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Contribution of acidification and eutrophication to declines in species richness of calcifuge grasslands along a gradient of atmospheric nitrogen deposition

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1 **The contribution of acidification and eutrophication to declines in species**
2 **richness of calcifuge grasslands along a gradient of atmospheric nitrogen**
3 **deposition**

4

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24 Running title: Acidification in relation to grassland species richness

25 **Abstract:**

- 26 1. Atmospheric deposition of nitrogen (N) is a global problem resulting in
27 negative consequences for biodiversity due to direct toxicity, increases in
28 invasive species, increased susceptibility to environmental stresses and soil-
29 mediated effects of acidification and eutrophication.
- 30 2. Reductions in plant species richness related to N deposition have been
31 observed in a number of habitats including calcifuge (acid) grasslands but the
32 mechanisms of this decline have not been fully investigated. We test the
33 hypotheses that along a large-scale gradient of N deposition there is a) an
34 increase in species tolerant of low pH conditions as a result of soil
35 acidification and b) an increase in competitive and nitrophilic species as a
36 result of soil eutrophication. As competitive species can occur in low pH
37 habitats both of these hypotheses could be true.
- 38 3. Using plant characteristics we examined changes in vegetation species
39 composition along the gradient of N deposition in the UK. Mean C-S-R
40 signatures were used to identify the competitive response of plant
41 communities together with Ellenberg N (nitrogen) scores to identify increases
42 in nitrophilic species. Ellenberg R (reaction, pH) scores were used to identify
43 change in response to soil pH together with an index of soil acidity preference
44 developed using regional survey data.
- 45 4. Mean C-S-R signatures showed no significant correlation with N deposition,
46 nor did mean Ellenberg N scores. Ellenberg R scores and the index of soil
47 acidity preference showed significant relationships with N deposition
48 indicating an increased dominance of acid tolerant species.

49 5. The results suggest that soil acidification as opposed to eutrophication and
50 consequent competition between species is contributing to shifts in species
51 composition and diversity linked to N deposition in calcifuge grasslands. Soil
52 acidification may be leading to reduced nutrient availability preventing the
53 effects of N addition from being apparent.

54

55 **Keywords:** Acid deposition, aluminium tolerance, C-S-R Strategies, Ellenberg
56 values, nitrogen cycling, nutrient availability, species composition.

57

58 **Introduction**

59

60 The creation of reactive nitrogen (N) has increased globally by 120% since 1970 and
61 continues to increase each year (Galloway et al., 2008). The global N cycle has now
62 reached the point where more N is fixed annually by human-driven than by natural
63 processes (Vitousek, 1994). There are important consequences of this increase for
64 deposition of reactive nitrogen. Deposition exceeds $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in many areas of
65 the world, 20 times more than the estimated deposition of $0.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ where
66 there is no human influence (Galloway et al., 2008). $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ is exceeded in
67 large parts of Europe, with only remote regions, such as northern Sweden and Norway
68 or northern Scotland, below this level.

69

70 The consequences of high N deposition for biodiversity are increasingly well known.
71 They include direct toxicity (e.g. van den Berg et al., 2008), increased susceptibility to
72 pests and disease (e.g. Brunstig & Heil, 1985), increased susceptibility to
73 environmental stresses (e.g. Caporn et al., 2000) and soil-mediated effects of

74 acidification and eutrophication. Amongst these, acidification and eutrophication are
75 believed to be the most important and may drive other changes listed above (Bobbink et
76 al., 1998).

77

78 Reductions in plant species richness at high levels of N deposition have been reported
79 from a number of experimental investigations (e.g. Bobbink et al 1998; Mountford et
80 al., 1993; Clark & Tilman, 2008; Suding et al. 2005) and in observational studies
81 (Stevens et al. 2004, Maskell et al, in press). Although the reduction in species
82 richness is widely reported, the mechanisms for this change have not been fully
83 investigated. There are at least two probable causes; eutrophication and acidification.

84

85 Eutrophication can lead to an increased vigour of potentially robust, nutrient-
86 responsive species (competitors, sensu Grime 1974). These species are better able to
87 take advantage of the additional nitrogen and out-compete the smaller, stress-tolerant
88 species for nitrogen and other resources, including light and water, leading to an
89 overall reduction in species richness as a result of competitive exclusion and reduced
90 recruitment (Stevens et al., 2004a). The most dramatic species losses are likely to
91 occur in communities initially at low-intermediate levels of nutrient availability (Al-
92 Mufti et al., 1977) and will not be as strong where systems are N-saturated.

93 Competitive exclusion and reduced recruitment has been supported by reported
94 increases in sward productivity (e.g. Wilson et al., 1995; Tilman, 1993) and increases
95 in species typical of more fertile conditions (e.g. Kirkham & Kent, 1997) following
96 experimental fertilization.

97

98 Atmospheric N deposition can also result in soil acidification both directly as a result

99 of acid deposition (nitric acid) and indirectly through processes and reaction in soil
100 and water. The other potential cause of species loss is a reduced species pool as a
101 result of increased soil acidity and consequent mobilisation of metals, loss of base
102 cations and changes in the balance between nitrogenous compounds (Crawley et al.,
103 2005; Jefferies & Maron, 1997). Few species are commonly found on soil with pH
104 below 4.5 (Grime & Hodgson, 1969; Grime et al., 2007), such that if soils are
105 acidified below this pH, the potential species pool is considerably reduced. This pH
106 threshold coincides with the aluminium (Al) buffering range where Al ions become
107 increasing available in the soil (Magistad, 1925). A number of heavy metals behave
108 in a similar manner to Al, becoming more biologically available in the soil as soil
109 becomes more acid. Once mobilised into solution, Al can be directly toxic to plants,
110 inhibiting root extension and resulting in abnormalities in lateral roots (Andersson,
111 1988). Additionally, at low pH, nitrate uptake is reduced by free Al^{3+} and this
112 increased Al can have a toxic effect on mycorrhizal symbionts (e.g. Lazof et al.,
113 1994). Other macro- and micro-nutrients including calcium and magnesium (base
114 cations) are commonly found at higher levels in higher pH soils and are among the
115 first ions to leach from the soil as pH declines. A key role for reductions in soil pH in
116 the mechanisms of declining species richness is suggested by the association between
117 acid deposition, reduced soil pH (e.g. Skiba et al., 1989; Blake et al., 1999; Stevens et
118 al., 2006; Horswill et al., 2008) and increased availability of metals (e.g. Stevens et
119 al., 2009; Roem et al., 2002).

