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 CO_2 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 CO_2 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 CO_2 assimilation was corrected for leaf area, system net CO_2 exchange (SNCE), integrated daily SNCE, and system respiration increased in response to elevated CO_2. The increases were mainly as a result of the larger leaf area developed during growth at high CO_2, before the short-term experiment; the observed decline in responses to SMS and high VPD treatments was partly because of leaf area reduction. Elevated CO_2 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 CO_2 under all treatments. Leaf-level net CO_2 assimilation rate and apparent quantum efficiency were consistently higher, and stomatal conductance and transpiration were significantly lower, under high CO_2 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 CO_2 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 CO_2 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 CO$_2$ assimilation
SGCA$_L$ = system gross CO$_2$ assimilation per unit leaf area at light saturation
SMS = soil moisture stress
SNCE$_{L \text{ or } D}$ = system net CO$_2$ exchange (soil area basis), L or D as subscripts refer to light or dark
VPD = atmospheric vapor pressure deficit

Introduction

Atmospheric CO$_2$ 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 CO$_2$ concentrations, because this interaction will eventually influence the productivity, sequestration capacity, species composition, and management of future forests under high CO$_2$.

There have been many measurements of leaf-level responses in trees to high CO$_2$, 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 CO$_2$, but high VPD diminished the effect of elevated CO$_2$ on g_s. Although there are now many flux tower studies of net system CO$_2$ 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 CO$_2$ on system net CO$_2$ exchange (SNCE). For example, Baldocchi (1997) reported a 65% decline in net ecosystem CO$_2$ 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 CO$_2$ 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 CO$_2$ 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 CO$_2$ 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 CO$_2$, increased leaf area also amplifies stand-level water use compared with ambient CO$_2$ (Pataki *et al.*, 1998). Changes in root-to-shoot biomass ratios have been observed in several tree species grown under elevated CO$_2$ (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 CO_2 (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) CO_2. 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 CO_2.

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 ($2 \times$ ambient) and 120 Pa ($3 \times$ ambient) CO_2 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 $41 \text{ m} \times 18 \text{ m}$ in a north–south orientation, had an approximate soil surface area of 550 m^2, soil volume of 550 m^3, air volume of 11 700 m^3, and average height available for plant growth of 12 m. The facility afforded independent control and measurement in each bay of atmospheric CO_2, 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), CO_2, relative humidity, and VPD) were measured near the center of each quarter every 15 s, and 15 min averages stored in dataloggers (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 CO_2 treatment bays, depending on the soil moisture treatment. Three CO_2 treatments were applied at the start of the third growing season. Atmospheric CO_2 concentration was measured and stabilized to its set point by varying the amount of pure CO_2 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 CO_2. 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 (SF_6), and CO_2 concentration in each bay was corrected for leak rate.

Experimental design

The experiment (Table 1, Fig. 1) in November–December 2001 comprised two CO_2 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 $\sim$ 35%,

VPD $\sim$ 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 $\sim$ 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 CO_2, 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 CO_2, 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 CO_2 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 μmol m^{-2} s^{-1}) values of environmental variables in the ambient and elevated CO_2 treatment bays of the cottonwood plantation at the Biosphere2 Laboratory during the four phases of the experiment

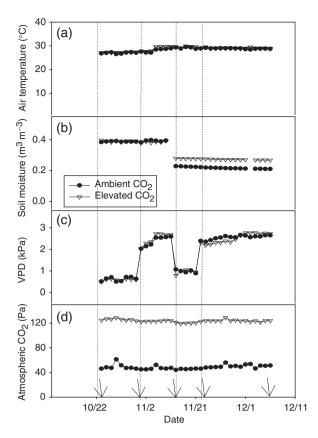| Environmental variable	Phase 1 High soil moisture low VPD (Wv)		Phase 2 High soil moisture high VPD (WV)		Phase 3 Low soil moisture low VPD (wv)		Phase 4 Low soil moisture high VPD (wV)	
Increasing stress	c*	C*	c	C	c	C	c	C
Air temperature (°C)	27.03	27.20	28.04	28.70	29.20	29.60	28.90	29.00
	(0.4)	(0.3)	(1.0)	(1.2)	(0.6)	(0.4)	(0.5)	(0.5)
Relative humidity (%)	83.00	84.00	38.00	37.00	76.00	77.00	36.00	38.00
	(2.9)	(2.4)	(4.3)	(4.2)	(2.6)	(2.9)	(4.3)	(6.1)
Soil moisture (m^3 m^{-3})†	0.39	0.39	0.39	0.39	0.23	0.28	0.22	0.27
VPD (kPa)	0.62	0.58	2.36	2.47	0.97	0.95	2.56	2.49
	(0.11)	(0.09)	(0.27)	(0.29)	(0.11)	(0.13)	(0.18)	(0.25)
CO_2 (Pa)	125.81	49.36	123.24	46.75	120.20	45.5	123.86	49.98
	(3.9)	(6.4)	(1.3)	(3.4)	(1.2)	(1.9)	(3.8)	(3.9)
Total daytime PPF (mol m^{-2} day^{-1})‡	14.7	13.6	10.6	9.9	10.3	9.7	11.5	10.9
	(1.8)	(1.6)	(1.8)	(1.5)	(1.5)	(1.5)	(0.9)	(0.8)

