

Increased leaf area dominates carbon flux response to elevated CO₂ in stands of *Populus deltoides* (Bartr.)

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Abstract

We examined the effects of atmospheric vapor pressure deficit (VPD) and soil moisture stress (SMS) on leaf- and stand-level CO₂ exchange in model 3-year-old coppiced cottonwood (*Populus deltoides* Bartr.) plantations using the large-scale, controlled environments of the Biosphere 2 Laboratory. A short-term experiment was imposed on top of continuing, long-term CO₂ treatments (43 and 120 Pa), at the end of the growing season. For the experiment, the plantations were exposed for 6–14 days to low and high VPD (0.6 and 2.5 kPa) at low and high volumetric soil moisture contents (25–39%). When system gross CO₂ assimilation was corrected for leaf area, system net CO₂ exchange (SNCE), integrated daily SNCE, and system respiration increased in response to elevated CO₂. The increases were mainly as a result of the larger leaf area developed during growth at high CO₂, before the short-term experiment; the observed decline in responses to SMS and high VPD treatments was partly because of leaf area reduction. Elevated CO₂ ameliorated the gas exchange consequences of water stress at the stand level, in all treatments. The initial slope of light response curves of stand photosynthesis (efficiency of light use by the stand) increased in response to elevated CO₂ under all treatments. Leaf-level net CO₂ assimilation rate and apparent quantum efficiency were consistently higher, and stomatal conductance and transpiration were significantly lower, under high CO₂ in all soil moisture and VPD combinations (except for conductance and transpiration in high soil moisture, low VPD). Comparisons of leaf- and stand-level gross CO₂ exchange indicated that the limitation of assimilation because of canopy light environment (in well-irrigated stands; ratio of leaf:stand = 3.2–3.5) switched to a predominantly individual leaf limitation (because of stomatal closure) in response to water stress (leaf:stand = 0.8–1.3). These observations enabled a good prediction of whole stand assimilation from leaf-level data under water-stressed conditions; the predictive ability was less under well-watered conditions. The data also demonstrated the need for a better understanding of the relationship between leaf water potential, leaf abscission, and stand LAI.

Nomenclature

A_{net} = leaf net photosynthetic CO₂ assimilation
B2L = Biosphere 2 Laboratory
 E = leaf transpiration
ECW = eastern cottonwoods
 g_s = stomatal conductance

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LAI = leaf area index
 PPF = photosynthetic photon flux
 SGCA = system gross CO₂ assimilation
 SGCA_L = system gross CO₂ assimilation per unit leaf area at light saturation
 SMS = soil moisture stress
 SNCE_{L or D} = system net CO₂ exchange (soil area basis), L or D as subscripts refer to light or dark
 VPD = atmospheric vapor pressure deficit

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Introduction

Atmospheric CO₂ concentrations are predicted to double by the middle of the 21st century and to be accompanied by an increase in air temperature (IPCC report, 2001) with effects on cloud cover, precipitation patterns, and atmospheric absolute humidity at the local or regional levels. If precipitation decreases and temperatures rise, as computer simulations predict for southern Europe and Central America (Rosenzweig & Hillel, 1993; Gregory *et al.*, 1997), atmospheric vapor pressure deficit (VPD) will also change. High VPD and low soil moisture are chiefly responsible for plant water stress (Kramer & Boyer, 1995) and consequently for effects on net carbon assimilation at the leaf and canopy levels. We investigated the interaction of carbon gain and water stress in leaves and stands of trees in ambient and elevated CO₂ concentrations, because this interaction will eventually influence the productivity, sequestration capacity, species composition, and management of future forests under high CO₂.

There have been many measurements of leaf-level responses in trees to high CO₂, soil moisture stress (SMS), and high VPD that showed large variability in plant responses between clones, at the species level, in mature trees vs. seedlings, and with age of a stand (Field *et al.*, 1995; Curtis, 1996). High VPD and low soil moisture tend to reduce g_s and decrease A_{net} . Will & Teskey (1997) reported a decrease in g_s in seedlings of several trees species under high CO₂, but high VPD diminished the effect of elevated CO₂ on g_s . Although there are now many flux tower studies of net system CO₂ exchange in natural communities (Wofsy *et al.*, 1993; Grant & Nalder, 2000; Clark *et al.*, 2001; Wallin *et al.*, 2001), we found few experimental evaluations of the interactive behavior of soil moisture, VPD, and elevated CO₂ on system net CO₂ exchange (SNCE). For example, Baldocchi (1997) reported a 65% decline in net ecosystem CO₂ exchange in a temperate hardwood forest as soil dried, and Law *et al.* (2001) measured a

larger decrease in net carbon flux with high VPD in a mature ponderosa pine ecosystem than in a younger plantation. Buchmann (2002) observed that only 14% of all eddy covariance flux studies in forests published in the decade before 2002 ($n = 196$) included plant ecophysiological measurements or biomass and growth estimates.

Scaling CO₂ assimilation from leaf to stand level is a complex process that ideally engages both 'bottom-up' and 'top-down' approaches (Norman, 1993) in the search for the transcending concepts that are needed to scale with confidence (Caldwell *et al.*, 1993). Ultimately, model outputs derived from leaf physiological measurements have to be tested against flux tower (or other) estimates of carbon assimilation (Baldocchi, 1993; Wilson *et al.*, 2001). Scaling leaf physiological responses to elevated CO₂ and water stress at the stand-level depends, among other things, on knowledge of tree architecture, on being able to account for variations in canopy leaf area and distributions, because of treatment effects on leaf development or on drought deciduousness, all of which are frequently overlooked in leaf-level measurements. For example, growth in elevated CO₂ often increases leaf area (Ferris *et al.*, 2001), thus amplifying leaf-level A_{net} at the stand level; although leaf level g_s has been reported to decrease under high CO₂, increased leaf area also amplifies stand-level water use compared with ambient CO₂ (Pataki *et al.*, 1998). Changes in root-to-shoot biomass ratios have been observed in several tree species grown under elevated CO₂ (Curtis & Wang, 1998), and these influence leaf- and stand-level responses to declining soil moisture or to increases in evaporative demand (Eamus *et al.*, 1995; Bunce & Ziska, 1998; Engel *et al.*, 2004).

Large-scale controlled environment experiments with enclosed stands of vegetation represent another approach to simultaneous evaluation of leaf- and stand-level assimilation processes. Just as controlled environment chamber gas exchange systems advanced

understanding of leaf photosynthesis, so also mass balance studies of enclosed model forest systems give insights into biological feedbacks such as the interactive effects of VPD and soil moisture on the carbon balance of forests under elevated CO₂ (Osmond *et al.*, 2004). To this end, we have extended previous studies of leaf-level photosynthetic and respiratory processes in a cottonwood forest plantation grown under controlled environmental conditions to the Biosphere 2 Laboratory (B2L) (Griffin *et al.*, 2002; Turnbull *et al.*, 2002) to stand-level measurements of SNCE in a 2 × 2 factorial treatment of high and low soil moisture and VPD in high (120 Pa) or ambient (43 Pa) CO₂. We took advantage of the fact that *Populus deltoides* is a riparian, phreatophytic species well known to be highly sensitive to water stress (Blake *et al.*, 1996), and somewhat unique to the extent of leaf senescence that occurs with loss of contact with the water table. We also measured some physiological responses of leaves to these controlled environmental perturbations and compared them with SNCE, taking into account changes in stand leaf area. We were particularly interested whether effects of SMS and VPD on SNCE might be associated with a shift from stand- to leaf-level limitations, and whether these interactions were modified by elevated CO₂.

Materials and methods

This study was done in the Eastern cottonwood (ECW) forest plantation of the B2L in Oracle, AZ, USA (32°37.13'N, 110°, 47.05'W, 1200 m a.s.l.). The mesocosm was partitioned into three sections (bays) that were separated by lightweight (0.30 mm thick) transparent polyvinylchloride curtains and maintained at 43 Pa (ambient), 80 Pa (2 × ambient) and 120 Pa (3 × ambient) CO₂ partial pressures during the photoperiod for the previous growing season. The center bay (80 Pa) was allocated to other experiments involving isoprene emissions at this time, and could not be included in the comparisons presented here. Each bay measured 41 m × 18 m in a north–south orientation, had an approximate soil surface area of 550 m², soil volume of 550 m³, air volume of 11 700 m³, and average height available for plant growth of 12 m. The facility afforded independent control and measurement in each bay of atmospheric CO₂, air and soil temperature, soil moisture, and absolute humidity level. Air handlers controlled temperature in each bay, and dew point was controlled by condensation of vapor on the cold-water manifold (Dempster, 1999). Water vapor was added to the atmosphere using fine-spray misters placed above the canopy near the top of the bays. Other details of the

structure, layout, and control of environmental parameters were given by Lin *et al.* (1998).

Plant material

A simple, model plantation forest system of 3-year-old coppiced stands (35 trees and 13 saplings averaging 8 m height) of cloned ECW (*P. deltoides* (Bartr.) genotype S7c8) were grown from cuttings originating from an east Texas source. The decision to use an ECW plantation was initially based on the rapid growth, simple canopy architecture, and the economic importance of these clones to the forest industry. Recent progress in mapping the *Populus* genome (Sterky *et al.*, 1998; Taylor, 2002; Tuskan *et al.*, 2004) has confirmed the wisdom of this decision. Trees were planted in May 1998 and stands were chilled at the end of the growing seasons to force dormancy before coppicing the canopy at 30 cm a.s.l. In growing seasons 1999 and 2000, regrowth from the stump was pruned to one leader per tree. The stands were litter free, all leaf fall being collected and dried as part of aboveground annual carbon budget and stand leaf area determination.

