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**Estimating Net Photosynthesis and Productivity of a Loblolly Pine
Forest Grown with Carbon Dioxide Enrichment**

Clint J. Springer

**Dissertation submitted to the
Eberly College of Arts and Sciences
at West Virginia University
in partial fulfillment of the requirements
for the degree of**

**Doctor of Philosophy
in
Environmental and Evolutionary Biology**

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2004**

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NPP**

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Clint J. Springer

Abstract

I examined the long-term effects of elevated CO₂ on the leaf chemistry and photosynthesis of four species growing in the understory and two species growing in the overstory at the Duke Forest FACE experiment. I then used these measurements to parameterize a process based forest productivity model, PnET-II, in order to model the net primary productivity of the portions of the forest growing under ambient and elevated CO₂. Finally, I performed a greenhouse study that examined the effects of elevated CO₂ and water availability on the growth and biomass allocation of loblolly pine seedlings from four distinct geographic locations. At the Duke FACE experiment I found a continued stimulation of photosynthesis with elevated CO₂ in each species I measured. However the effect of elevated CO₂ on photosynthesis of these species did depend on canopy position, season, and year. I observed no CO₂-induced changes in leaf chemistry or morphology. Modeled estimates of net primary productivity of the Duke Forest were in good agreement with those measured at the Duke FACE experiment. Estimates of net primary productivity of the portions of the forest grown under elevated CO₂ were much greater than the estimates of the portions of the forest grown under ambient CO₂. I also found that future climatic conditions expected in North Carolina, USA should alter forest net primary productivity overall but would not alter the response of forest productivity to elevated CO₂. In the study of the response of loblolly pine seedlings from different geographic locations to elevated CO₂, I found significantly higher biomass in elevated CO₂-grown seedlings but I found no evidence of provenance specificity in the growth response to elevated CO₂. Decreased water availability did decrease the plant growth and alter biomass allocation irrespective of geographic location and growth CO₂ concentration. However, I also concluded that a more detailed analysis is needed with respect to provenance specific responses of loblolly pine to variables of climate change such as CO₂ and water availability. Overall my studies revealed that the stimulation of photosynthesis and productivity at the Duke Forest FACE experiment has persisted into the latter years of the experiment and, with no changes in leaf chemistry, no loss of stimulation is expected.

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Chapter 1
General Introduction

Fossil fuel combustion and land use conversion have increased atmospheric carbon dioxide (CO₂) concentrations from approximately 280 μmol mol⁻¹ since the beginning of the industrial revolution to a current concentration of approximately 375 μmol mol⁻¹ CO₂. Final projections of future atmospheric CO₂ concentrations are uncertain and constantly changing; however, a doubling of the pre-industrial revolution value is expected some time in the mid-21st century (IPCC 2001). This doubling in CO₂ concentration and changes in the concentrations of other “greenhouse” gases in the atmosphere are expected to drive a concomitant 3-7 °C increase in global mean temperature and cause significant alterations to regional precipitation patterns (IPCC 2001).

Increased atmospheric CO₂ typically increases photosynthesis and productivity of plants when other factors, such as water and nutrient availability are not limiting (Bazzaz 1990). This is especially true for plants which utilize the C₃ photosynthetic pathway (Ward and Strain 1999), because CO₂, a primary substrate of photosynthesis, is typically available in sub-saturating quantities (Drake et al. 1997). Photosynthesis, or gross primary productivity, represents the uptake side of the global carbon (C) cycle, whereas respiration returns CO₂ to the atmosphere. The difference between uptake of CO₂ and the return of CO₂ to the atmosphere by plants in terrestrial ecosystems is known as terrestrial net primary productivity (*NPP*) (Schlesinger 1997). Because the uptake of carbon from the atmosphere is increased by elevated atmospheric CO₂, the net primary productivity of many ecosystems is also expected to increase in the future.

Forest ecosystems account for ~80% of annual terrestrial *NPP* (Schlesinger 1997). Because of the important role of forests in the global C cycle, developing reliable predictions about future forest productivity under increased atmospheric CO₂ and the associated changes in

the physical environment that control ecosystem productivity have been given a high priority by many scientists and policymakers. However, the ability to predict future concentrations of atmospheric CO₂ and ecosystem productivity rely heavily on our understanding of the physiological, biochemical, and biogeochemical process that control the response of forest ecosystem *NPP* to elevated atmospheric CO₂. Development of Free-Air Carbon Dioxide Enrichment (FACE) technology and its implementation at the FACTS-I Duke Forest FACE experiment (Hendrey et al. 1999) allows examination of the responses of an intact loblolly pine forest to elevated CO₂ while operating under the full suite of biotic and abiotic processes of a forest ecosystem. Since fumigation began in 1996, the Duke Forest FACE experiment has produced numerous studies on the response of forest ecosystems to elevated CO₂ from the molecular to the ecosystem level. However, after several years of CO₂ treatment at the Duke Forest FACE study, large uncertainties remain regarding the effects of CO₂ enrichment on carbon budgets of forest ecosystems. The primary objectives of this study are to provide a more complete understanding of the photosynthetic response of trees growing throughout the forest canopy at the Duke FACE experiment to elevated CO₂ and to provide a modeled estimate of the net primary productivity of the portion of the loblolly pine forest growing in both ambient and elevated CO₂ environments using PnET-II, a process-based forest productivity model (Aber and Federer 1992).

Modeled estimates of NPP using PnET-II rely on a fundamental relationship between net photosynthesis and leaf N concentrations (Field and Mooney 1986, Evans 1989, Evans and Seemann 1989, Reich et al. 1997). Thus, in order to use this model for the Duke Forest FACE study, I established these relationships in both the ambient and elevated CO₂-grown portions of the Duke Forest FACE experiment. These relationships were measured using the two dominant

overstory species and 4 dominant understory species in the Duke FACE experiment and were measured across multiple years to examine season-to-season and year-to-year variability.

Because of the environmental heterogeneity of the Duke FACE experiment, it was also necessary to determine if the response of photosynthesis to elevated CO₂ was affected by other environmental variables present in a long-term CO₂ enrichment experiment located in a natural field setting. Therefore, I examined several factors that may contribute to variation in the response of photosynthesis to elevated CO₂ in this experiment. The first factor I examined was a time-dependent loss of photosynthetic stimulation by elevated CO₂ that has been observed in many long-term CO₂ enrichment experiments. This decline in photosynthetic response to elevated CO₂ is typically associated with a decline in foliar N concentration. I performed an experiment to determine if either a decline in the photosynthetic response to elevated CO₂ or a CO₂-induced reduction in foliar N concentration had occurred after six years of CO₂ treatment. To complete this experiment, I measured photosynthesis-foliar N relationships at two common CO₂ concentrations over three measurement periods vertically through the forest canopy. I then used the comparison of these relationships measured at common CO₂ concentrations to detect if a loss of photosynthetic capacity had occurred in elevated CO₂-grown trees and if this loss of capacity was linked to changes in foliar N concentration. I hypothesized that after 6 years of CO₂ enrichment I would observe a decline in the photosynthetic capacity of elevated CO₂-grown trees versus ambient CO₂-grown trees and this decline would be related to a reduction in foliar N concentration.

The second environmental factor contributing a large amount of variation in the photosynthetic response to elevated CO₂ is the effect of the highly dynamic, and unpredictable, light environment in the forest understory on the CO₂ response of photosynthesis of species

growing in the forest understory. In this experiment I carried out a detailed analysis of the physiological mechanisms that control photosynthesis with respect to CO₂ and light availability. I then compared the results of this study to those of a study carried out in the first year of the CO₂ enrichment at the Duke FACE experiment using similar methods to understand the changes that may have occurred in the mechanisms controlling understory photosynthesis after 7 years of CO₂ enrichment. I hypothesized that after 7 years of CO₂ enrichment I would observe a change in the investment of resources in the leaf toward light harvesting and away from CO₂ uptake because of the decrease in CO₂ limitation of photosynthesis found in an elevated CO₂ environment and that this reinvestment would lead to a loss of photosynthetic stimulation by elevated CO₂.

The effects of two other factors, genetic variation and water availability that may contribute to the variability found in the response of tree growth to elevated CO₂, were examined simultaneously in a greenhouse experiment. This study investigated the biomass production of loblolly pine seedlings from four distinct geographic locations and three levels of water availability. In this study I hypothesized that I would observe a strong genotype by environment interaction where seedlings originating from wet areas would show a greater response to elevated CO₂ when grown under high water availability and seedlings originating from drier climates would show a greater response to elevated CO₂ when grown under drought conditions.-

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CHAPTER 2:

Photosynthesis and foliar nitrogen relationships after six years of CO₂ enrichment at the Duke Forest FACE experiment

Abstract

In this study I examined the effects of elevated CO₂ on the relationships between light-saturated photosynthesis (A_{sat}) and foliar nitrogen on an area basis (N_a) through the canopy of the Duke FACE experiment after six years of CO₂ treatment. Light-saturated photosynthesis and foliar N were measured on two overstory tree species and four understory tree species in ambient and elevated CO₂ FACE rings during early and late summer of 1999, 2001, and 2002. Net photosynthetic rates of every species examined in the overstory and understory were stimulated by elevated CO₂ at each measurement date. I found no effect of elevated CO₂ on foliar N_a of any of the species. The slope of the A_{sat} - N relationship was 81% greater in elevated CO₂ than in ambient CO₂ when averaged across all sample dates, which reflected a differential CO₂ effect at the top and bottom of the forest canopy on photosynthesis. A_{sat} - N relationships in both ambient and elevated CO₂-grown trees were compared at two common CO₂ concentrations during late summer 2001 and both measurement periods in 2002 to determine if the stimulatory effect of elevated CO₂ on photosynthesis had diminished over time. At all three sample times, neither the slopes nor the y-intercepts of A_{sat} - N relationships of ambient and elevated CO₂-grown trees differed when measured at common CO₂ concentrations. Thus, with the observation that foliar N was not affected by growth in elevated CO₂, I concluded that, after six years of CO₂ enrichment, overstory and understory trees growing at the Duke Forest FACE experiment show a sustained stimulation of photosynthesis by elevated CO₂. These relationships between foliar N and photosynthesis indicate that any alterations of N availability under elevated CO₂ will have a significant impact on the response of photosynthesis to elevated CO₂.

Introduction

As atmospheric CO₂ enrichment experiments increase in complexity from single plants in pots to intact forest ecosystems using FACE technology, the difficulty in determining the photosynthetic responses to elevated CO₂ also increases. For example, the photosynthetic responses of trees in forest ecosystems exposed to CO₂ enrichment exhibit a large amount of 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 the bottom of the canopy may respond differentially to elevated CO₂ (Herrick and Thomas 1999, Kubiske et al. 2002). In addition, the relative responses to CO₂ enrichment differ between 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 when determining the CO₂ enhancement of photosynthesis because approximations of future forest productivity rely on these values.

Another difficulty in predicting the response of photosynthesis to elevated CO₂ involves the common observation in long-term studies that the degree of photosynthetic stimulation by CO₂ enrichment sometimes declines over time. Several analyses of elevated CO₂ experiments have shown an approximate 20% reduction in the CO₂-stimulation of photosynthesis after long-term exposure to elevated CO₂ compared to short-term exposure (Gunderson and Wullschleger 1994, Medlyn et al. 1999). The loss of stimulation is typically characterized by a decline in leaf nitrogen and rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), which leads to the reduction of photosynthetic capacity (Long and Drake 1991, Stitt 1991, Bowes 1993, Sage 1994, Norby et al. 1999). Many forest ecosystems are N-limited (Vitousek and Howarth 1991) and greater growth rates and increased resource demands of plants growing in elevated CO₂ may

exacerbate nutrient limitations (Ingestad and Stoy 1982). Consequently, a reduction in the response of photosynthesis to elevated CO₂ may be a common occurrence in ecosystems that have a low soil N availability (Radoglou et al. 1992, Tissue et al. 1993, El Kohen and Mousseau 1994, Sage 1994, Curtis et al. 1995), such as piedmont loblolly pine forests (Piatek and Allen 2000, Richter et al. 2000).

One method that examines the response of photosynthesis to elevated CO₂ while including the variation in foliar N concentration throughout the forest canopy is to use the relationship between light-saturated photosynthesis and foliar N concentration (A_{sar} -N relationship). This method incorporates the variation caused by the light gradient through a canopy and can be used to examine differential species effects or the canopy as a whole. The A_{sar} -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 using both deciduous and evergreen tree species found a strong stimulation by elevated CO₂ (Peterson and CMEAL participants 1999). In addition, the A_{sar} -N relationship is indicative of photosynthetic capacity since foliar N concentrations strongly correlate with rubisco activity and electron transport (Terashima and Evans 1988), including plants grown in ambient and elevated CO₂ (Terashima and Evans 1988, Medlyn et al. 1999, Herrick and Thomas 2001). A_{sar} -N relationships of plants grown at different CO₂ concentrations but measured at a common CO₂ concentration may be used as a diagnostic tool to detect CO₂-related reductions in photosynthetic enhancement before employing a more time consuming and labor intensive examination of photosynthetic down regulation by means of CO₂ response curves (von Caemmerer and Farquhar 1981, Farquhar and Sharkey 1982, Sharkey 1985).

Loblolly pine trees growing in the prototype ring of the Duke Forest FACE experiment showed a diminished growth response to elevated CO₂ after four years of CO₂ treatment and it was concluded that this loss of stimulation by CO₂ was related to nutrient limitations (Oren et al. 2001). In the replicated Duke Forest FACE experiment, Finzi et al. (Finzi et al. 2002) found that the demand for N by trees under CO₂ enrichment exceeded the rate of N mineralization. These results suggest that we might expect a reduction in the stimulation of photosynthesis by elevated CO₂ in the replicated Duke Forest FACE experiment after six years of treatment. The objective of this study was to examine the effects of elevated CO₂ on the A_{sat} -N relationships through the canopy of the loblolly pine forest and to determine if the stimulatory effects of elevated CO₂ on photosynthesis have been sustained at the Duke Forest FACE experiment. I hypothesized that the slope of the A_{sat} -N relationship would be increased by CO₂ enrichment, indicative of greater nitrogen use efficiency by the tree species grown under elevated CO₂. Light-saturated photosynthetic rates (A_{sat}) and foliar N of two overstory and four understory tree species were measured at their growth CO₂ concentrations during the early summer and late summer of 1999, 2001, and 2002. In 2001 and 2002, A_{sat} -N relationships were compared at reciprocal CO₂ concentrations to determine if the stimulatory effects of elevated CO₂ had been reduced. I hypothesized that reduced foliar N in plants grown with elevated CO₂ would lead to a shift in the origin of the A_{sat} -N relationship when the relationships of the ambient and elevated CO₂ treatments were compared at similar CO₂ concentrations. However, a reduction in the stimulation of photosynthesis by elevated CO₂ without a concurrent loss of foliar N would lead to a reduction in the y-intercept of the A_{sat} -N relationship for plants grown in elevated CO₂.

Materials and Methods

Duke Forest Free-Air CO₂ 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 (*Pinus taeda* L.) plantation planted in 1983 after the forest was clear-cut in 1979. Although this forest is a plantation, it has had no management measures to prevent the growth of other tree species. Loblolly pine dominates the overstory, but there is a significant number of sweetgum trees (*Liquidambar styraciflua* L.) growing into the overstory and approximately 50 other woody species located mostly in the forest understory. The forest is growing on nutrient-poor, clay-rich Alfisols of the Enon series that is typical of many upland areas in the southeastern United States.

Six 30 m diameter experimental rings were established within the forest. Three of these rings are ambient controls and three are replicate elevated CO₂ treatments. Each FACE ring consists of 32 pipes that extend from the forest floor through the canopy and deliver a controlled amount of CO₂ 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 on the forest that occurs during operation. From August 1996 through the period of this study (summer 2002), the forest received 24 h d⁻¹ CO₂ fumigation except on days when the air temperature was below 5°C for more than one hour. During the first five years of the experiment (1997-2002), the daytime mean CO₂ concentration of the elevated rings was 572 µl l⁻¹ and the ambient rings was 376 µl l⁻¹. For a detailed description of the Duke Forest FACE experiment see Hendrey et al. (Hendrey et al. 1999).

Gas Exchange and Leaf Chemistry

Three overstory loblolly pine, two overstory sweetgum trees, and three trees from each of four species growing in the understory (sweetgum, redbud (*Cercis canadensis* L.), pignut 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 CO₂ concentration (A_{sat} , 380 or 580 $\mu\text{l l}^{-1}$ CO₂) on current year needles of loblolly pine and fully-expanded leaves of hardwood species during early summer (June-July) and late summer (August-September) of 1999, 2001, and 2002 with an open flow infrared gas analyzer equipped with an attached red-blue light source (LI-6400, LI-COR, Lincoln, NE USA). Measurements were made with 1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD) in the overstory and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD in the understory. During the late summer measurement period of 2001 and both measurement periods of 2002, light-saturated net photosynthetic rates were also measured at the reciprocal growth CO₂ concentrations, either 380 $\mu\text{l l}^{-1}$ CO₂ (A_{380}) or 580 $\mu\text{l l}^{-1}$ CO₂ (A_{580}). Photosynthesis was recorded when leaves had reached steady-state conditions with saturating light intensity. Needles and leaves of overstory loblolly pine and sweetgum trees were measured in upper (full sun), middle, and lower (shade) regions of the canopy. Measurements were made between 1000 h and 1600 h on sunny days in order to minimize diurnal effects. Minimum air temperatures during the sample period ranged from 13 to 19°C and maximum temperatures ranged from 30 to 34°C. During the 1999 and 2001 sample periods soil moisture averaged ~21% (Schafer et al. 2002). However, 2002 was much drier as the soil moisture during the two sample periods averaged only ~15% (H. Kim, unpublished data).

