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Future atmospheric CO_2 leads to delayed autumnal senescence

GAIL TAYLOR*[1], MATTHEW J. TALLIS*[1], CHRISTIAN P. GIARDINA†, KEVIN E. PERCY‡, FRANCO MIGLIETTA§, POOJA S. GUPTA¶, BENIAMINO GIOLI§, CARLO CALFAPIETRA‖**, BIRGIT GIELEN††, MARK E. KUBISKE‡‡, GIUSEPPE E. SCARASCIA-MUGNOZZA‖**, KATRE KETS§§, STEPHEN P. LONG¶¶ and DAVID F. KARNOSKY¶

*School of Biological Sciences, University of Southampton, Bassett Crescent East, S016 7PX, UK, †North Central Research Station, USDA Forest Service, Houghton, Michigan 49931-1295 USA, ‡Natural Resources Canada, Canadian Forest Service-Atlantic Forestry Centre, PO Box 4000, Fredericton, NB, Canada E3B 5P7, §Institute of Biometeorology-CNR, Via Caproni, 8 50145 Firenze, Italy, ¶School of Forest Resources & Environmental Science, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931-1295, USA, ‖Department of Forest Environment and Resources (DISAFRI), University of Tuscia, Via S. Camillo De Lellis, Viterbo 01100, Italy, **Institute of Agro-Environmental & Forest Biology (IBAF), National Research Council (CNR), Via Salaria km 29, 300, 00016 Monterotondo Scalo, Roma, Italy, ††Research Group of Plant and Vegetation Ecology, Department of Biology, University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, Wilrijk B-2610, Belgium, ‡‡Forestry Sciences Lab, North Central Research Station, USDA Forest Service, 5985 Highway K, Rhinelander, WI 54501 USA, §§Institute of Botany & Ecology, University of Tartu, 40 Lai Str, 51005 Tartu, Estonia, ¶¶190 Edward R. Madigan Laboratory, Departments of Plant Biology, University of IL at Urbana, 1201 West Gregory Drive, Champaign, Illinois 61801-4798, USA

Abstract

Growing seasons are getting longer, a phenomenon partially explained by increasing global temperatures. Recent reports suggest that a strong correlation exists between warming and advances in spring phenology but that a weaker correlation is evident between warming and autumnal events implying that other factors may be influencing the timing of autumnal phenology. Using freely rooted, field-grown *Populus* in two Free Air CO_2 Enrichment Experiments (AspenFACE and PopFACE), we present evidence from two continents and over 2 years that increasing atmospheric CO_2 acts directly to delay autumnal leaf coloration and leaf fall. In an atmosphere enriched in CO_2 (by $\sim$45% of the current atmospheric concentration to 550 ppm) the end of season decline in canopy normalized difference vegetation index (NDVI) – a commonly used global index for vegetation greenness – was significantly delayed, indicating a greener autumnal canopy, relative to that in ambient CO_2. This was supported by a significant delay in the decline of autumnal canopy leaf area index in elevated as compared with ambient CO_2, and a significantly smaller decline in end of season leaf chlorophyll content. Leaf level photosynthetic activity and carbon uptake in elevated CO_2 during the senescence period was also enhanced compared with ambient CO_2. The findings reveal a direct effect of rising atmospheric CO_2, independent of temperature in delaying autumnal senescence for *Populus*, an important deciduous forest tree with implications for forest productivity and adaptation to a future high CO_2 world.

Keywords: autumnal phenology, elevated CO_2, FACE, LAI, *Populus*, senescence

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Introduction

The timing of phenological events for many woody and herbaceous plants in mid to upper latitudes has changed, with significant advances in spring bud break and similarly significant delays in autumn leaf color change and leaf fall resulting in an extension of the growing season (Menzel & Fabian, 1999; Parmesan & Yohe, 2003; Root *et al*., 2003). Remote sensing of vegetation using the normalized difference vegetation index (NDVI – a measure of vegetation greeness) shows an 18-day

Correspondence: Gail Taylor, e-mail: G.Taylor@soton.ac.uk

[1]These authors contributed equally to this work.

extension of the growing season in Eurasia between 1982 and 1999 and a 12-day extension in North America (Zhou *et al*., 2001). These changes have been attributed to warmer temperatures causing longer growing seasons (Menzel & Fabian, 1999; Peñuelas & Filella, 2001; Zhou *et al*., 2001; Root *et al*., 2003). In the most comprehensive meta-analysis to date, consisting of 125 000 observations from 21 European countries, taken between 1971 and 2000 and using 542 plant species, Menzel *et al*. (2006) report the correlation between an advanced spring phenophase and warming patterns of 19 European countries was strong and significant ($r = -0.69$, $P < 0.001$). However, the association between warming and the autumnal phenophase was described by Menzel *et al*. (2006) as 'vague.' The correlation between leaf color change and fall and the temperature trends for 14 European countries was weak and non-significant ($r = 0.003$, $P = 0.99$). Moreover, of the leaf coloring events, only 52% were delayed and only 15% of these were significant. This contrasts sharply with that of spring events which showed 78% were earlier and that 31% of these were significant. Nevertheless, across Europe, during the last 30 years, autumnal senescence has been delayed by between 1.3 and 1.8 days decade^{-1} (Menzel *et al*., 2006 and Menzel & Fabian, 1999, respectively). It is notable that while warming has been unevenly distributed both spatially and temporally, it is the rise in global [CO_2] that has recently been more in synchrony with changing autumnal phenological patterns reported across wide geographic regions. For this reason, we hypothesized that elevated atmospheric [CO_2] could affect the timing of autumnal senescence directly and independent of temperature. We investigated this by utilizing two large-scale forest ecosystem experiments, one in the United States and one in Italy, where *Populus* trees were exposed to elevated CO_2, from planting to maturity, thus providing a unique experimental resource in which to determine the effect of future CO_2 on autumnal senescence.