Monitoring and control of environmental parameters

Each bay was divided conceptually into four quarters, and environmental parameters (air temperature, soil temperature, volumetric soil moisture content, and photosynthetic photon flux (PPF), CO₂, relative humidity, and VPD) were measured near the center of each quarter every 15 s, and 15 min averages stored in data-loggers (Campbell-CR10x, Campbell Scientific Inc., Logan, UT, USA). Air temperature and PPF at 3, 6, and 9 m from soil level were measured at the same four locations in each bay, and in one central location in each bay at 15 m. Volumetric soil moisture content was measured at 0–30, 20–50, and 50–80 cm depth at the central location using water content reflectometer probes (Model: CSI 615, Campbell Scientific Inc.). Soil temperatures were measured at 20, 50, and 80 cm depths with thermocouples. Surface soil temperature (10 cm depth) was measured with thermistors at two other locations in each bay.

Twenty-four-hour temperature was maintained at approximately 29 °C for the entire study period. The trees were drip-irrigated during the experiment, with volumetric soil water contents maintained from 22% to 39% and from 27% to 39% in the 43 and 120 Pa CO₂ treatment bays, depending on the soil moisture treatment. Three CO₂ treatments were applied at the start of the third growing season. Atmospheric CO₂ concentration was measured and stabilized to its set point by varying the amount of pure CO₂ injected into the air

handler units (Griffin *et al.*, 2002). The bays were operated in a closed mode during the day and in an open mode during the night when the fans were opened to prevent build up of CO₂. This had the concurrent effect of erratically varying the VPD at night. Leaks between bays and to the outside were determined before, during, and after the experiment using the tracer gas sulfur hexafluoride (SF₆), and CO₂ concentration in each bay was corrected for leak rate.

Experimental design

The experiment (Table 1, Fig. 1) in November–December 2001 comprised two CO₂ treatments: ambient (east bay; 43 Pa) or three times ambient (west bay; 120 Pa), combined in temporal sequence with high or low VPD, with or without SMS and resulting in a total of eight treatment combinations. The water stress treatments were applied for 7 days within each of the four treatment combinations of soil moisture and VPD. In phase 1, volumetric soil moisture content was kept high (39%) and VPD was set low (0.6 kPa). In phase 2, soil moisture content was kept high and VPD was raised to 2.4 kPa. Soil moisture content and VPD were returned to levels approximating the conditions under which the trees had been cultivated (soil moisture ~ 35%,

VPD ~ 1.3 kPa) for 7 days prior to phase 3, to overcome memory effects of high VPD in phase 2. During phase 3, irrigation was withheld, causing soil moisture content to decline, and VPD was controlled at 0.96 kPa. During phase 4, VPD was raised to ~ 2.5 kPa, while the soil column continued to dry, and irrigation was withheld. Phase 4 of the experiment was longer (2 weeks) than the other three phases so as to determine the effect of prolonged SMS on SNCE. The treatment combinations are designated as c, w, and v for the low-level treatments of CO₂, soil moisture, and VPD, and C, W, and V for the high-level treatments.

Measurements

Leaf gas exchange and water potential. To study leaf-level physiological responses to CO₂, soil moisture, and VPD, measurements of g_s , E , and A_{net} were made on two leaves from six randomly selected trees in each bay on at least 2 days during each soil moisture and VPD phase (Li-Cor Model 6400, Lincoln, NE, USA). On each of these days, measurements were taken once in the morning and once in the afternoon. The CO₂ and VPD conditions within the leaf gas exchange cuvette were maintained at prevailing bay treatment levels during measurements, and irradiance corresponded to that measured at midcanopy in the bays (mean PPF was

Table 1 Mean daytime (PPF > 100 $\mu\text{mol m}^{-2}\text{s}^{-1}$) values of environmental variables in the ambient and elevated CO₂ treatment bays of the cottonwood plantation at the Biosphere2 Laboratory during the four phases of the experiment

Environmental variable	Phase 1 High soil moisture low VPD (Wv)		Phase 2 High soil moisture high VPD (WV)		Phase 3 Low soil moisture low VPD (wv)		Phase 4 Low soil moisture high VPD (wV)	
	c*	C*	c	C	c	C	c	C
Air temperature (°C)	27.03 (0.4)	27.20 (0.3)	28.04 (1.0)	28.70 (1.2)	29.20 (0.6)	29.60 (0.4)	28.90 (0.5)	29.00 (0.5)
Relative humidity (%)	83.00 (2.9)	84.00 (2.4)	38.00 (4.3)	37.00 (4.2)	76.00 (2.6)	77.00 (2.9)	36.00 (4.3)	38.00 (6.1)
Soil moisture ($\text{m}^3 \text{m}^{-3}$) [†]	0.39	0.39	0.39	0.39	0.23	0.28	0.22	0.27
VPD (kPa)	0.62 (0.11)	0.58 (0.09)	2.36 (0.27)	2.47 (0.29)	0.97 (0.11)	0.95 (0.13)	2.56 (0.18)	2.49 (0.25)
CO ₂ (Pa)	125.81 (3.9)	49.36 (6.4)	123.24 (1.3)	46.75 (3.4)	120.20 (1.2)	45.5 (1.9)	123.86 (3.8)	49.98 (3.9)
Total daytime PPF ($\text{mol m}^{-2} \text{day}^{-1}$) [‡]	14.7 (1.8)	13.6 (1.6)	10.6 (1.8)	9.9 (1.5)	10.3 (1.5)	9.7 (1.5)	11.5 (0.9)	10.9 (0.8)

Values in parentheses denote standard errors.

*Lowercase c denotes ambient and uppercase C denotes elevated CO₂ treatment.

[†]Soil moisture measured at 20 cm depth in the soil.

[‡]PPF was measured 9 m above the soil surface.

PPF, photosynthetic photon flux.

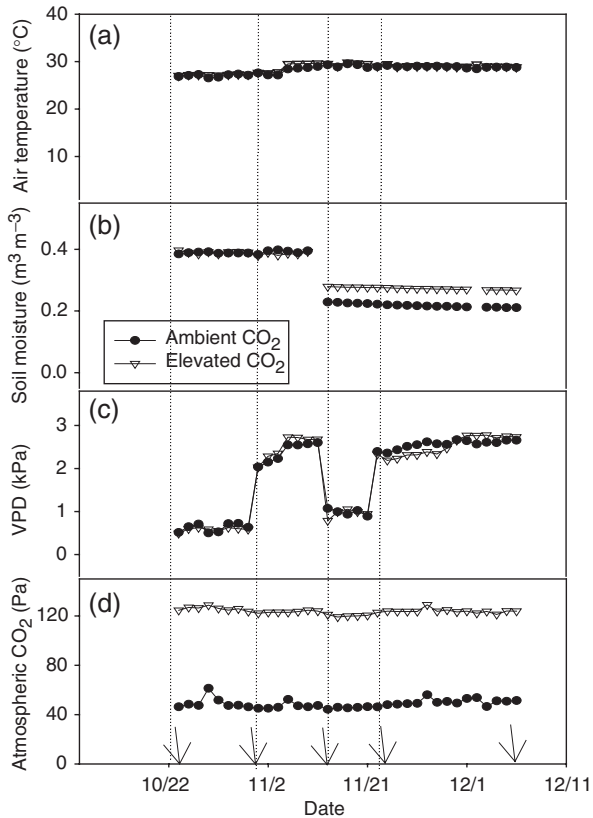


Fig. 1 Trends of daily means for the photoperiod when photosynthetic photon flux $> 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ of (a) air temperature, (b) soil moisture at 20 cm depth, (c) vapor pressure deficit (VPD), and (d) atmospheric CO_2 partial pressure, in the ambient and elevated CO_2 treatment bays for the duration of the study period. Dotted lines indicate the start and end of each phase of treatment, starting from Wv, WV, wv, to wV.

$627 \mu\text{mol m}^{-2} \text{s}^{-1}$). Light response curves were also measured on these leaves at these times. Leaf water potential was measured on one leaf from each of the six trees per bay twice during each phase using a pressure chamber (PMS Instrument Co., Corvallis, OR, USA).

System-level CO_2 exchange. We estimated SNCE ($\mu\text{mol m}^{-2}$ soil surface area s^{-1}) from changes in the bay CO_2 concentrations measured at 15 min intervals in the light (SNCE_L) and dark (SNCE_D):

$$\text{SNCE} = (C_{\text{change}} + C_{\text{in}} + C_{\text{inj}} - C_{\text{out}} - C_{\text{leak}}) / (\text{area} \times \text{time}),$$

where C_{change} is the mole fraction change in CO_2 concentration during a 15 min period, C_{in} is mole fraction of CO_2 entering the system, C_{inj} is mole fraction of pure CO_2 injected to maintain the desired set point, C_{out} is mole fraction of CO_2 exiting the

system, and C_{leak} is mole fraction of CO_2 leaking into or out of the system; area is soil surface area of the bays (m^2), and time is in seconds.

