

1 **Future atmospheric CO<sub>2</sub> leads to delayed autumnal**  
2 **senescence in *Populus* over two continents**

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35 **Running title** – Rising CO<sub>2</sub> delays autumnal senescence in *Populus*

36

37 **ABSTRACT**

38 Growing seasons are getting longer, a phenomenon partially explained by increasing  
39 global temperatures. Recent reports suggest that a strong correlation exists between  
40 warming and advances in spring phenology but that a weaker correlation is evident  
41 between warming and autumnal events implying that other factors may be influencing  
42 the timing of autumnal phenology. Using freely rooted, field-grown *Populus* in two  
43 Free Air CO<sub>2</sub> Enrichment Experiments (AspenFACE and PopFACE), we present  
44 evidence from two continents and over two years that increasing atmospheric CO<sub>2</sub>  
45 acts directly to delay autumnal leaf coloration and leaf fall.

46 In an atmosphere enriched in CO<sub>2</sub> (by ~ 45 % of the current atmospheric  
47 concentration to 550 ppm) the end of season decline in canopy Normalized Difference  
48 Vegetation Index (NDVI) - a commonly used global index for vegetation greenness -  
49 was significantly delayed, indicating a greener autumnal canopy, relative to that in  
50 ambient CO<sub>2</sub>. This was supported by a significant delay in the decline of autumnal  
51 canopy leaf area index (LAI) in elevated as compared to ambient CO<sub>2</sub>, and a  
52 significantly smaller decline in end of season leaf chlorophyll content. Leaf level  
53 photosynthetic activity and carbon uptake in elevated CO<sub>2</sub> during the senescence  
54 period was also enhanced compared to ambient CO<sub>2</sub>. The findings reveal a direct  
55 effect of rising atmospheric CO<sub>2</sub>, independent of temperature in delaying autumnal  
56 senescence for *Populus*, an important deciduous forest tree with implications for  
57 forest productivity and adaptation to a future high CO<sub>2</sub> world.

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59

60 **INTRODUCTION**

61

62 The timing of phenological events for many woody and herbaceous plants in mid to  
63 upper latitudes has changed, with significant advances in spring bud break and  
64 similarly significant delays in autumn leaf color change and leaf fall resulting in an  
65 extension of the growing season (Menzel & Fabian, 1999; Parmesan, & Yohe 2003;  
66 Root et al., 2003). Remote sensing of vegetation using the Normalized Difference  
67 Vegetation Index (NDVI – a measure of vegetation greenness) shows an 18-day  
68 extension of the growing season in Eurasia between 1982 to 1999 and a 12-day  
69 extension in North America (Zhou et al., 2001). These changes have been attributed to  
70 warmer temperatures causing longer growing seasons (Menzel & Fabian, 1999;  
71 Peñuelas, & Filella, 2001; Root et al., 2003; Zhou et al., 2001). In the most  
72 comprehensive meta-analysis to date, consisting of 125, 000 observations from 21  
73 European countries, taken between 1971 and 2000 and using 542 plant species,  
74 Menzel et al., (2006) report the correlation between an advanced spring phenophase  
75 and warming patterns of 19 European countries was strong and significant ( $r = -0.69$ ,  
76  $P < 0.001$ ). However the association between warming and the autumnal phenophase  
77 was described by Menzel et al., (2006) as ‘vague’. The correlation between leaf color  
78 change and fall and the temperature trends for 14 European countries was weak and  
79 non-significant ( $r = 0.003$ ,  $P = 0.99$ ). Moreover, of the leaf coloring events only 52 %  
80 were delayed and only 15 % of these were significant. This contrasts sharply with that  
81 of spring events which showed 78 % were earlier and that 31 % of these were  
82 significant. Nevertheless across Europe, during the last 30 years, autumnal senescence  
83 has been delayed by between 1.3 - 1.8 days decade<sup>-1</sup> (Menzel et al., 2006 and Menzel  
84 and Fabian, 1999, respectively). It is notable that while warming has been unevenly

85 distributed both spatially and temporally, it is the rise in global [CO<sub>2</sub>] that has recently  
86 been more in synchrony with changing autumnal phenological patterns reported  
87 across wide geographic regions. For this reason, we hypothesized that elevated  
88 atmospheric [CO<sub>2</sub>] could affect the timing of autumnal senescence directly and  
89 independent of temperature. We investigated this by utilizing two large-scale forest  
90 ecosystem experiments, one in the USA and one in Italy, where *Populus* trees were  
91 exposed to elevated CO<sub>2</sub>, from planting to maturity, thus providing a unique  
92 experimental resource in which to determine the effect of future CO<sub>2</sub> on autumnal  
93 senescence.

94

95 From previous research into the autumnal phenophase of forest trees, there has been  
96 large variability in response to elevated [CO<sub>2</sub>], with advances (Körner et al., 2005;  
97 Jach and Ceulemans, 1999; Norby, et al., 2003; Sigurdsson, 2001) delays (Karnosky  
98 et al., 2003; Körner et al., 2005; Li, et al., 2000; Rae et al., 2006), or no effect  
99 (Herrick, & Thomas, 2003) all reported. A similar variability in response is observed  
100 in annual plants, and has been linked to the determinate nature of plant development.  
101 Species with determinate growth often show early senescence in elevated [CO<sub>2</sub>] such  
102 as barley (Fangmeier, et al., 2000) and tobacco (Miller, et al., 1997) as the plant  
103 approaches maturity more quickly, whilst those with indeterminate growth such as  
104 soybean, show delayed senescence in elevated CO<sub>2</sub> (Miglietta, et al., 1993; Dermody,  
105 Long, & DeLucia, 2006). The determinate nature of growth may lead to the down-  
106 regulation of photosynthesis in elevated relative to ambient CO<sub>2</sub> as a consequence of  
107 reduced sink demand for photoassimilate (Ainsworth et al., 2004). Such plants may be  
108 considered ‘sink limited’ where this is defined as ‘an increased abundance of mobile  
109 carbon compounds associated with a reduced growth capacity or inability to utilize

110 mobile carbon compounds' after Hoch et al., (2003). By artificially manipulating the  
111 source-sink balance of *Arabidopsis* Wingler et al, (2004) showed for plants grown on  
112 2 % glucose and a 30mM N agar exhibited delayed senescence, whilst for plants  
113 grown on 2 % glucose with a reduced nutrient (4.7mM N) agar, senescence was  
114 advanced. It was concluded that increased sugar accumulation in the leaves of  
115 nitrogen deficient *Arabidopsis* (advanced senescence phenotype) could be the result  
116 of decreased sugar utilization for the synthesis of N demanding amino acids and  
117 proteins; an increased source to sink ratio promoting senescence and mediated  
118 through N availability (Pourtau et al., 2004). Herrick and Thomas (2003) hypothesize  
119 that for species which show an increased net photosynthesis in elevated CO<sub>2</sub>, the  
120 increased C:N ratio of such leaves will result in delayed autumnal senescence. In  
121 other words, a later season positive leaf carbon balance will result in delayed  
122 senescence when stimulated photosynthetic uptake in elevated CO<sub>2</sub> is sustained.  
123 These previous studies give a glimpse of the complexities of the senescence process  
124 but also begin to provide some mechanistic explanations that may enable us to make  
125 generalizations about autumnal senescence in a high CO<sub>2</sub> world.

126

127 In this study freely rooted *Populus* species were chosen which had been grown from  
128 initiation in elevated CO<sub>2</sub> using FACE technology for between six and seven years.  
129 The trees at these two sites have responded positively to growth in elevated CO<sub>2</sub>  
130 exhibiting increases in biomass (Karnosky et al., 2003; Liberloo et al., 2006) and  
131 limited down-regulation of photosynthesis (Karnosky et al., 2003; Davey et al., 2006,  
132 Calfapietra et al., 2005) and therefore were not considered to exhibit sink-limited  
133 growth. To determine whether an elevated [CO<sub>2</sub>] at that predicted for around 2050,  
134 extended the autumnal phenophase and increased canopy duration, we examined the

135 autumnal decline in leaf area index, NDVI and photosynthetic function at the  
136 AspenFACE site in Wisconsin, USA, and the PopFACE site in Tuscania, Italy during  
137 two consecutive years.

