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Gross primary production is stimulated for three *Populus* species grown under free-air CO_2 enrichment from planting through canopy closure

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Abstract

How forests will respond to rising [CO_2] in the long term is uncertain, most studies having involved juvenile trees in chambers prior to canopy closure. Poplar free-air CO_2 enrichment (Viterbo, Italy) is one of the first experiments to grow a forest from planting through canopy closure to coppice, entirely under open-air conditions using free-air CO_2 enrichment technology. Three *Populus* species: *P. alba*, *P. nigra* and *P. x euramericana*, were grown in three blocks, each containing one control and one treatment plot in which CO_2 was elevated to the expected 2050 concentration of 550 ppm. The objective of this study was to estimate gross primary production (GPP) from recorded leaf photosynthetic properties, leaf area index (LAI) and meteorological conditions over the complete 3-year rotation cycle. From the meteorological conditions recorded at 30 min intervals and biweekly measurements of LAI, the microclimate of leaves within the plots was estimated with a radiation transfer and energy balance model. This information was in turn used as input into a canopy microclimate model to determine light and temperature of different leaf classes at 30 min intervals which in turn was used with the steady-state biochemical model of leaf photosynthesis to compute CO_2 uptake by the different leaf classes. The parameters of these models were derived from measurements made at regular intervals throughout the coppice cycle. The photosynthetic rates for different leaf classes were summed to obtain canopy photosynthesis, i.e. GPP. The model was run for each species in each plot, so that differences in GPP between species and treatments could be tested statistically. Significant stimulation of GPP driven by elevated [CO_2] occurred in all 3 years, and was greatest in the first year (223–251%), but markedly lower in the second (19–24%) and third years (5–19%). Increase in GPP in elevated relative to control plots was highest for *P. nigra* in 1999 and for *P. x euramericana* in 2000 and 2001, although in 1999 *P. alba* had a higher GPP than *P. x euramericana*. Our analysis attributed the decline in stimulation to canopy closure and not photosynthetic acclimation. Over the 3-year rotation cycle from planting to harvest, the cumulative GPP was 4500, 4960 and 4010 g C m^{-2} for *P. alba*, *P. nigra* and *P. x euramericana*, respectively, in current [CO_2] and 5260, 5800 and 5000 g C m^{-2} in the elevated [CO_2] treatments. The relative changes were consistent with independent measurements of net primary production, determined independently from biomass increments and turnover.

Keywords: atmospheric change, canopy microclimate, elevated CO_2, FACE, global change, photosynthesis, POPFACE, *Populus*, short-rotation forestry

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Introduction

The global atmospheric concentration of CO_2 ($[CO_2]$) has increased 30% since preindustrial times, largely resulting from the burning of fossil fuels and land use change, and is expected to continue increasing from the current concentration of 370 ppm to approximately 550 ppm by 2050 (Prentice *et al.*, 2001). Understanding the impact of this unprecedented rate of increase in $[CO_2]$ on the global carbon cycle is crucial to predicting biosphere feedbacks. Photosynthesis is the primary process by which plants directly sense and respond to an increase in $[CO_2]$; understanding plant photosynthetic response to rising $[CO_2]$ is necessary to evaluate changes in the global carbon cycle (Drake *et al.*, 1997). Photosynthesis per unit land surface area, i.e. gross primary production (GPP), is the driving step of the global carbon cycle. Forests cover approximately 35% of global land area and account for 70% of terrestrial net primary production (NPP) (Melillo *et al.*, 1993; Geider *et al.*, 2001). NPP is the amount of carbon incorporated into plant biomass and available to other trophic levels or storage, i.e. GPP less autotrophic respiration (R_a). Forest GPP has the potential to affect the rate of increase in $[CO_2]$ and its accurate projection is critical in turn to projecting the future global carbon cycle (Cramer & Field, 1999; Hamilton *et al.*, 2002). As a consequence of the potential for elevated $[CO_2]$ to affect forest GPP, in many countries land is being reforested or afforested, and established forests are being managed, in an effort to meet the requirements of decreased net CO_2 emissions set out in the Kyoto Protocol (Schulze *et al.*, 2002).

It has been well documented that photosynthetic productivity of trees is enhanced by elevated $[CO_2]$ in the short term (Curtis & Wang, 1998), but these results have been limited by lack of studies of closed canopy forests under open-air conditions (DeLucia *et al.*, 1999; Karnosky, 2003). Photosynthetic rates of C_3 trees are limited by current $[CO_2]$, thus it is expected that at least in the short term, productivity will be enhanced under elevated $[CO_2]$ (Long *et al.*, 2004). A wealth of research has been conducted on tree species grown in greenhouse or chamber experiments, but results may be confounded by restricted growing conditions and altered microclimate (McLeod & Long, 1999). In addition, quantifying the response of mature forests to elevated $[CO_2]$ is difficult because of the longevity of tree species (Eamus & Jarvis, 1989). Consequently, most information available is for greenhouse and chamber studies on juvenile trees, life stages that behave very differently from trees that have reached canopy closure in the field (Lee & Jarvis, 1995; Norby *et al.*, 1999). Thus, many uncertainties exist when inferring the response of mature forest canopies from juvenile trees exposed to elevated $[CO_2]$.

Free-air CO_2 enrichment (FACE) technology provides the means to study the impacts of elevated $[CO_2]$ on a closed forest canopy in field conditions where there is no restriction to growth or alteration of microclimate (Hendrey *et al.*, 1999; McLeod & Long, 1999). There are four FACE experiments using replicated plots that have been investigating tree responses to elevated $[CO_2]$, however, results differ and contrast to studies of closed canopies in open-top chambers (Ainsworth & Long, 2005). After 6 years, the established loblolly pine (*Pinus taeda*) forest at the Forest Atmosphere Carbon Dioxide and Storage-I (FACTS-I) research site showed a stimulation of NPP with strong annual variation and no detectible response in the smallest nor largest size classes by year 6, indicating a loss in stimulation by the elevated $[CO_2]$ treatment over time (DeLucia *et al.*, 1999; Oren *et al.*, 2001; Hamilton *et al.*, 2002; Schafer *et al.*, 2003). After 3 years of treatment of a closed-canopy sweetgum (*Liquidambar styraciflua*) stand at Oak Ridge, NPP increased by 21% with no loss in response over time, however, most of the excess carbon was allocated to leaves and fine roots and not to long-term storage in woody tissues (Norby *et al.*, 2002). These FACE studies initiated CO_2 fumigation some years after the stands were established which might alter responses because of fundamental differences in juvenile compared with mature trees (Ceulemans & Mousseau, 1994; Norby *et al.*, 1999). This also makes it difficult to compare results to the response of trees grown throughout their lives under FACE (Norby *et al.*, 1999). The Aspen-FACE experiment, also known as FACTS-II, located in Rhinelander, Wisconsin, is one of two FACE sites that have grown trees from planting onward, entirely under elevated $[CO_2]$. Results from Aspen-FACE shown no loss in productivity with time under elevated $[CO_2]$ (Karnosky *et al.*, 2003), but do indicate that lower canopy leaves did not respond to the elevated $[CO_2]$ treatment, rather enhancement in growth was because of a contribution of light-saturated photosynthesis in the upper canopy (Takeuchi *et al.*, 2001). Open-top chamber experiments have previously shown that trees experience a reduction in stimulation by elevated $[CO_2]$ with long-term exposure (Tissue *et al.*, 1999; Ainsworth *et al.*, 2002). Theoretically, it is possible for acclimation to decrease the stimulation in growth under elevated $[CO_2]$ (Long & Drake, 1991). However, since the proportion of total photosynthesis contributed by shaded leaves will increase with canopy closure, this will independently decrease stimulation. Examination of the biochemical limitations to photosynthesis, and implicit in the widely validated steady-state model of leaf photosynthesis of Farquhar *et al.* (1980), shows that

