

# Temporal dynamics and spatial variability in the enhancement of canopy leaf area under elevated atmospheric CO<sub>2</sub>

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## Abstract

Increased canopy leaf area ( $L$ ) may lead to higher forest productivity and alter processes such as species dynamics and ecosystem mass and energy fluxes. Few CO<sub>2</sub> enrichment studies have been conducted in closed canopy forests and none have shown a sustained enhancement of  $L$ . We reconstructed 8 years (1996–2003) of  $L$  at Duke's Free Air CO<sub>2</sub> Enrichment experiment to determine the effects of elevated atmospheric CO<sub>2</sub> concentration ([CO<sub>2</sub>]) on  $L$  before and after canopy closure in a pine forest with a hardwood component, focusing on interactions with *temporal* variation in water availability and *spatial* variation in nitrogen (N) supply. The dynamics of  $L$  were reconstructed using data on leaf litterfall mass and specific leaf area for hardwoods, and needle litterfall mass and specific leaf area combined with needle elongation rates, and fascicle and shoot counts for pines. The dynamics of pine  $L$  production and senescence were unaffected by elevated [CO<sub>2</sub>], although  $L$  senescence for hardwoods was slowed. Elevated [CO<sub>2</sub>] enhanced pine  $L$  and the total canopy  $L$  (combined pine and hardwood species;  $P < 0.050$ ); on average, enhancement following canopy closure was ~16% and 14% respectively. However, variation in pine  $L$  and its response to elevated [CO<sub>2</sub>] was not random. Each year pine  $L$  under ambient and elevated [CO<sub>2</sub>] was *spatially* correlated to the variability in site nitrogen availability (e.g.  $r^2 = 0.94$  and  $0.87$  in 2001, when  $L$  was highest before declining due to droughts and storms), with the [CO<sub>2</sub>]-induced enhancement increasing with N ( $P = 0.061$ ). Incorporating data on N beyond the range of native fertility, achieved through N fertilization, indicated that pine  $L$  had reached the site maximum under elevated [CO<sub>2</sub>] where native N was highest. Thus closed canopy pine forests may be able to increase leaf area under elevated [CO<sub>2</sub>] in moderate fertility sites, but are unable to respond to [CO<sub>2</sub>] in both infertile sites (insufficient resources) and sites having high levels of fertility (maximum utilization of resources). The total canopy  $L$ , representing the combined  $L$  of pine and hardwood species, was constant across the N gradient under both ambient and elevated [CO<sub>2</sub>], generating a constant enhancement of canopy  $L$ . Thus, in mixed species stands,  $L$  of canopy hardwoods which developed on lower fertility sites (~3 g N inputs m<sup>-2</sup> yr<sup>-1</sup>) may be sufficiently enhanced under elevated [CO<sub>2</sub>] to compensate for the lack of response in pine  $L$ , and generate an appreciable response of total canopy  $L$  (~14%).

*Keywords:* broadleaf leaf area, drought, leaf area index, leaf area profile, *Liquidambar styraciflua*, nitrogen availability, *Pinus taeda*

Received 4 May 2005; revised version received 16 November 2006 and accepted 15 May 2007

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## Introduction

Canopy leaf area index (area of leaves above a unit of ground area;  $L$ ) affects light interception and thereby stand productivity (Jarvis & Leverenz, 1983; Waring, 1983; Vose & Allen, 1988). The  $L$  in the upper canopy strata determines the light availability at lower strata, and thus species dynamics in the subcanopy (Pearcy, 1990; Naumberg & Ellsworth, 2002; Augspurger & Bartlett, 2003). Furthermore,  $L$  affects rainfall interception and transpiration, and thus forest hydrology and soil moisture (Stogsdill *et al.*, 1989; Oren *et al.*, 1998), with impacts on soil respiration (Hanson *et al.*, 1993; Davidson *et al.*, 1998; Palmroth *et al.*, 2005).

