

CO₂ enrichment in a maturing pine forest: are CO₂ exchange and water status in the canopy affected?

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ABSTRACT

Elevated CO₂ is expected to reduce forest water use as a result of CO₂-induced stomatal closure, which has implications for ecosystem-scale phenomena controlled by water availability. Leaf-level CO₂ and H₂O exchange responses and plant and soil water relations were examined in a maturing loblolly pine (*Pinus taeda* L.) stand in a free-air CO₂ enrichment (FACE) experiment in North Carolina, USA to test if these parameters were affected by elevated CO₂. Current-year foliage in the canopy was continuously exposed to elevated CO₂ (ambient CO₂ + 200 μmol mol⁻¹) in free-air during needle growth and development for up to 400 d. Photosynthesis in upper canopy foliage was stimulated by 50–60% by elevated CO₂ compared with ambient controls. This enhancement was similar in current-year, ambient-grown foliage temporarily measured at elevated CO₂ compared with long-term elevated CO₂ grown foliage. Significant photosynthetic enhancement by CO₂ was maintained over a range of conditions except during peak drought.

There was no evidence of water savings in elevated CO₂ plots in FACE compared to ambient plots under drought and non-drought conditions. This was supported by evidence from three independent measures. First, stomatal conductance was not significantly different in elevated CO₂ versus ambient trees of *P. taeda*. Calculations of time-integrated c_i/c_a ratios from analysis of foliar δ¹³C showed that these ratios were maintained in foliage under elevated CO₂. Second, soil moisture was not significantly different between ambient and elevated CO₂ plots during drought. Third, pre-dawn and mid-day leaf water potentials were also unaffected by the seasonal CO₂ exposure, as were tissue osmotic potentials and turgor loss points. Together the results strongly support the hypothesis that maturing *P. taeda* trees have low stomatal responsiveness to elevated CO₂. Elevated CO₂ effects on water relations in loblolly pine-dominated forest ecosystems may be absent or small apart from those mediated by leaf area. Large photosynthetic enhancements in the upper canopy of *P. taeda* by elevated CO₂ indicate that this maturing forest may have a large carbon sequestration capacity with limiting water supply.

Key-words: *Pinus taeda* L.; CO₂ assimilation; drought; elevated CO₂; free-air CO₂ enrichment; loblolly pine photosynthesis; pine forest canopy; stable carbon isotopes; stomata; water stress.

Abbreviations: A_{net} , leaf net CO₂ assimilation; c_a , CO₂ concentration of air surrounding a leaf; c_i , leaf intercellular CO₂ concentration; Δ , ¹³C isotope discrimination; $\delta^{13}\text{C}$, relative stable carbon isotope content; ϵ , ratio of A_{net} at $c_a = 560 \mu\text{mol mol}^{-1}$ to A_{net} at $c_a = 360 \mu\text{mol mol}^{-1}$; FACE, free-air CO₂ enrichment; g_w , stomatal conductance to water vapour; Π_i , initial leaf osmotic potential; R_i , relative water content at incipient turgor loss; Ψ_l , xylem water potential of leaves; Ψ_m , soil matric potential.

INTRODUCTION

Native plants grown at elevated atmospheric CO₂ nearly always exhibit a sustained stimulation in photosynthesis due to effects of CO₂ on leaf biochemical processes (Sage 1994; Drake, González-Meler & Long 1997). Elevated CO₂ responses have been most often quantified under conditions of optimal water and nutrient availability even though such conditions are uncharacteristic of those of most native plants and may lead to unrealistic projections of CO₂ effects on natural vegetation (Amthor 1995; Curtis 1996). In natural environments, factors such as temperature, water and nutrient supply, and competitive interactions among trees interact simultaneously to impose stresses on plants. It is important to evaluate whether environmental limitations will diminish the magnitude of physiological responses to elevated CO₂ in forest trees, which usually occur on sites with significant resource limitations to productivity. In order to make inferences about CO₂ effects on trees in natural environments, the free-air CO₂ enrichment (FACE) approach has been developed to permit experimentation with elevated CO₂ *in situ* without impacting the forest microclimate (Hendrey *et al.* 1998).

In forest ecosystems, water availability is considered to be a major limitation to net primary production world-wide (Woodward 1987; Kozłowski & Pallardy 1996) and according to some global change scenarios drought periods may increase in warm temperate regions (Rind *et al.* 1990; Gregory, Mitchell & Brady 1997). Enhancements in the ratio of leaf net CO₂ assimilation (A_{net}) to evapotranspiration (E) in enriched CO₂, often termed instantaneous water-use efficiency (Eamus 1991), could be advantageous

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to tree growth and survival when water supply is limiting (Tschaplinski *et al.* 1995; Knapp *et al.* 1996). These enhancements are mediated through both a direct stimulation in rates of net photosynthesis by elevated CO₂, and through partial stomatal closure. The stimulation response of A_{net}/E to CO₂ enrichment, along with responses such as changes in leaf area, root water access, hydraulic conductivity and canopy-atmosphere coupling, will determine species performance with rising atmospheric CO₂ concentration, particularly in water-limited situations. Thus, drought–CO₂ interactions may affect tree carbon balance and survival during dry periods in an elevated CO₂ atmosphere (Miao, Wayne & Bazzaz 1992). While this would suggest that photosynthetic enhancements by elevated CO₂ would be greater under drought than under well-watered conditions as is often observed (Morison 1993), not all species will respond similarly in this respect (Dixon, LeThiec & Garrec 1995; Tschaplinski *et al.* 1995; Knapp *et al.* 1996). A greater understanding of how water availability may differentially affect plant responses to elevated CO₂ underlies the ability to understand CO₂ effects on forest productivity under different climate conditions and along soil moisture gradients in the landscape.

It is generally expected that elevated CO₂ will promote reductions in stomatal conductance that can ameliorate the negative effects of drought in many species through decreased water use and/or enhanced instantaneous and long-term water-use efficiency (Morison 1993). Moreover, reductions in water use as a result of partial stomatal closure could indirectly affect other important ecosystem processes and delay the onset of water stress during drying cycles (Field, Jackson & Mooney 1995; Hungate *et al.* 1997). However, growing experimental evidence suggests that many forest tree species show small or non-significant changes in stomatal conductance under long-term elevated CO₂ (Eamus & Jarvis 1989; Bunce 1992; Curtis 1996; Saxe, Ellsworth & Heath 1998), particularly conifers (Ellsworth *et al.* 1995; Teskey 1995; Picon, Guehl & Ferhi 1996). If long-term experiments confirm the hypothesis that elevated CO₂ has small or non-significant effects on stomatal conductance of coniferous forest trees, then there may be substantial errors in global modelling efforts that assume a larger response exists in conifer-dominated ecosystems and regions (Henderson-Sellers, McGuffie & Gross 1995; Sellers *et al.* 1996; Pan *et al.* 1998). On the other hand, significant long-term CO₂ responses of conifer stomata have been observed in some studies (Dixon *et al.* 1995; Tissue, Thomas & Strain 1997). Do conifers show a partial stomatal closure in response to year-long elevated CO₂ exposure, and if so, will this response affect tree–water relations? To date virtually no studies have comprehensively examined multiple aspects of stand–water relations in forest plots under elevated CO₂ beyond leaf-level gas exchange, and this hypothesis has remained largely untested.

