

## Article (refereed)

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**Williamson, Jennifer; Mills, Gina;** Freeman, Chris. 2010  
Species-specific effects of elevated ozone on wetland plants and  
decomposition processes. *Environmental Pollution*, 158 (5). 1197-  
1206. [10.1016/j.envpol.2010.01.019](https://doi.org/10.1016/j.envpol.2010.01.019)

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1 Species-specific effects of elevated ozone on wetland plants and decomposition  
2 processes

3

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10

11 Abstract

12 Seven species from two contrasting wetlands, an upland bog and a lowland fen in  
13 North Wales, UK, were exposed to elevated ozone (150 ppb for 5 days and 20 ppb for  
14 2 days per week) and low ozone (20 ppb) for four weeks in solardomes. The fen  
15 species were: *Molinia caerulea*, *Juncus subnodulosus*, *Potentilla erecta* and  
16 *Hydrocotyle vulgaris* and the bog species were: *Carex echinata*, *Potentilla erecta* and  
17 *Festuca rubra*. Senescence significantly increased under elevated ozone in all seven  
18 species but only *Molinia caerulea* showed a reduction in biomass under elevated  
19 ozone. Decomposition rates of plants exposed to elevated ozone, as measured by  
20 carbon dioxide efflux from dried plant material inoculated with peat slurry, increased  
21 for *Potentilla erecta* with higher hydrolytic enzyme activities. In contrast, a decrease  
22 in enzyme activities and a non-significant decrease in carbon dioxide efflux occurred  
23 in the grasses, sedge and rush species.

24

25

26 Key words

27 Tropospheric ozone, wetlands, senescence, chlorophyll content, enzyme activity,

28

29 Capsule:

30 Short-term, episodic ozone exposure increased senescence and changed short-term

31 decomposition processes in wetland plant species.

32

33

34 Introduction

35 Tropospheric ozone concentrations have been increasing for the past century from

36 pre-industrial levels of approximately 10ppb to current background concentrations of

37 35-50ppb (Laurila et al., 2004) and are predicted to continue to rise by between 0.5

38 and 2% per year in the northern hemisphere (Vingarzan, 2004). The effects of

39 ambient ozone on plants were first observed in the 1950s as an increase in the

40 incidence of plant injury in areas affected by photochemical smog (Laurence and

41 Andersen, 2003) and it is now known that the principle phytotoxic component of such

42 smog is ozone. Tropospheric ozone is a major secondary air pollutant formed during

43 a series of reactions between oxides of nitrogen and volatile organic compounds in the

44 presence of sunlight (NEG-TAP, 2001). Elevated concentrations are mainly associated

45 with periods of hot, sunny weather, which are predicted to increase as global warming

46 continues (Ashmore, 2005). Although regulations to control the emissions of ozone

47 precursor chemicals are in place in most European countries, the background

48 concentration of tropospheric ozone is continuing to increase, in part due to

49 transboundary transport of precursor compounds through the troposphere (Fiscus et

50 al., 2005). The current tropospheric ozone concentration of 35-50ppb found in

51 Northern Europe is considered to be high enough to be a significant threat to semi-  
52 natural vegetation and hence biodiversity (Ramo et al., 2006a). Ozone toxicity in  
53 plants causes visible injury to leaves, often coupled with reductions in photosynthesis  
54 and biomass accumulation (Ramo et al., 2006a). Current background levels of ozone  
55 in Europe have already been found to cause visible injury in over 80 species of crops  
56 and natural vegetation with yield/biomass reductions reported in some areas (Fuhrer et  
57 al., 1997; Hayes et al., 2007). As an ecosystem, peat-forming wetlands are of  
58 particular concern because of their ability to store large quantities of carbon with  
59 vegetated upland peat showing carbon sequestration values between 67 and 183 gCm<sup>-2</sup>  
60 yr<sup>-1</sup> depending upon the dominant vegetation type present (Bortoluzzi et al., 2006).  
61 The majority of these peat-forming wetlands in northern Europe are in upland areas  
62 where ozone concentrations are comparatively high compared to adjacent low-lying  
63 areas (Royal Society, 2008), and thus any changes that affect plant growth and  
64 physiology have the potential to affect carbon storage within peatlands.

65

66 Recent research has focused on a variety of semi-natural ecosystems but there is  
67 relatively little published information on the effects of ozone exposure on wetland  
68 plants. These species are likely to be relatively sensitive to ozone exposure as ozone  
69 sensitivity is associated with high levels of stomatal conductance, relatively high  
70 growth rates and specific leaf area; all characteristics shared by many wetland species  
71 (Power and Ashmore, 2002). Stomatal uptake of ozone is likely to be relatively high  
72 in wetland plants as they are not normally water-limited and as such may not close  
73 their stomata during the day (Busch, 2000; Koch and Rawlik, 1993; Li et al., 2004;  
74 Mann and Wetzel, 1999; Smith and Houppis, 2004) thereby taking up more ozone  
75 (Power and Ashmore, 2002). Studies on wetland plants have shown that elevated

76 ozone can cause specific visible ozone injury (Power and Ashmore, 2002), an increase  
77 in senescence and premature senescence (Franzaring et al., 2000) and decreases in  
78 above ground biomass (Power and Ashmore, 2002), below ground biomass and  
79 root:shoot ratio (Franzaring et al., 2000).

80

81 Research has shown that the carbon flux to soil is affected when plants are exposed to  
82 elevated ozone; both by altered rhizodeposition and changes in leaf litter quality and  
83 quantity (Andersen, 2003). Plants exposed to elevated ozone have been shown to  
84 contain a greater proportion of foliar nitrogen (Andersen et al., 2001; Berg and Staaf,  
85 1980) and are likely to decompose more rapidly, potentially releasing more carbon  
86 compounds to the microbial community. This increased level of foliar nitrogen has  
87 been found to be particularly present when exposure to elevated ozone has caused  
88 leaves to senesce prematurely. However, plants exposed to elevated ozone have also  
89 been shown to contain higher concentrations of protective compounds such as  
90 phenolics (Liu et al., 2005; Paakkonen et al., 1998; Saleem et al., 2001) which would  
91 reduce the rate of decomposition of leaf litter (Kim et al., 1998) as phenolic  
92 compounds inhibit the activity of hydrolase enzymes (Freeman et al., 2001). Ozone  
93 exposure has also been found to reduce the below ground biomass of some species  
94 (Grantz and Farrar, 2000; Grantz and Yang, 2000) which could lead to a reduction in  
95 the amount of labile carbon available to the bacterial community. This is further  
96 supported by results from Larson et al. (2002) who found that activity of extracellular  
97 hydrolase enzymes was reduced in soils that had been exposed to elevated ozone.  
98 However, McCrady and Andersen (2000) found that ozone exposure increased root  
99 exudation in spring wheat seedlings, which would lead to an increase in substrates  
100 available to soil microbes.