System gross CO_2 assimilation (SGCA, $\mu\text{mol m}^{-2} \text{s}^{-1}$ soil surface area basis) was calculated by adding SNCE_L and SNCE_D. System CO_2 exchange in the dark, the sum of both soil and leaf respiration, was assumed to proceed at the same rate throughout the 24 h cycle. We then obtained SGCA per unit leaf area (SGCA_L, $\mu\text{mol m}^{-2}$ leaf area s^{-1}) by dividing SGCA by the leaf area index (LAI).

LAI was estimated from the area of leaves on a subset of branches on each tree using equations relating leaf area to leaf length and width. Area of all leaves per branch was integrated and regressed against branch diameter and length to obtain leaf area by branch. Based on this, leaf area was estimated for all branches per tree for a subset of trees and leaf area per tree was regressed against tree diameter and height to obtain leaf area of each individual tree in the bay. Leaf area growth was estimated by applying tree diameter \times height–leaf area regression equations to monthly phenology data. At the end of each phase of the experiment, all leaves were gathered from the forest floor, oven dried, and converted to a leaf area estimate by multiplying the dry weight by a specific leaf area value estimated separately for each bay. Specific leaf areas were 178 and $157 \text{ cm}^2 \text{ g}^{-1}$ of dry leaf for the ambient and elevated CO_2 treatments.

Statistical analyses

We used a regression approach similar to the one adopted by Engel *et al.* (2004). This was appropriate for a nonreplicated design that tested specific pairs of treatments (CO_2 , soil moisture, or VPD) defined to be of interest. This approach used a regression of the SNCE of one treatment on the SNCE of another treatment. For example, to determine the effect of soil moisture under conditions of low VPD and ambient CO_2 , the SNCE for the treatment combination of high moisture, low VPD–ambient CO_2 (Wvc) was regressed against the SNCE for the treatment combination of low-moisture, low-VPD–ambient CO_2 (wvc). Similarly, the effects of CO_2 , soil moisture, and VPD on SNCE were tested. There were $4 + 4 + 4 = 12$ regressions. The null hypothesis was that the intercept is 0.0 and the slope is 1.0 (i.e. there is no difference between the SNCE for the treatments given).

We also tested for the effect of soil moisture and VPD by assuming a randomized block design analysis of variance (RCB-ANOVA). The two bays used in this study were each assigned a CO_2 concentration for the duration of the study and were considered as two

blocks that represent a random selection of the CO₂ concentrations to which inferences could be applied. Within each of the bays, two levels of soil moisture and two levels of VPD were applied to the stand of trees; each of these treatment combinations was used in each bay for a sequence of several days. Subsampling was done on the response variables every 15 min during the daylight hours. A randomized block analysis was performed where the soil moisture and VPD treatments formed a 2 × 2 treatment structure along with the soil moisture–VPD interaction. All interactions of the CO₂ blocks with soil moisture and VPD were assumed absent and were pooled to form the error term for testing the main effects of soil moisture and VPD and its interaction. SAS software (SAS Institute Inc., 1988) was used for all statistical analyses.

Results

Treatment parameters

Other than the designated CO₂ treatments, no differences in parameters (Fig. 1 and Table 1) were apparent between the two bays in any of the phases of the experiment. With the exception of 2 days, atmospheric CO₂ concentration was controlled within the set points (Fig. 1d). During phase 1 (Wv), average VPD was maintained at 0.5 kPa, causing excessive condensation of water vapor on the glass; so VPD was increased to 0.9 kPa during phase 3 (wv; Fig. 1c) to minimize water recycling to the soil from the glass during the dry down period. Total daytime PPF was higher during phase 1 (Wv) than in any of the subsequent treatments. During drying phases 3 (wV) and 4 (wv), average soil moisture content in the ambient CO₂ treatment was 18% lower than that in the elevated CO₂ treatment (Fig. 1b, Table 1).

Stand leaf area

Growth in high CO₂, prior to beginning the experiment (Table 2), increased average total leaf area by 49% relative to the ambient CO₂ treatment. The trees grown in elevated CO₂ maintained a higher leaf area throughout the experiment. Leaves were shed in response to increasing water stress (especially between treatments Wv and wv; Table 2), reducing canopy leaf area of the ambient CO₂ treatment by 31%, and of the elevated CO₂ treatment by 17% and by 50% in the low and high VPD treatments, respectively.

Leaf water potential (Ψ)

Predawn leaf water potential (Ψ; Fig. 2a) was more negative in response to low soil moisture. High VPD

Table 2 Stand total leaf area and leaf area indices estimated for the ambient and elevated CO₂ treatment bays of the cottonwood plantation Biosphere 2 laboratory during the course of the experiment for the various soil moisture, VPD, and CO₂ treatment combinations

Treatment	Leaf area (m ²)		Leaf area index (m ² m ⁻²)	
	Ambient CO ₂	Elevated CO ₂	Ambient CO ₂	Elevated CO ₂
Wv	1716	2549	3.14	4.69
WV	1716	2549	3.14	4.69
wv	1714	2105	3.13	3.87
wV	1177	12 654	2.15	2.33

Treatment combinations in the table are as in Table 1. VPD, vapor pressure deficit.

produced even more negative Ψ. Leaf water potential was less negative in the elevated CO₂ treatment than in ambient CO₂ under all soil moisture and VPD conditions. However, the only statistically significant difference (RCB-ANOVA approach) in predawn Ψ because of CO₂ concentration occurred under the most severe moisture stress (wV; Fig. 2a). Mid-day Ψ became more negative with increasing water stress treatments, well-irrigated plants at high VPD showing the most negative response. High VPD in the well-watered treatment (WV) produced almost the same low leaf water potential at mid-day as did the low soil moisture, low VPD treatment (wv; Fig. 2b). Although mid-day leaf water potentials in elevated CO₂ were consistently higher than in the ambient CO₂ treatment, there were no significant differences.

System-level CO₂ exchange

The 24-h patterns of SNCE for stands of ECW in each of the four soil moisture and VPD treatment combinations, and of PPF, are shown as hourly means with an SE for 5–10 days of each treatment (Fig. 3). In marked contrast to flux tower studies, the highest precision of CO₂ exchange was obtained nocturnally, and this efflux was always greater in elevated CO₂ treatments. Elevated CO₂ stimulated both system CO₂ uptake and respiratory CO₂ release in all treatments. Low soil moisture decreased the difference between the CO₂ treatments, with the combination of low soil moisture and high VPD bringing the SNCE curves closest together (Fig. 3c and d). Hysteresis, as a result of the volume–flow relationships in the large chambers, was similar in all treatments: the transition from system respiratory CO₂ loss to photosynthetic CO₂ uptake occurred at about the same time (07:30 hours) each

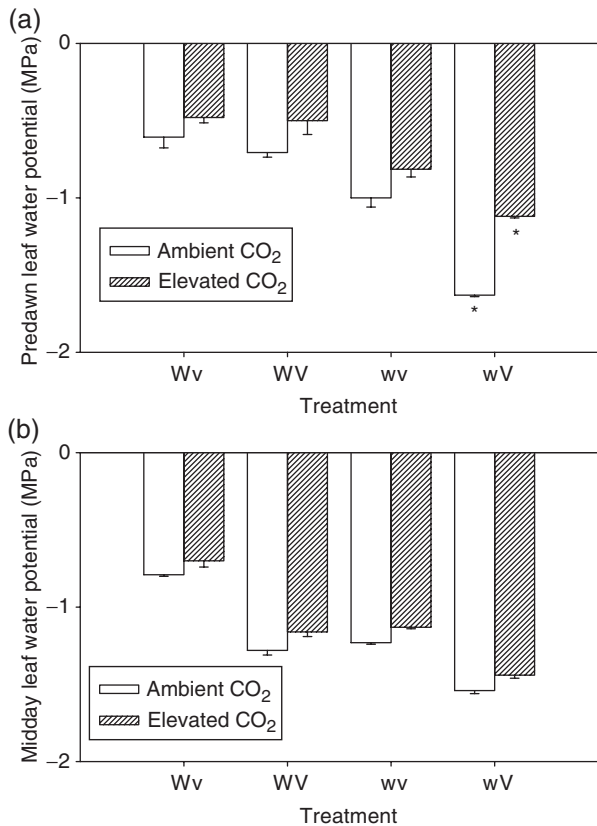


Fig. 2 Mean (a) predawn and (b) mid-day leaf water potential (MPa) for poplar leaves from trees exposed to various soil water and vapor pressure deficit (VPD) treatment combinations in ambient and elevated CO₂. Treatments are designated as W, w, high and low water (low and high soil moisture stress) and V, v, high and low VPD treatments, respectively. Asterisks within a treatment combination denote significant difference between CO₂ treatments at an α of 0.05.

morning, 1–2 h after sunrise, when PPF was about 100–200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. In the evening, the reverse transition was complete at dusk (about 19:00 hours). Integrated daily CO₂ exchange (D-CO₂; mol CO₂ day⁻¹) was calculated as the sum of all assimilation values between the respiration–photosynthesis transitions shown in Fig. 3. There were strong interactions between water stress treatments and system assimilation with a two- to sevenfold increase under high CO₂, the effect being found in the most severe water stress treatment (wV) (Fig. 3). The reduction in D-CO₂ with increasing water stress was always greatest in the ambient CO₂ treatments.