CO_2 uptake will be far more strongly stimulated at light saturation than in limiting light. As a result, even in the absence of acclimation of leaf level photosynthesis or nitrogen limitation, stimulation of GPP of a forest will decline as canopy size increases (Long *et al*., 2004). Thus, it is desirable to further elucidate the long-term response of trees to enrichment with elevated [CO_2] under open-air conditions and to critically evaluate the basis of any observed loss in stimulation with time.

An increase in NPP could be attributed to higher GPP, a reduction in R_a or a combination of the two. Although earlier studies suggested that R_a in trees declines with elevated [CO_2] (Curtis & Wang, 1998), more recent analyses suggest that these findings were an artifact of the method by which respiration was measured (Amthor, 2000; Jahnke, 2001; Jahnke & Krewitt, 2002; Davey *et al*., 2004). These more recent studies also show that leaf respiration is not decreased by elevated [CO_2], but rather increased per unit mass and per unit leaf area with long-term growth in elevated [CO_2] (Davey *et al*., 2004). If correct, this implies that NPP could only increase under elevated [CO_2] if there was a substantial increase in GPP. Understanding the response of GPP or of R_a to atmospheric changes is critical when attempting to interpret NPP (Cramer *et al*., 1999; Hamilton *et al*., 2002). One disadvantage of FACE, however, is that GPP cannot be measured directly. This contrasts with chamber experiments which can directly measure GPP by closing the chamber, but as consequence of this closure, suffer from an alteration of canopy microclimate (Dore *et al*., 2003). Enclosure of a FACE system would not only be very difficult, but would impose the microclimatic modifications that FACE was developed to avoid.

The Poplar free-air CO_2 enrichment (POPFACE, Viterbo, Italy) is a FACE experiment conducted on three fast growing *Populus* species: *P. alba*, *P. nigra* and *P. x euramericana*. The three POPFACE species vary by origin, branching and rooting habit, apical control and bud set (Calfapietra *et al*., 2001). The POPFACE experiment was managed as a short-rotation intensive forestry plantation where the trees were planted and then coppiced after 3 years of growth, thus capturing the transition from an open to closed canopy entirely under elevated [CO_2]. *Populus* in this context could be regarded as a model system for global change research because a 3-year study of fast-growing poplars includes a significant portion of the canopy development allowing research to infer potential responses of closed, mature forest canopies to rising [CO_2] (Ceulemans & Isebrands, 1996). While GPP cannot be measured directly, leaf photosynthesis, canopy structure and weather were measured at regular intervals throughout the first 3-year rotation cycle at POPFACE (Gielen *et al*., 2001, 2003; Bernacchi *et al*., 2003b). The basis was therefore provided for the calculation of GPP. By dynamic estimation of microclimate of different leaf classes and in turn leaf photosynthesis, and then by summing photosynthesis of the leaf classes GPP for each 30 min interval of the three growing seasons could be obtained (Farquhar *et al*., 1980; Long, 1991; Forseth & Norman, 1993; Humphries & Long, 1995; de Pury & Farquhar, 1997).

The objectives of this research were first to estimate the instantaneous GPP for the poplars grown in current ambient and elevated [CO_2] at POPFACE. Secondly, instantaneous GPP was integrated for each day, month and year of the POPFACE experiment to yield the total GPP for the entire rotation cycle. Finally, the total GPP estimates were analyzed to determine differences in species productivity and to test the hypothesis that, as a consequence of canopy closure and not photosynthetic acclimation, stimulation of GPP under elevated [CO_2] would be significantly reduced.

Methods

Site description

The POPFACE research facility is located near the city of Viterbo in central Italy (Tuscania, 42°22′N, 11°48′E, altitude 150 m). Six plots of 30 m diameter spaced 120 m apart were established on a 9 ha field planted with the hybrid *P. x euramericana* Dode (Gunier) (*P. deltoides* Bart. ex Marsh. × *P. nigra* L., I-214) at 2 m × 1 m spacing in late spring of 1999. Three *Populus* species, *P. alba* (genotype 2AS11), *P. nigra* (genotype Jean Pourtet) and *P. x euramericana* (genotype I-214), were planted within each plot at 1 m × 1 m spacing, totaling 350 trees per plot. Each plot was divided into six equal segments with two opposing segments per species. The plots were blocked in pairs, one of each pair was enriched with a target concentration of 550 ppm [CO_2]; the other remained in current ambient [CO_2] (370 ppm). The mean CO_2 concentration ($\pm$ the standard deviation) was 544 ± 48 ppm in 1999, 532 ± 83 ppm in 2000 and 554 ± 95 ppm in 2001 (Calfapietra *et al*., 2003b). Treatment was during the daylight hours from bud burst until leaf senescence using FACE technology (Miglietta *et al*., 2001). The experiment was a complete block design with three replicates, managed as a 3-year rotation intensive forestry plantation with drip irrigation and a scheduled coppice after the 2001 growing season. Detailed planting and site descriptions have been provided previously (Scarascia-Mugnozza *et al*., 2000; Miglietta *et al*., 2001).

