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Does Free-Air Carbon Dioxide Enrichment Affect Photochemical Energy Use by Evergreen Trees in Different Seasons? A Chlorophyll Fluorescence Study of Mature Loblolly Pine[1]

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Previous studies of the effects of growth at elevated CO_2 on energy partitioning in the photosynthetic apparatus have produced conflicting results. The hypothesis was developed and tested that elevated CO_2 increases photochemical energy use when there is a high demand for assimilates and decreases usage when demand is low. Modulated chlorophyll *a* fluorescence and leaf gas exchange were measured on needles at the top of a mature, 12-m loblolly pine (*Pinus taeda* L.) forest. Trees were exposed to ambient CO_2 or ambient plus 20 Pa CO_2 using free-air CO_2 enrichment. During April and August, periods of shoot growth, light-saturated photosynthesis and linear electron transport were increased by elevated CO_2. In November, when growth had ceased but temperatures were still moderate, CO_2 treatment had no significant effect on linear electron transport. In February, when low temperatures were likely to inhibit translocation, CO_2 treatment caused a significant decrease in linear electron transport. This coincided with a slower recovery of the maximum photosystem II efficiency on transfer of needles to the shade, indicating that growth in elevated CO_2 induced a more persistent photoinhibition. Both the summer increase and the winter decrease in linear electron transport in elevated CO_2 resulted from a change in photochemical quenching, not in the efficiency of energy transfer within the photosystem II antenna. There was no evidence of any effect of CO_2 on photochemical energy sinks other than carbon metabolism. Our results suggest that elevated CO_2 may increase the effects of winter stress on evergreen foliage.

Most previous studies of the effects of elevated pCO_2 on photosynthesis have focused on carbon assimilation and metabolism (for review, see Drake et al., 1997). Changes in carbon assimilation at elevated pCO_2 necessitate changes in the partitioning of absorbed energy between heat dissipation and photochemistry in the thylakoid membrane (Pammenter et al., 1993; Valentini et al., 1995; Drake et al., 1997). Modulated chlorophyll *a* fluorescence enables direct analysis of these processes (Ghashghaie and Cornic, 1994; Valentini et al., 1995). Previous fluorescence studies have shown contrasting effects of long-term elevation of pCO_2 on photochemistry.

For instance, in young wheat plants exposed to elevated pCO_2, a greater proportion of the absorbed light is used in photochemistry at high light (Habash et al., 1995). Such an increase in photochemical energy dissipation should diminish reversible photoinhibition, which would be evident as an increase in F_v/F_m. Consistent with this expectation, Jones et al. (1995) observed a higher midday F_v/F_m in the evergreen tree *Arbutus unedo* growing at elevated pCO_2 in the field under drought stress. In contrast, Scarascia-Mugnozza et al. (1996) showed decreased photochemistry and increased photoinhibition in *Quercus ilex* at elevated pCO_2 under drought in the field. Similarly, Roden and Ball (1996) observed a lower F_v/F_m in *Eucalyptus macrorhyncha* grown at elevated pCO_2 during a heat stress treatment. This variation might be explained by differences in limitations to photosynthesis by carbon metabolism.

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Abbreviations: A, net rate of CO_2 uptake per unit leaf area (μmol m^{-2} s^{-1}); α, leaf absorptance between 400–700 nm; FACE, free-air CO_2 enrichment; F_o, F_m, minimum and maximum dark-adapted fluorescence yield, respectively; F_o', F_m', F_s, minimum, maximum, and steady-state light-adapted fluorescence yield, respectively; F_v/F_m, quantum efficiency of PSII photochemistry in the dark-adapted state; F_v'/F_m', probability of an absorbed photon reaching an open PSII reaction center; J_{PSII}, estimated rate of linear electron flow through PSII (μmol m^{-2} s^{-1}); pCO_2, partial pressure of CO_2 (Pa); ϕ_{CO2}, quantum efficiency of CO_2 fixation corrected for leaf absorption; ϕ_{PSII}, quantum efficiency of linear electron transport through PSII; q_P, photochemical quenching coefficient; R_L, estimate of the rate of respiratory CO_2 efflux in the light (μmol m^{-2} s^{-1}); T_{leaf}, leaf temperature (°C); TPU, triose phosphate utilization; v_c, velocity of carboxylation; v_o, velocity of oxygenation.