Many forests do not reach their potential maximum  $L$  because of water or nutrient limitations (Brix, 1981; Linder, 1987; Vose & Allen, 1988; Albaugh *et al.*, 1998). It is expected that elevated atmospheric  $\text{CO}_2$  concentration ( $[\text{CO}_2]$ ) might alleviate certain limitations to  $L$  (e.g. Woodward, 1990). Seedlings and saplings grown under elevated  $[\text{CO}_2]$  often show dramatic increases in leaf area (Kellomäki & Wang, 1997; Tissue *et al.*, 1997), but this effect largely reflects accelerated ontogeny, in that larger plants have more leaves (Pataki *et al.*, 1998a; Norby *et al.*, 2003). These studies are unable to predict how elevated  $[\text{CO}_2]$  would affect  $L$  in a closed canopy forest where maximum  $L$  may be limited by water, nutrients or light.

The main mechanism by which elevated  $[\text{CO}_2]$  could alleviate water limitations to leaf area development would be through reductions in stomatal conductance leading to increased available soil water (e.g. Woodward, 1990; Drake & González-Meler, 1997). Overall, observed reductions in conductance have averaged around 20% (Drake & González-Meler, 1997; Medlyn *et al.*, 2001), although Curtis & Wang (1998) showed a nonsignificant 11% reduction. However, few studies have shown significant reductions in conifer species. Previous studies from the Duke Forest FACE site have failed to show reductions in loblolly pine (*Pinus taeda* L.) stomatal conductance with elevated  $[\text{CO}_2]$  (Ellsworth *et al.*, 1995; Ellsworth, 1999), as have other studies with loblolly pine (Lui & Teskey, 1995; Wang *et al.*, 1995; Murthy *et al.*, 1996; Maier *et al.*, 2002). Unchanging stand-level water-use under elevated  $[\text{CO}_2]$  at the Duke FACE is consistent with these findings (Schäfer *et al.*, 2002). Elevated  $[\text{CO}_2]$  could also help forests overcome nutrient limitations, if increased photosynthetic efficiency leads to increases in nitrogen use efficiency (i.e. less nitrogen being bound in the photosynthetic apparatus means more nitrogen for plant growth). While several studies find reductions in N concentrations on a mass basis (Murthy *et al.*, 1996; Curtis & Wang, 1998), few definitively find reductions on an area basis (Me-

lydly *et al.*, 1999; Maier *et al.*, 2002; Rogers & Ellsworth, 2002).

Another proposed mechanism for deeper canopies and higher  $L$  with elevated  $\text{CO}_2$  is the lower light compensation points (light level at which photosynthesis equals respiration) often predicted under elevated  $[\text{CO}_2]$  (e.g. Drake & González-Meler, 1997). Although some studies have found elevated  $[\text{CO}_2]$  to lower the light compensation point (Wang *et al.*, 1995; Maier *et al.*, 2002), others have found reductions only in the upper canopy where it is not likely to result in increased  $L$  (Norby *et al.*, 2003). Lower light compensation points have not been observed under elevated  $[\text{CO}_2]$  for loblolly pine in the Duke FACE experiment (as reported in Schäfer *et al.*, 2003). Furthermore, Maier *et al.* (2002) showed a decrease in shoot needle density, (and thus clumping) in loblolly pine trees under elevated  $[\text{CO}_2]$  which would decrease light penetration through the canopy (Stenberg, 1996a; Stenberg *et al.*, 2001; Palmroth *et al.*, 2002), potentially negating any positive influence of elevated  $[\text{CO}_2]$  on light compensation.

In addition, some studies have found  $[\text{CO}_2]$  effects on  $L$  dynamics, particularly changes in leaf senescence patterns (Jach & Ceulmans, 1999; Li *et al.*, 2000; Sigurdsson, 2001; Karnosky *et al.*, 2003; Tricker *et al.*, 2004). Such changes could offset or accentuate  $[\text{CO}_2]$  effects on  $L$  production.

