hylocomium splendens* to increased nitrogen  
436 deposition. *Annals of Botany* 99, 161-169.
- 437 Lee, J.A., 1998. Unintentional experiments with terrestrial ecosystems: ecological effects of  
438 sulphur and nitrogen pollutants. *Journal of Ecology* 86, 1-12.
- 439 Lee, J.A., Caporn, S.J.M. 1998. Ecological effects of atmospheric reactive nitrogen deposition on  
440 semi-natural terrestrial vegetation. *New Phytologist* 139, 127-134.
- 441 Legendre, P., Gallagher, E.D. 2001. Ecologically meaningful transformations for ordination of  
442 species data. *Oecologia* 129, 271-280.
- 443 Leith, I.D., Mitchell, R.J., Truscott, A.M., Cape, J.N., van Dijk, N., Smith, R.I., Fowler, D., Sutton,  
444 M.A., 2008. The influence of nitrogen in stemflow and precipitation on epiphytic bryophytes, *Isothecium*  
445 *myosuroides* Brid., *Dicranum scoparium* Hewd. and *Thuidium tamariscum* (Hewd.) Sschimp of Atlantic  
446 oakwoods. *Environmental Pollution* 155, 237-246.
- 447 Loranger, G.I., Pregitzer, K.S., King, J.S., 2004. Elevated CO<sub>2</sub> and O<sub>3</sub>t concentrations differentially  
448 affect selected groups of the fauna in temperate forest soils. *Soil Biology and Biochemistry* 36, 1521-  
449 1524.
- 450 Loya, W.M., Pregitzer, K.S., Karberg, N.J., King, J.S., Giardina, P., 2003. Reduction of soil carbon  
451 formation by tropospheric ozone under increased carbon dioxide levels. *Nature* 425, 705-707.
- 452 Mäkipää, R., Heikkinen, J., 2003. Large-scale changes in abundance of terricolous bryophytes  
453 and macrolichens in Finland. *Journal of Vegetation Science* 14, 497-508.
- 454 MAFF (1986) *The analysis of agricultural materials*. HMSO, London.
- 455 Maskell, L.C., Smart, S.M., Bullock, J.M., Thompson, K., Stevens, C.J., 2010. Nitrogen deposition  
456 causes widespread loss of species richness in British habitats. *Global Change Biology* 16, 671-679.
- 457 Mattock, A. (Ed.), 2008. *UK Biodiversity Action Plan: Priority habitat descriptions*. Joint Nature  
458 Conservation Committee, Peterborough.
- 459 McGeoch, M.A., Chown, S.L., 1998. Scaling up the value of bioindicators. *Trends in Evolution and*  
460 *Ecology* 13, 46-47.