I hypothesized that year-long CO₂ enrichment would have minimal effects on conductance to water vapour and water relations in the upper canopy of *Pinus taeda* L.

(loblolly pine), a dominant species in managed and unmanaged forests covering nearly 20 million ha of land in the south-eastern United States. As in many warm temperate regions, potential evapotranspiration in the growing range of *P. taeda* may exceed precipitation for extended periods. A strong summer drought in central North Carolina USA, site of the first forest FACE experiment, provided an opportunity to test (1) whether forest water relations and physiological function are improved in the canopy under elevated CO₂, and (2) whether photosynthetic enhancement by CO₂ is larger under water-limited conditions compared with well-watered conditions. The first year of elevated CO₂ exposure in this stand provided an opportunity to examine stomatal responses to elevated CO₂ prior to the onset of potential treatment differences in leaf area in the 15-year-old trees in the same stand, and hence separate these two potentially important mechanisms of altering water-use in elevated CO₂. The magnitude of leaf-level CO₂ and H₂O exchange responses to elevated CO₂ in conifers is important to increase understanding of possible feed-backs between climate and forest ecosystems affecting carbon sequestration at the forest stand scale.

MATERIALS AND METHODS

Study site and FACE treatment

The study was conducted in the Blackwood Division of Duke Forest in Orange County, NC, USA (35°58' N, 79°05' W). The site was described in Ellsworth *et al.* (1995) and consists of a 32-ha parcel of even-aged loblolly pine forest on a clay loam soil. Site elevation is 174 m with a nominal atmospheric pressure of 99.2 kPa for the location, so all molar ratios for CO₂ in air ($\mu\text{mol CO}_2 \text{ mol}^{-1}$) expressed here can be considered approximately equivalent to the partial pressure of CO₂ in air (Pa) \times 10. The *P. taeda* overstorey trees were approximately 12–13 m tall in the summer of 1997 at age 15 years. Within this stand, six circular plots were established, three replicates representing the CO₂ treatment and three representing experimental controls. The three treatment plots each consisted of a 30-m-diameter free-air CO₂ enrichment ring that was constructed and began treatment exposure in August 1996 (designated 'Elevated CO₂' or FACE plots hereafter). In addition to the three CO₂ treatment rings, rings were constructed at the same time in the three control plots and outfitted with blowers, vent pipes and towers configured and operated identically to the treatment rings but lacking CO₂ injection ('Ambient' control plots). Each ring had a central tower and a vertical personnel lift for access to the canopy. The FACE rings employed the design described in Hendrey *et al.* (1998), but were operated continuously (24 h a day) throughout the exposure period except when ambient temperature was below 5 °C for more than 1 h. Furthermore, instead of a constant set-point as used in the previous forest FACE experiment (Ellsworth *et al.* 1995), the treatment rings tracked ambient CO₂ concentration 24 h a day with a target of ambient + 200 $\mu\text{mol CO}_2 \text{ mol}^{-1}$.

The average 24 h CO₂ concentration at the centre of each FACE ring near the top of the canopy was 575, 574 and 573 $\mu\text{mol mol}^{-1}$ for the respective treatment rings during May–October 1997. This was the period of the growing season during which the current-year foliage developed and matured. Average daytime CO₂ concentration at 11–12 m height in each ring centre was 568, 564 and 567 $\mu\text{mol mol}^{-1}$ for the three treatment rings, respectively, between sunrise and sunset. Standard deviations of the 1 min exposure CO₂ concentration measured continuously over the season were ± 50 – $54 \mu\text{mol mol}^{-1}$, and the FACE system reliability was 99.8% of planned hours when treatment was applied. The corresponding ambient CO₂ concentration during the growing season was 380 $\mu\text{mol mol}^{-1}$ for 24 h, and 368 $\mu\text{mol mol}^{-1}$ for daytime. Each ring was located at least 90 m from any other ring and contamination of the ambient signal for controlling the FACE system was avoided by using the minimum of four monitored ambient signals in different cardinal directions.

The first full year of FACE operation included a dry summer. Over the main portion of the growing season (May–September), 38 cm of precipitation was recorded at the National Weather Service station 7 km from the study site, 30% lower than the 30 year normal for this period (National Weather Service, US Department of Commerce, unpublished results). Less than 3 cm of precipitation (80% below normal) was recorded over the 6 week period prior to 10 September when a drought-breaking rain occurred. Over the growing season, volumetric soil moisture was calculated on the basis of measurements of the soil dielectric constant in the upper 30 cm of the soil profile using modified time-domain reflectometry techniques (Topp, Davis & Annan 1980) with waveguides (CS615: Campbell Scientific, Ogden UT, USA). The upper 30 cm of soil at the study site comprised more than 90% of the stored soil water accessed by overstorey *P. taeda* trees (Oren *et al.* 1998). Within each ring the signals were recorded every half hour as an average for four locations within each plot. Continuous seasonal data were only available for two treatment and control plots ($n = 2$). Soil matric potential at pre-dawn was calculated according to Campbell (1985) using the moisture content data measured at pre-dawn and soil textural characteristics, and agreed well with the *in situ* soil moisture release curve parameters presented in Oren *et al.* (1998).

Leaf CO₂ assimilation and conductance measurements

At the end of the 1996 growing season, south to west-facing upper crown branches (approximately 12 m height above the ground) in all plots which had been produced during the growing season were selected for measurements. The selected trees were within 10 m of the centre of each plot. In the following growing season, net CO₂ assimilation (A_{net}) and conductance to water vapour (g_w) were measured at the growth CO₂ concentration (c_a) for the first cohort of current-year foliage on these branches. Daily maximum A_{net} at light-saturation was followed at

2–3 week intervals on clear, sunny days through the growing season beginning when current-year foliage was 80% elongated in early July. Elevated CO₂ treatment foliage had developed continuously under elevated CO₂ since the time when needle primordia were determined at bud set in the year previous to the reported measurements.

The measurements of A_{net} were made on single fascicles of foliage at ambient temperature and vapour pressure in the upper canopy (12–13 m height). Mid-day A_{net} measurements were designed to reflect the daily maximum A_{net} under ambient conditions, light saturation in natural sunlight (quantum flux density $> 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$), and natural leaf angles. Data from 10 full diurnal courses of leaf gas exchange were used to ensure that A_{net} was maximal around noon local time. Mid-day A_{net} measurements in all rings were completed within an hour of noon, except during peak drought when the measurements were made earlier in the morning to avoid mid-day stomatal closure. Measurements were made with a portable infra-red gas analyzer system for CO₂ and water vapour (CIRAS-1; PP-Systems, Hitchin, UK) operated in the open-flow mode with a 5.5-cm-long leaf chamber and an integrated gas CO₂ supply system. The chamber was modified with an attached Peltier cooling device to maintain chamber temperature near ambient air temperatures. Gas exchange rates are reported on a unit surface area basis calculated using a geometric approximation of the needle surface.

