Relationships between net photosynthesis and foliar nitrogen concentrations in a loblolly pine forest ecosystem grown in elevated atmospheric carbon dioxide

CLINT J. SPRINGER,[1–3] EVAN H. DeLUCIA[4] and RICHARD B. THOMAS[1]

[1] *Department of Biology, West Virginia University, Morgantown, WV 26506, USA*

[2] *Present address: Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045, USA*

[3] *Corresponding author (cjs@ku.edu)*

[4] *Department of Plant Biology, University of Illinois, Urbana, IL 61801, USA*

Summary   We examined the effects of elevated carbon dioxide concentration ($[CO_2]$) on the relationship between light-saturated net photosynthesis (A_{sat}) and area-based foliar nitrogen (N) concentration (N_a) in the canopy of the Duke Forest FACE experiment. Measurements of A_{sat} and N_a were made on two tree species growing in the forest overstory and four tree species growing in the forest understory, in ambient and elevated $[CO_2]$ FACE rings, during early and late summer of 1999, 2001 and 2002, corresponding to years three, five and six of CO_2 treatment. When measured at the growth $[CO_2]$, net photosynthetic rates of each species examined in the forest overstory and understory were stimulated by elevated $[CO_2]$ at each measurement date. We found no effect of elevated $[CO_2]$ on N_a in any of the species. The slope of the A_{sat}–N relationship was 81% greater in elevated $[CO_2]$ than in ambient $[CO_2]$ when averaged across all sample dates, reflecting a differential CO_2 effect on photosynthesis at the top and bottom of the canopy. We compared A_{sat}–N relationships in trees grown in ambient and elevated $[CO_2]$ at two common CO_2 concentrations, during late summer 2001 and both early and late 2002, to determine if the stimulatory effect of elevated $[CO_2]$ on photosynthesis diminishes over time. At all three sample times, neither the slopes nor the *y*-intercepts of the A_{sat}–N relationships of trees grown in ambient or elevated $[CO_2]$ differed when measured at common CO_2 concentrations, indicating that the responses of photosynthesis to long-term elevated $[CO_2]$ did not differ from the responses to a short-term increase in $[CO_2]$. This finding, together with the observation that N_a was unaffected by growth in elevated $[CO_2]$, indicates that these overstory and understory trees growing at the Duke Forest FACE experiment continue to show a strong stimulation of photosynthesis by elevated $[CO_2]$.

Keywords: elevated CO_2, foliar nitrogen, Free Air Carbon Enrichment, loblolly pine forest, net photosynthesis.

Introduction

As experiments with elevated atmospheric carbon dioxide concentration ($[CO_2]$) increase in complexity, from single plants in pots to intact forest ecosystems using free air carbon enrichment (FACE) technology, the difficulty in determining the photosynthetic responses to elevated $[CO_2]$ also increases. For example, the photosynthetic responses of trees in forest ecosystems exposed to elevated $[CO_2]$ exhibit wide seasonal variation, especially with respect to temperature (Myers et al. 1999) and precipitation (Gunderson et al. 2002). Light gradients through leaf canopies strongly affect leaf physiology, morphology and tissue chemistry. Thus, leaves at the top and bottom of the canopy may respond differently to elevated $[CO_2]$ (Herrick and Thomas 1999, Kubiske et al. 2002). In addition, the relative responses to elevated $[CO_2]$ differ among species and functional types within a forest ecosystem (Curtis and Wang 1998). It is important to incorporate as many of these sources of variation as possible into estimates of the CO_2 enhancement of photosynthesis because approximations of future forest productivity rely on these values.

Another difficulty in predicting the response of photosynthesis to elevated $[CO_2]$ involves the common observation that, in long-term studies, the degree of photosynthetic stimulation by elevated $[CO_2]$ sometimes declines by about 20% over time (Gunderson and Wullschleger 1994, Medlyn et al. 1999). The loss of stimulation is typically characterized by a decline in leaf nitrogen (N) and Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) that leads to a reduction in photosynthetic capacity (Stitt 1991, Long and Drake 1991, Bowes 1993, Sage 1994, Norby et al. 1999). Many forest ecosystems are N-limited (Vitousek and Howarth 1991) and higher growth rates and increased resource demands of plants growing in elevated $[CO_2]$ may exacerbate nutrient limitations (Ingestad and Stoy 1982). Consequently, a reduction in the response of photosynthesis to elevated $[CO_2]$ may be a common occurrence in ecosystems that have a low availability of soil N (Radoglou et al. 1992, Tissue et al. 1993, Sage 1994, El Kohen and Mousseau 1994, Curtis et al. 1995), such as piedmont loblolly pine forests (Piatek and Allen 2000, Richter et al. 2000).

One approach for examining the response of photosynthesis to elevated $[CO_2]$, while accounting for much of the variation in foliar N concentration through the forest canopy, is to use

the relationship between light-saturated photosynthesis and foliar N concentration (A_{sat}–N relationship). This method incorporates the variation caused by the light gradient through a forest canopy and can be used to examine differential species effects or the canopy as a whole. The A_{sat}–N relationship has been shown to be robust across a large number of species from a broad range of ecosystems (Field and Mooney 1986, Evans 1989, Reich et al. 1997) and a recent meta-analysis based on both deciduous and evergreen tree species found elevated [CO_2] increased the slope of this relationship (Peterson et al. 1999). In addition, the A_{sat}–N relationship is indicative of photosynthetic capacity because foliar N concentrations strongly correlate with Rubisco activity and electron transport (Terashima and Evans 1988), including plants grown in ambient and elevated [CO_2] (Medlyn et al. 1999, Herrick and Thomas 2001). Scaling A_{sat} to leaf N concentration has the additional benefit in that process-based models use this fundamental relationship to predict carbon flux in forest ecosystems (Aber and Federer 1992, Aber et al. 1996). The A_{sat}–N relationships of plants grown at different [CO_2] but measured at a common [CO_2] may be used as a diagnostic tool to detect reductions in the CO_2 enhancement of photosynthesis related to changes in foliar N concentration (Curtis et al. 2000) before employing a more time-consuming and labor-intensive examination of photosynthetic down-regulation by means of CO_2 response curves (von Caemmerer and Farquhar 1981, Farquhar and Sharkey 1982, Sharkey 1985). Because foliar N concentrations may be affected by elevated [CO_2] as well as by the light gradient through the forest canopy, the examination of A_{sat}–N relationships is valuable in describing the effects of elevated [CO_2] on photosynthesis because differences in foliar N concentration are accounted for as a covariate.

