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Elevated CO_2 concentration affects leaf photosynthesis–nitrogen relationships in *Pinus taeda* over nine years in FACE

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Summary To investigate whether long-term elevated carbon dioxide concentration ([CO_2]) causes declines in photosynthetic enhancement and leaf nitrogen (N) owing to limited soil fertility, we measured photosynthesis, carboxylation capacity and area-based leaf nitrogen concentration (N_a) in *Pinus taeda* L. growing in a long-term free-air CO_2 enrichment (FACE) facility at an N-limited site. We also determined how maximum rates of carboxylation (V_{cmax}) and electron transport (J_{max}) varied with N_a under elevated [CO_2]. In trees exposed to elevated [CO_2] for 5 to 9 years, the slope of the relationship between leaf photosynthetic capacity ($A_{net\text{-}Ca}$) and N_a was significantly reduced by 37% in 1-year-old needles, whereas it was unaffected in current-year needles. The slope of the relationships of both V_{cmax} and J_{max} with N_a decreased in 1-year-old needles after up to 9 years of growth in elevated [CO_2], which was accompanied by a 15% reduction in N allocation to the carboxylating enzyme. Nitrogen fertilization (110 kg N ha^{-1}) in the ninth year of exposure to elevated [CO_2] restored the slopes of the relationships of V_{cmax} and J_{max} with N_a to those of control trees (i.e., in ambient [CO_2]). The J_{max}:V_{cmax} ratio was unaffected by either [CO_2] or N fertilization. Changes in the apparent allocation of N to photosynthetic components may be an important adjustment in pines exposed to elevated [CO_2] on low-fertility sites. We conclude that fundamental relationships between photosynthesis or its component processes with N_a may be altered in aging pine needles after more than 5 years of exposure to elevated atmospheric [CO_2].

Keywords: carboxylation, down-regulation.

Introduction

Forests store large amounts of carbon derived from atmospheric carbon dioxide (CO_2), and thus play a central role in determining the atmospheric CO_2 concentration ([CO_2]) (Dixon et al. 1994, Barford et al. 2001). Currently, anthropogenic carbon emissions are causing atmospheric [CO_2] to rise by about 2 μmol mol^{-1} year^{-1} (Keeling and Whorf 2005), which raises the question of how this change in atmospheric [CO_2] affects the carbon storage capacity of forests.

Numerous studies have investigated the photosynthetic responses of various coniferous species to elevated atmospheric [CO_2] over the past decade (e.g., Wang et al. 1996, Jach and Ceulemans 2000, Ellsworth et al. 2004, Handa et al. 2005), and many reviews have addressed the responses of trees to elevated [CO_2] (e.g., Curtis and Wang 1998, Nowak et al. 2004, Ainsworth and Long 2005). However, the future sustainability of coniferous forest sinks for atmospheric CO_2 remains uncertain (White et al. 2000, Oren et al. 2001). To investigate this question, experimental exposures of trees to elevated [CO_2] have been udertaken at the single branch (Teskey 1997), whole tree (Maier et al. 2002), stand and ecosystem (Crous and Ellsworth 2004, Körner et al. 2005, Liberloo et al. 2007) levels, over periods from a year to a decade. Here we report effects of long-term elevated [CO_2] exposure on photosynthetic capacity of *Pinus taeda* L. in the longest running forest free-air CO_2 enrichment (FACE) experiment to date (Hendrey et al. 1999, Oren et al. 2001).

The early enhancement of photosynthesis by elevated atmospheric [CO_2] may not be sustained over time (Sage 1994, Poorter 1998, Oren et al. 2001, Poorter and Pérez-Soba 2001, Rogers and Ellsworth 2002, Norby and Iversen 2006). The lack of photosynthetic enhancement in elevated [CO_2] is especially apparent in ecosystems on low-nutrient sites (Oren et al. 2001, Norby et al. 2005, Reich et al. 2006*a*) and is strongly related to the availability and root exploitation of limiting nutrients (Zak et al. 2000, Oren et al. 2001, Finzi et al. 2002, Luo et al. 2004). The addition of nitrogen (N) to an N-limited ecosystem can, therefore, be expected to lengthen the long-term photosynthetic enhancement and growth in elevated [CO_2] (Oren et al. 2001, Finzi et al. 2006).