After gas exchange measurements were completed, each leaf was harvested and assayed for total N on a mass basis ($[N]_m$) and on a 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 (LM_a , g m⁻²) was calculated as the ratio of leaf dry mass to photosynthetic surface area.

Statistical Analysis

Leaf-level morphological and physiological measurements were analyzed for each species separately in this study. The two overstory species were tested individually for statistical significance using a four-way analysis of variance (ANOVA, $\alpha=0.05$) with growth CO₂ concentration (CO₂), canopy position, season, and year as the main effects. For leaf-level analyses, canopy position included only the upper (full sun) and lower (shade) measurements. In the understory, leaf-level measurements were analyzed using a three-way ANOVA ($\alpha=0.05$) for each species. The main effects in the understory statistical model were CO₂ concentration, season, and year and these effects were considered fixed.

A_{sat} - N relationships for each measurement date and an A_{sat} - N relationship across all measurement dates were calculated using photosynthesis and foliar N measured on a leaf area basis from all six tree species using least squares linear regression analysis. Heterogeneity of slopes of these relationships due to growth CO₂ concentration (C_a) or measurement date (D) was examined using an analysis of covariance ($\alpha=0.05$) with foliar N ($[N]_a$) concentration as the covariate, where a significant CO₂ x $[N]_a$ interaction would indicate a significant difference in the slope of the A_{sat} - N relationships, and provide evidence for adjustment in the photosynthetic nitrogen use efficiency of elevated CO₂-grown trees. Alternatively, a significant difference in

the main effect of CO₂ concentration would simply indicate only an overall CO₂ induced adjustment of photosynthesis that is unrelated to foliar N concentration. Furthermore, a significant $C_a \times [N]_a \times D$ interaction would indicate that the effect of CO₂ on the $A_{sat}-N$ relationship was dependent upon measurement date.

In order to determine whether the stimulatory effects of elevated CO₂ on photosynthesis had declined over time, $A_{sat}-N$ relationships calculated at common CO₂ concentration were analyzed using the same fixed and interactive effects as above. Significant differences in the CO₂ \times $[N]_a$ interactive effect would indicate a physiological adjustment in foliar N concentration that may impact the photosynthetic response to CO₂ enrichment. A significant difference in the main effect of CO₂ concentration (y-intercept of the relationship) would indicate a loss of photosynthetic capacity without a reduction or adjustment in foliar N concentration.

Results

Net Photosynthesis

Averaged across all measurements periods, elevated CO₂ strongly stimulated light-saturated net photosynthesis (A_{sat}) of loblolly pine (44%, $P < 0.0001$, Table 2.1) and sweetgum trees (40%, $P = 0.005$, Table 2.1) across both canopy positions in the forest overstory of the Duke Forest FACE experiment. A_{sat} of both overstory species was significantly higher in sun leaves than in shade leaves and the stimulation of photosynthesis for sweetgum trees by elevated CO₂ depended upon the position of the leaf in the overstory canopy ($P = 0.02$). Elevated CO₂ increased A_{sat} of sweetgum sun leaves approximately 60% whereas shade leaves were only stimulated by 37%. In loblolly pine, there was only a statistical trend for a greater stimulation of A_{sat} by CO₂ enrichment ($P = 0.095$). A_{sat} of loblolly pine sun needles was stimulated 48% by

elevated CO₂ compared to a 27% stimulation of A_{sat} in shade needles. For both loblolly pine and sweetgum, A_{sat} varied season to season (pine, P<0.0001; sweetgum, P=0.0247) and year to year (pine, P=0.0049; sweetgum, P=0.025) but the stimulation of A_{sat} by elevated CO₂ did not vary due to year or month in either species (Table 2.1).

In the Duke forest FACE understory A_{sat} was stimulated by elevated CO₂ in all four species measured (Table 2.2). The greatest stimulation of A_{sat} by CO₂ enrichment in the understory was for redbud leaves (61%, P=0.02) and the least stimulation was for red maple leaves (24%, P=0.049). A_{sat} of understory hickory and sweetgum leaves was stimulated by 50% and 44%, respectively, with CO₂ enrichment. The stimulation of A_{sat} by elevated CO₂ did not vary with season or year in any of the four understory species measured.

Leaf Morphology and Chemistry

Elevated CO₂ did not significantly affect foliar N concentrations of loblolly pine needles or sweetgum leaves in the sun or the shade canopy positions during any sample period of this study (Table 2.3). In addition, elevated CO₂ had no effect on leaf mass per unit area (LM_a) of these two species in the forest overstory. In the understory, LM_a was increased by CO₂ enrichment in all species except for hickory (Table 2.4). The largest stimulation of LM_a by elevated CO₂ was only 12% and was observed in understory-grown sweetgum trees. However, neither mass nor area based foliar N concentrations varied between ambient and elevated CO₂ in any of the understory species examined in this study.

Foliar N concentrations on a mass basis of overstory loblolly pine and sweetgum trees were not affected by canopy position. In both species, however, LM_a 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 LM_a ($P < 0.0001$) and 58% N_a ($P < 0.0001$) than shade needles when averaged across all seasons and years (Table 2.3). For overstory sweetgum trees, sun leaves of had 65% greater LM_a ($P = 0.017$) and 84% greater N_a ($P < 0.001$) than shade leaves when averaged across all measurement periods (Table 2.3).

In all of the understory species, LM_a and N_a increased between early summer and late summer in all species (Table 2.4). 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 2.4). In 2002, no seasonal change in N_m was observed for any of the species.

Net Photosynthesis-Nitrogen Relationships

A_{sat} - N relationships were developed by regressing light-saturated net photosynthesis measured at the growth CO_2 concentrations on foliar N concentration, using the two most dominant tree species in the forest overstory as well as the four tree species in the understory found in the greatest abundance in the ambient and elevated CO_2 FACE rings. Elevated CO_2 stimulated the A_{sat} - N relationship at each measurement date in 1999, 2001, and 2002 and the stimulation of the A_{sat} - N relationship by elevated CO_2 was not significantly altered by season or year of measurement (Table 2.5, Figure 2.1). Elevated CO_2 increased the slope of the A_{sat} - N relationships by approximately 81% ($CO_2 \times N$, $P = 0.005$) when calculated across all species and measurement dates (Table 2.5, Figure 2.2).

The slope of the A_{sat} - N relationships of ambient and elevated CO_2 -grown plants measured at common CO_2 concentrations were not significantly different when either $380 \pm 1 \mu\text{I}^1$ ($P = 0.556$) or $580 \pm 1 \mu\text{I}^1$ CO_2 ($P = 0.887$; Table 2.6) were used as common CO_2 concentrations (Figure 2.3). I

also found no significant changes in the y-intercepts of ambient or elevated CO₂-grown trees when relationships were determined at 380 $\mu\text{mol mol}^{-1}$ ($P=0.214$) or 580 $\mu\text{mol mol}^{-1}$ ($P=0.551$). These results were consistent across all measurement dates (CO₂ x D; $P=0.76$).

Discussion

Leaf-level photosynthesis of overstory and understory tree species in the Duke Forest FACE experiment was stimulated by CO₂ enrichment, but a large amount of variation in this response was found across species and canopy position. Photosynthesis was especially responsive to foliar N_a with greater CO₂ enhancement at higher N_a and, as a result, elevated CO₂ increased the slope of the $A_{\text{sat}}-N$ relationship by 81% when averaged across all sample dates (Figure 2.2). This increase by elevated CO₂ is remarkably close to the 74% increase in slope of the $A_{\text{sat}}-N$ relationship found by a meta-analysis of 10 studies examining the responses of pine and hardwood trees grown with CO₂ enrichment (Peterson *et al.* (1999). Furthermore, Takeuchi *et al.* (2001) found that the slope of the $A_{\text{sat}}-N$ relationship through the canopy of *Populus tremuloides* was increased 59% by elevated CO₂. The increase in slope of the $A_{\text{sat}}-N$ relationship of elevated CO₂-grown trees indicates an increase in photosynthetic nitrogen-use efficiency (PNUE) since there was a strong enhancement of leaf level photosynthesis but no reduction in leaf N by CO₂ enrichment. However, the increase in slope of the $A_{\text{sat}}-N$ relationship by elevated CO₂ also indicates that the stimulation in PNUE is not constant and reflects differential effects of CO₂ on photosynthesis in the sun and shade portions of the forest canopy.

Model estimates predict an increase in forest productivity with elevated CO₂. However, this increase relies on a sustained stimulation of photosynthesis by elevated CO₂ (Reynolds *et al.* 1996). This study using two overstory and five understory tree species clearly shows a sustained

positive response of photosynthesis to elevated CO₂ after 6 years of treatment in the Duke Forest FACE experiment. I found very little change in the CO₂ stimulation of photosynthesis across season or year despite the year 2002 being one of the driest summers on record for piedmont North Carolina (Waple and Lawrimore 2002). Furthermore, the effect of CO₂ on photosynthesis of loblolly pine needles growing at the top of the canopy was very similar in magnitude to the CO₂ enhancement of photosynthesis measured during the first year of CO₂ treatment (Myers et al. 1999). Also, the percent stimulation of photosynthesis by elevated CO₂ for sun and 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).

In order to further determine whether the stimulatory effect of elevated CO₂ on photosynthesis had been reduced over time, I compared the long term response to CO₂ to an immediate change in CO₂ concentration by examining A_{max} - N relationships in the elevated and ambient rings at common CO₂ levels (Curtis et al. 2000). Reductions in the CO₂ response of photosynthesis are often associated with a nearly proportional reduction in foliar N (Curtis and Wang 1998) and, thus I hypothesized that reduced foliar N in plants grown with elevated CO₂ would lead to a shift in the origin of the A_{sat} - N relationship when the relationships of the ambient and elevated CO₂ treatments were compared at similar CO₂ concentrations. If, however, there was a reduction in the stimulatory effect of elevated CO₂ on photosynthesis without a concurrent loss of foliar N, as has been observed in senescing loblolly pine needles (Rogers and Ellsworth 2002), I expected a reduction in the y-intercept of the A_{sat} - N relationship for plants grown in elevated CO₂. I found that neither the slope nor the y-intercept of the A_{sat} - N relationships of ambient and elevated CO₂ rings measured at common CO₂ concentrations were affected by

growth CO₂ concentration at any of the three sample periods (Figure 2.3). I also found no evidence of a shift in the origin of the A_{sat} - N relationships of ambient and elevated CO₂ rings since I observed no CO₂-induced decline of foliar nitrogen in the overstory or understory tree species (Tables 2.3 and 2.4). 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 strong sustained drought of 2002 when the concentrations of foliar N of every species in the Duke Forest FACE experiment were low. On the other hand, the concentration 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)). Interestingly, with the exception of 2002, the values of [N]_m and [N]_a in overstory and understory tree species that I observed were very similar to those that have been 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 of these species have changed very little over the six years of this experiment.

In conclusion, I found no CO₂-induced changes in foliar nitrogen concentration nor loss of stimulation of photosynthesis in two overstory and four understory tree species in the Duke Forest FACE experiment after six years of CO₂ enrichment. By using A_{sat} - N relationships, I incorporated a large amount of variation associated with a complex forest stand, including light and foliar N gradients, seasonal effects, and different species, into the photosynthetic responses to CO₂ and scaling net photosynthesis to leaf N 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 increase in slope of the A_{sat} - N relationships of elevated

CO₂-grown trees implies a large increase in photosynthetic nitrogen-use efficiency and may contribute to an increase in nitrogen use efficiency to at the ecosystem level, as has been found at the Duke Forest FACE experiment (Finzi et al. 2002). These relationships between foliar N and photosynthesis indicate that the photosynthetic responses of forest trees to elevated atmospheric CO₂ may be significantly altered by changes in N availability brought about by increased demand for N with growth under elevated CO₂.

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Figure Legends:

Table 2.1. Light-saturated net photosynthesis of needles and leaves located at the top (sun) and bottom (shade) of *P.taeda* (PITA) and *L. styraciflua* (LIST) growing in the overstory at the Duke Forest FACE experiment under ambient (AMB) and elevated (ELV) CO₂. Each value is the mean of three treatment rings for both loblolly pine and sweetgum (± 1 SE) measured at the growth CO₂ concentration in the early and late summers of 1999, 2001, and 2002.

				A_{sat}	
				AMB	ELV
PITA	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)
LIST	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 2.2. Light-saturated net photosynthesis understory *A. rubrum* (ACRU), *C. glabra* (CAGL), *C. canadensis* (CECA), and *L. styraciflua* (LIST) growing in the understory at the Duke Forest FACE experiment under ambient (AMB) and elevated (ELV) CO₂. Each value is the mean of three treatment rings (± 1 SE) measured at the growth CO₂ concentration in the early and late summers of 1999, 2001, and 2002.

			A_{sat}	
			AMB	ELV
ACRU	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)
CAGL	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)
CECA	1999	Early	11.0(0.7)	18.4(2.7)
		Late	10.1(0.3)	14.8(2.7)
	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)
LIST	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 2.3. Leaf mass per unit area (LM_a), mass-based foliar N concentration ($[N]_m$) and area based foliar N concentration ($[N]_a$) at the top and bottom of the canopy of loblolly pine (PITA) and sweetgum (LIST) trees growing in the forest overstory 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 plots. Each value is the mean of three treatment plots (± 1 SE).

				LM_a		$[N]_m$		$[N]_a$	
				AMB	ELV	AMB	ELV	AMB	ELV
PITA	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)
LIST	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)

Table 2.4. Leaf mass per unit area (LM_a), mass-based foliar N concentrations ($[N]_m$) and area based foliar N concentration ($[N]_a$) of red maple (ACRU), hickory (CAGL), redbud (CECA), and sweetgum (LIST) 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 plots. Each value is the mean of three treatment plots (± 1 SE).

			LM_a		$[N]_m$		$[N]_a$	
			AMB	ELV	AMB	ELV	AMB	ELV
ACRU	1999	Early	32.7(1.9)	34.1(2.0)	22(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)
CAGL	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)
CECA	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)
LIST	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 2.5. 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.

			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
<i>All Years</i>	<i>All Dates</i>	AMB	4.59	1.36	0.52
		ELV	8.32	0.68	0.66

Table 2.6. Regression components of A_{sat} - N relationships measured at two common CO_2 concentrations in the forest canopy of the Duke Forest FACE experiment during 1999, 2001, and 2002 in early and late summer.

			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

Figure 2.1. Light-saturated net photosynthetic rates (A_{sat}) measured at the growth CO_2 concentration versus foliar N concentrations ($[\text{N}_a]$) of loblolly pine, sweetgum, red maple, hickory, and redbud grown under ambient CO_2 (closed circles, solid line) and elevated CO_2 (open circles, dashed line). Measurements were conducted in (a) early summer 1999, (b) late summer 1999, (c) early summer 2001, (d) late summer 2001, (e) early summer 2002, (f) late summer 2002. Regression equations of these relationships can be found in Table 2.5.