Loblolly pine (*Pinus taeda* L.) trees growing in the prototype ring of the Duke Forest Free Air Carbon Enrichment (FACE) experiment showed a diminished growth response to elevated [CO_2] after four years of CO_2 treatment and it was concluded that this loss of stimulation by elevated [CO_2] was related to nutrient limitations (Oren et al. 2001). In the replicated Duke Forest FACE experiment, Finzi et al. (2002) found that the demand for N by trees grown in elevated [CO_2] exceeded the rate of N mineralization. These results suggest that we might expect a reduction in the stimulation of photosynthesis by elevated [CO_2] in the replicated Duke Forest FACE experiment after six years of CO_2 treatment. The objectives of this study were to examine the effects of elevated [CO_2] on the A_{sat}–N relationships through the canopy of the loblolly pine forest and to determine if the stimulatory effects of elevated [CO_2] on photosynthesis have been sustained at the Duke Forest FACE experiment. We hypothesized that the slope of the A_{sat}–N relationship would be increased by elevated [CO_2]. This increased slope would indicate a differential stimulation of photosynthetic N-use efficiency (PNUE) through the forest canopy, from areas of deep shade (lower canopy and understory) to areas of full sun (upper canopy), by the tree species grown in elevated [CO_2]. Light-saturated photosynthetic rates (A_{sat}) and foliar N concentrations of two overstory and four understory tree species were measured at their growth CO_2 concentrations during the early summer and late summer of 1999, 2001 and 2002. In 2001 and 2002, A_{sat}–N relationships

were compared at two common CO_2 concentrations to determine if the stimulatory effects of elevated [CO_2] on photosynthesis had declined.

Materials and methods

Duke Forest Free Air Carbon Enrichment (FACE) experiment

The Duke Forest FACE experiment is located in the Blackwood division of Duke Forest (35°97′ N, 79°09′ W), in a loblolly pine plantation planted in 1983 after the forest was clearcut in 1979. The plantation has not been subjected to any management measures to prevent growth of other tree species. Loblolly pine dominates the forest overstory, but there are a significant number of sweetgums (*Liquidambar styraciflua* L.) growing in the overstory and understory. In addition, about 50 other, primarily understory, woody species are present in this forest ecosystem. The forest is growing on nutrient-poor, clay-rich Alfisols of the Enon series that is typical of many upland areas in the southeastern USA.

Six 30-m-diameter experimental rings were established within the forest. Three of these rings are ambient controls and three are replicate elevated [CO_2] treatments. Each FACE ring consists of 32 pipes that extend from the forest floor through the canopy and deliver a controlled amount of CO_2 throughout the elevated rings with a target concentration of ambient + 200 µl l^{-1}. The three ambient control rings are equipped with blowers to deliver the same volume of air to the control rings to replicate any micrometeorological effects of the treatment rings on the forest. From August 1996 through the period of this study (summer 2002), the forest received CO_2 fumigation for 24 h day^{-1}, except on days when the air temperature was below 5 °C for more than 1 h. During the first five years of the experiment (1997–2002), the daytime mean [CO_2] of the elevated rings was 572 µl l^{-1} and of the ambient rings 376 µl l^{-1}. For a detailed description of the Duke Forest FACE experiment see Hendrey et al. (1999).

Gas exchange and leaf chemistry

Three loblolly pine trees and three sweetgum trees growing in the forest overstory along with three trees of each of four species growing in the understory (sweetgum, redbud (*Cercis canadensis* L.), hickory (*Carya glabra* Miller*)* and red maple (*Acer rubrum* L.)) were selected in each FACE ring. Light-saturated net photosynthetic rates were measured at the growth [CO_2] (A_{sat}, 380 or 580 µl l^{-1}) on fully expanded leaves and needles during early summer (June–July) and late summer (August–September) of 1999, 2001 and 2002 with an open-flow infrared gas analyzer equipped with a red-blue light source (LI-6400, Li-Cor, Lincoln, NE). Loblolly pine needles initiated during the previous year were measured in the early summer and current-year needles were measured during the late summer. Photosynthetic measurements were made at 1400 µmol m^{-2} s^{-1} photosynthetic photon flux (PPF) in the overstory and 400 µmol m^{-2} s^{-1} PPF in the understory. These PPFs have previously been found to saturate net photosynthesis of the overstory and the understory species studied (Herrick and Thomas 1999, Myers et al. 1999, DeLucia and Thomas 2000, C.J. Springer, unpublished data). During the late-sum-

mer measurement period of 2001 and both measurement periods of 2002, light-saturated net photosynthetic rates were also measured at the reciprocal growth [CO_2] (380 µl l^{-1} (A_{380}) or 580 µl l^{-1} (A_{580})). Photosynthesis was recorded when leaves had reached steady-state conditions with saturating light. Needles and leaves of overstory loblolly pine and sweetgum trees were measured in the upper (full sun), middle and lower (shade) regions of the canopy. To minimize diurnal effects, measurements were made between 1000 and 1600 h on sunny days. On any given day, trees in both ambient and elevated [CO_2] rings were measured to minimize the effect of day on measurements. Maximum ambient air temperatures during the measurement days ranged from 30 to 34 °C across the 3 years. Leaf temperatures were controlled in the cuvette to 30 °C. During the 1999 and 2001 sample periods, mean soil water content was ~21% (Schafer et al. 2002). However, 2002 was much drier and the mean soil water content during the two sample periods was only ~15% (H. Kim, Duke University, Durham, NC, unpublished data).

After gas exchange measurements were completed, each leaf was harvested and assayed for total N on a mass (N_m) and leaf-area basis (N_a). Leaf tissue for N analysis was dried at 65 °C and measured with a CN autoanalyzer (CE Instruments, Milan, Italy). Leaf mass per unit area (LMA; g m^{-2}) was calculated as the ratio of leaf dry mass to photosynthetic surface area.

Statistical analysis

Morphological and physiological measurements were analyzed for each species separately. The two species growing in the forest overstory (loblolly pine and sweetgum) were tested individually for statistical significance by four-way analysis of variance (ANOVA, α = 0.05) with growth [CO_2], canopy position, season and year as the main effects. For individual species analyses, canopy position included only the upper (full sun) and lower (shade) measurements. Data collected at the mid-canopy position was used only in the calculation of the A_{sat}–N relationships. The morphological and physiological measurements of each species growing in the forest understory (red maple, redbud, sweetgum and hickory) were analyzed by three-way ANOVA (α = 0.05) for each species individually. The main effects in the understory statistical model were [CO_2], season and year.

