Summary
We examined the photosynthetic responses of four species of saplings growing in the understory of the Duke Forest FACE experiment during the seventh year of exposure to elevated CO₂ concentration ([CO₂]). Saplings of these same species were measured in the first year of the Duke Forest FACE experiment and at that time showed only seasonal fluctuations in acclimation of photosynthesis to elevated [CO₂]. Based on observations from the Duke Forest FACE experiment, we hypothesized that after seven years of exposure to elevated [CO₂] significant photosynthetic down-regulation would be observed in these tree species. To test our hypothesis, photosynthetic CO₂-response and light-response curves, along with chlorophyll fluorescence, chlorophyll concentration and foliar N were measured twice during the summer of 2003. Exposure to elevated [CO₂] continued to increase photosynthesis in all species measured after seven years of treatment with the greatest photosynthetic increase observed near saturating irradiances. In all species, elevated [CO₂] increased electron transport efficiency but did not significantly alter carboxylation efficiency. Quantum yield estimated by light curves, chlorophyll concentration, and foliar nitrogen concentrations were unaffected by elevated [CO₂]. Contrary to our hypothesis, there is little evidence of progressive N limitation of leaf-level processes in these understory tree species after seven years of exposure to elevated [CO₂] in the Duke Forest FACE experiment.

Keywords: elevated CO₂ concentration, Free Air Carbon Enrichment, loblolly pine forest, photosynthetic down-regulation, progressive nitrogen limitation.

Introduction
Tree species growing in the forest understory contribute to the overall carbon balance of forest ecosystems and represent many of the species that occur in the overstory of mature forested ecosystems. A detailed understanding of the responses of forest understory tree species to increasing concentrations of atmospheric carbon dioxide ([CO₂]) is necessary, therefore, to predict forest structure and function in the future. In a review of the effects of elevated [CO₂] on plants grown under non-optimal conditions, Poorter and Perez-Soba (2001) concluded that an inadequate number of studies had been performed investigating the interactions of low-light availability and elevated [CO₂] to make a generalized statement about the responses of light-limited plants grown at elevated [CO₂]. Despite this, relatively few studies have examined the photosynthetic responses of understory trees to elevated [CO₂], with even fewer studies investigating these responses in a natural forest ecosystem (Osborne et al. 1997, Winter and Virgo 1998, DeLucia and Thomas 2000, Singsaas et al. 2000, Hattenschwiler 2001).

Forest canopies generate complex and variable light environments that expose plants growing in the forest understory to irradiances that range from a small portion of the incident solar radiation found at the top of the forest canopy to near direct sunlight. Generally, this variable light environment limits the growth and photosynthesis of understory species more than other environmental factors (Pearcy 1983, Denslow et al. 1990, Pacala et al. 1994, Kobe et al. 1995). Increases in atmospheric [CO₂] are likely to reduce carbon limitations and stimulate photosynthesis of understory species despite low light availability (DeLucia and Thomas 2000, Naumburg and Ellsworth 2000, Hattenschwiler 2001, Poorter and Perez-Soba 2001). Many studies of the photosynthetic response of tree species grown at low irradiances show that photosynthesis is nearly always increased by elevated [CO₂], though to varying degrees depending on nutrient status, time of year, species and other biotic and abiotic factors (Herrick and Thomas 1999, Kubiske et al. 2002, Springer et al. 2005).

Forest productivity models are based on the assumption of a sustained enhancement of photosynthesis by elevated [CO₂] (Reynolds et al. 1996) and, therefore, any long-term changes in the photosynthetic response of forest species, including understory species, must be quantified and incorporated in models to accurately predict forest ecosystem functioning in a future high [CO₂] world. However, many long-term experiments to determine the effects of atmospheric enrichment with
CO₂ report a time-dependent decline in the photosynthetic response to elevated [CO₂], which is often referred to as photosynthetic down-regulation. This loss in photosynthetic capacity is commonly accompanied by reductions in ribulose bisphosphate carboxylase-oxygenase (rubisco) activity and foliar nitrogen concentration (Long and Drake 1991, Stitt 1991, Bowes 1993, Sage 1994, Norby et al. 1999).