To investigate the long-term photosynthetic response of a coniferous species to elevated [CO_2] and its dependence on N availability, we studied *P. taeda* trees exposed to elevated [CO_2] for up to nine growing seasons in the Duke Forest FACE facility. In contrast to earlier work (Crous and Ellsworth 2004), here we focus on how photosynthetic metabolism as a function of leaf N is affected by long-term exposure to elevated atmospheric [CO_2] in combination with fertilization. Because photosynthesis serves as the first major coupling point between canopy carbon and nitrogen cycles, understanding the effects of long-term exposure to elevated [CO_2] on the photosynthesis–N relationship is critical (Peterson et al. 1999, Reich et al. 2006*b*). Our objectives were to: (1) quantify functional relationships between photosynthetic capacity and leaf N in ambient and elevated [CO_2]; (2) examine if and how the photosynthesis–N relationship of 1-year-old needles of *P. taeda* trees is affected by long-term exposure to elevated [CO_2] on a low-nutrient site; and (3) understand how increased N availability affects the photosynthetic response to long-term elevated [CO_2] in 1-year-old needles.

Materials and methods

Site description

Measurements were made at the Duke Forest FACE facility (35°58′36″ N, 70°05′36″ W, 174 m a.s.l., on the North Carolina Piedmont Plateau), which has been described in detail elsewhere (Ellsworth 1999, Hendrey et al. 1999, Schäfer et al. 2003). Briefly, the growing season in the vicinity of Duke Forest is from early March to mid-October with a mean annual temperature of 15.5 °C and a mean annual precipitation of 1154 mm. Loblolly pine (*P. taeda*) forests generally occur on acidic, nutrient-poor soils that are considered to be N-limited (Oren et al. 2001). Since August 1996, planted pine trees have been exposed to elevated [CO_2] by the FACE technique (Hendrey et al. 1999). The Duke forest FACE experiment consists of six large-diameter plots, with three replicates at ambient [CO_2] and three replicates at an elevated target [CO_2] of ambient + 200 μmol CO_2 mol^{-1}. Daytime exposure to elevated atmospheric [CO_2] was nearly continuous throughout the year except when temperatures were below 5 °C or windspeeds were greater than 6 m s^{-1}, which together represented less than 5% of the possible running time. In each plot, canopy access was gained by platform lifts (UL48, Upright, Charlotte, NC) or a walk-up tower in the center of each plot.

To examine potential interactions of plant responses to both enhanced [CO_2] and N supply, plots were divided in half by a 2-m deep root barrier and half of each plot was fertilized with ammonium nitrate (NH_4NO_3) in March 2005. The rate of fertilization was 110 kg N ha^{-1} $year^{-1}$ compared with the ambient N mineralization rates at the site of about 30 kg N ha^{-1} $year^{-1}$ (Matamala and Schlesinger 2000, Finzi et al. 2002) and a background N deposition of about 6.5 kg N ha^{-1} $year^{-1}$ (Oren et al. 2001). Data from the fertilization treatment were analyzed only in the first year of N fertilization.

Photosynthesis measurements

To quantify photosynthetic performance in ambient and elevated [CO_2], net CO_2 assimilation rates (A_{net}) of pine needles in each treatment were measured at different atmospheric [CO_2] with an LI-6400 (Li-Cor, Lincoln, NE) portable photosynthesis system. Measurements were made on leaves at the top of the canopy (upper locations; topmost 10% of total tree height) and the lowest living branch of the canopy (lower locations) as described by Crous and Ellsworth (2004). One-year-old needles were measured in both early and late summer, whereas current-year needles were only sufficiently developed to measure in late summer. Within each treatment, a single candidate tree was chosen for measurements. Leaves were carefully positioned in the leaf chamber where they were exposed to a saturating quantum flux of 1800 μmol m^{-2} s^{-1}, similar to full sunlight at the FACE site. Leaf temperatures were kept at 28 °C in early summer and 30 °C in late summer, reflecting ambient temperatures. Measurements were made from the second (1998) to the ninth (2005) growing season of experimental exposure to elevated [CO_2].

At least seven CO_2 concentrations were used in the stepwise photosynthetic CO_2 response curve, including the ambient and elevated [CO_2] that were the control and treatment target concentrations in FACE, respectively. Hence, A_{net} of all needles was measured at a common [CO_2] of 365 μmol mol^{-1}, which we refer to as $A_{net\text{-}Ca}$. The A_{net} of trees grown in ambient or elevated [CO_2] was analyzed by comparing A_{net} measured at the [CO_2] corresponding to the treatment target [CO_2]. Thus, treatment comparisons of A_{net} represent the overall response to increased atmospheric [CO_2], whereas $A_{net\text{-}Ca}$ represents photosynthetic capacity at a common [CO_2] for trees grown in ambient and elevated [CO_2]. Photosynthetic relationships are reported on a needle surface area basis (all-sided).

