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1 **Does a simulated upland grassland community respond to**
2 **increasing background, peak or accumulated exposure of**
3 **ozone?**

4

5

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7

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15

16 ***Abstract***

17 Tropospheric ozone concentrations are increasing, which may result in elevated
18 background concentrations at rural high-altitude sites. In this study simulated upland
19 grassland communities containing seven species were exposed to ozone treatments in
20 solardomes for 12 weeks in each of two consecutive summers. Ozone profiles, based
21 on future ozone predictions, were of elevated background concentrations, episodic
22 peaks of ozone and a combination of the two. During the winter between the two
23 exposures the communities were kept outdoors in ambient air. Whereas previous

24 studies have demonstrated that peaks of ozone cause detrimental effects to vegetation,
25 this study shows that for simulated grassland communities an increase in background
26 ozone concentration in the absence of peaks of ozone also corresponded with
27 increased senescence. In many cases senescence was further increased when peaks of
28 ozone were also present. The species used showed no acclimation to ozone and the
29 same relationship between senescence and ozone dose occurred in both years of the
30 study. A decrease in cumulative biomass was demonstrated for *Anthoxanthum*
31 *odoratum*, which contributed to a decrease in total community biomass. These results
32 indicate that current and future ozone concentrations could cause detrimental effects
33 on growth and vitality of natural grassland communities and that for some species the
34 consequences of increased background ozone concentration are as severe as that of
35 increased peaks.

36

37 **Key words**

38 Grassland; ozone profiles; senescence; biomass; competition

39 **1. Introduction**

40 Tropospheric ozone concentrations have been increasing during the last century due to
41 increasing anthropogenic emissions of ozone precursors (Volz and Kley, 1988). In
42 urban and lowland areas elevated ozone occurs as ozone episodes, where ozone
43 concentrations are increased for several days in a pronounced diurnal profile, with the
44 highest peak during the day and low concentrations at night (Garland and Derwent,
45 1979). In contrast, at remote, high-altitude sites ozone concentrations remain high at
46 night-time, because the air remains turbulent and losses of ozone from the lowest air
47 layers due to dry deposition are replaced from ozone-rich layers above (Coyle et al.,

48 2002; Ashmore et al., 2002). In the last 15 years, there has been evidence of a change
49 in the ozone profile over Europe. Peak concentrations have fallen (Vingarzan, 2004;
50 Szopa et al., 2006) whilst background ozone concentrations have steadily increased
51 (Solberg et al., 2005, Derwent et al., 2006). Current predictions indicate that the
52 rising background may stabilise by ca. 2030, but peak ozone concentrations may
53 again rise as global warming increases the incidence of climatic conditions conducive
54 to ozone formation (Royal Society, 2008). In this study, we investigated the
55 implications of the potential changes in ozone profile for an upland grassland since
56 the effects are likely to be felt first in remote upland regions (Coyle et al., 2003). For
57 these communities the diurnal cycles are less pronounced and the background ozone
58 concentration is already in the 30 to 40+ ppb range in many such areas of Europe (e.g.
59 Marchlyn Mawr, UK, altitude 610 m, www.welshairquality.co.uk, grid reference
60 SH604627).

61

62 Ozone has been shown to cause adverse effects on individual plant species, with
63 effects including visible injury and premature senescence (e.g. Bergmann et al., 1995)
64 and effects on growth (e.g. Franzaring et al., 2000; Gimeno et al., 2004a). Different
65 effects have been reported on different species and a wide range in sensitivity to
66 ozone has been observed (Hayes et al., 2006). In addition to these experimental
67 studies, effects of ozone on naturally occurring plants in ambient air have been
68 demonstrated across Europe (Hayes et al. 2007a). However, few studies have
69 investigated the effects of ozone at mean levels of 30 - 50 ppb without including some
70 peak exposures and this has been identified as a large gap in current knowledge that
71 makes it very difficult to predict the impact of changes in the background ozone
72 concentration on vegetation (Coyle et al., 2003). One of the few studies to investigate

73 the effects of elevated background ozone concentrations demonstrated that for the
74 grass species *Anthoxanthum odoratum*, elevated background ozone concentrations
75 induced premature senescence to a similar extent to episodic peaks superimposed on a
76 low background (Dawnay and Mills, 2009).