On a subset of sampling dates, gas exchange of ambient and elevated CO₂ trees was measured at a common c_a by temporarily changing the CO₂ supply delivered to the leaf chamber to produce the c_a level appropriate for the opposite treatment, immediately following the measurement of light-saturated A_{net} at growth c_a . Gas exchange measurements in full sunlight were continued once the c_a level was stable and the gas analyzers had been internally matched, typically about 3 min after the step change in c_a . For measurements carried out in this way the coefficient of variation over time for A_{net} was generally less than 2%. Chamber temperature was controlled to maintain constant ambient levels during the step change in c_a . The A_{net} measurements at the growth c_a for ambient trees will be referred to as A_{360} and those at the appropriate c_a similar to the elevated CO₂ treatment will be denoted A_{560} . Only A_{net} data from the reciprocal c_a measurements (A_{360} and A_{560}) were used in the data analysis since the step change in c_a was not long enough to permit possible adjustments in g_w under elevated CO₂. Photosynthetic enhancement ratio (e) is calculated as the instantaneous ratio of A_{560}/A_{360} for any given plot (ambient or elevated CO₂). The enhancement ratio for FACE A_{net} at growth c_a to ambient A_{net} at growth c_a is denoted e' .

Leaf stable carbon isotopes

At the end of September, a sample of current-year needles was collected from a branch marked for gas exchange measurements from one tree at the centre of each ambient and

elevated CO₂ ring ($n = 3$ per treatment). The foliage was collected at mid-day after peak daily light-saturated A_{net} was measured. Foliage was situated less than 3 m from a continuously monitored port used for measuring CO₂ concentration so the source air c_a and air $\delta^{13}\text{C}$ could be calculated (see Appendix). The foliage was stored on ice until oven-dried, then finely ground and homogenized and stored until analysed for stable carbon isotope ratio ($\delta^{13}\text{C}$ as defined by Farquhar, Ehleringer & Hubick 1989). Stable carbon isotope ratio was expressed as the ratio of $^{13}\text{C}/^{12}\text{C}$ relative to the Pee-dee Belemnite (PDB) standard, in per mil units. The $\delta^{13}\text{C}$ determinations were done on 2 mg subsamples from each tree within a plot and analysed at the Stable Isotope Ratio Facility for Environmental Research, University of Utah. From these data, the ratio c_i/c_a was calculated as described in the Appendix.

Xylem pressure potential measurements

Pre-dawn and mid-day measurements of xylem water potential were made during the season on single fascicles using the pressure chamber technique (Scholander *et al.* 1965). Previous year foliage was measured until current-year foliage was fully elongated in August. All foliage sampled was obtained from upper canopy positions at the third to fourth whorl from the top of the tree, where gas exchange measurements were made. Upon collection, fascicles were placed in a plastic bag with moist filter paper and taken to the field laboratory at the site for Ψ_1 measurements. Fascicles were placed in the pressure chamber with the cut end protruding, and the cut end was swabbed with 70% v/v alcohol and dried prior to measurements to prevent resin from obscuring the xylem under pressure (Schulte & Henry 1992). Samples were discarded in cases where the balance pressure could not be determined because of exuded resin.

Pressure–volume analysis was applied to data from dehydration isotherms for determining tissue water relations parameters (Koide *et al.* 1989) and test for possible effects of growth in elevated CO₂ on these parameters. Individual current-year fascicles of *P. taeda* were collected from the upper canopy at pre-dawn in mid-October toward the end of the growing season. Foliage was not rehydrated when collected due to concerns over artifacts (Schulte & Henry 1992), but samples were collected following a rainfall event so the initial portion of the pressure–volume curve could be resolved. Fascicles were allowed to transpire outside the pressure chamber and periodical measurements of fascicle mass and Ψ_1 were recorded as dehydration progressed. Pressure–volume curve parameters were derived as described in Koide *et al.* (1989). Initial tissue osmotic potential (Π_i) was predicted from the linear regression and extrapolation of $1/\Psi_1$ below turgor loss versus foliage relative-water content with regression fits of $r^2 = 0.93$ or better. Three pressure–volume curves were constructed per treatment with each replicate sample originating from a different treatment ring.

Data analysis

A repeated measures analysis of variance (ANOVA) model was employed to test for CO₂ effects on individual leaf gas exchange and water potential parameters across multiple sampling dates. For the repeated measures ANOVA, a probability level of 0.10 was considered significant due to the small number of replicates, whereas for other tests $P < 0.05$ was considered significant. Individual plots (e.g. rings) were considered as replicates for the purposes of the statistical analyses. Tukey's studentized range test was used to test for differences between treatment means for gas exchange parameters. For g_w , differences of at least 23% between means could be detected for the treatment versus control with a plot-to-plot coefficient of variation of 19% and the desired probability level. Differences between means for tissue water relations parameters were tested using Student's *t*-test. Soil moisture was analysed by fitting an exponential decay function to the time series of the soil drying cycle for each ring and testing for differences in the fitted parameters using Student's *t*-test. There was a complete seasonal record for two ambient and two elevated CO₂ rings. The model fits had $r^2 = 0.95$ or better in all cases. All data were analysed in the SAS statistical package (Release 6.12, SAS Institute, Cary, N.C.) and were normally distributed (Shapiro–Wilk *W* test).

RESULTS

There was a progressive development of drought during the growing season at the site culminating in volumetric soil moisture values reaching $0.15 \text{ m}^3 \text{ m}^{-3}$ in the upper 30 cm of the soil in early September (Fig. 1). For soils with a high clay content such as that of the Duke Forest site, most soil water at low moisture contents is held at high matric potential at low moisture contents and hence is unavailable for plant use (Fig. 1; Campbell 1985). Pre-dawn Ψ_1 declined throughout the dry period in August in parallel with the declines in soil moisture, and recovered following the rain events in mid-September. There was no significant effect of CO₂ treatment on soil moisture ($P > 0.1$, *t*-test) or pre-dawn Ψ_1 ($P > 0.1$, repeated-measures ANOVA) which is often considered a plant-integrated measure of soil moisture in the rooting zone. The CO₂ treatment also did not affect mid-day Ψ_1 ($P > 0.1$). Despite large variations in soil moisture content and pre-dawn Ψ_1 , there was no apparent seasonal variation in mid-day Ψ_1 , and values remained between -2.2 and -2.5 MPa on warm sunny days (Fig. 1).

The water potential at turgor loss (Ψ_l) was not significantly different between FACE and ambient foliage ($P > 0.1$; Table 1). There was also no treatment difference in the water content at turgor loss (R_l) or tissue osmotic potential at full hydration (Π_i). The Ψ_l -values in Table 1 were similar to mid-day Ψ_1 measured throughout the growing season (Fig. 1).