120

121 The extent to which each of these mechanisms is responsible for reductions in species
122 richness related to N deposition is not known, but is essential to our understanding of
123 N-deposition impacts. Here we use data from a national survey of acid grasslands

124 across the UK to test two hypotheses along a gradient of atmospheric N deposition,
125 where declines in species richness have been observed (Stevens et al., 2004). The
126 hypotheses are that there is an increase in species tolerant of low pH conditions as a
127 result of soil acidification and an increase in competitive and nitrophilic species as a
128 result of soil eutrophication. There are a number of potentially competitive species
129 found growing in these habitats so either or both of these mechanisms could be
130 responsible for species changes. Because pH and enrichment of soil N are both
131 potentially related to N deposition and are highly correlated with each other within
132 calcifuge grasslands (Stevens et al., 2004), we use plant community characteristics to
133 determine whether the plant community is responding to changes in pH, nutrient
134 enrichment or both. The data used in this study come from 68 grasslands with species
135 richness ranging from 6 to 27 species per 2 x 2 m quadrat. The results of earlier
136 investigations showed a significant decline in species richness related to increasing N
137 deposition along the gradient of deposition found in the UK (Stevens et al., 2004).
138 Changes in species composition, reduced pH, increased ammonia concentration
139 (Stevens et al., 2006) and changed soil metal concentrations (Stevens et al., 2009)
140 were also observed with increasing N deposition.

141

142 **Methods**

143

144 Calcifuge (acid) grasslands belonging to the community U4 *Festuca ovina-Agrostis*
145 *capillaris-Galium saxatile* grassland (Rodwell, 1992) (closely allied to the *Violion*
146 *caninae* association described in Swickerath, 1944) were surveyed along the gradient
147 of N deposition found in Great Britain. Sixty-eight sites were selected from Natural
148 England, Countryside Council for Wales and Scottish Natural Heritage databases

149 using stratified random sampling to cover the range of N deposition in the UK.
150 Grasslands surveyed consisted primarily of protected areas including Sites of Special
151 Scientific Interest (SSSI), National Nature Reserves (NNR) and National Parks.
152 Selected sites were in both upland and lowland areas and none were artificially
153 fertilised.

154

155 Deposition of inorganic N (oxidised and reduced) and acid deposition (N deposition
156 plus sulphur deposition) was modelled by the Centre for Ecology and Hydrology
157 (CEH) in Edinburgh, using the CEH National Atmospheric Deposition Model (Smith
158 et al., 2000). This provides values for both wet and dry deposition and oxidised and
159 reduced deposition at a 5 km resolution. Total inorganic N deposition at the surveyed
160 sites ranged from 6.2 to 36.3 kg N ha⁻¹ y⁻¹.

161

162 All of the sites were surveyed during the summers of 2002 and 2003, beginning in the
163 south of England and moving north. Full details of the field sites are given in Stevens
164 (2004). A full description of each site was made, including location
165 (latitude/longitude), altitude, aspect, slope, grazing intensity (on a scale of one to three
166 estimated by eye based on vegetation height and amount of animal faeces) and the
167 presence of any enclosures. Vegetation at each site was surveyed using five 2 x 2 m
168 quadrats randomly placed within a block 1 ha in extent, excluding areas of different
169 vegetation communities, footpaths or tracks and areas of intense animal activity such
170 as adjacent to feeding or water troughs. Sites with large point sources of ammonia in
171 the vicinity were avoided. Site characteristics are given in table 1. All higher plants
172 and bryophytes were identified to species level and their percentage cover, estimated

173 by eye, was recorded. These data were then compiled to give a species list for each
174 site.

175

176 Species lists from each site were used to calculate mean cover-weighted and
177 unweighted Ellenberg R (reaction - soil pH) and N (soil nutrient) scores (Ellenberg *et*
178 *al.*, 1991) using scores recalculated for plants in Great Britain (Hill *et al.*, 1999; Hill *et*
179 *al.*, 2007) and mean C-S-R signature (Grime *et al.*, 2008; Hunt *et al.*, 2004) for the
180 community. Ellenberg scores are indicator values based on Ellenberg's expert
181 opinion based on his own experiments and research to give a plants realised
182 ecological niche (Hill *et al.*, 1999). C-S-R scores provide a classification of the
183 external factors which affect vegetation grouped into two classes: stress (i.e.
184 phenomena which restrict production) and disturbance (i.e. phenomena which destroy
185 biomass). C-S-R scores are derived from vegetation surveys, knowledge of plant
186 functional traits and field and mesocosm experiments conducted by the Unit of
187 Comparative Plant Ecology at Sheffield University (Grime *et al.*, 2008). An index of
188 soil acidity preference was also calculated using data for over 600 sites from Grime
189 and Lloyd (1973). Although providing similar information to the Ellenberg score, this
190 provides a continuous scale and is tailored specifically to the threshold pH of 5. The
191 index of soil acidity preference was calculated as the proportion of sites on soils with
192 pH of 5 or below each species occurred on compared to the proportion of sites with
193 soils of a pH above 5 using the following formula:

194

195 Index of soil acidity preference = $a / (a + b)$

196 Where:

197 a Number of sites with soil of pH 5 or below on which the species occurs

198 b Number of sites with soils pH above 5 on which the species occurs

199

200 This is the probability of a species occurring on a soil with a pH < 5 relative to the
201 probability of a species occurring on any of the sampled grasslands. A score of 0
202 indicates a strong preference for soil with a pH above 5, a score of 1 indicates a strong
203 preference for soil with a pH of 5 or below and a score of 0.5 indicates no preference.
204 pH 5 was selected as the best value for use in this index because it is close to the point
205 of aluminium mobilisation and the division represented the largest change in species
206 preferences within this range. For example *Calluna vulgaris*, a strongly calcifuge
207 species, has a score of 0.98 whereas *Bellis perennis*, which is not found on the most
208 acid soils, has a score of zero. Values for the acid preference of species included in
209 this survey and some further examples can be found in the supporting online material.
210 Where there were no data on the distribution of a species, this species was not
211 included in the calculation.

212

213 For all scores, means were calculated without cover-weighting. Data were analysed
214 using Pearson correlation coefficients, simple and forward stepwise multiple
215 regression analysis in SPSS v17.0 with nitrogen deposition as the independent
216 variable and mean Ellenberg, CSR and soil acidity preference index scores as
217 dependant variables. In the stepwise multiple regression latitude, longitude, altitude,
218 topsoil pH, mean monthly rainfall, mean annual temperature, slope, and S deposition
219 were used as independent variables with the N deposition included as an independent
220 variable in all models and mean Ellenberg, CSR and soil acidity preference index
221 scores were the dependant variables.