77

78 It is possible that changes in community dynamics could occur with increased ozone
79 exposure due to the differential responses to ozone of the component species,
80 however, there have been comparatively few studies on the effects of ozone on plant
81 communities. Studies to date have been almost exclusively on grassland/pasture
82 vegetation. The majority of these studies carried out using two or three species
83 mixtures (e.g. Bender et al., 2002, 2006; Gimeno et al., 2004b; Tonneijck et al.,
84 2004), and a few additional studies used larger model communities or established
85 vegetation (e.g. Ashmore and Ainsworth, 1995; Rämö et al., 2006; Volk et al., 2006).
86 The studies have shown that some species respond differently to ozone depending on
87 which species they are growing in competition with, for example *Poa pratensis*
88 showed reduced growth with ozone exposure when growing with *Veronica*
89 *chamaedrys*, but not when grown with other species such as *Achillea millefolium*
90 (Bender et al., 2006). Elevated ozone has also been shown to have carry-over effects
91 the following spring in species that did not respond to summer ozone exposure (Hayes
92 et al., 2006). Longer-term studies involving field release of ozone in Switzerland have
93 indicated that effects on biomass can take some time to manifest. For a species-rich
94 hay pasture grassland, no effects were found in the first year of exposure, but after 5
95 years of exposure there was a significant decrease in total yield and the percentage of
96 legumes (Volk et al., 2006) with effects being cumulative with time. In a similar
97 ongoing study for sub-alpine pasture, no effects on biomass of the component species

98 was evident after three years (Bassin et al., 2007b), but some species had a reduced
99 chlorophyll content associated with yellowing (Bassin et al., 2009).

100

101 Despite the evidence for a changing ozone profile, with the exception of Dawnay and
102 Mills (2009), conducted in our laboratory, very few studies have investigated the
103 relative effects of increasing peak versus increasing background ozone concentration.
104 Oksanen and Holopainen (2001) exposed saplings of birch to three ozone profiles
105 with the same AOT40 (the sum of the differences between the hourly mean ozone
106 concentration (in ppb) and 40 ppb for each hour when the concentration exceeds 40
107 ppb) presented at 70 ppb for 24h d⁻¹, 100 ppb for 12h d⁻¹ or 200 ppb for 4.5 h d⁻¹. It
108 was found that high peak ozone concentration was important for visible injury and
109 reductions in stomatal conductance whilst growth reductions were more related to
110 total accumulated exposure. Exposure of mixtures of *Lolium perenne* and *Trifolium*
111 *repens* also indicated that clover yield was similar with an episodic profile compared
112 to a fairly constant ozone exposure with a similar AOT40 (Nussbaum et al., 1995),
113 although in this case the total forage yield was affected more by the episodic
114 treatment. More recently, Wang et al. (2008) reported that ozone with a diurnal
115 profile but the same overall mean concentration and accumulated dose as constant
116 ozone exposure had a greater negative effect on the growth and yield of oilseed rape.
117 Heath et al., 2009, reviewed evidence of the temporal responses to ozone and
118 provided some clues as to why different processes in plants respond differently to
119 different ozone profiles. He found that the time of maximum anti-oxidant defence
120 within plants was early-mid morning, well before the mid-afternoon peak ozone
121 concentration associated with a rural profile and suggested that due to higher
122 antioxidant activity morning ozone fluxes were less biologically effective than

123 afternoon fluxes. Heath et al (2009) re-iterated the importance of nocturnal fluxes
124 (see Musselman et al., 2000 for further details), and stressed the need for further
125 research into long-term effects of ambient exposures.