From previous research into the autumnal phenophase of forest trees, there has been large variability in response to elevated [CO_2], with advances (Jach & Ceulemans, 1999; Sigurdsson, 2001; Norby *et al*., 2003; Körner *et al*., 2005), delays (Li *et al*., 2000; Karnosky *et al*., 2003; Körner *et al*., 2005; Rae *et al*., 2006), or no effect (Herrick & Thomas, 2003) all reported. A similar variability in response is observed in annual plants, and has been linked to the determinate nature of plant development. Species with determinate growth often show early senescence in elevated [CO_2] such as barley (Fangmeier *et al*., 2000) and tobacco (Miller *et al*., 1997) as the plant approaches maturity more quickly, whilse those with indeterminate growth, such as soybean, show delayed senescence in elevated CO_2 (Miglietta *et al*., 1993; Dermody *et al*., 2006). The determinate nature of growth may lead to the down-regulation of photosynthesis in elevated relative to ambient CO_2 as a consequence of reduced sink demand for photoassimilate (Ainsworth *et al*., 2004). Such plants may be considered 'sink limited' where this is defined as 'an increased abundance of mobile carbon compounds associated with a reduced growth capacity or inability to utilize mobile carbon compounds' after Hoch & Körner (2003). By artificially manipulating the source-sink balance of *Arabidopsis* Wingler *et al*. (2005) showed that for plants grown on 2% glucose and a 30 mM nitrogen (N) agar exhibited delayed senescence, while for plants grown on 2% glucose with a reduced nutrient (4.7 mM N) agar, senescence was advanced. It was concluded that increased sugar accumulation in the leaves of N deficient *Arabidopsis* (advanced senescence phenotype) could be the result of decreased sugar utilization for the synthesis of N demanding amino acids and proteins; an increased source to sink ratio promoting senescence and mediated through N availability (Pourtau *et al*., 2004). Herrick & Thomas (2003) hypothesize that for species which show an increased net photosynthesis in elevated CO_2, the increased C : N ratio of such leaves will result in delayed autumnal senescence. In other words, a later season positive leaf carbon balance will result in delayed senescence when stimulated photosynthetic uptake in elevated CO_2 is sustained. These previous studies give a glimpse of the complexities of the senescence process but also begin to provide some mechanistic explanations that may enable us to make generalizations about autumnal senescence in a high CO_2 world.

In this study freely rooted *Populus* species were chosen which had been grown from initiation in elevated CO_2 using Free Air CO_2 Enrichment (FACE) technology for between 6 and 7 years. The trees at these two sites have responded positively to growth in elevated CO_2 exhibiting increases in biomass (Karnosky *et al*., 2003; Liberloo *et al*., 2006) and limited down-regulation of photosynthesis (Karnosky *et al*., 2003; Calfapietra *et al*., 2005; Davey *et al*., 2006) and, therefore, were not considered to exhibit sink-limited growth. To determine whether an elevated [CO_2] at that predicted for around 2050, extended the autumnal phenophase and increased canopy duration, we examined the autumnal decline in leaf area index (LAI), NDVI and photosynthetic function at the AspenFACE site in Wisconsin, USA, and the PopFACE site in Tuscania, Italy during 2 consecutive years.

Materials and methods

The AspenFACE experiment

Design. The AspenFACE experiment (32 ha) is situated on sandy loam glacial outwash soil in northern

Wisconsin, near Rhinelander (45°06′N, 89°07′W; 490 m a.s.l.; www.aspenface.mtu.edu). The system has been used to fumigate (1997–2006) aggrading trembling aspen (*Populus tremuloides* Michx.; five genotypes), mixed white birch (*Betula papyrifera*)/trembling aspen and mixed sugar maple (*Acer saccharum*)/trembling aspen stands with elevated atmospheric CO_2 at a concentration of 560 μmol mol^{-1} CO_2. Before planting soil N content was 15.06 μg N g^{-1} soil (NO_3^-–N) and 1.03 μg N g^{-1} soil (NH_4^+–N) mean for control plots and 15.24 μg N g^{-1} soil (NO_3^-–N) and 0.94 μg N g^{-1} soil (NH_4^+–N) mean for elevated CO_2 plots. The mean C : N ratio for the control and CO_2 enrichment FACE plots was 12.6 (Dickson *et al*., 2000). Complete design and performance characteristics of Aspen FACE and a summary of responses are available elsewhere (Karnosky *et al*., 2003). The mean minimum temperature throughout October 2003 was 2.2 °C and the mean maximum was 11.4 °C, whereas in 2004 for the same time period mean minimum temperature was 3.3 °C and maximum 12.0 °C. The lowest daily mean minimum temperature during October 2003 was on day 275 (−5.0 °C), and during October of 2004 was on day 279 (−2.6 °C).

Canopy and leaf characterization. Near hemispherical photographs with a 148° field of view were taken every 10–20 days during the 2001–2004 growing seasons using a fish-eye lens equipped Nikon LC-Erland Nikon E950 digital camera (Nikon Inc., Melville, NY, USA), analyzed with WinSCANOPY software (Regent Instruments, Quebec, Canada), and exported to XLScanopy (Regent Instruments) for conversion into LAI. We used software-provided Bonhomme assumptions (Welles & Norman, 1991) for estimating LAI for each of the five elevations from horizontal. Although errors are associated with the use of hemispherical images for LAI estimation, these estimates of LAI were strongly correlated with both harvest-based allometrically determined LAI and litter trap-based estimates of LAI ($R^2 = 0.67$, $P < 0.01$), and identified similar seasonal and treatment patterns for the trace gas treatments at AspenFACE (unpublished data of the authors). On each date, three estimates of LAI per ring section were averaged, resulting in six measurements for each of the four measurement periods [three ambient and three elevated (CO_2)]. A shift in the autumnal phenophase was calculated as the number of days from maximum to 50% of maximum LAI for CO_2 plots vs. control plots. Light-saturated photosynthesis was measured using a portable gas exchange system (Li-Cor 6400; Li-Cor Inc., Lincoln, NE, USA) for upper canopy aspen leaves (three leaves per clone, two clones, over nine time points during the senescence period). One block of each paired plot was examined at each time point. This was to restrict the diurnal influence on the *in situ* measurements and enable data from across the whole site to be collected during the time course of autumnal senescence.