Estimating GPP

Estimation of GPP was based on estimates of canopy properties including leaf area index (LAI), leaf photosynthetic properties including the maximum rate of carboxylation ($V_{c,max}$) and the maximum rate of electron transport (J_{max}), photosynthetic photon flux density (PPFD) and leaf temperature at 30 min intervals for every plot and species throughout each growing season. While PPFD and air temperature were measured every 30 min of each season, daily variation in LAI, $V_{c,max}$ and J_{max} were obtained by interpolation, as described below. GPP for each 30 min time-step was then estimated using a canopy radiation transfer and energy balance model to divide each canopy into sunlit and shaded foliage, and compute the PPFD and leaf temperature of each of the two dynamic foliage classes. Next, a steady-state model of leaf photosynthesis was used to compute the photosynthetic rate of the two leaf classes (shaded and sunlit) using $V_{c,max}$ and J_{max} obtained from previously published measurements on the leaves of these trees. Summing and then integrating the leaf photosynthetic rates, weighted by LAI, provided GPP. The following sections explain in detail the steps used to obtain GPP.

LAI. LAI was measured for each species in each plot once in 1999 and every 2 weeks in 2000 and 2001 with a fish-eye-type plant canopy analyzer (LAI-2000 PCA, Li-Cor Inc., Lincoln, NE, USA), as described previously (Gielen *et al*., 2001, 2003). These measurements were used to obtain daily estimates of LAI for each species in each plot. To accomplish this, the LAI was interpolated between the 2-week measurement periods for the 2000 and 2001 growing seasons (Gielen *et al*., 2001, 2003) beginning with a value of zero at the recorded date of bud-burst and terminated with a value of zero by the recorded date of completion of leaf death. Because planting was in June 1999, the first growing season was brief, and only the peak LAI was determined in this year (Gielen *et al*., 2001). It was therefore assumed for this season that LAI increased linearly from planting to the measured peak and then declined linearly to the date of total leaf senescence.

$V_{c,max}$ *and* J_{max}. Measurements of leaf photosynthetic CO_2 uptake (A) vs. intercellular CO_2 concentration (C_i) were made in each of the 1999, 2000 and 2001 growing seasons using a portable gas exchange measurement system (LI-COR 6400; Li-Cor Inc.; Bernacchi *et al*., 2003b). Maximum *in vivo* velocity of carboxylation of Rubisco ($V_{c,max,\ 25\,^\circ C}$) and the maximum *in vivo* rate of electron transport ($J_{max,\ 25\,^\circ C}$) were derived by fitting the equations of Farquhar *et al*. (1980) to the A vs. C_i relationship as described previously (Farquhar *et al*., 1980; Long & Bernacchi, 2003). These measurements suggested that values of $V_{c,max,\ 25\,^\circ C}$ and $J_{max,\ 25\,^\circ C}$ did not vary consistently over the growing season, except for a continuous decline once senescence began (Bernacchi *et al*., 2003b). It was therefore assumed in estimating the daily values of $V_{c,max,\ 25\,^\circ C}$ and $J_{max,\ 25\,^\circ C}$ for each species, plot and treatment that these values remained constant and at the average of all measurements made within a single year for each species within each plot until senescence began. After the date at which autumn senescence was first observed to occur in the plot, $V_{c,max,\ 25\,^\circ C}$ and $J_{max,\ 25\,^\circ C}$ were assumed to decline linearly to zero on the date of 100% leaf senescence.

Meteorological conditions. The air temperature (°C) and PPFD was recorded at 30-min intervals at the weather station located at Roccarespampani (Viterbo, Italy), approximately 10 km from the POPFACE site. Equipment or data storage failure resulted in missing values for temperature 1.5% of the time in 2000 and 4.5% in 2001. To fill these gaps, the average of the temperatures at the corresponding dates and times in the other 2 years of the rotation cycle were used. Leaf temperature (T_l) was estimated with an energy balance approach derived from the Penman–Monteith equation (Monteith, 1965), the stomatal conductance model of Ball *et al*. (1987), and radiation transfer model of Forseth & Norman (1993), as described previously (Humphries & Long, 1995). Using this approach, the recorded incoming PPFD and the air temperature were used to estimate leaf temperature separately for both sunlit and shaded leaves.

PPFD (I_{tot}) recorded at 30 min intervals was partitioned into potential direct and diffuse ($I_{dir,\,pot.}$ and $I_{diff,\,pot.}$) components by adapting the equations of Weiss & Norman (1985) for photosynthetically active radiation to PPFD as follows:

$$I_{dir,\,pot.} = I_s \alpha^{[(P/P_0)/\cos\theta]}, \tag{1}$$

$$I_{diff,\,pot.} = 0.5 I_s \{1 - \alpha^{[(P/P_0)/\cos\theta]}\} \cos\theta, \tag{2}$$

where I_s is the solar constant (2600, $\mu mol\,m^{-2}\,s^{-1}$), α is the assumed maximum atmospheric transmittance (0.85), P/P_0 is the ratio of actual to sea-level pressure and θ is the solar zenith angle (Long, 1991). Potential total PPFD ($I_{tot,\,pot.}$) was the sum of $I_{dir,\,pot.}$ and $I_{diff,\,pot.}$. The ratio of the recorded PPFD to the potential total PPFD was calculated as

$$\gamma = \frac{I_{tot}}{I_{tot,\,pot.}}. \tag{3}$$

These values were then partitioned into fractions of direct and diffuse radiation with the equations of Weiss

& Norman (1985) where the fraction of direct (f_{dir}) and diffuse (f_{diff}) beam radiation were calculated as

$$f_{dir} = \frac{I_{dir,pot.}}{I_{tot,pot.}}\left[1 - \left(\frac{A-\gamma}{B}\right)^{2/3}\right], \quad (4)$$

$$f_{diff} = 1 - f_{dir}, \quad (5)$$

where A (0.9) and B (0.7) are constants. Finally, the recorded PPFD was partitioned into direct (I_{dir}) and diffuse (I_{diff}) beam radiation as follows:

$$I_{dir} = f_{dir}\,\mathrm{PPFD}, \quad (6)$$

$$I_{diff} = f_{diff}\,\mathrm{PPFD}. \quad (7)$$

This allowed use of the recorded PPFD to calculate the mean photon flux for leaves in direct and diffuse beam radiation separately at each 30 min interval.

Calculating leaf photosynthesis, canopy photosynthesis and GPP

The following calculations were made from the interpolated values of LAI, $V_{c,max}$, J_{max}, and T_l, and recorded values of [CO_2] for each species in each plot, coupled with incoming diffuse and direct PPFD, estimated as above. The plot estimates of GPP provided the samples that were then used to statistically test for treatment, species and interaction effects within each year.