One way that photosynthetic down-regulation may occur is through progressive N limitation (Luo et al. 2004); this interpretation predicts that, as a higher atmospheric [CO₂] increases plant biomass production, resource demands increase, especially for N, and available nutrients in the soil become diminished more rapidly than under the current [CO₂]. A consequence of progressive N limitation is a decline in the availability of N for photosynthetic processes. This type of photosynthetic down-regulation may occur in ecosystems that have a low soil N availability, such as piedmont loblolly pine forests (Radoglou et al. 1992, Tissue et al. 1993, El Kohen and Mousseau 1994, Sage 1994, Curtis et al. 1995, Finzi et al. 2002). The enhancement of aboveground tree growth by elevated [CO₂] in the Duke FACE prototype ring declined over several years of treatment, but was restored when N fertilizer was added to the plot (Oren et al. 2001). This finding, as well as the observation that N demand by trees exposed to elevated [CO₂] in the Duke FACE experiment exceeds N supply (Finzi et al. 2002), suggest that the forest is becoming N-limited and that we may observe reduced foliar N and photosynthetic down-regulation in the near future.

A second condition that may lead to photosynthetic down-regulation in plants grown in elevated [CO₂] are imbalances between carbon sinks and carbon sources (Tissue et al. 1995) that may be a consequence of progressive N limitation or other limitations imposed on plant growth. In this type of elevated-[CO₂]-induced photosynthetic down-regulation, decreased strength of existing C sinks and low availability of new carbon sinks in the plant lead to carbohydrate accumulation in the leaves. This carbohydrate accumulation is sensed through hexokinase signaling and leads to reduced expression of photosynthetic genes, especially those encoding rubisco (Moore et al. 1999), resulting in decreased photosynthetic capacity until sink strength increases. This may be a particularly important mechanism of photosynthetic regulation for forest understory trees given the small growth response to elevated [CO₂] observed for these species in the Duke FACE experiment (Moore 2005).

Given the observations of Oren et al. (2001) and Moore (2005), we examined the photosynthetic responses of saplings of four understory species (Acer rubrum L., Carya glabra Mill., Cercis canadensis L., and Liquidambar styraciflua L.) during the seventh year of exposure to elevated [CO₂] at the Duke Forest FACE experiment to determine whether photosynthetic down-regulation had occurred. During the first year of the Duke Forest FACE experiment, DeLucia and Thomas (2000) examined the photosynthetic responses of saplings of the same four understory species to determine if elevated [CO₂] affected photosynthetic capacity and the allocation of resources between light-harvesting and carbon-fixing processes. Elevated [CO₂] increased photosynthesis of all the species measured but it also caused a small reduction in photosynthetic capacity. The results, however, were inconsistent and varied seasonally. In addition, it was concluded that the observed photosynthetic down-regulation in response to elevated [CO₂] was likely the result of a prolonged drought during the growing season prior to the measurements (DeLucia and Thomas, 2000). We reexamined the photosynthetic responses of saplings of the same four understory species to determine whether the enhancement of photosynthesis observed during the first year of exposure to elevated [CO₂] was sustained in the seventh year of the experiment.

Materials and methods

Site description

The FACTS-1 Duke Forest Free Air Carbon Enrichment (FACE) experiment is located in a loblolly pine (Pinus taeda L.) plantation in the Blackwood division of the Duke University Forest (35º97′N, 79º09′W). The site consists of six 30-m diameter experimental FACE rings established within the forest. Three of the rings deliver ambient +200 µl l⁻¹ [CO₂] during daylight hours to the portion of the loblolly pine forest within the ring. The remaining FACE rings serve as ambient [CO₂] control rings that are equipped to deliver the same volume of air as the treatment rings to replicate any micrometeorological effects on the forest that may occur during the operation of the FACE facility. Hendrey et al. (1999) has provided a detailed description of the entire Duke Forest FACE experiment and its operational procedures. Since establishment of the plantation in 1983, no management practices have been applied to the forest, allowing the natural establishment of ~50 species of trees within the forest. In the current study, saplings of A. rubrum (ACRU), C. glabra (CAGL), C. canadensis (CECA), and L. styraciflua (LIST) in the understory of the Duke Forest FACE experiment were selected for a detailed analysis of the responses of leaf gas exchange and fluorescence of understory tree species grown in elevated [CO₂] for seven years.