The PopFACE experiment

Design. The PopFACE experiment (9 ha) is situated on a nutrient rich, clay soil in Tuscania, Italy (42°22′N, 11°48′E; altitude 150 m a.s.l.; www.unitus.it/euroface.) Three species of *Populus, Populus alba*, *P. nigra*, and *P.* × *euramericana* were grown in the experiment which comprises three blocks containing six, 314 m^2 octagonal plots assigned to treatments of [CO_2] (ambient 372 and 550 μmol mol^{-1}). Complete design characteristics of PopFACE are available elsewhere (Miglietta *et al*., 2001). Briefly, planting was carried out in 1999 and trees coppiced 3 years later. After coppice, one sub-plot received N fertigation and the other remained at ambient soil N, only data from the ambient N fertigation treatments are reported here. For the unfertilized sub-plots and measured before planting, mean soil N content was 9.73 μg N g^{-1} soil (NO_3^-–N) and 0.73 μg N g^{-1} soil (NH_4^+–N) mean for control plots, and 7.17 μg N g^{-1} soil (NO_3^-–N) and 0.59 μg N g^{-1} soil (NH_4^+–N) mean for elevated CO_2 plots (Liberloo *et al*., 2006) and the mean C : N of the site was 9.3 (Hoosbeek *et al*., 2004). In 2004 the N content for the unfertilized sub-plots was 12.8 μg N g^{-1} soil (NO_3^-–N) and 1.86 μg N g^{-1} soil (NH_4^+–N) mean for control plots, and 6.65 μg N g^{-1} soil (NO_3^-–N) and 1.47 μg N g^{-1} soil (NH_4^+–N) mean for elevated CO_2 plots (Liberloo *et al*., 2006). During the period of study (2003–2004), trees had been planted for between 5 and 6 years and a closed canopy was evident. The mean minimum temperature throughout October 2003 was 10.8 °C and the mean maximum was 21.6 °C, whereas in 2004 for the same time period mean minimum temperature was 14.2 °C and mean maximum 25.0 °C. During the period of study in 2003 the lowest temperature was apparent on Julian day 300 (3.7 °C), whereas 2004 was characterized by an unseasonably warm autumn and the lowest temperature was noted on Julian day 273 (8.8 °C).

Canopy and leaf characterization. LAI measurements were made of the *P.* × *euramericana* and *P. nigra* canopies (2003, *P.* × *euramericana* only) using a Li-Cor LAI-2000 (Li-Cor Inc.). This technique has been previously correlated with harvest-based allometrically determined LAI for these species at this site (Liberloo *et al*., 2004). Following a reference value which was obtained in open skies clear of the canopy, 14 below canopy measures as described in Gielen *et al*. (2003) were

taken and this was replicated four times per sub-plot for each of the six experimental plots, at each time of measurement. Optical values of LAI (estimated using the Li-Cor LAI-2000) theoretically include stems and branches and are, therefore, considered an estimate of the above ground vegetation area index (VAI). Following total leaf fall an estimate of wood area index, (WAI) (m^2 of woody tissue m^{-2} of ground) was conducted in the same way for each species and year except that the function A/B = 1 was set to allow for no foliage (LI-COR, 1990). LAI* was re-estimated by subtracting the WAI from the VAI for the final two estimates of VAI in both years. A shift in the autumnal phenophase was calculated as the number of days difference for a 50% decline of the VAI (assuming decline was related with leaf fall) measured at bud-set for CO_2 plots vs. control plots.

Towards the end of the growing season (October 15, 2003, October 1 and October 23, 2004 for *P.* × *euramericana*, October 2 and October 26, 2004 for *P. nigra*), ground-based canopy reflectance was measured 1.0 m above the canopy using a field portable spectroradiometer (GER, Buffalo, NY, USA, Mod. 3700; range 350–1050 nm). Airborne measurements were made using a multispectral camera equipped with a single optic (Duncan Tech, Geospatial Systems Inc., Rochester, NY, USA, Mod. MS4100) operated at three wide bandwidths centered on 550, 680 and 800 nm. The camera (field of view of 60°) was mounted on a certified aircraft (Sky Arrow 650TCNS, Rome, Italy) flying at 200 m above the experimental area. NDVI was calculated as $(R_{NIR}-R_{RED})/(R_{NIR}+R_{RED})$ for the airborne. For the narrow bandwidth ground-based spectral measurements NDVI was calculated using $(R_{800}-R_{680})/(R_{800}+R_{680})$ (the center of the wavebands used for the airborne calculation) and a chlorophyll specific NDVI was calculated as $(R_{750}-R_{705})/(R_{750}+R_{705})$ (Gamon & Surfus, 1999), a robust predictor of *Populus* chlorophyll content ($r^2 = 0.98$, $P < 0.001$, $n = 97$; M. J. Tallis *et al*., unpublished results).

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

Estimating GPP. Data from Wittig *et al*. (2005) generated using the WIMOVAC model were extracted. The mean monthly GPP ($g\,C\,m^{-2}\,day^{-1}$) of *P.* × *euramericana* exposed to elevated [CO_2] at the PopFACE site and estimated in 2001 when a closed canopy existed were used in this analysis. The relationship between day of year (x axis) and GPP (y axis) was explained by a cubic function in the form $y = 2E^{-06}x^3 - 0.0021x^2 + 0.542x - 27.289$, $r^2 = 0.98$. The mean number of days shift in the autumnal phenophase (10 day advanced in control conditions) of *P.* × *euramericana* calculated in this study was applied to the model. During the time of the study reported here, no photosynthetic data profiles were available for *P.* × *euramericana* therefore the original model could not be run for this data. Instead, the parabola relationship from the 2001 data was shifted to account for a change in phenology. Assuming that any phenological shifts resulting from elevated CO_2 would be accounted for in this relationship, the GPP resulting from an elevated CO_2 stimulated shift in the autumnal phenophase was estimated as the difference between the areas under the two curves.