Leaf photosynthesis

Leaf photosynthesis was estimated with the steady-state biochemical model of leaf photosynthesis (Farquhar *et al*., 1980). This model assumes that photosynthetic uptake of CO_2 within a leaf is limited by the slower of two processes: (1) the rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzed carboxylation (Rubisco-limited photosynthesis); or (2) the rate of ribulose-1,5-bisphosphate regeneration (RuBP-limited photosynthesis), which is assumed to be limited by whole chain electron transport.

Leaf photosynthesis was thus calculated as

$$A = \left[1 - \frac{\Gamma^*}{C_i}\right]\min\{W_c, W_j\}, \quad (8)$$

where Γ^* is the CO_2 compensation point in the absence of dark respiration, C_i is the intercellular concentration of CO_2, calculated by iterative solution of the Ball *et al*. (1987) model of stomatal conductance and Eqn (8) as described previously (Ball *et al*., 1987; Humphries & Long, 1995), W_c is the Rubisco-limited rate of carboxylation and W_j is the RuBP-limited rate of carboxylation each calculated separately as

$$W_c = \frac{V_{c,max}C_i}{C_i + K_c\left[1 + \frac{O_i}{K_o}\right]}, \quad (9)$$

$$W_j = \frac{JC_i}{4.5C_i + 10.5\Gamma^*}, \quad (10)$$

where K_c is the Michaelis constant for CO_2 (404.9 μmol mol^{-1}) and K_o is the Michaelis constant for O_2 (278.4 mmol mol^{-1}) and J is the potential electron transport rate (Farquhar *et al*., 1980; Long, 1991; de Pury & Farquhar, 1997; Bernacchi *et al*., 2001, 2003a). The potential electron transport rate J, is calculated as

$$J = \frac{Q_2 + J_{max} - \sqrt{(Q_2 + J_{max})^2 - 4\Theta_{PSII}Q_2J_{max}}}{2\Theta_{PSII}}, \quad (11)$$

where Θ_{PSII} is the curvature factor and Q_2 is the maximum fraction of quanta that can be used in electron transport and is calculated as

$$Q_2 = I_{sun}\alpha_l\Phi_{PSII,max}\beta, \quad (12)$$

where I_{sun} is the PPFD received by sunlit leaves calculated separately for each plot and species from I_{dir}, I_{diff}, θ and LAI assuming a random inclination and orientation of foliage following Long (1991) and Forseth & Norman (1993), and α_l is leaf absorptance, $\Phi_{PSII,max}$ is the maximum quantum yield of photosystem II, β is the maximum fraction of quanta that reach photosystem II. Q_2 is calculated separately for shaded leaves using the mean PPFD received by shaded leaves (I_{shade}) as described by Long (1991) and Forseth & Norman (1993). To calculate $V_{c,max}$ and J_{max} at the actual leaf temperature, the following equations were used (Bernacchi *et al*., 2001, 2003a):

$$V_{c,max} = V_{c,max,25\,°C}\exp\left(26.35 - \frac{65.33}{RT_k}\right), \quad (13)$$

$$J_{max} = J_{max,25\,°C}\exp\left(17.57 - \frac{43.54}{RT_k}\right), \quad (14)$$

where R is the molar gas constant and T_k is the absolute leaf temperature. These values were substituted into Eqns (9)–(11) so that Eqn (8) could be solved to obtain leaf photosynthesis.

Canopy photosynthesis and GPP. At each 30 min interval, the total LAI that was sunlit (LAI_{sun}) and shaded (LAI_{shade}) was calculated from sun-leaf geometry following the equations of Long (1991) and Forseth & Norman (1993), as follows:

$$LAI_{sun} = (1 - \exp(-k\mathrm{LAI}/\cos\theta))\cos\theta/k, \quad (15)$$

$$LAI_{shade} = \mathrm{LAI} - LAI_{sun}, \quad (16)$$

where k is the foliar extinction coefficient. Photosynthetic rates (A_{sun} and A_{shade}) were calculated from

the photon flux and temperature at each leaf class at each 30 min interval (Eqns (1)–(5)). Canopy photosynthesis (A_c) was then calculated by summing the products of leaf photosynthetic rate and leaf area for each of the two leaf classes:

$$A_c = A_{sun}LAI_{sun} + A_{shade}LAI_{shade}. \quad (17)$$

The basic assumptions of this model of canopy photosynthesis are: (1) light is the dominant factor for calculations of canopy photosynthesis; (2) the dependence of photosynthesis on light is independent of depth in the canopy; and (3) the canopy leaves are randomly oriented and distributed in space (Forseth & Norman, 1993).

GPP, which is expressed in unit mass per unit area per unit time, was 0.27 times A_c, where 0.27 converts the estimate to mass C per unit ground area per unit time. GPP was estimated over longer periods by numerically integrating the daily course described by the 30 min time steps.

Statistical analysis

Monthly GPP for each year was analyzed using repeated measures, in a randomized complete block, mixed model analysis of variance (PROC MIXED SAS v8.01, The SAS Institute Inc., Cary, NC, USA) with month, treatment, species and subsequent interactions as fixed effects. To analyze annual GPP, each year (1999, 2000 and 2001) was analyzed separately with treatment, species and treatment by species as fixed effects in a randomized complete block mixed model. The best-fit variance/covariance matrices were chosen for each variable using Akaike's information criterion (Keselman *et al.*, 1998; Littell *et al.*, 1998, 2000). *A priori* pairwise linear comparisons were made between treatments within months (monthly GPP) and within years (annual GPP) to test for significant differences in monthly and annual GPP between treatments for each species.

Results

In total, approximately 4000 daily courses of GPP were computed. As examples, Fig. 1 shows the course of PPFD, air temperature and 30 min GPP in one control and one elevated [CO_2] plot for *P. nigra* after canopy closure on a mid-summer day of the second year after planting. Although a mid-day decline is indicated in both treatments, coinciding with decreased PPFD because of cloud cover, GPP was much higher in the elevated [CO_2] plot. The seasonal courses of mean monthly PPFD and mean monthly air temperature from 1999 to 2001, used as inputs for GPP estimation, are