Seasonal patterns in maximum A_{net} under ambient conditions and g_w at maximum A_{net} in current-year foliage

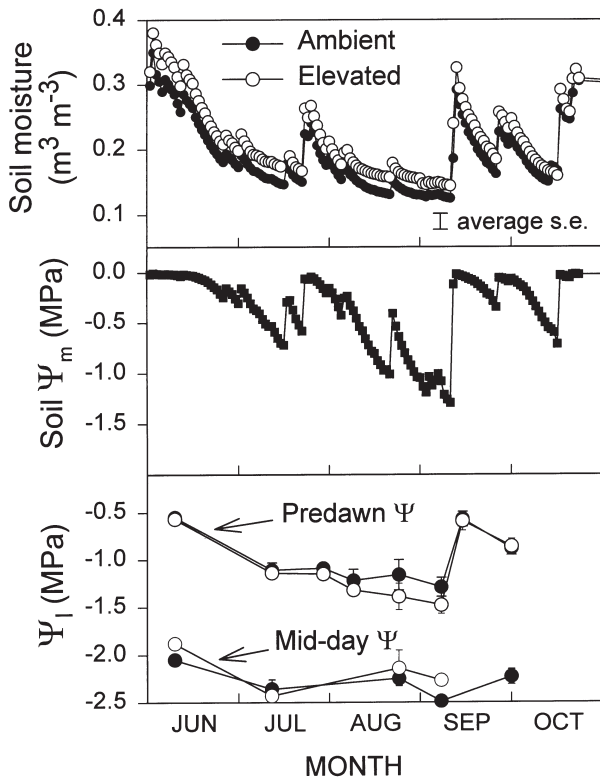


Figure 1. Seasonal course of soil moisture and mean xylem pressure potential (Ψ_1 , measured at pre-dawn or midday) in the study plots in Duke Forest, North Carolina, USA during the 1997 growing season. Data shown are treatment means for ambient (●) and elevated CO₂ (○) plots ('rings') in the stand $n = 2-3$ for Ψ_1 and $n = 2$ for soil moisture content, and bars indicate ± 1 standard error. Soil matric potential (Ψ_m) data are shown for both treatments combined. For soil moisture content data, the average standard error bar across all days is shown.

generally followed the pattern of soil moisture during July to October. The seasonal maximum A_{net} and g_w corresponded to a brief period of increased soil moisture following heavy rain in late July (Figs 1 and 2). With rapidly decreasing soil moisture following this rainfall, a large progressive decrease in maximum A_{net} and g_w at maximum A_{net} is apparent (Fig. 2). At peak drought in late August–early September, A_{net} was reduced to less than 60% of the seasonal maximum in both ambient and elevated CO₂ foliage, although there was recovery in A_{net} less than 1 week later with the increase in soil moisture after a drought-breaking rain event (Fig. 2). The decline and recovery of g_w during the drought cycle was similar in foliage in ambient versus elevated CO₂. The seasonal decrease in g_w with drought to 40% of the summer maximum was larger than that for A_{net} in both ambient and elevated CO₂ foliage. The declining g_w in July and August indicates that progressive stomatal limitations to A_{net} developed during the drying cycle. At peak drought the foliage c_i/c_a ratio (at growth c_a) was 0.42 ± 0.08 in elevated CO₂ trees and 0.49 ± 0.07 in ambient trees compared with 0.62 ± 0.02 in trees in both treatments during well-watered

periods (data not shown). Stomatal closure was also evident when comparing the diurnal course of gas exchange between a sunny day during peak drought and later in the same month under well-watered conditions (Fig. 3). During drought, daily maximum g_w was lower than under well-watered conditions and there was also a larger apparent decline in g_w through the day. The diurnal decrease in g_w during drought restricted the majority of CO₂ assimilation to times before solar noon.

Effects of CO₂ treatment on leaf gas exchange

Elevated CO₂ treatment had large apparent effects on A_{net} with instantaneous ϵ at mid-day varying between 1.45 and

Table 1. Tissue water relations parameters derived from pressure-volume analysis of current-year loblolly pine foliage developed under elevated CO₂ in FACE or under ambient CO₂. R_t is the relative water content at the turgor loss point, Π_i is the initial tissue osmotic potential from the portion of the curve near full saturation (MPa), and Ψ_t is the water potential at the point of incipient turgor loss (MPa). Data are for $n = 3$ plots per treatment

Parameter	Ambient	Elevated CO ₂
R_t	0.85 ± 0.02	0.84 ± 0.01
Π_i	-2.04 ± 0.22	-1.78 ± 0.02
Ψ_t	-2.38 ± 0.19	-2.33 ± 0.05

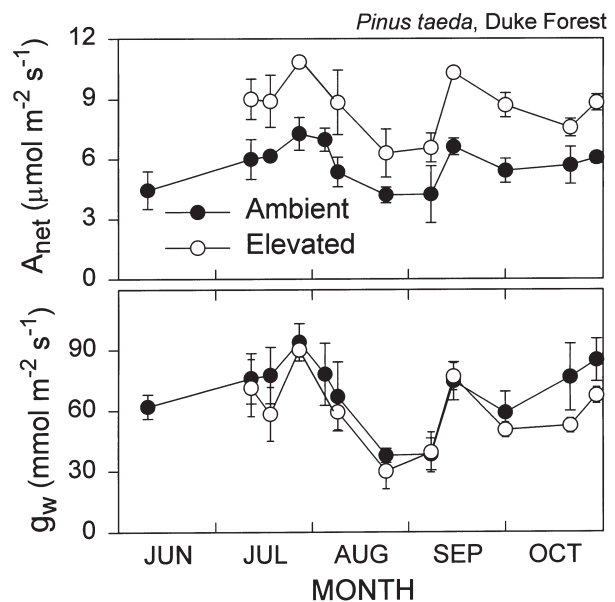


Figure 2. Seasonal patterns in light-saturated daily maximum net CO₂ assimilation (A_{net}) and stomatal conductance at maximum A_{net} (g_w) in current-year foliage in the canopy of *P. taeda* under ambient blower control plots and elevated CO₂ plots. A_{net} was measured under ambient conditions and growth c_a on sunny days. Foliage was 95% fully elongated by mid-July. Data shown are treatment means for trees in ambient control (●) and elevated CO₂ treatment (○) plots with ± 1 standard error