222

223 **Results**

224

225 For all of the scores used, the best-defined relationships were with un-weighted scores
226 as opposed to the cover-weighted scores. This may be because the dominance of a
227 few grass species in this community meant that differences in scores between sites
228 were reduced. Forbs of small stature and cover, common in this community, may also
229 have been insufficiently represented.

230

231 Correlations between the indices examined in this study showed that Ellenberg N and
232 R scores for sites were closely correlated ($r = 0.83$, $p < 0.001$) but there were no other
233 significant correlations between indices (table 2). Because C, S and R scores are
234 interrelated only C scores were considered.

235

236 Despite compositional shifts in the vegetation (reported in Stevens et al., 2004) very
237 little evidence was obtained of functional shifts in the vegetation. Calculating mean
238 C-S-R signatures for each site shows no significant change in the S score (stress
239 tolerance) with increasing N deposition ($p = 0.11$) nor any significant increase in the
240 C score (competitors) ($p = 0.81$). There was also no significant change in the R score
241 ($p = 0.06$) (Fig. 1). Results for acid deposition were very similar showing no change
242 in C or S score but with a weak but significant negative relationship between ruderals
243 and acid deposition ($r^2 = 0.07$, $p = 0.01$). C-S-R scores range from 0 to 1 so the
244 changes in competitor scores are small (range 0.12 to 0.36) indicating that none of the
245 communities are dominated by competitive species. There is a larger range of S scores
246 (0.36 to 0.79) but the range of R scores is also low (range 0.07-0.34). Adding other
247 measured variables (latitude, longitude, altitude, topsoil pH, mean monthly rainfall,

248 mean annual temperature, slope and S deposition) into the regression equations
249 improved the explanatory power of the relationships. The relationship between the C
250 score and N deposition was improved by the addition of soil pH, altitude and latitude
251 resulting in a model that accounted for 11% of the variation in the C score ($p < 0.05$).
252 The relationship between S score and N deposition was improved by the addition of S
253 deposition, latitude, longitude, altitude and soil pH to the model resulting in 40% of
254 the variation in S score being accounted for ($p < 0.001$). The addition of the same
255 variables also increased the explanatory power of the model for the R score resulting
256 in 44% of the variation in R score being accounted for ($p < 0.001$). A summary of
257 these models is provided in the supplemental material.

258

259 Mean Ellenberg N scores revealed a similar result to C scores. There was no
260 significant correlation between Ellenberg N score and N deposition ($r^2 = 0.03$, $p =$
261 0.14) (Fig. 2a). Ellenberg N scores ranged from 2.3 to 4.7 representing a shift from
262 indicators of infertile sites towards indicators of intermediate fertility. Mean
263 Ellenberg R scores showed a weak but significant negative correlation with N
264 deposition ($r^2 = 0.06$; $p = 0.02$) although very little of the variation is explained (Fig.
265 2b). There were no significant relationships between Ellenberg scores and acid
266 deposition (R $p = 0.35$; N $p = 0.12$). The addition of other variables measured in this
267 study did not improve the relationship between N deposition and Ellenberg scores.
268 Ellenberg R scores ranged from 3.3 to 5.3 representing a shift from acidity indicators
269 found mainly on acid soils to indicators of moderately acid soils where N deposition
270 was lower. The magnitudes of changes using Ellenberg scores were very small. This
271 is possibly because Ellenberg scores provide a discontinuous scale. As changes may
272 be only subtle as a result of N deposition and the pH range of the grasslands in this

273 investigation is small, the scale may not be sensitive enough to detect changes that are
274 occurring. Despite this limitation Ellenberg N scores have been used to identify
275 changes associated with N deposition in a number of other habitats (e.g. Bennie et al.,
276 2006 (calcareous grasslands); Smart et al., 2005 (infertile grasslands and moorland);
277 Pitcairn et al., 2002 (woodland)). Ellenberg scores are system-dependant to some
278 extent (Wamelink et al., 2002) but as this study was conducted in a single habitat and
279 region this problem is reduced.

280

281 The correlations using the soil acidity index yielded similar results as those using the
282 Ellenberg R scores, showing a significant correlation ($r^2 = 0.23$, $p < 0.001$) between
283 index score and N deposition with the species present at high N deposition showing a
284 stronger preference for acid habitats (Fig. 3). The relationship between index score
285 and acid deposition is very similar to that with N deposition ($r^2 = 0.28$, $p < 0.001$). The
286 addition of altitude, latitude and pH to the regression model resulted in a model which
287 explained 45% of the variation on the index of acidity preference score and N
288 deposition ($p < 0.001$) (see supplemental material).

289

290 **Discussion**

291

292 Soil pH shows a clear relationship with N deposition along the gradient used in this
293 study (Stevens et al., 2004). This seems to be reflected in the community
294 composition, which shows an increase in the proportion of species with a preference
295 for acid conditions. This is demonstrated by the Ellenberg R score and, to a greater
296 extent, by the index of soil acidity preference scores although this relationship is still
297 not strong. The slight improvement in the relationship by looking at total acid

298 deposition instead of N deposition against acid index score shows that acidification
299 from sulphur deposition and its historic impact may still be apparent. Given strong
300 evidence for mobilisation of Al and heavy metals (Stevens et al., 2009) along this
301 gradient and in experimental studies in similar communities (Blake et al., 1999;
302 Horswill et al., 2008), it is likely that the concentration of metal ions could play an
303 important role in reducing species richness.

304

305 Indeed, Grime and Hodgson (1969), working with many of the most abundant of the
306 species encountered in this investigation, showed a clear relationship between species
307 occurrence on acidic soils and resistance of the seedling root to aluminium toxicity.
308 Using an experimental approach, Roem et al. (2002) also conclude that aluminium
309 toxicity is the main driver of species reductions in heathland and acid grasslands in
310 the Netherlands. We conclude that such impacts of increasing acidity are limiting the
311 pool of species able to survive.