126

127 Using species from a typical upland grassland (National Vegetation Classification
128 (NVC) U4 community (*Festuca ovina*-*Agrostis capillaris*-*Galium saxatile* grassland,
129 Rodwell, 1992) growing together as a simulated community, this study investigated
130 the effects of increasing background ozone (by 20 – 27 ppb) and added peaks (by 50
131 ppb), singly and in combination over two consecutive summer exposures. The aim
132 was to determine the relative importance of these simulated current and future
133 ambient ozone concentrations on the development of senescence, above ground
134 biomass and competitiveness of the component species and functional groups. The
135 model communities were maintained in shallow containers to simulate a below-
136 ground competitive environment typical of upland grasslands.

137

138 **2. Methods**

139 **Plant communities**

140 Plants of *Anthoxanthum odoratum*, *Carex echinata*, *Carex bigelowii*, *Potentilla erecta*
141 and *Galium saxatile* were propagated from stock plants that originated from
142 Snowdonia, UK (grid reference SH646606), an area dominated by *Festuca ovina*-
143 *Agrostis capillaris*-*Galium saxatile* grassland (NVC U4, Rodwell, 1992). Seeds of
144 *Festuca ovina* and *Agrostis capillaris* were sown into cell trays from seed obtained
145 from a commercial seed supplier with seed originating from the UK (Emorsgate, UK).

146

147 Straight-sided containers (27cm diameter, 11 cm deep; LBS horticulture) were lined
148 with perforated plastic to discourage root growth through the drainage holes and filled
149 with a mixture of ericaceous compost and sharp sand (in a ratio of 40 litres to 25 kg).
150 Each pot was planted with *A. capillaris* (2 plants), *A. odoratum* (2 plants), *F. ovina* (2
151 plants), *C. echinata* (1 plant), *C. bigelowii* (1 plant), *P. erecta* (1 plant) and *G. saxatile*
152 (1 plant) to create a model plant community. Each pot had the same arrangement of
153 species, with plants of the lower growing species *P. erecta* and *G. saxatile* in the
154 centre of the pot and the other species around the pot edge. The communities were
155 established in a cool greenhouse (approximately 18°C) for 8 weeks, by which time the
156 plants had grown together to form a closed canopy. During this time the systemic
157 pesticide imidachloprid (Provado) was applied as a soil drench. Immediately prior to
158 the first exposure to ozone in the solardomes the vegetation was cut back to 11cm and
159 all leaves growing outside the pot perimeter were also removed.

160

161 Communities were categorised according to the size of the component species at the
162 end of the establishment phase. One community of the eight in each size category
163 was randomly allocated to each solardome. Five communities were exposed to ozone
164 in each solardome. Each community was moved within the solardome every two
165 weeks in year 1 and every three weeks in year 2 to avoid any potential confounding
166 effect of location.

167 **Over-wintering**

168 Following the first period of ozone exposure, the communities were left to over-
169 winter in sheltered but outdoor conditions. A weak nutrient solution was applied
170 every eight weeks over the winter period (half-strength “Phosphrogen”, PBI Home
171 and Garden). After cutting back the vegetation in June, the communities were

172 transferred to the solardomes on 4th July to acclimatise before ozone treatments started
173 on 8th July 2005.

174

175 Throughout both periods of ozone exposure the model communities were watered
176 during the early morning (4 am) using an automated misting system, with additional
177 hand-watering applied as necessary during periods of hot weather.

178 **Ozone exposure**

179 Eight solardomes (large, hemispherical glasshouses, 3m diameter, 2.1m high) were
180 used for ozone exposure. Ozone was generated by a G11 ozone generator (Dryden
181 Aqua, UK) using oxygen supplied by a Workhorse 8 oxygen generator (Dryden
182 Aqua, UK) and added to charcoal filtered air to give the required ozone
183 concentrations using a computer controlled (LabView version 6.0) mass-flow
184 controller system. The ozone concentration in each solardome was recorded every 30
185 minutes using two photometric ozone analysers of matched calibration
186 (Environmental Technology Services 400A). The first ozone exposure started on 14th
187 July 2004 and the second exposure started on 8th July 2005. Each exposure period
188 was for 12 weeks.