System-level data in Fig. 3 were dissected into key process components for later comparison with leaf-level data, to examine the relative responses of process components to high CO₂, and to evaluate the roles of leaf area differences between treatments on the balance

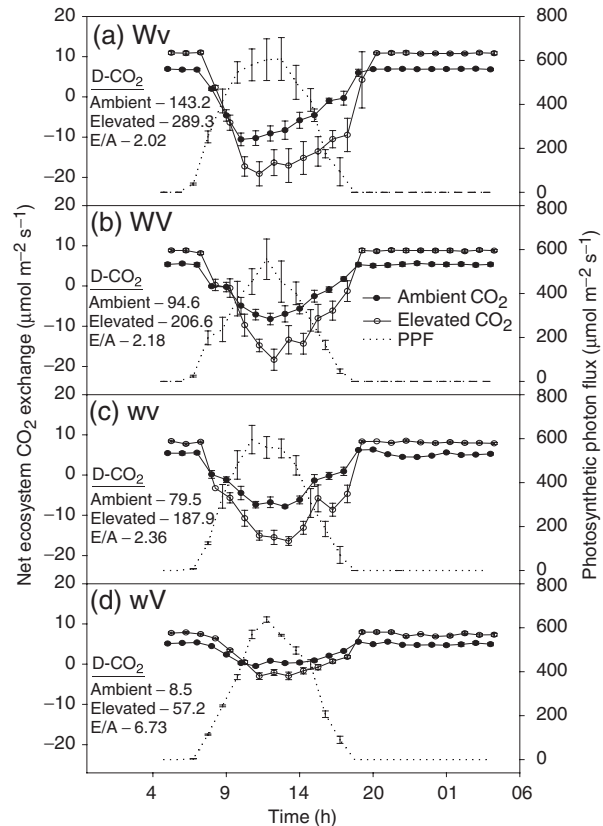


Fig. 3 Daytime trend of system net CO₂ exchange (SNCE) ($\mu\text{mol CO}_2\text{ m}^{-2}\text{ s}^{-1}$) for stands of poplar trees grown under a combination of ambient and elevated CO₂. The atmospheric convention is used, in which photosynthetic CO₂ assimilation is shown by a negative sign (treatment designations as in Fig. 2). Values are hourly averages (\pm SE) for data collected every 15 min each day for the duration of treatments shown in Fig. 1. The daytime trend of photosynthetic photon flux is denoted on the right-hand Y axes. Inset in each panel is the mean integrated daytime CO₂ exchange (D-CO₂; mol day⁻¹) calculated from SNCE_L for ambient and elevated CO₂ treatments. Also shown are the ratios of the elevated to ambient D-CO₂ values (E/A).

of CO₂ fluxes (Table 3). In all cases, mean mid-day SNCE (SNCE_L at PPF 600–700 $\mu\text{mol m}^{-2}\text{ s}^{-1}$) in elevated CO₂ was greater than in ambient CO₂, with the greatest stimulation in the most severe water stress treatment (wV). Mean SNCE_D was 50% higher in elevated than in ambient CO₂ in all treatments, and always declined in response to both high VPD and SMS (Table 3). Mean SGCA was nearly double in high CO₂. In the severe soil and atmospheric water stress treatments, the estimates of SGCA were dominated by SNCE_D.

Correcting SGCA for LAI (i.e. SGCA_L; Table 3) showed that larger leaf area developed in elevated CO₂ during the growing season before beginning the experiment, and the larger leaf fall responses of this canopy to high VPD and SMS accounted for most of

the effect of elevated CO₂ on system-level processes. Thus, SGCA on a leaf area basis (SGCA_L; $\mu\text{mol m}^{-2}\text{leaf area s}^{-1}$) was only slightly higher under elevated CO₂ in Wv and WV treatments, with stronger effects of elevated CO₂ evident in wv and wV treatments. On average, SGCA for treatment Wv in elevated CO₂ was 1.77 times that in ambient (Table 3). LAI in elevated CO₂ was 1.5 times that in ambient (4.69/3.14; Table 2). On a relative basis, leaf area alone accounted for 85% (1.5/1.77) of increased SGCA in high CO₂. The same type of calculation showed that leaf area accounted for 81%, 64%, and 61% of higher SGCA in the WV, wv, and wV treatments grown in high CO₂.

For convenience, statistical evaluations of the 24 h system-level data were based on mean SNCE, averaged throughout the period in which PPF exceeded $100 \mu\text{mol m}^{-2}\text{s}^{-1}$. At high soil moisture (W), elevated CO₂ resulted in greater mean SNCE: from 5.9 to $12.8 \mu\text{mol m}^{-2}\text{s}^{-1}$ at low VPD, and from 4.5 to $10.5 \mu\text{mol m}^{-2}\text{s}^{-1}$ at high VPD. Also under SMS (w), elevated CO₂ resulted in greater mean SNCE: from 4.6 to $11.5 \mu\text{mol m}^{-2}\text{s}^{-1}$ at low VPD, and from -0.86 to $0.74 \mu\text{mol m}^{-2}\text{s}^{-1}$ at high VPD. The consistently greater mean SNCE in elevated CO₂ was significant for all soil moisture and VPD treatment combinations as demonstrated by the *P*-value for the joint test of slope and intercept (Table 4). This was also clear in Fig. 4, and presumably reflected the effects of higher LAI in elevated CO₂ described above. The interaction of VPD and soil moisture on mean SNCE was nonsignificant in

wv treatments, in ambient and elevated CO₂ as shown by the joint test of intercept and slope (*P*-value of regressions 1 and 3, Table 5), but was significant for regressions 5 through 8 (Table 5). A randomized block analysis was done on mean SNCE for each treatment combination. The relative effects of soil moisture and VPD were similar for both CO₂ concentrations, with the main difference being that elevated CO₂ resulted in increased SNCE compared with ambient CO₂. ANOVA showed that soil moisture (*P*-value of 0.0433) and VPD (*P*-value of 0.0288) effects were significant, but the soil moisture–VPD interaction was not significant at the 0.05 level, supporting the independent results of the above regression analysis approach.

SGCA_L data derived from SNCE of Fig. 3 were used to construct light response curves; SGCA_L indicated the maximum gross assimilation capacity per unit leaf area (Fig. 5). Analyses of these curves showed that in elevated, compared with ambient CO₂, the mean initial slope ('apparent' quantum efficiency) across treatments was greater, that saturation was achieved at lower light in the absence of SMS (Table 6). Similarly, values of 'light compensation' were also lower in the elevated CO₂ treatment. Light-saturated SGCA_L and SNCE_D were greater in the elevated CO₂ treatment. Light saturation values decreased, and light compensation points increased with high SMS. No statistical tests were done on light response data. Although the system showed responses analogous to those expected from the CO₂–light interactions of leaves in terms of energy

Table 3 Comparisons of mean mid-day ($600 < \text{PPF} < 700$) SNCE_L, mean SNCE_D, and mean SGCA

Treatment	SNCE _L [*]	SNCE _D ($\mu\text{mol m}^{-2}\text{s}^{-1}$ soil surface area) [†]	SGCA [‡]	SGCA _L ($\mu\text{mol m}^{-2}\text{s}^{-1}$ leaf area) [§]
Wv	11.68 (0.42)	6.83 (0.09)	18.51 (0.41)	5.89 (0.13)
WV	10.93 (0.55)	5.36 (0.12)	16.29 (0.55)	5.19 (0.18)
wv	7.65 (0.21)	5.30 (0.10)	12.95 (0.21)	4.14 (0.07)
wV	0.19 (0.17)	4.37 (0.12)	4.56 (0.13)	2.12 (0.06)
Wv	22.43 (0.68)	10.37 (0.98)	32.80 (0.67)	6.99 (0.15)
WV	21.37 (0.64)	8.76 (0.13)	30.13 (0.64)	6.42 (0.14)
wv	16.95 (0.59)	8.14 (0.29)	25.09 (0.49)	6.48 (0.13)
wV	1.94 (0.32)	6.14 (0.30)	8.08 (0.39)	2.96 (0.17)
Ratio elevated CO ₂ /ambient CO ₂				
Wv	1.92	1.52	1.77	1.19
WV	1.96	1.63	1.85	1.24
wv	2.22	1.54	1.94	1.57
wV	10.21	1.41	1.77	1.40

The relative values obtained by dividing the rates of CO₂ exchange at elevated CO₂ by those at ambient CO₂ are also shown.

^{*}Mean mid-day system net CO₂ exchange in the light (SNCE_L; $700 > \text{PPF} > 600 \mu\text{mol m}^{-2}\text{s}^{-1}$).

[†]Mean nocturnal system net CO₂ exchange in the day (SNCE_D).

[‡]System gross CO₂ assimilation (SGCA = SNCE_L + SNCE_D).

[§]System gross CO₂ assimilation per unit leaf area (SGCA_L = SGCA/LAI).

Table 4 Parameters of the linear regression equations relating SNCE ($\mu\text{mol m}^{-2} \text{s}^{-1}$) obtained for the ambient (c) and the elevated (C) CO_2 treatments and various soil moisture and VPD treatment combinations designated as follows (W and w, high and low soil moisture; V and v, high and low VPD, respectively)

Regression*	<i>n</i>	<i>x</i> treatment mean	<i>y</i> treatment mean	Intercept	Slope	<i>P</i> -value†	<i>R</i> †
1	283	Wvc	WvC	3.6 (0.0001)	1.6 (0.0001)	<0.0001	0.83
2	223	WVc	WVC	2.5 (0.0001)	1.8 (0.0001)	<0.0001	0.83
3	124	wvc	wvC	5.4 (0.0001)	1.3 (0.0001)	<0.0001	0.75
4	448	wVc	wVC	2.3 (0.0001)	1.9 (0.0001)	<0.0001	0.63

*Denotes the regression number.