138

## 139 **MATERIALS AND METHODS**

### 140 *The AspenFACE Experiment*

#### 141 **Design**

142 The AspenFACE experiment (32 ha) is situated on sandy loam glacial outwash soil in  
143 northern Wisconsin, near Rhineland (45° 06' N, 89° 07' W; 490 m a.s.l.;  
144 [www.aspenface.mtu.edu](http://www.aspenface.mtu.edu)). The system has been used to fumigate (1997–2006)  
145 aggrading trembling aspen (*Populus tremuloides* Michx.; 5 genotypes), mixed white  
146 birch (*Betula papyrifera*)/trembling aspen and mixed sugar maple (*Acer*  
147 *saccharum*)/trembling aspen stands with elevated atmospheric CO<sub>2</sub> at a concentration  
148 of 560 μmol mol<sup>-1</sup> CO<sub>2</sub>. Prior to planting soil nitrogen content was 15.06 μg N g<sup>-1</sup> soil  
149 (NO<sub>3</sub><sup>-</sup> - N) and 1.03 μg N g<sup>-1</sup> soil (NH<sub>4</sub><sup>+</sup> - N) mean for control plots and 15.24 μg N g<sup>-1</sup>  
150 soil (NO<sub>3</sub><sup>-</sup> - N) and 0.94 μg N g<sup>-1</sup> soil (NH<sub>4</sub><sup>+</sup> - N) mean for elevated CO<sub>2</sub> plots. The  
151 mean C:N ratio for the control and CO<sub>2</sub> enrichment FACE plots was 12.6 (Dickson et  
152 al., 2000). Complete design and performance characteristics of Aspen FACE and a  
153 summary of responses are available elsewhere (Karnosky, et al., 2003). The mean  
154 minimum temperature throughout October 2003 was 2.2 °C and the mean maximum  
155 was 11.4 °C, whereas in 2004 for the same time period mean minimum temperature  
156 was 3.3 °C and maximum 12.0 °C. The lowest daily mean minimum temperature

157 during October 2003 was on day 275 (-5.0 °C), and during October of 2004 was on  
158 day 279 (-2.6 °C)

### 159 **Canopy and leaf characterization**

160 Near hemispherical photographs with a 148° field of view were taken every 10 to 20  
161 days during the 2001 to 2004 growing seasons using a fish-eye lens equipped Nikon  
162 LC-Erland Nikon E950 digital camera (Nikon, Inc., Melville, NY, USA), analyzed  
163 with WinSCANOPY software (Regent Instruments Quebec, Canada), and exported to  
164 XLScanopy (Regent Instruments Quebec, Canada) for conversion into LAI. We used  
165 software-provided Bonhomme assumptions (Welles, &, Norman, 1991) for estimating  
166 LAI for each of the 5 elevations from horizontal. Although errors are associated with  
167 the use of hemispherical images for LAI estimation, these estimates of LAI were  
168 strongly correlated with both harvest-based allometrically determined LAI and litter  
169 trap-based estimates of LAI ( $R^2 = 0.67$ ,  $P < 0.01$ ), and identified similar seasonal and  
170 treatment patterns for the trace gas treatments at AspenFACE (unpublished data of the  
171 authors). On each date, three estimates of LAI per ring section were averaged,  
172 resulting in 6 measurements for each of the 4 measurement periods (3 ambient and 3  
173 elevated [CO<sub>2</sub>]). A shift in the autumnal phenophase was calculated as the number of  
174 days from maximum to 50% of maximum LAI for CO<sub>2</sub> plots versus control plots.  
175 Light-saturated photosynthesis was measured using a portable gas exchange system  
176 (Li-Cor 6400; Li-Cor, Inc., Lincoln, NE, USA) for upper canopy aspen leaves (3  
177 leaves per clone, two clones, over 9 time points during the senescence period). One  
178 block of each paired plot was examined at each time-point. This was to restrict the  
179 diurnal influence on the *insitu* measurements and enable data from across the whole  
180 site to be collected during the time course of autumnal senescence.

181 ***The PopFACE Experiment***

182 **Design**

183 The PopFACE experiment (9 ha) is situated on a nutrient rich, clay soil in Tuscania,  
184 Italy (42° 22' N, 11° 48' E; altitude 150 m a.s.l.; [www.unitus.it/euroface](http://www.unitus.it/euroface).) Three  
185 species of *Populus*, *Populus alba*, *P. nigra*, and *P. x euramericana* were grown in the  
186 experiment which comprises three blocks containing six, 314 m<sup>2</sup> octagonal plots  
187 assigned to treatments of [CO<sub>2</sub>] (ambient 372 μmol mol<sup>-1</sup> and 550 μmol mol<sup>-1</sup>).  
188 Complete design characteristics of PopFACE are available elsewhere (Miglietta, et  
189 al., 2001). Briefly, planting was carried out in 1999 and trees coppiced three years  
190 later. After coppice one sub-plot received nitrogen fertigation and the other remained  
191 at ambient soil nitrogen, only data from the ambient nitrogen fertigation treatments  
192 are reported here. For the unfertilized sub-plots and measured prior to planting, mean  
193 soil nitrogen content was 9.73 μg N g<sup>-1</sup> soil (NO<sub>3</sub><sup>-</sup> - N) and 0.73 μg N g<sup>-1</sup> soil (NH<sub>4</sub><sup>+</sup> -  
194 N) mean for control plots and 7.17 μg N g<sup>-1</sup> soil (NO<sub>3</sub><sup>-</sup> - N) and 0.59 μg N g<sup>-1</sup> soil  
195 (NH<sub>4</sub><sup>+</sup> - N) mean for elevated CO<sub>2</sub> plots (Liberloo et al., 2006) and the mean C:N of  
196 the site was 9.3 (Hoosbeek et al., 2004). In 2004 the N content for the unfertilized  
197 sub-plots was 12.8 μg N g<sup>-1</sup> soil (NO<sub>3</sub><sup>-</sup> - N) and 1.86 μg N g<sup>-1</sup> soil (NH<sub>4</sub><sup>+</sup> - N) mean for  
198 control plots and 6.65 μg N g<sup>-1</sup> soil (NO<sub>3</sub><sup>-</sup> - N) and 1.47 μg N g<sup>-1</sup> soil (NH<sub>4</sub><sup>+</sup> - N) mean  
199 for elevated CO<sub>2</sub> plots (Liberloo et al., 2006). During the period of study (2003 –  
200 2004) trees had been planted for between five and six years and a closed canopy was  
201 evident. The mean minimum temperature throughout October 2003 was 10.8 °C and  
202 the mean maximum was 21.6 °C, whereas in 2004 for the same time period mean  
203 minimum temperature was 14.2 °C and mean maximum 25.0 °C. During the period of  
204 study in 2003 the lowest temperature was apparent on Julian day 300 (3.7 °C),

205 whereas 2004 was characterized by an unseasonably warm autumn and the lowest  
206 temperature was noted on Julian day 273 (8.8 °C).

### 207 **Canopy and leaf characterization**

208 LAI measurements were made of the *P. x euramericana* and *P. nigra* canopies (2003,  
209 *P. x euramericana* only) using a Licor LAI-2000 (Licor Inc., Nebraska, USA). This  
210 technique has been previously correlated with harvest-based allometrically  
211 determined LAI for these species at this site (Liberloo et al., 2004). Following a  
212 reference value which was obtained in open skies clear of the canopy, 14 below  
213 canopy measures as described in Gielen et al., (2003) were taken and this was  
214 replicated four times per sub-plot for each of the six experimental plots, at each time  
215 of measurement. Optical values of LAI (estimated using the Li-cor LAI-2000)  
216 theoretically include stems and branches and are therefore considered an estimate of  
217 the above ground vegetation area index (VAI). Following total leaf fall an estimate of  
218 wood area index, WAI (m<sup>2</sup> of woody tissue / m<sup>2</sup> of ground) was conducted in the  
219 same way for each species and year except that the function A/B = 1 was set to allow  
220 for no foliage (*LI-COR*, 1990). LAI\* was re-estimated by subtracting the WAI from  
221 the VAI for the final two estimates of VAI in both years. A shift in the autumnal  
222 phenophase was calculated as the number of days difference for a 50% decline of the  
223 VAI (assuming decline was related with leaf fall) measured at bud-set for CO<sub>2</sub> plots  
224 versus control plots.