After measuring each [CO_2] response curve, needles were excised and stored at 0 °C for later measurement of surface area and determination of dry mass as described by Crous and Ellsworth (2004). Homogenized subsamples of dried and ground needle tissue were analyzed for N concentration on an area basis (N_a) with an NA-1500 elemental analyzer (Carlo-Erba, Milan, Italy).

Statistical analyses

To assess the response of pines to long-term elevated [CO_2] exposure and potentially elucidate the role of N in this response, we focused our analysis on the relationship between leaf photosynthesis and N_a. This relationship is hypothesized to be general (Field and Mooney 1986, Reich et al. 1997) and any changes in it identified as differences in the slopes of the regressions, could be considered robust and, hence, useful in modeling photosynthesis on the basis of N_a (Ollinger et al. 2002).

Fitting the model of Farquhar et al. (1980) to the photosynthetic CO_2 response curve data as described by Ellsworth et al. (2004) was expected to provide insight into the specific photosynthetic components (e.g., maximum rates of carboxylation (V_{cmax}) and electron transport (J_{max})) that responded to long-term exposure to elevated [CO_2] (Rogers and Ellsworth 2002). To minimize artifacts of the fitting procedure for V_{cmax} and J_{max} in the data, we removed data likely associated with a leaky chamber (Pons and Welschen 2002; e.g., day respiration > 1.5 mmol m^{-2} s^{-1}), low stomatal conductance (< 3 mmol m^{-2} s^{-1} during measurements) or failure to meet nutrient analysis QA/QC standards. Less than 10% of the dataset failed these criteria.

The fraction of N allocated to active-state Rubisco (fN_{Rub}) was estimated as described by Niinemets and Tenhunen (1997), assuming that all activated Rubisco participates in carboxylation and is an estimate of the N-use efficiency for carboxylation in the absence of mesophyll diffusional limitations.

Linear regressions were conducted to determine V_{cmax}, J_{max}, A_{net} and N_a relationships with [CO_2], needle age and duration of exposure to elevated [CO_2].

To test the effect of the duration of elevated [CO_2] treatment on photosynthetic parameters in 1-year-old needles, the dataset was divided into three periods: an early period (Years 2 and 3 of elevated [CO_2] treatment); a middle period (Years 5–7); and a late period (Years 8 and 9). The results were insensitive to variation in these year groupings by inclusion or removal of a year as long as the sample size was not unduly restricted. Differences in slope between [CO_2] or fertilization treatments were tested by a dummy variable representing the interaction term between the independent variable (N_a) and [CO_2] in the regression analyses.

Results

After almost 10 years of exposure to elevated [CO_2], A_{net} of trees in elevated [CO_2] remained above that of control trees in both current-year (Figure 1a) and 1-year-old (Figure 1b) needles across a twofold range in N_a. Photosynthetic enhancement in elevated [CO_2] was characterized by an increase in the intercept of the A_{net} versus N_a relationship for both current-year and 1-year-old needles, and a significant increase in the slope of the relationship for current-year needles (Figures 1a and 1b). In ambient [CO_2], the A_{net} versus N_a relationships of current-year and 1-year-old needles had similar slopes and intercepts ($P > 0.1$). In contrast, in elevated [CO_2], 1-year-old needles had significantly lower slopes of A_{net} as a function of N_a than current-year needles ($P = 0.003$). To illustrate the magnitude of this [CO_2] effect, at a standardized mean N_a of 0.9 g m^{-2}, enhancement averaged +68 ± 6% (mean ± 95% confidence interval) for current-year needles and only 40 ± 3% for 1-year-old needles.

The effect of elevated [CO_2] on A_{net} (Figures 1a and 1b) is the result of a direct stimulation of photosynthesis by CO_2 combined with offsetting reductions in photosynthetic capacity from enzyme down-regulation. In turn, changes in photosynthetic capacity could be caused by changes in N_a or in capacity per N_a, or both. Changes in capacity can be estimated from changes in photosynthetic capacity of leaves developed in elevated [CO_2] versus ambient [CO_2] but measured at a common ambient [CO_2] of 365 µmol mol^{-1} ($A_{net\text{-}Ca}$). Significantly lower intercepts of $A_{net\text{-}Ca}$ as a function of N_a indicated that growth in elevated [CO_2] decreased photosynthetic capacity in 1-year-old foliage (Figures 1c and 1d). In addition, the nearly significant difference in slopes ($P = 0.058$) for 1-year-old foliage suggests that reductions in $A_{net\text{-}Ca}$ in response to elevated [CO_2] are greater at higher N_a. Leaf N concentration did not vary with CO_2 treatment for any of the periods examined (Table 1; $P > 0.10$). In combination, these results indicate that elevated [CO_2] decreased photosynthetic capacity by decreasing photosynthesis per unit N_a rather than by decreasing N_a.