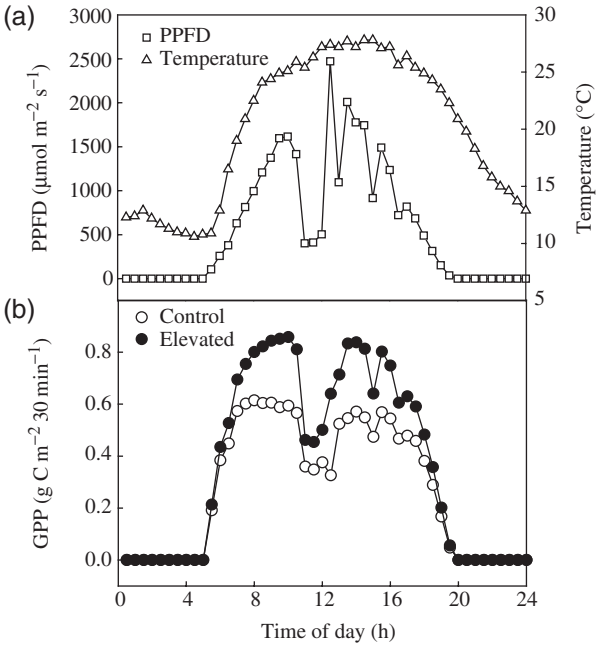

Fig. 1 (a) An example of the photosynthetic photon flux density (PPFD, open squares) and air temperature (open triangles) record for one day (July 18, 2000). (b) The corresponding estimated diurnal course of gross primary production (GPP) for *Populus nigra* in one elevated (filled circles) and control (open circles) plot for the same day. Each point represents the GPP for each 30 min interval of the day. The mean CO_2 concentration was 370 ppm in the control and 550 ppm in the elevated plot.

shown in Fig. 2. Monthly $V_{c,max,25\,°C}$, $J_{max,25\,°C}$ and LAI averaged across ambient and elevated [CO_2] treatments for each species from 1999 to 2001 and used as inputs to the model, are shown in Fig. 3. Each parameter differed for each species in the respective years with the most notable differences observed in LAI. This showed a dramatic increase in canopy size with time, i.e. from a maximum of 1 in 1999 to a maximum of 6 in 2001, as reported previously (Gielen *et al.*, 2001, 2003).

Monthly GPP averaged across plots showed a persistent stimulation in elevated [CO_2] relative to ambient [CO_2] treatments for all species (Fig. 4). GPP increased dramatically from 1999 to 2000 and the treatment effect was highly significant for the majority of the growing season for all species in 1999 and during mid-season in 2000 and 2001 (Fig. 4). This increase in GPP from 1999 to 2000 parallels the increase in LAI from a maximum of 1 in 1999 to a maximum of 6 in 2000 (Fig. 3). The interaction of treatment × species and treatment × species × month was only significant in 1999, although all other factors and interactions were significant throughout (Table 1). Despite the dramatic increase in GPP in 2000, relative to 1999, only a small

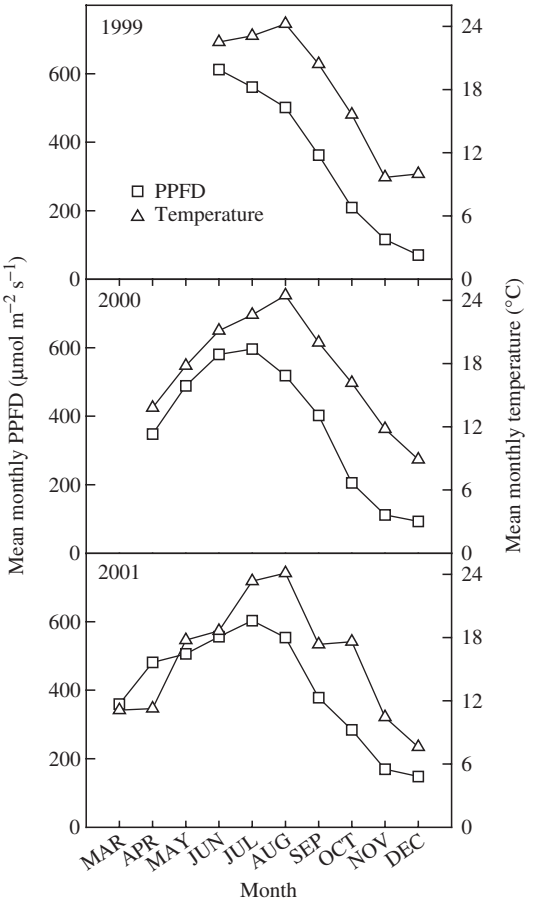

Fig. 2 Mean monthly photosynthetic photon flux density (PPFD, open squares) and mean monthly air temperature (open triangles) recorded near Poplar free-air CO_2 enrichment from 1999 to 2001.

further increase occurred in 2001. An exception was the early season, April and May, where monthly GPP in 2001 was substantially more than in the same period in 2000. In all years, absolute GPP and the relative stimulation was greatest in the summer months. Although stimulation was often indicated in spring and autumn, these differences were often nonsignificant, but always significant in summer with the widest margins of significance observed in 1999 and 2000 (Fig. 4).

Annual GPP was significantly greater for all species in all years with the exception of *P. nigra* in 2001 (Fig. 5). Stimulation of GPP by elevated [CO_2] was clearly greatest in the first year (1999), declining into 2000 and 2001 in all species (Fig. 5; Table 2). The interaction of treatment × species was only significant in 1999 (Table 2). The stimulation in 1999 was greatest in *P. nigra*. However, by 2001 *P. x euramericana* showed the highest stimulation. During 2000, all species showed approximately the same magnitude of stimulation (Fig. 5). The monthly patterns did suggest that absolute GPP was least in *P. alba* in 1999, but had become least in *P. x euramericana* by the final year (Fig. 4). This is confirmed when the annual GPP are compared (Fig. 5). This also confirms the marked decline in percent stimulation because of elevated [CO_2] with canopy closure, which although most marked between the first and second year, continues into the third year (Fig. 5). By the final year, the species with the lowest GPP in the control, *P. x euramericana*, showed the highest relative stimulation of GPP by elevated [CO_2] (Fig. 5). Over the 3-year rotation cycle from planting to harvest, the cumulative GPP was 4500, 4960 and 4010 g C m^{-2} for *P. alba*, *P. nigra* and *P. x euramericana*, respectively, in current [CO_2] and 5260, 5800 and 5000 g C m^{-2} in the elevated [CO_2] treatment.

Discussion

This study is the first calculation of GPP for a forest from planting through canopy closure to harvest entirely under fully open-air elevation of [CO_2] at expected 2050 levels of 550 ppm. As hypothesized, this study shows a significant and sustained increase in absolute GPP across the 3 years in all three species, and a significant interaction of elevated [CO_2] and species in the first year. Over the 3-year growth cycle, elevated [CO_2] increased GPP for *P. alba*, *P. nigra* and *P. x euramericana* from 4500, 4960 and 4010 g C m^{-2}, respectively, to 5260, 5800 and 5000 g C m^{-2}. These 3-year totals represent relative stimulations of GPP of 17%, 17% and 25%, respectively. The relative percent stimulation, analyzed on an annual basis, was greatest in the first year declining sharply into the second and third years. Averaged across all species and plots, the relative percent stimulation of annual GPP was 234%, 22% and 11% in 1999, 2000 and 2001, respectively. In spite of this sharp decline in the relative percent stimulation, the absolute GPP was always greater under elevated [CO_2]. As shown below this decline in the percent stimulation with time is not evidence for acclimation, but simply a normal result of the transition from an open to closed canopy.