225 Towards the end of the growing season (October 15<sup>th</sup>, 2003, October 1<sup>st</sup> and October  
226 23<sup>rd</sup> 2004 for *P. x euramericana*, October 2<sup>nd</sup> and October 26<sup>th</sup> 2004 for *P. nigra*)  
227 ground-based canopy reflectance was measured 1.0 m above the canopy using a field  
228 portable spectroradiometer (GER, Buffalo, NY, USA Mod. 3700; range 350-1050

229 nm). Airborne measurements were made using a multispectral camera equipped with  
230 a single optic (Duncan Tech, USA Mod. MS4100) operated at three wide bandwidths  
231 centered on 550, 680 and 800 nm. The camera (field of view of 60°) was mounted on  
232 a certified aircraft (Sky Arrow 650TCNS, Rome, Italy) flying at 200 m above the  
233 experimental area. Normalized Difference Vegetation Index (NDVI) was calculated  
234 as  $(R_{\text{NIR}} - R_{\text{RED}}) / (R_{\text{NIR}} + R_{\text{RED}})$  for the airborne. For the narrow bandwidth ground  
235 based spectral measurements NDVI was calculated using  $(R_{800} - R_{680}) / (R_{800} + R_{680})$   
236 (the centre of the wavebands used for the airborne calculation) and a chlorophyll  
237 specific NDVI was calculated as  $(R_{750} - R_{705}) / (R_{750} + R_{705})$  (Gamon, & Surfus,  
238 1999), a robust predictor of *Populus* chlorophyll content ( $r^2 = 0.98$ ,  $P < 0.001$ ,  $n = 97$ ;  
239 Tallis *et al.*, unpublished).

240 Leaf material of known fresh weight was collected from mainstems and between the  
241 10<sup>th</sup> to 12<sup>th</sup> leaf down from the closed apical bud. Chlorophyll was extracted using  
242 DMF (N,N-dimethylformamide; analytical grade; Fisher Scientific) and chlorophyll  
243 content was assessed using the coefficients of Wellburn, (1994). Gas exchange  
244 measurements were carried out on 6 leaves per plot of *P. nigra* using a portable gas  
245 exchange system (Li-Cor 6400; Li-Cor, Inc., Lincoln, NE, USA). Leaves were  
246 selected from the same canopy position as those taken for chlorophyll extraction and  
247 harvested on 06<sup>th</sup> November 2004. The protocol described in Calfapietra *et al.* (2005)  
248 was used for measurements on detached, rehydrated leaves held in controlled  
249 conditions.

## 250 **Estimating GPP**

251 Data from Wittig *et al.*, (2005) generated using the WIMOVAC model were  
252 extracted. The mean monthly GPP ( $\text{g C m}^{-2} \text{ d}^{-1}$ ) of *P. x euramericana* exposed to

253 elevated [CO<sub>2</sub>] at the PopFACE site and estimated in 2001 when a closed canopy  
254 existed were used in this analysis. The relationship between day of year (X axis) and  
255 GPP (Y axis) was explained by a cubic function in the form  $y = 2E^{-06}x^3 - 0.0021x^2 +$   
256  $0.542x - 27.289$ ,  $r^2 = 0.98$ . The mean number of days shift in the autumnal  
257 phenophase (10 day advanced in control conditions) of *P. x euramericana* calculated  
258 in this study was applied to the model. During the time of the study reported here no  
259 photosynthetic data profiles were available for *P. x euramericana* therefore the  
260 original model could not be run for this data. Instead the parabola relationship from  
261 the 2001 data was shifted to account for a change in phenology. Assuming that any  
262 phenological shifts resulting from elevated CO<sub>2</sub> would be accounted for in this  
263 relationship, the GPP resulting from an elevated CO<sub>2</sub> stimulated shift in the autumnal  
264 phenophase was estimated as the difference between the areas under the two curves.

## 265 **Statistical analysis**

266 The AspenFACE experiment has three replications for each species, organized in  
267 three randomized complete blocks. The PopFACE experiment has three replications  
268 for each species, organized in a factorial block design. Late season canopy data from  
269 the two FACE sites were analyzed in two ways, with both approaches showing highly  
270 significant differences between ambient and elevated [CO<sub>2</sub>] treatments. In the first  
271 approach, we relied on univariate ANOVA to examine treatment effects on LAI at the  
272 end of the growing season for AspenFACE, and PopFACE. Late season LAI effects  
273 for the two sites were quantified by integrating the area under the curve for the last  
274 three measurement periods for each site and year (Figure 1) for control and elevated  
275 [CO<sub>2</sub>] treatments. The presence of site heterogeneity at a single time point of  
276 measurement at the PopFACE site lead to type I error and this was reduced by

277 including random block as a tested factor. As both CO<sub>2</sub> treatment and random block  
278 were now tested factors the CO<sub>2</sub> effect was sought from average within-block  
279 differences, therefore it was not obscured by the natural heterogeneity which existed  
280 between blocks as discussed in Tricker *et al.*, (2005). Where no treatment interaction  
281 with block was evident at  $P \leq 0.25$  (Underwood, 1997) *post hoc* pooling was carried  
282 out as detailed in Tricker *et al.* (2005). Here the F ratio was constructed by summing  
283 the block x CO<sub>2</sub> interaction sum of squares with that of the error, and the denominator  
284 df adjusted accordingly. Both the F ratio and *P* values are given for the average within  
285 block differences and the pooled data. Data were analyzed using analysis of variance  
286 (ANOVA) carried out in Minitab 14.0 (Minitab Inc., Philadelphia). To further reduce  
287 the influence of site heterogeneity (particular evident during senescence) the  
288 percentage change of the response variables of canopy reflectance and leaf  
289 chlorophyll content of each plot over the time course of the autumnal phenophase  
290 were calculated, therefore normalizing the initial value of the plot to zero. Following  
291 arcsine transformation the percentage change data were analyzed using the same  
292 model, and plot was the unit of replication.

293

## 294 **RESULTS**

295 Across the two FACE experiments, elevated [CO<sub>2</sub>] enhanced late season LAI by 20%  
296 to 50% compared with ambient [CO<sub>2</sub>] (Figure. 1A and B). In Wisconsin, elevated  
297 [CO<sub>2</sub>] extended autumn leaf retention by 10-40% in pure stands of trembling aspen  
298 (*Populus tremuloides*) (Figure 1A and B), by 8-48% in mixed stands of aspen and  
299 birch (*Betula papyrifera*) (Figure 2A-B), and by 17-32% in mixed stands of aspen and  
300 maple (*Acer saccharum*) (Figure 2C-D). In Tuscania, stands of *P. x euramericana*

301 grown under elevated [CO<sub>2</sub>] exhibited a 10% enhancement in LAI in the late  
302 summer/early autumn, and a 15% to 35% enhancement at the close of the growing  
303 season (Figure 1B). Late season LAI effects at AspenFACE and VAI effects at  
304 PopFACE were quantified by integrating the area under the curve for the last three  
305 measurement periods for each site and year (Figure 1B) for control and elevated  
306 [CO<sub>2</sub>] treatments. These analyses showed that the effects of elevated [CO<sub>2</sub>] on late  
307 season phenology were significant ( $P = 0.016$ ) at the two FACE sites for 2003 and  
308 2004. There were no CO<sub>2</sub> by site or CO<sub>2</sub> by year interactions ( $P = 0.535$ ,  $P = 0.585$ ,  
309 respectively) – data not shown. Interestingly it appears from Figure 1A that in  
310 AspenFACE, leaf fall in both 2003 and 2004 was increased dramatically at  
311 approximately DOY 280, an effect that was not apparent in PopFACE. It seems likely  
312 that this represents a response to differences in the rate of photoperiod and  
313 temperature decline at the two sites.