Presently, few studies in closed canopy forests have assessed the response of  $L$  to  $[\text{CO}_2]$  enrichment, and none have shown any sustained or consistent effect of elevated  $[\text{CO}_2]$  on the magnitude or dynamics of  $L$  (Hättenschwiler *et al.*, 1997; Gielen *et al.*, 2003; Norby *et al.*, 2003). These studies have been conducted in deciduous forests and may not be indicative of the potential for response in coniferous forests. Thus, the objective of this study was to quantify the effect of  $[\text{CO}_2]$  on  $L$  in a loblolly pine plantation growing at a site with substantial *spatial* variation in hardwood presence and N availability and *temporal* variation in water availability.

In practice, estimating  $L$  in loblolly pine is difficult. Loblolly pine – unlike many evergreen conifers – has a rapid needle turnover rate (~19 months; Zhang & Allen, 1996), resulting in highly dynamic seasonal  $L$ , with the maximum  $L$  reaching as much as twice the minimum  $L$  (Kinerson *et al.*, 1974; Dougherty *et al.*, 1994; Vose *et al.*, 1994). Commonly used methods cannot generate accurate  $L$  estimates in such canopies where more than one cohort of needles is present at most times, yet a large proportion of  $L$  is replaced annually. Estimates based on allometry do not capture the effects of interannual variation in climate such as droughts or wind and ice storms, and optical estimates frequently have a bias, particularly in coniferous canopies (Gower

& Norman, 1991; Sampson & Allen, 1995; Stenberg, 1996b).

Our study, performed at the Duke Free Air CO<sub>2</sub> Enrichment (FACE) experiment, used data on litterfall to estimate leaf production. We combined leaf production estimates with specific leaf area (SLA) values, and for the pine with measurements of needle elongation, and fascicle and shoot counts, to reconstruct an 8-year record of seasonal dynamics of *L* (see overview of the procedure in Fig. 1). This record of *L* estimates, spanning a broad range of climatic conditions and natural disturbance events, was used to address hypotheses on the effect of elevated [CO<sub>2</sub>] on *L*, and to provide input for use in modeling carbon and water cycle processes.

Our objective was to examine the potential effects of elevated [CO<sub>2</sub>] on pine and hardwood leaf area dynamics, focusing on the following questions: Can elevated [CO<sub>2</sub>] increase peak and mean *L* in a closed canopy conifer forest? Does elevated [CO<sub>2</sub>] affect the timing of leaf production or loss? Is spatial variation in the [CO<sub>2</sub>]-induced enhancement of *L* related to nitrogen availability? Does elevated [CO<sub>2</sub>] change the response of *L* to environmental stress? Based on results from comparable studies in hardwood forests (Hättenschwiler *et al.*, 1997; Gielen *et al.*, 2003; Norby *et al.*, 2003), we

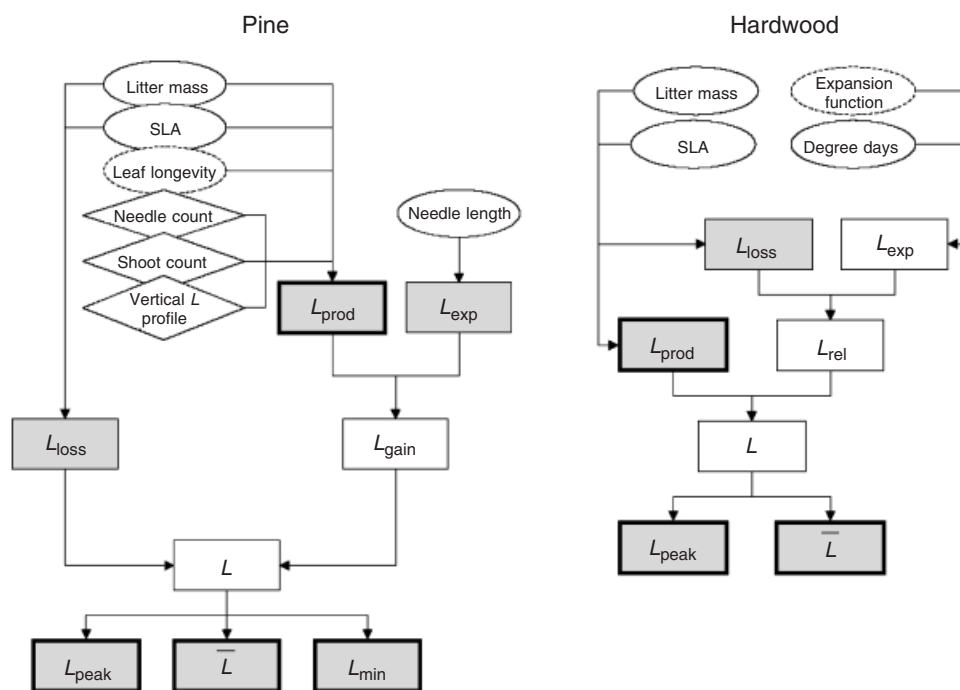
hypothesized that elevated [CO<sub>2</sub>] would have little impact on stand *L*, either in terms of magnitude or dynamics, once our forest canopy closed.