In 1999, following planting in June, peak LAI was more than doubled by elevated [CO_2] relative to control (Fig. 3; Gielen *et al*., 2001). This large effect following planting could be explained by increased leaf photosynthesis providing more carbohydrate for the production of more leaf area, in turn feeding forward in a compound interest manner (Long *et al*., 2004). However, the peak LAI was well under 1, and so most of this new leaf area would be in full sun. As the canopies closed in early 2000, most sunlight was intercepted, and the addition of further leaf area later in 2000 and 2001 could have little impact on GPP in contrast to 1999. In 2000

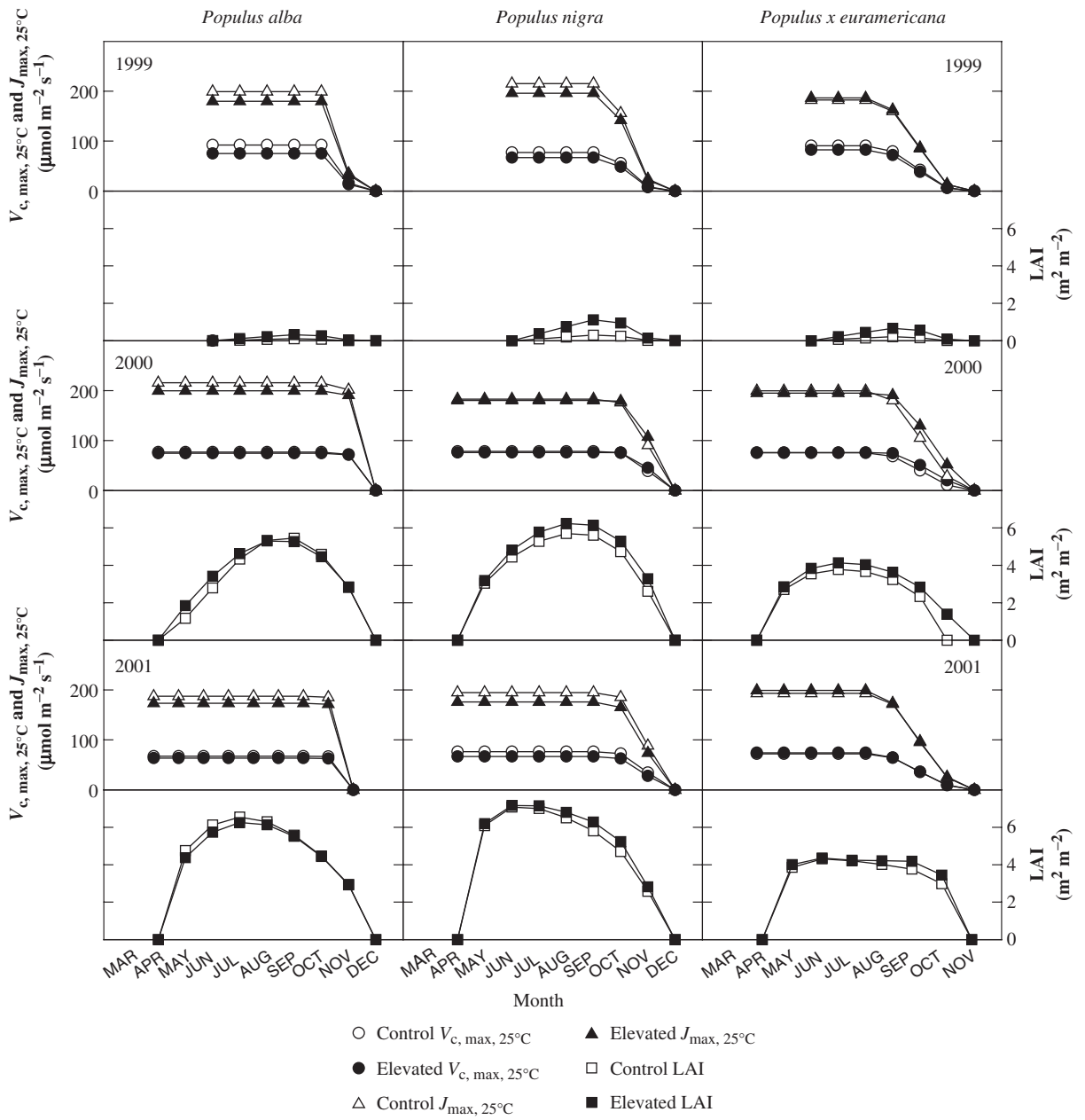

Fig. 3 Monthly mean of leaf photosynthetic and area parameters interpolated from the regular measurements made at the site: $V_{c,max,25\,°C}$ (open circles), $J_{max,25\,°C}$ (open triangles) and leaf area index (LAI) (open squares) in control treatments and $V_{c,max,25\,°C}$ (closed circles), $J_{max,25\,°C}$ (closed triangles) and LAI (closed squares) in elevated treatments for each species and year of the Poplar free-air CO_2 enrichment rotation cycle. Concentrations of CO_2 in the control and elevated plots are as in Fig. 1.

and 2001, LAI reached ca. 6, creating closed canopy conditions. Under closed canopy conditions the model predicts that over the course of a day approximately 50% of GPP is contributed from photosynthesis under shade conditions. Light-saturated photosynthesis is more strongly increased by elevated [CO_2] than light-limited photosynthesis, typically by a factor of 2–3 × (Long *et al.*, 2004). From the kinetic data published previously (Bernacchi *et al.*, 2001, 2003a) it may be shown that increase in [CO_2] from 370 to 550 ppm at 25 °C increases leaf CO_2 uptake by 41% at light saturation, but only 12% under light-limiting conditions (Long *et al.*, 2004). This is because light-saturated photosynthesis is predominantly limited by the amount of active Rubisco (Rogers & Humphries, 2000). Rubisco-limited photosynthesis is increased strongly by elevated [CO_2], primarily because the velocity of carboxylation is increased because of increased binding of CO_2