189

190 Ozone treatment was allocated to each solardome in a randomised block arrangement,
191 with two blocks of four solardomes. An individual solardome received the same
192 treatment in both years. The treatments applied and abbreviations for these treatments
193 were:

194 LL: Low background (15 ppb), low peaks (20 ppb)

195 LH: Low background (15 ppb), high peaks (65 ppb)

196 HL: High background (35 ppb), low peaks (40 ppb)

197 HH: High background (35 ppb), high peaks (85 ppb)

198

199 'Peaks' of ozone were applied for eight hours per day, automated to start to increase

200 at 09:00, reaching a peak at 11:00, then decreasing over 2 hours starting at 18:00.

201 These peaks were applied for four consecutive days in each 7-day period. The

202 concentrations indicated for these peaks are the maximum target ozone concentration

203 during the peak. The ozone concentrations remained at the appropriate 'background'

204 levels for the treatment at all other times.

205

206 The mean ozone concentration during the establishment of the communities was 24.8

207 ppb, with an AOT40 over the establishment period of approximately 80 ppb.h. Ozone

208 concentrations of the ambient air were not measured on site throughout the

209 overwintering period, but started on 8th July in 2005 (year 2), however, the AOT40

210 prior to 8th July was negligible, based on measurements from a nearby monitoring

211 station.

212 **Simulated meadow cuts and final harvest**

213 The plant canopies were cut back to 7 cm to simulate meadow cuts at the end of the

214 ozone exposure period in Year 1, after over-wintering and early season growth (June

215 2005), and to soil level after exposure to ozone in Year 2. The above-ground

216 vegetation was separated into the component species prior to drying at 65°C. Below-

217 ground biomass was not determined. Due to a system fault in one of the replicate

218 solardomes for the 'HH' treatment on 25th August (week six), plants from this dome

219 were discarded in Year 1. The five original communities and five spare communities

220 (which had received the same ozone treatment) from the replicate dome were

221 randomly allocated between the two HH treatments for the exposure in Year 2.

222 **Visible injury and senescence assessments**

223 Communities were checked weekly for ozone injury. The percentage of ozone-
224 injured leaves and senesced leaves (a leaf was classified as senesced if >25% of the
225 leaf was senesced) per species per pot was recorded fortnightly in Year 1 and three-
226 weekly in Year 2.

227 **Statistical analysis**

228 All senescence data were arcsine transformed prior to analysis. Data from the
229 discarded plants from the HH treatment in year 1 were included in this assessment for
230 the weeks 0-6 (prior to system fault); after this in year 1, for weeks 7-12, data from
231 the additional pots in the replicate solardome were used. Senescence at harvest and
232 biomass were separately analysed using two-way analysis of variance in GenStat
233 (Version 8) using the mean value per solardome. Data was analysed to investigate
234 whether 'background', 'peak' or an interaction between 'background' and 'peak'
235 ozone concentrations influenced plant response. Differences in the rates of
236 progression of senescence between treatments were assessed for each species using a
237 Repeated Measures test in SPSS (Version 12), based on the mean per replicate
238 solardome at each assessment. Comparison of the development of senescence
239 between Year 1 and Year 2 was made using the general linear model function within
240 Minitab Version 14, using AOT0 and AOT40 as the ozone parameter.

241 **3. Results**

242 **Ozone exposure**

243 Figure 1 illustrates the mean weekly profile in the solardomes in years 1 and 2. In
244 both years, the difference in mean concentration between the two replicate solardomes