101

102 Below ground microbial biomass has been found to be significantly reduced by  
103 elevated ozone in crop systems (Islam et al., 2000) and microbial respiration is also  
104 reduced by elevated ozone (Phillips et al., 2002). This is unlikely to be a direct effect  
105 of ozone, even though it is toxic to bacteria, because ozone reacts with vegetation and  
106 the soil surface meaning very little will diffuse into the soil and reach the bacterial  
107 community (Turner et al., 1974). Furthermore, recent isotopic studies using  $^{18}\text{O}$  have  
108 found that, following an 11 hour exposure to 100ppb ozone, there was no ozone  
109 derived  $^{18}\text{O}$  in root tissue of white clover (*Trifolium repens*) (Toet et al., 2009).  
110 However, the diversity of bacterial communities found when plants had been exposed  
111 to elevated ozone was not significantly reduced (Dohrmann and Tebbe, 2005) and, in  
112 peatlands, exposure to elevated ozone was found to increase total microbial biomass  
113 by 24% (Morsky et al., 2008).

114

115 This study sets out to investigate the effects of short term (four weeks) ozone  
116 exposure on seven wetland vascular plant species commonly found in fen and bog  
117 systems in Central and Northern Europe. Percentage senescence and chlorophyll  
118 content were assessed throughout the experimental period, above and below ground  
119 biomass were measured at the end of exposure and the short-term decomposability of  
120 the above ground plant material exposed to elevated ozone was determined post-  
121 ozone exposure. The tested hypotheses are that: exposure to elevated ozone will  
122 increase senescence and decrease chlorophyll content; exposure to elevated ozone will  
123 cause a decrease in both above and below ground biomass with a relatively larger  
124 decrease being seen in the below ground biomass and exposure to ozone will reduce  
125 the rates of decomposition of leaves within wetland soil.

126

127 Methods

128

129 Plant selection and propagation

130 Plants were collected from two wetland sites in North Wales, UK: Cors Erddreiniog, a

131 low-lying fen site on Anglesey (SH 465 822) just above sea level and Marchlyn

132 Mawr, an upland bog site in Snowdonia (SH 611 624) at 550m altitude. Cors

133 Erddreiniog is an alkaline fen and is part of the Anglesey Fens special area of

134 conservation (SAC). The national vegetation classification (NVC) communities

135 found at this site are M22 (*Juncus subnodulosus* – *Cirsium dissectum* fen meadow)

136 and M25 (*Molinia caerulea* - *Potentilla erecta* mire) ([www.jncc.gov.uk](http://www.jncc.gov.uk)). Marchlyn

137 Mawr is on the border of the Snowdonia National Park and contains typical upland

138 bog vascular plant species although *Sphagnum* mosses dominate the area. The site

139 has not had its NVC classification published, the flora dominant at the site place it as

140 being M6 (*Carex echinata* - *Sphagnum recurvum/auriculatum* mire) ([www.eryri-](http://www.eryri-)

141 [npa.gov.uk](http://npa.gov.uk)). Four species from Cors Erddreiniog and three from Marchlyn Mawr that

142 were representative of the dominant vegetation at each site were used in this

143 experiment. The fen species were *Molinia caerulea*, *Juncus subnodulosus*,

144 *Hydrocotyle vulgaris* and *Potentilla erecta*. The species from the bog site were *Carex*

145 *echinata*, *Festuca rubra* and *Potentilla erecta*.

146

147 Individual plants of each species were collected from the field and potted up using

148 peat compost (HUMAX 100% peat) in a greenhouse with controlled lighting and

149 heating (day 18°C, night 16°C) until they were large enough for propagation. One

150 month before plants were placed in the solardomes, 24 individual plants of each

151 species were planted into one-litre pots (10x10x10cm). The plants were matured for  
152 three weeks in the greenhouse and were moved to a sheltered outdoor location a week  
153 prior to being placed in the solardomes. Plants of each species were then allocated  
154 into three size classes with eight individuals in each size class. Within each group of  
155 eight individuals, one plant was randomly allocated to each solardome so there was  
156 one “small”, one “medium” and one “large” plant per replicate solardome.

157

#### 158 Experimental Design

159 Plants were exposed to elevated ozone at the CEH solardome facility at  
160 Abergwyngregyn from 22nd August 2006 to 19th September 2006. This facility  
161 consists of eight hemispherical, glass domes 2.2 metres high and 3 metres in diameter,  
162 situated on an East-West line to minimise differences in shading (Rafarel et al., 1995)  
163 and receiving two complete air changes per minute. The experiment was designed to  
164 see how plants reacted to a relatively short term, high dose ozone exposure, with peak  
165 values matching those found in some parts of the UK during the summer of 2006  
166 ([www.welshairquality.co.uk](http://www.welshairquality.co.uk)). Four solardomes were set to receive a constant ozone  
167 concentration of 20ppb throughout the experiment (control) and the other four were  
168 set to an episodic regime with ozone concentrations increasing from 20ppb to 150ppb  
169 over one day, remaining at 150ppb for three days and returning to 20ppb on the fifth  
170 day and remaining at 20ppb for two days (elevated ozone). This profile was repeated  
171 over the four weeks of the experiment. The solardomes were arranged as a split block  
172 design with two blocks of four domes. Within each block, two domes with high  
173 ozone concentrations and two with low ozone concentrations were randomly assigned.  
174 Ozone was generated by passing oxygen (from a Workhorse 8 oxygen generator,  
175 Ozone Industries Ltd.) through a G11 ozone generator (Ozone Industries Ltd.) where



176 electricity was used to dissociate oxygen molecules that recombined to form ozone.  
177 A computer-controlled (Lab-VIEW version 7) mass-flow controller system delivered  
178 the correct amount of ozone to the solardomes. The ozone concentration within the  
179 domes was measured on a 30 minute cycle by two API400 ozone analysers  
180 (Envirotech) with matched calibrations. Ozone concentrations in one dome were  
181 continually sampled to provide a feedback system using a Model 49C ozone analyser  
182 (Thermo Electron) and the ozone supply to all domes was adjusted accordingly.

183

#### 184 Plant measurements

185 Whole plant necrotic senescence was measured when the plants were first placed in  
186 the solardomes and weekly throughout the experiment. Senescence was recorded as  
187 the percentage of senesced leaves on a plant. A leaf was counted as senesced if more  
188 than 25% of the leaf showed necrotic senescence. Senescence was chosen as a  
189 measure of ozone stress as it is a general response to photo-oxidant stress and the  
190 symptoms are not species-specific. Relative senescence was calculated as the  
191 difference between the mean senescence under elevated ozone and the mean  
192 senescence from the control.

193

194 An estimate of leaf chlorophyll content of non-senesced leaves was taken weekly  
195 using a Minolta SPAD meter. Measurements were taken on the second youngest,  
196 fully expanded leaf and only leaves with no visible senescence or ozone damage were  
197 used. *Festuca rubra* and *Juncus subnodulosus* were not included in this analysis as  
198 their leaves were too narrow and did not fill the sample window.