Lower photosynthetic capacity in elevated [CO_2] might have resulted from lower electron transport capacity or lower

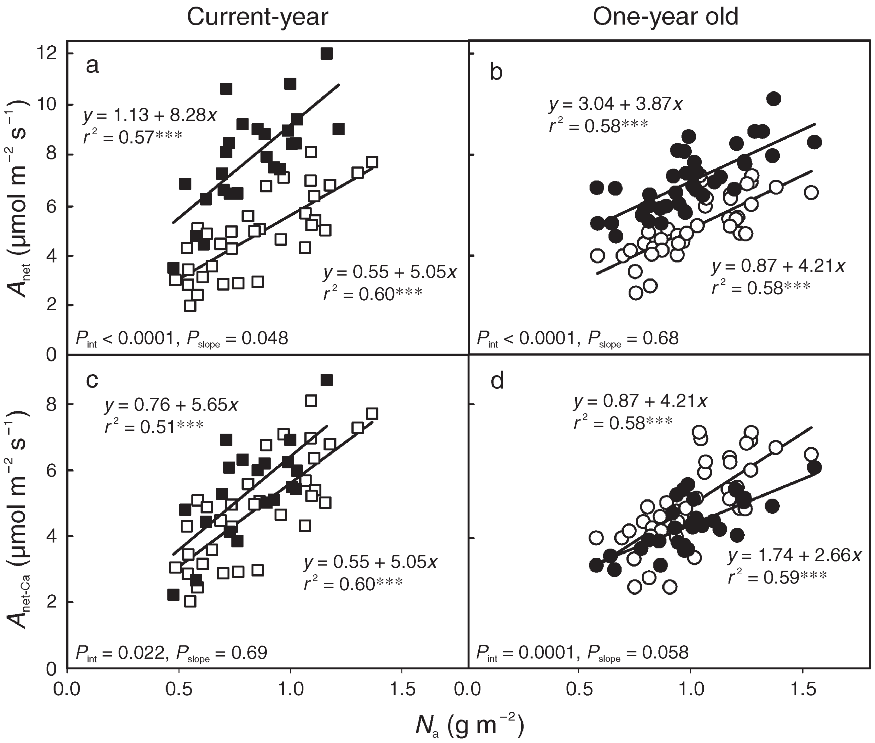

Figure 1. Relationships of net photosynthesis (a, b) in current growth conditions (A_{net}) and (c, d) at a common CO_2 concentration (365 µmol mol^{-1}; $A_{net\text{-}Ca}$) with leaf nitrogen concentration on an area basis (N_a) for current-year (□, ■) and 1-year-old (○, ●) foliage of *Pinus taeda* trees grown in ambient (open symbols) and elevated (closed symbols) CO_2 concentrations across Years 5 to 9 of the experiment without fertilization. Shown are linear regression equations, all of which are significant at $P < 0.001$ (***), and the significance of the effect of CO_2 treatment on the intercept (P_{int}) and slope (P_{slope}).

Table 1. Analysis of variance of the effects of needle age class (current-year versus 1-year-old needles), CO_2 concentration (ambient versus ambient + 200 μmol mol^{-1}) and time period (Years 2 and 3 versus Years 5–7 versus Years 8 and 9) on the predicted fraction of nitrogen allocated to Rubisco (fN_{Rub}) and total leaf nitrogen on an area basis (N_a) for upper-canopy *Pinus taeda* foliage at the Duke FACE site. Abbreviations: MSE is mean square error; df is degrees of freedom; and ns denotes non-significant variables ($P > 0.1$).

Source	df	fN_{Rub}		N_a	
		MSE	P	MSE	P
Age class	1	0.0236	< 0.0001	0.32077	0.0036
CO_2	1	0.00044	ns	0.00280	ns
Period	2	0.0041	0.0332	0.06586	ns
Age class × CO_2	1	0.0052	0.0036	0.00733	ns
Age class × Period	2	0.00079	ns	0.03452	ns
CO_2 × Period	2	0.0007	ns	0.06456	ns
Age × CO_2 × Period	2	0.0011	0.0866	0.04658	ns
Residual error	83	0.00032	–	0.03578	–