**Materials and methods**

*Site description*

The study site is within a loblolly pine (*Pinus taeda* L.) plantation established in 1983 on moderately low fertility, acidic clay-loam (Enon Series) in Duke Forest in Orange County, North Carolina (35°58'N, 79°08'W; elevation 163 m). The climate is warm and humid in summer and moderate in winter with a mean annual temperature of 15.8 °C. Precipitation is distributed approximately evenly throughout the year, with a 111-year average of 1145 mm. In early 2002 pines reached ~18 m in height and made up 90% of the basal area. Common broadleaf species include sweetgum (*Liquidambar styraciflua*) in the mid to upper canopy, and *Acer rubrum*, *Ulmus alata* and *Cornus florida* in the mid to lower canopy.

In 1994, the FACE prototype (Plot 7), a 15 m radius plot, and an adjacent untreated reference plot (Plot 8) were established. CO<sub>2</sub> enrichment (550 ppm during



**Fig. 1** Overview of methodology for calculating leaf area and leaf area derived variables presented in this paper. Ovals indicate inputs (measured inputs with solid borders, parameters with dashed borders) and diamonds represent measured input variables necessary only under disturbance conditions (see ‘Materials and methods’). Squares are calculated variables, with annual values in thick borders, and daily time steps in thin borders. Gray shading highlights the leaf area variables that will be the focus of analysis and discussion in this paper.  $L_{prod}$  = leaf area production;  $L_{exp}$  = leaf area expansion;  $L_{loss}$  = leaf area loss;  $L_{peak}$  = annual maximum leaf area;  $\bar{L}$  = average (annual or functional) leaf area index;  $L_{min}$  = annual minimum leaf area index. SLA, specific leaf area.

daylight hours of the growing season) commenced in 1994 according to the FACE protocol (Hendrey *et al.*, 1999). The replicated FACE (Plots 1–6; also 15 m radius) was subsequently established in 1996 when CO<sub>2</sub> enrichment (+ 200 ppm) was initiated in three of the plots. In 1998, Plots 7 and 8 were each split in half, and one half of each received yearly nitrogen fertilization (11.2 g N m<sup>-2</sup> yr<sup>-1</sup>) which was also applied through a 7.5 m buffer arcing outside the fertilized half. Four pairs of auxiliary plots (10 m × 10 m with 10 m wide buffer) were established nearby, and one member of each pair was fertilized (Oren *et al.*, 2001). The average actual CO<sub>2</sub> concentration during 1996–2003 was 570 ppm, with >90% of 1-min [CO<sub>2</sub>] averages within 20% of the target (average target = 572 ppm). Figure 2 shows the timeline for the application of [CO<sub>2</sub>] and fertilization treatments to each experimental plot.

### Canopy and leaf measurements

In this section we describe the measurements that allowed the characterization of the seasonal and inter-annual development of leaf area (refer also to Fig. 1).