Gas exchange and chlorophyll fluorescence measurements

Photosynthetic light-response curves, photosynthetic CO₂-response curves and chlorophyll fluorescence were measured on leaves of four to six saplings of each species in two ambient and two elevated [CO₂] FACE rings in early June 2003 and early September 2003. Gas exchange was measured with an open-flow gas exchange system (LI-6400, Li-Cor, Lincoln, NE) with an environmentally controlled cuvette. All gas exchange measurements were made between 1000 and 1600 h (EST) on clear days. During gas exchange measurements, mean leaf temperature and mean relative humidity was 24.82 ± 0.6 °C and 58.8 ± 0.2 % in June and 20.8 ± 0.9 °C and 58.1 ± 0.3 % in September. Ambient photosynthetic photon flux (PPF) in the forest understory measured at the time of the photosynthetic measurements with a quantum sensor (Li-Cor 190SA) attached to the photosynthesis measurement system.
CO₂ EFFECTS ON UNDERSTORY PHOTOSYNTHESIS

Averaged 57.2 ± 11.2 µmol m⁻² s⁻¹ during the June measurements and 50.8 ± 9.8 µmol m⁻² s⁻¹ during the September measurements. Daily soil water during the June and September measurement periods averaged 31.6 ± 0.5 and 23.9 ± 0.7% (v/v), respectively (Figure 1). No treatment effects were observed on leaf temperature, relative humidity or understory PPF during the two measurement periods.

Steady-state photosynthesis was measured across nine incident irradiances between 0 and 1200 µmol m⁻² s⁻¹ (PPF) on one fully expanded leaf per sapling and photosynthetic light-response curves were plotted. A red-blue LED light source attached to the LI-6400 supplied the incident irradiance. Cuvette CO₂ concentrations were held at 380 µmol mol⁻¹ for ambient-grown saplings and at 580 µmol mol⁻¹ for saplings grown in elevated [CO₂]. Apparent quantum efficiency (Qₑ), respiration (Rₒ), light-saturated maximum photosynthesis (Aₛₐₜ), and light compensation point (Γ) were estimated from the light response curves with Photosynthesis Assistant software (Dundee Scientific Ltd., U.K.) that employs the calculations of Prioul and Chartier (1977).

The relationship between net photosynthesis (A) and calculated intercellular CO₂ concentration (Cᵢ) was determined based on rates of photosynthesis measured over 10 external concentrations of CO₂ ranging from 50 to 1500 µl l⁻¹. Measurements were made at a saturating PPF of 1000 µmol m⁻² s⁻¹. The A–Cᵢ curves were analyzed with the two-factor leaf photosynthesis model of Farquhar et al. 1980, with modifications by Harley et al. 1992. Light-saturated carboxylation efficiency (Vₑₘₐₓ) of ribulose-bisphosphate carboxylase oxygenase (rubisco) and electron transport efficiency (Jₑₘₐₓ) were calculated based on the assumptions of Wullschleger 1993.

Chlorophyll fluorescence of the upper surface of the same leaves used for gas exchange measurements was measured with a pulse-modulated fluorometer (PAM-2000, Walz, Germany). Attached leaves were dark adapted for about 10 min before exposure to a weak modulating light beam that allowed measurement of baseline fluorescence (Fₒ). The leaves were then exposed to a 0.6 s flash of saturating light to measure maximal fluorescence yield (Fₘ). The optimal efficiency of photosystem II (PSⅡ, Fₒ/Fₘ) was calculated as (Fₘ − Fₒ)/Fₘ.

Leaf characteristics

For the gas exchange measurements leaves were harvested for determination of specific leaf area (SLA), foliar N concentration on a mass ([N]ₘₐ₉₉) and an area ([N]ₚ₉₉) basis, and chlorophyll concentration on a mass (Chlₘₐ₉₉) and area (Chlₚ₉₉) basis. Specific leaf area was calculated from the mass of oven-dried leaf disks with an area of 3.14 cm². The disks were then ground and combusted with an elemental analyzer (NC 2500, CE Instruments, Milan, Italy) to determine foliar N concentration. Disks frozen at the time of collection were ground in liquid N and extracted with 80% aqueous acetone for chlorophyll measurement according to the procedure of Porra et al. (1989).

