Open Research Online

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

5 Stevens, C.J.[1,2], Thompson, K.[3], Grime, J.P.[3], Long, C.J.[4] and Gowing, D.J.G.[1]

6

7 [1] Department of Life Sciences, The Open University, Walton Hall, Milton Keynes,

8 MK7 6AA, UK

9 [2] Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK

10 [3] Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10

11 2TN, UK

12 [4] 42 Canterbury Avenue, Lancaster, LA1 4AU

13

14 Dr Carly Stevens

15 Department of Life Sciences,

16 The Open University,

17 Walton Hall,

18 Milton Keynes,

19 MK7 6AA

20

21 01908 658342

22 c.j.stevens@open.ac.uk

23

24 Running title: Acidification in relation to grassland species richness

Abstract:

1. Atmospheric deposition of nitrogen (N) is a global problem resulting in negative consequences for biodiversity due to direct toxicity, increases in invasive species, increased susceptibility to environmental stresses and soil-mediated effects of acidification and eutrophication.

2. Reductions in plant species richness related to N deposition have been observed in a number of habitats including calcifuge (acid) grasslands but the mechanisms of this decline have not been fully investigated. We test the hypotheses that along a large-scale gradient of N deposition there is a) an increase in species tolerant of low pH conditions as a result of soil acidification and b) an increase in competitive and nitrophilic species as a result of soil eutrophication. As competitive species can occur in low pH habitats both of these hypotheses could be true.

3. Using plant characteristics we examined changes in vegetation species composition along the gradient of N deposition in the UK. Mean C-S-R signatures were used to identify the competitive response of plant communities together with Ellenberg N (nitrogen) scores to identify increases in nitrophilic species. Ellenberg R (reaction, pH) scores were used to identify change in response to soil pH together with an index of soil acidity preference developed using regional survey data.

4. Mean C-S-R signatures showed no significant correlation with N deposition, nor did mean Ellenberg N scores. Ellenberg R scores and the index of soil acidity preference showed significant relationships with N deposition 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

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 kg N ha^{-1} yr^{-1} in many areas of

65   the world, 20 times more than the estimated deposition of 0.5 kg N ha^{-1} yr^{-1} where

66   there is no human influence (Galloway et al., 2008). 10 kg N ha^{-1} 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

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 NO_2

448   and SO_2 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 15NO3- 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. & Futty, 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 *Jounral 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.

617

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 (^{o})	0 - 60
Aspect (^{o})	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

Figure legends

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).