†Probability value for the joint test of the slope and intercept comparing the two treatment combinations within a row.

SNCE of the ambient and elevated treatments were the dependent and independent variables, respectively ($\text{SNCE}_{\text{AMB}\text{CO}_2} = \text{intercept} + \text{slope} \times \text{SNCE}_{\text{EL}\text{CO}_2}$). Values in parentheses are *P*-values for testing the two individual hypotheses that the intercept is equal to zero and the slope is equal to 1.0. Mean NSE values for the CO_2 treatments, for the different soil moisture and VPD combinations are also included.

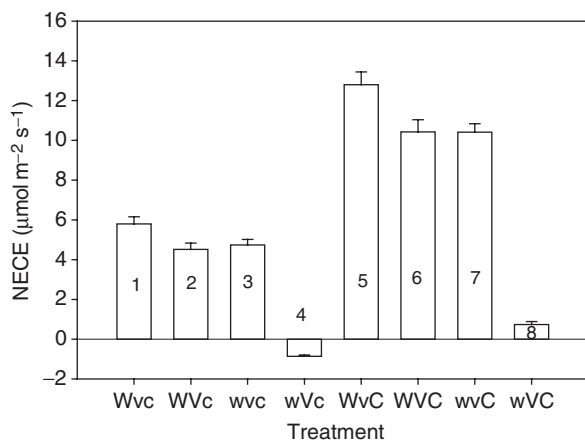


Fig. 4 Light response curves for system gross CO_2 assimilation per unit leaf area at light saturation (SGCA_L) ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) in stands of poplar trees, constructed from data in Fig. 3. The physiological convention is used in which photosynthetic CO_2 assimilation is shown with positive sign, and respiration with a negative sign. The parameters derived from these graphs are shown in Table 6a.

costs of assimilation by Rubisco (von Caemmerer & Farquhar, 1981), the data cannot be converted to absolute values without more detailed analyses of the diffuse light environment in the canopy that is beyond the scope of the present study.

Leaf-level gas exchange

Spot measurements, under selected conditions, were used to detect whether major discontinuities emerged in our understanding of leaf- and system-level pro-

cesses in the course of these experiments. For example, leaf net CO_2 assimilation rate (A_{net} ; Fig. 6a) was consistently greater in elevated CO_2 than in ambient in all soil moisture and VPD combinations. The mean relative increase of A_{net} ($A_{\text{net, elev}}/A_{\text{net, amb}}$) across all treatments was 1.4. Relative increases of A_{net} in response to elevated CO_2 were greatest when soil moisture was low and VPD was high. Stomatal conductance and E were significantly lower under elevated CO_2 in all soil moisture, VPD combinations except the well-watered reference Wv (Fig. 6b and c), with g_s decreasing by 40–50%. Although there appeared to be an anomaly in treatment Wv, in which transpiration did not track leaf stomatal conductance (Fig. 6b and c), E decreased and A_{net} increased in response to high CO_2 , thereby significantly increasing instantaneous water use efficiency (WUE; Fig. 6d) at high, but not at low, soil moisture.

The light response curves of leaf photosynthesis (Fig. 7) showed an additive impact, via stomatal limitation, of SMS and high VPD leading to earlier light saturation and lower photosynthetic rate (Table 6). Elevated CO_2 greatly stimulated photosynthesis in the SMS, VPD treatments. The light compensation point in all cases was below $50 \mu\text{mol m}^{-2} \text{s}^{-1}$. Although measurement protocols were not intended to yield precise values for light use efficiency, estimates ranged from 0.10 to 0.04 (uncorrected for reflectance). As expected, leaves from well-watered trees showed an increase in light use efficiency with high CO_2 , the relative effect being more pronounced at high VPD. Under SMS, elevated CO_2 treatment facilitated maximum light use efficiency at low VPD, but this mitigating effect did not extend to treatment wV.

Table 5 Parameters of the linear regression equations relating SNCE obtained for the various soil water, VPD, and CO₂ treatment combinations

Regression*	<i>n</i>	<i>x</i> treatment mean	<i>y</i> treatment mean	Effect tested	Intercept	Slope	<i>P</i> -value [†]	<i>R</i> [†]
1	123	wvc 4.71	Wvc 5.22	Water	0.90 (0.2760)	0.92 (0.5473)	0.5043	0.27
2	216	wVc -0.11	WVc 4.69	Water	4.89 (0.0001)	1.78 (0.0008)	0.0001	0.22
3	125	wvC 11.69	WvC 11.34	Water	0.33 (0.8740)	0.94 (0.7185)	0.8681	0.22
4	217	wVC 2.62	WVC 10.64	Water	6.16 (0.0001)	1.71 (0.0001)	0.0001	0.33
5	219	WVc 4.54	Wvc 6.70	VPD	3.41 (0.0001)	0.73 (0.0001)	0.0001	0.36
6	124	wVc 0.13	wvc 4.80	VPD	4.60 (0.0001)	1.61 (0.0070)	0.0001	0.30
7	220	WVC 10.54	WvC 13.98	VPD	7.09 (0.0001)	0.65 (0.0001)	0.0001	0.36
8	125	wVC 3.38	wvC 11.75	VPD	7.95 (0.0001)	1.13 (0.2964)	0.0001	0.41

The treatment combinations from which the SNCE were obtained, which are the independent (*x*) or dependent (*y*) variables, are also indicated. Treatment combinations are designated as follows (W and w = high and low soil moisture, V and v = high and low VPD, respectively). Values in parentheses denote probability values.

*Denotes the regression number.

[†]Probability value for the joint test of the slope and intercept comparing the two treatment combinations within a row. SNCE, system net CO₂ exchange; VPD, vapor pressure deficit.

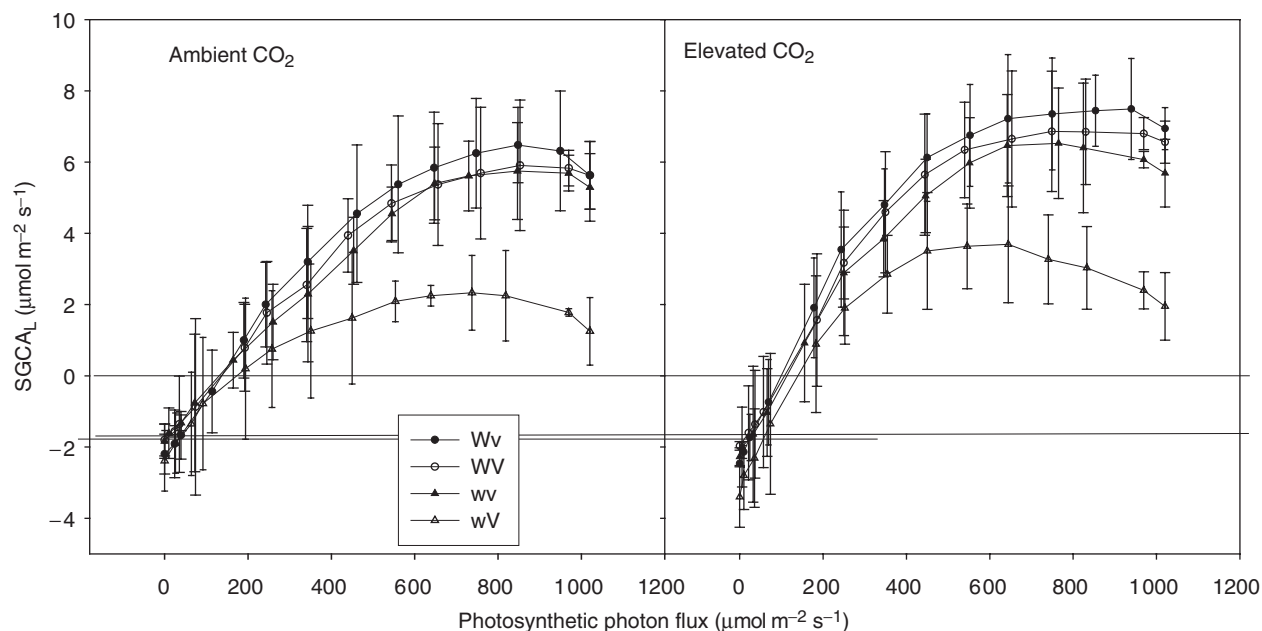

Fig. 5 Interactions of elevated CO₂ (C) and ambient CO₂ (c) with low and high vapor pressure deficit (VPD) (v and V) and soil moisture stress (W and W) on mean system net CO₂ exchange (SNCE) in stands of poplars.