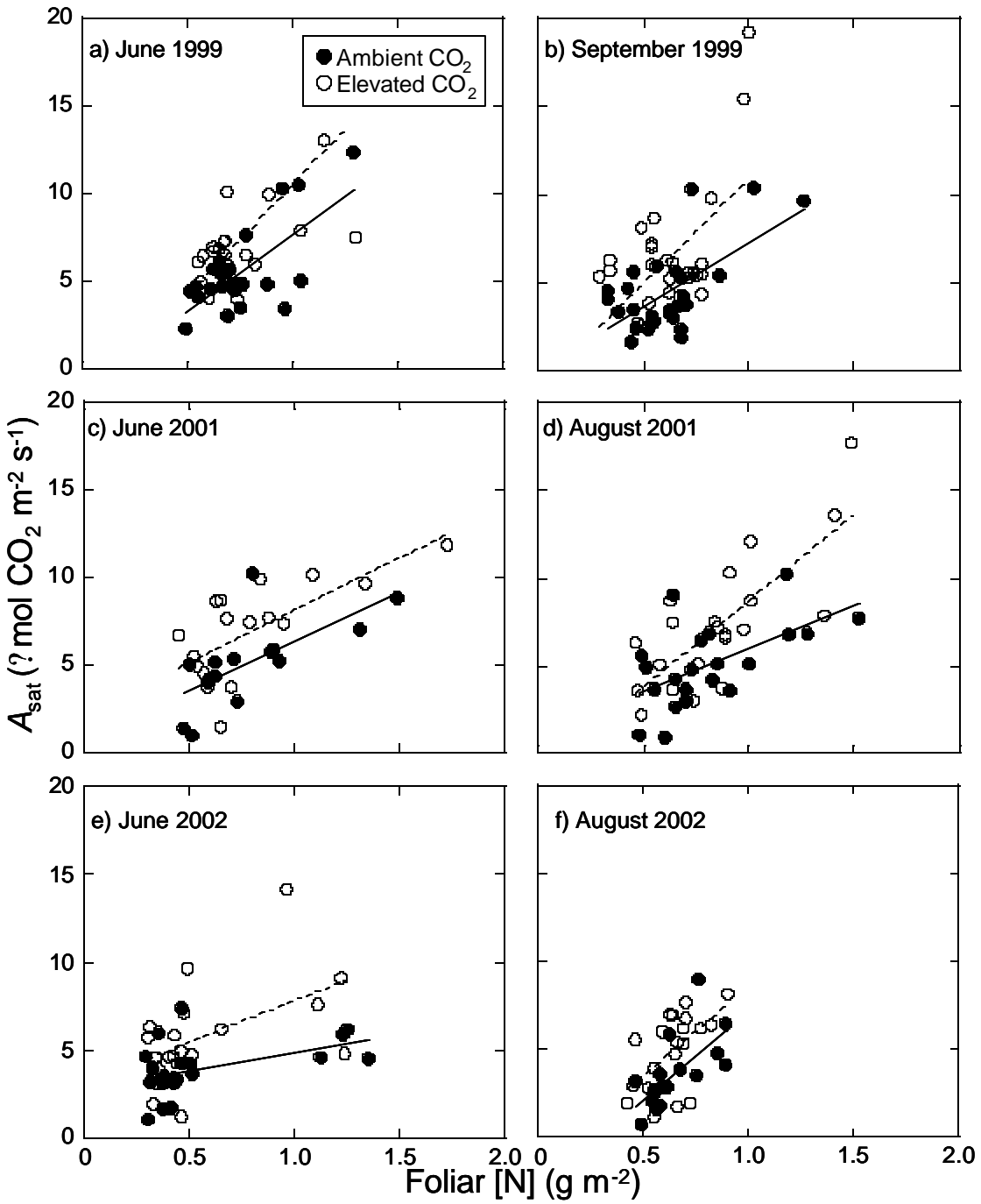


Figure 2.2. Light-saturated net photosynthetic rates (A_{sat}) measured at the growth CO_2 concentration versus foliar N concentrations ($[\text{N}_a]$) of loblolly pine, sweetgum, red maple, hickory, and redbud grown under ambient CO_2 (closed circles, solid line) and elevated CO_2 (open circles, dashed line). Data were pooled to calculate a single relationship for each CO_2 treatment across all measurement dates. Regression equations of these relationships can be found in Table 2.5.

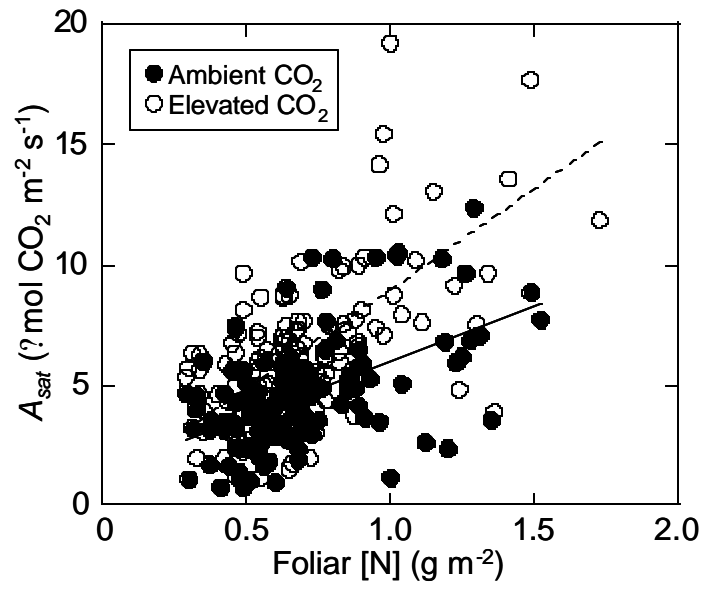
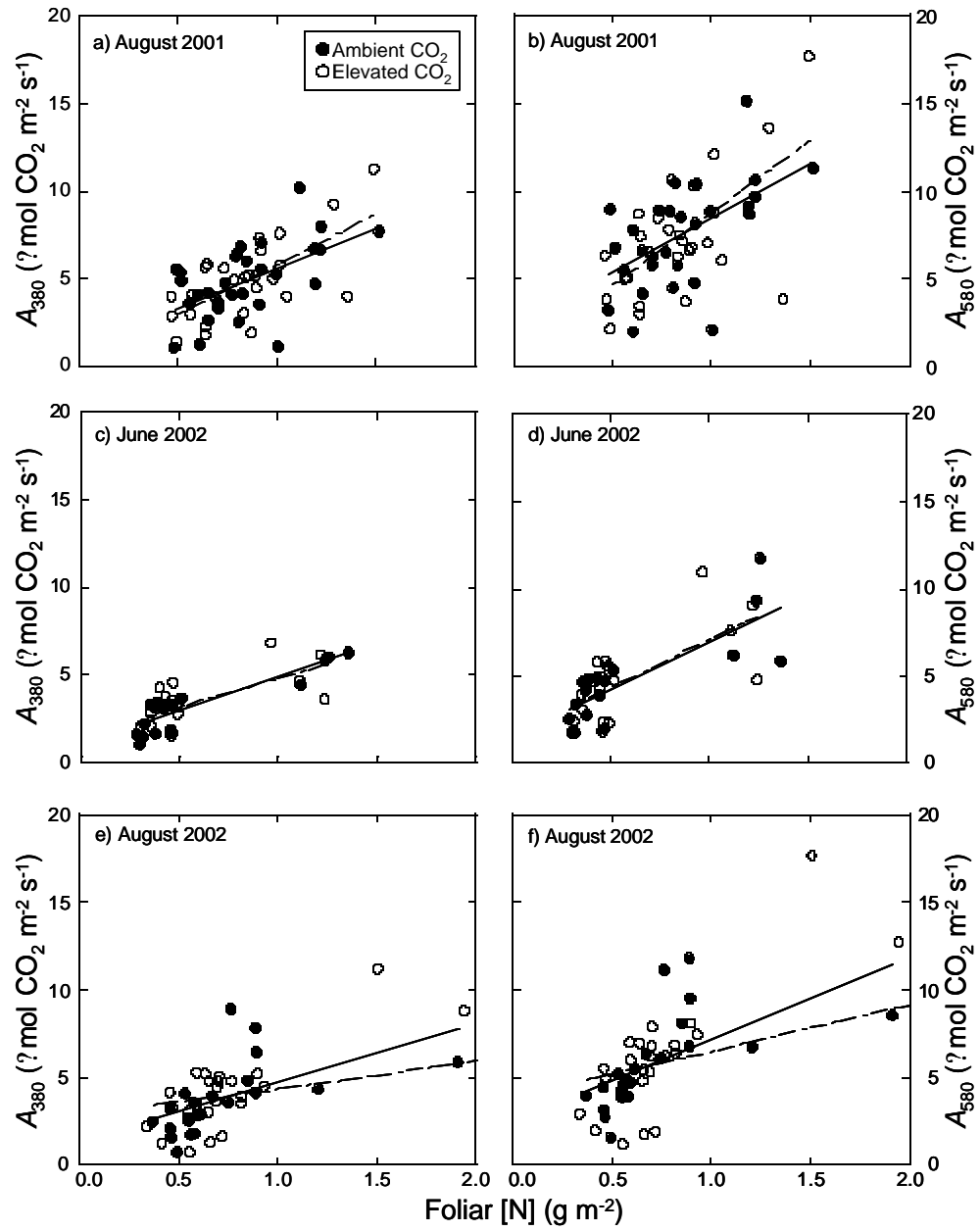


Figure 2.3. Light-saturated net photosynthetic rates (A_{sat}) versus foliar N concentrations ($[N_a]$) of loblolly pine, sweetgum, red maple, hickory, and redbud grown under ambient CO_2 (closed circles, solid line) and elevated CO_2 (open circles, dashed line). Measurements were made at two common CO_2 concentrations of $380 \mu\text{l l}^{-1} \text{CO}_2$ (A_{380}) and $580 \mu\text{l l}^{-1} \text{CO}_2$ (A_{580}) during late summer 2001 (a,b) and early summer 2002 (c,d) and late summer 2002 (e,f). Regression equations of these relationships can be found in Table 2.6.



CHAPTER 3:

Photosynthetic responses of four understory tree species after 7 years of CO₂ enrichment in the Duke Forest FACE experiment

Abstract

In this study I examined the photosynthetic response of four species of saplings growing in the understory of the Duke Forest FACE experiment after seven years of CO₂ enrichment. These same four species of saplings were measured in the first year of the Duke Forest FACE experiment and showed only seasonal fluctuations in acclimation of photosynthesis to elevated CO₂. I hypothesized that after seven years of CO₂ enrichment I would observe photosynthetic down-regulation in the saplings measured. I measured photosynthetic CO₂-response and light response curves, along with chlorophyll fluorescence, chlorophyll concentration, and foliar N to diagnose potential elevated CO₂-induced photosynthetic down-regulation twice during the summer of 2003. I found a continued stimulation of photosynthesis (A_{sat}) by elevated CO₂ in all four species measured. Elevated CO₂ stimulated the electron transport efficiency (J_{max}) of two of the species measured but did not alter the carboxylation efficiency ($V_{\text{C}_{\text{max}}}$). I found no effect of CO₂ enrichment on quantum yield measured by light curves, chlorophyll fluorescence, chlorophyll concentration, or foliar nitrogen concentration. Elevated CO₂ continues to stimulate photosynthesis in the understory of the Duke Forest FACE experiment after seven years of CO₂ enrichment. I found that the stimulation of photosynthesis was primarily driven by a reduction in the carbon limitation imposed on low-light grown plants, where plants acclimated by increasing the electron transport efficiency of leaves. This study represents one of the first long-term comparisons of photosynthesis of understory trees growing in elevated CO₂.

Introduction

Understory species experience a continuously changing, unpredictable light environment that can range from a small proportion of the incident solar radiation found at the top of the canopy to near direct sunlight (Chazdon and Pearcy 1991). Variability in the light environment in a forest understory typically limits the growth and photosynthesis of plant species more so than other environmental factors (Pearcy 1983, Denslow et al. 1990, Pacala et al. 1994, Kobe et al. 1995). Under low light conditions, understory leaves are light-limited and, when compared to sun leaves, shade leaves have excess capacity to fix carbon in the Calvin-Benson cycle because of an increased investment of foliar nitrogen in light harvesting processes rather than carbon fixation (Evans 1989). Understory species contribute a significant amount to the overall carbon balance of many forest ecosystems; any changes that may occur with increased atmospheric CO₂ could have an impact on forest succession and on the role of forests in the global carbon cycle in the future.

Increases in atmospheric CO₂ concentrations are likely to reduce carbon limitations and stimulate photosynthesis of understory species despite low light availability. Relatively few studies, however, have examined the photosynthetic responses of understory plant species to elevated CO₂ and very few have investigated these responses in a natural forest ecosystem (Osborne et al. 1997, Winter and Virgo 1998, Hattenschwiler 2001). In a review of the effects of elevated CO₂ on plants growing under non-optimal conditions, Poorter & 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 general statement about the response of plants to elevated CO₂ when growing in a light-limited environment. In forest understory species, most studies have observed increased photosynthesis and subsequent increases in biomass production

under elevated CO₂ (DeLucia and Thomas 2000, Naumburg and Ellsworth 2000, Hattenschwiler 2001, Poorter and Perez-Soba 2001).

Most studies investigating understory species responses to elevated CO₂ have been performed over short time periods but, long-term responses of understory species are not well understood. Many long-term CO₂ enrichment experiments report a time dependent decline in the photosynthetic response of plants to elevated CO₂ often referred to as photosynthetic down-regulation. This loss in photosynthetic enhancement by elevated CO₂ is sometimes accompanied by reductions in foliar N concentration and ribulose biphosphate carboxylase-oxygenase (rubisco) activity (Long and Drake 1991, Stitt 1991, Bowes 1993, Sage 1994, Norby et al. 1999). With increased atmospheric CO₂ and subsequent increased plant growth rates comes the possibility of increased resource demands, especially N, which may exhaust the available nutrients in the soil more rapidly than under current atmospheric CO₂ (Ingestad and Stoy 1982). As less N is available for photosynthetic processes, photosynthetic down-regulation may occur. Many forest ecosystems are N-limited (Vitousek and Howarth 1991) and this type of photosynthetic down-regulation might be a common occurrence in forests, such as piedmont loblolly pine forests, that have a low soil N availability (Radoglou et al. 1992, Tissue et al. 1993, El Kohen and Mousseau 1994, Sage 1994, Curtis et al. 1995). For example, a loss in the growth enhancement by elevated CO₂ in the Duke Forest FACE prototype ring was restored when N fertilizer additions were made to the plot (Oren et al. 2001). This suggests that photosynthetic down-regulation due to reduced foliar N may soon be observed in the fully-replicated Duke FACE study. Furthermore, future forest productivity models depend on the assumption of a sustained increase of photosynthesis by elevated CO₂ (Reynolds et al. 1996) and a complete

understanding of the photosynthetic response of not only overstory species to elevated CO₂ but also the photosynthetic response of understory species as well.

During the first year of the Duke Forest FACE experiment, DeLucia & Thomas (2000) examined the photosynthetic responses of four species of understory saplings to elevated CO₂. Elevated CO₂ stimulated photosynthesis of all species but a small degree of down-regulation of photosynthesis in the high CO₂ treatment was observed. These results, however, were inconsistent; they varied seasonally and observations of photosynthetic down regulation were explained by a long period of severe drought during the growing season prior to the measurements (DeLucia & Thomas, 2000). The current study re-examines the photosynthetic responses of the same four understory species (*Acer rubrum*, *Carya glabra*, *Cercis canadensis*, and *Liquidambar styraciflua*) after seven years of CO₂ enrichment at the Duke Forest FACE experiment. These measurements were made during early and late summer of 2003, one of the wettest years on record in piedmont, NC and after a winter ice storm removed approximately 26% of the canopy leaf area. To understand the long-term response of photosynthesis in these understory species to elevated CO₂, I measured photosynthetic light response curves, CO₂ response curves and parameters associated with chlorophyll fluorescence and concentration on at two times during the seventh growing season under CO₂ enrichment. I hypothesized that, as N becomes more limiting under elevated CO₂ due to increased demand by elevated CO₂-grown trees, I will observe photosynthetic-down regulation in the four species measured. I also hypothesized that increased light availability due to a loss in leaf area in the forest overstory that elevated CO₂-grown saplings may exhibit an up-regulation of photosynthesis.

Materials and Methods

Site Description

The Duke University FACE experiment is located in the Blackwood division of Duke Forest (35°97' N, 79°09' W) in a loblolly pine (*Pinus taeda* L.) plantation that has undergone no management measurements since establishment in 1983. The site consists of six 30 m diameter experimental FACE rings established within the forest. Three of these rings deliver ambient + 200 $\mu\text{l l}^{-1}$ CO₂ during daylight hours to the portion of the loblolly pine forest within the ring. The remaining FACE rings serve as ambient experiment control rings, which 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) provides a detailed description of the entire Duke Forest FACE experiment and its operational procedures. Since the beginning of CO₂ fumigation (1997-2003), the daytime mean CO₂ concentration of the elevated rings has been 576 $\mu\text{mol mol}^{-1}$ and in the ambient rings the daytime mean CO₂ concentration has been 376 $\mu\text{mol mol}^{-1}$. Saplings of the species *Acer rubrum* (ACRU), *Carya glabra* (CAGL), *Cercis canadensis* (CECA), and *Liquidambar styraciflua* (LIST) growing in the understory of Duke Forest FACE experiment were selected to perform a detailed analysis of the response of leaf gas exchange and fluorescence of understory tree species to elevated atmospheric CO₂.

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 measurements were made with an open-flow gas exchange system (LI-6400, LiCor Inc., USA) with an environmentally controlled cuvette. All gas exchange measurements were made between 1000 h and 1600 h (EST) on clear days. During gas exchange measurements neither leaf temperature, nor relative humidity significantly differed between the June ($T_{\text{air}}=24.8\pm0.6$ °C, RH=58.8 \pm 0.2 %) and September ($T_{\text{air}}=20.8\pm0.9$ °C, RH=58.1 \pm 0.3 %) measurement periods.

Photosynthesis measurements were recorded across nine incident irradiance levels between 0 and 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (photosynthetic-photon flux density, PPF) on one fully expanded leaf per sapling and were used to calculate photosynthetic-light response curves. A red-blue LED light source ($\lambda=650$ nm) attached to the LI-6400 supplied the incident irradiance. Cuvette CO_2 concentrations were held at 380 $\mu\text{mol mol}^{-1} \text{CO}_2$ for ambient grown saplings and 580 $\mu\text{mol mol}^{-1} \text{CO}_2$ for elevated CO_2 -grown saplings. Apparent quantum efficiency (Q_e), respiration (R_d), light saturated maximum photosynthesis (A_{sat}), and light compensation point (Γ), were estimated from light response curves using the Photosynthesis Assistant software package (Dundee Scientific Ltd., UK), which employs the calculations of Prioul & Chartier (Prioul and Chartier 1977).