The A_{sat}–N relationships for each measurement date and a single A_{sat}–N relationship across all measurement dates were calculated based on photosynthesis and area-based foliar N (N_a) from all tree species by least-squares linear regression analysis. Heterogeneity of slopes of these relationships associated with growth [CO_2] or measurement date (*D*) was examined by an analysis of covariance (α = 0.05) with N_a as the covariate, where a significant $CO_2 \times N_a$ interaction would indicate a significant difference in the slope of the A_{sat}–N relationships. This difference in slope would show that elevated [CO_2] has a reduced effect on A_{sat} at lower N_a and a greater effect at higher foliar N concentrations. Alternatively, a significant difference in the main effect of [CO_2] would indicate a significant difference in the *y*-intercept of the A_{sat}–N relationships, reflecting a consistent effect of elevated [CO_2] on net photosynthesis across all foliar N concentrations.

To determine if the stimulatory effects of elevated [CO_2] on photosynthesis declined over time, A_{sat}–N relationships were calculated from photosynthesis measurements made at common concentrations and analyzed using the same main and interactive effects as above. In designing this method, we took into account that the effect of elevated [CO_2] on foliar N concentrations may be independent of its effect on photosynthesis, and vice versa. Specifically, a statistically significant interaction between CO_2 and N_a would indicate a nonuniform CO_2-induced photosynthetic adjustment across all N_a values. However, if the $CO_2 \times N_a$ term is not statistically significant, then the CO_2 term in the statistical model would describe the effects of elevated [CO_2] on photosynthesis independently of N_a. Therefore, if we observe no treatment effects on A_{sat}–N relationships measured at a common [CO_2] and no effects of elevated [CO_2] on N_a, we can conclude that no physiological adjustment to elevated [CO_2] occurred. In addition, we can conclude that the stimulation of photosynthesis by elevated [CO_2] when measured at the growth [CO_2] is simply the consequence of increased CO_2 substrate at the Rubisco binding site.

Results

Net photosynthesis

Averaged across all measurements periods, elevated [CO_2] strongly stimulated A_{sat} of loblolly pine (44%, $P < 0.0001$; Table 1) and sweetgum trees (40%, $P = 0.005$; Table 1) across both canopy positions in the forest overstory of the Duke Forest FACE experiment. In both overstory species, A_{sat} was significantly higher in sun leaves than in shade leaves. In sweetgum trees, the stimulation of A_{sat} by elevated [CO_2] depended on leaf position in the overstory canopy ($P = 0.02$): A_{sat} of sun leaves was stimulated by about 60%, whereas that of shade leaves was stimulated by only 37%. In loblolly pine, there was a similar statistical trend, but not as strong ($P = 0.095$): elevated [CO_2] stimulated the A_{sat} of sun needles by 48%, compared with a 27% stimulation of shade needles. For both loblolly pine and sweetgum, A_{sat} varied from season to season (loblolly pine, $P < 0.0001$; sweetgum, $P = 0.0247$) and from year to year (loblolly pine, $P = 0.0049$; sweetgum, $P = 0.025$), but the stimulation of A_{sat} by elevated [CO_2] did not vary with year or month in either species (Table 1).

In the Duke forest FACE understory, A_{sat} was stimulated by elevated [CO_2] in all four species (Table 2). The greatest stimulation of A_{sat} by elevated [CO_2] in the understory was in redbud (61%, $P = 0.02$) and the least stimulation was in red maple (24%, $P = 0.049$). Elevated [CO_2] stimulated A_{sat} of understory hickory and sweetgum by 50 and 44%, respectively. Stimulation of A_{sat} by elevated [CO_2] did not vary with time in any of the understory species measured.

Leaf morphology and chemistry

Growth in elevated [CO_2] did not significantly affect N_a of overstory loblolly pine needles or sweetgum leaves in the sun or the shade canopy positions during any sampling period (Table 3). In addition, elevated [CO_2] had no effect on LMA of these two species in the forest overstory. In the understory, LMA was increased by elevated [CO_2] in all species except for

Table 1. Light-saturated net photosynthesis (A_{sat}; µmol CO_2 m^{-2} s^{-1}) of needles and leaves located at the top (sun) and bottom (shade) of *P. taeda* and *L. styraciflua* overstory trees in the Duke Forest FACE experiment. Each value is the mean of three treatment rings for both loblolly pine and sweetgum (± 1 SE) measured at the growth CO_2 concentration ([CO_2]) during early and late summer of 1999, 2001 and 2002. Abbreviations: AMB = ambient [CO_2]; and ELV = elevated [CO_2].

Species	Year	Early/Late	A_{sat}	
			AMB	ELV
P. taeda				
Sun	1999	Early	4.4 (0.5)	8.5 (0.8)
		Late	5.4 (0.4)	8.0 (0.4)
	2001	Early	7.9 (0.9)	10.5 (0.6)
		Late	6.3 (0.6)	10.9 (1.4)
	2002	Early	5.9 (0.9)	7.8 (1.0)
		Late	7.7 (1.3)	9.8 (0.4)
Shade	1999	Early	4.3 (0.6)	5.5 (0.8)
		Late	4.0 (1.3)	5.7 (0.3)
	2001	Early	7.1 (1.6)	8.3 (0.8)
		Late	5.6 (0.8)	7.1 (1.1)
	2002	Early	3.9 (0.4)	5.6 (0.5)
		Late	5.9 (0.7)	6.8 (0.4)
L. styraciflua				
Sun	1999	Early	11.0 (0.7)	18.4 (2.7)
		Late	10.1 (0.3)	14.8 (2.7)
	2001	Early	10.4 (0.8)	15.0 (1.2)
		Late	9.0 (1.2)	14.9 (2.8)
	2002	Early	6.1 (0.1)	11.6 (2.5)
		Late	7.8 (0.3)	12.3 (5.4)
Shade	1999	Early	6.4 (0.6)	7.2 (0.2)
		Late	4.6 (0.7)	5.5 (0.3)
	2001	Early	7.4 (0.6)	7.7 (0.8)
		Late	4.2 (0.6)	7.1 (0.5)
	2002	Early	3.1 (0.5)	6.2 (1.4)
		Late	2.4 (0.2)	4.9 (0.3)

Table 2. Light-saturated net photosynthesis (A_{sat}; µmol CO_2 m^{-2} s^{-1}) of red maple (*A. rubrum*), hickory (*Carya glabra*), redbud (*Cercis canadensis*) and sweetgum (*L. styraciflua*) in the understory of the Duke Forest FACE experiment. Each value is the mean of three treatment rings (± 1 SE) measured at the growth CO_2 concentration ([CO_2]) during early and late summer of 1999, 2001 and 2002. Abbreviations: AMB = ambient [CO_2]; and ELV = elevated [CO_2].