245 for each treatment was < 2 ppb. Ozone concentrations in all treatments were reduced
246 to 10-20 ppb on day 7 allowing access for plant measurements. In year 1 mean
247 background ozone concentrations were 20.3 ppb in both the LL and LH treatments
248 and 48.7 ppb and 47.6 ppb in the HL and HH treatments respectively, with mean peak
249 heights of an additional 2.9 ppb for the low peaks, and 49.8 ppb for the high peaks
250 treatments. In year 2, the mean background ozone concentrations were 17.2 ppb and
251 18.1 ppb in the LL and LH treatments and 37.1 ppb and 39.8 ppb in the HL and HH
252 treatments respectively, with mean peak heights of an additional 5.8 ppb for the low
253 peaks and 52.3 ppb for high peaks treatments. The mean ozone concentrations for the
254 background and peaks for each treatment, and the AOT0 (the sum of the differences
255 between the hourly mean ozone concentration (in ppb) and 0 ppb for each hour when
256 the concentration exceeds 0 ppb) and AOT40 in each year of the study are shown in
257 Table 1. There was good replication between the solardomes for each treatment in
258 each year (Figure 1), however, the ozone concentrations were generally lower in year
259 2 than in year 1, particularly the background ozone concentrations for the 'HL' and
260 'HH' ozone treatments.

261 **Ozone-specific visible injury**

262 No ozone-specific visible injury was observed on any of the component species in any
263 treatment during either of the twelve-week exposures of the communities to ozone.

264 **Senescence**

265 At harvest in year 1, *P. erecta*, *C. echinata* and *F. ovina* showed increased senescence
266 in response to increasing background ozone concentrations at $p < 0.05$ (Figure 2a),
267 with *A. odoratum* and *A. capillaris* showing strong trends towards the same effect
268 ($p < 0.1$). However, in year 2 the only species to show a significant increase in

269 senescence in response to increasing background ozone concentrations was *F. ovina*
270 ($p < 0.01$; Figure 2b). Significant increases in senescence corresponding with
271 increased peaks of ozone at harvest in year 1 were shown for *P. erecta* ($p < 0.01$) and
272 *F. ovina* ($p < 0.05$; Figure 2c). Strong trends for increased senescence with increased
273 peaks of ozone in year 1 were also observed for *C. echinata* and *A. odoratum* ($p < 0.1$).
274 At harvest in year 2, the only species that showed a significant effect of increased
275 peaks of ozone was *F. ovina* ($p < 0.01$; Figure 2d).

276

277 In year 1, the extent of senescence at harvest of both *P. erecta* and *A. odoratum*
278 showed a significant interaction between increased background and peaks of ozone
279 ($p < 0.05$ for both species (Table 2), with a greater increase in senescence than the sum
280 of effects of background and peaks individually. In year 2, the only species to show
281 such an interaction was *F. ovina* ($p < 0.05$), where the combination of increased
282 background and peak ozone concentrations again corresponded with a synergistic
283 increase in senescence.

284

285 The difference in the extent of senescence between the 'HL' and 'LL' treatments for
286 *F. ovina* was larger in year 1 (19.1% and 8.5% respectively) than in year 2 (8.9% and
287 7.0% respectively). This corresponded with a larger difference in mean ozone
288 concentration between these treatments in year 1 than in year 2 (Table 1).

289

290 The AOT40 (calculated over 24 h per day) for the LH treatment and the HL
291 treatments were similar in Year 1 (16.0 and 13.3 ppm.h respectively). There were no
292 significant differences in the extent of senescence between these two treatments in

293 Year 1 for any of the species (data not presented). Comparisons were not appropriate
294 in Year 2 because the difference in AOT40 between the two treatments was larger.

295

296 Repeated measures analysis, comparing the rate of development of senescence to that
297 of the 'LL' treatment for each species based on the time*treatment effect showed that
298 for *F. ovina* there was accelerated senescence during the exposure period in year 1 for
299 the HL ($p<0.05$), LH ($p<0.05$) and the HH ($p<0.05$) treatments (Figure 3). In year 2
300 for *F. ovina* there was a significant increase in the rate of development of senescence
301 in the HH treatment compared to LL only ($p<0.05$). *P. erecta* showed accelerated
302 senescence during the exposure period in year 1 for the HL ($p<0.01$), LH ($p<0.05$)
303 and the HH ($p<0.05$) treatments compared to the LL ozone treatment (Figure 3).