Statistical analysis. The AspenFACE experiment has three replications for each species, organized in three randomized complete blocks. The PopFACE experiment has three replications for each species, organized in a factorial block design. Late season canopy data from the two FACE sites were analyzed in two ways, with both approaches showing highly significant differences between ambient and elevated [CO_2] treatments. In the first approach, we relied on univariate analysis of variance (ANOVA) to examine treatment effects on LAI at the end of the growing season for AspenFACE, and PopFACE. Late season LAI effects for the two sites were quantified by integrating the area under the curve for the last three measurement periods for each site and year (Fig. 1) for control and elevated [CO_2] treatments. The presence of site heterogeneity at a single time point of measurement at the PopFACE site lead to type I error and this was reduced by including random block as a tested factor. As both CO_2 treatment and random block were now tested factors, the CO_2 effect was sought from average within-block differences, therefore, it was not obscured by the natural heterogeneity which existed between blocks as discussed in Tricker *et al*. (2005). Where no treatment interaction with block was evident at $P \leq 0.25$ (Underwood, 1997), *post hoc* pooling was carried out as detailed in Tricker *et al*. (2005). Here, the F ratio was constructed by summating the block × CO_2 interaction sum of squares with that of the error, and the denominator df adjusted accordingly. Both the F ratio and P values are given for the average within block

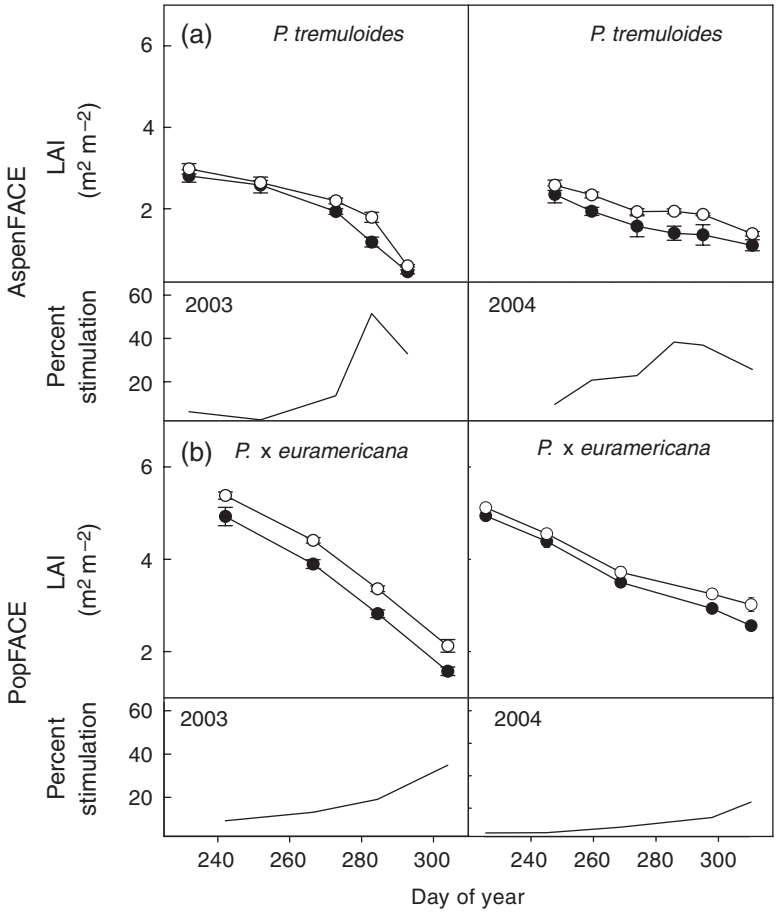

Fig. 1 The late season effects of elevated CO_2 on leaf area index (LAI). Stand LAI in elevated CO_2 (open circles) and control plots (closed circles) at the AspenFACE (a), and PopFACE (b) experiments. Also shown for each site is the % stimulation of late season stand LAI, where stimulation = $[(LAI_{Elevated\,CO_2} - LAI_{Control}/LAI_{Control}) \times 100]$.

differences and the pooled data. Data were analyzed using ANOVA carried out in Minitab 14.0 (Minitab Ltd, Coventry, UK). To further reduce the influence of site heterogeneity (particular evident during senescence) the percentage change of the response variables of canopy reflectance and leaf chlorophyll content of each plot over the time course of the autumnal phenophase were calculated, therefore, normalizing the initial value of the plot to zero. Following arcsine transformation the percentage change data were analyzed using the same model, and plot was the unit of replication.

Results

Across the two FACE experiments, elevated [CO_2] enhanced late season LAI by 20–50% compared with ambient [CO_2] (Fig. 1a and b). In Wisconsin, elevated [CO_2] extended autumn leaf retention by 10–40% in pure stands of trembling aspen (*P. tremuloides*) (Fig. 1a and b), by 8–48% in mixed stands of aspen and birch (*B. papyrifera*) (Fig. 2a and b), and by 17–32% in mixed stands of aspen and maple (*A. saccharum*) (Fig. 2c and d). In Tuscania, stands of *P.* × *euramericana* grown under elevated [CO_2] exhibited a 10% enhancement in LAI in the late summer/early autumn, and a 15–35% enhancement at the close of the growing season (Fig. 1b). Late season LAI effects at AspenFACE and VAI effects at PopFACE were quantified by integrating the area under the curve for the last three measurement periods for each site and year (Fig. 1b) for control and elevated [CO_2] treatments. These analyses showed that the effects of elevated [CO_2] on late season phenology were significant ($P = 0.016$) at the two FACE sites for 2003 and 2004. There were no CO_2 by site or CO_2 by year interactions ($P = 0.535$, $P = 0.585$, respectively) – data not shown. Interestingly it appears from Fig. 1a that in ApsenFACE, leaf fall in both 2003 and 2004 was increased dramatically at approximately DOY 280, an effect that was not apparent in PopFACE. It seems likely