Species	Year	Early/Late	A_{sat}	
			AMB	ELV
A. rubrum	1999	Early	4.4 (0.4)	6.0 (0.7)
		Late	3.2 (0.5)	5.1 (0.4)
	2001	Early	4.6 (0.9)	6.0 (0.7)
		Late	6.2 (1.7)	5.1 (0.7)
	2002	Early	3.4 (0.1)	4.5 (0.3)
		Late	3.0 (0.3)	4.1 (2.2)
Carya glabra	1999	Early	4.7 (0.4)	6.4 (0.2)
		Late	2.7 (0.6)	4.7 (0.3)
	2001	Early	4.6 (0.4)	5.4 (1.1)
		Late	3.0 (0.9)	4.4 (1.2)
	2002	Early	3.4 (0.2)	4.4 (0.1)
		Late	2.7 (0.6)	4.7 (0.3)
Cercis canadensis	1999	Early	5.3 (0.8)	7.0 (0.7)
		Late	3.5 (0.1)	5.3 (0.5)
	2001	Early	15.0 (1.2)	10.4 (0.8)
		Late	9.0 (1.2)	14.9 (2.8)
	2002	Early	6.1 (0.1)	11.6 (2.5)
		Late	7.8 (0.3)	12.3 (5.4)
L. styraciflua	1999	Early	5.1 (0.7)	6.8 (0.2)
		Late	5.1 (0.9)	6.4 (0.6)
	2001	Early	4.9 (0.9)	7.0 (0.3)
		Late	6.6 (1.4)	7.1 (0.4)
	2002	Early	4.6 (0.9)	7.2 (1.2)
		Late	3.9 (0.2)	6.2 (0.2)

hickory (Table 4). The largest stimulation of LMA by elevated [CO_2] was only 12% and was observed in understory-grown sweetgum trees. Elevated [CO_2] had no effect on either N_a or mass-based leaf N concentration (N_m) in any of the understory species examined.

Canopy position had no effect on N_m of overstory loblolly pine and sweetgum trees. In both species, however, LMA was greater in sun leaves than in shade leaves and, as a result, N_a of overstory loblolly pine and sweetgum trees showed similar differences in the upper and lower canopy. Sun needles of loblolly pine trees had 41% greater LMA ($P < 0.0001$) and 58% higher N_a ($P < 0.0001$) than shade needles when averaged across all seasons and years (Table 3). For overstory sweetgum trees, sun leaves were 65% thicker ($P = 0.017$) and had 84% higher N_a ($P < 0.001$) than shade leaves when averaged across all measurement periods (Table 3).

In all of the understory species, LMA, and therefore N_a, in-creased between early summer and late summer (Table 4). On the other hand, N_m of all species except understory sweetgum decreased between early summer and late summer during the years 1999 and 2001 (Table 4). In 2002, no seasonal changes in N_m were observed for any of the species.

Net photosynthesis–nitrogen relationships

The A_{sat}–N relationships were developed by regressing light-saturated net photosynthesis measured at the growth [CO_2] on N_a for the two most dominant tree species in the forest overstory as well as for the four most prominent tree species in the understory in the ambient and elevated [CO_2] FACE rings. Elevated [CO_2] increased the slope of the A_{sat}–N relationship at each measurement date in 1999, 2001 and 2002, and this increase resulting from elevated [CO_2] was not significantly altered by measurement date (Table 5, Figure 1). Elevated [CO_2] increased the slope of the A_{sat}–N relationships by about 81% ($P = 0.005$) when averaged across all measurement dates (Table 5, Figure 2).

Table 3. Leaf mass per unit area (LMA; $g\,m^{-2}$), mass-based foliar N concentration (N_m; $mg\,g^{-1}$) and area-based foliar N concentration (N_a; $g\,m^{-2}$) at the top and bottom of overstory loblolly pine (*P. taeda*) and sweetgum (*L. styraciflua*) trees growing in the Duke Forest FACE experiment. Measurements were made twice each year (early and late summer) during 1999, 2001 and 2002 in both ambient (AMB) and elevated (ELV) [CO₂] FACE rings. Each value is the mean of three treatment plots (± 1 SE).