The relationship between net photosynthesis (A) and calculated intercellular CO_2 concentration (C_i) was used to determine if acclimation of photosynthesis to elevated CO_2 had occurred in the measured saplings. Photosynthesis was measured over 10 external concentrations of CO_2 ranging between 50 and 1500 $\mu\text{l l}^{-1} \text{CO}_2$. Measurements were made with a saturating light level of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF. $A-C_i$ curves were analyzed with the two-factor leaf photosynthesis model of Farquhar *et al.* (Farquhar *et al.* 1980) with modifications by Harley *et al.* (Harley *et al.* 1992). Light-saturated carboxylation efficiency ($V_{c_{\text{max}}}$) of ribulose-

bisphosphate carboxylase oxygenase (rubisco) and the electron transport efficiency (J_{\max}) were calculated using the assumptions of Wullschleger (Wullschleger 1993).

A pulse-modulated fluorometer (PAM-2000, Walz, Germany) was used to measure the chlorophyll fluorescence of the upper surface of the same leaves used for gas exchange measurements. Attached leaves were turned over and dark adapted for approximately three minutes before exposure to a weak modulating light beam that allowed measurement of baseline fluorescence (F_o). The leaves were then exposed to a 0.6 s flash of saturating light to measure maximal fluorescence yield (F_m). The optimal efficiency of photosystem II (PS_{II} , F_v/F_m) was then calculated as $(F_m - F_o)/F_m$. After determination of F_v/F_m , the same leaf was exposed to four intensities of light provided by an actinic light source ranging from 25 to 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD in 60 s intervals. After each level of light intensity, a 0.6 s flash of saturating light was used to determine light-adapted maximum fluorescence yield (F_m'). Between each measurement leaves were exposed to 0.6 s of far-red light to estimate light induced adjustments of baseline fluorescence (F_o'). The quantum yield of PS_{II} ($\Phi_{PS_{II}}$) was then estimated using $(F_m' - F_o')/F_m'$ derived from Schreiber *et al.* (Schreiber *et al.* 1998).

Leaf Characteristics

Leaves used for gas exchange measurements were harvested for determination of specific leaf area (SLA), foliar N concentration on a mass ($[N]_{\text{mass}}$) and an area ($[N]_{\text{area}}$) basis, and chlorophyll concentration on a mass (Chl_{mass}) and area (Chl_{area}) basis. The mass of oven dried leaf disks of an area of 3.14 cm^2 were used to calculate SLA. These 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 as in Porra *et al.* (1989).

Statistics

All gas exchange, chlorophyll fluorescence measurements, and measurements of leaf morphology, with the exception of FPS_{II} data were analyzed using a three-way analysis of variance (ANOVA, $\alpha=0.05$) with growth CO_2 concentration (C), measurement date (D), and species (S) as the main effects and C X D, S X C, S X D, and C X D X S as the interactive effects. When necessary to agree with the assumptions of the ANOVA test, a log-transformation was performed to assure normality. The relationship between FPS_{II} and PPFD were evaluated using least squares linear regression analysis for each species individually. Heterogeneity of slopes of these relationships due to growth CO_2 concentration (C) was examined using an analysis of co-variance ($\alpha=0.05$) with PPFD as the covariate, where a significant C x PPFD interaction indicated a significant difference in the slope of the FPS_{II} -PPFD relationship measured.

Results

Light Response Curves

Elevated CO_2 stimulated light-saturated photosynthetic rates in all four species measured ($P=0.0005$, Figures 3.1 & 3.2, Table 3.1). The largest stimulation of A_{sat} (120%) was found in *A. rubrum* in June; however this stimulation decreased to 65% in September. *C. canadensis* also showed a decrease in the amount of stimulation of A_{sat} by elevated CO_2 from June to September, in which A_{sat} was stimulated by 67% in June and only by 28% in September. However, the stimulation of A_{sat} by elevated CO_2 remained relatively constant throughout the summer for *C.*

glabra (17-22%) and *L. styraciflua* (41%-52%). A_{sat} also significantly declined ($P=0.0021$) throughout the summer irrespective of species or CO₂ treatment (Table 3.1). Neither the apparent quantum efficiency (Q_e), respiration (R_d), nor the light compensation point (G) derived from the photosynthetic-light response curves differed significantly between ambient and elevated CO₂ or date in any of the four species (Table 3.1).

CO₂ Response Curves

Elevated CO₂-grown saplings showed no differences in $V_{c_{\text{max}}}$ in June or September (Table 3.2). However, $V_{c_{\text{max}}}$ of all species except *C. canadensis* did decline from June to September in both ambient and elevated CO₂. The $V_{c_{\text{max}}}$ of *A. rubrum*, *C. glabra*, and *L. styraciflua* declined the most (62-77%) from June to September, with the $V_{c_{\text{max}}}$ of *C. canadensis* only declining 33% across the same time period (Table 3.2). J_{max} was significantly higher under elevated CO₂ in *A. rubrum* ($P=0.04$) and *C. glabra* ($P=0.02$). *C. glabra* ($P=0.0005$) and *L. styraciflua* ($P=0.0002$) were the only species in which I observed a significant decrease in J_{max} from June to September (Table 3.2). I found no significant differences due to CO₂ treatment or date in the ratio of $V_{c_{\text{max}}}$ to J_{max} (Table 3.2) in any of the saplings measured.

Chlorophyll Fluorescence & Concentration

The F_v/F_m of elevated CO₂-grown saplings did not significantly differ from ambient CO₂-grown saplings in June or September (Table 3.3). Also, the relationships between Ψ_{PSII} and PPFD did not significantly differ between CO₂ treatments or dates, in any of the species measured (Figures 3.3 & 3.4). Area-based chlorophyll concentrations did significantly increase from June to September in all species, except *C. glabra* (Table 3.3). I observed the largest increase (28%) in Chl_{area} from June to September in *L. styraciflua* ($P=0.0045$) with *A. rubrum*

($P=0.04$) and *C. canadensis* ($P=0.08$) increasing approximately 21% each. Elevated CO₂ did not significantly affect the area or mass-based chlorophyll concentration, or the chlorophyll *a* to chlorophyll *b* ratios of any of the four species measured (Table 3.3).

Leaf Properties

Elevated CO₂ did not significantly alter SLA (Table 3.3) in any species. SLA did decrease slightly from early summer to late summer in all species measured except *L. styraciflua* where SLA slightly increased, however neither were statistically significant (Table 3.3).

Elevated CO₂ did not significantly alter [N]_{mass} or [N]_{area} in any species; however [N]_{mass} did significantly decrease in all species from June to September (Table 3.3). The largest decrease in [N]_{mass} was observed in *C. glabra* (22%, $P=0.0001$) and the smallest decrease in *A. rubrum* (16%, $P=0.0038$). When expressed on an area basis foliar N concentration was not significantly different between ambient and elevated CO₂ in June or September (Table 3.3).

Discussion

After seven years of CO₂ enrichment the stimulation of photosynthesis by elevated CO₂ of the four species of understory saplings measured was sustained (Table 3.1, Figures 3.1 & 3.2) when compared to the results of DeLucia and Thomas (2000) performed in the first year of the Duke FACE experiment. There was no evidence of photosynthetic down-regulation that might occur as a result of increased N limitation with growth in elevated CO₂. This result is surprising because in many long-term elevated CO₂ studies a loss in the enhancement of photosynthesis is observed (Curtis and Wang 1998). However, some studies have shown a sustained increase in the response of photosynthesis to long-term CO₂ enrichment (Herrick and Thomas 2001). Another unexpected result of the current study is the change in species that had the greatest

photosynthetic response to elevated CO₂. DeLucia & Thomas (DeLucia and Thomas 2000) found the greatest stimulation of photosynthesis in *C. canadensis*, whereas the largest stimulation of photosynthesis was found in *A. rubrum* during this study. Similarly, in another study, *A. rubrum* and *L. styraciflua* also showed a significant stimulation of photosynthesis by elevated CO₂ in the understory of the Duke FACE experiment (Naumburg and Ellsworth 2000). One explanation for this variation may be the dry months prior to the study of DeLucia & Thomas (DeLucia and Thomas 2000) before measurements were taken in 1997 and the relatively wet months prior to the measurement period of this study in 2003. Another possible explanation was the occurrence of a large ice storm which destroyed approximately 26% of the *Pinus taeda* canopy at the Duke FACE experiment in the winter of 2002. This disturbance event may have increased light availability enough to decrease the light-limitation of photosynthesis of the understory trees and possibly increased the available N through the increase in leaf litter during the winter of 2002. As in the previous study, I found that elevated CO₂ stimulated photosynthesis the most near saturating irradiances and if the loss in canopy mass increased the occurrence of direct sunlight reaching the understory this may be a partial explanation for the shift in species response observed from 1997 to 2003. As in DeLucia & Thomas (2000), I found no CO₂-induced differences in Q_e or Ψ_{PSII} in any of the species measured.

I observed no significant changes in the $V_{C_{max}}$ of any of the elevated CO₂-grown saplings in June or September. $V_{C_{max}}$ did significantly decrease 33 to 77% from June to September in all species irrespective of growth CO₂ concentration (Table 3.2). This decrease in $V_{C_{max}}$ can be attributed to a decrease in foliar N concentration from June to September (Table 3.3). DeLucia & Thomas (2000) found no difference in $V_{C_{max}}$ in June between CO₂ treatments, but did find a significant decrease in $V_{C_{max}}$ in September. This observed change in $V_{C_{max}}$ was attributed to the

persistence of a severe drought during the summer of 1997 at the Duke FACE experiment, which may have induced fall senescence earlier than in the current study. Our results also contrast those found in shade leaves of *A. saccharum* and *P. tremuloides*, where photosynthetic acclimation occurred and coincided with a decrease in $V_{C_{max}}$ and foliar N concentration (Takeuchi et al. 2001, Kubiske et al. 2002).

In the current study J_{max} significantly increased under elevated CO_2 in each species (Table 3.2), with the increase ranging from 18 to 33%. *A. rubrum* showed the largest increase at 33%, this increase in electron transport efficiency coincides with *A. rubrum* yielding the largest observed stimulation of A_{sat} by elevated CO_2 . Similarly, DeLucia & Thomas (2000) also observed a stimulation of J_{max} by elevated CO_2 , however this stimulation was only 13% across all of the species measured. The increase in J_{max} observed in the earlier study caused an overall 10% increase in the ratio of $V_{C_{max}}$ to J_{max} . However, in our study, because $V_{C_{max}}$ slightly increased and J_{max} significantly increased under elevated CO_2 , I observed no changes in the ratio of $V_{C_{max}}$ to J_{max} . Despite the higher electron transport efficiency of elevated CO_2 -grown saplings, I found no differences in F_v/F_m for any of the four species measured indicating no differences in the efficiency of PSII. Elevated CO_2 also did not significantly alter the concentration of chlorophyll in any of the saplings measured, but chlorophyll concentration did decrease from June to September in all species measured (Table 3.3).

I observed a sustained stimulation of the photosynthesis of *A. rubrum*, *C. glabra*, *C. canadensis*, and *L. styraciflua* growing in the understory of the Duke Forest FACE experiment after seven years of CO_2 enrichment. Relieving the carbon limitation imposed on shade plants by increasing the atmospheric CO_2 concentration primarily controlled this stimulation of photosynthesis. As in DeLucia & Thomas (2000), I observed the greatest stimulation in species

that showed an acclimatory response of the efficiency of ribulose-bisphosphate regeneration.

However, these species were not the same species that showed the similar response in the year following initiation of CO₂ enrichment at the Duke FACE experiment.

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Table 3.1. Dark respiration (R_d), apparent quantum efficiency (Q_e , mol mol⁻¹), light saturated maximum photosynthesis (A_{sat} , mol CO₂ m⁻² s⁻¹), and the light compensation point (G , mol m⁻² s⁻¹) of *A. rubrum* (ACRU), *Carya glabra* (CAGL), *Cercis canadensis* (CECA), and *Liquidambar styraciflua* (LIST) grown in ambient and elevated CO₂. Measurements were made in June and September 2003 and each value is the mean of 4 to 6 individuals (± 1 SE).

			R_d	Q_e	A_{sat}	G	
ACRU	June	Amb	0.33 (0.05)	0.058 (0.013)	3.7 (0.9)	7.8 (2.7)	
		Elv	0.24 (0.08)	0.079 (0.007)	8.2 (0.9)	4.1 (1.4)	
	Sept	Amb	0.31 (0.12)	0.067 (0.007)	2.6 (0.6)	2.6 (1.7)	
		Elv	0.18 (0.08)	0.061 (0.012)	4.3 (0.5)	2.6 (0.9)	
	CAGL	June	Amb	0.31 (0.05)	0.065 (0.015)	5.3 (0.5)	5.8 (1.3)
			Elv	0.20 (0.05)	0.070 (0.021)	6.2 (0.3)	4.0 (2.2)
Sept		Amb	0.30 (0.06)	0.060 (0.008)	3.5 (0.4)	5.7 (1.6)	
		Elv	0.16 (0.03)	0.062 (0.008)	4.3 (0.4)	1.9 (1.1)	
CECA	June	Amb	0.30 (0.05)	0.049 (0.011)	4.2 (0.3)	7.3 (1.5)	
		Elv	0.42 (0.05)	0.079 (0.017)	7.0 (0.6)	6.5 (1.5)	
	Sept	Amb	0.40 (0.08)	0.079 (0.005)	4.3 (0.3)	5.1 (1.0)	
		Elv	0.19 (0.11)	0.103 (0.017)	5.5 (0.2)	1.6 (1.2)	
LIST	June	Amb	0.57 (0.13)	0.077 (0.010)	5.1 (1.1)	7.3 (0.7)	
		Elv	0.43 (0.09)	0.084 (0.005)	7.2 (1.3)	5.2 (1.2)	
	Sept	Amb	0.43 (0.03)	0.070 (0.004)	3.8 (0.6)	5.3 (1.0)	
		Elv	0.30 (0.05)	0.074 (0.009)	5.8 (0.7)	4.5 (1.1)	

Table 3.2. Carboxylation efficiency ($V_{c_{max}}$, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), electron transport efficiency (J_{max} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), and the ratio of $V_{c_{max}} / J_{max}$ of *A. rubrum* (ACRU), *Carya glabra* (CAGL), *Cercis canadensis* (CECA), and *Liquidambar styraciflua* (LIST) grown in ambient and elevated CO_2 . Measurements were made in June and September 2003 and each value is the mean of 4 to 6 individuals (± 1 SE).

Table 3.3. Specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$), foliar nitrogen concentration ($[\text{N}]_{\text{mass}}$, mg g^{-1} ; $[\text{N}]_{\text{area}}$, g m^{-2}) total chlorophyll concentration (Chl_{mass} , mg g^{-1} ; Chl_{area} , g cm^{-2}), chlorophyll a/b ratio ($\text{Chl}_{a/b}$) and dark-adapted chlorophyll fluorescence (F_v/F_m , Rel.) of *A. rubrum* (ACRU), *Carya glabra* (CAGL), *Cercis canadensis* (CECA), and *Liquidambar styraciflua* (LIST) grown in ambient and elevated CO_2 . Measurements were made in June and September 2003 and each value is the mean of 4 to 6 individuals (± 1 SE).

			SLA	$[\text{N}]_{\text{mass}}$	$[\text{N}]_{\text{area}}$	Chl_{mass}	Chl_{area}	$\text{Chl}_{a/b}$	F_v/F_m	
ACRU	June	Amb	292.0 (16.0)	14.4 (0.5)	0.50 (0.04)	7.8 (0.7)	26.8 (2.1)	4.5 (0.3)	0.72 (0.02)	
		Elv	283.1 (13.3)	14.5 (0.8)	0.52 (0.03)	7.7 (1.2)	27.1 (3.6)	3.5 (0.4)	0.76 (0.01)	
	Sept	Amb	286.1 (21.8)	13.1 (0.5)	0.46 (0.04)	9.1 (2.4)	32.2 (3.9)	2.5 (0.2)	0.73 (0.01)	
		Elv	259.8 (6.9)	11.9 (0.5)	0.48 (0.04)	9.0 (0.3)	34.5 (1.7)	2.5 (0.2)	0.71 (0.02)	
	CAGL	June	Amb	282.1 (17.4)	16.5 (0.7)	0.59 (0.02)	5.9 (0.6)	22.5 (2.9)	4.1 (0.6)	0.75 (0.02)
			Elv	317.7 (31.0)	18.4 (0.6)	0.60 (0.05)	9.2 (2.2)	28.6 (2.7)	3.7 (0.3)	0.74 (0.01)
Sept		Amb	268.0 (21.0)	14.4 (0.4)	0.54 (0.03)	7.3 (0.6)	25.4 (2.1)	2.3 (0.2)	0.72 (0.02)	
		Elv	242.0 (20.2)	14.2 (0.6)	0.57 (0.07)	5.7 (1.1)	23.0 (3.3)	3.0 (0.6)	0.74 (0.02)	
CECA	June	Amb	432.9 (40.6)	19.8 (1.2)	0.47 (0.04)	8.6 (1.5)	20.6 (3.6)	3.9 (0.9)	0.71 (0.02)	
		Elv	501.7 (35.3)	23.6 (1.0)	0.49 (0.06)	11.4 (1.7)	22.8 (3.3)	2.7 (0.2)	0.77 (0.01)	
	Sept	Amb	377.5 (32.5)	16.6 (0.5)	0.45 (0.03)	10.4 (2.1)	27.3 (2.2)	2.4 (0.2)	0.71 (0.02)	
		Elv	404.3 (30.0)	18.4 (0.3)	0.46 (0.02)	11.2 (1.4)	26.5 (2.1)	2.6 (0.1)	0.73 (0.02)	
LIST	June	Amb	368.6 (32.9)	15.6 (0.7)	0.44 (0.04)	6.7 (0.5)	18.8 (1.9)	4.8 (1.4)	0.75 (0.03)	
		Elv	342.4 (34.3)	16.3 (0.5)	0.50 (0.05)	6.4 (1.5)	19.2 (3.4)	3.9 (0.4)	0.75 (0.01)	
	Sept	Amb	403.7 (37.2)	13.3 (0.4)	0.34 (0.03)	9.3 (0.7)	23.3 (1.0)	2.6 (0.4)	0.74 (0.01)	
		Elv	405.4 (12.8)	13.2 (0.3)	0.39 (0.02)	9.6 (0.5)	29.4 (1.7)	2.9 (0.3)	0.76 (0.02)	

Figure 3.1. Photosynthetic-light response curves of four species of understory saplings growing under ambient ($\sim 380 \mu\text{l l}^{-1} \text{CO}_2$; *closed circles*) and elevated atmospheric CO_2 ($\sim 580 \mu\text{l l}^{-1}$; *open circles*) in June of 2003 at the Duke Forest FACE experiment. Each point is a mean (± 1 SE) of four to six measurements.