314 In order to test our hypothesis that rising [CO<sub>2</sub>] was primarily responsible for  
315 increasing growing season length as opposed to air temperature (warming), we  
316 examined 2002-2004 AspenFACE data from our three control and three CO<sub>2</sub> rings.  
317 We determined a positive, statistically significant ( $P = 0.001$ ) Pearson correlation  
318 between cumulative seasonal CO<sub>2</sub> exposure (ppm hrs) and aspen canopy, end of  
319 season LAI (LAI<sub>end</sub>; one week prior to initiation of leaf fall). We found no  
320 relationship ( $P = 0.797$ ) between LAI<sub>end</sub> and temperature summed as growing degree  
321 days (924 days, 2002; 807 days, 2003 and 749 days, 2004 to base 10<sup>0</sup>C) or between  
322 LAI<sub>end</sub> ( $P = 0.419$ ) and precipitation amount (499 mm, 2002; 240 mm, 2003; 307 mm,  
323 2004) – data not shown.

324

325 The digital photography from the AspenFACE site confirms the stimulation of LAI  
326 calculated from optical estimates and also identifies a delay in foliar senescence in the  
327 canopies exposed to elevated CO<sub>2</sub> (Figure 2, A-D). After removal of the contribution  
328 of WAI to the VAI estimates, the above ground stimulation of LAI\* at the PopFACE  
329 site during the autumnal phenophase was also identified as resulting from an increase  
330 in LAI in elevated CO<sub>2</sub> for both species and both years. This increased LAI\* was  
331 significant on day 287 of 2003 ( $F_{1,2} 61.1, P \leq 0.05$ ), and day 296 of 2004 ( $F_{1,2} 29.8, P$   
332  $\leq 0.05$ ) for *P. x euramericana* and day 308 of 2004 ( $F_{1,2} 18.59, P \leq 0.05$ ) for *P. nigra*  
333 (Figure 2E).

334

335 At PopFACE the phenological indicator NDVI also showed that both *P. x*  
336 *euramericana* and *P. nigra* canopy greenness was likewise extended under elevated  
337 [CO<sub>2</sub>]. Using false color imagery, we were able to visually display the stimulation of  
338 NDVI as the brightest red color in both 2003 and 2004 (Figure 3A), and at PopFACE  
339 the decline in late-season NDVI characteristic of autumnal senescence was  
340 significantly reduced ( $F_{1,2} 13.27, P = 0.068$ , from average within block differences  
341 and  $F_{1,8} 11.18, P \leq 0.01$ , from *post-hoc* pooling, Figure 3B). The decline in the  
342 chlorophyll specific NDVI (Gamon & Surfus, 1999) was greater than that of NDVI  
343 during the autumnal phenophase. The decline in chlorophyll specific NDVI was also  
344 significantly reduced ( $F_{1,2} 25.71, P \leq 0.05$ , from average within block differences and  
345  $F_{1,8} 32.85, P \leq 0.001$ , from *post-hoc* pooling) by growth in elevated [CO<sub>2</sub>] (data not  
346 shown) a trend that was supported by the decline in extracted leaf chlorophyll content  
347 which was also significantly reduced ( $F_{1,2} 5.20, P = 0.15$ , from average within block  
348 differences and  $F_{1,8} 45.16, P \leq 0.001$ , from *post-hoc* pooling) by growth in elevated

349 [CO<sub>2</sub>] (Figure 3C). A similar finding was identified in 2003. Between 24<sup>th</sup> September  
350 and 16<sup>th</sup> October 2003, only two of the three blocks were harvested, and over this time  
351 a 37 % (*P. x euramericana*) and a 19 % (*P. nigra*) increase in leaf chlorophyll content  
352 in elevated CO<sub>2</sub>, respective to control, was observed.

353

354 Late season carbon uptake was stimulated in elevated [CO<sub>2</sub>] at both sites for all  
355 species (Figure 4A-C). However, the duration of this stimulation appeared genotype  
356 specific at the AspenFACE site (Figure 4A-B). This was evident by measurements of  
357 light-saturated photosynthesis for upper canopy leaves of aspen genotypes 271 and  
358 42E. Elevated CO<sub>2</sub> stimulated photosynthesis throughout the early-mid autumnal  
359 phenophase (day of year 246 -273) for both genotypes. The late autumn stimulation  
360 of photosynthesis was between 30 % (42E) and 86 % (271) on the 06<sup>th</sup> October 2004,  
361 a stimulation resulting from extended canopy greenness (Figure 2A-D). This  
362 stimulation was sustained by Clone 271 resulting in ~ 300 % increased leaf carbon  
363 uptake in elevated [CO<sub>2</sub>] compared to control late into the autumn (12<sup>th</sup> October  
364 2004) (Figure 4A), but was now absent in clone 42E (Figure 4B). At PopFACE late  
365 season (06<sup>th</sup> November 2004) light saturated photosynthetic capacity of single leaves  
366 was also enhanced in elevated [CO<sub>2</sub>] for *P. nigra*, the only species measured at this  
367 site (Figure 4C). This resulted from a large stimulation in both  $J_{\max}$  (maximum  
368 electron flow through photosystem II,  $F_{1,2}$  11.97,  $P = 0.074$ , from average within  
369 block differences and  $F_{1,13}$  17.0,  $P \leq 0.001$ , from *post-hoc* pooling) and an equally  
370 large stimulation in  $V_{\max}$  (maximum velocity of carboxylation of Rubisco,  $F_{1,2}$   
371 12.17,  $P = 0.072$ , from average within block differences and  $F_{1,13}$  11.65,  $P \leq 0.005$ ,  
372 from *post-hoc* pooling) (Figure 4C). The ratio between  $V_{\max}$  and  $J_{\max}$  was 0.45 and  
373 remained the same between both treatments.

374

375 At PopFACE *P. x euramericana* sets bud some 30 days before *P. nigra* (Calfipetria  
376 et al., 2003). The extended functionality of the *P. x euramericana* canopy in elevated  
377 [CO<sub>2</sub>] during the autumnal phenophase was estimated by the number of days  
378 difference for a 50 % decline in VAI relative to control conditions. This was  
379 calculated to be between 5 and 15 days for 2003 and 2004 respectively. Taking a  
380 mean of 10 days this extension was estimated to contribute 2 % to the total annual  
381 GPP of *P. x euramericana* exposed to elevated [CO<sub>2</sub>] at the PopFACE site (Figure 5).

382

### 383 **DISCUSSION**

384 These data proved compelling evidence at the scale of the leaf and canopy, that  
385 autumnal senescence in such forest ecosystems will be delayed, as the atmospheric  
386 concentration of CO<sub>2</sub> continues to rise. We have shown that delayed autumnal  
387 senescence may occur in forests as a direct response to elevated CO<sub>2</sub>, independent of  
388 temperature. This effect could explain the poor correlations observed previously  
389 between autumn phenology and rising temperatures, in contrast to the strong  
390 correlations between spring phenology and rising temperatures. We hypothesized  
391 that, with no sink limitation, photosynthesis and canopy greenness would be  
392 maintained for longer in elevated CO<sub>2</sub> and data collected across different continents  
393 and *Populus* species grown both in pure and mixed stands, support this hypothesis and  
394 show a strong effect of elevated [CO<sub>2</sub>] with a significant extension of late season LAI  
395 (Figure 1A-B and 2E). Whole canopy greenness also persisted for longer at both sites  
396 during the autumnal phenophase (Figure 2A-D, and Figure 3A and B). Furthermore,  
397 late-season measurements of photosynthesis indicate that function of the canopy was

398 retained and carbon uptake maintained (Figure 4). The decline of chlorophyll content  
399 used as an estimate of individual leaf senescence was also significantly reduced  
400 indicating a delay or slowing of the senescence process (Figure 3C). We have shown  
401 that these trees continue to photosynthesize after bud-set and that this process is  
402 enhanced in elevated CO<sub>2</sub>.