Following the theoretical model of Farquhar et al. (1980) and subsequent modification by Sharkey (1985), photosynthesis at light saturation may be limited by: (a) the amount of active Rubisco; (b) the rate of regeneration of RubP; and (c) the rate of Pi release by TPU (Harley et al., 1992). If photosynthesis is limited by the amount of active Rubisco, elevation of pCO_2 will increase energy use in photochemistry and therefore electron flux through PSII (J_{PSII}). Because Rubisco is not CO_2 saturated at the present atmospheric pCO_2, an increase in pCO_2 results in an increase in v_c that is larger than the decrease in v_o. Therefore, there will be an increase in the use of NADPH and in turn an increase in J_{PSII}. When RubP regeneration is limiting, an increase in pCO_2 will result in an increase in v_c, which is exactly offset by a decrease in v_o (Drake et al., 1997). Therefore, although there will be a net increase in CO_2 uptake, the rate of NADPH utilization and J_{PSII} will be unaffected. When TPU is limiting, v_c will not increase with an increase in pCO_2, but v_o will be decreased by inhibition of the oxygenation reaction (Sharkey, 1985). CO_2 uptake will be unaffected by elevated pCO_2, but the use of NADPH, and in turn J_{PSII}, will be decreased. For example, at 25°C and using the parameters of Harley et al. (1992), an increase in pCO_2 from 36 to 56 Pa would result in a 16% increase in J_{PSII} if Rubisco was limiting, no change in J_{PSII} if RubP was limiting, and a 14% decrease in J_{PSII} if TPU was limiting. This analysis assumes that elevated pCO_2 does not alter the rate of electron use by other processes such as Mehler reactions and nitrogen metabolism. However, there is no evidence that substantial changes in sinks for J_{PSII} occur in elevated pCO_2 (Epron et al., 1994; Habash et al., 1995; Bartak et al., 1996). Acclimatory losses of Rubisco or capacity for RubP regeneration have been observed in situ under elevated pCO_2 (e.g. Gunderson and Wullschleger, 1994; Oechel et al., 1994; Curtis, 1996; Bryant et al., 1998; Rogers et al., 1998) and would complicate this conceptual model.

In evergreen species the limitation to light-saturated photosynthesis is likely to change with season. In these species photosynthesis continues throughout the times of the year when growth is environmentally restricted, e.g. by low temperature. At low temperatures, in which translocation may be inhibited, TPU limitation may occur (Socias et al., 1993). During periods of active growth, demand for carbohydrates may be high and photosynthesis limited by the amount of active Rubisco. From this conceptual framework, we developed the following hypothesis.

Elevated pCO_2 has different effects on photochemistry, depending on the season. During the major periods of growth, light-saturated photosynthesis is limited by the amount of active Rubisco and elevated pCO_2 increases J_{PSII}, leading to decreased photoinhibition. During times of the year when growth has ceased and translocation may be inhibited by low temperature, photosynthesis is limited by TPU and elevated pCO_2 decreases J_{PSII}, leading to increased photoinhibition.

The FACE facility at the Duke Forest in North Carolina provided an opportunity to test these hypotheses. This experiment exposed mature, 12-m evergreen loblolly pine (*Pinus taeda*) trees to a pCO_2 elevated 20 Pa above the current ambient level in open air (Hendrey et al., 1999). The lack of an enclosure was ideal for studying photoinhibition, which could be substantially decreased by the lower light levels within chamber enclosures (McLeod and Long, 1999). Moreover, mature trees have a large sink capacity and defined seasonal patterns of growth, yet have received little attention in terms of their response to elevated pCO_2 (Lee and Jarvis, 1995; Saxe et al., 1998).

MATERIALS AND METHODS

The study site was a 32-ha even-aged *P. taeda* (loblolly pine) plantation in Duke Forest, NC (35°58′N, 79°05′W). The forest was located on clay-rich soils with low nitrogen and phosphorus availability (Ellsworth et al., 1995). The pine trees were 15 years old and 12 m tall in the summer of 1997. FACE technology was used to elevate ambient pCO_2 by 20 Pa in three 30 m diameter circular forest plots (Lewin et al., 1994). The system has been described in detail elsewhere and is only briefly outlined here (Hendrey et al., 1999). Each ring was surrounded by a plenum connected via computer-controlled valves to 15-m vertical vent pipes. According to windspeed and direction, jets of air enriched in CO_2 are released at a range of heights to maintain a uniform enriched pCO_2 through the canopy within each ring.

Each elevated pCO_2 ring was paired with an identical control ring in which air was added at the same volume and direction, but without pCO_2 enrichment. Elevated pCO_2 fumigation was maintained over 24 h except when ambient air temperatures dropped below 5°C (December–March). The mean pCO_2 recorded at 1-min intervals throughout 1997 was 54.6 Pa in the treatment rings and 38 Pa over a 24-h period in the controls. Access to the canopy surface was via a central tower and telescopic platform (UL40, Upright, Charlotte, NC) within each ring.

Our first measurements were made within days of the start of CO_2 fumigation in this FACE facility in September 1996. This foliage had appeared in April 1996 and had therefore developed fully under ambient pCO_2. Subsequent measurements on this foliage cohort were made in February and April 1997. Measurements in August 1997, November 1997, and February 1998 were on foliage that had appeared in April of 1997 and had therefore developed fully under elevated pCO_2. Measurements were also made in September 1996 in a FACE-prototype ring of the design described above, which had been established in 1993. The vegetation had been fumigated at a pCO_2 of 55 Pa during each growing season (May–October) since 1993 (Ellsworth et al., 1995; Hendrey et al., 1999).

