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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 (NO_x , NH_y , 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 measurable 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_x 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⁻¹ yr⁻¹ 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_{ANOSIM}>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_x 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 NO_x deposition, which is included in forward selection,
210 may be serving as a proxy for the legacy of both cumulative 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 O₃ 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⁻¹ yr⁻¹ lies close to the mid-point of the critical load range of 10-20
288 kg ha⁻¹ yr⁻¹, (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

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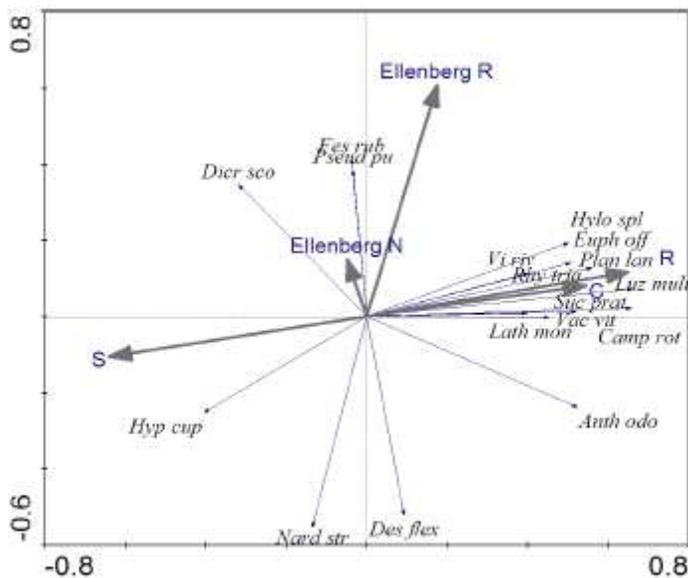
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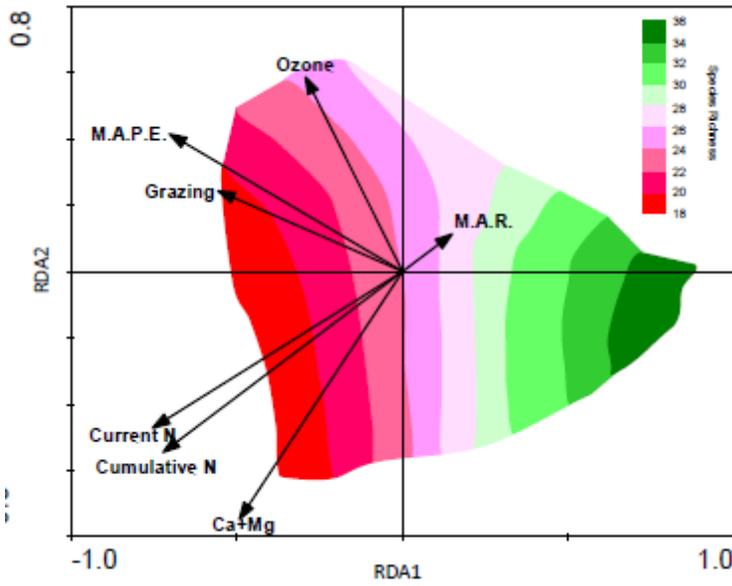
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553 FIGURES AND TABLES

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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566

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

Variable	Marginal effect (%)	Conditional effect (%)
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

597

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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