Table 6 Parameters from fitting of light response curves for net CO₂ uptake measured at the leaf (A_{net}) and stand-level (SGCA_L) at ambient and elevated CO₂ for each soil moisture and VPD treatment combination

Treatment*					
Stand-level	Respiration [†]	SGCA _L max	Q _{SGCAL}	L _{comp}	L _{sat}
Wvc	-2.26	8.8	0.017	133	647
WVc	-1.84	7.9	0.014	133	700
wvc	-1.69	7.3	0.012	144	769
wVc	-2.22	4.3	0.013	168	494
WvC	-2.4	10.2	0.025	95	499
WVC	-2.07	9.1	0.021	100	539
wvC	-2.19	8.6	0.019	113	558
wVC	-3.09	6.1	0.021	149	444

Leaf-level	Respiration	A _{max}	Q _e	L _{comp}	L _{sat}
Wvc	-2.23	37.9	0.091	24.4	439
WVc	-0.42	27.1	0.069	6.11	402
wvc	-1.04	5.76	0.034	26.9	175
wVc	-1.16	3.98	0.056	20.8	92.3
WvC	-0.91	49.9	0.102	8.92	499
WVC	-1.06	21.1	0.084	12.5	262
wvC	-1.26	12.3	0.103	12.3	132
wVC	-0.81	8.99	0.044	18.4	224

*Treatments are as specified in Table 1.

[†]Across the row, abbreviations are respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$), maximum net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$), quantum flux efficiency, light compensation point ($\mu\text{mol m}^{-2} \text{s}^{-1}$), and light saturation point ($\mu\text{mol m}^{-2} \text{s}^{-1}$).

SGCA_L, system gross CO₂ assimilation per unit leaf area at light saturation; VPD, vapor pressure deficit.

Comparison of CO₂ exchange rates at the system- and leaf-level

Estimates of SGCA_L derived from system-level SGCA (Table 3) gave robust estimates of canopy gross assimilation on a leaf area basis for comparisons with leaf-level data (Table 7). We reasoned that A_{gross} (A_{net} + leaf respiration) was a valid basis for comparison with SGCA_L. These comparisons seem legitimate because SGCA_L presented here was estimated at mid-day and at light saturation (Table 3), and because A_{net} data were obtained *in situ* on midcanopy leaves at near-saturating PPF values. The CO₂ fertilization response was evident at system- and leaf-level in all treatments, and on average, the relative responses were similar. The most striking feature of the comparisons in Table 7 is the way the relative magnitudes of system- and leaf-level estimates of CO₂ assimilation responded to SMS. The ratio of A_{gross} /SGCA_L ranged from 3.2 to 3.5 in well-watered treatments at low and high VPD, in

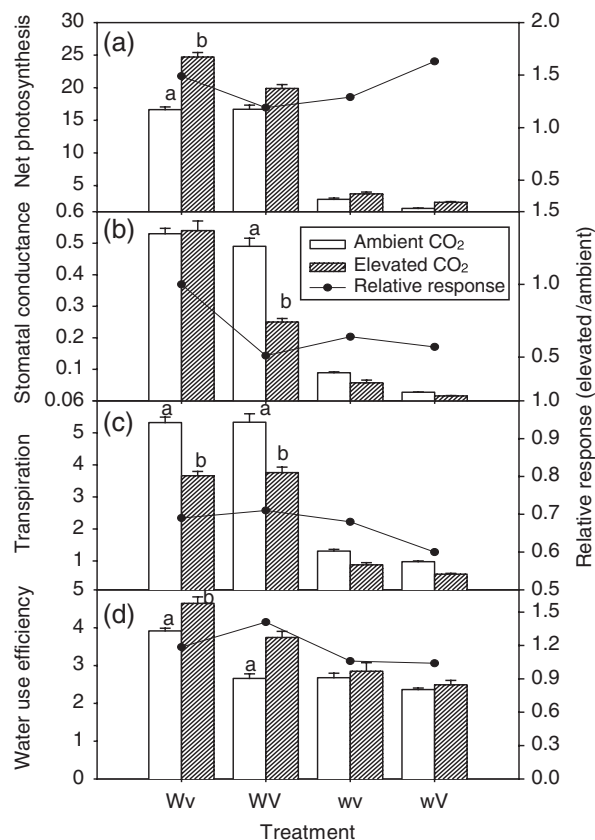


Fig. 6 Mean leaf-level gas exchange parameters (a) net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), (b) stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$), (c) transpiration ($\text{mmol m}^{-2} \text{s}^{-1}$), and (d) instantaneous water use efficiency (E/A_{net}). Relative responses; the ratio of elevated to ambient CO₂ treatment for each of the above variables is shown on the right-hand Y axes. Different letters within a treatment combination denote significant difference between CO₂ treatments at an α of 0.05.

ambient or high CO₂. Under SMS these ratios were always about equal (0.8–1.3).

Discussion

Increased aboveground biomass, as well as increased leaf area, was reported in poplar plantations exposed to elevated CO₂ (Ceulemans *et al.*, 1995; Tognetti *et al.*, 1999; Ferris *et al.*, 2001). Our experiments, with enclosed model cottonwood plantations in controlled environments, confirmed the previously reported response of biomass and leaf area (Engel *et al.*, 2004) and go further in permitting direct assessment of the mitigation of atmospheric and SMS on system-level photosynthesis and respiration by elevated CO₂. System-level measurements of nocturnal CO₂ efflux from soil, roots and

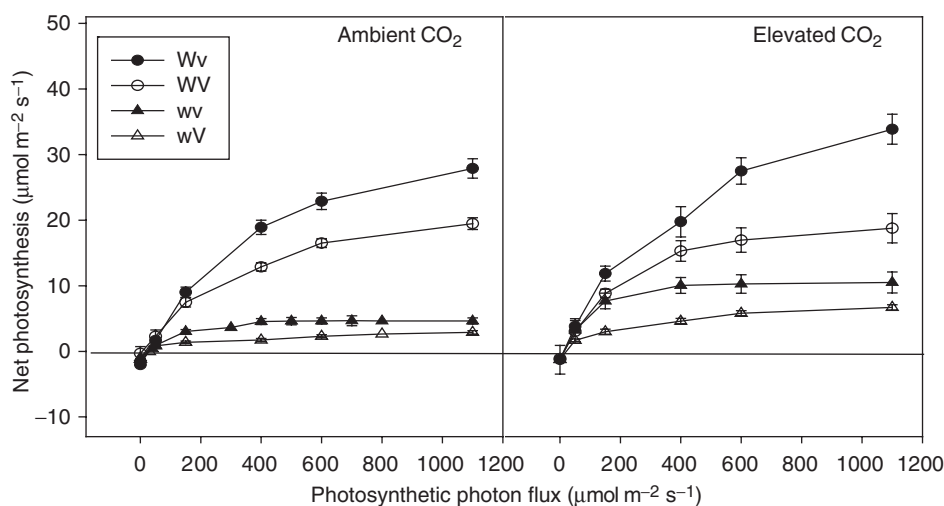


Fig. 7 Response of poplar leaf net photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) to photosynthetic photon flux ($\mu\text{mol photons } \mu\text{m}^{-2} \text{ s}^{-1}$). Treatment designations as in Fig. 3.

Table 7 Comparisons of system- and leaf-level photosynthesis on a leaf area basis (SE shown in parentheses)

Treatment	SGCA _L [*] ($\mu\text{mol m}^{-2} \text{ s}^{-1}$ leaf area)	$A_{\text{gross}}^{\dagger}$	$A_{\text{gross}}/\text{SGCA}_L$
<i>Ambient CO₂</i>			
Wv	5.89 (0.13)	16.6 (0.04) + 2.3	3.2
WV	5.19 (0.18)	16.7 (0.10) + 0.4	3.3
wv	4.14 (0.07)	2.9 (0.12) + 1.0	0.9
wV	2.12 (0.06)	1.6 (0.16) + 1.2	1.3
<i>Elevated CO₂</i>			
Wv	6.99 (0.15)	24.7 (0.07) + 0.9	3.5
WV	6.42 (0.14)	19.9 (0.12) + 1.1	3.3
wv	6.48 (0.13)	3.73 (0.14) + 1.3	0.8
wV	2.96 (0.17)	2.44 (0.17) + 0.8	1.1
<i>Ratio elevated CO₂/ambient CO₂</i>			
Wv	1.19	1.49	1.1
WV	1.24	1.19	1.0
wv	1.57	1.29	0.9
wV	1.40	1.63	0.8

The relative values obtained by dividing the rates of CO₂ exchange at elevated CO₂ by those at ambient CO₂ are also shown.

^{*}From Table 3; rates of gross assimilation per unit leaf area derived from SGCA.

[†]From Fig. 6; rates of gross photosynthesis ($A_{\text{net}} + \text{respiration}$) as measured by the Li-Cor.

SGCA, system gross CO₂ assimilation.

aboveground biomass were highly reproducible, were always greater in elevated CO₂, and did not respond much to water stress treatments (Fig. 3, Table 3). Previous experiments showed that after 12 months, growth of the cottonwood plantation at ambient CO₂, belowground (roots + soil) respiration was comparable in each bay (Murthy *et al.*, 2003). The stimulation in system respiration in elevated CO₂ reported here reflects the greater biomass in this treatment and the provision of more respiratory substrates from stimulated photo-

synthesis that was confirmed over subsequent growing seasons (Barron-Gafford *et al.*, 2004; in press).

The development of higher LAI during growth in elevated CO₂ prior to the application of treatments, and the loss of LAI in response to SMS, altered system level SNCE and D-CO₂ by increasing total light intercepted. With the sun directly overhead, the fraction of light (F) absorbed by a canopy is given by

$$F = 1 - e^{-k \times \text{LAI}},$$

where e is the base of the natural logarithm, k is an extinction coefficient (depending on solar angle and leaf angle distribution, usually averaging = 0.5). We can assume that the glass structure of B2 laboratory had a similar effect on light penetration in both treatments, so that with LAI = 4.7 in elevated CO₂ treatment the stand would absorb 90.5% of the incident light, and at LAI = 2.3 the ambient treatment would absorb 67%. As the solar angle decreased, and the path length through the canopy increased during these experiments conducted after the Fall solstice, the difference in light absorption in the CO₂ treatments decreased. However, leaf fall in response to SMS and VPD treatments wv and wV (Table 2) brought light interception closer together as the experiment progressed.