Species	Year	Early/Late	LMA		N_m		N_a	
			AMB	ELV	AMB	ELV	AMB	ELV
P. taeda								
Sun	1999	Early	95.3 (4.3)	98.2 (6.4)	10.1 (0.6)	10.9 (0.5)	0.96 (0.05)	1.08 (0.12)
		Late	42.4 (1.3)	44.0 (1.9)	11.2 (0.8)	12.0 (0.7)	0.48 (0.04)	0.53 (0.02)
	2001	Early	107.4 (3.4)	130.0 (5.3)	13.1 (0.4)	10.9 (1.7)	1.40 (0.09)	1.39 (0.19)
		Late	90.8 (7.6)	96.6 (5.5)	12.2 (0.6)	11.3 (0.8)	1.11 (0.13)	1.11 (0.15)
	2002	Early	71.1 (4.7)	72.6 (8.9)	8.4 (0.4)	7.4 (0.3)	0.43 (0.04)	0.53 (0.06)
		Late	90.4 (4.7)	88.1 (1.4)	8.8 (0.2)	8.6 (0.5)	0.80 (0.03)	0.77 (0.05)
Shade	1999	Early	69.7 (2.7)	72.2 (1.9)	10.5 (0.4)	10.8 (0.5)	0.72 (0.02)	0.78 (0.02)
		Late	33.3 (1.7)	30.2 (1.0)	10.5 (0.3)	10.8 (0.5)	0.35 (0.02)	0.32 (0.02)
	2001	Early	79.6 (2.4)	82.8 (3.8)	11.0 (0.4)	10.7 (0.5)	0.80 (0.03)	0.77 (0.05)
		Late	67.2 (5.7)	68.8 (4.3)	9.9 (0.2)	9.0 (0.6)	0.65 (0.09)	0.62 (0.02)
	2002	Early	40.3 (1.0)	43.6 (2.0)	7.6 (0.1)	7.3 (0.2)	0.31 (0.01)	0.32 (0.01)
		Late	72.4 (4.3)	68.8 (3.1)	7.6 (0.5)	7.1 (0.4)	0.56 (0.06)	0.48 (0.02)
L. styraciflua								
Sun	1999	Early	55.1 (6.3)	55.4 (3.8)	19.9 (0.6)	17.2 (1.3)	1.09 (0.10)	0.95 (0.15)
		Late	54.3 (7.8)	66.7 (5.8)	18.5 (0.4)	15.3 (1.4)	1.00 (0.15)	0.94 (0.06)
	2001	Early	84.4 (3.7)	70.1 (2.8)	19.4 (0.9)	19.0 (0.4)	1.64 (0.07)	1.32 (0.45)
		Late	67.7 (7.3)	67.0 (5.6)	19.8 (0.5)	16.0 (3.9)	1.34 (0.17)	1.07 (0.24)
	2002	Early	59.9 (2.7)	61.5 (2.6)	12.4 (0.1)	10.9 (1.3)	1.24 (0.01)	1.09 (0.13)
		Late	66.2 (9.3)	95.6 (8.4)	13.5 (0.8)	12.3 (0.5)	0.88 (0.08)	1.16 (0.20)
Shade	1999	Early	39.9 (2.2)	35.7 (3.7)	17.5 (0.3)	18.2 (1.0)	0.70 (0.05)	0.65 (0.03)
		Late	42.8 (1.1)	48.2 (4.7)	16.0 (1.9)	14.9 (0.7)	0.68 (0.69)	0.71 (0.04)
	2001	Early	41.1 (1.9)	40.5 (4.7)	21.2 (0.5)	20.1 (0.1)	0.87 (0.04)	0.81 (0.16)
		Late	45.8 (3.5)	45.8 (6.5)	17.9 (0.6)	19.1 (2.2)	0.82 (0.09)	0.86 (0.02)
	2002	Early	39.1 (1.4)	40.6 (7.3)	12.4 (1.2)	11.7 (0.6)	0.48 (0.12)	0.48 (0.08)
		Late	31.4 (2.6)	39.7 (4.7)	11.6 (0.3)	11.6 (1.5)	0.36 (0.02)	0.46 (0.11)

The slopes of the A_{sat}–N relationships of trees grown in ambient and elevated [CO₂] were not significantly different when either 380 μl l^{-1} ($P = 0.556$) or 580 μl l^{-1} ($P = 0.887$; Table 6) was the common [CO₂] (Figure 3). We also found no significant changes in the *y*-intercepts of trees grown in ambient or elevated [CO₂] when relationships were determined at 380 μl l^{-1} ($P = 0.214$) or 580 μl l^{-1} ($P = 0.551$). These results were consistent across all measurement dates (CO₂ × D; $P = 0.76$).

Discussion

Leaf-level net photosynthetic rates of overstory and understory tree species in the Duke Forest FACE experiment were stimulated by elevated [CO₂], but there was much variation in this response across species and canopy position. Photosynthesis was especially responsive to N_a, with greater enhancement by [CO₂] at higher N_a with the result that elevated [CO₂] increased the slope of the A_{sat}–N relationship by 81% when averaged across all sample dates (Figure 2). This is remarkably close to the 74% increase in the slope of the A_{sat}–N relation-

ship found by a meta-analysis of 10 studies examining the responses of pine and hardwood trees grown with elevated [CO₂] (Peterson et al. 1999). In addition, Takeuchi et al. (2001) found that elevated [CO₂] increased the slope of the A_{sat}–N relationship through the canopy of *Populus tremuloides* Michx. by 59%. The increase in slope of the A_{sat}–N relationship in response to elevated [CO₂] indicates an increase in PNUE, because there was a strong enhancement of leaf-level photosynthesis but no reduction in N_a by elevated [CO₂]. However, the increase in slope of the A_{sat}–N relationship by elevated [CO₂] also indicates that the increase in PNUE is not constant and reflects differential effects of CO₂ on photosynthesis at the top and bottom of the forest canopy. This differential effect of elevated [CO₂] on PNUE at the top and bottom of the forest canopy is likely caused by the distinctly different allocation of foliar N in sun- and shade-adapted leaves. Specifically, sun-adapted leaves typically allocate a larger proportion of foliar N to the dark reactions of photosynthesis (i.e., Rubisco) than shade-adapted leaves (Boardman 1977). Elevated [CO₂] pri-

Table 4. Leaf mass per unit area (LMA; g m^{-2}), mass-based foliar N concentration (N_m; mg g^{-1}) and area-based foliar N concentration (N_a; g m^{-2}) of red maple (*A. rubrum*), hickory (*Carya glabra*), redbud (*Cercis canadensis*) and sweetgum (*L. styraciflua*) trees growing in the forest understory of the Duke Forest FACE experiment. Measurements were made twice each year (early and late summer) during 1999, 2001 and 2002 in both ambient (AMB) and elevated (ELV) CO_2 FACE rings. Each value is the mean of three treatment plots (± 1 SE).