312

313 One reason why the data do not appear to support the hypothesis that the loss of
314 species with increasing deposition is related to eutrophication effects, may be the
315 interaction between soil pH and nutrient availability in the soil. Availability of a
316 number of nutrients is reduced by declining pH. Maximum availability of phosphorus
317 in the soil occurs between pH 5.5 and 7.5, so as pH is reduced below 5.5 as a result of
318 acidification, there is less phosphorus available. Base cations (including the macro-
319 and micro-nutrients calcium, magnesium and potassium) are readily leached from
320 acidified soils and concentrations along this deposition gradient are significantly
321 related to pH (Stevens et al., 2009). Long term investigation of the unfertilized,
322 unlimed plots at the Park Grass experiment at Rothamsted, UK, has shown reductions

323 in concentrations of exchangeable calcium and reductions in cation exchange capacity
324 and base saturation over the 120 years of atmospheric deposition (Blake et al., 1999).
325
326 Nitrification is inhibited at low soil pH because the *Nitrosomas* bacteria, responsible
327 for nitrification, have optimum pH requirements of 7 to 8. This has been
328 demonstrated in several habitats including grasslands and heathlands (e.g. Dorland *et*
329 *al.*, 2004; Roelofs *et al.*, 1985). If ammonium accumulates in the soil due to low
330 nitrification this could also reduce denitrification activity (Sanchez-Martin *et al.*,
331 2008). At low pH nitrate uptake is reduced by free Al^{3+} which can also have a
332 negative impact on mycorrhizal symbionts (e.g. Lazof et al., 1994). As a result of the
333 changes in processing the predominant form of N available in the soil some plants
334 have become adapted to using one form or another according to their preferred
335 growing conditions. Plants adapted to acidic soils with low nitrification rates (more N
336 available as ammonium rather than nitrate) use ammonium as their preferred N
337 source. They can tolerate high ammonium concentrations without toxic effects and are
338 less efficient at using nitrate (Britto & Kronzucker, 2002). The consequence of all
339 these changes in nutrient availability with pH is that N, and other important nutrients,
340 may be less available to plants growing in acidified soils even though N is added.
341 Even if there is additional N available to plants other nutrients could become limiting
342 and responses to nutrient enrichment may not become manifest. As N deposition
343 increases and the soil becomes increasingly acidic, those species less tolerant of acid
344 conditions are no longer found and the community becomes increasingly dominated
345 by acid specialists.
346

347 Ellenberg R and N scores for sites were closely correlated with each other but
348 Ellenberg R scores were not correlated with the index of soil acidity preference as
349 might have been expected. This is likely to be because the scores are derived in a
350 very different manner and the index of soil acidity preference scores are based on data
351 from grasslands only whereas the Ellenberg scores represent a tolerance across the
352 range of habitats a species occurs in.

353

354 The lack of an increase in the Ellenberg N score suggests that any enrichment of the
355 soil by atmospherically deposited N is not available to the vegetation and/or is not of
356 sufficient magnitude to bring about significant changes in the plant community
357 composition. Increased KCl-extractable ammonium in the soil samples noted by
358 Stevens et al. (2006) may be a result of growth and therefore uptake being limited by
359 a lack of other resources. The range of Ellenberg N scores reported in this study is
360 small and the values remain at the lower end of the scale. This is because the
361 Ellenberg scores encompass the whole range of situations plants grow in including the
362 most fertile. In this study we were only concerned with semi-natural grasslands
363 where nutrient inputs are low. These levels of input are considerably lower than the
364 rates of fertiliser application to agricultural grasslands. Experimental evidence in
365 some communities has shown that over time, sites with high atmospheric deposition
366 might be expected to show a slow increase in Ellenberg N score (e.g. Smart et al.,
367 2005).

368

369 The lack of a significant change in the C score with increasing N deposition further
370 implies that competition for nitrogen is not the prime mechanism controlling
371 community composition. Under our eutrophication hypothesis, we might expect to see

372 reductions in stress tolerant species with increasing N deposition, but this is not the
373 case in this grassland community. Although large amounts of N are deposited in some
374 of these grasslands, Phoenix et al (2003) demonstrated in the same community type
375 that even at high N inputs, large amounts of N are immobilized in the soil, potentially
376 in forms that are inaccessible to plants. Experimental N additions have shown mixed
377 responses in terms of vegetation productivity, but Horswill et al. (2008) cite declines
378 in productivity at high levels of N addition as evidence that competition is not
379 responsible for declines in species richness. Although we know that soil KCl-
380 extractable ammonium increases with increasing N deposition, neither KCl-
381 extractable nitrate or total nitrogen increased along the N deposition gradient (Stevens
382 et al., 2006).

383

384 The results from this study contrast with those found in calcareous grasslands. For
385 example, Bennie et al. (2006) report increases in the Ellenberg N score of calcareous
386 grasslands between 1953 and 2003 which they relate to N deposition. The change in
387 Ellenberg N suggests that in calcareous grasslands, where soils are well buffered
388 against changes in pH, eutrophication may be a more significant driver of change than
389 in acid soils. In calcareous soils pH will not be reduced to a sufficient level to cause
390 the changes in soil chemistry reported above. This has important implications for
391 semi-natural communities sensitive to N deposition suggesting that in communities
392 with base rich soils eutrophication may be the most important driver of species change
393 whereas in communities with acid soils, acidification may be more important. In very
394 resource poor communities this relationship may not hold as other nutrients or
395 limiting resources may be more important. Further work is needed to investigate the
396 relative importance of acidification and eutrophication in driving species composition

397 changes in response to N deposition across a range of habitats. Management options
398 to mitigate the effects of N deposition will differ depending if the change is driven by
399 acidification or eutrophication and indicators used to detect damage from N
400 deposition must also be different.

401

402 Stevens et al. (2004) reported that relationships between species richness and total
403 inorganic N deposition were slightly stronger than those with soil pH, indicating that
404 acidification alone does not explain the full effect of N deposition. Although the
405 evidence from this study supports the hypothesis that soil acidification is the dominant
406 process responsible for a decline in species richness with increasing N deposition, it is
407 likely that there is an element of eutrophication and other soil chemical changes are
408 involved too, the effects of which may be partly missed by shifts in the limiting
409 nutrient.