199

200 Above-ground material was removed from the pot and weighed immediately after  
201 harvest to determine fresh weight before being dried to constant weight at 65°C. Root  
202 weight was determined by washing the root mass through a sieve, removing attached  
203 soil and substrate particles and drying to constant weight at 65°C. From this data, the  
204 above ground to below ground biomass ratio was calculated. After measurements of  
205 the dry biomass were made, above ground plant material was mixed to provide  
206 composite dome samples for each species and ground using a ball mill.

207

208 Decomposition assay and sampling

209 A microbial inoculum slurry was prepared using 2kg of fresh fen peat from Cors  
210 Erddreiniog (SH 465 822) and 6 litres of deionised water and filtered to remove large  
211 particulate matter. Approximately 1g of dried, ground plant material of each species  
212 (except *H. vulgaris*) was accurately weighed and put in individual 125ml glass bottles  
213 with 80ml of the pre-prepared slurry. Blank samples consisted of 80ml of slurry  
214 without the addition of any plant material. Immediately after sample inoculation,  
215 bottles were sealed and gases were allowed to accumulate for 1 hour. During the  
216 accumulation of gases, bottles were kept in the dark and constantly shaken at 50rpm  
217 to encourage mixing. Background samples of laboratory air were taken at the start of  
218 the gas accumulation and samples of the gases within the bottles were taken after 1  
219 hour. Gas samples were taken using the same method after 3, 5, 7 and 10 days of  
220 incubation. Carbon dioxide was measured using a Perkin Elmer Gas Chromatograph  
221 (GC) fitted with a flame ionisation detector (FID) to detect methane and a methaniser  
222 to convert carbon dioxide to methane. The GC was calibrated using bottled gas with a  
223 known concentration of carbon dioxide (BOC gases) and this gas was used for quality  
224 control (QC) at set points throughout each sample run.

225

226 Twenty ml water samples were taken and filtered through a 0.45µm filter after the 10-  
227 day incubation period. These were analysed for total dissolved carbon (TC),  
228 phenolics and dissolved nitrogen. TC was measured using a Thermalox™ elemental  
229 analyser. Samples were injected over a platinum-coated, mesh catalyst. Oxygen was  
230 used as the carrier gas and thermal catalytic oxidation was used to oxidise carbon  
231 compounds in the sample to carbon dioxide. Carbon dioxide was detected and  
232 measured using a non-dispersive infrared detector. Standards consisted of Potassium  
233 Hydrogen Phthalate dissolved in distilled, de-ionised water and known concentrations  
234 were used to create the calibration curve and for QC standards. The concentration of  
235 total soluble phenolics was measured using Folin-Ciocalteu reagent following the  
236 methods of Box (1983). This measures polyphenolic compounds including phenolics,  
237 tannins and lignin. Dissolved organic nitrogen was measured using the Thermalox™  
238 machine used for TC measurements and ammonium ions were measured using a  
239 SKALAR. After 10 days of decomposition, unfiltered water samples were taken and  
240 analysed for phenol oxidase, beta glucosidase and N-acetylglucosaminidase activities.  
241 Phenol oxidase assays followed the procedure of Pind et al. (1994) except the liquid  
242 from the assay was used rather than creating a slurry from peat samples. Beta  
243 glucosidase and N-acetylglucosaminidase were assayed fluorimetrically following the  
244 method of Freeman et al. (1995).

245

#### 246 Statistical Analysis

247 Values from the three plants per species per dome were averaged to provide four  
248 replicates per ozone treatment at each time point prior to analysis. The effects of  
249 ozone were assessed using general analysis of variance (GENSTAT version 7). Data

250 measured as a percentage was arc-sine transformed in Minitab ver14 prior to analysis  
251 and back-transformed for presentation. Ozone dose-response for each species was  
252 analysed in Sigma-Plot by linear regression of relative senescence (as difference from  
253 the control) against AOT<sub>024hr</sub> using treatment means from each week of ozone  
254 exposure. The significance of the regression and the percentage variation in  
255 senescence explained by ozone were analysed using GENSTAT version 8. Carbon  
256 dioxide emissions were calculated to give cumulative results over the 10 days of  
257 decomposition. General analysis of variance was used to calculate the significance of  
258 any differences at each time point and repeated measures ANOVA was used to  
259 analyse the change in gas exchange over time. Analysis of variance was used to  
260 calculate any differences in enzyme activity and water chemistry after the 10 days of  
261 incubation.

262

## 263 Results

264

### 265 Ozone Exposure in the Solardomes

266 Average ozone concentrations measured in the solardomes over the four week  
267 experiment are shown in Table 1. Mean peak ozone concentrations were within 10%  
268 of the target value of 150ppb and background concentrations were 20ppb for the  
269 elevated ozone treatment and 13-14ppb for the control treatment. AOT<sub>024hr</sub> values  
270 after 28 days showed a mean value of 12 ppmh in the control treatment and a mean of  
271 76ppmh in the elevated ozone treatment while AOT<sub>40</sub> (daylight hours) ranged from  
272 0ppmh in the control treatment to a mean of 27ppmh in the elevated ozone treatment  
273 (Table 1). Temperatures in the solardomes followed ambient temperatures but were

274 generally 1-2°C higher, with a mean daytime temperature of 20°C and a range of 15-  
275 28°C and a mean overnight temperature of 14.9°C and a range of 10-20°C.

276

277 Senescence

278 All of the species included in this experiment showed an increase in senescence  
279 during the four weeks of ozone exposure compared to those under control conditions  
280 (Figures 1 and 2). *M. caerulea* showed a significant increase in senescence under  
281 elevated ozone in weeks two, three, and four with time also being a significant factor,  
282 suggesting the difference in mean senescence values became more pronounced over  
283 time ( $P < 0.001$ ). *J. subnodulosus* showed the same pattern with a significant increase  
284 in senescence under elevated ozone from week two of the experiment ( $P < 0.05$ ) and  
285 time through the experiment also being a highly significant factor ( $P < 0.001$ ). *P.*  
286 *erecta* plants from the fen exposed to high ozone showed a significant increase in  
287 senescence over the four week experimental period ( $P < 0.05$ ) although the difference  
288 in senescence measured weekly was only significant in week two. In weeks three and  
289 four the data showed a trend towards significance ( $P < 0.1$ ) but variation within  
290 treatments was too high for a statistically significant difference to be measured. *H.*  
291 *vulgaris* showed a trend towards a significant increase in senescence under elevated  
292 ozone by the fourth week of the experiment ( $P < 0.1$ ) but it did not senesce in the first  
293 two weeks suggesting that it was slower to respond to ozone than other species.