In Situ Chlorophyll *a* Fluorescence

A modulated chlorophyll fluorimeter and leaf clip (PAM 2000, Walz) were used to measure diurnal variation in F_o', F_m', and F_s following the method of Nogues et al. (1998). ϕ_{PSII}, q_P, and F_v'/F_m' were determined from each measurement of F_o', F_m', and F_s (Genty et al., 1989). Fascicle absorptance of α was determined using a quantum sensor and an external integrating sphere (LI-1800–12, LI-COR) following the procedures of Rackham and Wilson (1968). J_{PSII} was estimated from ϕ_{PSII} using measured values of α

and assuming that 50% of absorbed photon flux was distributed to PSII (Krall and Edwards, 1992; Ghashghaie and Cornic, 1994). Measurements of F_o and F_m were made following dark adaptation for 10 min to determine F_v/F_m. All of the above measurements were made under prevailing light conditions on two fully expanded fascicles from sun-exposed, upper-crown branches (10–12 m high) of each of three trees in each of the six rings at approximately 2-h intervals from sunrise to sunset. Trees sampled were within 10 m of the center of the ring, where pCO_2 is most homogeneous (Hendrey et al., 1999). Measurements were also made in the prototype FACE ring in September 1996 by the procedures described above for the other rings.

The recovery of F_v/F_m was monitored in situ as follows. In full sun between 12 and 2 PM, F_v'/F_m' was measured, the branch was shaded (PPFD $<$ 50 μmol m^{-2} s^{-1}), and F_v/F_m was measured at intervals for 60 min. Three fascicles on one branch in one control and one elevated ring were measured.

Photosynthetic Gas Exchange

A was measured at ambient PPFD using a portable open gas-exchange system (CIRAS-1, PP Systems, Hitchin, UK) as described by Ellsworth (1999). As with fluorescence measurements, fascicles at the top of the canopy were selected and their natural orientation and inclination were retained during measurement. The pCO_2 within the leaf chamber was maintained at the growth pCO_2. Temperature and leaf-air vapor pressure differences within the leaf chamber were maintained near ambient levels. Fascicle surface area was calculated using the method of Johnson (1984). On each date the fluorescence was measured, leaf gas exchange was measured between 11 AM and 3 PM, sampling one fascicle from one to three trees in each of the six rings. Measurements were made in parallel with the above fluorescence measurements on separate fascicles but from the same populations.

Light Response of CO_2 Uptake and Fluorescence

To separate developmental differences and long-term effects due to elevated pCO_2 from any change induced by exposure to high light during the day, measurements were also made on fascicles collected around dawn. Fascicles of the populations used in the diurnal studies described above were cut under water, transferred to a controlled environment, and maintained in low light until measured. Measurements were made within 2 h of collection. The responses of A, J_{PSII}, ϕ_{PSII}, F_v'/F_m', and q_P to PPFD were determined simultaneously on individual fascicles in April, August, and November 1997 and in February 1998. A leaf gas exchange system (LI 6400, LI-COR) incorporating a controlled environment cuvette modified to accept the fiber optics from a modulated fluorimeter (PAM 2000) was used.

Measurements were made at PPFD from 0 to 1,600 μmol m^{-2} s^{-1} provided by a quartz iodide source, and were made at the mean midday T_{leaf} and humidities observed in the diurnal measurements. Measurements in April, August, and November were made at T_{leaf} = 22.0°C ± 0.1°C, 26.0°C ± 0.1°C, and 18.0°C ± 0.2°C, respectively. In February 1998 the measurements were made at T_{leaf} = 19.0°C ± 0.2°C. In all months, measurements were made in both 21 and 1 kPa pO_2 (Scott Specialty Gases, Durham, NC). At least two fascicles from all six rings were measured on each occasion. From the response of A to PPFD, ϕ_{CO2} was calculated as $(A + R_L)/(\text{PPFD} \times \alpha)$, where R_L is the rate of CO_2 evolution after 2 to 3 min in the dark. The relationship of ϕ_{PSII} to ϕ_{CO2} in 1 kPa pO_2 was used to determine any effect of growth pCO_2 on the magnitude of alternative sinks for electron flux (Genty et al., 1989; Edwards and Baker, 1993).

Statistical Analysis

Two-way ANOVA was used to test the effect of growth pCO_2 and time of year on chlorophyll fluorescence and gas exchange parameters at light saturation both in situ and on the excised fascicles (SYSTAT Inc., Evanston, IL). To avoid pseudoreplication, means for each parameter were calculated for every ring and subsequently treated as the individual, giving a sample size of n = 3 per treatment for statistical analyses. For measurements made in the FACE-prototype experiment individual fascicles were the replicates. The effect of pCO_2 treatment on the slope and intercept of the relationship between ϕ_{PSII} and ϕ_{CO2} was examined by regression ANOVA. The derived chlorophyll fluorescence parameters ϕ_{PSII}, F_v'/F_m', F_v'/F_m', and q_P, were arcsine-transformed prior to statistical analysis (Sokal and Rohlf, 1981).

RESULTS

Skies were clear throughout the measurement days. With the exception of February 1997, temperatures were typical of the season (Fig. 1, p–t). In February 1997 the maximum day temperature was high (19°C), however the previous night had been −5°C and the average daily minimum for the month was zero, with below-zero minimum temperatures on 20 d within the month. Minima were similar in February 1998, although the lower daily maximum illustrated for 1998 is more typical of this month.