403

404 Senescence is a complex process, for which a mechanistic understanding is still  
405 emerging, but is often studied under laboratory conditions using model annual plant  
406 species (Buchanan-Wollaston, *et al.*, 2003; Wingler *et al.*, 2005). These systems may  
407 not entirely represent the complexity of true autumnal senescence for which in  
408 *Populus* response to changing photoperiod is the dominant trigger for the onset of  
409 dormancy (Keskitalo *et al.*, 2005), and this response is dependent upon the latitudinal  
410 origin and genotype of the tree (Böhlenius *et al.*, 2006). Nevertheless, model plants  
411 have enabled us to identify specific aspects of the senescence process that may be  
412 relevant in explaining our data since a number of other environmental variables  
413 influence the progression of senescence and these are also known to influence plant  
414 response to elevated [CO<sub>2</sub>]. They include temperature, light, nitrogen supply, soil  
415 moisture and within plant variables such as physiological responses to carbon and  
416 nitrogen status and the balance between source and sink tissue (Wingler *et al.*, 2006).  
417 This complexity of interactions, combined with the use of small plants in pots or trees  
418 that are sink-limited, as well as varying spatial and temporal estimates of canopy  
419 longevity may help to explain some of the different responses and confusion observed  
420 in the literature to date. However, some consistencies are also apparent, linking  
421 features known to increase leaf longevity with growth in elevated CO<sub>2</sub>. These include  
422 decreased SLA (specific leaf area, indicative of increased leaf thickness), and

423 decreased leaf N<sub>mass</sub> (leaf N on a mass basis) (Reich, Walters, & Ellsworth, 1997;  
424 Wright et al., 2004); increased photosynthetic nitrogen use efficiency (PNUE)  
425 (Escudero, & Mediavilla, 2003) and decreased oxidative stress (Woo *et al.*, 2004).  
426 These changes are commonly observed in elevated CO<sub>2</sub> and are known to have the  
427 potential to extend leaf longevity. Both decreased SLA and leaf N<sub>mass</sub> in response to  
428 elevated CO<sub>2</sub> have been reported for all species studied here (Karnosky et al., 2003;  
429 Tricker et al., 2004) and recently increased PNUE has been documented for *P. x*  
430 *euramericana* in elevated CO<sub>2</sub> at PopFACE during this period of study (Liberloo et  
431 al., 2006). Decreased oxidative stress has been linked with long-term growth in  
432 elevated CO<sub>2</sub> as inferred from a decreased leaf antioxidant pool (Karnosky et al.,  
433 2003; Schwanz, & Polle, 1998). Furthermore climatic conditions during the autumnal  
434 phenophase have previously been shown to result in an increased J<sub>max</sub>:V<sub>cmax</sub>  
435 (Onoda et al., 2005) implying that autumnal photosynthesis was more dependent on  
436 [CO<sub>2</sub>] explaining enhanced carbon uptake for younger leaves in elevated [CO<sub>2</sub>] for  
437 the perennial herb *Polygonum cuspidatum*, during the autumnal period (Onoda et al.,  
438 2005). Thus our data strongly suggest that delayed autumnal senescence may be  
439 linked to a positive photosynthetic C-fixation being maintained in the absence of sink  
440 limitation in elevated CO<sub>2</sub>, particularly so in the fertile soils of these two experiments  
441 exhibiting a typically low C:N for forest soils. This is unlike the nutrient poor soil of  
442 Duke FACE where no senescence response to CO<sub>2</sub> was observed for indeterminate  
443 sweet gum trees (Herrick and Thomas, 2003).

444

445 A second mechanism may also be involved in explaining this phenomenon. Reduced  
446 stomatal conductance is frequently observed in elevated CO<sub>2</sub> (Long, et al., 2004;  
447 Tricker et al., 2005); an effect which may indirectly result in enhanced canopy

448 temperature as the partitioning of absorbed solar radiation between sensible heat and  
449 evapotranspiration is altered (Sellers, et al., 1996). This response of canopy  
450 temperature to elevated CO<sub>2</sub> is observed at SoyFACE, where a significant increase in  
451 temperature by approximately 1 °C was observed in the FACE plots (Long et al.,  
452 2006) and where delayed autumnal decline of LAI was also apparent (Dermody et al.,  
453 2006). However, in a recent open top chamber study using a *Populus* mapping  
454 population, where such temperature effects may be negated between CO<sub>2</sub> and control  
455 treatments, autumnal senescence was delayed by elevated CO<sub>2</sub>. Moreover, areas of the  
456 *Populus* genome were identified as QTL (quantitative trait loci) to explain this  
457 delayed senescence, suggesting that in future it will be possible to identify the genes  
458 underlying this response and that delayed senescence induced by elevated CO<sub>2</sub> is not  
459 the result of enhanced leaf temperature (Rae et al., 2006). A suite of genes associated  
460 with senescence in autumn trees has already been identified in *Populus*. This revealed  
461 a shift from gene expression associated with anabolism to that of catabolism and an  
462 increased role of mitochondria for energy generation as photosynthesis breaks down  
463 (Andersson et al., 2004). Furthermore, in *Populus* during autumnal senescence leaf  
464 pigment contents declined with the most rapid decline for chlorophyll. All pigments  
465 declined except for the flavonoid anthocyanin - this photoprotective compound  
466 increased in concentration during senescence (Keskitalo et al., 2005). Preliminary  
467 data from *P. x euramericana* leaf material described here has identified a number of  
468 gene transcripts that are differentially expressed during the autumnal phenophase,  
469 including those associated with phenylpropanoid metabolism, and anthocyanin  
470 biosynthesis (Tallis et al., pers com), again suggesting a mechanism related to altered  
471 plant metabolism and C:N balance rather than altered leaf temperature. Taking into  
472 account the assumptions of the extended growth/differentiation balance model

473 (GDB<sub>e</sub>) of carbon partitioning discussed in Mattson, Julkunen-Tiitto and Herms  
474 (2005), the autumnal stimulation of photosynthesis reported here may allow excess  
475 carbon to be partitioned to carbon rich secondary metabolites as the demand from  
476 growth reduces in the autumn. This portioning to secondary metabolites may also  
477 have a positive influence on leaf retention and carbon uptake during senescence  
478 particularly considering the increased synthesis of photoprotective compounds as  
479 discussed above.

480 Using a <sup>14</sup>CO<sub>2</sub> label Nelson and Isebrands (1983) showed that late season leaves  
481 retained on trees after bud-set in a short rotation poplar coppice exhibited  
482 photosynthetic rates high enough to contribute important quantities of photosynthate  
483 for continued radial stem growth, root growth and reserve storage in the stems and  
484 roots. Therefore and irrespective of mechanism, we were keen to understand how this  
485 extended autumnal phenophase might affect seasonal gross primary productivity. A  
486 delay in the 50 % decline of VAI after bud set of between 5 to 15 days for *P. x*  
487 *euramericana* in 2003 and 2004 respectively, and 12 days for *P. nigra* in 2004 was  
488 calculated and confirmed that estimated by an independent analysis of canopy leaf  
489 retention (Tricker et al., 2004, An elevated CO<sub>2</sub> extension to the autumnal phenophase  
490 by 10 days was estimated to contribute approximately 2 % to the annual GPP of *P. x*  
491 *euramericana* growing in a managed SRC plantation in central Italy (Figure 5). This  
492 is similar to data reported by Goulden et al., (1996) for a mature mixed oak and maple  
493 stand in New England when gross ecosystem exchange of carbon for a 5 to 10 day  
494 delay in senescence was 0.5 - 0.9 % that of the whole season. Contemporary climatic  
495 changes have already been reported to reduce the carbon sink strength of many  
496 northern hemisphere forests during recent hot dry summer months (Bunn & Goetz  
497 2006; Angert et al., 2005). Here, an extension in the growing season through

498 increased canopy longevity and carbon gain may provide an increased sink for  
499 atmospheric carbon (Keeling et al., 1996; Lucht et al., 2002), although this requires  
500 further investigation. In contrast, on a global scale an extended growing season may  
501 contribute to global warming due to decreasing surface albedo (Betts, 2000).