Values in parentheses denote standard errors.
*Lowercase c denotes ambient and uppercase C denotes elevated CO_2 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 $> 100 \, \mu mol \, m^{-2} \, s^{-1}$ 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.

$627 \, \mu mol \, m^{-2} \, s^{-1}$). 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 ($\mu mol \, m^{-2}$ 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 ($SNCE_D$):

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

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, $\mu mol \, m^{-2} \, s^{-1}$ 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 ($SGCA_L$, $\mu mol \, m^{-2}$ 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 × 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 $157 \, cm^2 \, g^{-1}$ 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 CO_2 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 CO_2 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 CO_2 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 CO_2 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 CO_2 treatment was 18% lower than that in the elevated CO_2 treatment (Fig. 1b, Table 1).

Stand leaf area

Growth in high CO_2, prior to beginning the experiment (Table 2), increased average total leaf area by 49% relative to the ambient CO_2 treatment. The trees grown in elevated CO_2 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 CO_2 treatment by 31%, and of the elevated CO_2 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 CO_2 treatment bays of the cottonwood plantation Biosphere 2 laboratory during the course of the experiment for the various soil moisture, VPD, and CO_2 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 CO_2 treatment than in ambient CO_2 under all soil moisture and VPD conditions. However, the only statistically significant difference (RCB-ANOVA approach) in predawn Ψ because of CO_2 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 CO_2 were consistently higher than in the ambient CO_2 treatment, there were no significant differences.

System-level CO_2 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 CO_2 exchange was obtained nocturnally, and this efflux was always greater in elevated CO_2 treatments. Elevated CO_2 stimulated both system CO_2 uptake and respiratory CO_2 release in all treatments. Low soil moisture decreased the difference between the CO_2 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 CO_2 loss to photosynthetic CO_2 uptake occurred at about the same time (07:30 hours) each
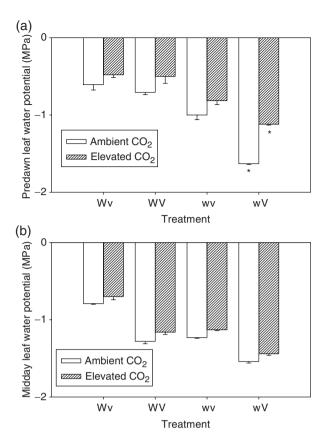
Fig. 2 Mean (a) predawn and (b) mid-day leaf water potential (MPa) for poplar leaves from trees exposed to various soil water and vapor pressure deficit (VPD) treatment combinations in ambient and elevated CO_2. Treatments are designated as W, w, high and low water (low and high soil moisture stress) and V, v, high and low VPD treatments, respectively. Asterisks within a treatment combination denote significant difference between CO_2 treatments at an α of 0.05.

Fig. 3 Daytime trend of system net CO_2 exchange (SNCE) ($\mu mol\, CO_2\, m^{-2}\, s^{-1}$) for stands of poplar trees grown under a combination of ambient and elevated CO_2. The atmospheric convention is used, in which photosynthetic CO_2 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 CO_2 exchange (D-CO_2; $mol\, day^{-1}$) calculated from $SNCE_L$, for ambient and elevated CO_2 treatments. Also shown are the ratios of the elevated to ambient D-CO_2 values (E/A).