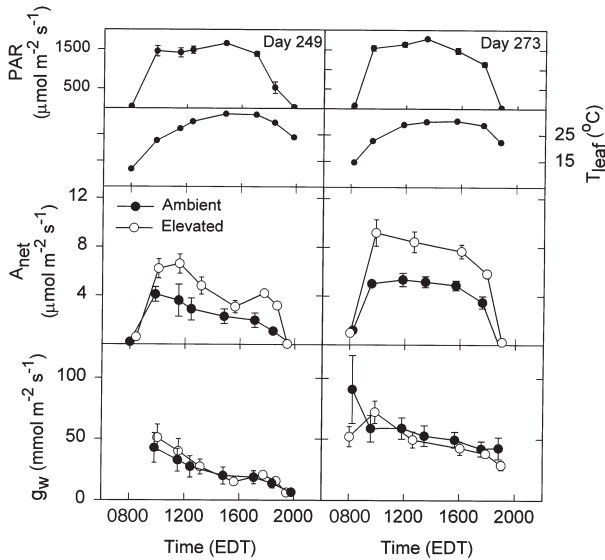


Figure 3. Diurnal course of leaf-level net CO₂ assimilation (A_{net}) and stomatal conductance (g_w) in current-year foliage of *P. taeda* at 12 m height in the canopy under ambient (●) and elevated (○) CO₂ treatments. Environmental parameters (T_{leaf} , leaf temperature; PAR, quantum flux density in the photosynthetically active radiation wavebands) are shown in the upper two panels and physiological measures are shown in the bottom panels. Data are for clear, sunny days at peak drought (left panels; DOY 249) and under well-watered conditions (right panels; DOY 273). For pooled ambient and elevated CO₂ trees Ψ_l was -1.4 ± 0.1 MPa at pre-dawn on DOY 249, -2.58 ± 0.1 MPa at mid-day on DOY 249, and -0.8 ± 0.1 MPa on DOY 273. Data are for foliage from $n = 3-5$ trees per treatment (\pm SE) at each time point within representative plots of each treatment.

1.65 across the period shown (Fig. 2). However, in Fig. 2 there was not complete replication of experimental units (plots) across the entire seasonal time series, and hence a more limited data-set with full replication was used for statistical tests. For the smaller data-set representing sampling dates with treatment replication (Fig. 4), the daily maximum A_{net} at growth c_a was significantly higher ($P < 0.05$; studentized range test) in FACE treatment plots compared with the ambient control plots for four out of five sampling dates. The sampling date where this contrast was not significant was toward the peak of the drought in late August. The overall difference between A_{net} at growth c_a in ambient versus elevated CO₂ plots was marginally significant ($P < 0.08$; repeated-measures ANOVA) across all dates and significant at $P < 0.05$ across all dates eliminating the data collected around peak drought in late August. When A_{net} at growth c_a was significantly different between treatments, indicated by * in Fig. 4, the ratio of A_{560} of elevated CO₂ foliage to A_{360} in ambient foliage (ϵ') varied between 1.5 and 1.8.

Integrated over the day, cumulative photosynthetic performance was enhanced by 84% by elevated CO₂ at peak drought and by 69% on a well-watered day (Fig. 3). The daily sum of photosynthetic enhancement was somewhat larger than the instantaneous ϵ at mid-day on these days.

Using the integrated daily A_{net} values for drought versus well-watered conditions, an estimate of the potential loss in photosynthesis due to drought can be calculated and compared between ambient and elevated CO₂ conditions. The two days in Fig. 3 were comparable in maximum air temperature and vapour pressure deficit, although duration of sunny conditions was approximately 45 min less in late September due to decreasing day-length which would reduce daily A_{net} by less than 5%. Considering this, the estimated reduction in integrated daily A_{net} attributable to drought was 45% for upper canopy foliage under ambient CO₂ and 40% for the elevated CO₂ treatment.

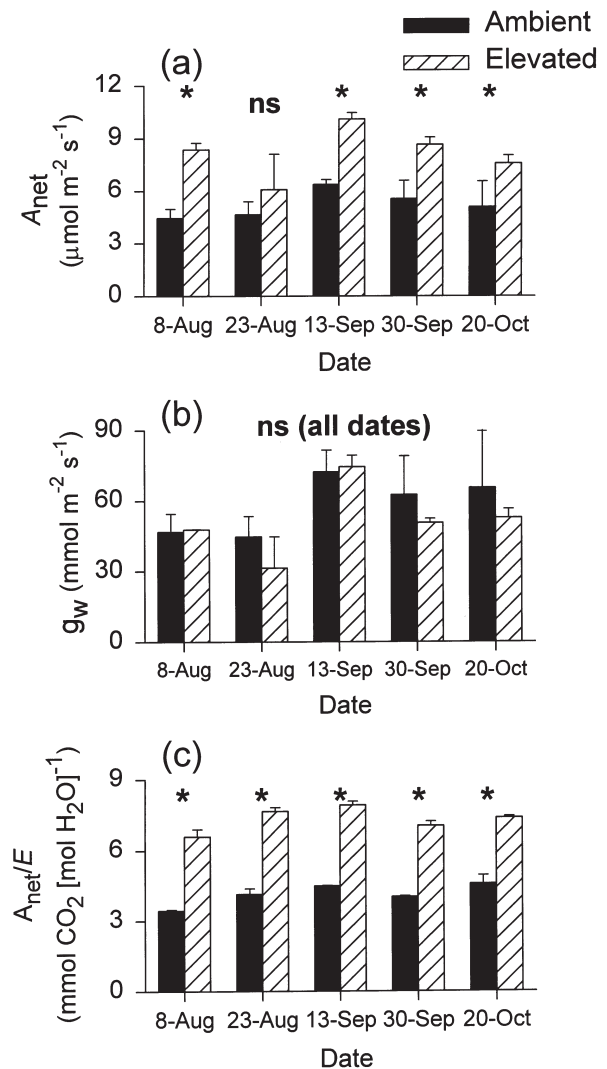


Figure 4. Treatment effects on gas exchange parameters [A_{net} at growth c_a and light saturation, upper panel; leaf conductance (g_w), middle panel; instantaneous photosynthesis to transpiration (A_{net}/E) ratio, bottom panel] of upper canopy pine foliage over five sampling dates during the growing season. Data shown are treatment means \pm SE for ambient control (dark bars) and elevated CO₂ (FACE) treatment plots (hatched bars) in the stand. Treatment comparisons within a sampling date were not significant (NS) or significant at the $P = 0.05$ level (*; Tukey range test) for each date as indicated.

In contrast to the large effect of elevated CO₂ on A_{net} , there was no evidence of differences in g_w in elevated CO₂ compared with ambient CO₂ either seasonally (Fig. 2) or with time of day under conditions of different water availability (Fig. 3). There was no significant difference in g_w in FACE treatment versus ambient controls across all sampling dates ($P > 0.10$; repeated-measures ANOVA), or on any individual sampling date (Fig. 4). Thus enhancement in A_{net} alone was responsible for highly significant differences in photosynthesis to transpiration ratio (A_{net}/E) across the season ($P < 0.003$; repeated-measures ANOVA). Leaf temperatures and leaf–air vapour pressure difference for ambient and elevated CO₂ measurements were not significantly different ($P > 0.10$), so no treatment bias in A_{net}/E attributable to measurement conditions other than ambient c_a existed.