Under the warm conditions of August 1997, when substantial growth was occurring, the total electron flux through PSII (J_{PSII}), and therefore the proportion of absorbed light energy used in photochemisty, was strongly and significantly enhanced by elevated pCO_2. This only applied to the period when photon flux was saturating, i.e. above 700 μmol m^{-2} s^{-1} (Fig. 1, h and m; Table I). At lower photon fluxes J_{PSII} was unaffected by pCO_2, which is consistent with the expected transition in limitation of photosynthesis from Rubisco to RubP regeneration rate (Fig. 1h).

The increase in J_{PSII} at light saturation corresponded to a highly significant 65% enhancement of A around noon of the same day (Fig. 1c; Table I). Similar enhancements in J_{PSII} were observed in September 1996 in both the full experiment, 1 week after pCO_2 elevation began, and in the

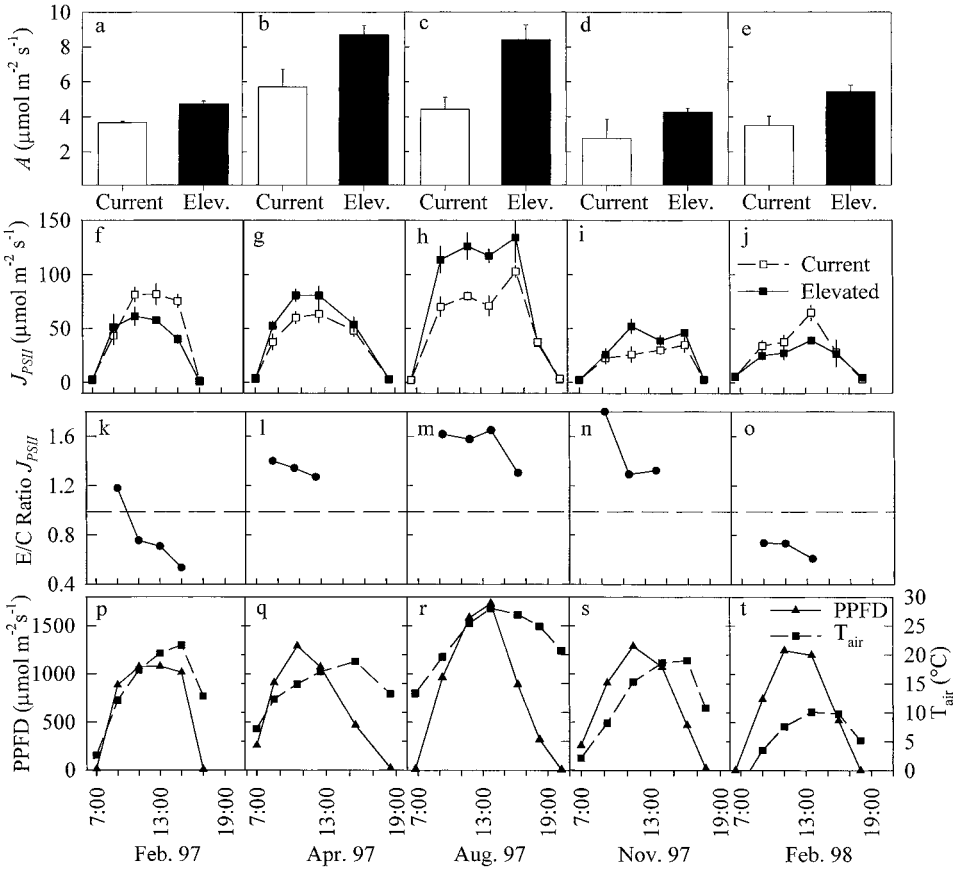

Figure 1. a to e, *A* at midday; f to j, diurnal variation in J_{PSII}; k to o, ratio of elevated/current (E/C) pCO_2 measurements of J_{PSII} at light saturation; and p to t, T_{air} and PPFD for 5 sunny days in different seasons from February 1997 to February 1998. White bars and symbols are for trees growing at current ambient pCO_2 and black bars and symbols are for trees growing at elevated pCO_2. Symbols shown are the means ± 1 SE.

parallel prototype experiment (Hendrey et al., 1999), in which the trees had been exposed to elevated pCO_2 for the three preceding summers (Table II). A smaller but significant enhancement of J_{PSII} and *A* was observed in April in the early part of the growing season (Fig. 1, b, g, and l; Table I). By sharp contrast, in February of both years there were significant decreases in J_{PSII} under elevated pCO_2, showing that less of the absorbed energy was being utilized by photochemistry (Fig. 1, f and j; Table I). Moreover, there was a progressive decrease in J_{PSII} over the course of the day at elevated pCO_2 relative to controls, which may indicate development of increased TPU limitation at elevated pCO_2 (Fig. 1k). During February there was no growth, and freezing temperatures probably inhibited translocation. A slight increase in *A* due to elevated pCO_2 was observed at midday in February 1997; the indicated increase in February 1998 was not significant (Fig. 1, a and e; Table I). Although enhancement of J_{PSII} was indicated for November 1997, soon after the end of the growing season, this was not significant (Fig. 1i).