capacity for enzymatic CO_2 fixation, or both. Both V_{cmax} (a measure of Rubisco activity for CO_2 fixation) and J_{max} were generally strongly correlated with N_a in 1-year-old needles ($P < 0.001$; Figure 2). The exception was that neither V_{cmax} nor J_{max} was related to N_a early in the experiment (Years 2 and 3; Figures 2a and 2d) when variation in canopy N_a was small. Trees grown in ambient [CO_2] showed no significant variation in V_{cmax}–N_a and J_{max}–N_a relationships over time (Figure 2) and no significant time-dependent change in slopes, based on pairwise comparisons of slopes across time periods (Figure 3 and data not shown). Growth in elevated [CO_2] affected the V_{cmax}–N_a and J_{max}–N_a relationships. In general, compared with needles grown in ambient [CO_2], needles grown in elevated [CO_2] tended toward lower V_{cmax} and J_{max} at a given N_a. Such differences were greater at higher N_a values (cf. slope term) and with longer duration of CO_2 exposure (Figures 2 and 3). In Years 8 and 9, this difference resulted in a 64% reduction in the V_{cmax}–N_a slope in elevated [CO_2] versus ambient [CO_2] and a 52% reduction in the J_{max}–N_a slope (Figure 3).

To further examine N allocation to carboxylation capacity, we calculated fN_{Rub} from V_{cmax} and N_a (Ellsworth et al. 2004). Apparent fN_{Rub} differed significantly between needle age classes ($P < 0.0001$, Table 1); however, there was also a significant needle age class × [CO_2] interaction ($P = 0.0036$). Moreover, a weak 3-way interaction of needle age class × [CO_2] × Period ($P < 0.087$, Table 1) indicated lower fN_{Rub} in 1-year-old needles of trees in Years 5–9 of the elevated [CO_2] treatment, consistent with the decrease in the V_{cmax}–N_a slope observed in the later years of the experiment (Figures 2c and 3a). The apparent fN_{Rub} in 1-year-old foliage in the upper crown of mature *P. taeda* trees exposed to 8–9 years of elevated [CO_2] in FACE declined 15%, from 10.3 ± 0.6% (mean ± SE) to 8.7 ± 0.5%.

For all three measures of photosynthetic and biochemical capacity in 1-year-old needles (i.e., $A_{net\text{-}Ca}$, V_{cmax} and J_{max}), the decrease in the slopes of their relationships with N_a in response to long-term exposure to elevated [CO_2] (Figures 1 and 2) was completely reversed by N fertilization (Figure 4). Relationships in needles from fertilized trees in the ambient and elevated [CO_2] treatments had similar slopes ($P > 0.1$, Figure 4 and data not shown). Slopes of both the V_{cmax}–N_a and J_{max}–N_a relationships in 1-year-old needles grown in elevated [CO_2] with N fertilization recovered to values similar to those measured in ambient [CO_2] (Figure 4, green dashed line versus solid blue line).

The J_{max}:V_{cmax} ratio was constant over time and did not differ between the elevated [CO_2] and elevated [CO_2] + N fertilization treatments. There was no difference in the slopes of the J_{max}–V_{cmax} relationships in 1-year-old (Figure 5) or current-year (data not shown) needles after 9 years of exposure to elevated [CO_2]. No effect of N fertilization on the J_{max}:V_{cmax} ratio was observed (Figure 5).