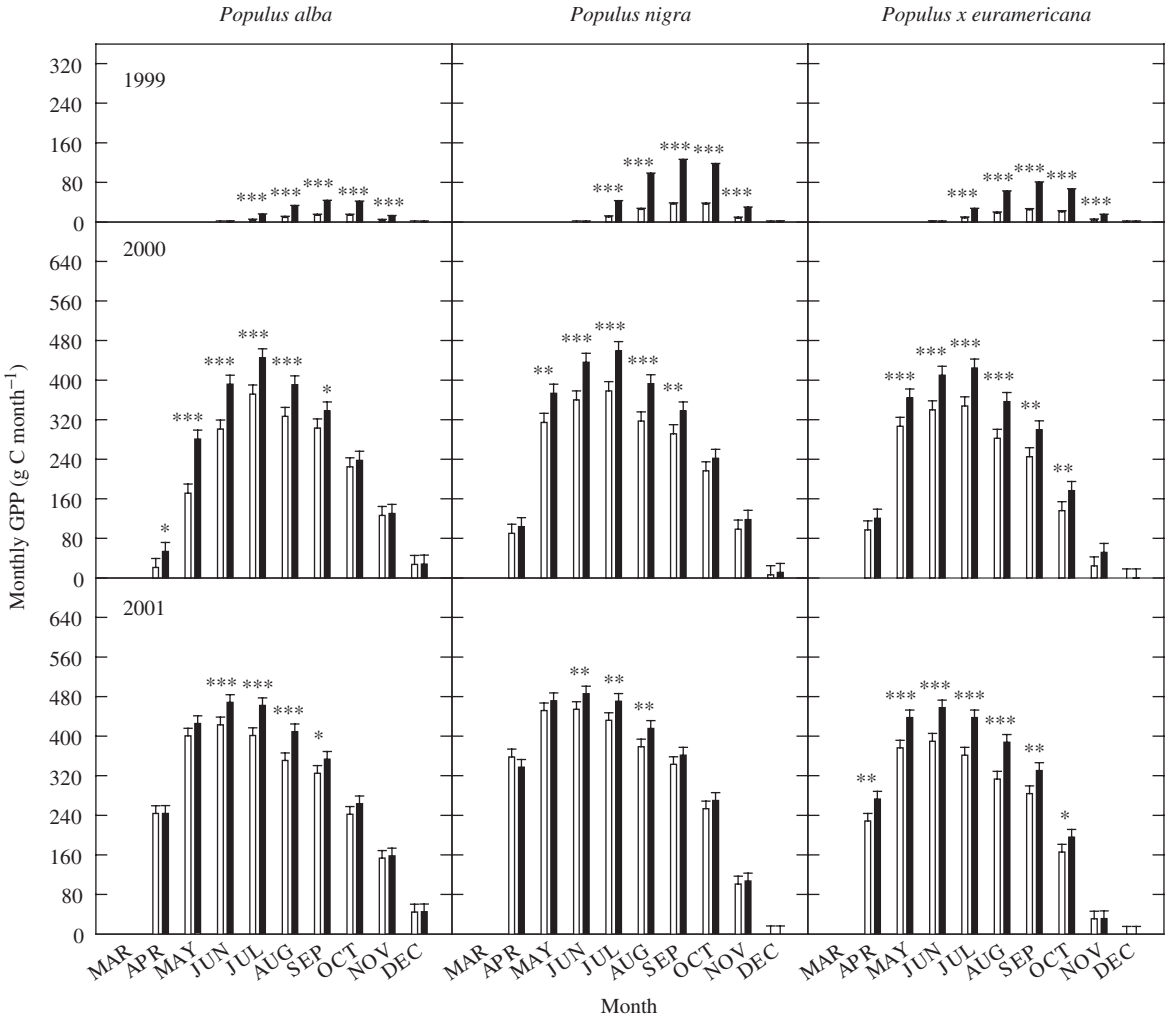

Fig. 4 Monthly gross primary production (GPP) in grams carbon for three *Populus* species grown in control (open bars) or elevated (filled bars) [CO_2] plots for each year of the rotation cycle (1999–2001). Each bar represents the mean of the summed monthly GPP for the three replicate plots in each month of the rotation cycle. Concentrations of CO_2 in the control and elevated plots are as in Fig. 1. Standard error bars represent the standard error of the differences of least squared means. Unmarked treatment comparisons indicate that there was no statistically significant difference; marked bars are significant at the $^*P<0.05$ level, $^{**}P<0.01$ level or $^{***}P<0.001$ level.

to Rubisco, but also because of increased [CO_2] inhibition of oxygenase activity (Long, 1991). Under light-limiting conditions, photosynthesis is limited by the rate of regeneration of the CO_2 acceptor, RuBP. Here, the velocity of carboxylation that Rubisco can support is irrelevant, because it is limited by RuBP supply and independent of [CO_2]; however, a small increase still occurs, because oxygenation of RuBP is partially inhibited by the increased [CO_2] making more RuBP available for carboxylation. This explains the absolute increase in GPP in all years, but a decline in the relative stimulation after closure of the light environment within the canopy. However, Bernacchi *et al*. (2001) also noted some acclimation of leaf photosynthetic capacity. Does photosynthetic acclimation also contribute to decreased stimulation of GPP? This is analyzed below.

To numerically determine the effect of acclimation in photosynthetic capacity, the model run was repeated using the photosynthetic input data (i.e. $V_{c,\,max,\,25\,^\circ C}$ and $J_{max,\,25\,^\circ C}$) from the ambient plots combined with the LAI and [CO_2] for the elevated plots. The output generated represents the potential GPP in the absence of any acclimation of photosynthesis. Using leaf photosynthetic parameters from control plots to estimate GPP of the elevated plots resulted in an increase of annual GPP of less than 1.5% and was not statistically significant, as determined with a two-tailed *t*-test ($P>0.2$). This suggests that the effect of acclimation of leaf photosynthesis was negligible.

Table 1 A mixed model analysis of variance of the effects of [CO_2] treatment, species, month and their interaction on monthly GPP for three *Populus* species in each year of the POPFACE experiment

Year	Effects	*F*-value	*P*
1999	Treatment	729.29	<0.0001
	Month × treatment	633.42	<0.0001
	Species	210.02	<0.0001
	Month × species	204.5	<0.0001
	Treatment × species	68.94	<0.0001
	Month × treatment × species	62.24	<0.0001
2000	Treatment	27.71	0.0096
	Month × treatment	10.75	<0.0001
	Species	7.89	0.0006
	Month × species	18.22	<0.0001
	Treatment × species	0.02	0.9846
	Month × treatment × species	0.97	0.4918
2001	Treatment	13.99	0.0201
	Month × treatment	15.98	<0.0001
	Species	18.44	<0.0001
	Month × species	47.16	<0.0001
	Treatment × species	1.14	0.3242
	Month × treatment × species	1.17	0.3054

GPP, gross primary production; POPFACE, Poplar free-air CO_2 enrichment.