Statistics

Measurements of gas exchange, chlorophyll fluorescence and leaf morphology and chemistry were analyzed by a three-way analysis of variance (ANOVA, α = 0.05) with growth CO₂ concentration, measurement date, and species as the main effects. When necessary, data were log transformed to achieve conformity with the assumptions of the ANOVA test.

Results

Light response curves

Elevated [CO₂] increased light-saturated photosynthetic rates in saplings of all species studied (P = 0.0005, Table 1, Figures 2 and 3). Although there was no statistically significant CO₂ × species interaction (P = 0.275), we found great variation in the photosynthetic responses of the individual species to growth in elevated [CO₂]. For example, the largest increase in Aₛₐₜ (120%) was observed in A. rubrum in June; however, this response decreased to 65% in September. Cercis canadensis also showed a smaller enhancement in Aₛₐₜ in response to elevated [CO₂] between June and September (67% in June but only 28% in September). The effect of elevated [CO₂] on Aₛₐₜ remained relatively constant throughout the summer for C. glabra (17% in June, 22% in September) and L. styraciflua (41% in June, 52% in August). There was a seasonal decline in Aₛₐₜ between June and September (P = 0.0021, Table 1) regardless of species or CO₂ treatment.

 Saplings grown at elevated [CO₂] had a 54% lower light compensation point of photosynthesis than saplings grown at ambient [CO₂] (P = 0.0225, Table 1); however, the reduction did not depend on species (P = 0.9316). Neither apparent quantum efficiency (Qₑ) nor respiration (Rₒ) derived from the photosynthetic-light response curves differed significantly between CO₂ treatments or between measurements made in June and September in any of the species (Table 1).

Figure 1. Mean monthly daily soil water (% v/v) for 1997, 1998 and 2003 at the Duke Forest FACE experiment. Data not available for January through April of 1997.
Leaves of saplings grown at elevated 
\[\text{CO}_2\] showed no significant differences in \(V_{\text{c max}}\) compared with saplings grown at ambient \[\text{CO}_2\], but there was a statistical trend for 9% higher \(V_{\text{c max}}\) in elevated \[\text{CO}_2\] (\(P = 0.0933\), Figure 4). In addition, growth at elevated \[\text{CO}_2\] produced a 17% increase in \(J_{\text{max}}\) averaged over the growing season. The dry mass of tree biomass increased by 38% (Figure 5). Measurements were made in June and September 2003 and each value is the mean for 4–6 individuals (± 1 SE).

### Table 1. Dark respiration (\(R_d\)), apparent quantum efficiency (\(Q_e\), mol mol\(^{-1}\)), light saturated maximum photosynthetic rate (\(\mu\text{mol CO}_2\text{ m}^{-2}\text{ s}^{-1}\)), dark-adapted chlorophyll fluorescence (\(F_{v}/F_{m}\), Rel.), and the light compensation point (\(\Gamma\), \(\mu\text{mol m}^{-2}\text{ s}^{-1}\)) of \(A.\ rubrum\) (ACRU), \(Carya\ glabra\) (CAGL), \(Cercis\ canadensis\) (CECA), and \(Liquidambar\ styraciflua\) (LIST) grown in an ambient (Amb) or elevated (Elev) concentration of \[\text{CO}_2\]. Measurements were made in June and September 2003 and each value is the mean for 4–6 individuals (± 1 SE).