502

503 The need to incorporate a dynamic growing season length in predictive models of  
504 forest productivity has previously been identified (White et al., 1999). Data presented  
505 here supports that need and further identifies the effects of changing [CO<sub>2</sub>] on plant  
506 phenology as a variable to be considered when modeling forest productivity and  
507 biosphere-atmosphere interactions. In the case of Kyoto forests, future sink capacity  
508 may be influenced by phenological responses to elevated CO<sub>2</sub> that are independent of  
509 response to temperature and have not previously been recognized as important. This  
510 study has provided clear evidence that future rising CO<sub>2</sub> affects autumnal phenology  
511 directly. The mechanisms remain to be elucidated, but the phenomenon should be  
512 considered in future predictive models on the effects of climate change on temperate  
513 forest productivity.

514

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799 **Figure Legends**

800 **Figure 1. The late season effects of elevated CO<sub>2</sub> on Leaf Area Index (LAI).**

801 Stand LAI in elevated CO<sub>2</sub> (open circles) and control plots (closed circles) at the  
802 AspenFACE (A), and PopFACE (B) experiments. Also shown for each site is the %  
803 stimulation of late season stand LAI, where stimulation =  $[(LAI_{Elevated\ CO_2} -$   
804  $LAI_{Control}) / LAI_{Control}] * 100$ ].

805

806 **Figure 2. The effect of elevated CO<sub>2</sub> on foliar senescence and abscission at**

807 **AspenFACE and PopFACE.** In a mixed aspen/maple stand at AspenFACE (A,  
808 Control; B, elevated CO<sub>2</sub> with images taken on October 5, 2004) and in a mixed  
809 aspen/birch stand at AspenFACE, from hemispherical fisheye photographs (C,  
810 Control; D, elevated CO<sub>2</sub>) taken late in the growing season (early October, 2002). (E)  
811 The mean and SE are shown representing canopy Leaf Area Index (LAI\*) for both *P.*  
812 *x euramericana* and *P. nigra* in 2004 at PopFACE Tuscania. Leaf Area Index (LAI\*)  
813 was calculated as the difference between optical estimates of both VAI (Vegetation  
814 Area Index) and WAI (Woody Area Index).

815

816 **Figure 3. Remote sensing of senescence in *Populus* at PopFACE, central Italy.**

817 (A) NDVI (the Normalized Differential Vegetation Index) measured using a wide  
818 bandwidth tri-band multispectral camera from a Sky Arrow aircraft on 1<sup>st</sup> November  
819 2003 and 25<sup>th</sup> October 2004. NDVI is represented for *P. nigra* (x) and *P. x*  
820 *euramericana* (y) in both years and treatments, by the colour scale, as shown. (B) The  
821 mean decline of canopy NDVI taken between 1<sup>st</sup> October and 26<sup>th</sup> October 2004  
822 measured with the GER 3700 at 1 m above the canopy in both elevated CO<sub>2</sub> (open)

823 and control (closed) treatments. (C) The mean decline in extracted leaf chlorophyll.  
824 Decline in leaf chlorophyll was measured between 21<sup>st</sup> September and 18<sup>th</sup> October  
825 for *P. x euramericana* and 21<sup>st</sup> September to 2<sup>nd</sup> November for *P. nigra*.

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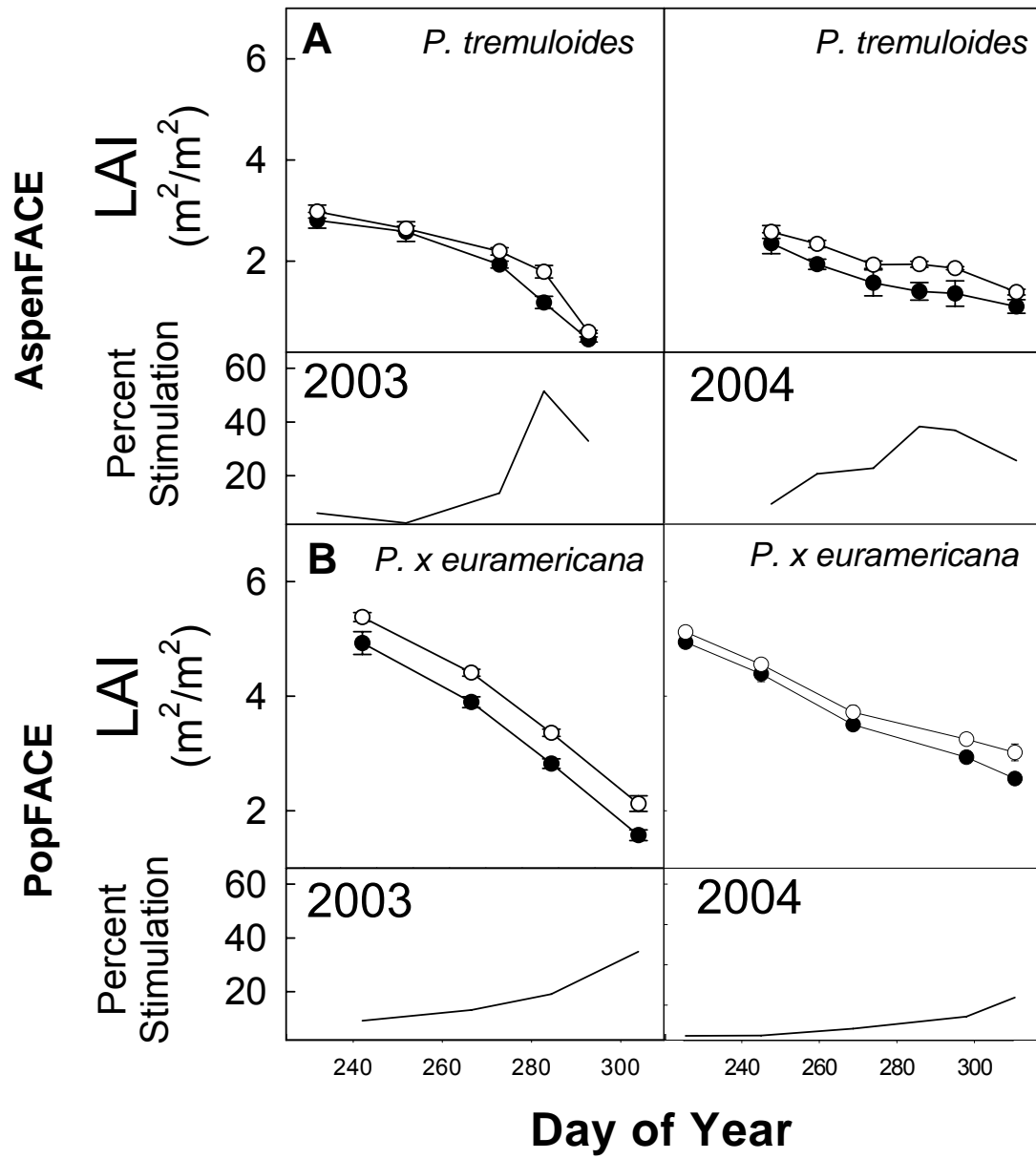
827 **Figure 4. The effects of elevated CO<sub>2</sub> on late season light saturated**  
828 **photosynthesis.** Light saturated photosynthesis in elevated CO<sub>2</sub> (open) and control  
829 treatments for *P. nigra* at PopFACE (A) and for *P. tremuloides* genotype 42E (B) and  
830 *P. tremuloides* genotype 271 (C) at AspenFACE.