651

652

Figure 1

654

655

656
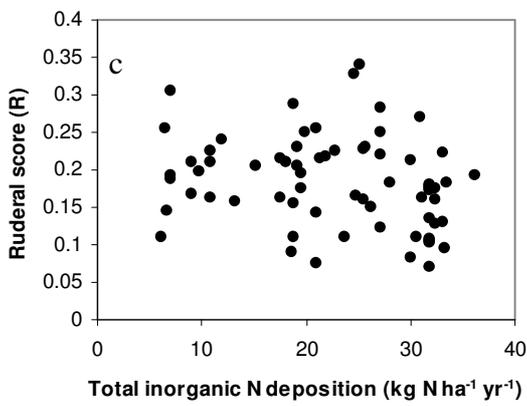
657

658

659

660

661

662
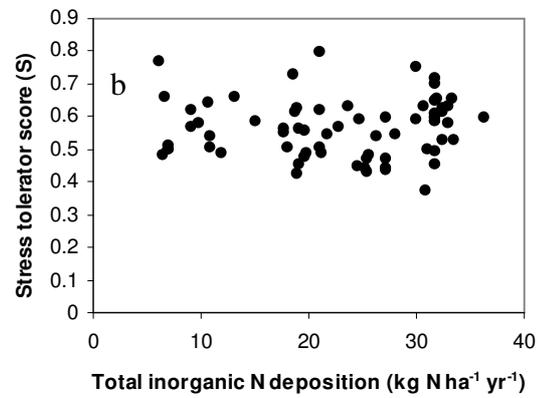
663

664

665

666

667

668
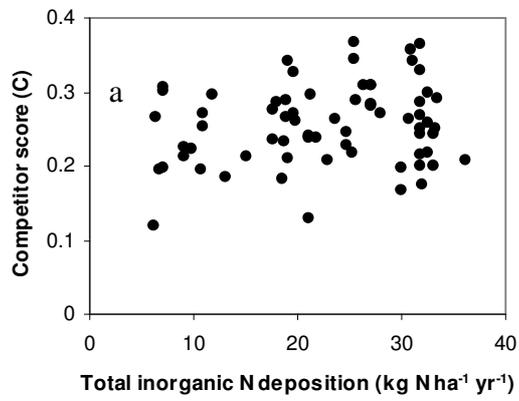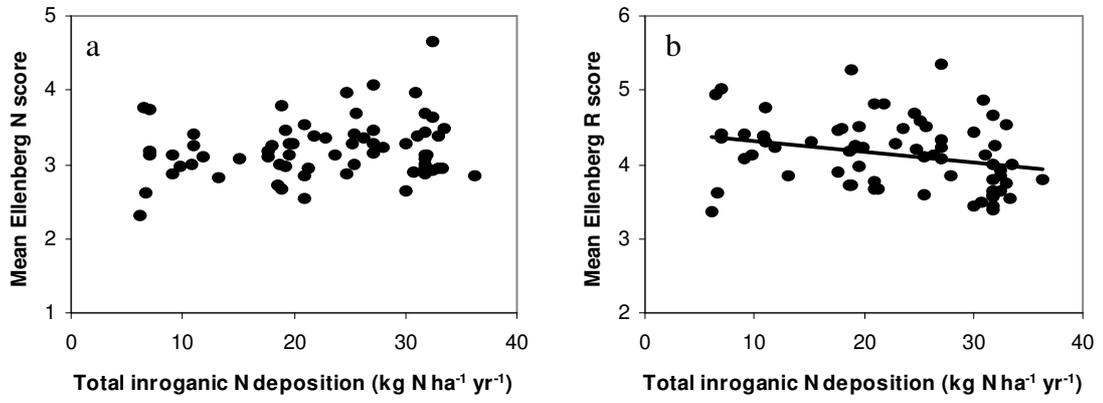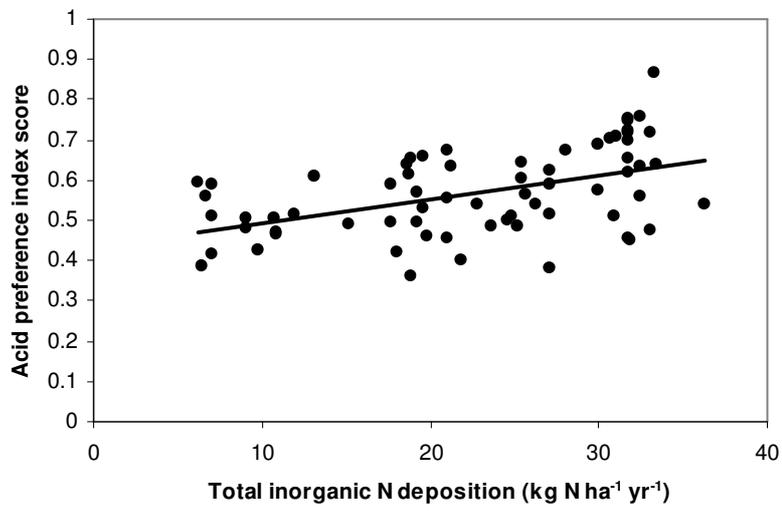
669

670

671

672

673

674   Figure 2.

675

676   Figure 3

677

678

679

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

692