- 461 Mills, G., Hayes, F., Jones, M.L.M., Cinderby, S., 2007. Identifying ozone-sensitive communities of  
462 (semi-)natural vegetation suitable for mapping exceedance of critical levels. *Environmental Pollution*  
463 146, 736-743.
- 464 Mitchell, R.J., Sutton, M.A., Truscott, A.M., Leith, I.D., Cape, J.N., Pitcairn, C.E.R., van Dijk, N.,  
465 2004. Growth and tissue nitrogen of epiphytic Atlantic bryophytes: effects of increased and decreased  
466 atmospheric N deposition. *Functional Ecology* 18, 332-329.
- 467 Mitchell, R.J., Truscot, A.M., Leith, I.D., Cape, J.N., van Dijk, N., Tang, Y.S., Fowler, D., Sutton,  
468 M.A., 2005. A study of the epiphytic communities of Atlantic oak woods along an atmospheric nitrogen  
469 deposition gradient. *Journal of Ecology* 93, 482-492.
- 470 Morecroft, M.D., Sellers, E.K., Lee, J.A., 1994. An experimental investigation into the effects of  
471 atmospheric nitrogen deposition on two semi-natural grasslands. *Journal of Ecology* 82, 475-483.
- 472 Mortenson, L.M., 1992. Effects of ozone on growth of seven grass and one clover species. *Acta*  
473 *Agriculturae Scandinavica, Section B- Plant Soil Science* 42, 235-239.
- 474 Mountford, J.O., Lakhani, K.H., Kirkham, F.W., 1993. Experimental assessment of the effects of  
475 nitrogen addition under hay-cutting and aftermath grazing on the vegetation of meadows on a Somerset  
476 peat moor. *Journal of Applied Ecology* 30, 321-332.
- 477 NEGTA, 2001. Transboundary air pollution: acidification, eutrophication and ground-level  
478 ozone in the UK. Department for Environment, Food and Rural Affairs, London, UK.
- 479 Olsson, B.A., Kellner, O., 2006. Long-term effects of nitrogen fertilization on ground vegetation  
480 in coniferous forests. *Forest Ecology and Management* 237, 458-470.
- 481 Olszyk, D.M., Johnson, M.G., Phillips, D.L., Seidler, R.J., Tingey, D.T., Watrud, L.S., 2001.  
482 Interactive effects of CO<sub>2</sub> and O<sub>3</sub> on a ponderosa pine plant/litter/soil mesocosm. *Environmental*  
483 *Pollution* 115, 447-462.
- 484 Oke, T.R. (1978) *Boundary layer climates*. Methuen & Co., London.
- 485 Pitcairn, C.E.R., Leith, I.D., Sheppard, L.J., Sutton, M.A., Fowler, D., Munro, R.C., Tang, S., Wilson,  
486 D. 1998. The relationship between nitrogen deposition, species composition and foliar nitrogen  
487 concentrations in woodland flora in the vicinity of livestock farms. *Environmental Pollution* S1, 41-48.

- 488 Phoenix, G.K., Hicks, W.K., Cinderby, S., Kuylenstierna, J.C.I., Stock, W.D., Dentener, F.J., Giller,  
489 K.E., Austin, A.T., Lefroy, R.D.B., Gimeno, B.S., Ashmore, M.R., Ineson, P., 2006. Atmospheric nitrogen  
490 deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N  
491 deposition impacts. *Global Change Biology* 12, 470-476.
- 492 Podani, J., Csányi, B. 2010. Detecting indicator species: Some extensions to the IndVal measure.  
493 *Ecological Indicators* 10, 1119-1124.
- 494 Potter, J.A., Press, M.C., Callaghan, T.V., Lee, J.A. 1995. Growth responses of *Polytrichum*  
495 *commune* and *Hylocomium splendens* to simulated environmental change in the sub-Arctic. *New*  
496 *Phytologist* 131, 533-541.
- 497 Power, S.A., Ashmore, M.R., Cousins, D.A., Sheppard, L.J., 2008. Effects of nitrogen addition on  
498 the stress sensitivity of *Calluna vulgaris*. *New Phytologist* 138, 663-673.
- 499 Rao, C.R., 1995. A review of canonical coordinates and an alternative to correspondence analysis  
500 using Hellinger distance. *Qüestiió* 19, 23-63.
- 501 Reiling, K., Davison, A.W., 1992. The response of native, herbaceous species to ozone: Growth  
502 and fluorescence screening. *New Phytologist* 120, 29-37.
- 503 RoTAP, in press. Review of transboundary air pollution: Acidification, eutrophication, ground  
504 level ozone and heavy metals in the UK. Centre for Ecology and Hydrology, UK.
- 505 Rodwell, J.S. (Ed.), 1992. *British plant communities: Volume 3, grasslands and montane*  
506 *communities*, Cambridge University Press.
- 507 Sala, O.E., 18 others, 2000. Global biodiversity scenarios for the year 2100. *Science* 287, 1770-  
508 1774.
- 509 Salemaa, M., Mäkipää, R., Oksanen, J., 2008. Differences in the growth response of three  
510 bryophyte species to nitrogen. *Environmental Pollution* 152, 82-91.
- 511 Silverside, A.J., 1991. The identity of *Euphrasia officinalis* L. and its nomenclatural implications.  
512 *Watsonia* 18, 343-350.
- 513 Silvertown, J., Dodd, M.E., Gowing, D.J., Mountford, J.O., 1999. Hydrologically defined niches  
514 reveal a basis for species richness in plant communities. *Nature* 400, 61-63.