410

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418

419 **References**

420 Al-Mufti, M.M., Sydes, C.L., Furness, S.B., Grime, J.P. & Band, S.R. (1977) A
421 quantitative analysis of shoot phenology and dominance in herbaceous vegetation.
422 *Journal of Ecology*, **65**(759-791).
423

424 Andersson, M. (1988) Toxicity and tolerance of aluminium in vascular plants. *Water,*
425 *Air and Soil Pollution*, **39**, 439-62.
426

427 Bennie, J., Hill, M., Baxter, R. & Huntley, B. (2006) Influence of slope and aspect on
428 long-term vegetation change in British chalk grasslands. *Journal of Ecology*, **94**, 355-
429 68.
430

431 Blake, L., Goulding, K.W.T., Mott, C.J.B. & Johnston, A.E. (1999) Changes in soil
432 chemistry accompanying acidification over more than 100 years under woodland and
433 grass at Rothamsted Experimental Station, UK. *European Journal of Soil Science*, **50**,
434 401-12.
435

436 Bobbink, R., Hornung, M. & Roelofs, J.G.M. (1998) The effects of air-borne nitrogen
437 pollutants on species diversity in natural and semi-natural European vegetation.
438 *Journal of Ecology*, **86**, 717-38.
439

440 Britto, D.T. & Kronzucker, H.J. (2002) NH₄⁺ toxicity in higher plants: a critical
441 review. *Journal of Plant Physiology*, **159**, 567-84.
442

443 Brunsting, A.M.H. & Heil, G.W. (1985) The role of nutrients in the interactions
444 between a herbivorous beetle and some competing plant species in heathlands. *Oikos*,
445 **44**, 23-26.

446

447 Caporn, S.J.M., Ashenden, T.W. & Lee, J.A. (2000) The effect of exposure to NO₂
448 and SO₂ on frost hardiness in *Calluna vulgaris*. *Environmental and Experimental*
449 *Botany*, **43**, 111-19.

450

451 Clark, C.M. & Tilman, D. (2008) Loss of plant species after chronic low-level
452 nitrogen deposition to prairie grasslands. *Nature*, **451**, 712-15.

453

454 Crawley, M.J., Johnston, A.E., Silvertown, J., Dodd, M., de Mazancourt, C., Heard,
455 M.S., Henman, D.F. & Edwards, G.R. (2005) Determinants of species richness in the
456 Park Grass Experiment. *The American Naturalist*, **165**(2), 179-92.

457

458 Dorland, E., Van den Berg, L.J.L., Van den Berg, A.J., Vermeer, M., Roelofs, J.G.M.
459 & Bobbink, R. (2004) The effects of sod cutting and additional liming on potential net
460 nitrification in heathland soils. *Plant and Soil*, **265**, 267-77.

461

462 Ellenberg, H., Weber, H. E., Düll, R., Wirth, V., Werner, W. & Paulißen D. (1991)
463 Zeigerwerte von Pflanzen in Mitteleuropa. *Scripta Geobot*, **18**, 9-166.

464

465 Francis, A.J. (1982) Effects of acidic precipitation and acidity on soil microbial
466 processes. *Water, Air and Soil Pollution*, **18**, 375-94.

467

468 Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R.,
469 Martinelli, L.A., Seitzinger, S.P. & Sutton, M.A. (2008) Transformation of the
470 nitrogen cycle: Recent trends, questions and potential solutions. *Science*, **320**, 889-92.
471

472 Grime, J.P., Fridley, J.D., Askew, A.P., Thompson, K., Hodgson, J.G. & Bennett,
473 C.R. (2008) Long-term resistance to simulated climate change in an infertile
474 grassland. *Proceedings of the National Academy of Sciences of the United States of*
475 *America*, **150**(29), 10028-32.
476

477 Grime, J.P., Hodgson, J.G. & Hunt, R. (2007) *Comparative plant ecology: a*
478 *functional approach to common British species*, 2nd edn. Unwin Hyman, London.
479

480 Grime, J.P. & Hodgson, J.G. (1969). An investigation of the ecological significance of
481 lime-chlorosis by means of large-scale comparative experiments. *Ecological aspects*
482 *of the mineral nutrition of plants*. (ed I.H. Rorison), pp. 67-99. Blackwell Scientific
483 Publications, Oxford.
484

485 Grime, J.P. & Lloyd, P.S. (1973) *An ecological atlas of grassland plants* Edward
486 Arnold, London.
487

488 Hill, M.O., Mountford, J.O., Roy, D.B. & Bunce, R.G.H. (1999). Ellenberg's indicator
489 values for British plants. ECOFACT Volume 2. Technical Annex. In. Institute of
490 Terrestrial Ecology.
491

492 Hill, M.O., Preston, C.D., Bosanquet, S.D.S. & Roy, D.B. (2007) *BRYOATT:*
493 *Attributes of British and Irish Mosses, Liverworts and Hornworts*. Centre for Ecology
494 and Hydrology, Huntingdon.
495

496 Horswill, P., O'Sullivan, O., Phoenix, G.K., Lee, J.A. & Leake, J.R. (2008) Base
497 cation depletion, eutrophication and acidification of species-rich grasslands in
498 response to long-term simulated nitrogen deposition. *Environmental Pollution*, **155**,
499 336-49.
500

501 Hunt, R., Hodgson, J.G., Thompson, K., Bungener, P., Dunnett, N.P. & Askew, A.P.
502 (2004) A new practical tool for deriving a functional signature for herbaceous
503 vegetation. *Applied Vegetation Science*, **7**, 163-70.
504

505 Jefferies, R.L. & Maron, J.L. (1997) The embarrassment of riches: atmospheric
506 deposition of nitrogen and community and ecosystem processes. *Trends in Ecology*
507 *and Evolution*, **12**(2), 74-78.
508

509 Kirkham, F.W. & Kent, M. (1997) Soil seed bank composition in relation to the
510 above-ground vegetation in fertilized and unfertilized hay meadows on a Somerset
511 peat moor. *Journal of Applied Ecology*, **34**, 889-902.
512

513 Lazof, D.B., Rincon, M., Rufty, T.W., Carter, T.E. & MacKown, C.A. (1994)
514 Aluminum accumulation and associated effects on $^{15}\text{NO}_3^-$ influx in roots of two
515 differentially-sensitive soybean genotypes. *Plant and Soil*, **164**, 291-97.
516

517 Magistad, O.C. (1925) The aluminium content of the soil solution and its relation to
518 soil reaction and plant growth. *Soil Science*, **20**, 181-226.

519

520 Morecroft, M.D., Sellers, E.K. & Lee, J.A. (1994) An experimental investigation into
521 the effects of atmospheric deposition on two semi-natural grasslands. *Journal of*
522 *Ecology*, **82**, 475-83.

523

524 Maskell, L.C., Smart, S.M., Bullock, J.M., Thompson, K. & Stevens, C.J. Nitrogen
525 Deposition causes widespread species loss in British habitats. *Global Change*
526 *Biology*, in press.