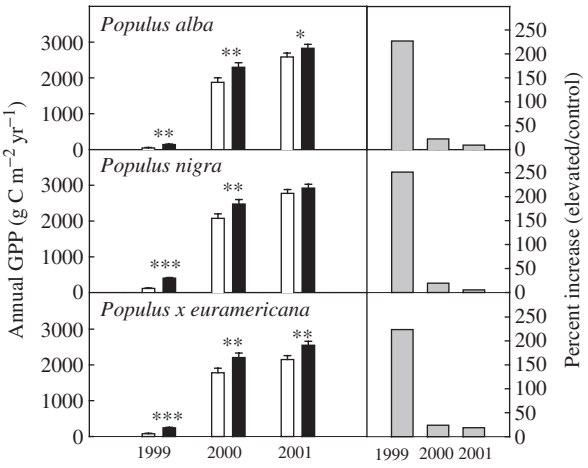

Fig. 5 Annual gross primary production (GPP) in grams carbon for three *Populus* species grown in the control (open bars) or elevated (filled bars) plots for each year of the rotation cycle (1999–2001). Each bar represents the mean of three replicate plots for 1 year of the rotation cycle. Concentrations of CO_2 in the control and elevated plots are as in Fig. 1. Standard error bars represent the standard error of the differences of least-squared means. Bars on the right-hand side represent the percent increase in GPP of elevated relative to ambient treatments for each year. All comparisons were statistically significant at either the $^*P<0.05$ level, $^{**}P<0.01$ level or $^{***}P<0.001$ level.

Table 2 A mixed model analysis of variance of the effects of [CO_2] treatment, species and their interaction on annual GPP for three *Populus* species in each year of the POPFACE experiment

Year	Effects	*F*-value	*P*
1999	Treatment	508.64	0.002
	Species	146.48	0.0002
	Treatment × species	48.08	0.0016
2000	Treatment	17.01	0.0541
	Species	7.81	0.0416
	Treatment × species	0.02	0.9814
2001	Treatment	18.21	0.0508
	Species	22.81	0.0065
	Treatment × species	1.41	0.3443

GPP, gross primary production; POPFACE, Poplar free-air CO_2 enrichment.

Consequently, the decline in stimulation of GPP is predominantly a function of canopy closure and not a result of any systematic downregulation of photosynthetic capacity.

While based entirely on measurements, the calculation of GPP requires several assumptions. What are the most likely errors and are there independent measures that corroborate the findings? The canopy model divides the foliage into two dynamic classes, shaded and sunlit leaves. Although more sophisticated models that divide the canopy into multiple layers exist, theoretical comparisons suggest that these provide little improvement in accuracy (Forseth & Norman, 1993). In practice, such models also require detailed information about each layer for parameterization. The most significant potential error in the approach used to obtain GPP here is the assumption that $V_{c,max}$ and J_{max}, which were derived from mature upper canopy leaves (Bernacchi *et al.*, 2003b), apply throughout the canopy. It is likely that both decline into the canopy (Osborne *et al.*, 1998). However, in low light, predicted photosynthesis depends predominantly on the maximum quantum yield and this has been shown to be remarkably constant across species, leaf age and sun vs. shade environments (Long *et al.*, 1993). To test the potential error of the assumption, the simulation was repeated for one sunny day, dividing the canopy into an upper and lower three units of LAI. It was assumed that $V_{c,max}$ and J_{max} were (1) the same in the upper and lower canopy and (2) decreased by 70% in the lower canopy. The latter case decreased the daily GPP by 2%, which resulted largely from overestimation of photosynthesis when lower canopy leaves received direct radiation from sunflecks. This suggests that any error from this assumption was

small. A further assumption was that $V_{c,max}$ and J_{max} declined linearly in the autumn following the beginning of canopy senescence through to leaf death. If a constant rather than declining $V_{c,max}$ and J_{max} are assumed, the effect on GPP is also likely very small, because a large decline in GPP will result from the concurrent large decreases in LAI and temperature (Figs 2 and 3).

Although the estimated GPP could not be directly validated, NPP has already been determined completely independently from increments in total woody biomass, cumulative leaf fall, and fine root mass and turnover (Lukac *et al*., 2003; Calfapietra *et al*., 2003a). NPP could also be estimated from the GPP values given here, if total plant respiration (R_a) for the same plots and intervals were known. Practically, this is a measure that can rarely be obtained, since *in situ* root respiration must be separated from that of other soil organisms. Controlled environment studies in hydroponics have shown that for a wide range of temperature treatments, R_a remained a remarkably constant fraction of GPP; 0.4 (Gifford, 1995). In general, environmental conditions that increase GPP cause a similar increase in R_a. If then an R_a/GPP of 0.4 is assumed, extrapolating from the GPP estimates given earlier, NPP for *P. alba*, *P. nigra* and *P. x euramericana* would be 2700, 2814 and 2406 $g\,C\,m^{-2}$, respectively, rising to 3156, 3480 and 3000 $g\,C\,m^{-2}$ in elevated [CO_2]. These values deduced from the photosynthetic gas exchange and canopy characteristics, compared with NPP derived from shoot and root biomass increments and turnover for *P. alba*, *P. nigra* and *P. x euramericana* of 2640, 3480 and 2840 $g\,C\,m^{-2}$, respectively, in the control plots, and 3080, 3640 and 3160 $g\,C\,m^{-2}$ in elevated [CO_2] (Lukac *et al*., 2003; Calfapietra *et al*., 2003a). The authors' original estimates were given as dry biomass; here we have assumed that C constitutes 40% of that biomass. Although there are some differences in these estimates, they agree well in absolute magnitude and the relative stimulation of NPP by elevated [CO_2]. Given the low replicate size ($n = 3$ blocks) and the assumptions involved in both estimates of NPP, the agreement is remarkable and provides some validation of the GPP estimates.

In conclusion, elevated [CO_2] substantially increased the GPP of all three *Populus* species. Although the stimulation declined sharply over the 3 years, this was attributed to the transition from open to closed canopy, and was not the result of loss because of photosynthetic acclimation. Although direct validation of GPP estimates were not possible, assuming a constant R_a/GPP provided estimates of NPP close to those estimated independently from biomass accumulation and turnover. The results suggest that with selection, nutrient and moisture supply, coppice managed plantation poplars have the potential for large and sustained increases in GPP.

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