Differences in the relationship between A_{net} and g_w between the ambient and elevated CO₂ treatments (Fig. 5) are consistent with enhanced A_{net}/E under elevated CO₂. The slopes of the lines shown are significantly different ($P < 0.05$). While the regression relationship may imply that ϵ will vary with the magnitude of g_w , ϵ calculated in this way is biased by the non-zero intercepts of the relationships. The ratio of the slope for the elevated CO₂ regression line to that of the ambient was 1.5, suggesting an overall seasonal photosynthetic enhancement of this magnitude for a similar range in g_w between ambient and elevated CO₂ foliage. Despite this enhancement, the long-term ratio of c_i/c_a for the ambient foliage was apparently maintained in the elevated CO₂ treatment since neither the c_i/c_a ratio nor the ¹³C isotope discrimination (Δ) was different between treatments (Table 2). The short-term stimulation of current-year foliage developed under ambient CO₂ and measured at $c_a = 560 \mu\text{mol mol}^{-1}$ (e.g. ϵ for ambient foliage) was 1.57 when averaged over the season (Fig. 6a). There was no significant difference in the slope of this rela-

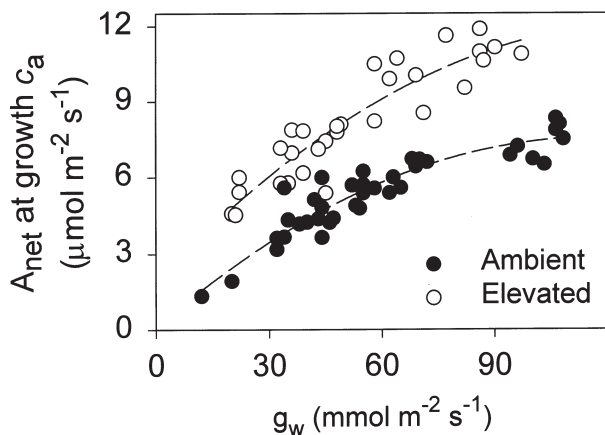


Figure 5. Relationship between leaf conductance (g_w) and daily maximum A_{net} at growth c_a for upper canopy foliage in ambient (●) and elevated (○) CO₂ plots. The lines shown are $Y = 1.94 + 0.15 \times X + 5.8 \times 10^{-4} \times X^2$ ($r^2 = 0.84$) for elevated CO₂ foliage and $Y = 0.12 + 0.13 \times X + 5.5 \times 10^{-4} \times X^2$ ($r^2 = 0.86$) for ambient foliage.

Table 2. Carbon isotope discrimination and c_i/c_a ratio of current-year, upper-canopy loblolly pine (*Pinus taeda*) foliage developed under ambient and elevated CO₂. Time-integrated c_i/c_a was calculated from foliar and air stable carbon isotope data (Appendix). Data are for $n = 3$ plots per treatment

Parameter	Ambient	Elevated CO ₂
Δ (‰)	19.2 ± 0.4	19.3 ± 1.0
Integrated c_i/c_a	0.66 ± 0.02	0.66 ± 0.04

tionship between foliage from ambient and FACE plots ($P > 0.1$), so the photosynthetic enhancement ϵ of foliage that developed under elevated CO₂ in FACE was similar to that of ambient foliage (Fig. 6a). The relationship between A_{560} and A_{360} was also similar when values from paired Elevated CO₂ and ambient plots were used rather than instantaneous ϵ values, although the variance was larger (data not shown). The enhancement ratio for single leaves is approximately constant over the seasonal range in A_{net} values from July to September with a limited range in temperature (Fig. 6a).

To evaluate the possibility of an intrinsic physiological change under elevated CO₂ that would reduce carboxylation efficiency or photosynthetic enhancement (Sage 1994), I compared A_{net} measurements in ambient and FACE plots made at a common c_a . For the sampling dates shown in Fig. 4, there were no significant differences between treatments for A_{net} measured at a common c_a (data not shown). The composite $A_{\text{net}}-c_i$ curve across sampling dates also indicates that all the data follow the same intrinsic relationship over the range of c_i values (Fig. 6b). For the CO₂ supply function lines shown in Fig. 6(b) illustrating the same g_w among treatments, the c_i/c_a ratios were also the same for ambient and elevated CO₂ foliage. The c_i/c_a ratio depicted (0.63) was similar to values from the $\delta^{13}\text{C}$ data in Table 2.

DISCUSSION

Elevated CO₂ exposure in free-air had substantial impacts on canopy leaf carbon assimilation in the upper canopy but little apparent effect on water relations or water supply over a season with a strong drought event. Theoretical predictions of enhanced water supply under elevated CO₂ are supported by observations in crop ecosystems (Pinter *et al.* 1996) and in grasslands (Jackson *et al.* 1994; Knapp *et al.* 1996; Owensby *et al.* 1996) but have rarely been tested in forest ecosystems. A general prediction supported by these studies is that partial stomatal closure elicited by increased intercellular CO₂ under elevated CO₂ (Morison 1987; Mott 1988) improves plantwater relations, which may affect ecosystem function during water-limited conditions and soil drying cycles (Morison 1993; Field *et al.* 1995). However, for the dominant forest species *P. taeda* examined in this study, a lack of significant differences in g_w between ambient and elevated CO₂-grown foliage at daily

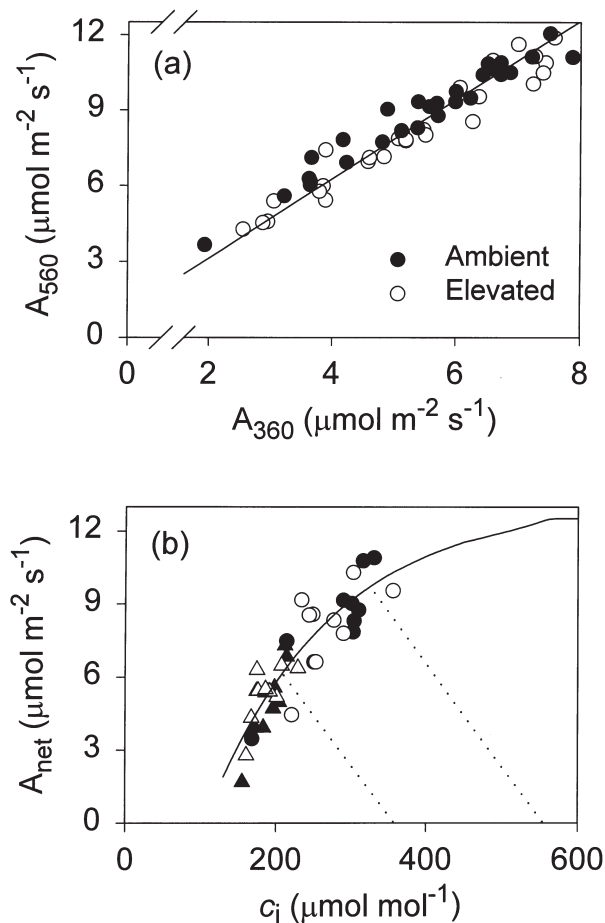


Figure 6. (a) Relationship between light-saturated A_{560} (leaf net CO_2 assimilation rate at $c_a = 560 \mu\text{mol mol}^{-1}$) and light-saturated A_{360} (CO₂ assimilation rate at current ambient c_a) for current-year canopy foliage in ambient (●) and elevated (○) CO₂ plots during the growing season. Data shown are means \pm SE for each treatment on a given sampling date. The line shown is $Y = 1.57 \times X$. (b) Composite relationship between light-saturated leaf net CO₂ assimilation rate (A_{net}) and calculated intercellular CO₂ concentration (c_i) for current-year canopy foliage of *P. taeda* in ambient (closed symbols) and FACE plots (open symbols) under drought and well-watered conditions. Measurements were made at $c_a = 360 \mu\text{mol mol}^{-1}$ (triangles) and $c_a = 560 \mu\text{mol mol}^{-1}$ (circles). Data are daily maximum A_{net} from July to September, and leaf temperature varied between 29 and 32 °C for the measurements. The mean CO₂ supply function corresponding to foliage from each treatment for well-watered conditions (13 September) is indicated by the parallel dashed lines showing the CO₂ gradient from c_a to c_i .