294

295 Senescence on *Potentilla erecta* plants from the bog showed a trend towards a  
296 significant increase with elevated ozone over the four week experimental period  
297 ( $P < 0.1$ ) and a highly significant effect of time, meaning that senescence increased in  
298 plants from both the treatment and the control. However, percentage senescence

299 values only differed significantly in week two ( $P < 0.05$ ) suggesting a transient increase  
300 in senescence. *C. echinata* plants showed a significant increase in senescence under  
301 elevated ozone by week four of the experiment ( $P < 0.05$ ) and time was again a highly  
302 significant factor in the senescence measurements ( $P < 0.001$ ). *F. rubra* showed the  
303 same pattern as *M. caerulea* and *J. subnodulosus* with plants exposed to elevated  
304 ozone showing significantly more senescence by week two ( $P < 0.05$ ) and the  
305 difference becoming progressively more significant over time ( $P < 0.001$  for  
306 ozone\*time interaction).

307

308 Six of the seven species exposed showed a significant ( $P < 0.05$ ) increasing linear  
309 relationship with AOT0<sub>24hr</sub> (Table 2). The only species not to show a significant  
310 relationship was *P. erecta* from the fen. When considering the percentage variance in  
311 the relative senescence that could be explained by ozone dose for the six species that  
312 did show a significant difference, at least 70% of the variation could be explained by  
313 the increasing ozone dose (Table 2).

314

315 Chlorophyll content of non-senescing leaves

316 Species tested for their chlorophyll content over the course of the experiment differed  
317 in their response to ozone exposure (Figure 2). *M. caerulea* showed no significant  
318 difference between individuals exposed to high or low ozone but both sets of plants  
319 showed a significant decrease in chlorophyll content over the four week exposure  
320 period ( $P < 0.001$ ). *P. erecta* plants from both the fen and the bog showed a significant  
321 reduction in chlorophyll content when they had been exposed to elevated ozone by  
322 week four of the experiment ( $P < 0.05$ ). *C. echinata* plants showed a transient increase  
323 in week three in chlorophyll content in plants exposed to elevated ozone but this did

324 not continue to week four. *H. vulgaris* plants showed significantly reduced  
325 chlorophyll contents in plants exposed to elevated ozone in weeks two, three and four.

326

327 Plant biomass

328 In contrast to the increase in senescence, only *M. caerulea* showed a significant  
329 decrease in above-ground fresh and dry weight at the end of the exposure period  
330 ( $P < 0.05$ ) (Table 3). Of the other species tested, the above ground biomass of *P.*  
331 *erecta* from the bog exposed to high ozone was slightly lower and *C. echinata*  
332 biomass exposed to high ozone was slightly higher when compared to their respective  
333 controls ( $P < 0.1$ ).

334

335

336 Plant decomposition

337 Cumulative carbon dioxide emissions from the decomposition of the five species used  
338 in the assay are shown in Figure 3. *Potentilla erecta* plants from the fen that had been  
339 exposed to elevated ozone caused a significant increase in carbon dioxide emissions  
340 from peat after five days of aerobic decomposition ( $P < 0.05$ ) and emissions continued  
341 to be higher for the remainder of the assay ( $P < 0.1$ ). Carbon dioxide emissions from  
342 *P. erecta* plants from the bog showed a similar trend although the differences were not  
343 large enough to be significant. Carbon dioxide emissions from the other four species  
344 did not differ according to past ozone exposure.

345

346 Total carbon and phenolic compounds after 10 days of decomposition were very  
347 similar from plants that had and hadn't been exposed to elevated ozone (Table 4) with  
348 only total carbon from *Festuca rubra* showing a trend towards a reduction under

349 elevated ozone ( $P < 0.1$ ). This reduction in total carbon led to the proportion of carbon  
350 as phenolic compounds being increased under elevated ozone for *F. rubra* ( $P < 0.1$ ).  
351 The concentration of ammonium ions after 10 days of aerobic decomposition did not  
352 change for any of the five species and the only difference in the concentrations of total  
353 nitrogen compounds was a trend towards a reduction under elevated ozone seen in the  
354 *Carex echinata* decomposition assay ( $P < 0.1$ ).

355

356 Of the three enzymes whose activity was measured after ten days of decomposition,  
357 beta glucosidase and N-acetylglucosaminidase showed significant differences with  
358 ozone treatment (Table 5). Phenol oxidase activity did not show any variation under  
359 elevated ozone, but within treatment variation was high and enzyme activity was very  
360 low (data not presented). Beta glucosidase activity showed a significant reduction  
361 under elevated ozone in *Molinia caerulea* and *Juncus subnodulosus* ( $P < 0.05$  and  
362  $P < 0.01$  respectively) and a non-significant reduction in *C. echinata* and *F. rubra*.

363 However, beta glucosidase activities increased under elevated ozone for the  
364 decomposition assays using *P. erecta* from the fen and the bog ( $P < 0.1$  and  $P < 0.05$   
365 respectively). A similar pattern was seen with N-acetylglucosaminidase; activities  
366 increased in the assays for plants exposed to elevated ozone for *P. erecta* from the fen  
367 and the bog ( $P < 0.1$  and  $P < 0.01$ ) but decreased significantly for plants exposed to  
368 elevated ozone for *J. subnodulosus* ( $P < 0.001$ ), *C. echinata* ( $P < 0.05$ ), and *F. rubra*  
369 ( $P < 0.05$ ). Enzyme activities in the slurry containing *M. caerulea* exposed to elevated  
370 ozone showed a non-significant decrease (Table 5).

371

372 Discussion



373 Plant senescence is defined as “the deteriorative processes that are the natural causes  
374 of death” (Leopold, 1980) and is characterised by a decrease in leaf chlorophyll  
375 content and photosynthetic activity (Wingler et al., 2006). Accelerated foliar  
376 senescence is a common response for many plant species treated with elevated ozone  
377 (e.g. (Bergmann et al., 1999; Gielen et al., 2007; Mikkelsen and HeideJorgensen,  
378 1996; Paakkonen et al., 1996; Pell et al., 1997) and is often coupled with biochemical  
379 changes within the plant such as increases in ethylene emission, a cause of senescence  
380 (Schraudner et al., 1997). The link between ozone exposure and premature  
381 senescence has been found to be more marked in Northern latitudes because summer  
382 nights are shorter meaning there is less time for plants to recover from ozone injury  
383 through the repair processes that are driven by dark respiration (De Temmerman et al.,  
384 2002). Northern latitudes are also characterised by cooler and more humid  
385 conditions, both of which tend to lead to higher levels of stomatal conductance and  
386 hence higher ozone uptake (Yamaji et al., 2003), meaning that wetland plants in  
387 Northern latitudes are likely to be particularly affected by elevated ozone as they are  
388 characterised by high levels of stomatal conductance and leaf area (Power and  
389 Ashmore, 2002). This is shown in this experiment as all seven species showed an  
390 increase in senescence over the 28 days of exposure. For *H. vulgaris* and *P. erecta*  
391 plants from the bog, weekly differences in senescence were not significant over the  
392 experimental period but the correlation with AOT0<sub>24hr</sub> was significant, showing that  
393 as ozone dose increased the amount of senescence also increased. This is in  
394 agreement with other experiments on the effects of ozone exposure on wetland plants  
395 with five out of ten wet meadow species tested showing increased senescence  
396 (Franzaring et al., 2000) and five wetland species also showing increased injury under  
397 elevated ozone (Power and Ashmore, 2002). The effects of elevated ozone on plant