<table>
<thead>
<tr>
<th>Species</th>
<th>Month</th>
<th>[CO(_2)]</th>
<th>(R_d)</th>
<th>(Q_e)</th>
<th>(A_{\text{sat}})</th>
<th>(F_{v}/F_{m})</th>
<th>(\Gamma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACRU</td>
<td>June</td>
<td>Amb</td>
<td>0.33 (0.05)</td>
<td>0.058 (0.013)</td>
<td>3.7 (0.9)</td>
<td>0.72 (0.02)</td>
<td>7.8 (2.7)</td>
</tr>
<tr>
<td></td>
<td>Elev</td>
<td></td>
<td>0.24 (0.08)</td>
<td>0.079 (0.007)</td>
<td>8.2 (0.9)</td>
<td>0.76 (0.01)</td>
<td>4.1 (1.4)</td>
</tr>
<tr>
<td></td>
<td>Sept</td>
<td>Amb</td>
<td>0.31 (0.12)</td>
<td>0.067 (0.007)</td>
<td>2.6 (0.6)</td>
<td>0.73 (0.01)</td>
<td>2.6 (1.7)</td>
</tr>
<tr>
<td></td>
<td>Elev</td>
<td></td>
<td>0.18 (0.08)</td>
<td>0.061 (0.012)</td>
<td>4.3 (0.5)</td>
<td>0.71 (0.02)</td>
<td>2.6 (0.9)</td>
</tr>
<tr>
<td>CAGL</td>
<td>June</td>
<td>Amb</td>
<td>0.31 (0.05)</td>
<td>0.065 (0.015)</td>
<td>5.3 (0.5)</td>
<td>0.75 (0.02)</td>
<td>5.8 (1.3)</td>
</tr>
<tr>
<td></td>
<td>Elev</td>
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<td>0.20 (0.05)</td>
<td>0.070 (0.021)</td>
<td>6.2 (0.3)</td>
<td>0.74 (0.01)</td>
<td>4.0 (2.2)</td>
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<tr>
<td></td>
<td>Sept</td>
<td>Amb</td>
<td>0.30 (0.06)</td>
<td>0.060 (0.008)</td>
<td>3.5 (0.4)</td>
<td>0.72 (0.02)</td>
<td>5.7 (1.6)</td>
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<tr>
<td></td>
<td>Elev</td>
<td></td>
<td>0.16 (0.03)</td>
<td>0.062 (0.006)</td>
<td>4.3 (0.4)</td>
<td>0.74 (0.02)</td>
<td>1.9 (1.1)</td>
</tr>
<tr>
<td>CECA</td>
<td>June</td>
<td>Amb</td>
<td>0.30 (0.05)</td>
<td>0.049 (0.011)</td>
<td>4.2 (0.3)</td>
<td>0.71 (0.02)</td>
<td>7.3 (1.5)</td>
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<tr>
<td></td>
<td>Elev</td>
<td></td>
<td>0.42 (0.05)</td>
<td>0.079 (0.017)</td>
<td>7.0 (0.6)</td>
<td>0.77 (0.01)</td>
<td>6.5 (1.5)</td>
</tr>
<tr>
<td></td>
<td>Sept</td>
<td>Amb</td>
<td>0.40 (0.08)</td>
<td>0.079 (0.005)</td>
<td>4.3 (0.3)</td>
<td>0.71 (0.02)</td>
<td>5.1 (1.0)</td>
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<tr>
<td></td>
<td>Elev</td>
<td></td>
<td>0.19 (0.11)</td>
<td>0.103 (0.017)</td>
<td>5.5 (0.2)</td>
<td>0.73 (0.02)</td>
<td>1.6 (1.2)</td>
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<td>LIST</td>
<td>June</td>
<td>Amb</td>
<td>0.57 (0.13)</td>
<td>0.077 (0.010)</td>
<td>5.1 (1.1)</td>
<td>0.75 (0.03)</td>
<td>7.3 (0.7)</td>
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<tr>
<td></td>
<td>Elev</td>
<td></td>
<td>0.43 (0.09)</td>
<td>0.084 (0.005)</td>
<td>7.2 (1.3)</td>
<td>0.75 (0.01)</td>
<td>5.2 (1.2)</td>
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<td></td>
<td>Sept</td>
<td>Amb</td>
<td>0.43 (0.03)</td>
<td>0.070 (0.004)</td>
<td>3.8 (0.6)</td>
<td>0.74 (0.01)</td>
<td>5.3 (1.0)</td>
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<tr>
<td></td>
<td>Elev</td>
<td></td>
<td>0.30 (0.05)</td>
<td>0.074 (0.009)</td>
<td>5.8 (0.7)</td>
<td>0.76 (0.02)</td>
<td>4.5 (1.1)</td>
</tr>
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</table>