June 2003

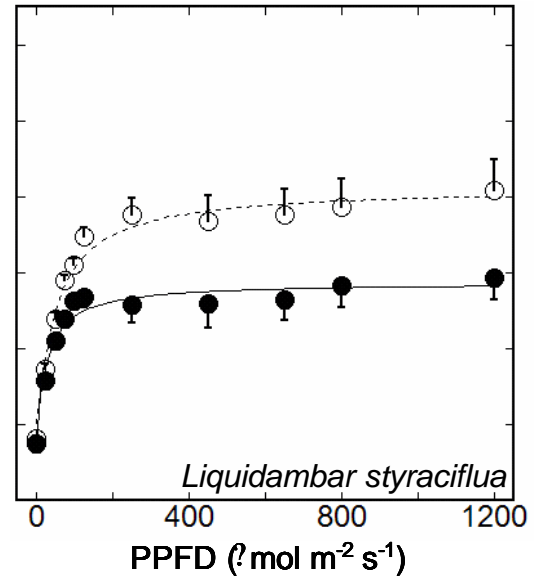
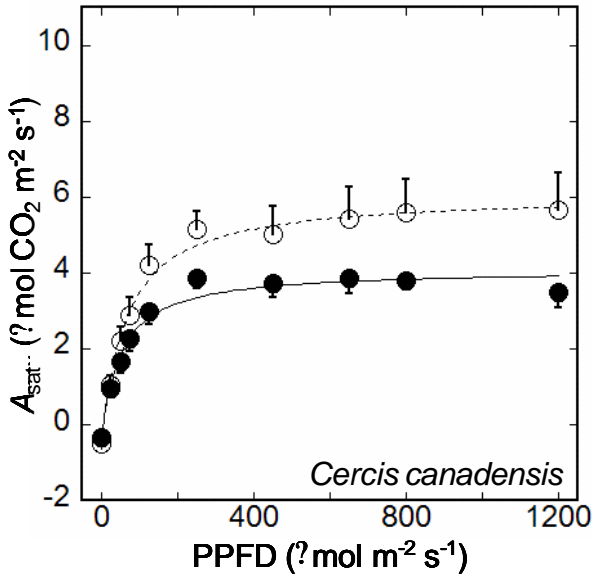
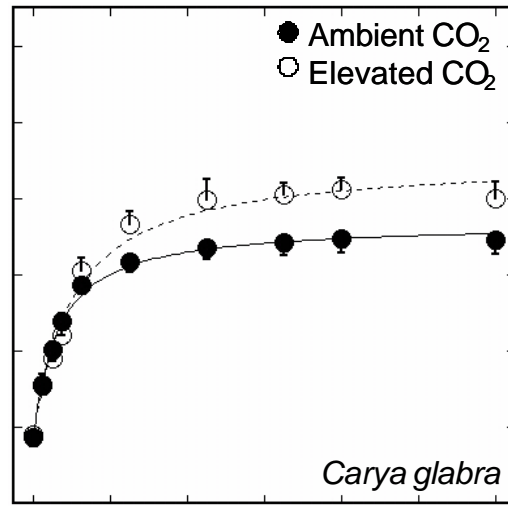
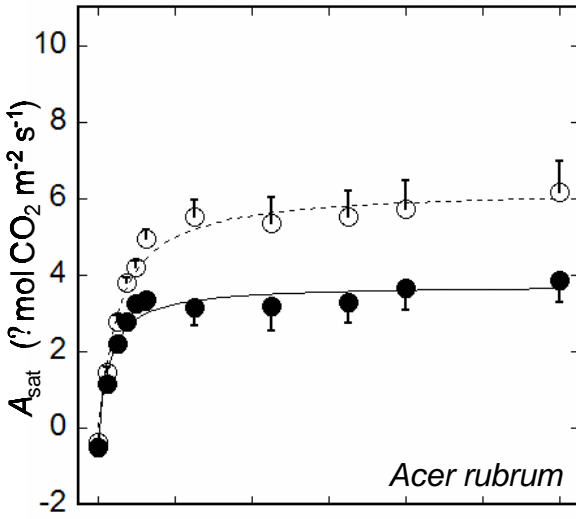


Figure 3.2. Photosynthetic-light response curves of four species of understory saplings growing under ambient ($\sim 380 \mu\text{l l}^{-1} \text{CO}_2$; *closed circles*) and elevated atmospheric CO_2 ($\sim 580 \mu\text{l l}^{-1}$; *open circles*) in September of 2003 at the Duke Forest FACE experiment. Each point is a mean (± 1 SE) of four to six measurements.

September 2003

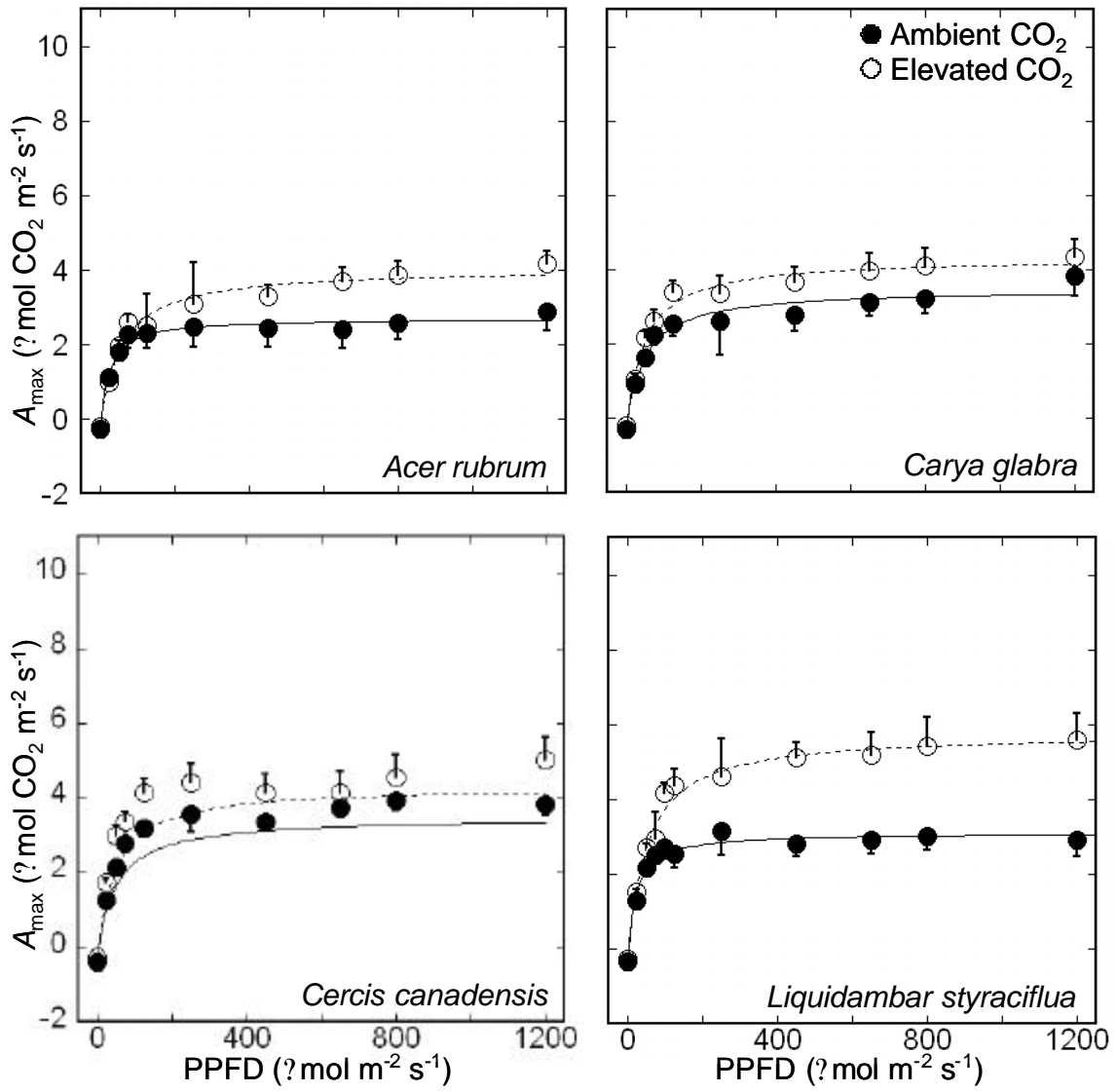


Figure 3.3. Chlorophyll fluorescence-PPFD relationships of four species of understory saplings growing under ambient ($\sim 380 \mu\text{l l}^{-1} \text{CO}_2$; *closed circles*) and elevated atmospheric CO_2 ($\sim 580 \mu\text{l l}^{-1}$; *open circles*) in June of 2003 at the Duke Forest FACE experiment. Each point is a mean (± 1 SE) of four to six measurements.

June 2003

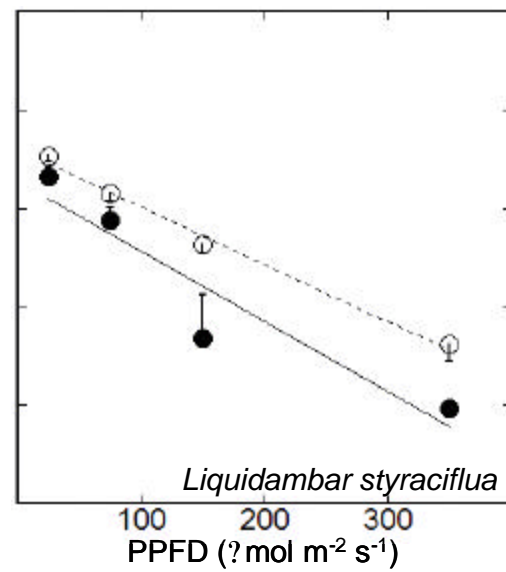
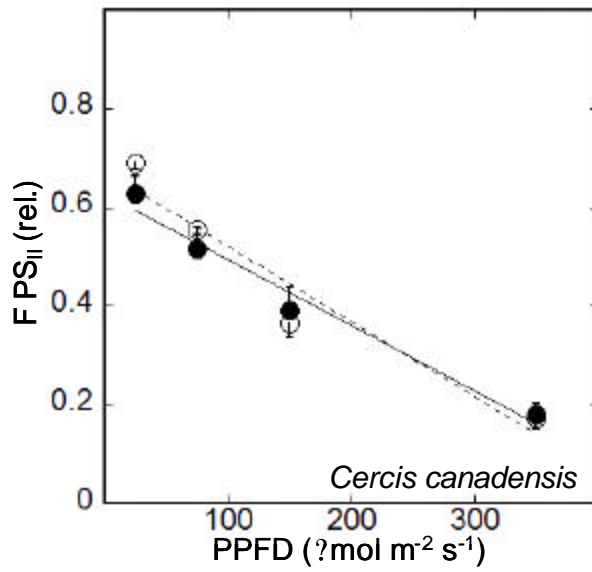
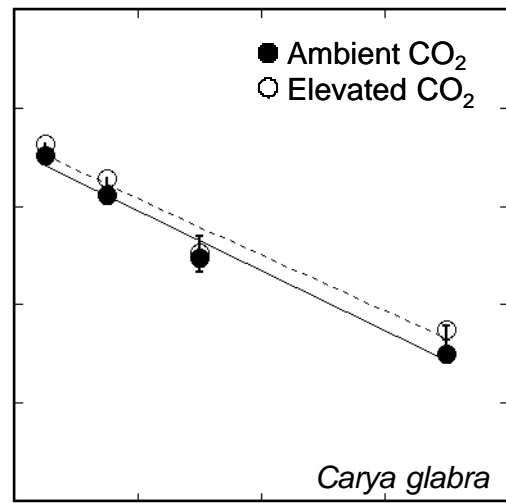
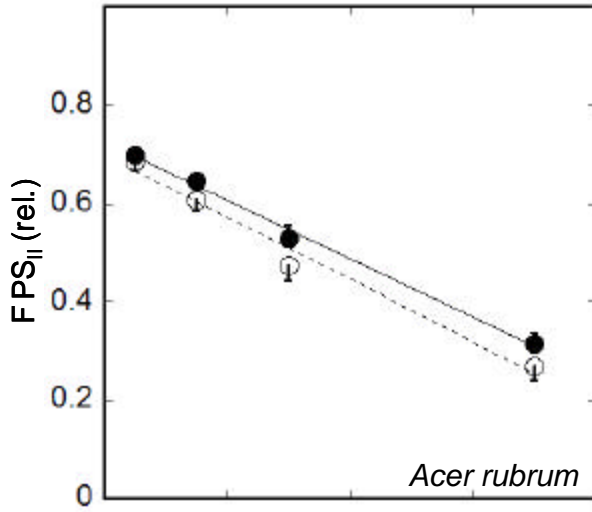
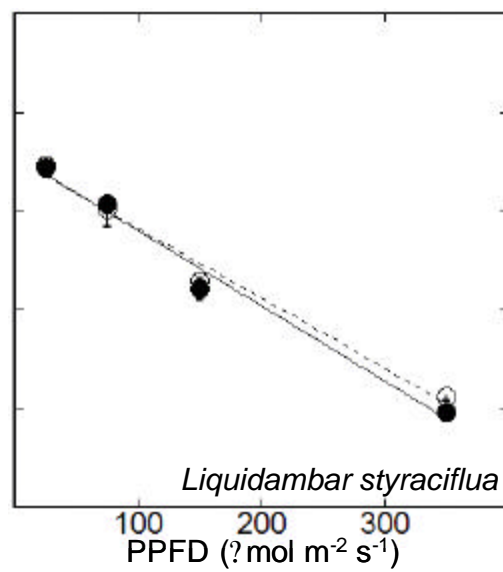
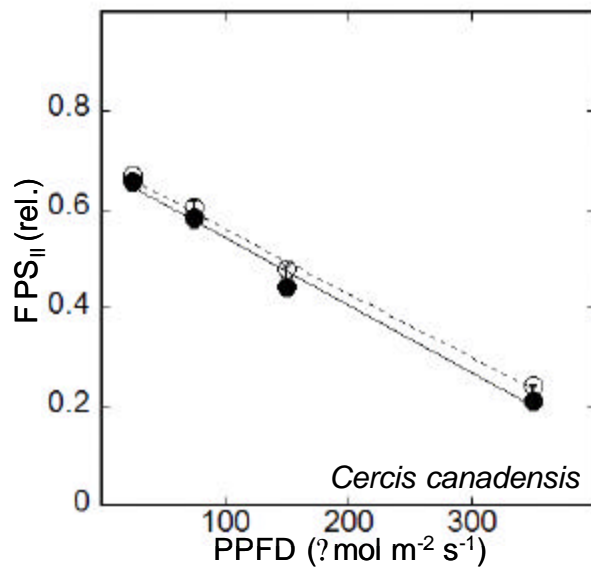
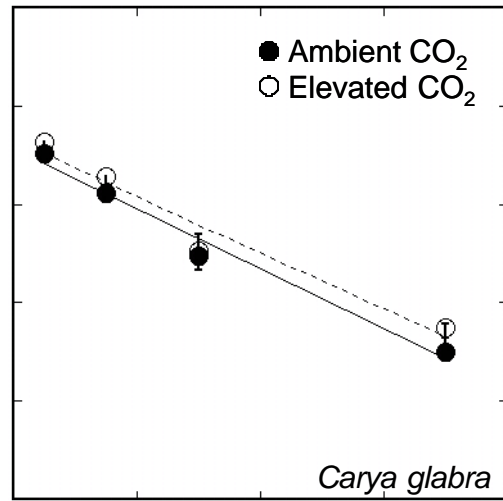
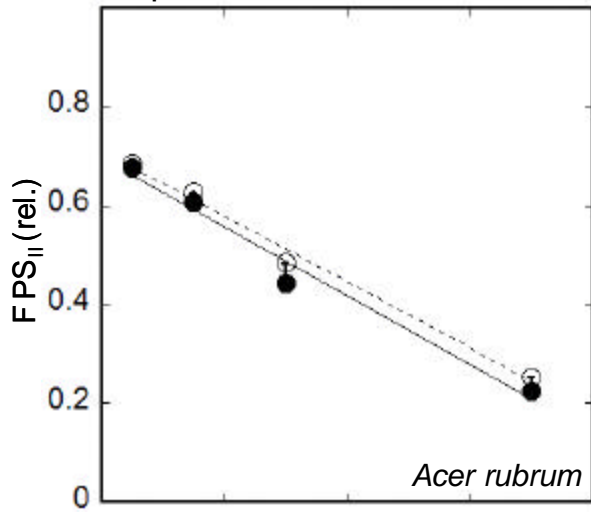


Figure 3.4. Chlorophyll fluorescence-PPFD relationships of four species of understory saplings growing under ambient ($\sim 380 \mu\text{l l}^{-1} \text{CO}_2$; *closed circles*) and elevated atmospheric CO_2 ($\sim 580 \mu\text{l l}^{-1}$; *open circles*) in September of 2003 at the Duke Forest FACE experiment. Each point is a mean (± 1 SE) of four to six measurements.