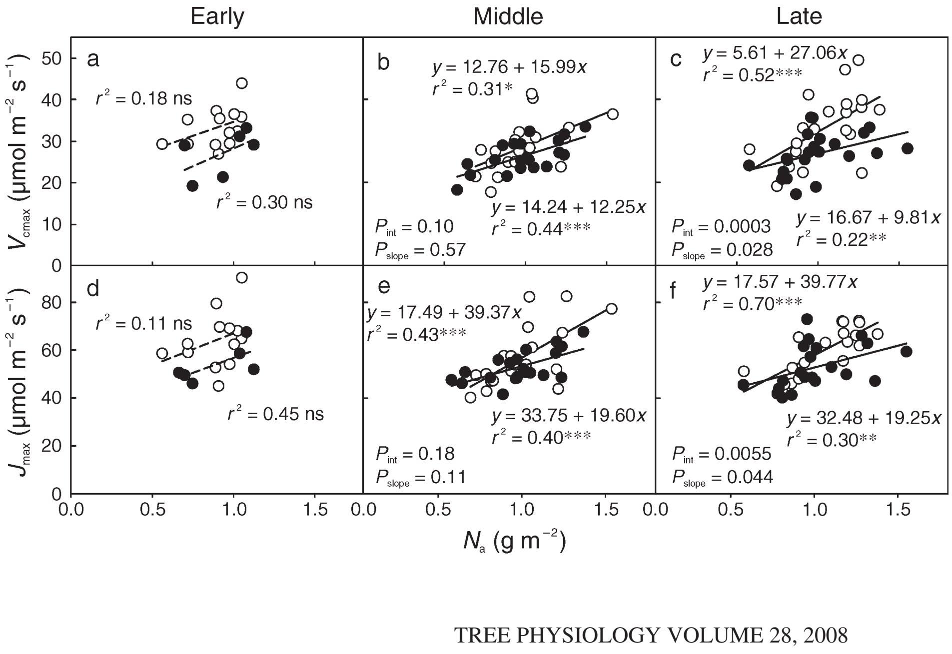

Figure 2. Relationships in 1-year-old needles of maximum rates of (a–c) carboxylation (V_{cmax}) and (d–f) electron transport (J_{max}) as functions of leaf nitrogen on an area basis (N_a) in *Pinus taeda* trees grown in ambient (○) and elevated (●) CO_2 concentrations in early (Years 2 and 3 of treatment), middle (Years 5–7) and late (Years 8 and 9) time periods of the experiment without fertilization. Shown are linear regression equations, with their significance (*** = $P < 0.001$; ** = $P < 0.05$; and ns = not significant), and the significance of the effect of CO_2 treatment on the intercept (P_{int}) and slope (P_{slope}).

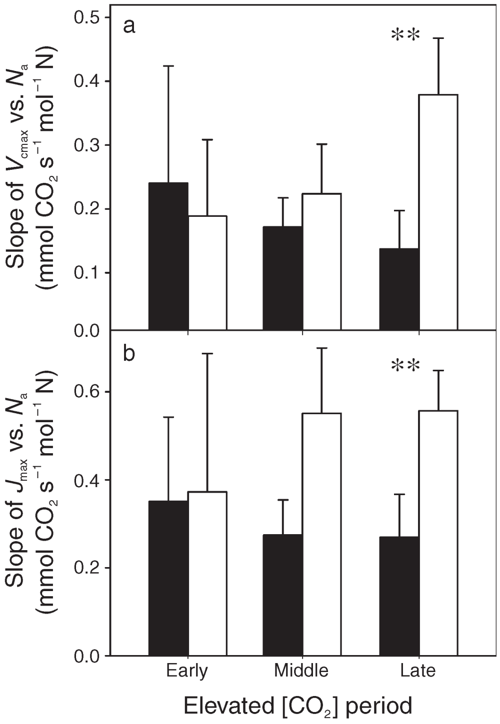

Figure 3. Slope values with estimated standard errors for the relationships of maximum rates of (a) carboxylation (V_{cmax}) and (b) electron transport (J_{max}) with leaf nitrogen on an area basis (N_a) for *Pinus taeda* trees grown in ambient (open bars) and elevated (closed bars) CO$_2$ concentrations ([CO$_2$]) in early (Years 2 and 3 of treatment), middle (Years 5–7) and late (Years 8 and 9) time periods of the experiment without fertilization. Significant differences ($P < 0.05$) between [CO$_2$] treatments are indicated by asterisks (**).

Discussion

We found evidence of photosynthetic down-regulation in 1-year-old foliage of *P. taeda* trees exposed to elevated [CO$_2$] for 9 years in FACE (Figure 1). These findings support previous results from the Duke FACE experiment (Rogers and Ellsworth 2002, Crous and Ellsworth 2004) and are consistent with results for other conifers exposed to elevated [CO$_2$] (Turnbull et al. 1998, Tissue et al. 1999, Jach and Ceulemans 2000). The observed down-regulation in 1-year-old needles was not caused by changes in N_a, because only the steepness of the slopes of the V_{cmax}–N_a and J_{max}–N_a relationships was affected by elevated [CO$_2$] (Figure 2). The relationship between $A_{net\text{-}Ca}$ and N_a, another measure of photosynthetic capacity, was similarly affected (Figure 1d). The decrease in the slope of the V_{cmax}–N_a relationship in 1-year-old needles in response to elevated [CO$_2$] became more pronounced over time (23 to 64% reduction in slope) resulting in a statistically significant CO$_2$ effect on 1-year-old needles late in the experiment (Figures 2 and 3). These changes indicate significant down-regulation of V_{cmax} over time (Figure 3), which was mirrored by similar down-regulation in J_{max}, resulting in a decreased photosynthetic enhancement by 37% in 1-year-old needles in the elevated [CO$_2$] treatment.