morning, 1–2 h after sunrise, when PPF was about 100–200 μ mol photons $m^{-2}\, s^{-1}$. In the evening, the reverse transition was complete at dusk (about 19:00 hours). Integrated daily CO_2 exchange (D-CO_2; $mol\, CO_2\, day^{-1}$) 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 CO_2, the effect being found in the most severe water stress treatment (wV) (Fig. 3). The reduction in D-CO_2 with increasing water stress was always greatest in the ambient CO_2 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 CO_2, and to evaluate the roles of leaf area differences between treatments on the balance
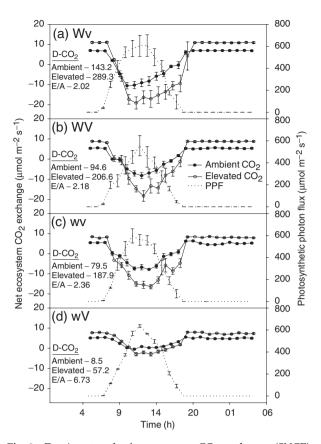
of CO_2 fluxes (Table 3). In all cases, mean mid-day SNCE ($SNCE_L$ at PPF 600–700 $\mu mol\, m^{-2}\, s^{-1}$) in elevated CO_2 was greater than in ambient CO_2, with the greatest stimulation in the most severe water stress treatment (wV). Mean $SNCE_D$ was 50% higher in elevated than in ambient CO_2 in all treatments, and always declined in response to both high VPD and SMS (Table 3). Mean SGCA was nearly double in high CO_2. 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 CO_2 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 CO_2 on system-level processes. Thus, SGCA on a leaf area basis (SGCA$_L$; μmol m^{-2} leaf area s^{-1}) was only slightly higher under elevated CO_2 in Wv and WV treatments, with stronger effects of elevated CO_2 evident in wv and wV treatments. On average, SGCA for treatment Wv in elevated CO_2 was 1.77 times that in ambient (Table 3). LAI in elevated CO_2 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 CO_2. 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 CO_2.

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 μmol m^{-2} s^{-1}. At high soil moisture (W), elevated CO_2 resulted in greater mean SNCE: from 5.9 to 12.8 μmol m^{-2} s^{-1} at low VPD, and from 4.5 to 10.5 μmol m^{-2} s^1 at high VPD. Also under SMS (w), elevated CO_2 resulted in greater mean SNCE: from 4.6 to 11.5 μmol m^{-2} s^{-1} at low VPD, and from -0.86 to 0.74 μmol m^{-2} s^{-1} at high VPD. The consistently greater mean SNCE in elevated CO_2 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 CO_2 described above. The interaction of VPD and soil moisture on mean SNCE was nonsignificant in wv treatments, in ambient and elevated CO_2 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 CO_2 concentrations, with the main difference being that elevated CO_2 resulted in increased SNCE compared with ambient CO_2. 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 CO_2, 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 CO_2 treatment. Light-saturated SGCA$_L$ and SNCE$_D$ were greater in the elevated CO_2 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 CO_2–light interactions of leaves in terms of energy

Table 3 Comparisons of mean mid-day (600 < PPF < 700) SNCE$_L$, mean SNCE$_D$, and mean SGCA

Treatment	SNCE$_L$*	SNCE$_D$ (μmol m^{-2} s^{-1} soil surface area)[†]	SGCA[‡]	SGCA$_L$ (μmol m^{-2} 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 CO_2 exchange at elevated CO_2 by those at ambient CO_2 are also shown.
*Mean mid-day system net CO_2 exchange in the light (SNCE$_L$; 700 > PPF > 600 μmol m^{-2} s^{-1}).
[†]Mean nocturnal system net CO_2 exchange in the day (SNCE$_D$).
[‡]System gross CO_2 assimilation (SGCA = SNCE$_L$ + SNCE$_D$).
[§]System gross CO_2assimilation per unit leaf area (SGCA$_L$ = SGCA/LAI).
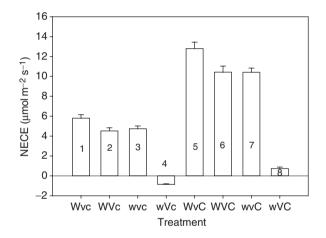
Table 4 Parameters of the linear regression equations relating SNCE ($\mu mol\, m^{-2}\, s^{-1}$) obtained for the ambient (c) and the elevated (C) CO_2 treatments and various soil moisture and VPD treatment combinations designated as follows (W and w, high and low soil moisture; V and v, high and low VPD, respectively)