September 2003



CHAPTER 4:
Growth response of *Pinus taeda* seedlings from four provenances to water availability and elevated carbon dioxide

Abstract

The current study investigated the response of loblolly pine seedlings from four geographic regions to water availability and elevated atmospheric carbon dioxide. Loblolly pine seedlings originated from coastal North Carolina, the piedmont region of the southeastern United States, east Texas, and the Lost Pines region of Texas. Seedlings were grown in either ambient (368 ± 5 ppm) or elevated (616 ± 17 ppm) CO_2 and three levels of water availability. The study was carried out in greenhouses over 16 weeks. Elevated CO_2 stimulated the total biomass of loblolly pine seedlings. Water availability did not significantly affect seedling growth but did change the allocation of biomass. Seedlings grown under low water availability allocated more biomass to roots at the expense of stem tissue. Growth CO_2 concentration and water availability did not affect the biomass production or allocation of seedlings interactively. Although seedling biomass was different between seed sources, biomass allocation was not. The effects of elevated CO_2 and water availability on biomass accumulation and allocation were not dependent on seed source. Previous studies have exhibited a seed-source specific response of loblolly pine seedlings to water availability, however these studies were performed over a longer period of time. Further investigation of intra-specific responses of trees to environmental changes is necessary for future regional and global modeling projections of forest productivity.

Introduction

Water stress typically reduces the growth and photosynthesis of plants by decreasing leaf area production and the exchange of carbon dioxide between the leaf and the atmosphere (Kramer and Boyer 1995). Moderate water stress reduces net photosynthesis as a result of decreased stomatal conductance, thus decreasing the intercellular CO₂ (*C_i*) concentration of the leaf. Under more severe water stress, plants can exhibit metabolic inhibition and changes in cellular activities associated with decreased water potential. Elevated atmospheric CO₂ primarily stimulates leaf-level photosynthesis by competitively inhibiting the oxygenation of ribulose-1,5-bisphosphate (RubP), thus reducing photorespiration and increasing the net efficiency of photosynthesis (Drake et al. 1997). Elevated CO₂ also reduces stomatal conductance in many species, thus decreasing water loss and improving the water-use efficiency of elevated CO₂-grown plants (Curtis and Wang 1998; Drake et al. 1997; Medlyn et al. 1999). Consequently, elevated atmospheric CO₂ concentration may partially offset the negative effects of water stress by increasing the CO₂ gradient that exists between the atmosphere and the intercellular spaces of the leaf and reducing water loss through the stomata (Groninger et al. 1996; Liang and Maruyama 1995; Samuelson and Seiler 1994; Tolley and Strain 1984; Tschaplinski et al. 1995). In a quantitative review of 123 observations, Poorter and Soba (2001) found that drought was the only stress condition where plants exhibited a higher response to elevated CO₂ than under optimal conditions. For example, Tolley & Strain (1984) and Tschaplinski et al. (1995) reported a greater relative stimulation of loblolly pine biomass by elevated CO₂ under low water conditions than under optimal water conditions.

Several studies have examined the effects of elevated CO₂ on different genotypes of the same species. Herbaceous species exhibit a wide range of intra-specific differences in their

physiological responses to elevated CO₂ (Curtis et al. 1994; Hunt et al. 1991; Steinger et al. 1997) but few studies have examined intra-specific variation in the responses of woody species to elevated CO₂. Wang et al. (1994), using five genotypes of *Populus tremuloides*, reported a strong genotypic effect on photosynthetic responses to elevated CO₂, but the interaction of genotype and CO₂ treatment was not significant for biomass production.

The distribution of loblolly pine extends from eastern Maryland south to the northern portions of Florida and west to eastern Texas. Across this distribution, loblolly pine trees show geographic differentiation with respect to photosynthesis and biomass production (Boltz et al. 1986; Bongarten and Teskey 1987). In a study of loblolly pine seedlings from seven different seed sources, Bongarten and Teskey (1987) found strong seed source differences in the allocation of biomass such that loblolly pine seedlings originating from drier locations had higher root-shoot ratios than those originating from wetter locations. In another study determining the biomass allocation of loblolly pine seedlings from four seed sources, Barnes (2002) reported differential allocation of biomass between seedlings from the different seed sources under drought conditions. Trees of coastal origin, an environment that receives abundant rainfall, consistently outgrow trees of northern origin in their juvenile and adult stages (Wakeley and Bercaw 1965; Wells and Wakeley 1966). Apparent differences between coastal and interior trees have been attributed to geographic differences in drought tolerance. These geographic differences in photosynthesis and biomass allocation of loblolly pine suggest that loblolly pine will show intra-specific variation in the response to elevated atmospheric CO₂ with respect to water availability. Therefore, I hypothesized that the biomass and biomass allocation of loblolly pine seedlings from interior seed sources would respond more to elevated CO₂ when grown in

decreased water availability and seedlings from coastal seed sources would respond more to elevated CO₂ at high levels of water availability.

Materials and Methods

Loblolly pine seeds from four distinct geographic regions across the natural distribution of loblolly pine were obtained from the Tree Improvement Program at North Carolina State University and the Forest Science Lab of the Texas Forest Service. The four geographic regions used in this study were coastal North Carolina, the piedmont region of the southeastern United States, east Texas, and the Lost Pines region of Texas. For a full description of the geographic differences in loblolly pine see Schmidting (2001). After a 90 d period of cold stratification at 4°C, seeds were planted in 10 x 15 cm cylindrical pots in a mixture of coarse sand and natural soil (9:1 v/v). Natural soil was collected in a loblolly pine forest at the Duke University Forest (Durham, NC) to ensure inoculation of seedlings with mycorrhizal fungi. At harvest, mycorrhizal colonization was not quantified but was evident by the presence of abundant bifurcated root tips in the seedlings. Prior to germination, pots were assigned to one of two atmospheric CO₂ concentrations (ambient, ~370 ppm or elevated, ~600 ppm) and low, intermediate, or high water availability. The elevated treatment concentration of 600 ppm was selected to approximate the projected atmospheric concentration near the mid-point of the current century (Schimel et al. 1996). Atmospheric carbon dioxide concentration was monitored and controlled by a commercially available CO₂ monitoring system (CDMC-6, Green Air Products, Gresham Oregon, USA) with an attached solenoid valve that dispensed pure CO₂ through polyethylene tubing (6 mm) with 1.5 mm holes placed every 1 m around the entire perimeter of the greenhouse. To ensure adequate mixing of CO₂, four multi-speed fans (20 inch diameter) were placed in the elevated CO₂ greenhouse. Fans were also placed in the ambient

CO₂ greenhouse to mimic any micro-meteorological effects caused by the fans. Seedlings were randomly placed within each greenhouse and rotated within and between greenhouses to minimize the effect of position within a greenhouse and the effect of greenhouse on the seedlings during the study period. The average daytime atmospheric CO₂ concentration in the ambient treatment was 368± 5 ppm CO₂ and in the elevated treatment the average concentration was 616± 17 ppm CO₂. The study was performed from late June through the end of October of 2003. The average daytime temperature through the study period was 26.7± 0.2°C and the average nighttime temperature was 21.1± 0.1°C. A total of eight seedlings were present in each treatment combination of seed source, CO₂ concentration, and water availability. After germination, seedlings in all treatment combinations were watered to saturation once a day with a nutrient solution containing 0.5 mM N; 1 mM P; 3 mM K; 3 mM Ca; 1.5 mM Mg; 3 mM S; 0.16 mM Fe; 0.05 mM B; 0.01 mM Mn; 0.001 mM Cu; 0.05 μM Mo; and 0.16 μM Co for the first four weeks of the experiment. At week four, water treatments were started. Water availability treatments were maintained by watering high water level seedlings once each day to saturation, intermediate water level seedlings were watered once every three days, and low water seedlings were watered once each week. Watering regimes were maintained from week four of the experiment until termination of the experiment at week 16. At week 16, seedlings were harvested and divided into stems, needles, and roots. All plant parts were then dried to a constant mass at 65°C for 48 hours before being weighed for final biomass.

Data was analyzed for statistical significance using a three-way analysis of variance (ANOVA, $\alpha=0.05$) to test the main effects and interactions of CO₂ concentration, water availability, and geographic seed source. Data were transformed as necessary to conform with the assumptions of ANOVA.

Results

Elevated CO₂ significantly stimulated ($F=15.68$, $P=0.0001$) the total biomass of loblolly pine seedlings 26%, regardless of water availability and seed source (Figure 4.1). The total biomass did differ significantly ($F=4.37$, $P=0.006$) between seed sources. Seedlings originating from east Texas grew the most and seedlings from coastal North Carolina grew the least (Figure 4.1). There were no interactive effects of CO₂, water availability, or seed source on the total biomass of seedlings. Elevated CO₂ did not significantly affect biomass allocation to roots ($F=0.33$, $P=0.56$), stems ($F=1.44$, $P=0.23$), or needles ($F=0.96$, $P=0.33$; Table 4.1). However, the amount of biomass allocated to roots and stems was significantly altered by changes in water availability. Loblolly pine seedlings allocated 23% ($F=10.95$, $P=0.0001$) more biomass to stems when grown under high water availability. However under low water availability, loblolly pine seedlings allocated 12% ($F=2.75$, $P=0.06$) more biomass to roots. Neither CO₂ nor water availability altered the allocation of biomass to needles in the loblolly pine seedlings. There were also no significant interactions of water availability, CO₂, or seed source on any of the biomass allocation parameters measured (Table 4.1)

Discussion

Generally elevated atmospheric CO₂ stimulated the biomass of the loblolly pine seedlings. Although, seedling biomass increased with increasing CO₂ concentration; biomass allocation did not change with increasing CO₂ concentration. However, decreased water availability did increase the root mass of the seedlings at the expense of stem mass. This is similar to the findings of Bongarten and Teskey (1987) where loblolly pine seedlings exhibited no significant differences in the amount of biomass allocated to leaves under optimal or drought stress conditions. Many studies have observed similar increases in loblolly pine seedling

biomass with increasing CO₂ (Groninger et al. 1996; Sionit et al. 1985; Tolley and Strain 1984; Tschaplinski et al. 1993). One previous study, Tschaplinski et al. (1993), also found that loblolly pine seedling biomass increased with elevated CO₂ without altering the root to shoot ratio. In contrast to the current study, both Tolley and Strain (1984) and Groninger et al. (1996) found no evidence of CO₂ or water stress-induced changes in biomass allocation in loblolly pine seedlings. Interestingly, the effect of elevated CO₂ and water stress on the biomass of the seedling organs was not differential with respect to seed source. This follows the findings of Egli et al. (1998) where no provenance-specific responses were found in the above-ground growth and gas exchange of *Fagus sylvatica* and *Picea abies*. However, Houpis et al. (1999) found that the response of aboveground growth of *Pinus ponderosa* to elevated CO₂ was provenance-specific in 8 of 15 seed sources examined.

Loblolly pine seedling biomass differed between seed sources but the allocation of biomass did not. This result is in contrast with that of Bongarten and Teskey (1987) who found differential biomass allocation in loblolly pine seedlings from different seed sources. The study also attributed the variation in allocation patterns to differences in growing season temperature and water availability in the source of origin of the seedlings. One difference between the current study and Bongarten and Teskey (1987) is the length of the experiment. The current study lasted only 16 weeks where the study of Bongarten and Teskey (1987) was carried out across a full year. As in the current study, Ledig (1983) found a lack of biomass allocation differences between seed sources of loblolly pine but this experiment was carried out over a relatively short period of time also. In the current study, the effect of elevated CO₂ on seedling biomass was also not dependent on water availability, which contrasts the results of other studies (Groninger et al. 1996; Tolley and Strain 1984; Tschaplinski et al. 1993) where elevated CO₂

increased biomass of tree seedlings more under high water availability than when the seedlings were grown under low water availability.

Although, the current study did not support the hypothesis that elevated CO₂ will have geographic region-specific effects on the growth of loblolly pine, the topic deserves further investigation based on the more long-term work of Bongarten and Teskey (1987). The possibility of geographic specificity in the response of loblolly pine growth to changing environmental conditions, such as atmospheric CO₂ and water availability, could have major impacts on the forestry industry by altering the intense management of the species that is already taking place. Long-term work investigating the geographic specific responses of loblolly pine and other tree species is needed to fully understand forest response in the future and to improve the accuracy of our regional and global modeling efforts.

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Figure 4.1. Total biomass of loblolly pine seedlings grown in ambient and elevated CO₂ under three levels of water availability (low, intermediate, and high) originating from a) the lost pines region, b) coastal North Carolina, c) the piedmont of the southeastern United States, and d) east Texas. Values are the means \pm 1 SE.

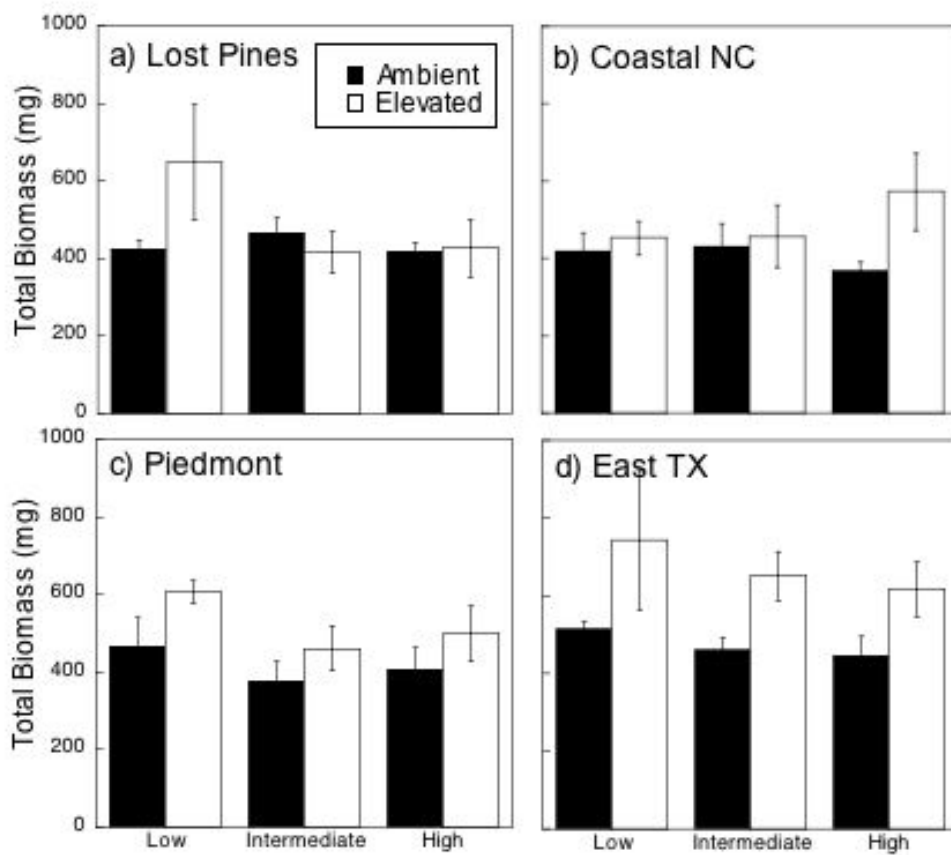


Table 4.1. Biomass allocation of loblolly pine seedlings from four distinct provenances grown under ambient and elevated CO₂ and three levels of water availability (high, intermediate (Int), and low). Each value represent the mean of each group ± 1 SE.