304 However, there were no significant differences in the rate of development of
305 senescence in year 2 for this species. For both *F. ovina* and *P. erecta* differences in
306 the extent of senescence were first apparent after exposure to the ozone regime for 8
307 weeks ($p<0.05$ and $p<0.01$ respectively). Although some effects of elevated ozone
308 had been shown in cumulative biomass for *A. odoratum*, no differences in the rate of
309 development of senescence were apparent in either year (Figure 3), although this may
310 have been higher in the HH treatment in year 1. There were no significant differences
311 in the rate of development of senescence in response to ozone in either year 1 or year
312 2 for the other species (data not presented).

313

314 Although there was reduced senescence in the HH treatment in year 2 compared to
315 year 1, the ozone concentrations and AOT40 in year 2 were also lower. Regression
316 analysis showed that there was no significant difference in the sensitivity to
317 cumulative ozone (AOT0 or AOT40) between the two years of study for any of the

318 species used. Using the data for both years, the relationship between cumulative
319 ozone (AOT0) and senescence showed linear relationships for *F. ovina* ($r^2=0.79$,
320 $p<0.001$), *A. odoratum* ($r^2=0.12$, $p=0.106$), and *P. erecta* ($r^2=0.57$, $p=0.001$), with
321 similar relationships using AOT40 (Figure 4).

322 **Species abundance based on above-ground biomass**

323 The above-ground biomass of each species at each harvest, and the total harvested
324 biomass are shown in Table 3. There were no significant differences in the total
325 above-ground biomass of the communities between the different treatments at harvest
326 in year 1, after overwintering or at harvest in year 2. However, the total cumulative
327 above-ground biomass showed a significant effect of increasing peaks of ozone
328 ($p<0.05$) and a significant interaction between increasing background and increasing
329 peaks ($p<0.5$), with a synergistic decrease in biomass. These effects on total
330 harvested biomass corresponded with effects on the biomass of *A. odoratum*, which
331 had the largest contribution to the total biomass of the communities. *A. odoratum* had
332 a total cumulative biomass which showed a significant effect of increasing peaks of
333 ozone ($p<0.05$) and a significant interaction between increasing background and
334 increasing peaks ($p<0.05$), corresponding with a further decrease in biomass
335 amounting to 15% in the HH treatment compared to LL. In addition to the decrease in
336 *A. odoratum* biomass, there was also a decrease in the grass:forb ratio with increasing
337 background ($p<0.1$ for year 1; $p<0.05$ using total cumulative biomass), but there was
338 no significant effect of increasing peak concentration on this ratio.

339

340 Some differences in above-ground biomass were apparent for individual species. *F.*
341 *ovina* showed significant reductions in biomass at the harvest after exposure to ozone
342 in year 1 due to both the influence of increased ‘background’ ($p<0.05$) and ‘peaks’

343 ($p < 0.05$) of ozone exposure. There was no significant interaction between increased
344 'background' and increased 'peaks' for this species. The differences in biomass
345 between treatments for *F. ovina* did not persist for the duration of the experiment and
346 there were no significant differences after over-wintering, at the final harvest or in
347 cumulative biomass.

348

349 After overwintering, significant alterations in biomass due to the influence of
350 increases in both 'peaks' ($p < 0.05$) and 'background' ($p < 0.05$) were shown for *P.*
351 *erecta* (Table 3). Although these alterations in biomass were no longer evident after
352 exposure to ozone in Year 2 there was an effect on the cumulative biomass for this
353 species with a significant influence of increasing background ($p < 0.05$) and a strong
354 trend for an influence of peaks ($p < 0.1$).

355

356 **4. Discussion**

357 The relationship between the extent of senescence with both AOT0 and AOT40
358 showed no difference in the sensitivity of the communities to ozone between the two
359 years detected, indicating that the plants responded to the ozone dose received in the
360 growing season with no carry-over effect or acclimation to ozone. This is in contrast
361 to some other studies, such as Tonneijck et al. (2004), Barbo et al (1998) and
362 Bungener et al (1999), which have all observed a decrease in response of perennial
363 plants over years in multi-year experiments, attributed to physiological or
364 morphological adaptations that had occurred within the three years. It has been
365 suggested that the limitations for root growth during the second and subsequent years
366 of study may decrease sensitivity to ozone by reducing plant relative growth rates, as
367 higher relative growth rates may be associated with ozone sensitivity (Bassin et al.,

368 2007a). However, it was not possible to determine whether the relative growth rates
369 of the communities changed between exposure seasons in this study, because the
370 harvested biomass at the end of the exposures was at different cutting heights in the
371 different years.