- 515 Stevens, C.J., Dise, N.B., Mountford, J.O., Gowing, D.J., 2004. Impact of nitrogen deposition on  
516 the species richness of grasslands *Science* 303, 1876-1879.
- 517 Stevens, C.J., Dise, N.B., Gowing, D.J., Mountford, J.O., 2004. Loss of forb diversity in relation to  
518 nitrogen deposition in the UK: regional trends and potential controls. *Global Change Biology* 12, 1823-  
519 1833.
- 520 Stevens, C.J., Maskell, L.C., Smart, S.M., Caporn, S.J.M., Dise, N.B., Gowing, D.J., 2009. Identifying  
521 indicators of atmospheric nitrogen deposition in acid grasslands. *Biological Conservation* 142, 2069-  
522 2075.
- 523 Stevens, C.J., Dise, N.B., Gowing, D.J., 2009b. Regional trends in soil acidification and  
524 exchangeable metal concentrations in relation to acid deposition rates. *Environmental Pollution* 157,  
525 313-319.
- 526 Stevens, C.J., Thompson, K., Grime, J.P., Long, C.J., Gowing, D.J., 2010. Contribution of  
527 acidification and eutrophication to declines in species richness of calcifuge grasslands along a gradient of  
528 atmospheric nitrogen deposition. *Functional Ecology* 24, 478-484.
- 529 Suttle, K.B., Thomsen, M.A., Power, M.E., 2007. Species interactions reverse grassland responses  
530 to changing climate. *Science* 315, 640-642.
- 531 Ter Braak, C., Šmilauer, P., 1997-2004. CANOCO for Windows. Biometris-Plant Research, The  
532 Netherlands.
- 533 Terry, A.C., Ashmore, M.R., Power, S.A., Allchin, E.A., Heil, G.W., 2004. Modelling the impact of  
534 atmospheric nitrogen on *Calluna*-dominated ecosystems in the UK. *Journal of Applied Ecology* 41, 897-  
535 909.
- 536 Thompson, N., Barrie, I.A., Ayles, M. 1981. The meteorological office rainfall and evaporation  
537 calculation system: Morecs. The Meteorological Office, Bracknell, UK.
- 538 UNECE Convention on Long-range Transboundary Air Pollution, 2004. Manual on methodologies  
539 and criteria for modelling and mapping critical loads and levels. Federal Environmental Agency, Berlin,  
540 Germany.

541 van Breemen, N., van Dijk, H.F.G., 1988. Ecosystem effects of atmospheric deposition of  
542 nitrogen in the Netherlands. *Environmental Pollution* 54, 249-274.

543 Ward, J.H., 1963. Hierarchical grouping to optimize an objective function. *Journal of the*  
544 *American Statistical Association* 58 236-244.

545 Watkinson, A.R., Ormerod, S.J., 2001. Grasslands, grazing and biodiversity: editors' introduction.  
546 *Journal of Applied Ecology* 38, 233-237.

547 Williamson, J., Mills, G., Freeman, C., 2010. Species-species effects of elevated ozone on wetland  
548 plants and decomposition processes. *Environmental Pollution* 158, 1197-1206.