Thus, it is clear that differences in LAI and light interception alone cannot account for the 2-fold increase in SNCE and D-CO₂ in elevated CO₂ (Fig. 3), and a response to CO₂ fertilization is evident. When system-level gas exchange is corrected for respiration and leaf area, the stimulation of system assimilation by elevated CO₂ increased from only 18% in well-irrigated treatments to 98% in the most severe water stress (Table 3). It seems likely that CO₂ fertilization was mediated by direct effects on carbon assimilation via Rubisco, which compensated the indirect effects of reduced stomatal conductance. This is indicated by the large effects of elevated CO₂ on the initial slope, and maximum rate, of light response curves for SGCA_L (Table 6). It is clear that elevated CO₂ also led to a 50% increase in system respiration (SNCE_p) in all treatments, but how much of this can be ascribed to the effects of elevated CO₂ on leaf area (more respiring plant material) and to CO₂ fertilization (more substrate available for plant and soil respiration) is difficult to estimate. Following this experiment all trees were coppiced leaving only the tree stump aboveground, and the system CO₂ efflux measured were -1.3 and -0.9 μmol m⁻² s⁻¹ for the ambient and elevated CO₂ treatments, respectively. These data suggest that most of the stand-level respiration was because of aboveground biomass. However, during the water stress treatments, aboveground respiratory values were probably underestimated, because belowground respiration was lower (treatments having reduced the soil microbial load and respiration; Barron-Gafford *et al.*, 2004; Lipson *et al.*, 2004) than the carbohydrate input from the aboveground component. Nevertheless, the values still provided a good indication of system level, aboveground respiration, which was greater in the elevated CO₂ treatment, presumably because of the higher leaf area.

Making the widely used assumption that system-level respiration continues unabated in the light, and that system-level gross assimilation can be estimated by

adding respiration to SNCE, we obtained SGCA on a leaf area basis, that could be compared with leaf-level gross CO₂ assimilation data ($A_{\text{gross}} = A_{\text{net}} + \text{leaf respiration}$). Although we did not set out to gather comprehensive leaf-level data to test models against measured system-level CO₂ exchange, our gas exchange data from attached midcanopy leaves were obtained at incident values of PPF corresponding to light saturation in the stands (Figs 4, 6). These data exposed marked differences between leaf- and system-level process responses to elevated CO₂ and water stress treatments that may be useful in scaling future measurement and modeling studies. Although some researchers believe that relative responses to elevated CO₂ should be used with caution, especially during conditions of drought (Gunderson *et al.*, 2002), we find it convenient for purposes of discussion (Figs 3 and 6, Tables 3 and 6), but in all cases, absolute values are available in the data sets.

Relative to well-irrigated controls, our continuous measurements of SNCE revealed large decreases in D-CO₂ (Fig. 3) at ambient and elevated CO₂ in response to controlled atmospheric water stress at high VPD (means of 36% and 67%). Although greater stand LAI contributed most to the increase in D-CO₂ because of high CO₂, there was no response of LAI to VPD in well-irrigated trees (Table 2); other explanations have to be found for the large decline in D-CO₂ in the WV treatments compared with Wv. This was a surprisingly large response, given the small effects of VPD on leaf-level assimilation (Fig. 6), and presumably reflected the 27% decrease in average daily PPF in this treatment (Table 1). The light environment remained remarkably stable at the lower value in subsequent treatments, so the decline in assimilation because of lower PPF needs to be taken into account in all comparisons of treatments with Wv. Controlled SMS at low VPD produced some further reduction in D-CO₂ (56% and 65% at ambient and high CO₂, respectively), whereas exposure to water stress simultaneously in the soil and atmosphere had a large effect, reducing D-CO₂ to only 6% and 20% of Wv. In all cases, the application of high VPD and SMS had smaller relative effects in elevated CO₂ treatments than in ambient CO₂ treatments.

The effects of elevated CO₂, high VPD, and SMS on leaf-level gas exchange were comparable with responses obtained from other C₃ plant leaves, even though the elevated CO₂ treatment in our experiments was three times ambient. Across all treatments, the mean relative increase of A_{net} because of elevated CO₂ was 1.4, comparable with the lower range of other reported values (1.5–1.8; Gunderson & Wullschleger, 1994; Curtis, 1996; Ellsworth, 1999; Medlyn *et al.*, 1999; Norby *et al.*, 1999). Elevated CO₂ had no effect on g_s in the well-irrigated reference treatment Wv, as found in

some other trees (Teskey, 1995; Murthy *et al.*, 1997; Ellsworth, 1999), but in all other treatments, the relative value in elevated CO₂ declined to 0.5–0.6 (Fig. 6), a little larger than reported for some trees (Field *et al.*, 1995; Curtis, 1996; Drake *et al.*, 1997; Curtis & Wang, 1998; Medlyn *et al.*, 2001; Gunderson *et al.*, 2002) and possibly reflecting the higher CO₂ concentration used here. The effects of elevated CO₂ and moisture stress (via stomatal conductance) on leaf-level light response curves (Table 6) show the well-known interactions of CO₂ assimilation kinetics in air (via Rubisco) with leaf internal CO₂ concentrations and the energy costs of CO₂ recycling in photorespiration (von Caemmerer & Farquhar, 1981).

In general, leaf-level responses to high CO₂ and VPD were poor indicators of system-level responses in well-watered treatments Wv and WV. Although the relative increase in system-level SGCA_L and leaf-level A_{net} to elevated CO₂ at the same incident PPF was more or less similar across treatments, the ratio of $A_{\text{net}}/\text{SGCA}_L$ was about three under well-irrigated conditions. In contrast, the large effects of wv and wV treatments on leaf-level g_s and A_{net} (Fig. 6) brought A_{net} into close proximity with SGCA_L. The ratio change was strongly driven by the most direct and least equivocal measurement, A_{net} , and leaving aside the possibility that measurements were made on unrepresentative leaves, the ratio change presumably signaled major buffering of leaf gas exchange processes in the context of canopy assimilation. In well-irrigated treatments, Wv and WV, it is unlikely that SNCE, SGCA, and SGCA_L were limited by leaf-level gas exchange capacity, but rather, by canopy light environment, with an estimated 70–90% of the incident photosynthetically active radiation absorbed. This is consistent with the fact that the response of SGCA_L to PPF showed the same saturation curve in all except the most severe treatment (wV). Clearly, detailed evaluations of PPF gradients and of A_{net} light response profiles in the canopy are needed to properly test this possibility. The large effects of wv and wV treatments on leaf-level g_s and A_{net} suggest that individual leaf properties, rather than canopy architecture, dominated stand-level responses under SMS.

Complexity emerged in other leaf-to-stand-level scaling comparisons, especially under SMS. For example, the leaf ψ of well-irrigated trees in ambient CO₂ became more negative in mid-day (but not predawn) in response to high VPD (Fig. 2), with effects on A_{net} and g_s (Fig. 6a and b) that were not simply reflected in the 24 h measurements of SNCE (Fig. 3), or in calculated SGCA and SGCA_L at the system level (Table 3). Predawn values of leaf ψ in the most extreme stress (wV) were significantly lower under ambient CO₂, but mid-day leaf ψ did not change compared with WV

treatments (Fig. 2) and yet g_s and A_{net} declined fivefold (Fig. 6). Explanations of these interactions are beyond the scope of this paper, but the drastic drop in SNCE values with the wV treatment suggests a catastrophe in the transpiration system, consistent with xylem cavitation, possibly similar to the sudden decreases observed for SNCE in *Pinus ponderosa* stands (Law *et al.*, 2001). The reasons for extensive leaf fall in response to SMS in elevated CO₂ treatments are not obvious. In both CO₂ treatments, predawn leaf ψ in SMS treatments was only a little lower than in well-irrigated treatments, and the mid-day excursion was less in high CO₂, presumably because of the large decline in stomatal conductance. Larger LAI, in the elevated CO₂ treatment prior to the experiment led to greater shading, and this might have favored greater senescence and abscission under conditions of SMS.

With these limitations in mind, we submit that these preliminary, large-scale, but still relatively short-term, top-down experiments in controlled environments illustrate the potential for evaluating the effects of system-level processes, such as changes in LAI, on responses of system-level assimilation to elevated CO₂ and its interactions with water stress. At the same time they illustrate the need to comprehensively document leaf-level responses to light and VPD environments, and to define these environments, before attempting bottom-up simulations of system-level processes, even if changes in LAI because of treatments are taken into account. We seem to be some way from the goal of predicting these second-order effects of tree water relations on leaf retention and function, let alone predicting biological feedbacks in climate models from leaf-level responses.

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References

- Baldocchi D (1997) Measuring and modelling carbon dioxide and water vapor exchange over a temperate broad-leaved forest during the 1995 summer drought. *Plant, Cell and Environment*, **20**, 1108–1122.
- Baldocchi DD (1993) Scaling water vapor and carbon dioxide exchange from leaves to canopies: rules and tools. In: *Scaling Physiological Processes Leaf to Globe* (eds Ehleringer JH, Field CB), pp. 77–114. Academic Press, San Diego.