**CO\(_2\)** response curves

Leaves of saplings grown at elevated \[\text{CO}_2\] showed no significant differences in \(V_{\text{c max}}\) compared with saplings grown at ambient \[\text{CO}_2\]., but there was a statistical trend for 9% higher \(V_{\text{c max}}\) in elevated \[\text{CO}_2\] (\(P = 0.0933\), Figure 4). In addition, growth at elevated \[\text{CO}_2\] produced a 17% increase in \(J_{\text{max}}\) averaged over the growing season.
aged across all species ($P = 0.025$, Figure 4). The ratio of $J_{\text{max}}$ to $V_{\text{c max}}$ of all species was unaffected by elevated $[\text{CO}_2]$ (data not shown, $P = 0.6442$). There was a seasonal decline in $V_{\text{c max}}$ and $J_{\text{max}}$ between June and September: $V_{\text{c max}}$ decreased 53% from June to September ($P = 0.0002$), whereas $J_{\text{max}}$ decreased 38% from June to September ($P < 0.0001$). However, this see-

Table 2. Specific leaf area (SLA, cm$^2$ g$^{-1}$), foliar nitrogen concentration ([N]$_{\text{mass}}$, mg g$^{-1}$), ([N]$_{\text{area}}$, g m$^{-2}$) total chlorophyll concentration (Chl$_{\text{mass}}$, mg g$^{-1}$), Chl$_{\text{area}}$, mg cm$^{-2}$), and chlorophyll a/b ratio (Chl$_{\text{a/b}}$) of A. rubrum (ACRU), Carya glabra (CAGL), Cercis canadensis (CECA) and Liquidambar styraciflua (LIST) grown in an ambient (Amb) or elevated (Elev) concentration of CO$_2$. Measurements were made in June and September 2003 and each value is the mean of 4–6 individuals ($\pm$ 1 SE).

<table>
<thead>
<tr>
<th>Species</th>
<th>Month</th>
<th>[CO$_2$]</th>
<th>SLA [cm$^2$ g$^{-1}$]</th>
<th>[N]$_{\text{mass}}$ [mg g$^{-1}$]</th>
<th>[N]$_{\text{area}}$ [g m$^{-2}$]</th>
<th>Chl$_{\text{area}}$ [mg g$^{-1}$]</th>
<th>Chl$_{\text{mass}}$ [mg g$^{-1}$]</th>
<th>Chl$_{\text{a/b}}$</th>
</tr>
</thead>
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<tr>
<td>ACRU</td>
<td>June</td>
<td>Amb</td>
<td>292.0 (16.0)</td>
<td>14.4 (0.5)</td>
<td>0.50 (0.04)</td>
<td>26.8 (2.1)</td>
<td>7.8 (0.7)</td>
<td>4.5 (0.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elev</td>
<td>283.1 (13.3)</td>
<td>14.5 (0.8)</td>
<td>0.52 (0.03)</td>
<td>27.1 (3.6)</td>
<td>7.7 (1.2)</td>
<td>3.5 (0.4)</td>
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<td></td>
<td>Sept</td>
<td>Amb</td>
<td>286.1 (21.8)</td>
<td>13.1 (0.5)</td>
<td>0.46 (0.04)</td>
<td>32.2 (3.9)</td>
<td>9.1 (2.4)</td>
<td>2.5 (0.2)</td>
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<tr>
<td></td>
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<td>Elev</td>
<td>259.8 (6.9)</td>
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<td>0.48 (0.04)</td>
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<td>9.0 (0.3)</td>
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<td>CAGL</td>
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<td>Amb</td>
<td>282.1 (17.4)</td>
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<td>268.0 (21.0)</td>
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<td>0.54 (0.03)</td>
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<td>2.3 (0.2)</td>
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<td></td>
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<td>Elev</td>
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<td>0.57 (0.07)</td>
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<td>3.0 (0.6)</td>
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<td>CECA</td>
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<td>Amb</td>
<td>432.9 (40.6)</td>
<td>19.8 (1.2)</td>
<td>0.47 (0.04)</td>
<td>20.6 (3.6)</td>
<td>8.6 (1.5)</td>
<td>3.9 (0.9)</td>
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<td></td>
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<td>Elev</td>
<td>501.7 (35.3)</td>
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<td>0.49 (0.06)</td>
<td>22.8 (3.3)</td>
<td>11.4 (1.7)</td>
<td>2.7 (0.2)</td>
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<td>26.5 (2.1)</td>
<td>11.2 (1.4)</td>
<td>2.6 (0.1)</td>
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<td>368.6 (32.9)</td>
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<td>6.7 (0.5)</td>
<td>4.8 (1.4)</td>
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<tr>
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<td></td>
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<td>16.3 (0.5)</td>
<td>0.50 (0.05)</td>
<td>19.2 (3.4)</td>
<td>6.4 (1.5)</td>
<td>3.9 (0.4)</td>
</tr>
<tr>
<td></td>
<td>Sept</td>
<td>Amb</td>
<td>403.7 (37.2)</td>
<td>13.3 (0.4)</td>
<td>0.34 (0.03)</td>
<td>23.3 (1.0)</td>
<td>9.3 (0.7)</td>
<td>2.6 (0.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elev</td>
<td>405.4 (12.8)</td>
<td>13.2 (0.3)</td>
<td>0.39 (0.02)</td>
<td>29.4 (1.7)</td>
<td>9.6 (0.5)</td>
<td>2.9 (0.3)</td>
</tr>
</tbody>
</table>
sonal decline in photosynthetic capacity was unaffected by growth [CO₂] or species.