527

528 Mountford, J.O., Lakhani, K.H. & Kirkham, F.W. (1993) Experimental assessment of
529 the effects of nitrogen addition under hay-cutting and aftermath grazing on the
530 vegetation of meadows on a Somerset peat moor. *Journal of Applied Ecology*, **30**,
531 321-32.

532

533 Phoenix, G.K., Booth, R.E., Leake, J.R., Read, D.J., Grime, P. & Lee, J.A. (2003)
534 Effects of enhanced nitrogen deposition and phosphorus limitation on nitrogen
535 budgets of semi-natural grasslands. *Global Change Biology*, **9**, 1309-21.

536

537 Pitcairn, C. E. R., Skiba, U. M., Sutton, M. A., Fowler, D., Munro, R. & Kennedy, V.
538 (2002) Defining the spatial impacts of poultry farm ammonia emissions on species
539 composition of adjacent woodland groundflora using Ellenberg Nitrogen Index,
540 nitrous oxide and nitric oxide emissions and foliar nitrogen as marker variables.
541 *Environmental Pollution*, **119**, 9-21.

542

543 Rodwell, J.S. (1992) *Grasslands and montane communities*. University Press,
544 Cambridge.

545

546 Roelofs, J.G.M., Kempers, A.J., Houdijk, A.L.F.M. & Jansen, J. (1985) The effect of
547 air-borne ammonium sulphate on *Pinus nigra* var. *maritima* in the Netherlands. *Plant*
548 *and Soil*, **84**, 45-56.

549

550 Roem, W.J. & Berendse, F. (2000) Soil acidity and nutrient supply ratio as possible
551 factors determining changes in plant species diversity in grassland and heathland
552 communities. *Biological Conservation*, 92, 151-61.

553

554 Roem, W.J., Klees, H. & Berendse, F. (2002) Effects of nutrient addition and
555 acidification on plant species diversity and seed germination in heathland. *Journal of*
556 *Applied Ecology*, **39**, 937-48.

557

558 Sanchez-Martin, L., Vallejo, A., Dick, J. & Skiba, U.M. (2008) The influence of
559 soluble carbon and fertilizer nitrogen on nitric oxide and nitrous oxide emissions from
560 two contrasting agricultural soils. *Soil Biology and Biochemistry*, **40**, 142-51.

561

562 Schwickerath, M. (1944) Das Hohe Venn und seine Randgebiete. *Pflanzensoziologie*,
563 **6**, 1-278.

564

565 Skiba, U., Cresser, M.S., Derwent, R.G. & Futton, D.W. (1989) Peat acidification in
566 Scotland. *Nature*, **337**(5), 68-70.

567

568 Smart, S.M., Bunce, R.G.H., Marrs, R., LeDuc, M., Firbank, L.G., Maskell, L.C.,
569 Scott, W.A., Thompson, K., Walker, K.J. (2005) Large-scale changes in the
570 abundance of common higher plant species across Britain between 1978, 1990 and
571 1998 as a consequence of human activity: Tests of hypothesized changes in trait
572 representation. *Biological Conservation*, **124**, 355-71.

573

574 Smith, R.I., Fowler, D., Sutton, M.A., Flechard, C. & Coyle, M. (2000) Regional
575 estimation of pollutant gas dry deposition in the UK: model description, sensitivity
576 analyses and outputs. *Atmospheric Environment*, **34**, 3757-77.

577

578 Stevens, C.J. (2004) *Ecosystem properties of acid grasslands along a gradient of*
579 *nitrogen deposition*, PhD thesis, The Open University, Milton Keynes.

580

581 Stevens, C.J., Dise, N. B., Gowing, D. J., Mountford, J. O. (2006) Loss of forb
582 diversity in relation to nitrogen deposition in the UK: regional trends and potential
583 controls. *Global Change Biology*, **12**, 1823-33.

584

585 Stevens, C.J., Dise, N.B. and Gowing, D.J. (2009) Regional trends in soil acidification
586 and metal mobilisation related to acid deposition. *Environmental Pollution*, **157**, 313-
587 19.

588

589 Stevens, C.J., Dise, N.B., Mountford, J.O. & Gowing, D.J. (2004) Impact of nitrogen
590 deposition on the species richness of grasslands. *Science*, **303**(5665), 1876-79.

591

592 Stevens, M.H.H., Bunker, D.E., Schnitzer, N.A., Carson, D.E. (2004a) Establishment
593 limitation reduces species recruitment and species richness as soil resources rise.
594 *Journal of Ecology*, **92** (2), 339-347.

595

596 Suding, K.N., Collins, S.L., Gough, L., Clark, C., Cleland, E.E., Gross, K.L.,
597 Milchunas, D.G. & Pennings, S. (2005) Functional- and abundance-based
598 mechanisms explain diversity loss due to N fertilization. *Proceedings of the National*
599 *Academy of Sciences of the United States of America*, **102**(12), 4387-92.

600

601 Tilman, D. (1993) Species richness of experimental productivity gradients: How
602 important is colonization limitation. *Ecology*, **74**, 2179-91.

603

604 van den Berg, L.J.L., Peters, C.J.H., Ashmore, M.R. & Roelofs, J.G.M. (2008)
605 Reduced nitrogen has a greater effect than oxidised nitrogen on dry heathland
606 vegetation. *Environmental Pollution*, **154**(3), 359-69.

607

608 Vitousek, P.M. (1994) Beyond global warming: Ecology and global change. *Ecology*,
609 **75**(7), 1861-76.

610 Wamelink, G.W.W., Joosten, V., van Dobben, H.F. & Berendse, F. (2002) Validity
611 of Ellenberg indicator values judged from physico-chemical field measurements.
612 *Journal of Vegetation Science*, **13**, 269-278.

613

614 Wilson, E.J., Wells, T.C.E. & Sparks, T.H. (1995) Are calcareous grasslands in the
615 UK under threat from nitrogen deposition? - An experimental determination of a
616 critical load. *Journal of Ecology*, **83**, 823-32.

618 **Tables**

619

620 Table 1. Site characteristics of the 68 sites surveyed.

621

| Site Characteristic | Range |
|--|--------------|
| Total N deposition (kg N ha ⁻¹ yr ⁻¹) | 6.2 – 36.3 |
| Total S deposition (kg N ha ⁻¹ yr ⁻¹) | 6.6 - 28.7 |
| Altitude (m) | 15 - 692 |
| Slope (°) | 0 - 60 |
| Aspect (°) | 0 - 315 |
| Topsoil pH | 3.7 – 5.5 |
| Subsoil pH | 3.3 – 5.7 |
| C : N (by mass) | 13.3 - 30.2 |
| % N | 0.1 – 1.6 |
| Mean species richness (per 2 x 2 m quadrat) | 6.4 – 26.4 |

622

623 Table 2. Correlation coefficients and significance values for comparison between

624 indices.