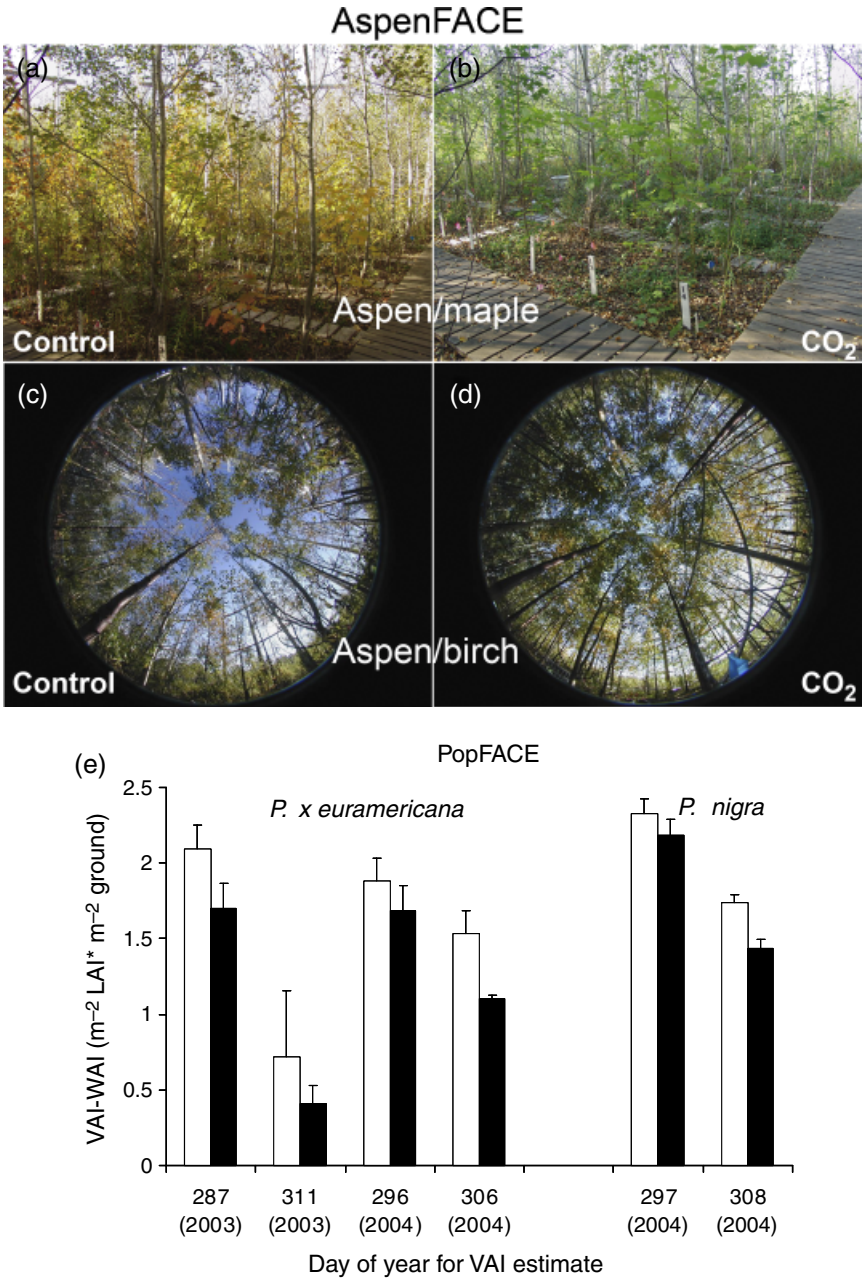

Fig. 2 The effect of elevated CO_2 on foliar senescence and abscission at AspenFACE and PopFACE. In a mixed aspen/maple stand at AspenFACE (a, control; b, elevated CO_2 with images taken on October 5, 2004) and in a mixed aspen/birch stand at AspenFACE, from hemispherical fisheye photographs (c, control; d, elevated CO_2) taken late in the growing season (early October, 2002). (e) The mean and SE are shown representing canopy Leaf Area Index (LAI*) for both *P.* × *euramericana* and *Populus nigra* in 2004 at PopFACE Tuscania. leaf area index (LAI*) was calculated as the difference between optical estimates of both vegetation area index (VAI) and woody area index (WAI).

that this represents a response to differences in the rate of photoperiod and temperature decline at the two sites.

In order to test our hypothesis that rising [CO_2] was primarily responsible for increasing growing season length as opposed to air temperature (warming), we examined 2002–2004 AspenFACE data from our three control and three CO_2 rings. We determined a positive, statistically significant ($P = 0.001$) Pearson's correlation between cumulative seasonal CO_2 exposure (ppm h) and aspen canopy, end of season LAI (LAI_{end}; 1 week before initiation of leaf fall). We found no relationship ($P = 0.797$) between LAI_{end} and temperature summed

as growing degree days (924 days, 2002; 807 days, 2003 and 749 days, 2004 to base 10 °C) or between LAI_{end} ($P = 0.419$) and precipitation amount (499 mm, 2002; 240 mm, 2003; 307 mm, 2004) – data not shown.

The digital photography from the AspenFACE site confirms the stimulation of LAI calculated from optical estimates and also identifies a delay in foliar senescence in the canopies exposed to elevated CO_2 (Fig. 2a and d). After removal of the contribution of WAI to the VAI estimates, the above ground stimulation of LAI* at the PopFACE site during the autumnal phenophase was also identified as resulting from an increase in LAI in elevated CO_2 for both species and both years. This increased LAI* was 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, $P \leq 0.05$) for *P.* × *euramericana* and day 308 of 2004 ($F_{1,2}$ 18.59, $P \leq 0.05$) for *P. nigra* (Fig. 2e).

At PopFACE, the phenological indicator NDVI also showed that both *P.* × *euramericana* and *P. nigra* canopy greenness was likewise extended under elevated [CO_2]. Using false color imagery, we were able to visually display the stimulation of NDVI as the brightest red color in both 2003 and 2004 (Fig. 3a), and at PopFACE the decline in late-season NDVI characteristic of autumnal senescence was significantly reduced ($F_{1,2}$ 13.27, $P = 0.068$, from average within block differences and $F_{1,8}$ 11.18, $P \leq 0.01$, from *post hoc* pooling, Fig. 3b). The decline in the chlorophyll-specific NDVI (Gamon & Surfus, 1999) was greater than that of NDVI during the autumnal phenophase. The decline in chlorophyll-specific NDVI was also significantly reduced ($F_{1,2}$ 25.71, $P \leq 0.05$, from average within block differences and $F_{1,8}$ 32.85, $P \leq 0.001$, from *post hoc* pooling) by growth in elevated [CO_2] (data not shown) a trend that was supported by the decline in extracted leaf chlorophyll content which was also significantly reduced ($F_{1,2}$ 5.20, $P = 0.15$, from average within block differences and $F_{1,8}$ 45.16, $P \leq 0.001$, from *post hoc* pooling) by growth in elevated [CO_2] (Fig. 3c). A similar finding was identified in 2003. Between September 24 and October 16, 2003, only two of the three blocks were harvested, and over this time a 37% (*P.* × *euramericana*) and a 19% (*P. nigra*) increase in leaf chlorophyll content in elevated CO_2, respective to control, was observed.