- Barron-Gafford G, Martens D, Grieve K *et al.* (2005) Growth of eastern cottonwoods (*Populus deltoides*) in elevated CO₂ stimulates stand-level respiration and rhizodeposition of carbohydrates, accelerates soil nutrient depletion, yet stimulates above and belowground biomass production. *Global Change Biology* (in press).
- Blake TJ, Sperry JS, Tschaplinski TJ *et al.* (1996) Water relations. In: *Biology of Populus and its Implications for Management and Conservation* (eds Stettler RF, Bradshaw Jr HD Jr, Heilman PE, Hinckley TM, Field CR), pp. 401–419. NRC Research Press, Ottawa, ON, Canada.
- Buchmann N (2002) Plant ecophysiology and forest response to global change. *Tree Physiology*, **22**, 1177–1184.
- Bunce JA, Ziska LH (1998) Decreased hydraulic conductance in plants at elevated carbon dioxide. *Plant, Cell and Environment*, **21**, 121–126.
- Caldwell MM, Matson PA, Wessman C *et al.* (1993) In: *Scaling Physiological Process from the Leaf to the Globe* (eds Ehleringer J, Field CR), pp. 223–230. Academic Press, San Diego.
- Ceulemans R, Jiang XN, Shao BY (1995) Growth and physiology of one-year old poplar (*Populus*) under elevated atmospheric CO₂ levels. *Annals of Botany*, **75**, 609–617.
- Clark KL, Cropper WP Jr, Gholz HL (2001) Evaluation of modeled carbon fluxes for a slash pine ecosystem: SPM2 simulations. *Forest Science*, **47**, 52–59.
- Curtis PS (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant, Cell and Environment*, **19**, 127–137.
- Curtis PS, Wang X (1998) A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia*, **113**, 299–313.
- Dempster WF (1999) Biosphere 2 engineering design. *Ecological Engineering*, **13**, 31–42.
- Drake BG, Gonzalez-Meler M, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO₂? *Annual Review of Plant Physiology and Plant Molecular Biology*, **48**, 609–639.
- Eamus D, Duff GA, Berryman CA (1995) Photosynthetic responses to temperature, light flux-density, CO₂ concentration and vapor pressure deficit in *Eucalyptus tetrodonta* grown under CO₂ enrichment. *Environmental Pollution*, **90**, 41–49.
- Ellsworth DS (1999) CO₂ enrichment in a maturing pine forest: are CO₂ exchange and water status in the canopy affected? *Plant, Cell and Environment*, **22**, 461–472.
- Engel VC, Griffin KL, Murthy R *et al.* (2004) Growth CO₂ modifies the transpiration response of *Populus deltoides* to drought and vapor pressure deficit. *Tree Physiology*, **24**, 1137–1145.
- Ferris R, Sabatti M, Miglietta F *et al.* (2001) Leaf area is stimulated in *Populus* by free air CO₂ enrichment (POPFACE), through increased cell expansion and production. *Plant, Cell and Environment*, **24**, 305–315.
- Field CB, Jackson RB, Mooney HA (1995) Stomatal responses to increased CO₂: implications from the plant to the global scale. *Plant, Cell and Environment*, **18**, 1214–1225.
- Gregory JM, Mitchell JFB, Brady AJ (1997) Summer drought in northern midlatitudes in a time-dependent CO₂ climate experiment. *Journal of Climate*, **10**, 662–686.
- Griffin KL, Turnbull MH, Murthy R *et al.* (2002) Leaf respiration is differentially affected by leaf vs. ecosystem night-time warming. *Global Change Biology*, **8**, 479–485.
- Gunderson CA, Sholtis JD, Wullschlegel SD *et al.* (2002) Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO₂ enrichment. *Plant, Cell and Environment*, **25**, 379–393.
- Gunderson CA, Wullschlegel SD (1994) Photosynthetic acclimation in trees to rising atmospheric CO₂: a broader perspective. *Photosynthesis Research*, **39**, 369–388.
- Grant RF, Nalder IA (2000) Climate change effects on net carbon exchange of a boreal aspen–hazelnut forest: estimates from the ecosystem model ecosys. *Global Change Biology*, **6** (2), 183–200.
- IPCC report 2001. IPCC (Intergovernmental Panel on Climate Change) (2001) Third Assessment Report of Working Group I. United Nations Environmental Programme, Geneva, Switzerland.
- Kramer PJ, Boyer JS (1995) *Water Relations of Plants and Soils*. Academic Press, San Diego.
- Law BE, Goldstein AH, Anthoni PM *et al.* (2001) Carbon dioxide and water vapor exchange by young and old ponderosa pine ecosystems during a dry summer. *Tree Physiology*, **21**, 299–308.
- Lin G, Marino BDV, Wei Y *et al.* (1998) An experimental and modeling study of responses in ecosystems carbon exchanges to increasing CO₂ concentrations using a tropical rainforest mesocosm. *Australian Journal of Plant Physiology*, **25**, 547–556.
- Lipson DA, Blair M, Barron-Gafford G *et al.* (2004) Relationships between microbial diversity and soil processes under elevated atmospheric carbon dioxide and drought. *Applied and Environmental Microbiology* (submitted).
- Medlyn BE, Badeck F-W, De Pury DGG *et al.* (1999) Effects of elevated CO₂ on photosynthesis in European forest species: a meta-analysis of model parameters. *Plant Cell and Environment*, **22**, 1475–1495.
- Medlyn BE, Barton CVM, Broadmeadow MSJ *et al.* (2001) Stomatal conductance of forest species after long-term exposure to elevated CO₂ concentration: a synthesis. *New Phytologist*, **149**, 247–264.
- Murthy R, Griffin KL, Zarnoch SJ *et al.* (2003) Carbon dioxide-efflux from a 550 m³ soil across a range of soil temperatures. *Forest Ecology and Management*, **178**, 311–317.
- Murthy R, Zarnoch SJ, Dougherty PM (1997) Seasonal trends of light-saturated net photosynthesis and stomatal conductance of loblolly pine trees grown in contrasting environments of nutrition, water and carbon dioxide. *Plant, Cell and Environment*, **20**, 558–568.
- Norby RJ, Wullschlegel SD, Gunderson CA *et al.* (1999) Tree responses to rising CO₂ in field experiments: implications for the future forest. *Plant Cell and Environment*, **22**, 683–714.
- Norman JM (1993) Scaling processes between leaf and canopy levels. In: *Scaling Physiological Processes Leaf to Globe* (eds Ehleringer JH, Field CB) pp. 41–76. Academic Press, San Diego.
- Osmond B, Ananyev G, Berry J *et al.* (2004) Changing the way we think about global change research: scaling up in experimental ecosystem science. *Global Change Biology*, **10**, 393–407.

- Pataki DE, Oren R, Tissue DT (1998) Elevated carbon dioxide does not affect stomatal conductance of *Pinus taeda* L. *Oecologia*, **117**, 47–52.
- Rosenzweig C, Hillel D (1993) The Dust Bowl of the 1990s: analog of greenhouse effect in Great Plains? *Journal of Environmental Quality*, **22**, 9–22.
- SAS Institute Inc. (1988) *SAS Procedures Guide. Release 6.03 Edition*. SAS Institute Inc., Cary, NC.
- Sterky F, Regan S, Karlsson J (1998) Gene discovery in the wood-forming tissues of poplar: analysis of 5692 expressed sequence tags. *Proceedings of the National Academy of Sciences USA*, **95**, 13330–13335.
- Taylor G (2002) Poplar: Arabidopsis for forestry. Do we need a model tree? *Annals of Botany*, **90**, 681–689.
- Teskey RO (1995) A field study of the effects of elevated CO₂ on carbon assimilation, stomatal conductance and leaf and branch growth of *Pinus taeda* trees. *Plant Cell and Environment*, **18**, 565–573.
- Tognetti R, Longobucco A, Miglietta F *et al.* (1999) Water relations, stomatal response and transpiration of *Quercus pubescens* trees during summer in a Mediterranean carbon dioxide spring. *Tree Physiology*, **19**, 261–270.
- Turnbull MH, Murthy R, Griffin KL (2002) The relative impacts of daytime and night-time warming on photosynthetic capacity in *Populus deltoides*. *Plant Cell Environment*, **25**, 1729–1737.
- Tuskan GA, DiFazio SP, Teichmann T (2004) Poplar genomics is getting popular. *Plant Biology*, **6**, 2–5.
- von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, **153**, 376–387.
- Wallin G, Linder S, Lindroth A *et al.* (2001) Carbon dioxide exchange in Norway spruce at the shoot, tree, scale and ecosystem scale. *Tree Physiology*, **b**, 969–976.
- Will RE, Teskey RO (1997) Effect of elevated carbon dioxide concentration and root restriction on net photosynthesis, water relations and foliar carbohydrate status of loblolly pine seedlings. *Tree Physiology*, **17**, 655–661.
- Wilson KB, Baldocchi DD, Hanson PJ (2001) Leaf age affects the seasonal pattern of photosynthetic capacity and net ecosystem exchange of carbon in a deciduous forest. *Plant Cell and Environment*, **24**, 571–583.
- Wofsy SC, Goulden ML, Munger JW *et al.* (1993) Net exchange of CO₂ in a mid-latitude forest. *Science*, **260**, 1314–1317.