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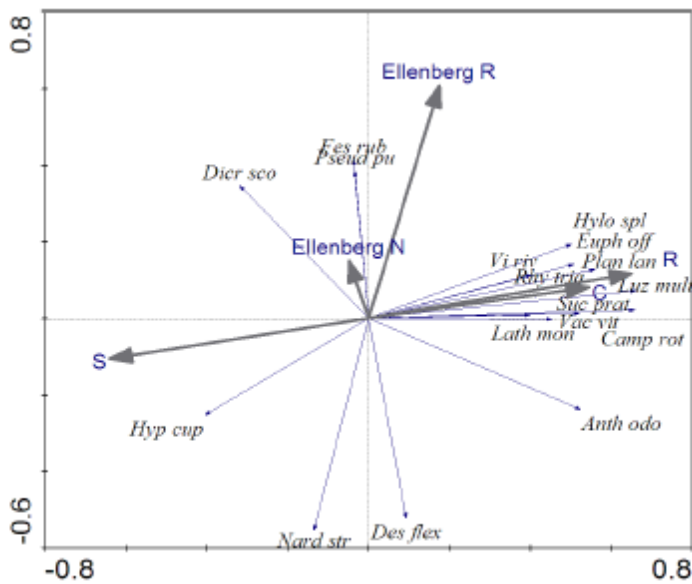
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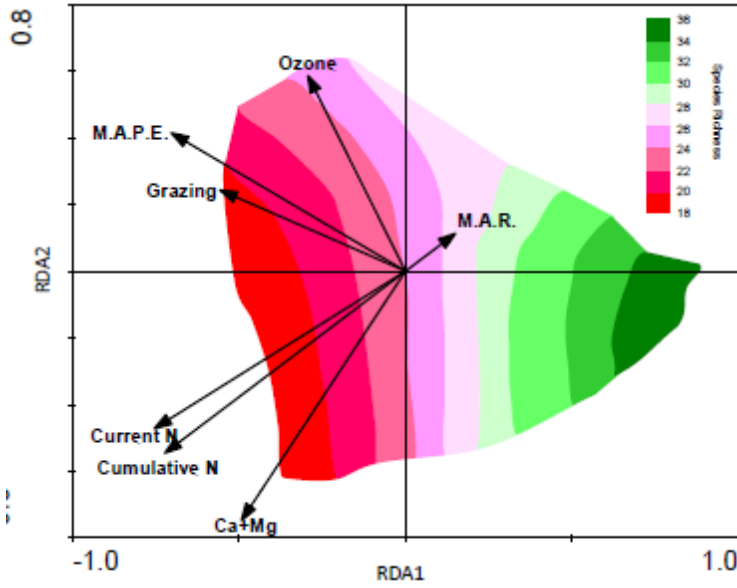
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## 553 FIGURES AND TABLES

554 Figure 1. Ordination bi-plots showing redundancy analysis results including all environmental variables  
 555 identified in forward selection for a) position of selected species well-fitted by the ordination and  
 556 (passively projected) mean C-S-R scores and Ellenberg R and N (see Stevens et al. 2010), and b) selected  
 557 environmental variables with contours of species richness fitted using LOESS regression (span  $\alpha=0.8$ ) in  
 558 CANOCO vers. 4.53 (ter Braak and Šmilauer 1997-2004). Environmental variable abbreviations given in  
 559 Table 1, species abbreviations: *Fes rub*= *Festuca rubra*, *Pseud pu*= *Pseudoscleropodium purum*, *Hylo spl*=  
 560 *Hylocomium splendens*, *Euph off*= *Euphrasia officinalis*, *Plan lan*= *Plantago lanceolata*, *Vi riv*= *Viola*  
 561 *riviniana*, *Rhy triq*= *Rhytidiadelphus triquetrus*, *Luz mult*= *Luzula multiflora*, *Suc prat*= *Succisa pratensis*,  
 562 *Vac vit*= *Vaccinium vitis-idaea*, *Lath mon*= *Lathyrus montanus*, *Camp rot*= *Campanula rotundifolia*, *Anth*  
 563 *odo*= *Anthoxanthum odoratum*, *Des flex*= *Deschampsia flexuosa*, *Nard str*= *Nardus stricta*, *Hyp cup*=  
 564 *Hypnum cupressiforme* agg., *Dicr sco*= *Dicranum scoparium*.



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567 Table 1. Results of redundancy analyses of Hellinger-transformed grassland species cover data showing  
 568 marginal effect (i.e. without co-variables) and conditional effect (i.e. using variance partitioning) of  
 569 minimal suite of environmental variables identified using forward selection. Variables listed in order of  
 570 inclusion in forward selection, with exception of nitrogen deposition-base cation deposition interaction  
 571 variable which was tested separately. Results show % variance explained, and significance level by  
 572 Monte Carlo permutation tests (999 unrestricted permutations). Names in parentheses are abbreviated  
 573 forms used in Fig. 1.