Variations in J_{PSII} may be analyzed by examining the causes of the change in ϕ_{PSII}, which, assuming an equal

Table I. *In situ measurements of photosynthesis*

Summary of the two-way ANOVA to test for the effects of growth pCO_2 ($F_{1,20}$), time of year ($F_{4,20}$), and their interaction ($F_{4,20}$) on light-saturated J_{PSII}, *A*, q_P, F_v'/F_m', and PPFD; * denotes significance at $P > 0.05$. When the interaction between growth pCO_2 and time of year was significant ($P > 0.05$), the effect of pCO_2 was tested for each sampling date using Tukey's pairwise comparison; bold text indicates significance at $P < 0.1$.

Parameter	F-Statistic			Probability				
	pCO_2	Time	Interaction	February 1997	April 1997	August 1997	November 1997	February 1998
J_{PSII}	**7.4***	**89.8***	**21.5***	**0.03**	**0.04**	**<0.01**	0.26	**0.06**
A	**55.3***	**22.4***	**3.2***	**0.02**	**0.01**	**<0.01**	0.31	0.10
q_P	**3.8***	**72.7***	**25.5***	**0.01**	**0.06**	**<0.01**	0.23	**0.01**
F_v'/F_m'	**4.6***	**164***	0.61	–	–	–	–	–
PPFD	0.1	**41.2***	1.0	–	–	–	–	–

Table II. *Midday mean chlorophyll fluorescence parameters in September 1996*

J_{PSII}, F_v'/F_m' and q_P were measured on sun-exposed branches in two FACE experiments during September 1996: (a) The FACE prototype, which was a single elevated pCO_2 ring and control that had been operated over three consecutive growing seasons prior to these measurements. (b) The adjacent full experiment of three replicate elevated and three replicate current pCO_2 rings that had been operated for just 1 week prior to these measurements. Values are the means (SE) for two fascicles measured on each of three trees in the prototype ring and a control ring; and means (SE) for the three replicate elevated and control pCO_2 rings in the full experiment. Two-way ANOVA tested the effect of pCO_2 ($F_{1,14}$), experiment ($F_{1,14}$), and their interaction ($F_{1,14}$) on each parameter. F values for the effect of pCO_2 are shown, *indicates $P < 0.05$; n.s. indicates $P > 0.05$. No interaction was found for any of the parameters ($F_{1,14} < 3.2$; $P > 0.05$). E/C indicates the ratio of J_{PSII} and q_P measured at elevated pCO_2 to that measured at the current ambient pCO_2. J_{PSII} is expressed in μmol m^{-2} s^{-1}, F_v'/F_m' and q_P are dimensionless.

Parameter	FACE Prototype		Full Experiment		F Statistic
	Current	Elevated	Current	Elevated	
J_{PSII}	**227.0 (12.0)**	**297.0 (17.0)**	**170.3 (3.5)**	**241.9 (15.8)**	**19.9***
E/C of J_{PSII}	1.31		1.42		
F_v'/F_m'	0.73 (0.03)	0.67 (0.02)	0.65 (0.05)	0.72 (0.01)	0.03, n.s.
q_P	**0.47 (0.02)**	**0.67 (0.04)**	**0.43 (0.04)**	**0.56 (0.02)**	**22.8***
E/C of q_P	1.43		1.30		

distribution of absorbed energy between the two photosystems, is equal to the ratio of J_{PSII} to twice the absorbed photon flux (Fig. 2, a–e). Variation in ϕ_{PSII} is the product of variation in q_P and F_v'/F_m'. Over the year, elevated pCO_2 has little effect on F_v'/F_m', with variation in ϕ_{PSII} resulting from a change in q_P (Fig. 2, f–o). This would be consistent with a limitation downstream of PSII, as would occur if the demand for NADPH in carbon metabolism changed. In February 1997 elevated pCO_2 depressed q_P relative to controls, with the converse effect in August (Fig. 2, f and h). Although elevated pCO_2 produced no obvious effect on F_v'/F_m', the diurnal minimum F_v/F_m determined after 10