625

| Indicies | R | P |
|-----------------------------|----------|----------|
| Ellenberg R and Ellenberg N | 0.83 | <0.001 |
| Ellenberg R and Acid Index | 0.07 | 0.575 |
| Ellenberg R and C score | 0.10 | 0.425 |
| Ellenberg N and Acid Index | 0.10 | 0.438 |
| Ellenberg N and C score | 0.10 | 0.431 |
| Acid Index and C score | 0.01 | 0.911 |

626

627

628 **Figure legends**

629

630 Figure 1. Mean competitor (a), stress tolerator (b) and ruderal (c) scores (Grime et al.,
631 2008; Hunt et al., 2004) against nitrogen deposition in 68 calcifuge grasslands
632 surveyed along a nitrogen deposition gradient in the UK. A high score shows a strong
633 tendency towards the trait being measured. Regression analysis showed no significant
634 relationship between C, S and R scores and nitrogen deposition (C $p = 0.81$; S $p =$
635 0.11 , R $p = 0.06$).

636

637 Figure 2. Mean Ellenberg scores for (a) N (soil nutrients) and (b) R (soil reaction)
638 against nitrogen deposition in 68 calcifuge grasslands surveyed along a nitrogen
639 deposition gradient in the UK. Low scores represent indicators of low nutrient status
640 for N and indicators of acid environments for R. Regression analysis showed no
641 significant relationship between Ellenberg N score and nitrogen deposition ($p = 0.14$)
642 and a significant relationship between Ellenberg R score and nitrogen deposition ($r^2 =$
643 0.06 , $p < 0.02$).

644

645

646 Figure 3. Mean acidity index score against nitrogen deposition in calcifuge
647 grasslands. A score of 1 indicates a strong preference for soil with a pH below 4.5, a
648 score of 0 indicates a strong preference for soil with a pH of 4.5 or above and a score
649 of 0.5 indicates no preference. Regression analysis showed a significant relationship
650 between acidity index score and nitrogen deposition ($r^2 = 0.23$, $p < 0.001$).

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652

653 Figure 1

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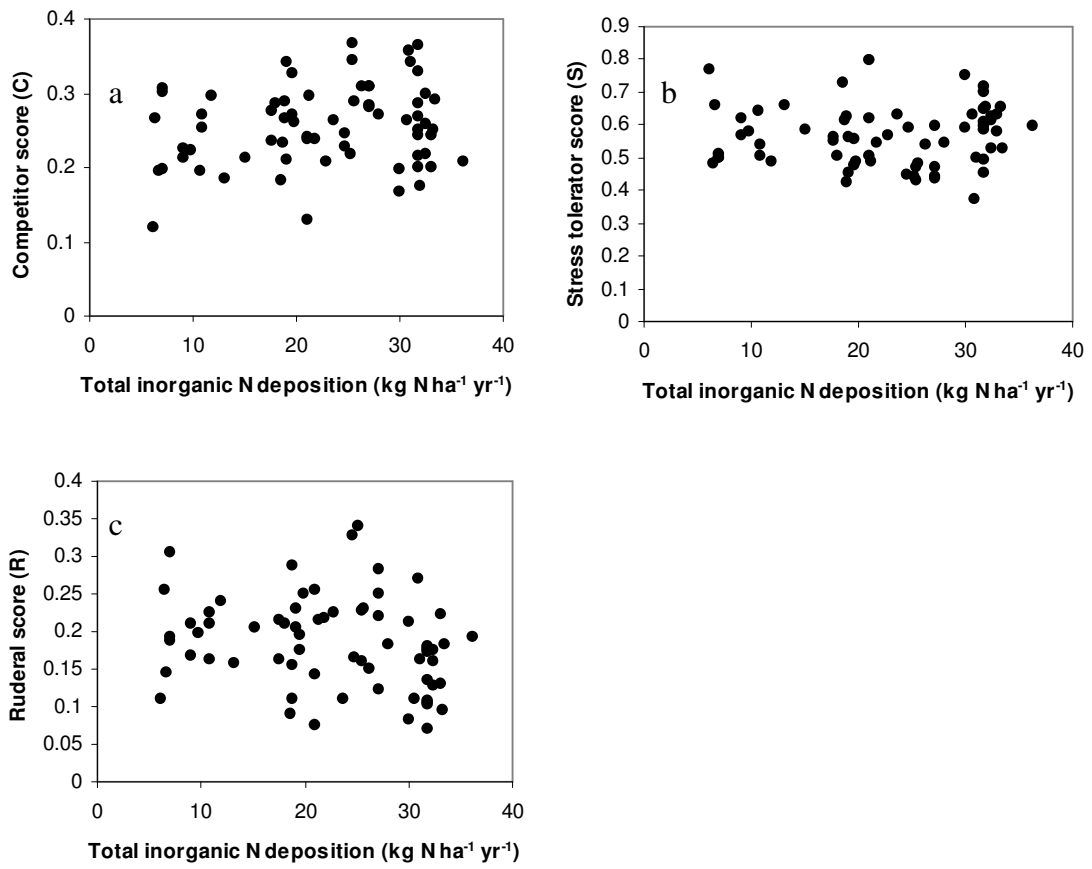
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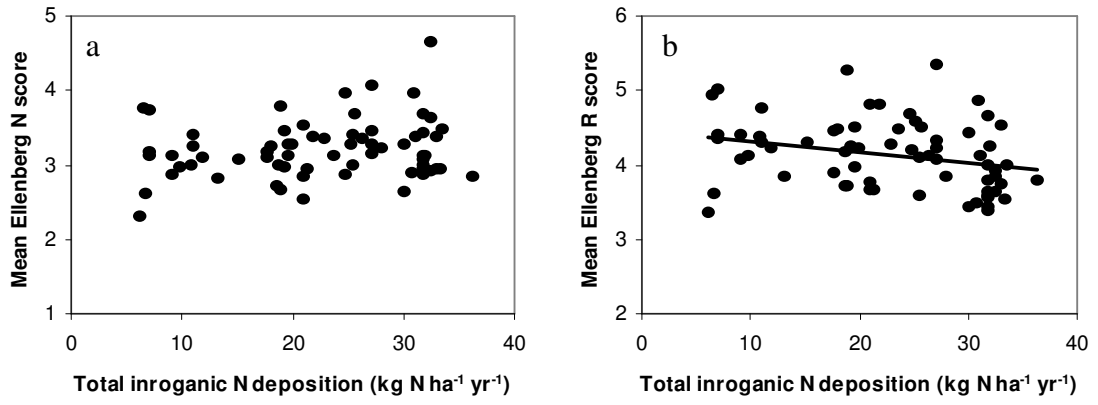
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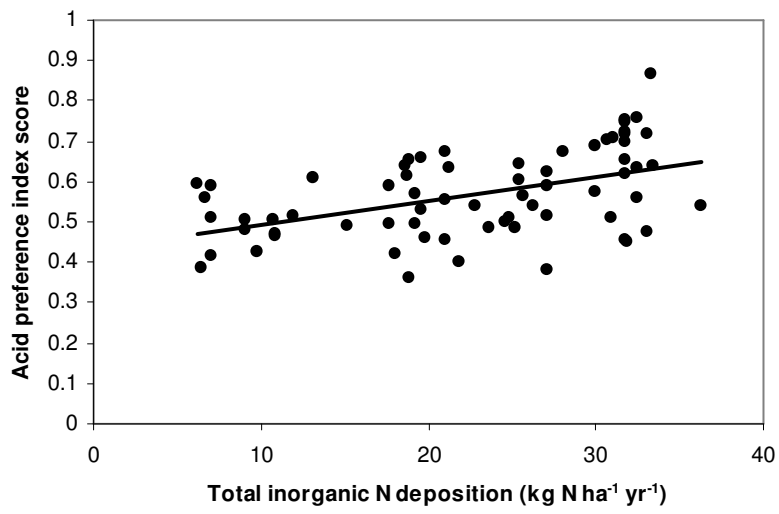
674 Figure 2.