		LMR		SMR		RMR	
		Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Lost Pines	High	0.41 ± (0.02)	0.31 ± (0.04)	0.18 ± (0.02)	0.16 ± (0.01)	0.41 ± (0.04)	0.53 ± (0.04)
	Int	0.38 ± (0.04)	0.41 ± (0.03)	0.11 ± (0.01)	0.12 ± (0.01)	0.51 ± (0.05)	0.47 ± (0.03)
	Low	0.41 ± (0.04)	0.43 ± (0.06)	0.11 ± (0.01)	0.12 ± (0.02)	0.48 ± (0.05)	0.45 ± (0.08)
Coastal NC	High	0.43 ± (0.02)	0.44 ± (0.08)	0.14 ± (0.02)	0.17 ± (0.02)	0.43 ± (0.03)	0.39 ± (0.10)
	Int	0.36 ± (0.04)	0.41 ± (0.02)	0.16 ± (0.02)	0.14 ± (0.02)	0.48 ± (0.03)	0.45 ± (0.04)
	Low	0.40 ± (0.03)	0.40 ± (0.06)	0.16 ± (0.02)	0.17 ± (0.03)	0.44 ± (0.02)	0.43 ± (0.04)
Piedmont	High	0.44 ± (0.02)	0.43 ± (0.02)	0.17 ± (0.01)	0.15 ± (0.02)	0.39 ± (0.03)	0.41 ± (0.02)
	Int	0.38 ± (0.04)	0.46 ± (0.02)	0.14 ± (0.02)	0.16 ± (0.01)	0.47 ± (0.03)	0.38 ± (0.02)
	Low	0.41 ± (0.02)	0.37 ± (0.02)	0.08 ± (0.01)	0.11 ± (0.01)	0.51 ± (0.02)	0.51 ± (0.03)
Texas	High	0.45 ± (0.02)	0.42 ± (0.03)	0.17 ± (0.01)	0.17 ± (0.01)	0.38 ± (0.02)	0.41 ± (0.04)
	Int	0.47 ± (0.07)	0.41 ± (0.03)	0.11 ± (0.02)	0.13 ± (0.01)	0.41 ± (0.06)	0.46 ± (0.04)
	Low	0.46 ± (0.02)	0.32 ± (0.13)	0.14 ± (0.01)	0.15 ± (0.02)	0.40 ± (0.03)	0.53 ± (0.13)

CHAPTER 5:

Net primary productivity of the first seven years of treatment at the Duke Forest FACE experiment estimated using PnET-II

Abstract

The net primary productivity (*NPP*) of forest ecosystems is expected to increase with increasing atmospheric CO₂. However this increase in *NPP* may be modulated by changes in other climate variables, such as air temperature and precipitation that are also expected to change in the near future. In this study I modeled the *NPP* of the portions of the Duke Forest FACE experiment growing under ambient and elevated CO₂. I also used three climate change scenarios to determine how the response of forest *NPP* to elevated CO₂ may change with future changes in precipitation and temperature. I then systematically decreased the foliar N concentration in both ambient and elevated CO₂ to determine the foliar N concentration at which the stimulation of *NPP* by elevated CO₂ would equal zero. I parameterized PnET-II, a monthly time step lumped-parameter forest productivity model with site specific and generalized physiological and physical variables. Model output is compared to measured estimates of *NPP* at the Duke Forest FACE experiment. I found good agreement between measured *NPP* and model estimated *NPP* in both ambient and elevated CO₂. Climate change scenarios showed an additive stimulation of *NPP* by increased temperature and increased precipitation, but did not alter the stimulation of *NPP* by elevated CO₂. I also found that the foliar N concentration where elevated CO₂ no longer increases forest *NPP* may be an unrealistic value in a field setting. This modeling effort suggests that other changes in physiological parameters other than the photosynthesis-foliar N relationship may be important in determining future forest productivity in changing climatic conditions.

Introduction

Forest ecosystems cover approximately 43% of the land area on Earth and account for nearly 70% of annual terrestrial net primary productivity (*NPP*) (Schlesinger 1997). *NPP* is the annual accumulation of plant biomass available to consumers and may represent an important sink for atmospheric carbon dioxide. Since the beginning of the industrial revolution, the concentration of atmospheric CO₂ has been increasing at a rate of approximately 1.5 ppm yr⁻¹ and coupled with this increase in atmospheric CO₂ is an expected 3 -7 °C increase in global mean temperature as well as changes in regional precipitation patterns (IPCC 2001). The Hadley 2 climate model, a general circulation model, has projected an increase in the average air temperature and a 0-10% increase in precipitation for North Carolina, USA, as the pre-industrial revolution atmospheric CO₂ concentration (280 ppm) doubles (Kittel et al. 1997).

Increased atmospheric CO₂ has been shown to stimulate the *NPP* of forest ecosystems (DeLucia et al. 1999), but this stimulation of *NPP* may be affected by other climatic variables (Hamilton et al. 2002). The Duke Forest Free-Air CO₂ Enrichment (FACE) experiment was developed to investigate the physiological and biogeochemical processes that regulate the response of an intact loblolly pine forest to elevated atmospheric CO₂. A secondary objective of the Duke FACE experiment is to use this data to develop or parameterize models of carbon flux and storage that can be used to predict responses of forests to future changes in atmospheric CO₂ (Hendrey et al. 1999). In the first two years (1997-1998) of the FACE study, *NPP* of the forest grown under elevated CO₂ was stimulated by approximately 26% (DeLucia et al. 1999). This CO₂ induced stimulation of *NPP* has continued throughout the experiment although inter-annual variation in the response of *NPP* to CO₂ enrichment has been observed (Dave Moore, *unpublished data*). The most recent analysis of tree growth at the Duke Forest FACE experiment

suggests that this inter-annual variation in the response of *NPP* to elevated CO₂ is related to growing season temperature and annual precipitation, acting either independently or interactively (Dave Moore, *unpublished data*). Therefore, understanding the response of forest productivity to elevated CO₂ under varying climatic conditions is necessary in order to predict the consequences of climate change on the loblolly pine forests of the southeastern United States.

In this study, I utilize a process-based forest productivity model, PnET-II, to estimate net primary productivity of the loblolly pine forest at the Duke FACE experiment grown under elevated CO₂ for the first seven years of the experiment. I used three climate change scenarios that simulate climatic conditions that are expected to occur in the southeastern United States near the end of the 21st century to examine the interactive effects of elevated CO₂ with temperature or precipitation on *NPP*. Because the response of forest growth to elevated CO₂ is limited by nitrogen availability (Oren et al. 2001), I also determined the foliar nitrogen concentration at which elevated CO₂ no longer increases forest *NPP*.

Methods and Materials

Study Site

The Duke Forest FACE experiment is located in a *Pinus taeda* plantation planted in 1983 after the re-generating forest was clear-cut in 1979. Since establishment, the plantation has not been managed to prevent the growth of other tree species. The forest is dominated by loblolly pine (*Pinus taeda*, 1733 stems ha⁻¹) along with a large number of sweetgum (*Liquidambar styraciflua*, 620 stems ha⁻¹) and yellow poplar (*Liriodendron tulipifera* L., 68 stems ha⁻¹) as well as other hardwood species present in the canopy and the understory. The forest is growing on a

nutrient-poor, clay-rich loam soil that is typical of many upland areas in the southeastern United States.

The Duke Forest FACE experiment consists of six 30-m diameter FACE rings. Three of these rings serve as experimental control plots while the remaining three are replicate CO₂ treatment plots. Each FACE ring consists of 32 pipes that extend from the forest floor through the canopy. Within each elevated CO₂ treatment ring, these pipes deliver a controlled amount of CO₂ with a target concentration of ambient + 200 $\mu\text{l l}^{-1}$ CO₂. The three remaining ambient control rings are equipped with blowers and receive the same volume of air as the elevated CO₂ treatment rings to replicate any micrometeorological effects on the forest that occurs during the operation. Since the beginning of full operation of the Duke Forest FACE experiment in 1996 the forest has received 24 h d⁻¹ CO₂ fumigation except on days when the air temperature was below 5 °C for more than one hour. Starting in the winter of 2003, the forest was fumigated during daylight hours and when wind speeds are less than 15 m.p.h. For a complete description of the operating procedures of the Duke Forest FACE experiment see Hendrey et al. (1999).

PnET-II Model

The PnET-II model is a monthly time-step, lumped-parameter model that simulates the carbon and hydrologic cycles of forest ecosystems (Aber and Federer 1992). PnET-II was originally developed for use in northern forests but has been adapted for use in pine forests of the southeastern United States (McNulty et al. 1996). Data used to parameterize the PnET-II model is derived either from literature values or measurements recorded at the site of interest. These parameters include characterizations of the vegetation, soil, and climate of the forest. The vegetation parameters thoroughly characterize the physiological and physical processes which

control photosynthesis, light capture, and carbon allocation through the forest canopy. The single soil parameter characterizes the water holding capacity of the soil located at study site. Climate inputs include minimum and maximum temperature, photosynthetically active radiation (PAR) at the top of the canopy, and total precipitation. Climate data for the current study was collected at the Duke Forest FACE experiment and obtained from the lab of Dr. Ram Oren.

The physiological data used to parameterize PnET-II for the Duke Forest FACE experiment were mostly derived from data collected directly at the study site in the loblolly pine plantation growing under both ambient and elevated atmospheric CO₂ (Table 5.1). The primary physiological parameter used in PnET-II, the photosynthesis-foliar N relationship, was obtained from data presented in Chapter 2 of this document. The standard error of this relationship was used to calculate a 95% confidence interval to provide an estimate of error for the modeled productivity values. Data for light interception and the respiration Q₁₀ were obtained through personal communication with the lab of Dr. Evan DeLucia and other literature values derived at the Duke FACE experiment (Table 5.1). When data were not available from the study site, literature values were used, primarily from Sun et al. (2000) (Table 5.1).

Climate & Foliar N Change Scenarios

I developed a control climate scenario by averaging the minimum and maximum air temperature of each month of the year, as well as the annual precipitation recorded from 1997-2003 at the Duke Forest FACE experiment. The Hadley 2 global climate model was used to create 3 additional scenarios of climate change that may occur in North Carolina, USA near the end of the 21st century. The first climate change scenario increased the mean minimum air temperature from 2.5-2.8 °C and the mean maximum air temperature by 1.6-2.0 °C, depending

on the month of the year, while not altering the annual precipitation. The second climate change scenario increased the monthly precipitation from 0-10% depending on season but did not change temperature. The final climate change scenario combined increased air temperatures with increased precipitation as stated above. To determine the foliar nitrogen concentration at which elevated CO₂ no longer increased forest *NPP*, I systematically decreased the foliar N concentration and estimated the stimulation of *NPP* by elevated CO₂ until the stimulation equaled zero.

Statistics

Least squares linear regression models were applied to the relationships between the stimulation of modeled *NPP* due to CO₂ enrichment and mean growing season temperature and total annual precipitation. Relationships were considered significantly different than zero at a significance level of $\alpha = 0.05$.

Results & Discussion

Modeled estimates of *NPP* for the Duke Forest FACE experiment indicate that *NPP* was stimulated by elevated CO₂ in each year of treatment (Figure 5.1). Averaged across all years of the experiment, the stimulation of *NPP* by elevated CO₂ was 15%. The largest stimulation was found in 1997, the first full year of treatment at the Duke FACE experiment, where elevated CO₂ stimulated *NPP* by 21%. The smallest stimulation of *NPP* was in the 2000, where CO₂ enrichment led to a 9% stimulation of *NPP*.

These modeled values of *NPP* show close agreement with allometric measurements of *NPP* at the Duke Forest FACE experiment during the second year of treatment (1988) (Hamilton et al. 2002) and fall into the range of *NPP* measurements of an 11 year old pine forest in NC, USA (500-1235 g C m⁻² yr⁻¹, Maier and Kress 2000). For the second year of treatment, Hamilton

et al. (2002) reported that the *NPP* of the ambient CO₂-grown portion of the forest was 705 g C m⁻² yr⁻¹ and 897 g C m⁻² yr⁻¹ in the elevated CO₂ plots. Modeled estimates of *NPP* for the same year of the study were 759±113 g C m⁻² yr⁻¹ under ambient CO₂ and 907±181 g C m⁻² yr⁻¹ in the elevated CO₂-grown portion of the forest (Figure 5.1).

Current unpublished analyses of productivity at the Duke Forest FACE experiment show that the amount of stimulation of tree growth by elevated CO₂ is correlated to growing season temperature (D. Moore, *unpublished data*). Modeled results of *NPP* by elevated CO₂ were not related to growing season temperature (Figure 5.2). This difference was unexpected because of the strong negative effect that temperature has on photosynthesis and strong positive effect temperature has on the stimulation of photosynthesis by elevated CO₂ (Myers and Thomas 1999). Modeled results of *NPP* by elevated CO₂ was also not found to be related to annual precipitation (Figure 5.3). This result is consistent with the most current growth data analysis where a relationship was not found between precipitation and the CO₂ stimulation of growth (D. Moore, *unpublished data*). It should be noted that the modeled results are for the seven years of the Duke FACE experiment and a relationship between the degree of stimulation of *NPP* by elevated CO₂ and growing season temperature may be observed as the experiment progresses.

Modeled *NPP* was stimulated under each of the climate change scenarios of the Hadley 2 model (Figure 5.3). Growth in elevated CO₂ stimulated the modeled *NPP* of the forest approximately 17% regardless of changes in climate. However, the modeled estimates of *NPP* in the temperature change scenario and the precipitation scenario resulted in much higher estimates of *NPP* in both ambient and elevated CO₂ grown portions of the forest. This response of *NPP* to climate change was further increased when both precipitation and temperature were changed simultaneously. This simultaneous change in temperature and precipitation resulted in a 57%

increase of *NPP* across all CO₂ treatments (Figure 5.3). This additive effect of precipitation and temperature on *NPP* in the current study is substantially larger than an increase in modeled *NPP* of a loblolly pine forest growing in eastern NC performed by Sun et al. (2000), where a 25% increase in modeled forest *NPP* was observed under similar climate change scenarios. One explanation for this observation may be that the increased temperature moved the average day-time temperature nearer the optimal range for photosynthesis, thus increasing the average daily net photosynthesis. However, this phenomenon may not be realized in an actual field-grown loblolly pine forest because of the ability of loblolly pine to alter the optimal operating temperature of photosynthesis (Strain et al. 1976). Also, a longer growing season may explain this large difference because the PnET-II model utilizes growing degree days to initiate and terminate tree growth and warmer winter days with more available water from increased precipitation may lead to an extension of the growing season. The foliar N concentration at which the stimulation of *NPP* is no longer increased by CO₂ enrichment was found at a critical level of 0.7% foliar N. This value of foliar N concentration may be unrealistic in a field setting. Allen (1987) determined that at 1.1% foliar N, the productivity of loblolly pine forests is critically limited by N availability. Therefore, the stimulation of *NPP* by elevated CO₂ may never diminish to zero.

In conclusion, the PnET-II model was able to provide a good estimate of *NPP* of the loblolly pine forest growing under both ambient and elevated CO₂ at the Duke Forest FACE experiment. However estimates of forest *NPP* under future climatic conditions may be unrealistic because of physiological adjustments not accounted for in the PnET-II model. Future efforts to model forest productivity with the PnET-II model should follow an increased understanding of the physiological mechanisms that control the response of trees to elevated CO₂

and the interaction of elevated CO₂ and other abiotic factors such as water availability, temperature, and light availability on the growth and productivity of forest ecosystems.

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Table 5.1. Duke Forest FACE experiment specific parameters that were changed from earlier modeling efforts of Aber and Federer (1992) and Sun et al. (2000) used to estimate net primary productivity of the portions of the forest growing under ambient and elevated CO₂ using the PnET-II forest productivity model.

Parameter Definition	Ambient CO₂	Elevated CO₂	Source
Intercept of Amax-N Relationship	5.80	8.84	Chapter 2
Slope of Amax-N Relationship (mmol CO ₂ m ⁻² s ⁻¹)	32.38	52.68	Chapter 2
Optimum Temperature for Photosynthesis (°C)	24	24	Sun et al. 2002 Hamilton et al.
Q ₁₀ for Leaf Respiration	2.1	2.7	2001
Light Attenuation Constant	0.5	0.5	E.H. DeLucia
Foliar N Concentration (%)	1.32	1.32	Chapter 2
Specific Leaf Weight (g m ⁻²)	100	100	Chapter 2

Figure 5.1. Net primary productivity (NPP) of portions of the Duke Forest FACE experiment growing under ambient (closed circles, solid line) and elevated (open circles, dashed line) CO₂ from 1997 to 2003 estimated using the PnET-II forest productivity model. Values represent model output \pm 1 S.E. determined by using the lower and upper standard errors of model parameters measured by the investigator.