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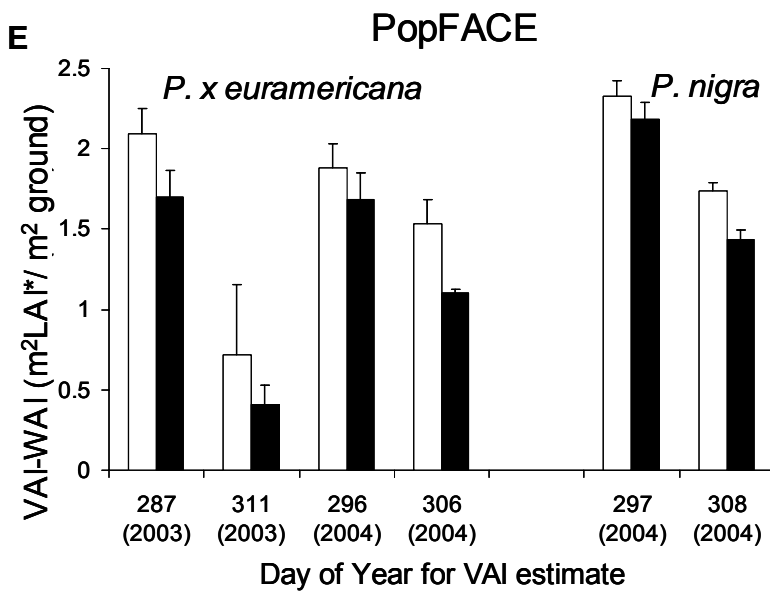
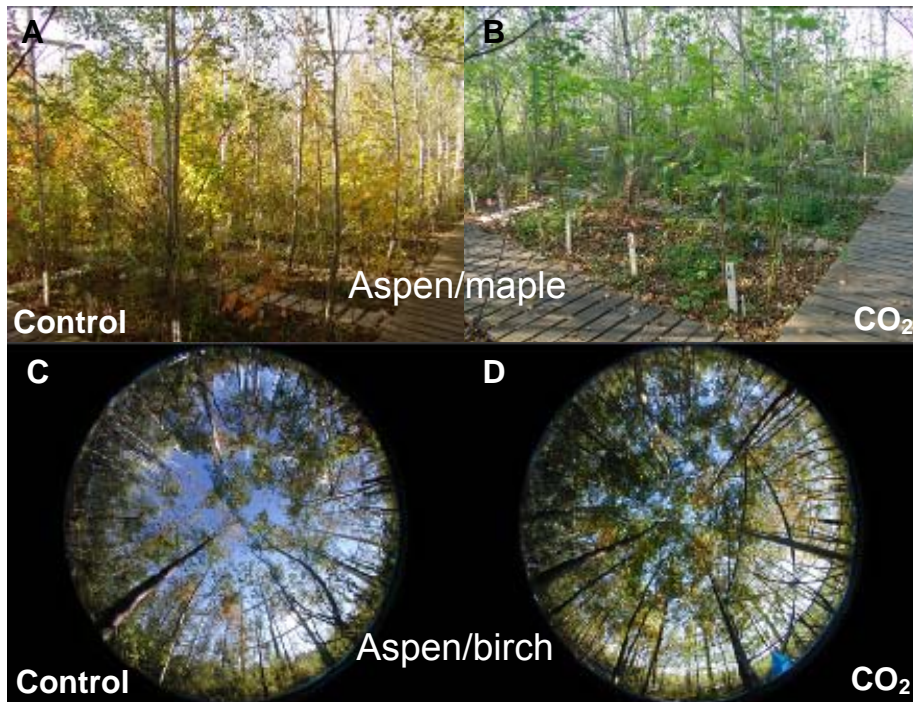
832 **Figure 5. The daily gross primary production for a closed canopy *P. x***  
833 ***euramericana* growing at 550 ppm [CO<sub>2</sub>] from the modeled data of Wittig *et al***  
834 **(2005).** The cubic relationship between daily GPP and day of year in elevated [CO<sub>2</sub>]  
835 is displayed (----). Advancing the end date by 10 days is represented (—) and the  
836 influence of this on seasonal GPP is calculated as the difference between the areas  
837 under the two curves. The result is an estimate of the seasonal GPP contributed from  
838 lengthening of the autumnal phenophase as a consequence of increased atmospheric  
839 [CO<sub>2</sub>].

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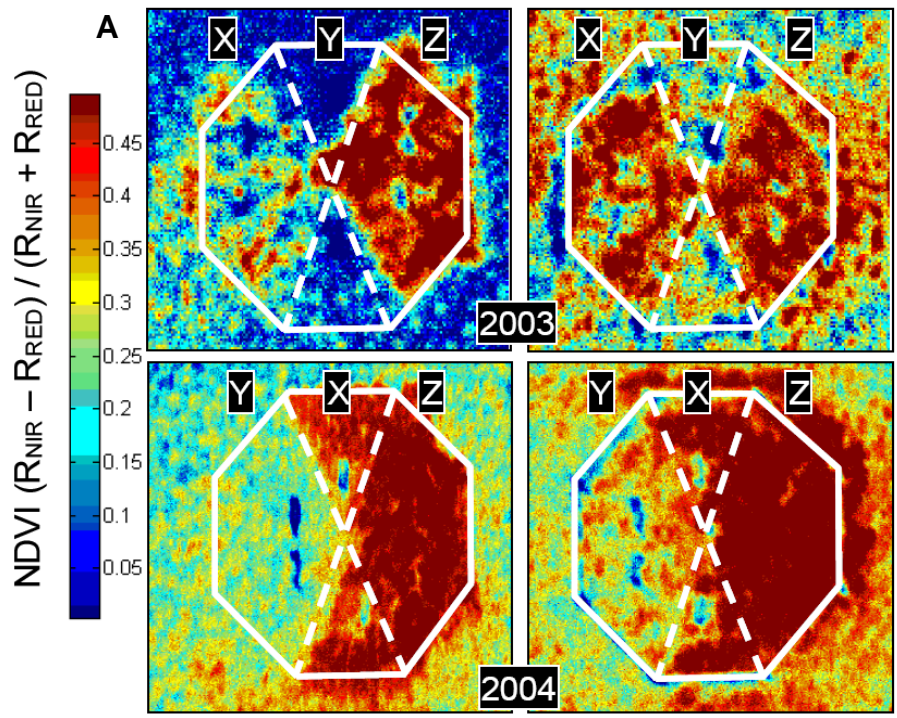
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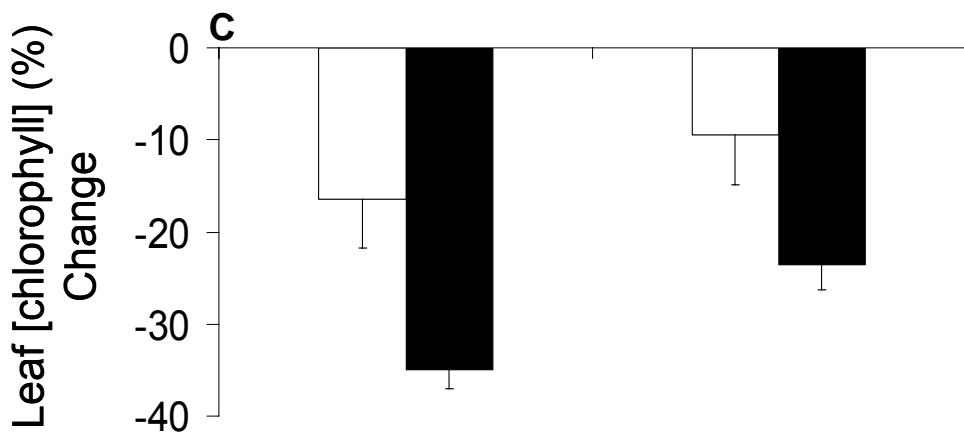
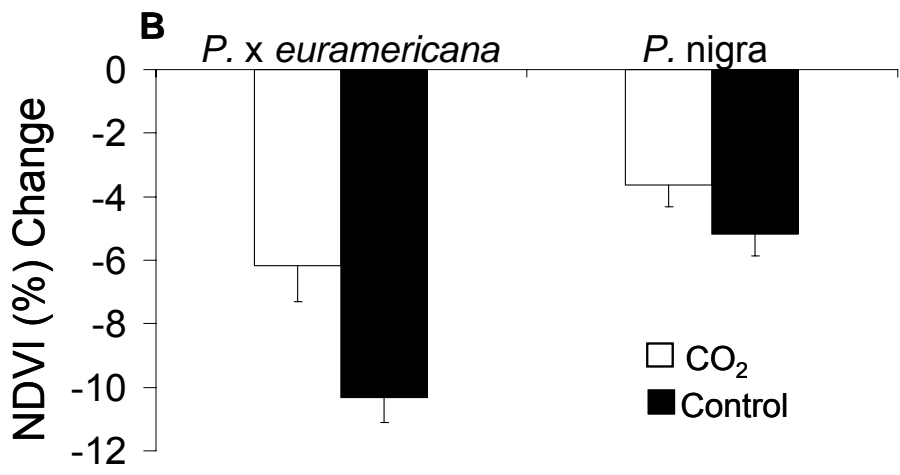
### AspenFACE