Species	Year	Early/Late	LMA		N_m		N_a	
			AMB	ELV	AMB	ELV	AMB	ELV
A. rubrum	1999	Early	32.7 (1.9)	34.1 (2.0)	22.0 (1.8)	19.0 (0.6)	0.71 (0.03)	0.65 (0.04)
		Late	40.6 (3.9)	44.0 (2.4)	15.4 (0.2)	16.1 (0.5)	0.62 (0.09)	0.70 (0.02)
	2001	Early	33.7 (3.6)	34.9 (4.1)	20.8 (1.3)	17.5 (0.4)	0.71 (0.11)	0.62 (0.09)
		Late	36.7 (2.4)	40.8 (2.8)	13.7 (0.5)	16.9 (5.3)	0.70 (0.04)	0.86 (0.26)
	2002	Early	30.9 (0.5)	35.2 (2.9)	12.2 (0.3)	12.5 (0.6)	0.38 (0.01)	0.44 (0.05)
		Late	48.1 (4.1)	58.4 (1.6)	12.0 (1.2)	12.0 (1.5)	0.58 (0.02)	0.70 (0.01)
Carya glabra	1999	Early	32.8 (3.2)	34.0 (0.4)	20.0 (1.5)	19.7 (0.8)	0.65 (0.05)	0.66 (0.02)
		Late	40.3 (1.5)	43.9 (2.1)	17.0 (0.5)	16.4 (0.9)	0.68 (0.01)	0.72 (0.05)
	2001	Early	31.3 (2.1)	33.3 (2.0)	20.6 (0.8)	18.4 (0.6)	0.64 (0.09)	0.61 (0.03)
		Late	34.4 (2.6)	30.1 (5.7)	14.8 (1.2)	15.7 (3.5)	0.84 (0.04)	0.70 (0.12)
	2002	Early	32.6 (1.7)	33.7 (0.4)	13.8 (0.4)	13.3 (0.6)	0.45 (0.03)	0.45 (0.02)
		Late	53.1 (9.1)	44.2 (3.1)	18.0 (0.3)	15.0 (0.2)	0.92 (0.15)	0.64 (0.09)
Cercis canadensis	1999	Early	24.1 (5.0)	28.5 (3.4)	24.7 (2.2)	23.1 (1.8)	0.58 (0.07)	0.65 (0.03)
		Late	30.5 (1.7)	31.6 (4.9)	21.1 (1.9)	18.6 (1.6)	0.64 (0.02)	0.57 (0.04)
	2001	Early	24.5 (2.1)	22.2 (0.2)	25.7 (2.2)	25.5 (2.6)	0.62 (0.03)	0.58 (0.06)
		Late	23.8 (2.2)	27.7 (3.6)	11.7 (2.4)	19.9 (0.5)	0.53 (0.02)	0.92 (0.06)
	2002	Early	26.0 (0.2)	27.0 (1.9)	18.1 (1.4)	16.1 (0.8)	0.47 (0.03)	0.43 (0.02)
		Late	37.5 (5.1)	38.2 (2.4)	18.1 (0.1)	16.2 (0.2)	0.66 (0.06)	0.59 (0.04)
L. styraciflua	1999	Early	26.4 (3.5)	34.5 (2.9)	20.8 (1.2)	20.1 (0.9)	0.55 (0.08)	0.70 (0.07)
		Late	32.7 (2.6)	37.3 (2.5)	17.8 (0.9)	17.3 (0.9)	0.57 (0.04)	0.64 (0.05)
	2001	Early	28.7 (2.3)	30.9 (1.8)	15.9 (3.1)	15.2 (3.1)	0.48 (0.10)	0.58 (0.14)
		Late	26.9 (6.3)	30.2 (1.2)	15.8 (3.7)	15.5 (3.9)	0.55 (0.15)	0.76 (0.09)
	2002	Early	25.9 (1.6)	27.4 (6.5)	13.7 (0.8)	13.3 (0.2)	0.35 (0.03)	0.44 (0.06)
		Late	38.3 (2.6)	40.9 (3.0)	15.0 (0.9)	15.6 (1.2)	0.57 (0.04)	0.64 (0.08)

Table 5. Regression components of the A_{sat}–N relationships in the forest canopy of the Duke Forest FACE experiment measured during 1999, 2001 and 2002 in early and late summer. Abbreviations: AMB = ambient CO_2 concentration ([CO_2]); and ELV = elevated [CO_2].

Year	Early/Late	CO_2 Treatment	Slope	*y*-Intercept	r^2
1999	Early	AMB	8.71	−0.99	0.69
		ELV	12.66	−2.12	0.85
	Late	AMB	7.22	0.02	0.62
		ELV	11.81	−0.95	0.58
2001	Early	AMB	4.00	2.47	0.58
		ELV	5.51	2.81	0.74
	Late	AMB	4.91	1.08	0.58
		ELV	9.82	−1.05	0.77
2002	Early	AMB	2.08	2.80	0.47
		ELV	4.70	3.12	0.50
	Late	AMB	9.95	−2.85	0.66
		ELV	9.99	−1.46	0.57
All years and times		AMB	4.59	1.36	0.52
		ELV	8.32	0.68	0.66

marily increases photosynthesis by decreasing the competitive inhibition by oxygen at the Rubisco binding-site, thereby reducing photorespiration (Drake et al. 1997). Thus, sun leaves in a forest ecosystem would be expected to show a greater increase in PNUE in response to elevated [CO_2] than shade leaves.