Chlorophyll fluorescence and leaf properties
The theoretical maximum quantum yield of PSII \( (F_v/F_m) \) of leaves of saplings of each species was not significantly affected by elevated [CO₂] in either June or September \( (P = 0.4780, \text{Table 1}) \). Area-based chlorophyll concentrations \( (\text{Chl}_{\text{area}}) \) increased significantly (14%) when averaged across all species during the September measurement period compared with the June measurement period \( (P = 0.0049, \text{Table 2}) \). Additionally, \( \text{Chl}_{\text{area}} \) varied significantly between species \( (P = 0.0068, \text{Table 2}) \), with \( \text{A. rubrum} \) having the highest concentration and \( L. \text{styraciflua} \) the lowest. Growth at elevated [CO₂] did not significantly affect mass-based chlorophyll concentration, area-based chlorophyll concentration, or the chlorophyll a/b ratio in the species studied (Table 2).

Specific leaf area (SLA) was not significantly affected by elevated [CO₂] \( (P = 0.8395, \text{Table 2}) \) in any species. In addition, neither mass-based \( ([\text{N}]_{\text{mass}}) \) nor area-based \( ([\text{N}]_{\text{area}}) \) foliar nitrogen concentration of saplings was altered by elevated [CO₂]. Averaged across all species and CO₂ treatments, \( [\text{N}]_{\text{mass}} \) decreased 20% between June and September (Table 2), whereas \( [\text{N}]_{\text{area}} \) did not exhibit a seasonal decline.

Discussion
During the seventh year of exposure to elevated [CO₂] at the Duke FACE experiment, light-saturated net photosynthetic rates of the understory tree species we examined were increased by elevated [CO₂] and we found no evidence of photosynthetic down-regulation in any species in either early or late summer. Based on the conclusions of Oren et al. (2001) and Finzi et al. (2002) that the Duke FACE experimental forest may be entering a phase of N limitation, and the observations of Moore (2005) that exposure to elevated [CO₂] is not increasing aboveground growth of the understory trees at the Duke FACE experimental forest, we had hypothesized that the understory trees in this forest grown at elevated [CO₂] would exhibit a reduction in photosynthetic capacity when compared to understory trees grown at ambient [CO₂]. On the contrary, we observed a small increase in the photosynthetic capacity of all of the study species in response to elevated [CO₂]. Although photosynthetic up-regulation by elevated [CO₂] is not a common observation (Curtis and Wang 1998), it has been demonstrated in several studies (Campbell et al. 1988, Ziska and Teramura 1992, Idso et al. 1991). Idso et al. (1991) suggested that increased photosynthetic capacity exhibited by sour orange trees grown at elevated [CO₂] was associated with an increase in sink capacity for carbon. The slight photosynthetic up-regulation that we observed in the understory trees grown at elevated [CO₂] may have also been related to an increase in sink strength as a result of a small increase in light availability created by an ice storm during the winter of 2002 that destroyed approximately 7% of the overstory canopy in the elevated [CO₂] rings and 11% in the ambient [CO₂] rings (McCarthy et al. In review).