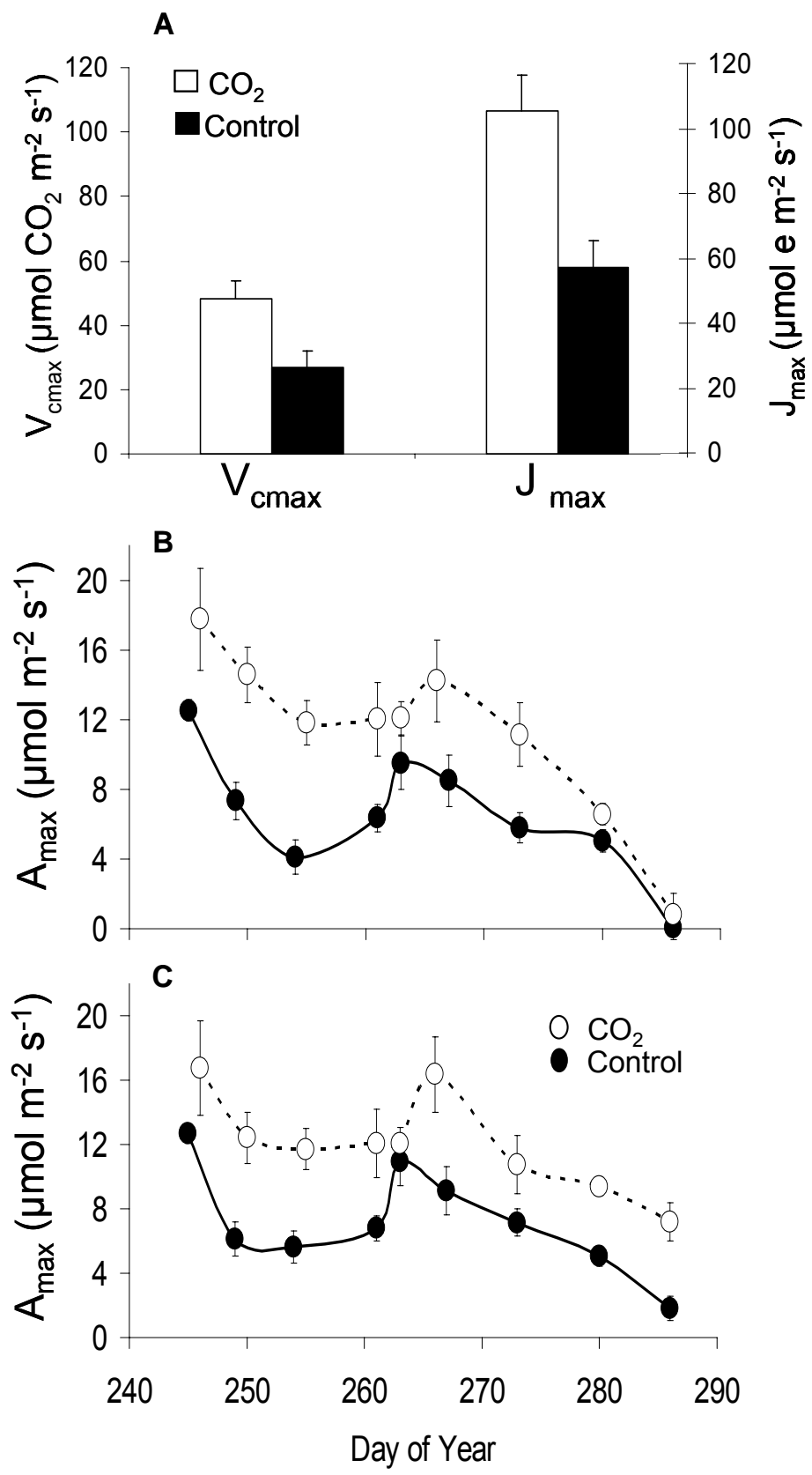
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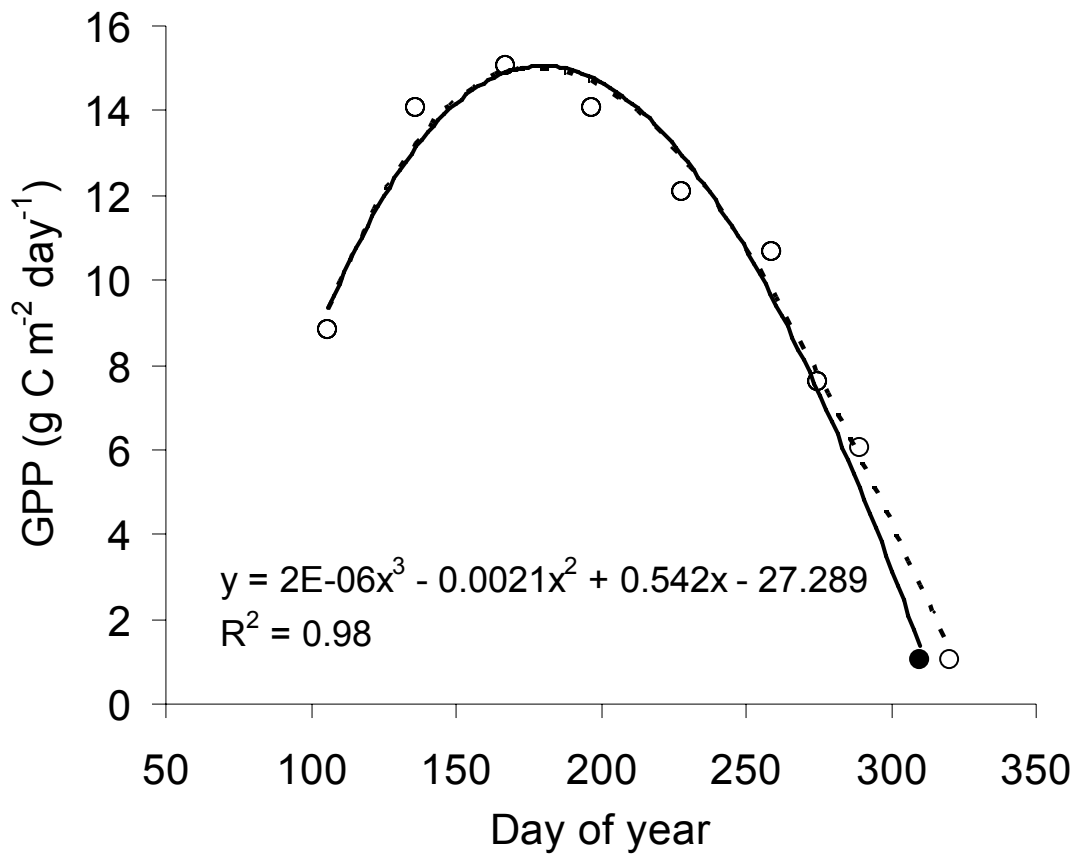


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