**Litterfall.** For the six replicated FACE plots we used litterfall data previously published only as annual values (Finzi *et al.*, 2002, 2006). Litter falling into 12 0.16 m<sup>2</sup> baskets in each plot was collected bimonthly during peak litterfall (September–December) and monthly otherwise. Samples were dried (65 °C, 4 days), separated into pine needles or hardwood leaves, and weighed. Beginning in fall 2001, each plot within the FACE prototype complex (Plots 7 and 8 and

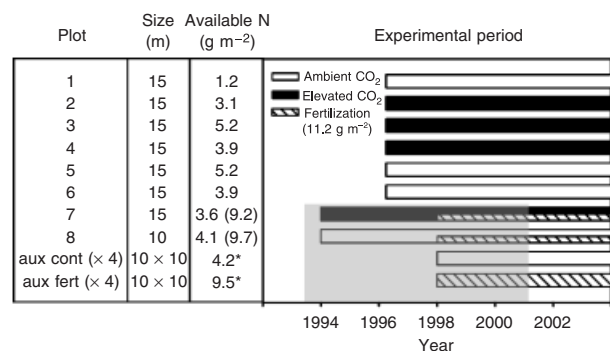
the auxiliary plots; see Fig. 2) was equipped with four 0.5 m<sup>2</sup> wood frame litter baskets. The processing protocol was as described above, but sweetgum leaves were assigned an independent category. No differences were observed between pine and hardwood foliage mass collected with the two types of collectors ( $P = 0.600$  and  $0.888$ , respectively) during a 5-month calibration period in which the collectors were colocated in all plots.

**Specific leaf area.** In the FACE prototype complex, from each autumn collection (September through December, 2001–2003), before litter drying, five needles/leaves each of pine, sweetgum, and all other hardwoods combined were used to determine SLA (cm<sup>2</sup> g<sup>-1</sup>). Projected leaf area was measured optically (DIAS, Decagon Devices Inc., Pullman, WA, USA), samples were oven dried (70 °C; ≥3 days) and weighed. Although previous work at this site did not find a significant difference between litter and green leaf/needle SLA (Finzi *et al.*, 2002), SLA did differ between senesced and green needles collected after the 2002 ice storm ( $P = 0.003$ ), and therefore green needle SLA was employed for green needles dislodged in that event. None of the species groups showed any trend in litter SLA within a year, or any significant differences between treatments (all  $P > 0.050$ ). Therefore, litter SLA values were averaged within years and across treatments. For pine, litter SLA values measured in 2001 were used for conversions of leaf litterfall to leaf area for all years before 2001.

To account for the effect of light environment on SLA of sweetgum and other hardwoods mostly present in the lower and subcanopy, SLA from 2001 was reduced by multiplying by the ratio of pine  $L$  at time of hardwood bud-break in each year before 2001 and pine  $L$  in 2001.

**Optical measurements.** In order to contrast our estimates with values from a known, albeit biased, method, optical gap fraction measurements were taken using a LAI-2000 canopy analyzer (Li-Cor, Lincoln, NE, USA). In the FACE prototype complex, measurements were taken at four locations per plot, every 1–2 months during the periods of August 1999–July 2000 and October 2001–December 2003. In the replicated FACE plots, measurements were taken every 1–2 months at eight locations per ring between August 2002 and December 2003. Viewcaps (90° for FACE prototype complex and 180° for replicated FACE) were used for all measurements.

The LAI-2000 was also used to generate vertical  $L$  profiles (leaf area density at 2 m resolution; LAD) for the pine canopy during periods in which hardwoods were



**Fig. 2** Overview of experimental design and treatment regime throughout the experimental period. Plot size is given as either plot radius or dimensions. Auxiliary control and fertilized plots are comprised of four plots each, with the available nitrogen values given representing the average of the four plots. Available nitrogen values in parenthesis are for the fertilized halves of the plots, assuming half of the applied fertilizer is available. Gray shading indicates the period of time during which a subset of the plots were exposed to treatment, but not measured.

leafless. These measurements were taken in four cardinal directions on four dates (16 November 2002; 7 March 2003; 26 November 2003 and 12 March 2004) in the replicated FACE and the FACE prototype plots from the central walkup towers, and on two dates (7 March 2003 and 12 March 2004) in the reference and auxiliary plots.