398 senescence had been previously assessed for three of the species used in this  
399 experiment: *P. erecta*, *F. rubra* and *C. echinata* (Hayes et al., 2006). In that study, *F.*  
400 *rubra* and *C. echinata* had significant increases in senescence after ten weeks of  
401 ozone exposure but *P. erecta* did not show as high an increase in senescence as found  
402 in this experiment (Hayes et al., 2006). This is possibly because Hayes et al. (2006)  
403 used an episodic regime with a maximum concentration of 100ppb ozone over four  
404 days per week whereas in this experiment the ozone concentration was around 140ppb  
405 in the treatment domes for five days out of seven. As a further comparison, the  
406 elevated ozone treatment of Hayes et al (2006) had an AOT 40 (daylight hours) of  
407 18.3 ppmh accumulated over ten weeks, whereas in this experiment the AOT 40  
408 (daylight hours) was 24.8 ppmh accumulated over only four weeks.

409

410 The increase in senescence caused by elevated ozone has also been shown to be  
411 accompanied by an increase in the nitrogen content of abscised leaves (Findlay and  
412 Jones, 1990) which could have an effect on the subsequent decomposition of plant  
413 biomass as more fertilisation has been found to speed up the decomposition of plant  
414 litter (Allison and Vitousek, 2004). In the current study, carbon dioxide efflux was  
415 increased from plant material exposed to elevated ozone during the *P. erecta*  
416 decomposition with a corresponding increase in hydrolase activity suggesting that  
417 initial rates of decomposition had increased. However, there was no change in the  
418 nitrogen content of the slurry. Extra-cellular enzyme activity was found to decrease  
419 in the slurries containing *M. caerulea*, *J. subnodulosus*, *Carex echinata* and *F. rubra*  
420 that had been exposed to elevated ozone suggesting a reduction in the decomposition  
421 of the plant material. This is in agreement with previous work carried out on  
422 blackberry and broomsedge (Kim et al., 1998) that found exposure to elevated ozone

423 reduced litter decomposition. Exposure to elevated ozone also caused a reduction in  
424 decomposition of soybean residues (Booker et al., 2005). However, although  
425 hydrolytic enzyme activities were changed by exposure of the plants to elevated  
426 ozone, phenol oxidase activity was unaffected, there were no significant changes in  
427 phenolic concentrations after ten days of decomposition and only *F. rubra* showed a  
428 decrease in TC concentrations after elevated ozone. This suggests that, in contrast to  
429 previous results (Booker et al., 2005; Iglesias et al., 2006; Saleem et al., 2001), these  
430 plants did not upregulate their production of anti-oxidant compounds such as  
431 ascorbate and phenolics.

432

433 In this experiment, the chlorophyll content of healthy leaves of *P. erecta* from the fen  
434 and the bog and *H.vulgaris* was reduced under elevated ozone. In contrast, exposure  
435 to elevated ozone increased the chlorophyll content of healthy leaves in *C. echinata*.  
436 A reduction in chlorophyll content in leaves exposed to elevated ozone was also found  
437 for birch (*Betula pendula*) (Paakkonen et al., 1996) and strawberry (*Fragaria vesca*)  
438 (Ramo et al., 2007). However, there was no change in leaf chlorophyll content in  
439 *Centaurea jacea* after exposure to elevated ozone (Ramo et al., 2006b). This suggests  
440 that reduction in chlorophyll content of healthy leaves is not always a symptom of  
441 ozone damage. Chlorosis, or the bleaching of chlorophyll during cell damage, has  
442 been seen under elevated ozone as a precursor to elevated senescence (Heath, 2008);  
443 over a longer experimental period it is possible that the percentage of senesced leaves  
444 would have increased further.

445

446 Only *M. caerulea* plants exposed to elevated ozone showed a reduction in fresh and  
447 dry above-ground biomass compared to plants that received a constant 20ppb ozone.

448 This is in contrast to previous published results (Franzaring et al., 2000) that found the  
449 biomass of *M. caerulea* increased under elevated ozone. The difference in findings  
450 could be due to the type of ozone regime experienced by the plants; growth could be  
451 stimulated by moderate ozone exposure but reduced by higher ozone concentrations.  
452 *P. erecta* showed a trend towards a reduction in dry above-ground biomass under  
453 elevated ozone, which is in contrast to the results of Hayes et al (2006) where *P.*  
454 *erecta* showed a non-significant increase in biomass under elevated ozone. The  
455 results of this experiment show that, as in previous studies (Davison and Barnes,  
456 1998), increases in senescence are not necessarily associated with reduction in plant  
457 growth, making it difficult when considering the wider ecological significance of  
458 elevated ozone. This result has also been found for some herbs and grasses; enhanced  
459 visible injury and senescence under elevated ozone did not necessarily lead to a  
460 reduction in biomass (Pleijel and Danielsson, 1997). The overall lack of change to  
461 above and below-ground biomass is unexpected as ozone exposure has been found to  
462 inhibit growth in a variety of species e.g. (Grantz, 2003; Grantz and Yang, 2000;  
463 Hayes et al., 2006; Peltonen et al., 2005). Inhibition of plant growth by ozone  
464 exposure may have been absent in this experiment because of the short-term nature of  
465 the experiment and it could be that it takes longer for changes in biomass to appear.  
466 In some experiments, it has been found that the biomass allocation to plant roots is  
467 reduced under elevated ozone (Andersen, 2003; Grantz and Yang, 2000). This may  
468 be because allocation to the roots is dependent on the source strength (Andersen,  
469 2003) and plant repair after ozone exposure requires the diversion of fixed  
470 carbohydrate from other plant sinks (Dizengremel, 2001). This was not seen in this  
471 experiment; again possibly because of the short time scale of the exposure period.  
472

473 Conclusions

474 From this experiment it can be seen that wetland plant species are affected by ozone,  
475 with senescence being increased under elevated ozone in all species studied.  
476 However, plant biomass was only negatively affected in one species (*M. caerulea*),  
477 suggesting that over short-term exposures, increases in senescence do not lead to  
478 decreases in plant growth. Chlorophyll content was affected in some species, with *P.*  
479 *erecta* plants from the fen and bog and *H. vulgaris* showing a decrease in chlorophyll  
480 content and *C. echinata* showing a transient increase. This could have a negative  
481 effect on carbon dioxide fixation during photosynthesis if the chlorophyll content of  
482 healthy leaves is reduced prior to visible senescence. The results of the plant  
483 decomposition suggest that the effects of elevated ozone on forb decomposition differ  
484 from the effects of elevated ozone on grass and sedge decomposition. *P. erecta* plants  
485 showed higher carbon dioxide efflux and higher rates of hydrolase activity whereas  
486 the other species tested showed a non-significant decrease in carbon dioxide efflux  
487 and a decrease in hydrolytic enzyme activity. If exposure to elevated ozone does  
488 change the decomposition rates and enzyme activities in wetland areas it could change  
489 the potential for wetlands to act as carbon sinks. If plant decomposition increases  
490 more carbon could be released at the end of the growing season; whereas if plant  
491 decomposition is reduced more carbon fixed during plant growth could be stored.  
492 However, for overall carbon storage to increase, it would be necessary for plant  
493 biomass to be unaffected by exposure to elevated ozone over a longer period. This  
494 seems unlikely and further studies are needed to fully comprehend the implications of  
495 rising ozone concentrations for wetland carbon cycling and storage.