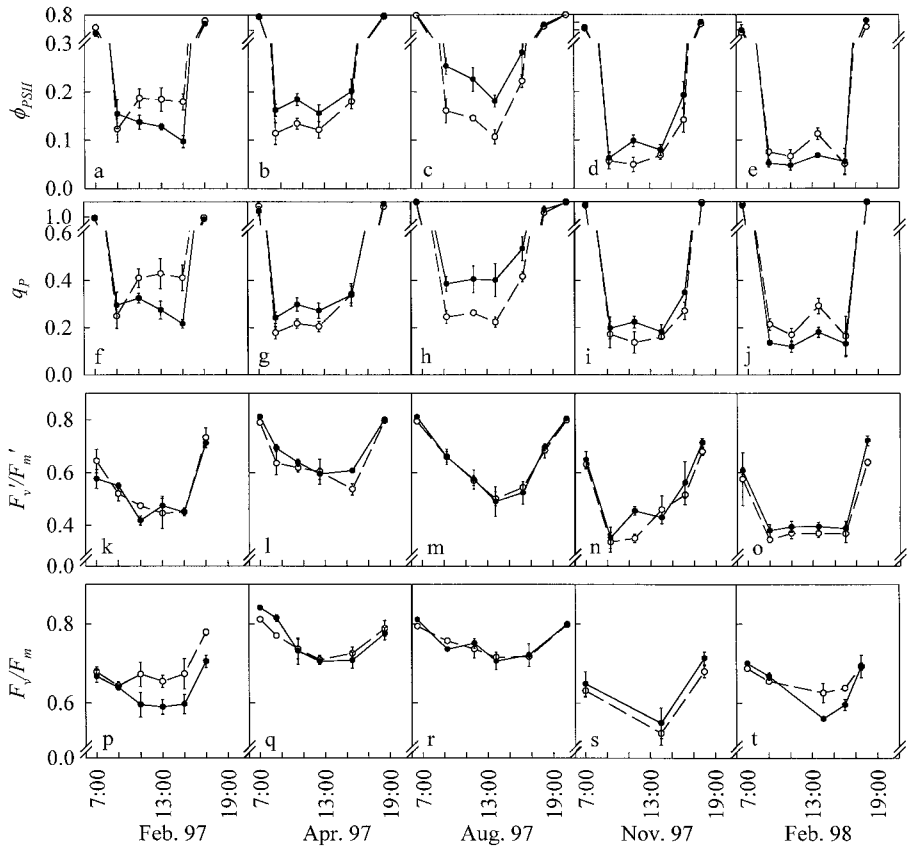

Figure 2. Diurnal variation in ϕ_{PSII} (a–e), q_P (f–j), F_v'/F_m' (k–o), and $F_{v/Fm}$ (p–t) for elevated (●) and current (○) pCO_2 measured on the same tissue and at the same times as the measurements illustrated in Figure 1.

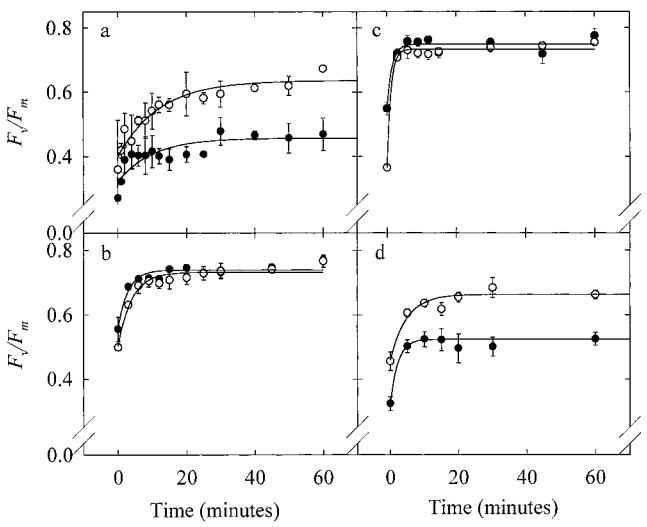

Figure 3. Effect of elevated pCO_2 on the recovery of F_v/F_m after transfer to shade in the early afternoon. Days shown are in: February 1997 (a), April 1997 (b), August 1997 (c), and February 1998 (d). Points shown are the means of three measurements made on sun-exposed branches sampled from the same population measured in Figures 1 and 2. A negative exponential curve was fitted to the points illustrated. Symbols are as in Figure 1.

min of dark adaptation was significantly lower at elevated pCO_2 in February ($F_{1,8} = 21.5$; $P < 0.05$), yet was unaffected in other months (Fig. 2, p–t).

There was no significant change in F_o between dawn and the point at which F_v/F_m was minimal, in February 1997 ($F_{1,8} = 3.1$; $P < 0.05$) and February 1998 ($F_{1,8} = 0.3$; $P < 0.05$) (data not shown). Recovery of F_v/F_m was slower at elevated pCO_2 in February, giving a significant separation between pCO_2 treatments after about 3 min of recovery. This was still clearly evident after 60 min of dark adaptation (Fig. 3). Had there been any systematic difference in PPFD between the FACE and control samples, this could have caused differences in fluorescence parameters. However, the PPFD was almost identical between CO_2 treatments ($F_{1,20} = 0.1$; $P = 0.76$; Table I).

Samples of fascicles were excised before dawn and measured later in a controlled-environment cuvette. This revealed potential photosynthesis in the absence of photoinhibition, water stress, or the TPU limitation that might develop over a diurnal course. Significant enhancement of A and J_{PSII} in the elevated pCO_2 treatment was seen in these excised fascicles regardless of the time of year (Table III). These increases showed that both the lack of enhancement of A and the inhibition of J_{PSII} observed in the same tissues in situ was a temporary property developed on exposure to light during the day, and was not the result of long-term acclimation to elevated pCO_2. Enhancement of J_{PSII} in the excised fascicles again corresponded to a significant enhancement of q_P but not F_v'/F_m' (Table III). In all seasons, light saturation of A occurred at photon flux densities of about 700 μmol m^{-2} s^{-1} (data not shown). Regression of ϕ_{PSII} against ϕ_{CO2} showed no significant effect of elevated pCO_2 on the ratio of the rates of whole-chain electron transport through PSII to CO_2 assimilation in the absence of photorespiration (Fig. 4). There was no significant effect of elevated pCO_2 on either the slope or the intercept of this relationship in November 1997 (Fig. 4), April 1997 (data not shown), or February 1998 (data not shown). On no occasion was the intercept significantly greater than zero.