Regression*	n	x treatment mean	y treatment mean	Intercept	Slope	P-value[†]	R[†]
1	283	Wvc	WvC	3.6	1.6	<0.0001	0.83
		5.94	12.88	(0.0001)	(0.0001)		
2	223	WVc	WVC	2.5	1.8	<0.0001	0.83
		4.55	10.51	(0.0001)	(0.0001)		
3	124	wvc	wvC	5.4	1.3	<0.0001	0.75
		4.68	11.59	(0.0001)	(0.0001)		
4	448	wVc	wVC	2.3	1.9	<0.0001	0.63
		−0.86	0.74	(0.0001)	(0.0001)		

*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 ($SNCE_{AMBCO2}$ = intercept + slope × $SNCE_{ELCO2}$). 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 ($SGCA_L$) ($\mu mol\, CO_2\, m^{-2}\, s^{-1}$) 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} ($A_{net\ elev}/A_{net\ amb}$) 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 $50\, \mu mol\, m^{-2}\, s^{-1}$. 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 CO_2 treatment combinations

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

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.
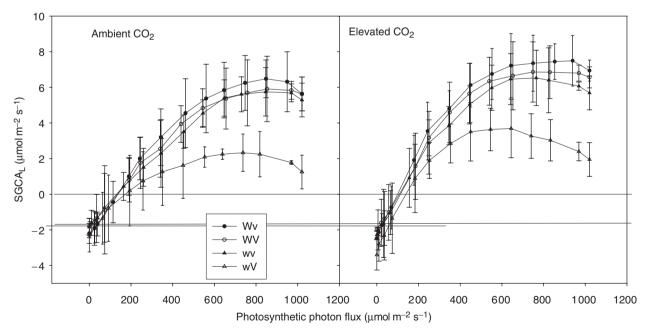SNCE, system net CO_2 exchange; VPD, vapor pressure deficit.

Fig. 5   Interactions of elevated CO_2 (C) and ambient CO_2 (c) with low and high vapor pressure deficit (VPD) (v and V) and soil moisture stress (W and W) on mean system net CO_2 exchange (SNCE) in stands of poplars.

Table 6 Parameters from fitting of light response curves for net CO_2 uptake measured at the leaf (A_{net}) and stand-level ($SGCA_L$) at ambient and elevated CO_2 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

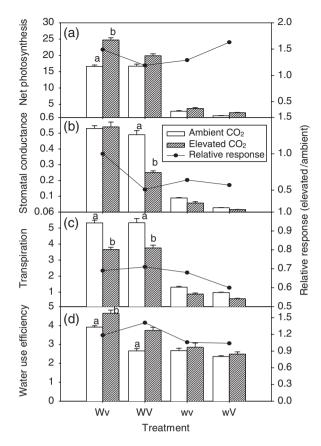| Leaf-level	Respiration	A_{max}	Q_e	L_{comp}	L_{sat}
Wvc	−2.23	37.9	0.091	24.4	439
WVc	−0.42	27.1	0.069	6.11	402
wvc	−1.04	5.76	0.034	26.9	175
wVc	−1.16	3.98	0.056	20.8	92.3
WvC	−0.91	49.9	0.102	8.92	499
WVC	−1.06	21.1	0.084	12.5	262
wvC	−1.26	12.3	0.103	12.3	132
wVC	−0.81	8.99	0.044	18.4	224

*Treatments are as specified in Table 1.
[†]Across the row, abbreviations are respiration ($\mu mol\,m^{-2}\,s^{-1}$), maximum net photosynthesis ($\mu mol\,m^{-2}\,s^{-1}$), quantum flux efficiency, light compensation point ($\mu mol\,m^{-2}\,s^{-1}$), and light saturation point ($\mu mol\,m^{-2}\,s^{-1}$).
$SGCA_L$, system gross CO_2 assimilation per unit leaf area at light saturation; VPD, vapor pressure deficit.