675



676 Figure 3

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678

679

680 **Supporting Online Material**681 Table 1. Soil acidity index scores for species occurring in the survey of acid
682 grasslands used in this study.

| Species | Number of records in Grime and Lloyd (1973) | Acidity index score |
|---------------------------------|--|----------------------------|
| <i>Achillea millefolium</i> | 53 | 0.31 |
| <i>Agrostis canina</i> | 82 | 0.70 |
| <i>Agrostis capillaris</i> | 268 | 0.64 |
| <i>Anemone nemorosa</i> | 26 | 0.31 |
| <i>Anthoxanthum odoratum</i> | 128 | 0.39 |
| <i>Arrhenatherum elatius</i> | 131 | 0.11 |
| <i>Bellis perennis</i> | 24 | 0.00 |
| <i>Briza media</i> | 89 | 0.06 |
| <i>Calluna vulgaris</i> | 62 | 0.98 |
| <i>Campanula rotundifolia</i> | 173 | 0.14 |
| <i>Carex caryophylla</i> | 96 | 0.17 |
| <i>Carex flacca</i> | 113 | 0.05 |
| <i>Centaurea nigra</i> | 84 | 0.05 |
| <i>Cerastium holosteoides</i> | 80 | 0.11 |
| <i>Chamnerion angustifolium</i> | 19 | 0.58 |
| <i>Cirsium arvense</i> | 42 | 0.31 |
| <i>Cirsium palustre</i> | 35 | 0.23 |
| <i>Conopodium majus</i> | 26 | 0.27 |
| <i>Dactylis glomerata</i> | 175 | 0.14 |
| <i>Deschampsia cespitosa</i> | 38 | 0.47 |
| <i>Deschampsia flexuosa</i> | 322 | 0.96 |
| <i>Euphrasia officinalis</i> | 28 | 0.00 |
| <i>Festuca ovina</i> | 356 | 0.60 |
| <i>Festuca rubra</i> | 253 | 0.21 |
| <i>Galium saxatile</i> | 160 | 0.93 |
| <i>Hieracium pilosella</i> | 95 | 0.09 |
| <i>Holcus lanatus</i> | 131 | 0.36 |
| <i>Holcus mollis</i> | 72 | 0.94 |
| <i>Hypochoeris radicata</i> | 39 | 0.18 |
| <i>Leontodon hispidus</i> | 83 | 0.02 |
| <i>Lotus corniculatus</i> | 159 | 0.11 |
| <i>Luzula campestris</i> | 159 | 0.48 |
| <i>Molinia caerulea</i> | 20 | 1.00 |
| <i>Nardus stricta</i> | 130 | 0.98 |
| <i>Pimpinella saxifraga</i> | 75 | 0.04 |
| <i>Plantago lanceolata</i> | 137 | 0.10 |
| <i>Poa pratensis</i> | 156 | 0.30 |
| <i>Potentilla erecta</i> | 85 | 0.54 |
| <i>Prunella vulgaris</i> | 42 | 0.05 |
| <i>Pteridium aquilinum</i> | 57 | 0.93 |
| <i>Ranunculus acris</i> | 23 | 0.30 |
| <i>Ranunculus bulbosus</i> | 44 | 0.07 |
| <i>Rumex acetosa</i> | 111 | 0.38 |

683

684 A score of 0 indicates a strong preference for soil with a pH above 4.5, a score of 1

685 indicates a strong preference for soil with a pH of 4.5 or below and a score of 0.5

686 indicates no preference.

687

688 Table 2. Results of stepwise multiple regression to identify optimal models with the
 689 aim of increasing the explanatory power of the relationship between N deposition and
 690 the index scores.
 691

| Index | Model | R ² |
|--------------------------|--|----------------|
| Ellenberg R | No variables explain additional variation | -- |
| Ellenberg N | No variables explain additional variation | |
| Acidity Preference Index | Index = (0.626 + 0.002 (Ndep) - 0.114 (pH) + 0.005 (latitude) + 0.001 (altitude) | 0.45 |
| c score | c = 0.443 + 0.002 (Ndep) - 0.000093 (altitude) - 0.006 (latitude) + 0.024 (pH) | 0.11 |
| s score | s = 0.279 - 0.002 (Ndep) + 0.001 (altitude) - 0.125 (pH) + 0.014 (latitude) + 0.014 (longitude) - 0.002 (Sdep) | 0.40 |
| r score | r = 0.288 + 0.001 (Ndep) + 0.001 (altitude) + 0.088 (pH) - 0.008 (latitude) - 0.008 (longitude) + 0.001 (Sdep) | 0.44 |

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