DISCUSSION

Our results agree with our initial hypothesis that the effects of elevated pCO_2 on photochemistry will differ in a predictable manner with the time of year. In August and April, periods of significant growth for these trees, J_{PSII} was increased at elevated pCO_2 when light was saturating (Fig. 1, l and m). Conversely, in February of both years, elevated pCO_2 depressed J_{PSII} at light saturation (Fig. 1, k and o). These results agree with the theoretical prediction that the amount of active Rubisco will limit light-saturated photo-

Table III. *Light-saturated photosynthetic characteristics*

Mean (SE) A_{sat}, J_{PSII}, ϕ_{PSII}, q_P, and F_v'/F_m' at a PPFD of 1,600 μmol m^{-2} s^{-1} for April, August and November 1997 and February 1998 of the three replicate rings for each pCO_2 treatments. Fascicles were excised under water around dawn and maintained in low light until measured in a controlled-environment gas exchange cuvette. Measurements were made at a pCO_2 of 36 Pa for the controls and at 55 Pa for the elevated CO_2 grown fascicles. The effects of pCO_2 ($F_{1,16}$), time of year ($F_{3,16}$), and their interaction ($F_{3,16}$) were tested for significance using two way ANOVA. *** denotes $P < 0.001$; * denotes $P < 0.05$. A_{sat} and J_{PSII}, are expressed in μmol m^{-2} s^{-1}. ϕ_{PSII}, q_P, and F_v'/F_m' are dimensionless.

Parameter	April		August		November		February		pCO_2	Time	Interaction
	36 Pa	55 Pa	36 Pa	55 Pa	36 Pa	55 Pa	36 Pa	55 Pa			
A_{sat}	4.94	7.52	4.8	8.97	4.18	7.21	3.61	5.66	**57.0**	**5.8**	0.3
	(0.2)	(0.6)	0.6	0.5	(0.5)	(0.9)	(0.24)	(0.6)	***	*	
J_{PSII}	68.9	126	70	109	36.1	63.1	25.4	59.1	**32.1**	**16.1**	0.5
	(1.5)	(14.8)	6.5	5.7	(9.6)	(14.8)	(4.8)	(12.1)	***	***	
ϕ_{PSII}	0.12	0.21	0.10	0.16	0.06	0.10	0.04	0.09	**27.5**	**46.2**	0.9
	(0.01)	(0.02)	0.01	0.01	(0.01)	(0.02)	(0.01)	(0.02)	***	***	
q_P	0.26	0.42	0.21	0.34	0.14	0.24	0.12	0.25	**21.6**	**20.4**	0.9
	(0.03)	(0.04)	0.01	0.04	(0.04)	(0.05)	(0.03)	(0.05)	***	***	
F_v'/F_m'	0.44	0.51	0.48	0.49	0.40	0.41	0.34	0.37	4.9	**97.2**	0.5
	(0.01)	(0.02)	0.01	0.04	(0.01)	(0.01)	(0.01)	(0.01)		***	

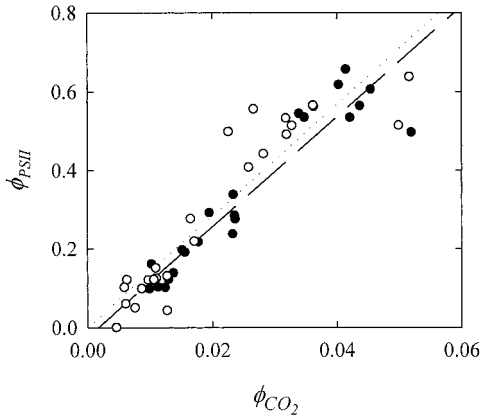

Figure 4. Relationship of ϕ_{PSII} to ϕ_{CO2} determined simultaneously on fascicles from each elevated and each control pCO_2 ring in November 1997. The line indicates the least-square best fit to the data for each pCO_2 treatment. White symbols and the dashed line are for trees growing at current ambient pCO_2 and black symbols and the solid line are for trees growing at elevated pCO_2. The intercept of each regression was not significantly different from zero ($F_{1,46} = 1.7$; $P < 0.05$), and no significant effect of pCO_2 was found on the relationship between ϕ_{PSII} and ϕ_{CO2} ($F_{1,46} = 0.1$; $P < 0.05$).

synthesis during the major periods of photosynthate demand and that TPU will limit it during the winter, when growth has ceased and translocation may be limited by the low mean temperature. In February of both years the daily minima were at or below 0°C and were therefore likely to restrict translocation. The suggestion that the amount of active Rubisco is limiting during August and April is consistent with the loss of the pCO_2-dependent increase in J_{PSII}, as light decreases over the diurnal course (Fig. 1, g and h).