496

497

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676

677

		week 1	week 2	week 3	week 4
high ozone (ppb)	peak background	147±1.51 20±0.17	139±1.90 19±0.89	146±1.93 20±0.80	146±1.14 19±0.97
low ozone (ppb)	peak background	19±0.37 13±0.74	20±0.43 14±0.44	20±0.29 13±2.7	20±0.47 13±0.65
high ozone (ppm.h)	AOT0 AOT40	20.1±0.2 7.1±0.1	38.9±0.2 13.9±0.2	55.8±0.4 19.8±0.3	75.7±0.5 27.0±0.3
low ozone (ppm.h)	AOT0 AOT40	3.1±0.1 0	6.2±0.1 0	9.1±0.3 0	12.3±0.4 0

678 **Table 1:** Peak and background ozone concentrations, cumulative AOT<sub>0</sub><sub>24hr</sub> and  
679 AOT<sub>40</sub> (daylight hours) for the two ozone treatments over the 28 day ozone exposure  
680 period. Values are the means of the four domes in each treatment and are shown ± 1  
681 standard error.

682

683

Plant	Regression analysis			Repeated Measures ANOVA
	R <sup>2</sup> value	P value	% variance accounted for	P values for repeated measures ANOVA
<i>M. caerulea</i>	0.967	<b>0.003</b>	95.5	<b>0.004</b>
<i>J. subnodulosus</i>	0.959	<b>0.004</b>	94.6	<b>0.012</b>
<i>P. erecta</i> (fen)	0.442	0.221	25.5	<b>0.045</b>
<i>H. vulgaris</i>	0.835	<b>0.030</b>	78.0	0.162
<i>C. echinata</i>	0.895	<b>0.015</b>	86.0	0.589
<i>F. rubra</i>	0.964	<b>0.003</b>	95.0	<b>0.005</b>
<i>P. erecta</i> (bog)	0.796	<b>0.042</b>	72.7	<i>0.076</i>

684 **Table 2:** Regression analysis of relative senescence against AOT0<sub>24hr</sub> for each species  
685 together with repeated measures ANOVA for weekly senescence measurements.  
686 Significant P values at P<0.05 are in bold and values 0.05<P<0.1 are in italics.  
687

	Species	Above ground fresh weight			Above ground dry weight			Root dry weight			Root:shoot ratio		
		High ozone	Low ozone	Sig?	High ozone	Low ozone	Sig?	High ozone	Low ozone	Sig?	High ozone	Low ozone	Sig?
Fen	<i>M. caerulea</i>	1.91±0.3	2.53±0.3	*	0.85±0.1	1.08±0.1	*	1.44±0.2	1.79±0.2	NS	0.61±0.1	0.63±0.1	NS
	<i>J. subnodulosus</i>	1.91±0.2	1.97±0.2	NS	0.52±0.1	0.53±0.1	NS	0.75±0.1	0.95±0.1	NS	0.73	0.58	NS
	<i>P. erecta</i>	0.95±0.1	0.96±0.1	NS	0.36±0.1	0.41	NS	0.52±0.1	0.61±0.1	NS	0.84±0.1	1.28±0.6	NS
	<i>H. vulgaris</i>	0.88±0.1	1.18±0.2	NS	0.11	0.15	NS	0.19	0.31	(*)	0.65±0.1	0.52±0.1	NS
Bog	<i>C. echinata</i>	0.97±0.1	0.75±0.1	NS	0.32	0.25	(*)	0.41	0.32	NS	0.80	0.81±0.1	NS
	<i>F. rubra</i>	0.87±0.1	0.87±0.1	NS	0.26	0.3	NS	0.20	0.28	NS	0.48±0.1	1.22±0.2	NS
	<i>P. erecta</i>	0.72±0.1	0.95±0.1	NS	0.24	0.33±0.1	(*)	0.41±0.1	0.56±0.1	NS	2.09±1.4	0.68±0.1	NS

689 **Table 3:** The mean biomass for the 7 species exposed to elevated ozone. Figures are shown as the mean for each treatment ± 1 standard

690 deviation. Where standard deviations are not shown they were less 0.05g. \* P<0.05 (\*) P<0.1 NS non-significant

691

	Total dissolved carbon			Total dissolved nitrogen		
	High ozone	Low ozone	P value	High ozone	Low ozone	P value
<i>M. caerulea</i>	266.4±3.0	264.0±18.4	NS	8.64±1.0	8.88±1.1	NS
<i>J. subnodulosus</i>	364.8±16.4	414.3±45.0	NS	16.70±1.1	18.16±1.5	NS
<i>P. erecta</i> (fen)	861.3±38.6	831.0±39.2	NS	11.60±1.0	11.86±0.9	NS
<i>P. erecta</i> (bog)	892.0±59.0	802.8±38.4	NS	11.83±0.5	10.66±0.4	NS
<i>C. echinata</i>	283.6±10.3	286.3±27.6	NS	8.32±0.4	10.46±0.9	<0.1
<i>F. rubra</i>	339.7±14.6	522.7±80.9	<0.1	10.96±0.7	10.98±0.9	NS

692 **Table 4:** Total carbon and total nitrogen in the slurries after 10 days of aerobic  
693 decomposition. Values are concentrations in mg/l and are shown as the treatment  
694 mean ± 1 standard error.

695

	Beta Glucosidase activity			N-acetylglucosaminidase activity		
	High ozone	Low ozone	P value	High ozone	Low ozone	P value
<i>M. caerulea</i>	1.14±0.62	2.41±0.22	<0.05	0.35±0.10	1.56±0.29	NS
<i>J. subnodulosus</i>	8.17±2.10	14.55±0.70	<0.01	6.35±0.43	13.62±0.80	<0.0001
<i>P. erecta</i> (fen)	10.24±1.48	7.71±0.78	<0.1	9.34±0.81	6.49±0.99	<0.1
<i>P. erecta</i> (bog)	13.93±8.50	0.60±0.16	<0.05	11.74±2.51	0.13±0.12	<0.01
<i>C. echinata</i>	6.13±4.50	10.29±1.88	NS	1.32±0.53	6.08±1.22	<0.05
<i>F. rubra</i>	0	10.52±5.62	NS	0	1.12±0.35	<0.05

696 **Table 5:** Beta glucosidase and N-acetylglucosaminidase activities after 10 days of  
697 aerobic decomposition. Values are the enzyme activity per gram of plant weight and  
698 are shown as the mean ± 1 standard error.