Earlier studies on the understory tree species at the Duke FACE experiment have all shown a significant enhancement of photosynthesis in response to elevated [CO₂] (DeLucia and Thomas 2000, Naumburg and Ellsworth 2000, Singsaas et al. 2000, Springer et al. 2005) and our study indicates that this trend has continued through the seventh year of elevated [CO₂] exposure. DeLucia and Thomas (2000) examined the photosynthetic responses of the four understory species that we studied during the first year of exposure to elevated [CO₂] at the Duke FACE experiment and found that the greatest increase in photosynthesis was at saturating irradiances, but that elevated [CO₂] had little effect on apparent quantum yield. In our study, elevated [CO₂] appeared to increase apparent quantum yield in all species but one, although the effect was not statistically significant. This trend contrasts with previous studies showing little evidence of changes in apparent quantum yield in response to elevated [CO₂] (Osborne et al. 1997, Singsaas et al. 2000). We did, however, observe a decrease in the light
compensation point of saplings exposed to elevated [CO₂], corroborating previous reports that understory plants show no significant acclimation of quantum yield to elevated [CO₂] but show a reduction in the light compensation point via a decrease in photorespiration (Osborne et al. 1997, Singsaas et al. 2000). There was also a seasonal decline in photosynthetic enhancement by elevated [CO₂] between early and late summer (cf. DeLucia and Thomas 2000). DeLucia and Thomas (2000) found little evidence to support the hypothesis that resources from carbon fixation processes are reallocated to light-harvesting processes in leaves of plants grown at elevated [CO₂] and low irradiances (Sage 1994, Medlyn 1996).

Our study of the long-term effects of elevated [CO₂] confirms the short-term effects of elevated [CO₂] found by DeLucia and Thomas (2000). Thus, we found the greatest response to elevated [CO₂] at saturating light and a strong seasonal effect on the enhancement of photosynthesis by elevated [CO₂] that was associated with reductions in photosynthesis and foliar N, but we found no effect of long-term exposure to elevated [CO₂] on apparent quantum yield or on the ratio of Jₘₐₓ to Vₖₐₜₐₜₜ. These data indicate that, over the seven years of exposure to elevated [CO₂], there were few acclimatory responses by these species that increased the efficiency of the photosynthetic processes in the shaded forest understory. The primary effect of elevated [CO₂] on photosynthesis is to decrease competitive inhibition by oxygen at the binding site of Rubisco, thereby decreasing photorespiration (Drake et al. 1997) and the light compensation points. The main difference between our study and the study by DeLucia and Thomas (2000) was a lack of consistent rank order of the stimulatory effect of elevated [CO₂] on photosynthesis of the four understory species. For example, in our study, elevated [CO₂] had the greatest effect on the photosynthetic rates of A. rubrum, whereas DeLucia and Thomas (2000) found that C. canadensis had the greatest enhancement of photosynthesis by elevated [CO₂]. In addition, we found lower concentrations of foliar N across the four species than DeLucia and Thomas (2000), a surprising result given the dry months prior to the study of DeLucia and Thomas (2000) before measurements were taken in 1997 and 1998 and the relatively wet months prior to the measurement period of our study in 2003 (Figure 1).

The progressive N limitation hypothesis predicts a diminished response of plant productivity to elevated [CO₂] as N availability decreases because of the increased nutrient demands of greater plant biomass production (Luo et al. 2004). Reductions in photosynthetic capacity and foliar N in plants that are grown in long-term elevated [CO₂] are an important early diagnostic at the leaf level of potential progressive N limitations caused by increased atmospheric [CO₂]. It is clear from many studies completed in greenhouses and controlled environment growth chambers that limiting N reduces plant response to elevated [CO₂] (Curtis and Wang 1998). We examined the photosynthetic responses of four understory tree species to seven years of exposure to elevated [CO₂] and found no reduction in foliar N and no loss in the enhancement of photosynthesis. Thus, after seven years of elevated [CO₂] treatment in the Duke Forest FACE experiment, we see little evidence of progressive N limitation in the leaf-level processes of these four species of understory trees.

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References