Model estimates predict an increase in forest productivity in response to elevated [CO_2]. However, this increase relies on a sustained stimulation of photosynthesis by elevated [CO_2] (Reynolds et al. 1996). We examined two overstory and four understory tree species and found a sustained positive response of photosynthesis to elevated [CO_2] after 6 years of treatment in the Duke Forest FACE experiment. There was little change in the CO_2-stimulation of photosynthesis across season or year, even though the year 2002 was one of the driest summers on record for piedmont North Carolina (Waple and Lawrimore 2002). Furthermore, the effect of elevated [CO_2] on photosynthesis of loblolly pine needles growing at the top of the canopy was very similar in magnitude to its effect during the first year of treatment (Myers et al. 1999). Also, the percent stimulation of photosynthesis by elevated [CO_2] for sun and
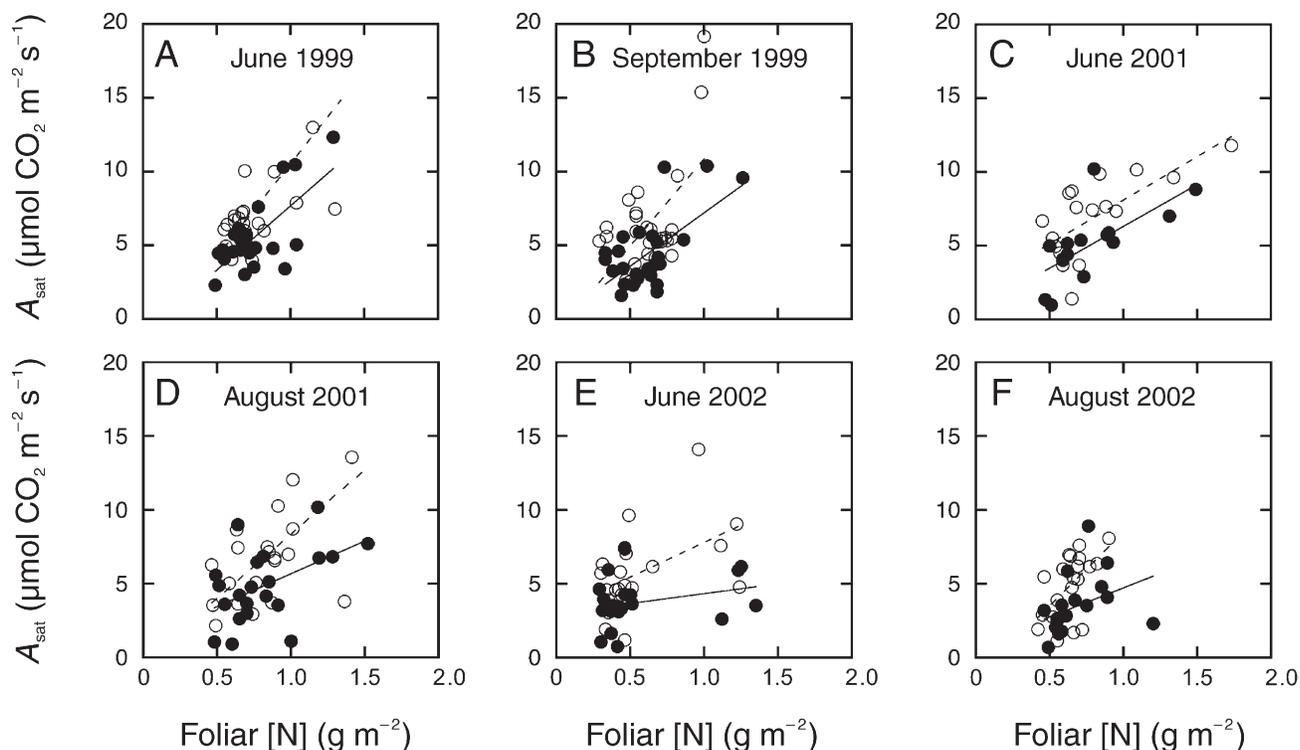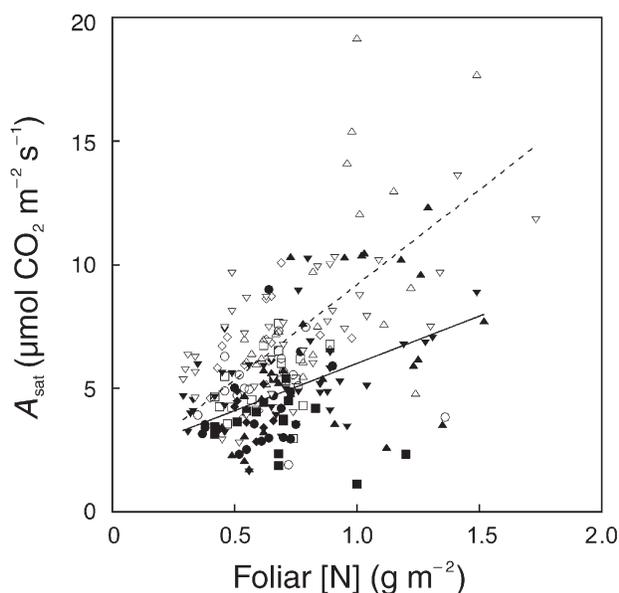
Figure 1. Light-saturated net photosynthetic rates (A_{sat}) measured at the growth CO_2 concentration versus area-based foliar N concentrations (N_a) of loblolly pine, sweetgum, red maple, hickory and redbud in ambient [CO_2] (●, solid line, $n = 17–29$) and elevated [CO_2] (○, dashed line, $n = 19–28$). Measurements were conducted in (A) early summer 1999, (B) late summer 1999, (C) early summer 2001, (D) late summer 2001, (E) early summer 2002 and (F) late summer 2002. Regression equations of these relationships are given in Table 3.

Figure 2. Light-saturated net photosynthetic rates (A_{sat}) measured at the growth CO_2 concentration ([CO_2]) versus area-based foliar N concentrations ([N]) of loblolly pine (▼, ▽), sweetgum (▲, △), red maple (●, ○), hickory (■, □) and redbud (◆,◇) grown at ambient [CO_2] (closed symbols, solid line, $n = 68$) and elevated [CO_2] (open symbols, dashed line, $n = 72$). Data were pooled to calculate a single relationship for each CO_2 treatment across all measurement dates. Regression equations of these relationships are given in Table 3.

shade sweetgum leaves was comparable to the stimulation of photosynthesis in overstory sweetgum trees averaged across the first three years of the Duke Forest FACE experiment (Herrick and Thomas 1999).

To determine whether the stimulatory effects of elevated [CO_2] on photosynthesis had been reduced over time, we compared the long-term response to elevated [CO_2] to an immediate change in [CO_2] by examining A_{sat}–N relationships in the elevated and ambient rings at common [CO_2] (Curtis et al. 2000). Reductions in the stimulatory effect of elevated [CO_2] on photosynthesis are often associated with a nearly proportional reduction in foliar N (Curtis and Wang 1998); thus, we hypothesized that reduced foliar N in plants grown with elevated [CO_2] would lead to a shift toward the origin of the A_{sat}–N relationship when the relationships of the ambient and elevated [CO_2] treatments were compared at similar CO_2 concentrations. If, however, there was a reduction in the stimulatory effect of elevated [CO_2] on photosynthesis without a concurrent loss of foliar N, as has been observed in senescing loblolly pine needles (Rogers and Ellsworth 2002), we expected differences in slope or y-intercept of the A_{sat}–N relationship for plants grown in elevated [CO_2]. We found no differences in the A_{sat}–N relationships of trees grown in ambient and elevated [CO_2] and measured at common [CO_2] in any of the three measurement periods (Figure 3). We also found no evidence of a shift along the x-axis of the A_{sat}–N relationships of trees in the ambient and elevated [CO_2] treatments, because we

Table 6. Regression components of A_{sat}–N relationships grown at ambient (AMB) and elevated (ELV) CO_2 concentration and measured at two common CO_2 concentrations (A_{380} and A_{580}) in the forest canopy of the Duke Forest FACE experiment during 2001 and 2002 in early and late summer.