372

373 The accelerated rates of senescence with increasing ozone treatment usually did not
374 correspond to reductions in biomass during the exposure period within an individual
375 growing season. The exception was *F. ovina*, which showed reductions in biomass in
376 year 1 only. Senescence was also more frequently detected than changes in biomass
377 in species from wetlands (Franzaring et al., 2000) and from upland grasslands (Hayes
378 et al., 2006). Similarly, visible injury in the absence of biomass changes has
379 frequently been demonstrated (e.g. Pleijel and Danielsson, 1997). However, although
380 not always considered to be an ecological impact on plants, premature and enhanced
381 senescence could be detrimental because this would mean reduced assimilation of
382 resources in a growing season, resulting in a more gradual reduction in the overall
383 ability of a plant species to survive and withstand other stresses.

384

385 Small increases in background ozone concentration in the absence of any peaks of
386 ozone, from pre-industrial to current levels, were sufficient to induce a significant
387 increase in senescence of *F. ovina*. The effect was larger at the higher ozone
388 concentrations used in year 1 (backgrounds of 20.3 and 48.7 ppb for LL and HL
389 respectively) compared to those of year 2 (background concentration of 17.2 ppb and
390 37.1 ppb for LL and HL respectively). Other species such as *C. echinata* and *P. erecta*
391 were less sensitive to the small increase in background during year 2, but did show
392 enhanced senescence in the larger concentration range used in year 1. There was also

393 a linear response of increasing senescence with increasing ozone exposure (AOT0 and
394 AOT40), indicating that there was no threshold for response in the species studied.
395 Increasing background ozone in the absence of peaks has also been demonstrated to
396 accelerate senescence in *A. odoratum* (Dawnay and Mills, 2009). The current study
397 and that of Dawnay and Mills (2009) both show effects that are at relatively low
398 ozone concentrations that are within the range already experienced within upland
399 areas of Europe.

400

401 Effects of ozone on the competitive balance of this community were small despite the
402 reduction in total biomass. There was a decrease in cumulative above-ground
403 biomass with increased background ozone concentration for *A. odoratum*, with
404 decreased biomass of similar magnitude in the highest ozone treatments observed
405 throughout the study for this species which only reached significance by the end of
406 the second exposure. This decrease in *A. odoratum* biomass contributed to a
407 reduction in grass:forb ratio. Previous studies have demonstrated an increase in the
408 grass:forb ratio with ozone exposure (e.g. Wilbourn et al., 1995) however, this is
409 dependant on the relative sensitivity to ozone of the species involved. *A. odoratum*
410 has been shown to be more sensitive to ozone than some other grasses such as *Lolium*
411 *perenne* (Hayes et al., 2007b), which was frequently used in the earlier studies.

412

413 The responses of the species to ozone when grown as a community were not as large
414 as expected based on their responses when they were grown and exposed to ozone as
415 individual plants in a previous study, where widespread visible injury was observed
416 on *C. echinata* and *P. erecta* when the plants were grown individually (Hayes et al.,
417 2006). Reductions in ozone concentration of up to 30% have been demonstrated

418 within a grassland canopy (e.g. Jäggi et al., 2006). Together with increased boundary
419 layer thickness due to reduced windspeed within a canopy this could combine to give
420 lower uptake of ozone into the plants when grown as part of a community rather than
421 as individuals, particularly for species such as *P. erecta*, which were protected by
422 other taller vegetation.