Comparison of CO_2 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 midday and at light saturation (Table 3), and because A_{net} data were obtained *in situ* on midcanopy leaves at near-saturating PPF values. The CO_2 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 CO_2 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 ($\mu mol\,m^{-2}\,s^{-1}$), (b) stomatal conductance ($mmol\,m^{-2}\,s^{-1}$), (c) transpiration ($mmol\,m^{-2}\,s^{-1}$), and (d) instantaneous water use efficiency (E/A_{net}). Relative responses; the ratio of elevated to ambient CO_2 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 CO_2 treatments at an α of 0.05.

ambient or high CO_2. 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 CO_2 (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 CO_2. System-level measurements of nocturnal CO_2 efflux from soil, roots and
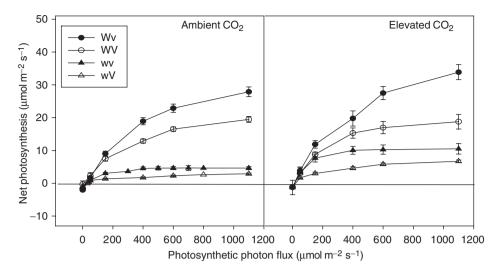
Fig. 7 Response of poplar leaf net photosynthetic rate ($\mu mol\,CO_2\,m^{-2}\,s^{-1}$) to photosynthetic photon flux ($\mu mol\,photons\,\mu m^{-2}\,s^{-1}$). Treatment designations as in Fig. 3.

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

Treatment	SGCA$_L$* ($\mu mol\,m^{-2}\,s^{-1}$ leaf area)	A_{gross}[†]	A_{gross}/SGCA$_L$
Ambient CO_2			
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
Elevated CO_2			
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
Ratio elevated CO_2/ambient CO_2			
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 CO_2 exchange at elevated CO_2 by those at ambient CO_2 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_{net} + respiration) as measured by the Li-Cor.

SGCA, system gross CO_2 assimilation.

aboveground biomass were highly reproducible, were always greater in elevated CO_2, 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 CO_2, belowground (roots + soil) respiration was comparable in each bay (Murthy *et al.*, 2003). The stimulation in system respiration in elevated CO_2 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 CO_2 prior to the application of treatments, and the loss of LAI in response to SMS, altered system level SNCE and D-CO_2 by increasing total light intercepted. With the sun directly overhead, the fraction of light (F) absorbed by a canopy is given by

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

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 CO_2 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 CO_2 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-CO_2 in elevated CO_2 (Fig. 3), and a response to CO_2 fertilization is evident. When system-level gas exchange is corrected for respiration and leaf area, the stimulation of system assimilation by elevated CO_2 increased from only 18% in well-irrigated treatments to 98% in the most severe water stress (Table 3). It seems likely that CO_2 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 CO_2 on the initial slope, and maximum rate, of light response curves for $SGCA_L$ (Table 6). It is clear that elevated CO_2 also led to a 50% increase in system respiration ($SNCE_D$) in all treatments, but how much of this can be ascribed to the effects of elevated CO_2 on leaf area (more respiring plant material) and to CO_2 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 CO_2 efflux measured were -1.3 and $-0.9 \, \mu\text{mol m}^{-2}\text{s}^{-1}$ for the ambient and elevated CO_2 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 CO_2 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 CO_2 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 CO_2 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 CO_2 and water stress treatments that may be useful in scaling future measurement and modeling studies. Although some researchers believe that relative responses to elevated CO_2 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-CO_2 (Fig. 3) at ambient and elevated CO_2 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-CO_2 because of high CO_2, 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-CO_2 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-CO_2 (56% and 65% at ambient and high CO_2, respectively), whereas exposure to water stress simultaneously in the soil and atmosphere had a large effect, reducing D-CO_2 to only 6% and 20% of Wv. In all cases, the application of high VPD and SMS had smaller relative effects in elevated CO_2 treatments than in ambient CO_2 treatments.

The effects of elevated CO_2, high VPD, and SMS on leaf-level gas exchange were comparable with responses obtained from other C_3 plant leaves, even though the elevated CO_2 treatment in our experiments was three times ambient. Across all treatments, the mean relative increase of A_{net} because of elevated CO_2 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 CO_2 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 CO$_2$ 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 CO$_2$ concentration used here. The effects of elevated CO$_2$ and moisture stress (via stomatal conductance) on leaf-level light response curves (Table 6) show the well-known interactions of CO$_2$ assimilation kinetics in air (via Rubisco) with leaf internal CO$_2$ concentrations and the energy costs of CO$_2$ recycling in photorespiration (von Caemerer & Farquhar, 1981).

In general, leaf-level responses to high CO$_2$ 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 CO$_2$ at the same incident PPF was more or less similar across treatments, the ratio of A_{net}/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 CO$_2$ 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 CO$_2$, 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 CO$_2$ treatments are not obvious. In both CO$_2$ 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 CO$_2$, presumably because of the large decline in stomatal conductance. Larger LAI, in the elevated CO$_2$ 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 CO$_2$ 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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