(Fig. 3) and seasonal time scales (Figs 2, 4) after more than 12 months of CO₂ exposure was probably responsible for the similarity in soil moisture and leaf–water relations parameters between CO₂ treatments (Fig. 1, Table 1). Thus a major primary mechanism for elevated CO₂ effects on ecosystem water exchange with the atmosphere via stomatal appears to be relatively unimportant in the short term or on the seasonal time scale for this conifer forest. If the responses of conifer forest in the field differ significantly from those established in field-based grassland studies,

then such differences must be considered in models projecting future forest–climate CO₂ and H₂O feed-back and exchange processes (Sellers *et al.* 1996). There is a paucity of field data from elevated CO₂-exposed forest stands for making a general comparison with grasslands. However, many current modelling efforts consider relative stomatal responses to elevated CO₂ to be similar among diverse herbaceous and tree species (Henderson-Sellers *et al.* 1995; Haxeltine, Prentice & Cresswell 1996; Pan *et al.* 1998) and may misrepresent the magnitude of CO₂ responses in some ecosystems with a wide geographic range.

Evidence from species with high photosynthetic rates such as crops, ruderal herbs and grasses suggests that the guard cells of stomata generally respond to intercellular CO₂ concentration (Mott 1988). The lack of such a response in other C₃ plants may be considered exceptional, although until recently stomatal responses of native woody species have been largely overlooked (Mansfield, Hetherington & Atkinson 1990). In literature comparisons, Field *et al.* (1995) found that the stomatal responses of trees were generally smaller than those documented for crops, although Curtis (1996) concluded that there were small but significant CO₂ effects on stomatal conductance based on a meta-analysis of 16 studies on unstressed trees. Even so, there are exceptions where a lack of a stomatal response to CO₂ has been noted (Eamus & Jarvis 1989; Ellsworth *et al.* 1995; Heath & Kerstiens 1997). Robinson (1994) and Saxe *et al.* (1998) speculated that some plant groups might be less responsive in this regard than those with high rates of metabolism, especially woody species and coniferous trees. The hypothesis that coniferous trees show small or non-significant stomatal responses to elevated CO₂ is supported by results from field studies on different species of Pinaceae studied in chambers by Beerling & Woodward (1996), Dixon *et al.* (1995), Hogan *et al.* (1996) and Picon *et al.* (1996) (see also references in Saxe *et al.* 1998). Studies on *P. taeda* itself have yielded inconsistent results in this respect for young plants (cf. Tolley & Strain 1985; Tissue *et al.* 1997), although mature individuals have consistently not shown significantly elevated CO₂ effects on g_w with long-term CO₂ exposures of isolated branches (Teskey 1995; Murthy, Zarnoch & Dougherty 1997) or entire trees in FACE (Ellsworth *et al.* 1995; this study). Experiments employing containerized or root-restricted young trees with limited rooting space may artificially induce large stomatal responses to elevated CO₂ in seedlings as a result of size-dependent water stress as was shown for *P. taeda* seedlings (Will & Teskey 1997). Thus apart from a mechanistic interpretation of CO₂-induced partial stomatal closure, growth conditions and plant size can also have a strong indirect effect on the magnitude of stomatal responses to elevated CO₂ (Talbot, Srivastava & Zeiger 1996; Will & Teskey 1997). Results from studies that utilize artificial plant growth conditions such as root restrictions or chamber artifacts need to be interpreted carefully against the context of actual field conditions.

In addition to effects of elevated CO₂ on plant–water relations that are mediated by stomata, other response

mechanisms can be considered. For instance, indirect effects of elevated CO₂ on plant–water relations may occur via CO₂-induced differences in plant leaf area or leaf area to water-absorbing surface (Eamus 1991; Heath & Kerstiens 1997). In mature trees exposed to elevated CO₂ as in this study, such indirect effects are negligible in the first year of CO₂ exposure compared with the size-induced differences between ambient and elevated CO₂ trees that are common in seedling studies (Saxe *et al.* 1998). Elevated CO₂ may also directly alter leaf dehydration tolerance in cases where CO₂-induced excess carbohydrates serve as osmotica (Morse *et al.* 1993; Tschaplinski, Norby & Wullschleger 1993). There was no evidence of CO₂ effects on tissue water relations parameters in *P. taeda* in this study (Table 1) nor in chamber-grown seedlings (Tschaplinski *et al.* 1993) which again supports the contention that water relations and dehydration tolerance in this conifer species were largely unaffected by CO₂ exposure. Results from *P. taeda* have important implications for understanding forest CO₂ responses under water-limited conditions, which are predicted to intensify in its growing region under climate radiative-forcing scenarios (Rind *et al.* 1990) and with increasing urban water demands in the region.

A tendency for similarity in c_i/c_a ratios between ambient and elevated CO₂ foliage has been noted by Morison (1987), Sage (1994) and Drake *et al.* (1997). This may suggest that stomatal responses to elevated CO₂ are largely not independent of responses in A_{net} (Sage 1994). Well-watered *P. taeda* maintained c_i/c_a ratios in elevated CO₂ although at peak drought the lower c_i/c_a ratios in elevated CO₂ versus ambient trees suggests that there are greater stomatal limitations during severe stress in elevated CO₂. However, the seasonally integrated c_i/c_a ratios indicated by ¹³C isotope discrimination remained identical in ambient and elevated CO₂ (Table 2). As long as c_i is maintained within the nearly linear region of the initial portion of the $A_{\text{net}}-c_i$ relationship (Fig. 6b), a decrease in g_w at elevated CO₂ is not necessary to maintain a constant c_i/c_a with increasing c_a and adequate water availability. The constancy of c_i/c_a ratios forms the basis for empirical models for predicting stomatal conductance (Jarvis & Davies 1998) but does not necessarily presuppose partial stomatal closure with long-term exposure to elevated CO₂ in species where g_w is intrinsically low.