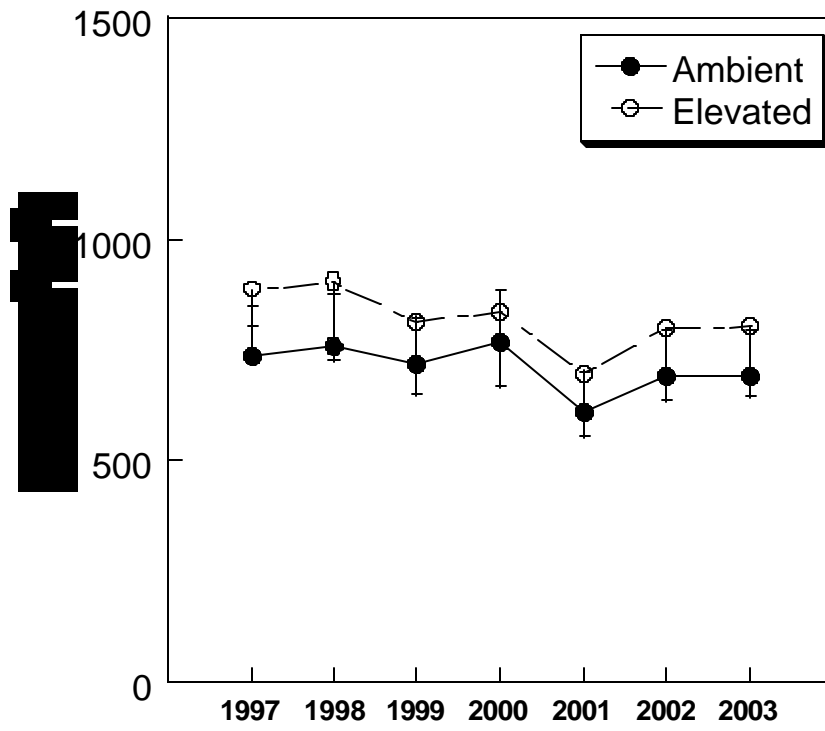


Figure 5.2. Relationship between the stimulation of NPP due to elevated CO₂ versus mean growing season temperature. Regression equation: $y=1.05+0.005x$, $R^2=0.18$, $P=0.1689$.

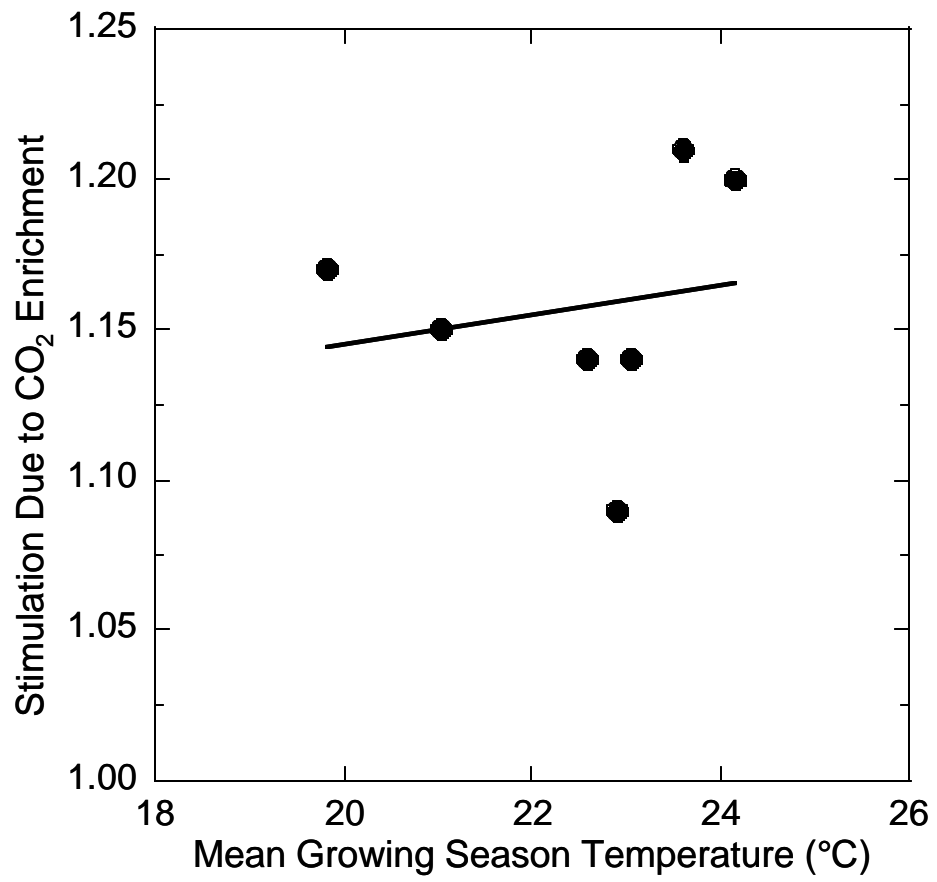


Figure 5.2. Relationship between the stimulation of NPP due to elevated CO₂ versus annual precipitation. Regression equation: $y=1.14 +0.00016x$, $R^2=0.17$, $P=0.1459$.

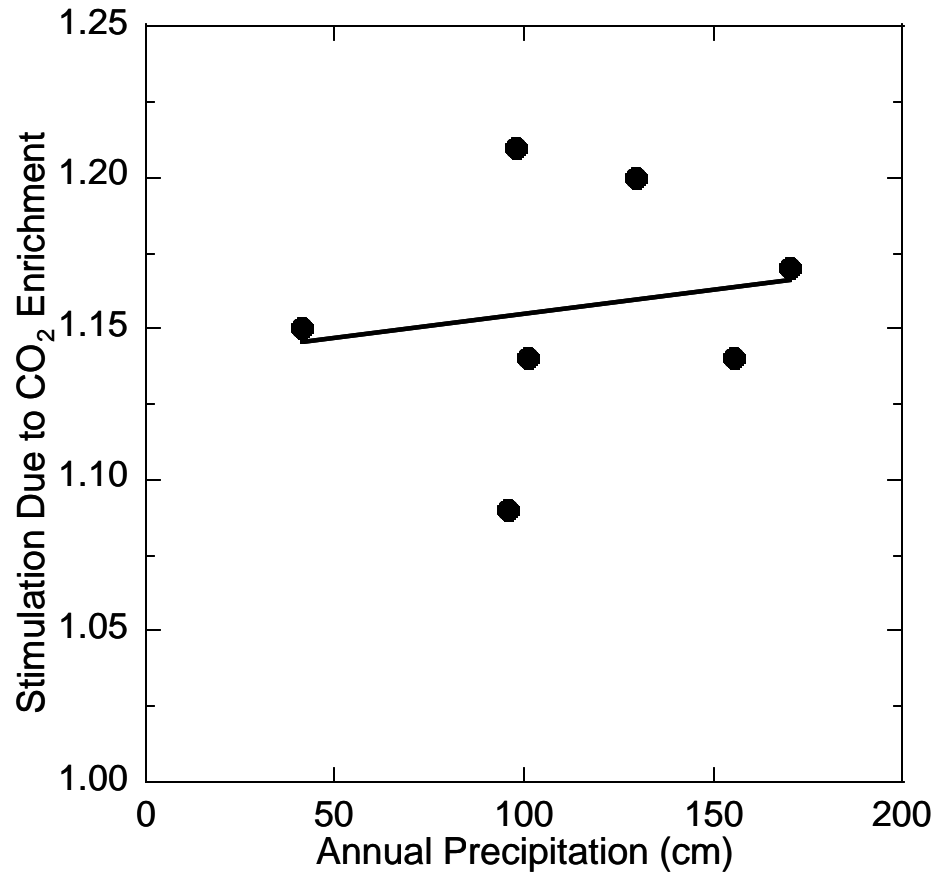


Figure 5.3. Modeled NPP of the Duke Forest FACE experiment grown under ambient (closed bars) and elevated (open bars) CO₂ using a control climate scenario and three climate change scenarios, temperature only (Temp), precipitation only (Precip), and both temperature and precipitation (Both).

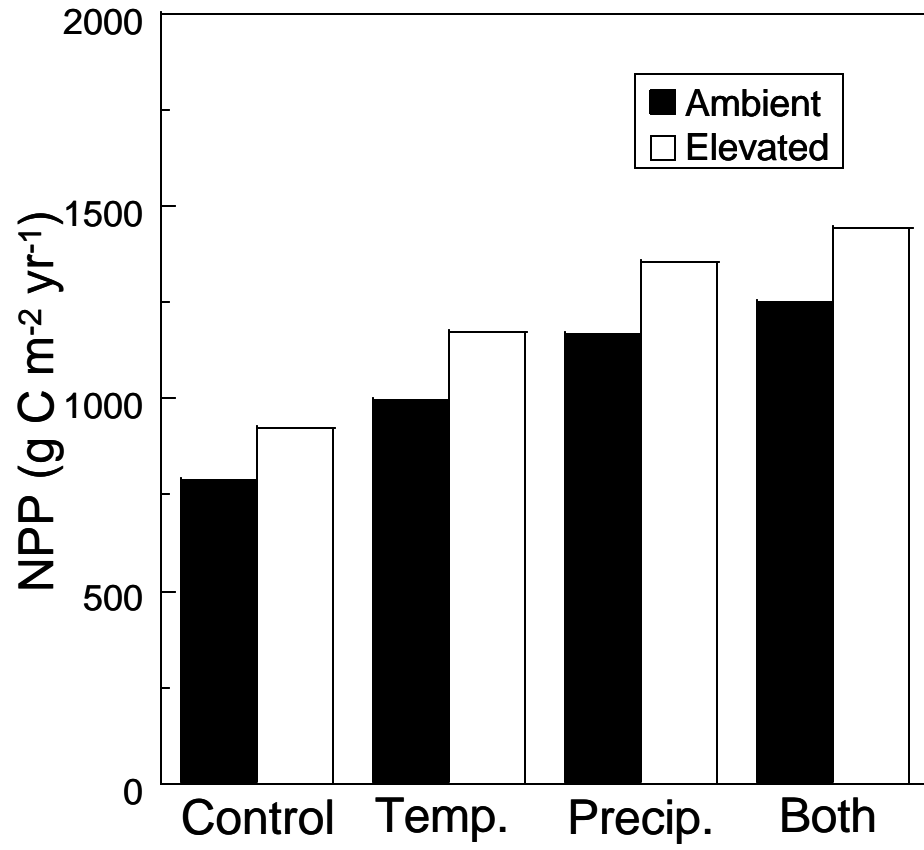
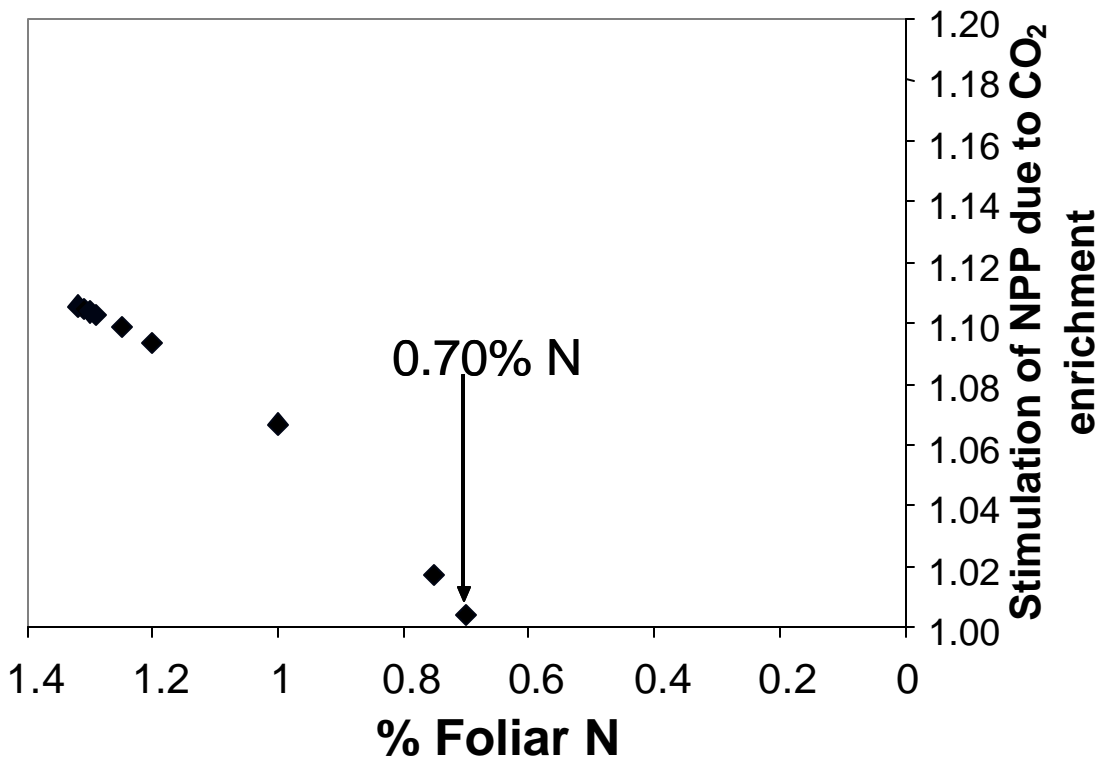


Figure 5.4. The stimulation of NPP by CO₂ enrichment versus % foliar N concentration. The stimulation of NPP by CO₂ enrichment reaches zero at 0.70% foliar N concentration.



General Conclusions

Global climate change has the potential to alter forest ecosystem functioning and productivity, therefore understanding the physiological mechanisms that control forest function and productivity, such as photosynthesis, is extremely important. Also of great importance is our understanding of the interactions of these controlling mechanisms with the large degree of environmental heterogeneity found in a natural ecosystems and how these mechanisms change with time. The purpose of the research in this dissertation is to provide insight into the interactions of the response of photosynthesis to elevated CO₂ with factors that may vary in a forest ecosystem, such as foliar N concentration, light availability, water availability, genetic differentiation, and intra- and inter-annual climate variability and to provide an early modeled estimate of a loblolly pine forest net primary productivity growing under ambient and elevated CO₂.

My first experiment found that the strong relationship that exists between foliar N concentration and photosynthesis is stimulated by growth in elevated CO₂ at the Duke FACE experiment. The slope of this relationship was increased by 81%. I hypothesized that as the increased demand for N with growth in elevated CO₂ exceeded the rate of supply of N that the response of photosynthesis to elevated CO₂ and ultimately the photosynthesis-foliar N relationship would be altered. This hypothesis was based on the observation found in many long-term elevated CO₂ studies of a decreased response of photosynthesis to elevated CO₂ that is typically associated with a decrease in foliar N (Curtis and Wang 1998). However, the increase of the slope of this relationship was sustained through the study period and photosynthesis-foliar N relationships measured at common CO₂ concentrations provided no evidence of changes in the photosynthetic response to elevated CO₂ or foliar N concentration with growth in elevated CO₂.

The increased slope of this relationship under elevated CO₂ represents an increased PNUE of trees growing in elevated CO₂. This higher PNUE is the consequence of a reduction of the competitive inhibition of oxygen on RUBISCO activity and hence, photorespiration, at the carboxylation site.

My second study was a detailed analysis of the photosynthetic response of four species growing in the forest understory to elevated CO₂ and light availability at the Duke FACE experiment. This study compared the photosynthetic response of these four species after seven years of CO₂ enrichment to a study that investigated the same topic in the first year of treatment. I hypothesized that after 7 years of CO₂ enrichment I would observe a change in the investment of resources in the leaf toward light harvesting and away from CO₂ uptake because of the decrease in CO₂ limitation of photosynthesis found in an elevated CO₂ environment and that this reinvestment would lead to a loss of photosynthetic stimulation by elevated CO₂. I found no evidence of a loss of stimulation and in some species an up-regulation of the response of photosynthesis was observed. I also found no CO₂ induced changes in light harvesting capacity of trees growing in elevated CO₂. The current study and the previous study support the hypothesis that ground-rooted trees may not exhibit a decrease in the photosynthetic response to elevated CO₂ as do trees growing in pots (Curtis and Wang 1998, Norby et al. 1999, Medlyn et al. 1999).

The objective of my third study was to determine if loblolly pine seedlings exhibit geographic differentiation in their response to elevated CO₂ and water availability. To carry out this experiment I grew loblolly pine seedlings from four distinct geographic regions in ambient or elevated CO₂ and three levels of water availability in a greenhouse. I hypothesized that a genotype by environment interaction would be evident where seedlings originating from wet

areas would respond more to elevated CO₂ when grown under high water availability and seedlings originating from drier climates would respond more to elevated CO₂ when grown under drought conditions. I found no significant interactions between growth in elevated CO₂ and geographic origin. However, I did observe increased biomass production irrespective of geographic origin. I also observed changes in biomass allocation under varying levels of water availability. I concluded, because of strong support for geographic patterns of biomass allocation and production observed in earlier more long-term studies, that the topic deserves more study under long-term elevated CO₂ and drought conditions.

My final study was performed to provide model estimates of the net primary productivity (*NPP*) of the portions of the loblolly pine forest growing under ambient and elevated CO₂ at the Duke FACE experiment and to determine the effects of projected future climate changes on the CO₂ response of forest *NPP* using the PnET-II (Aber and Federer 1992) forest productivity model. I found good agreement between modeled *NPP* and measured *NPP* at the Duke FACE experiment. I also observed a 15% stimulation of *NPP* across all years of the experiment. Increased temperature and precipitation did not alter the stimulation of *NPP* by elevated CO₂. However, increased temperature precipitation did result in much higher *NPP*. My estimates of *NPP* under future climate conditions however, were much larger than those predicted by others (Sun et al. 2000). I attributed the differences observed between my model estimates and those of others to changes in leaf level physiology not accounted for by the PnET-II model that may occur with large fluctuations in climate.

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