Given the magnitude of the positive x-intercepts of the V_{cmax}–N_a and J_{max}–N_a relationships in 1-year-old needles (Figure 2), the net effect of the relatively large CO$_2$-induced changes in slopes resulted in a smaller proportional decrease in photosynthetic capacity at a common [CO$_2$] (i.e., about –14%; Figure 1d). Because the effects of elevated [CO$_2$] were observed in 1-year-old needles but not in current-year needles

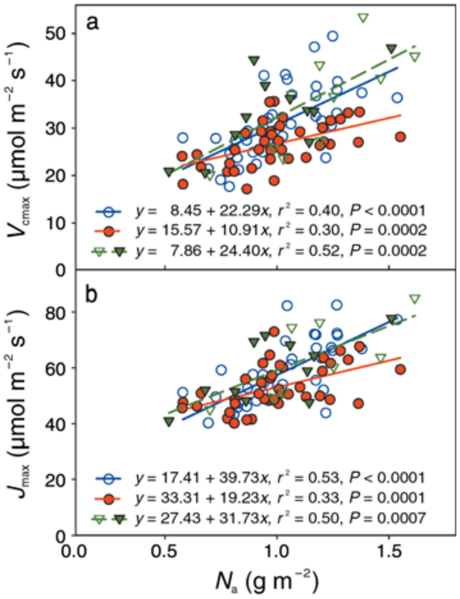

Figure 4. Maximum rates of (a) carboxylation (V_{cmax}) and (b) electron transport (J_{max}) as functions of leaf nitrogen on an area basis (N_a) in 1-year-old foliage of nitrogen-fertilized (▽,▼) and unfertilized (○,●) *Pinus taeda* trees in ambient (open symbols) and elevated (closed symbols) CO$_2$ concentrations ([CO$_2$]) during Years 5–9 of the experiment. Trees were fertilized only in Year 9. Linear regressions are shown for each treatment combination. Relationships in fertilized trees were similar for both [CO$_2$] treatments, so the data were pooled for regression.

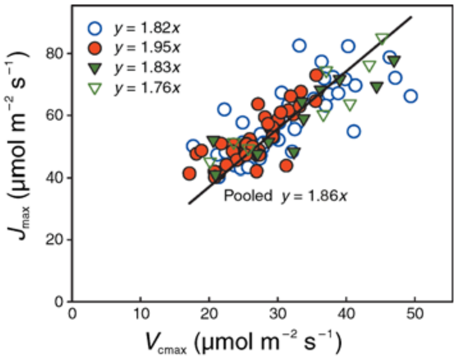

Figure 5. Relationship between maximum rates of electron transport (J_{max}) and carboxylation (V_{cmax}) in 1-year-old foliage of nitrogen-fertilized (▽,▼) and unfertilized (○,●) *Pinus taeda* trees grown in ambient (open symbols) and elevated (closed symbols) CO$_2$ concentrations during Years 5–9 of the experiment. Trees were fertilized only in Year 9. Linear regression equations are listed for each treatment combination, and the regression for all pooled data is shown. All regressions were significant at $P < 0.0001$.

measured at the same time, it appears that needle-age-related declines in photosynthetic capacity are enhanced by long-term exposure to elevated [CO_2] (Jach and Ceulemans 2000, Rogers and Ellsworth 2002, Crous and Ellsworth 2004) rather than by declines in N_a.

The change in form of the V_{cmax}–N_a relationship in response to elevated [CO_2] likely represents a reduction in N allocated to Rubisco (Table 1). This reduction is compensated for by the well-documented stimulation of carboxylation rates by elevated [CO_2] and suppression of photorespiration (Long et al. 2004, Ainsworth and Rogers 2007). Therefore the overall A_{net} enhancement by elevated [CO_2] of 40% in 1-year-old needles after 8 to 9 years of elevated [CO_2] exposure still results in increased photosynthetic N-use efficiency in elevated [CO_2]. Given that new foliage represents a large N sink for the canopy, the inferred reduction in N allocated to photosynthetic capacity in 1-year-old needles may be a determinant of how much foliage can be supported in the *P. taeda* stand in elevated [CO_2] on these infertile soils (Finzi et al. 2002).

In highly N-limited systems where pine species are often important, N reallocation from 1-year-old to current-year foliage occurs by mobilization of leaf soluble protein N (Fife and Nambiar 1984, Cherbuy et al. 2001). Reallocation of N from old foliage to current-year foliage can provide a mechanism to supply N to growing foliage at branch apices and thereby maximize whole-plant carbon gain (Field 1983, Hirose and Werger 1987). We expected the reduction in photosynthetic capacity in 1-year-old needles in elevated [CO_2] to be alleviated when N availability was increased (Farage et al. 1998). In our study, there was a large response to N fertilization in the first year of application, even in needles that had developed before fertilization. Nitrogen fertilization prevented the CO_2-induced down-regulation of photosynthetic capacity in 1-year-old foliage. This was shown as a recovery of the slopes of both the V_{cmax}–N_a and J_{max}–N_a relationships in the fertilized plus elevated [CO_2] treatment (Figure 4). Given that the reduction in photosynthetic capacity in 1-year-old foliage resulted in a reduction in the apparent fraction of N in Rubisco (Table 1), the fertilization-induced increase in N-use efficiency is achieved by increased N allocation to the photosynthetic apparatus with increased available N (Hikosaka and Hirose 1998, Poorter and Evans 1998, Westbeek et al. 1999). A strong response to N fertilization suggests that there were N limitations to growth via changes in photosynthetic functioning in the long-term elevated [CO_2] treatment, supporting earlier observations of reduced N mineralization in elevated [CO_2] (Finzi et al. 2006).