Late season carbon uptake was stimulated in elevated [CO_2] at both sites for all species (Fig. 4a–c). However, the duration of this stimulation appeared genotype specific at the AspenFACE site (Fig. 4a and b). This was evident by measurements of light-saturated photosynthesis for upper canopy leaves of aspen genotypes 271 and 42E. Elevated CO_2 stimulated photosynthesis throughout the early-mid autumnal phenophase (day of year 246–273) for both genotypes. The late autumn stimulation of photosynthesis was between 30% (42E)

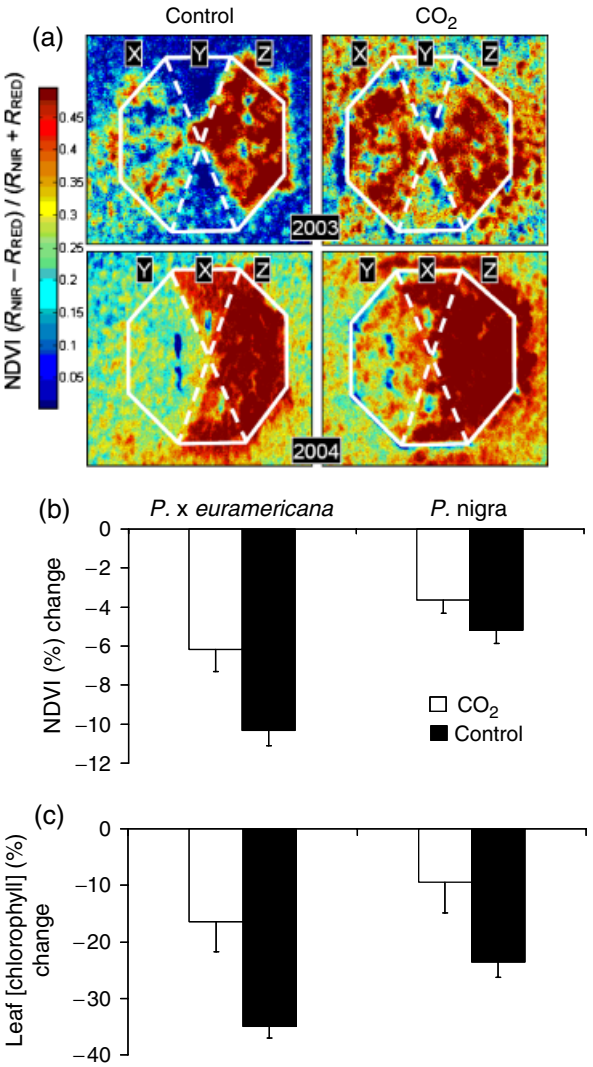

Fig. 3 Remote sensing of senescence in *Populus* at PopFACE, central Italy. (a) The normalized differential vegetation index (NDVI) measured using a wide bandwidth tri-band multispectral camera from a Sky Arrow aircraft on November 1, 2003 and October 25, 2004. NDVI is represented for *Populus nigra* (*x*) and *P.* × *euramericana* (*y*) in both years and treatments, by the color scale, as shown. (b) The mean decline of canopy NDVI taken between October 1 and October 26 2004 measured with the GER 3700 at 1 m above the canopy in both elevated CO_2 (open) and control (closed) treatments. (c) The mean decline in extracted leaf chlorophyll. Decline in leaf chlorophyll was measured between September 21 and October 18 for *P.* × *euramericana* and September 21 to November 2 for *P. nigra*.

and 86% (271) on the October 6, 2004, a stimulation resulting from extended canopy greenness (Fig. 2a–d). This stimulation was sustained by Clone 271 resulting in ~300% increased leaf carbon uptake in elevated [CO_2] compared with control late into the autumn (October 12, 2004) (Fig. 4a), but was now absent in clone 42E (Fig. 4b). At PopFACE late season (November

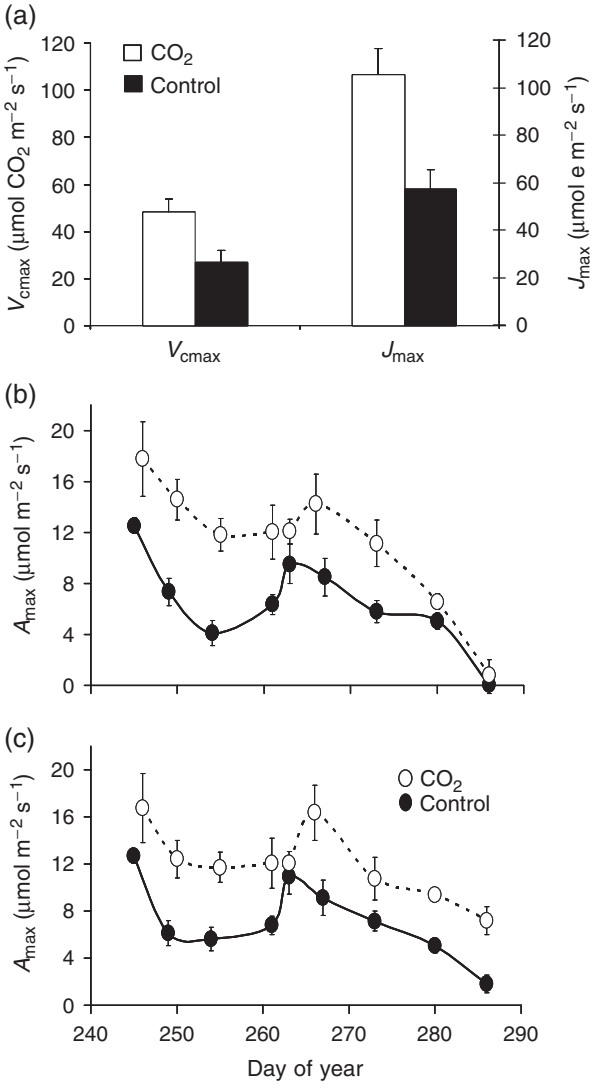

Fig. 4 The effects of elevated CO_2 on late season light saturated photosynthesis. Light saturated photosynthesis in elevated CO_2 (open) and control treatments for *Populus nigra* at PopFACE (a) and for *Populus tremuloides* genotype 42E (b) and *P. tremuloides* genotype 271 (c) at AspenFACE.