423

424 The contrasting ozone profiles with similar AOT40 during Year 1 of this study
425 indicate that an increase in background ozone concentration, such as that predicted
426 from increased hemispheric ozone concentrations, is as damaging to plants as an
427 episodic profile from regional ozone pollution. Similar increases in senescence
428 compared to the lowest ozone treatment were found for both *F. ovina* and *P. erecta*.
429 This is in agreement with a previous study using mixtures of *L. perenne* and *Trifolium*
430 *repens*, which indicated that clover yield was similar with an episodic profile
431 compared to a fairly constant ozone exposure with a similar AOT40 (Nussbaum et al.,
432 1995), although in this case the total forage yield was more affected by the episodic
433 treatment.

434

435 The largest effects on both senescence and biomass were found with a combination of
436 increased peaks and increased background ozone concentration simulating that
437 predicted for future decades. In some cases a synergistic effect of increased
438 background with increased peaks of ozone was apparent, including for total biomass.
439 Previously critical levels for ozone have been based on data obtained using episodic
440 ozone treatments (LRTAP Convention, 2004). AOT40 (the sum of the differences
441 between the hourly mean ozone concentration (in ppb) and 40 ppb for each hour when
442 the concentration exceeds 40 ppb, accumulated during daylight hours) has been used

443 to establish critical levels for ozone (LRTAP Convention, 2004, 2006). However,
444 small changes in background ozone concentration around the 40 ppb threshold would
445 have a large influence on the cumulative AOT40, therefore this index may not be
446 appropriate for regions where the background is already at or close to 40 ppb and is
447 likely to increase in the future. The predicted decrease in peak height during ozone
448 episodes over recent years (NEG-TAP, 2001) coupled with the predicted rise in
449 background ambient ozone levels over the next 50 years suggests that data from
450 experiments such as that described here would need to be taken into account in future
451 revisions of the critical level.

452

453 **5. Conclusions**

454 The ozone treatments used in this study simulated changes in background ozone that
455 may occur in future decades, with or without modest peaks of ozone that were well
456 within the current normal ambient range. The effects of these treatments on the seven
457 species within the simulated communities were mixed and were species-dependant,
458 making generalisations difficult. Significant effects were observed on both the
459 grasses and the forbs and occurred in response to increased background and/or peaks
460 of ozone. Increased senescence of *F. ovina* and *P. erecta* was observed when the
461 background ozone concentrations were increased in the absence of any additional
462 peaks of ozone. These effects were also seen when the background ozone
463 concentrations were raised in addition to peaks of ozone. Overall, the species used
464 showed no acclimation to ozone and the same relationship between senescence and
465 ozone dose occurred in the second year as the first year of this study.

466

467 In this community, *A. odoratum* was most affected in terms of above-ground biomass,
468 but other species such as *F. ovina* showed transient significant differences between
469 ozone treatments. It is likely that effects on biomass were occurring more slowly due
470 to a gradual reduction in overall plant vitality. This study highlights the need for
471 long-term experiments to study the effects of ozone on plant communities, because
472 some of the significant effects were not observed until after exposure to ozone in the
473 second year.

474

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479

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644 **Figure Legends**

645 Figure 1: Season average weekly profile of ozone data in a) 2004 and b) 2005.

646

647 Figure 2: Senescence of the component species of the community at harvest in
648 response to A) background ozone in year 1 B) background ozone in year 2 C) peaks
649 of ozone in year 1 D) peaks of ozone in year 2. Bars are standard errors. **, * and
650 (*) indicate differences at $p < 0.01$, $p < 0.05$ and $p < 0.1$.

651

652 Figure 3: Development of senescence for species in the community in the different
653 ozone treatments. A) *A. odoratum*, year 1. B) *A. odoratum*, year 2. C) *F. ovina*, year
654 1. D) *F. ovina*, year 2. E) *P. erecta*, year 1. F) *P. erecta*, year 2
655 Bars are standard errors.

656

657 Figure 4: Senescence at harvest in year 1 and year 2 in relation to AOT0 and AOT40
658 for A and B) *A.odoratum* C and D) *F.ovina* and E and F) *P. erecta*.

659

660