574 Table2. Indicator species of a) high- and low-N deposition, and b) high- and low-ozone exposure sites as  
 575 identified by IndVal (Dufrêne and Legendre 1997). Showing species with  $P < 0.01$  and indicator values  
 576 (IV)  $> 40\%$ . Two sets of analyses were conducted using a cut-off at either the mid-point of the critical  
 577 level/load range (CL:  $15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , Achermann & Bobbink 2003;  $3000 \text{ ppb hrs AOT}_{40}$  ozone, UNECE-  
 578 CLRTAP 2004) or the first division suggested by CONISS (see text).

579 Appendix 1. Environmental data included in ordinations.



580 Table 1. Results of redundancy analyses of Hellinger-transformed grassland species cover data showing  
 581 marginal effect (i.e. without co-variables) and conditional effect (i.e. using variance partitioning) of  
 582 minimal suite of environmental variables identified using forward selection. Variables listed in order of  
 583 inclusion in forward selection, with exception of nitrogen deposition-base cation deposition interaction  
 584 variable which was tested separately. Results show % variance explained, and significance level by  
 585 Monte Carlo permutation tests (999 unrestricted permutations). Names in parentheses are abbreviated  
 586 forms used in Fig. 1.

587

<b>Variable</b>	<b>Marginal effect (%)</b>	<b>Conditional effect (%)</b>
Current total inorganic N deposition [Current N]	6.3 (P=0.001)	1.9 (ns)
Mean annual potential evapotranspiration [MAPE]	5.2 (P=0.001)	2.5 (P=0.02)
Ozone exposure [Ozone]	4.5 (P=0.001)	3.5 (P=0.001)
Mean annual rainfall [MAR]	2.7 (P=0.028)	2.7 (P=0.001)
Deposition of non-marine Ca and Mg [Ca+Mg]	6.1 (P=0.001)	2.4 (P=0.018)
Cumulative dry deposition of oxidised N (1900-) [Cumulative N]	6.3 (P=0.001)	2.2 (P=0.035)
Grazing index [Grazing]	4.4 (P=0.001)	2.2 (P=0.041)
All above variables	24.8	-
N deposition*base cation deposition interaction	6.4 (P=0.001)	2.4 (P=0.018)

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590

591 Table2. Indicator species of a) high- and low-N deposition, and b) high- and low-ozone exposure  
 592 sites as identified by IndVal. Showing species with  $P < 0.01$  and indicator values (IV)  $> 40\%$ . Two sets of  
 593 analyses were conducted using a cut-off at either the mid-point of the critical level/load range (CL: 15 kg  
 594  $\text{N ha}^{-1} \text{ yr}^{-1}$ , Achermann & Bobbink 2003; 3000 ppb hrs AOT40 ozone, UNECE-CLRTAP 2004) or the first  
 595 sample division suggested by CONISS (see text).

596 a)

High N sites			Low N sites		
Species	IV (CONISS)	IV (CL)	Species	IV (CONISS)	IV (CL)
<i>Hypnum cupressiforme</i> agg.	73.13	73.42	<i>Plantago lanceolata</i>	67.53	64.42
			<i>Calluna vulgaris</i>	66.3	56.73
			<i>Lotus corniculatus</i>	64.22	61.79
			<i>Euphrasia officinalis</i>	52.61	45.40
			<i>Hylocomium splendens</i>	49.1	41.94
			<i>Polygala serpyllifolia</i>	42.23	36.63
			<i>Campanula rotundifolia</i>	42.13	43.39

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598 b)

High ozone sites			Low ozone sites		
Species	IV (CONISS)	IV (CL)	Species	IV (CONISS)	IV (CL)
<i>Pseudoscleropodium purum</i>	57.68	60.05	<i>Nardus stricta</i>	63.34	59.81
<i>Festuca rubra</i>	55.42	55.14	<i>Deschampsia flexuosa</i>	57.77	65.91
<i>Dicranum scoparium</i>	39.06	40.81	<i>Juncus effusus</i>	-	48.72

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