699

700

701 **Figure 1:** Weekly percentage senescence measured on the four fen species.

702 Statistical tests were performed on arc-sine transformed data and data was back-  
703 transformed for presentation. \* P<0.05, \*\* P<0.01, \*\*\* P<0.001 for differences  
704 between ozone treatments at each time point.

705

706 **Figure 2:** Weekly percentage senescence measured on the three bog species.

707 Statistical tests were performed on arc-sine transformed data and data was back-

708 transformed for presentation. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  for differences  
709 between ozone treatments at each time point.

710

711 **Figure 3:** Changes in chlorophyll content for five species over the 4 week exposure  
712 period. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  for differences between ozone treatments  
713 at each time point.

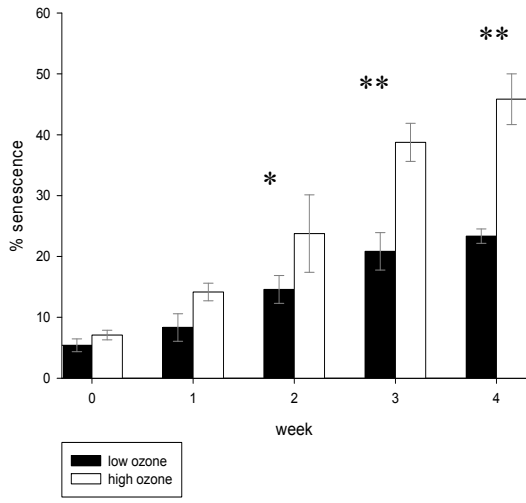
714

715 **Figure 4:** Cumulative carbon dioxide efflux during the 10 day decomposition assay.  
716 (\*)  $P < 0.1$ , \*  $P < 0.05$  for differences between ozone treatments at each time point.

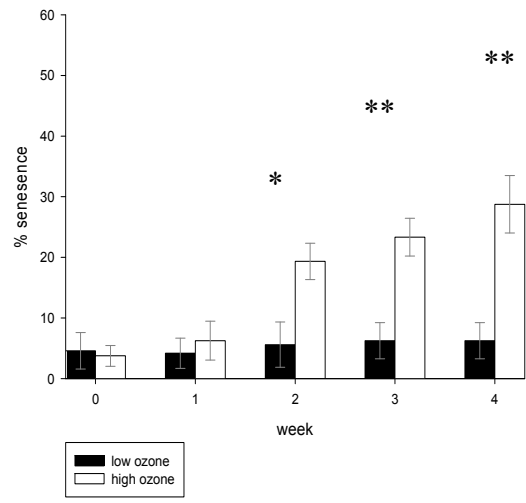
717



*M. caerulea*

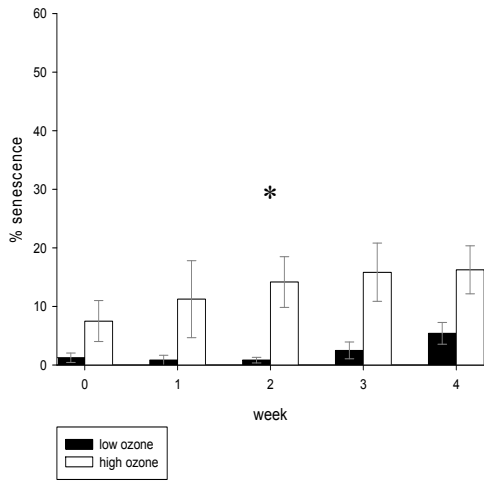


*J. subnodulosus*

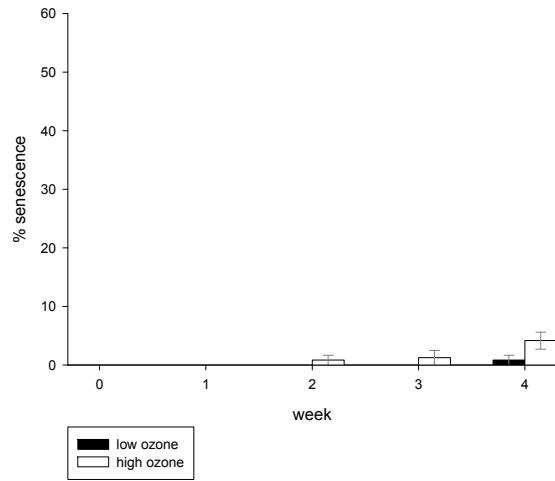


718

*P. erecta* (fen)



*H. vulgaris*

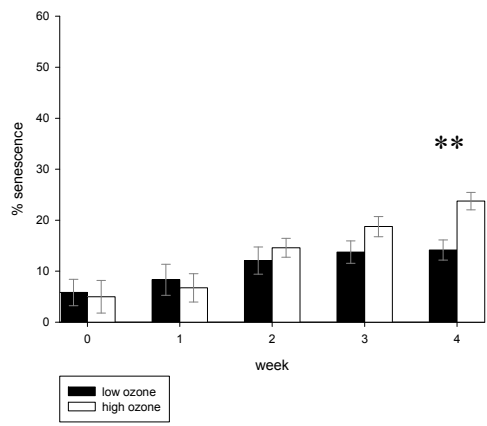


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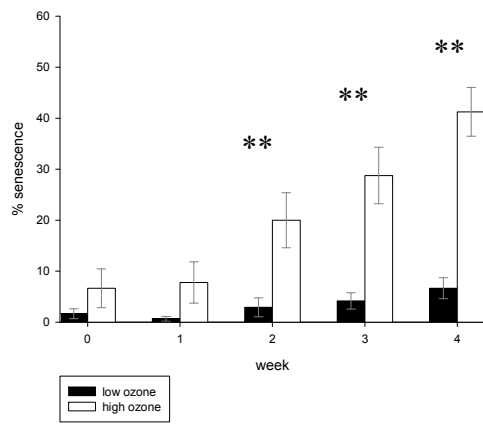
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721