CO₂ assimilation in canopy foliage under elevated CO₂ and drought

In spite of the lack of observable CO₂ effects on water balance, photosynthetic CO₂ assimilation was consistently enhanced in elevated CO₂-grown foliage over the course of a growing season with drought (Figs 2, 3 & 4). The photosynthetic enhancement ratio ϵ in upper canopy foliage that developed under continuous exposure to ambient + 200 $\mu\text{mol mol}^{-1}$ CO₂ in FACE was large (approximately 1.5 to 1.8 on different days, mean of 1.57; Figs 4, 6a). This was similar to the ϵ of 1.65 reported in a single-season

FACE exposure in Ellsworth *et al.* (1995) in the same forest stand, using a similar exposure CO₂ concentration in FACE with daytime-only fumigation. There is considerable evidence for a lack in photosynthetic adjustments to elevated CO₂ in previous experiments lasting one to several growing seasons (Ellsworth *et al.* 1995; Teskey 1995; Tissue *et al.* 1997). This is supported by the maintenance of similar A_{560}/A_{360} ratios in FACE and ambient foliage (Fig. 6a) and by the similarity in the $A_{\text{net}}-c_i$ curve, composited across sampling dates (Fig. 6b). Myers, Thomas & DeLucia (1999) presented evidence that there were no biochemical adjustments in photosynthetic capacity during summer in ambient-grown foliage of the previous year's cohort in this stand when exposed to free-air CO₂ enrichment. Single-season or single-year CO₂ exposures are unlikely to be sufficiently long to induce physiological adjustments in the photosynthetic apparatus in trees (Ceulemans *et al.* 1997; Drake *et al.* 1997), particularly those in a mature stand that developed under current ambient CO₂ (Körner 1995).

Photosynthetic enhancement of upper canopy foliage was largely maintained despite drought conditions during a portion of the growth period (Figs 2, 4). A lack of significant CO₂ effects on A_{net} at growth c_a toward peak drought was observed, suggesting greater sensitivity of A_{net} to drought in elevated CO₂-grown trees (Fig. 4). However, both ambient and elevated CO₂ foliage both showed similar and large instantaneous enhancements in photosynthesis, even when daily maximum A_{net} was low due to drought (Figs 3, 6a). Thus there is not sufficient evidence to conclude that photosynthetic enhancement by elevated CO₂ is intrinsically smaller under drought than under well-watered conditions in mature *P. taeda*. The effects of CO₂ treatment on the magnitude of water-stress induced repression of A_{net} at a given c_a were not large, since the instantaneous photosynthetic enhancement was nearly constant across the range of A_{net} values over well-watered and drought conditions (Fig. 6a). The constant ϵ for a given temperature can be expected if intrinsic leaf biochemical processes in photosynthesis are relatively unaffected during drought (Fig. 6b), and stomatal conductance largely limits photosynthesis with decreasing soil moisture (Fig. 2). Given the lack of strong curvature in the $A_{\text{net}}-c_i$ curve relationship below calculated c_i values of 400 $\mu\text{mol mol}^{-1}$ (Fig. 6b), near-constant ϵ values are expected for the CO₂ supply function corresponding to a typical g_w for pine. At summertime temperatures, light-saturated A_{net} in *P. taeda* operates in the responsive initial portion of the $A_{\text{net}}-c_i$ curve, which explains why photosynthetic enhancement by elevated CO₂ is large.

Much of the seasonal and diurnal decrease in A_{net} with drought was apparently controlled by stomata (Figs 2, 3) as would be expected in drought sensitive species like some *Pinus* species (Green & Mitchell 1992; Picon *et al.* 1996). The maintenance of consistent mid-day Ψ_1 over the season with declining pre-dawn Ψ_1 in both treatments during the drought period (Figs 1, 2) indicates that as drought progressed, the opportunity for photosynthesis over the course of a day would decrease in proportion to the available drop

in Ψ to a threshold value corresponding to the turgor loss point (Table 1). Due to a lack of partial stomatal closure with elevated CO_2 , the relative reductions in A_{net} at growth c_a by water-stress induced stomatal closure in ambient and FACE trees are similar. The overall results from this FACE experiment support earlier evidence that maturing *P. taeda* canopies have a large capacity for CO_2 responses in photosynthesis even under drought, but low stomatal responsiveness to elevated CO_2 . This observation points to the need for reconsideration of elevated CO_2 effects on water relations in conifer-dominated forest ecosystems in warm temperate regions, and highlights an important difference from warm temperate grassland ecosystems. While evidence from other coniferous forest ecosystems and longer-term experiments is needed, the results suggest that in some forests, feed-backs to the climate system in an elevated CO_2 atmosphere may not occur as has been predicted by the current generation of coupled biosphere-atmosphere models (Sellers *et al.* 1996). Understanding the mechanisms of short-term and long-term regulation of stomatal conductance, stomatal density and leaf area per unit stem basal area in woody plant species in elevated CO_2 underlie our ability to predict forest function and its feed-backs with climate and rising atmospheric CO_2 .

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APPENDIX

Calculation of c_i/c_a from leaf $\delta^{13}\text{C}$

Stable C isotope discrimination (Δ) can be calculated from leaf $\delta^{13}\text{C}$ values (in ‰) as

$$\Delta = (\delta_a - \delta_p) / (1 + \delta_p / 1000) \quad (1)$$

where δ_a is the $\delta^{13}\text{C}$ of the source air and δ_p is the $\delta^{13}\text{C}$ of the plant tissue (Farquhar *et al.* 1989). The source air (δ_a) was assumed to be approximately -8.5‰ for the daytime CO₂ concentration of $368 \mu\text{mol mol}^{-1}$ for ambient rings. In

FACE rings, a two-ended mixing ratio model was used to calculate the isotope ratio of the source air in elevated CO₂ (δ_e) using the 1 min mean CO₂ concentration of the sampling port near the foliage and the equation

$$\delta_e = (c_a - c_{\text{amb}})/c_a \times \delta_c + c_{\text{amb}}/c_a \times \delta_a \quad (2)$$

where c_{amb} is the air CO₂ concentration outside the FACE plot (equivalent to c_a in ambient plots) and δ_c is the stable isotope signature of the pure CO₂ added. The calculation was performed using daytime CO₂ concentration data only from the point of leaf emergence in April to the end of September when foliage was collected. All CO₂ used for enrichment in the FACE rings was from a constant fossil-fuel source with a δ_c of

$-43.7 \pm 0.6\text{‰}$ over the study period (J. A. Andrews, unpublished results). From Δ , the ratio of c_i to c_a can be calculated by solving the equation

$$\Delta = a + (b - a) \times c_i/c_a \quad (3)$$

where a is the coefficient for diffusion through the stomatal pore (4.4‰) and b is the fixation of gaseous CO₂ with respect to c_i/c_a (27‰) as described in Farquhar *et al.* (1989).

Rearranging (3) and (1) to solve for c_i/c_a yields

$$c_i/c_a = [(\delta_a - \delta_p)/(1 + \delta_p/1000) - a]/(b - a) \quad (4)$$

which can be solved for c_i/c_a of ambient and elevated CO₂ foliage. Mean δ_p was 27.4 and 39.3‰ for foliage from ambient and elevated CO₂ plots, respectively.