As PPFD drops below the saturating level, a transition from limitation of Rubisco to RubP regeneration would be expected, eliminating any effect on J_{PSII}. Responses of A to c_i determined for these needles also indicated that in the absence of TPU limitation the amount of active Rubisco rather than the capacity for RubP regeneration is the major limitation to light-saturated photosynthesis (data not shown) (Myers et al., 1999). Fascicles excised under water around dawn during February and transferred to an illuminated, controlled-environment cuvette showed stimulation of both A and J_{PSII} in elevated pCO_2 (Table III). This suggests that the fascicles grown in elevated pCO_2 had the capacity to respond to elevated pCO_2 with increased A and J_{PSII}, but that this was not realized in the in situ diurnal measurements. One explanation of this result would be that sugar phosphates could accumulate more rapidly in the early part of the day, leading to TPU limitation in the elevated pCO_2 treatment. This explanation is consistent with the progressive decline in the ratio of J_{PSII} at elevated to ambient pCO_2 observed in February 1997 (Fig. 1k). In the absence of photosynthetic acclimation and within the context of the three potential limitations to light-saturated photosynthesis of the model of Farquhar et al. (1980) as modified by Sharkey (1985), these changes could only be explained by TPU limitation.

An acclimatory loss of Rubisco or capacity for RubP regeneration would also cause a decrease in J_{PSII}. However, parallel studies of the responses of CO_2 uptake to intercellular CO_2 concentration (A/C_i) gave no evidence in vivo of any loss of Rubisco activity or capacity for RubP regeneration in any season (Ellsworth, 1999; Myers et al., 1999). Growth at elevated pCO_2 could also affect J_{PSII} if it decreases alternative sinks for electrons, in particular Mehler reactions or photosynthetic nitrogen metabolism. For example, Polle et al. (1993, 1997) showed a decrease in the activity of enzymes associated with the metabolism of active oxygen species under elevated pCO_2. A significant alternative sink would be apparent as a change in the relationship between the efficiencies of electron transport and CO_2 uptake. However, there was no effect of pCO_2 on this relationship when measured in the absence of photorespiration (Fig. 4), as has been observed previously (Epron et al., 1994; Habash et al., 1995; Bartak et al., 1996).

Although F_v'/F_m' declined with increasing photon flux, it was unaffected by elevated pCO_2 despite significant effects on J_{PSII}. Increased J_{PSII} in the summer and decreased J_{PSII} in the winter were paralleled by changes in q_P (Fig. 2). This shows that variations in electron flux due to growth in elevated pCO_2 at different times of the year result from variations in the proportion of open PSII reaction centers rather than from any effect on the efficiency with which absorbed quanta are transferred to the reaction center. This is consistent with altered rates of electron transport at light saturation resulting from variations in the capacity of processes downstream of PSII to accept electrons. These results also suggest that F_v'/F_m' is not simply driven by electron flux, since significant changes in J_{PSII} caused by elevated pCO_2 do not appear to affect the efficiency of energy transfer to the reaction center. Habash et al. (1995) found that increased electron transport in young wheat plants growing at elevated pCO_2 in a controlled environment corresponded to increased q_P without an effect on F_v'/F_m'.

Photoinhibition has been defined as a reversible decrease in the efficiency of excitation energy transfer to PSII reaction centers. This serves to protect the reaction centers from photoinactivation and damage when the rate of excitation of PSII is in excess of the rate at which the reaction centers can use excitation energy for photochemistry (Osmond, 1994). The cost of this protection is that when a leaf is in low light after photoinhibition, the efficiency of photosynthesis remains low for many minutes, and sometimes hours, with the loss of potential carbon fixation (Long et al., 1994). We anticipated that a decrease in PSII photochemistry in the winter due to elevated pCO_2 would increase photoinhibition and decrease the maximum potential for carbon fixation. Elevated pCO_2 produced significant reductions in F_v/F_m in February of both years, however, no effect was observed on F_v'/F_m'. This was assumed to result from an increase in light-induced quenching processes being considerably greater than the quenching remaining in the dark-adapted fascicles used for the F_v/F_m measurements (Fig. 2). Since there were no significant effects of elevated pCO_2 on F_o, the reduced F_v/F_m was almost certainly associated with

zeaxanthin quenching. A slow recovery of F_v/F_m (requiring more than 10 h) in maize leaves growing at chilling temperature was shown previously to be associated with the conversion of zeaxanthin to violaxanthin (Fryer et al., 1995). Therefore, the slower recovery of F_v/F_m in plants grown at elevated pCO_2 is indicative of the imposition of an additional stress during the winter, which is not experienced by the control plants. Other evidence that overwintering leaves may be subjected to increased stress at elevated pCO_2 is provided by Lutze et al. (1998) who showed increased frost damage to *Eucalyptus pauciflora* seedling leaves at elevated pCO_2.

In conclusion, this study has shown that for mature trees, elevated pCO_2 can cause both decreases and increases in the use of absorbed light energy in photochemistry, which is consistent with seasonal changes in limitations on photosynthetic carbon metabolism. In the summer, elevated pCO_2 results in significantly more of the absorbed light being used in photochemistry and a decreased potential for photoinhibition, although no significant effect of pCO_2 on photoinhibition was observed. Utilization of absorbed energy within the photosynthetic apparatus appears to be strongly inhibited under elevated pCO_2 during the winter period, and this correlates with a slower recovery from photoinhibition compared with ambient trees. These results suggest that elevated pCO_2 may add a further stress to overwintering evergreen vegetation in temperate regions.

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