Year	Early/ Late	CO_2 treatment	A_{380}			A_{580}		
			Slope	y-Intercept	r^2	Slope	y-Intercept	r^2
2001	Late	AMB	4.54	1.08	0.56	5.98	2.46	0.54
		ELV	5.68	0.20	0.66	8.24	0.51	0.62
2002	Early	AMB	8.21	−0.61	0.62	5.52	1.46	0.80
		ELV	3.19	1.60	0.68	5.47	1.60	0.68
	Late	AMB	3.36	1.33	0.55	4.71	2.46	0.60
		ELV	5.24	0.27	0.78	8.29	0.00	0.80

observed no CO_2-induced decline in foliar N in the overstory or understory tree species (Tables 3 and 4). Many of the tree species in the understory and sweetgum in the overstory had foliar N concentrations above values that are typically found in N-limiting conditions (Blinn and Buckner 1989). The exception to this was during the severe, sustained drought of 2002 when foliar N concentrations of every species in the Duke Forest FACE experiment were low. However, the concentrations

of foliar N in loblolly pine needles at all measurement periods were close to those values where N begins to limit loblolly pine productivity (< 11 mg g^{-1}; Allen 1987). With the exception of 2002, the values of N_m and N_a in overstory and understory trees that we studied were similar to those reported in earlier studies performed at the Duke Forest FACE experiment (Herrick and Thomas 1999, Myers et al. 1999, DeLucia and Thomas 2000, Rogers and Ellsworth 2002), indicating that foliar N concen-
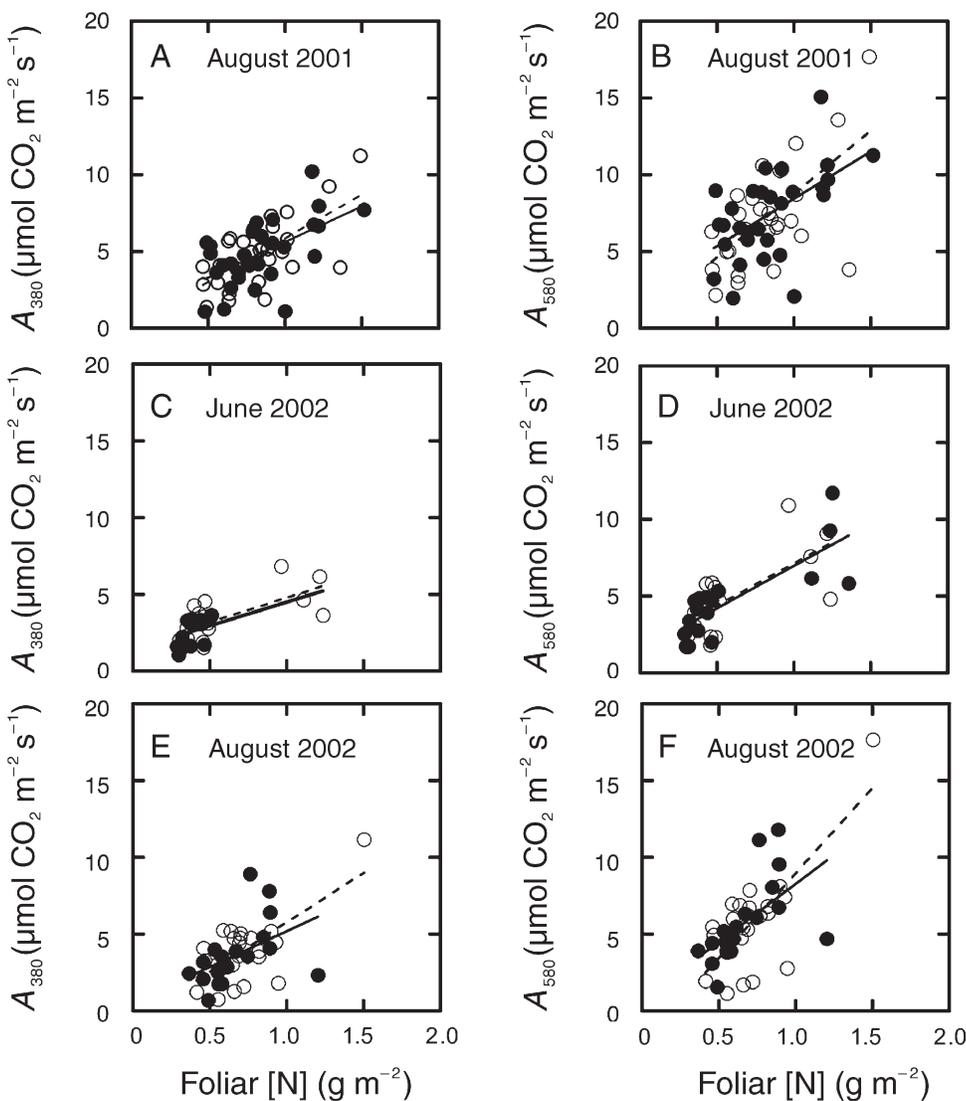

Figure 3. Light-saturated net photosynthetic rates (A) versus area-based foliar N concentrations ([N]) of loblolly pine, sweetgum, red maple, hickory and redbud grown at ambient (●, solid line, $n = 17$–29) and elevated CO_2 concentrations ([CO_2]) (○, dashed line, $n = 19$–28) measured at common CO_2 concentrations of 380 µl l^{-1} (A_{380}) and 580 µl l^{-1} (A_{580}). Measurements were made during late summer 2001 (A, B), early summer 2002 (C, D) and late summer 2002 (E, F). Regression equations of these relationships ($n = 18$–28) are given in Table 4.

trations of these species have changed very little over the six years of this experiment.

In summary, foliar N concentration varies widely in the forest canopy at the Duke Forest FACE experiment. The A_{sat}–N relationships incorporate much of this variation associated with the light gradient through the canopy and can incorporate differential species effects and seasonal effects into estimates of the photosynthetic responses to elevated $[CO_2]$. Based on three years of measurements on two overstory tree species and four understory tree species, we found that foliar N accounted for 52% of the variation in net photosynthesis in the ambient $[CO_2]$ FACE rings and 66% of the variation in net photosynthesis in the elevated $[CO_2]$ rings. Foliar N concentrations were greater at the top of the canopy than at the bottom and the increased slope of the A_{sat}–N relationship in trees grown in elevated $[CO_2]$ indicates differential effects of elevated $[CO_2]$ on photosynthesis at the top and bottom of the forest canopy. Thus, this study illustrates the importance of canopy position in a forest ecosystem as a source of variation when determining the responses of photosynthesis to elevated $[CO_2]$. Few studies have examined the photosynthetic responses of trees in a forest such as the Duke Forest FACE experiment that has been treated with elevated $[CO_2]$ for as many as six years. Previous studies suggest that we might expect some loss of the photosynthetic enhancement by elevated $[CO_2]$ because of reductions in leaf N concentrations. However, we found little evidence of CO_2-induced changes in foliar N concentration or loss of stimulation of photosynthesis by elevated $[CO_2]$ in the study trees. Thus, we conclude that, with the exception of some photosynthetic down-regulation observed in senescing loblolly pine needles (Rogers and Ellsworth 2002), the primary effect of long-term exposure to elevated $[CO_2]$ in the Duke Forest FACE experiment has been the strong sustained enhancement of photosynthesis of the trees growing in this forest.

Acknowledgments

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