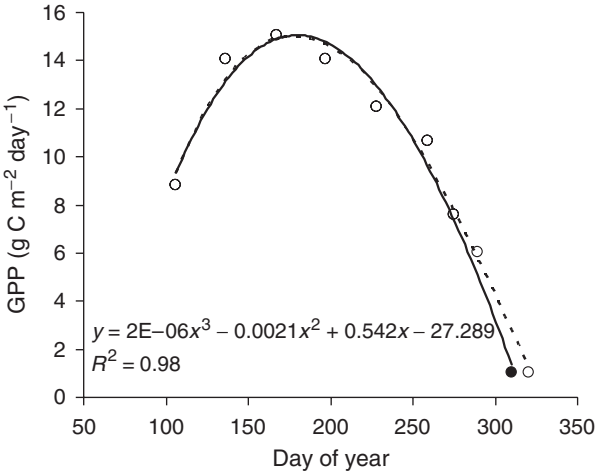

Fig. 5 The daily gross primary production for a closed canopy *P.* × *euramericana* growing at 550 ppm [CO_2] from the modeled data of Wittig *et al*. (2005). The cubic relationship between daily GPP and day of year in elevated [CO_2] is displayed (---). Advancing the end date by 10 days is represented (—) and the influence of this on seasonal GPP is calculated as the difference between the areas under the two curves. The result is an estimate of the seasonal GPP contributed from lengthening of the autumnal phenophase as a consequence of increased atmospheric [CO_2].

6, 2004) light saturated photosynthetic capacity of single leaves was also enhanced in elevated [CO_2] for *P. nigra*, the only species measured at this site (Fig. 4c). This resulted from a large stimulation in both J_{max} (maximum electron flow through photosysytem II, $F_{1,2}$ 11.97, $P = 0.074$, from average within block differences and $F_{1,13}$ 17.0, $P \leq 0.001$, from *post hoc* pooling) and an equally large stimulation in V_{cmax} (maximum velocity of carboxylation of Rubisco, $F_{1,2}$ 12.17, $P = 0.072$, from average within block differences and $F_{1,13}$ 11.65, $P \leq 0.005$, from *post-hoc* pooling) (Fig. 4c). The ratio between V_{cmax} and J_{max} was 0.45 and remained the same between both treatments.

At PopFACE, *P.* × *euramericana* sets bud some 30 days before *P. nigra* (Calfapietra *et al*., 2003). The extended functionality of the *P.* × *euramericana* canopy in elevated [CO_2] during the autumnal phenophase was estimated by the number of days difference for a 50% decline in VAI relative to control conditions. This was calculated to be between 5 and 15 days for 2003 and 2004, respectively. Taking a mean of 10 days this extension was estimated to contribute 2% to the total annual GPP of *P.* × *euramericana* exposed to elevated [CO_2] at the PopFACE site (Fig. 5).

Discussion

These data provide compelling evidence at the scale of the leaf and canopy, that autumnal senescence in such forest ecosystems will be delayed, as the atmospheric concentration of CO_2 continues to rise. We have shown that delayed autumnal senescence may occur in forests as a direct response to elevated CO_2, independent of temperature. This effect could explain the poor correlations observed previously between autumn phenology and rising temperatures, in contrast to the strong correlations between spring phenology and rising temperatures. We hypothesized that, with no sink limitation, photosynthesis and canopy greenness would be maintained for longer in elevated CO_2 and data collected

across different continents and *Populus* species grown both in pure and mixed stands support this hypothesis and show a strong effect of elevated [CO_2] with a significant extension of late season LAI (Figs 1a, b and 2e). Whole canopy greenness also persisted for longer at both sites during the autumnal phenophase (Figs 2a–d and 3a, b). Furthermore, late-season measurements of photosynthesis indicate that function of the canopy was retained and carbon uptake maintained (Fig. 4). The decline of chlorophyll content used as an estimate of individual leaf senescence was also significantly reduced indicating a delay or slowing of the senescence process (Fig. 3c). We have shown that these trees continue to photosynthesize after bud-set and that this process is enhanced in elevated CO_2.