*C. echinata*

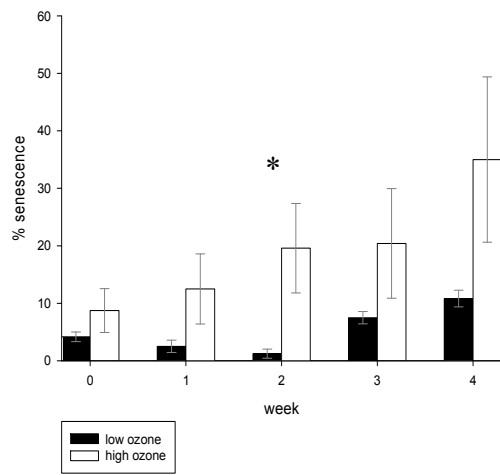


*F. rubra*



722

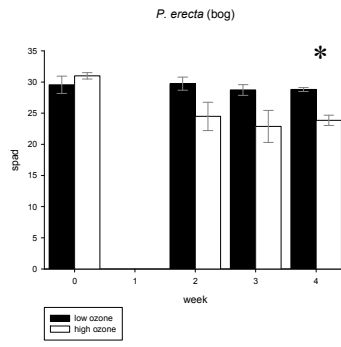
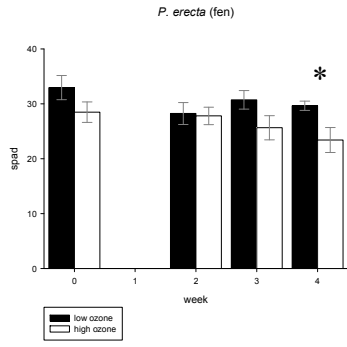
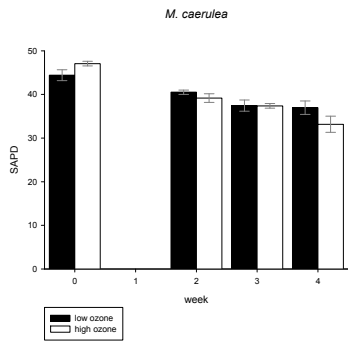
*P. erecta* (bog)



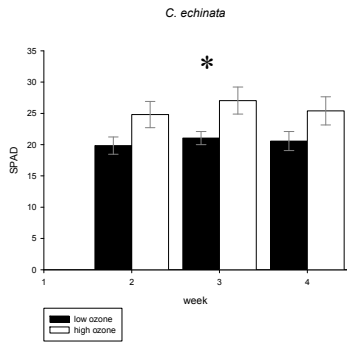
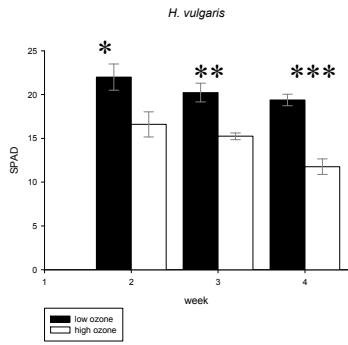
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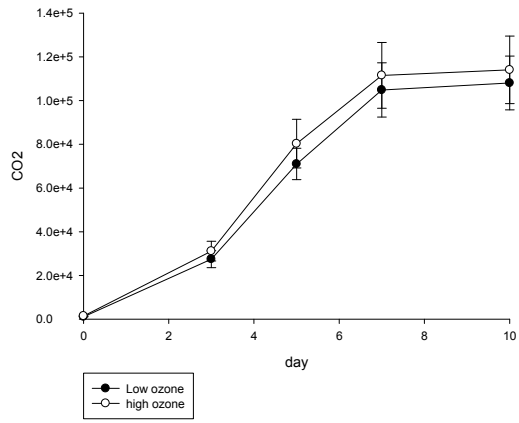


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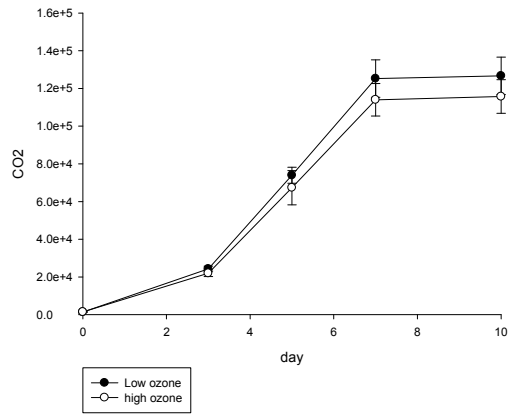
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729

*Molinia caerulea*



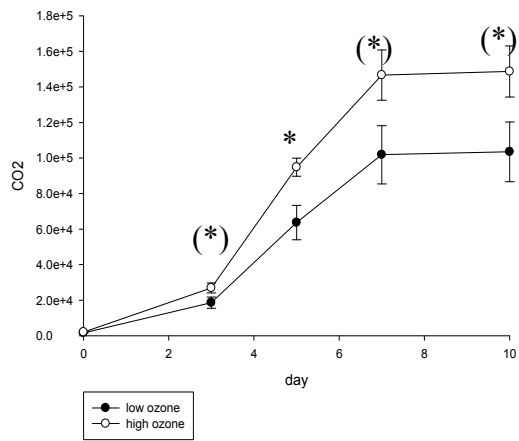
*Juncus subnodulosus*



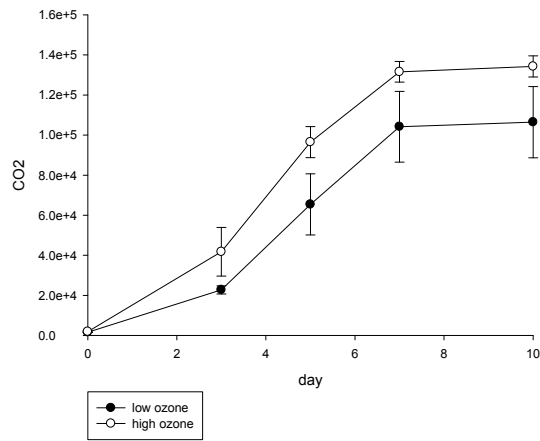
730

731

*Potentilla erecta* (fen)

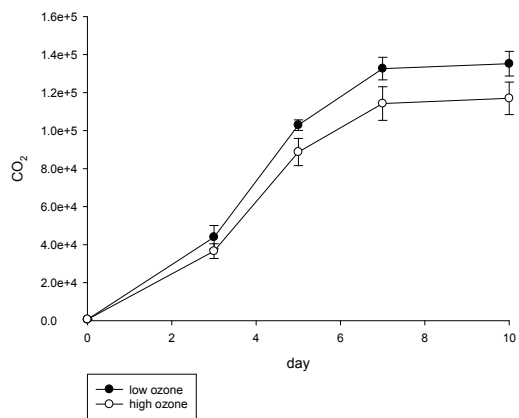


*Potentilla erecta* (bog)

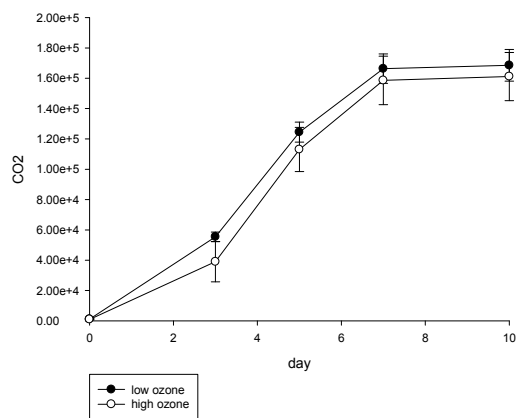


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*Carex echinata*



*Festuca rubra*



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