Our results suggest changes in N allocation in response to environmental conditions, with N allocated away from carboxylation and RUBP regeneration in response to long-term exposure to elevated [CO_2] (Figure 3), whereas, N fertilization induced N investment in photosynthetic components (Figure 4). Therefore, we speculate that N invested in the carboxylation enzyme acts as an indicator of the physiological N demand of plants.

The ratio between J_{max} and V_{cmax} was unaffected by long-term exposure of trees to elevated [CO_2] or by short-term N fertilization. A conservative J_{max}:V_{cmax} ratio (Figure 5) is consistent with findings across many plant species (Medlyn 1996, Leuning 1997, Medlyn et al. 1999, Warren et al. 2003). In contrast, the meta-analysis by Ainsworth and Rogers (2007) showed that V_{cmax} was reduced by about twice that of J_{max} in response to long-term elevated [CO_2]. A constant J_{max}:V_{cmax} ratio with environmental conditions that force adjustments in photosynthetic capacity such as shade (Kull and Niinemets 1998, Hikosaka 2005), low nutrient availability (Ainsworth et al. 2003) and elevated [CO_2] (Medlyn 1996, Midgley et al. 1999, Onoda et al. 2005) implies coordination of the activities of photosynthetic components (Reynolds et al. 1992, Chen et al. 1993, Medlyn 1996). A constant J_{max}:V_{cmax} ratio also suggests no N reallocation between photosynthetic components (Medlyn et al. 1999), which is consistent with our results showing a comparable magnitude of down-regulation in carboxylation and electron transport components manifested by a similar reduction in slopes in response to long-term exposure to elevated [CO_2] (Figures 2c, 2f and 3). Coordination of the different components of the photosynthetic apparatus is maintained via N allocation to avoid an imbalance between limitations by the light-dependent and light-independent portions of photosynthesis (Chen et al. 1993). Insight into N reallocation and partitioning within the photosynthetic apparatus may elucidate the mechanism enabling plants to adapt to changing environmental conditions (Field et al. 1992, Onoda et al. 2004).

In conclusion, after almost a decade of exposure to elevated [CO_2] in FACE, photosynthesis of current-year and 1-year-old needles of *P. taeda* was still stimulated in elevated [CO_2] compared with ambient [CO_2]. However, reductions in photosynthetic capacity in 1-year-old needles in response to elevated [CO_2] were evident in the *P. taeda* trees in FACE. Strong reductions in the slope of the relationship between $A_{net\text{-}Ca}$ and N_a (by 40 ± 3%) were evident in 1-year-old needles of trees exposed to elevated [CO_2] for 5–9 years, whereas no significant reduction was observed in current-year needles. We found evidence of changes in V_{cmax}–N_a and J_{max}–N_a relationships in 1-year-old needles of trees exposed to elevated [CO_2] for 8–9 years, with slopes declining by about 50–60%. Decreasing photosynthetic capacity, evident as reductions in the slopes of V_{cmax} and J_{max} as functions of N_a, may suggest limited N pools for foliage growth at the Duke Face site after nearly a decade of exposure to elevated [CO_2]. Because N fertilization increased the slopes of these relationships in trees exposed to elevated [CO_2] to values similar to those in ambient [CO_2] control trees, we attributed the elevated-[CO_2]-induced reductions in photosynthetic capacity to reductions in the allocation of N to Rubisco and proteins involved in electron transport. Decreases in N allocation to photosynthesis may serve to increase mobile and available N for new foliage growth. Reallocation of N pools among foliage cohorts (e.g., from 1-year-old to current-year foliage) could provide a mechanism for plant adaptations to environmental perturbations such as rising atmospheric [CO_2] or increasing N availability.

Relationships between leaf photosynthetic capacity, V_{cmax} and J_{max} as functions of N_a are widely used in scaling leaf responses to the canopy and for gaining insight into ecosystemscale responses to elevated atmospheric [CO_2] (McMurtrie and Wang 1993, Friend et al. 1997, White et al. 2000). Consideration of dynamic photosynthetic N allocation along with inclusion of canopy N dynamics in pine may improve physiological process models in order to estimate future atmospheric [CO_2] and plant responses to these CO_2 concentrations.

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