Senescence is a complex process, for which a mechanistic understanding is still emerging, but is often studied under laboratory conditions using model annual plant species (Buchanan-Wollaston *et al*., 2003; Wingler *et al*., 2005). These systems may not entirely represent the complexity of true autumnal senescence for which in *Populus* response to changing photoperiod is the dominant trigger for the onset of dormancy (Keskitalo *et al*., 2005), and this response is dependent upon the latitudinal origin and genotype of the tree (Böhlenius *et al*., 2006). Nevertheless, model plants have enabled us to identify specific aspects of the senescence process that may be relevant in explaining our data since a number of other environmental variables influence the progression of senescence and these are also known to influence plant response to elevated [CO_2]. They include temperature, light, N supply, soil moisture and within plant variables such as physiological responses to carbon and N status and the balance between source and sink tissue (Wingler *et al*., 2005). This complexity of interactions, combined with the use of small plants in pots or trees that are sink limited, as well as varying spatial and temporal estimates of canopy longevity may help to explain some of the different responses and confusion observed in the literature to date. However, some consistencies are also apparent, linking features known to increase leaf longevity with growth in elevated CO_2. These include decreased specific leaf area (SLA) (indicative of increased leaf thickness), and decreased leaf Nmass (leaf N on a mass basis) (Reich *et al*., 1997; Wright *et al*., 2004); increased photosynthetic N use efficiency (PNUE) (Escudero & Mediavilla, 2003) and decreased oxidative stress (Woo *et al*., 2004). These changes are commonly observed in elevated CO_2 and are known to have the potential to extend leaf longevity. Both decreased SLA and leaf Nmass in response to elevated CO_2 have been reported for all species studied here (Karnosky *et al*., 2003; Tricker *et al*., 2004) and recently increased PNUE has been documented for *P.* × *euramericana* in elevated CO_2 at PopFACE during this period of study (Liberloo *et al*., 2006). Decreased oxidative stress has been linked with long-term growth in elevated CO_2 as inferred from a decreased leaf antioxidant pool (Schwanz & Polle, 1998; Karnosky *et al*., 2003). Furthermore, climatic conditions during the autumnal phenophase have previously been shown to result in an increased $J_{max}:V_{cmax}$ (Onoda *et al*., 2005) implying that autumnal photosynthesis was more dependent on [CO_2] explaining enhanced carbon uptake for younger leaves in elevated [CO_2] for the perennial herb *Polygonum cuspidatum*, during the autumnal period (Onoda *et al*., 2005). Thus, our data strongly suggest that delayed autumnal senescence may be linked to a positive photosynthetic C-fixation being maintained in the absence of sink limitation in elevated CO_2, particularly so in the fertile soils of theses two experiments exhibiting a typically low C : N for forest soils. This is unlike the nutrient poor soil of Duke FACE where no senescence response to CO_2 was observed for indeterminate sweet gum trees (Herrick & Thomas, 2003).

A second mechanism may also be involved in explaining this phenomenon. Reduced stomatal conductance is frequently observed in elevated CO_2 (Long *et al*., 2004; Tricker *et al*., 2005); an effect which may indirectly result in enhanced canopy temperature as the partitioning of absorbed solar radiation between sensible heat and evapotranspiration is altered (Sellers *et al*., 1996). This response of canopy temperature to elevated CO_2 is observed at SoyFACE, where a significant increase in temperature by approximately 1 °C was observed in the FACE plots (Long *et al*., 2006) and where delayed autumnal decline of LAI was also apparent (Dermody *et al*., 2006). However, in a recent open top chamber study using a *Populus* mapping population, where such temperature effects may be negated between CO_2 and control treatments, autumnal senescence was delayed by elevated CO_2. Moreover, areas of the *Populus* genome were identified as quantitative trait loci to explain this delayed senescence, suggesting that in future it will be possible to identify the genes underlying this response and that delayed senescence induced by elevated CO_2 is not the result of enhanced leaf temperature (Rae *et al*., 2006). A suite of genes associated with senescence in autumn trees has already been identified in *Populus*. This revealed a shift from gene expression associated with anabolism to that of catabolism and an increased role of mitochondria for energy generation as photosynthesis breaks down (Andersson *et al*., 2004). Furthermore, in *Populus* during autumnal senescence leaf pigment contents declined with the most rapid decline for chlorophyll. All pigments declined except for the flavonoid anthocyanin – this photoprotective compound increased in concentration during senescence (Keskitalo *et al*.,

2005). Preliminary data from *P.* × *euramericana* leaf material described here has identified a number of gene transcripts that are differentially expressed during the autumnal phenophase, including those associated with phenylpropanoid metabolism, and anthocyanin biosysnthesis (M. J. Tallis *et al*., personal communication), again suggesting a mechanism related to altered plant metabolism and C : N balance rather than altered leaf temperature. Taking into account the assumptions of the extended growth/differentiation balance model (GDB_e) of carbon partitioning discussed in Mattson *et al*. (2005), the autumnal stimulation of photosynthesis reported here may allow excess carbon to be partitioned to carbon rich secondary metabolites as the demand from growth reduces in the autumn. This portioning to secondary metabolites may also have a positive influence on leaf retention and carbon uptake during senescence particularly considering the increased synthesis of photoprotective compounds as discussed above.

Using a $^{14}CO_2$ label, Nelson & Isebrands (1983) showed that late season leaves retained on trees after bud-set in a short rotation poplar coppice exhibited photosynthetic rates high enough to contribute important quantities of photosynthate for continued radial stem growth, root growth and reserve storage in the stems and roots. Therefore and irrespective of mechanism, we were keen to understand how this extended autumnal phenophase might affect seasonal gross primary productivity. A delay in the 50% decline of VAI after bud set of between 5 and 15 days for *P.* × *euramericana* in 2003, and 2004, respectively, and 12 days for *P. nigra* in 2004 was calculated and confirmed that estimated by an independent analysis of canopy leaf retention (Tricker *et al*., 2004). An elevated CO_2 extension to the autumnal phenophase by 10 days was estimated to contribute approximately 2% to the annual GPP of *P.* × *euramericana* growing in a managed SRC plantation in central Italy (Fig. 5). This is similar to data reported by Goulden *et al*. (1996) for a mature mixed oak and maple stand in New England when gross ecosystem exchange of carbon for a 5–10 days delay in senescence was 0.5–0.9% that of the whole season. Contemporary climatic changes have already been reported to reduce the carbon sink strength of many northern hemisphere forests during recent hot dry summer months (Angert *et al*., 2005; Bunn & Goetz, 2006). Here, an extension in the growing season through increased canopy longevity and carbon gain may provide an increased sink for atmospheric carbon (Keeling *et al*., 1996; Lucht *et al*., 2002), although this requires further investigation. In contrast, on a global scale an extended growing season may contribute to global warming due to decreasing surface albedo (Betts, 2000).

The need to incorporate a dynamic growing season length in predictive models of forest productivity has previously been identified (White *et al*., 1999). Data presented here supports that need and further identifies the effects of changing [CO_2] on plant phenology as a variable to be considered when modeling forest productivity and biosphere–atmosphere interactions. In the case of Kyoto forests, future sink capacity may be influenced by phenological responses to elevated CO_2 that are independent of response to temperature and have not previously been recognized as important. This study has provided clear evidence that future rising CO_2 affects autumnal phenology directly. The mechanisms remain to be elucidated, but the phenomenon should be considered in future predictive models on the effects of climate change on temperate forest productivity.

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