In March of 2002, crown length for all pines in the FACE prototype complex plots, and within a subplot of each replicated FACE plot (1.5 m extending from plot boardwalks) was determined as the difference between total tree height and height to crown base measured with a survey laser (Criterion 400, Laser Technology Inc., Englewood, CO, USA).

*Elongation of needles.* Needle elongation in the FACE prototype complex was quantified in 2002, and in the replicated FACE in 1998 and 1999 (Rogers & Ellsworth, 2002). Needles at lower, middle and upper canopy positions of three trees were monitored in each plot. Needle length was obtained at 1–3 week intervals on all current year flushes. The trajectory of relative expansion of new foliage in each third of the canopy was scaled to relative leaf area expansion ( $L_{exp}$ ) for the pine canopy by weighting the trajectories according to the proportion of leaf area in each canopy third. For years in which elongation measurements were not conducted, the relative growth trajectory from the year that was most climatically similar (in terms of precipitation and temperature) was employed.

*Needle and shoot counts.* Between mid-July and mid-September 2002, fascicles were counted monthly on each shoot measured for needle elongation. Counts were made of needles in all flushes of 2002, and the last flush of 2001. The fraction of 2002 needles per shoot ( $N_{f02}$ ) at each measurement time was related to the vertical position within the canopy. In November of 2004, counts of current and previous year flushes were made at 2 m intervals through the crowns of trees (three to seven trees) of five plots spanning the [CO<sub>2</sub>] × fertilization treatments. The fraction of shoots with current year flushes ( $S_{fc}$ ) was regressed against the relative height within the canopy.

*Base of live crown sapwood area.* In April 2002, diameter at breast height (dbh, cm) and at the base of the live crown (dblc; cm) was measured on 48 trees in plots of the FACE prototype complex and replicated FACE, using towers and canopy lifts. These measurements were used to develop a regression between dblc and dbh ( $r^2 = 0.87$ ,  $P < 0.001$ ). This regression,  $dblc = 0.74 \times (dbh) - 16.40 \times (\text{stand density}) + 0.18$ , where stand density is trees per m<sup>2</sup>, was then used to calculate the

diameter at the base of live crown of all pines within the replicated FACE and FACE prototype complex plots. Sapwood area was taken to be 85% of base of live crown total cross sectional area (Schäfer *et al.*, 2002).

*Calculation of leaf area index (1996–2003)*

$L$  at any point in time is the balance between growth and loss of foliage, and requires accounting for both dynamics and magnitude. Due to differences in foliage dynamics, procedures for characterizing pine and hardwood leaf area index will be described separately, beginning with pine (refer also to Fig. 1).

*Pine.* Annual leaf area production of pine ( $L_{prodP}$ ; m<sup>2</sup> m<sup>-2</sup>) was derived as

$$L_{prodP} = LM \times SLA, \tag{1}$$

where LM is annual pine needle litter mass (g) (Table 1). For loblolly pine, the process of equating needlefall mass to yearly production is complicated by an average needle longevity of 19 months (Zhang & Allen, 1996), such that most of the needlefall within a given year represents the needles produced during the previous year. To estimate  $L_{prodP}$  from the timing and amount of leaf area loss ( $L_{lossP}$ ), we calculated the annual  $L_{prodP}$  for a given year as the sum of litterfall  $L$  collected during the period of time spanning May of the

**Table 1** Frequently used symbols

	Definition	Units
<i>Subscripts</i>		
c	Canopy (pine + hardwood)	
h	Hardwood	
i	Canopy layer ( $i = 2$ m interval or canopy third)	
P	Pine	
t	Time (day of year)	
<i>Symbols</i>		
$A_s$	Sapwood area	m <sup>2</sup> m <sup>-2</sup>
$L$	Leaf area index	m <sup>2</sup> m <sup>-2</sup>
$L_{exp}$	Relative leaf expansion	
$L_{gain}$	New leaf area gain	m <sup>2</sup> m <sup>-2</sup>
LM	Annual litter mass of leaves or needles	g
$L_{loss}$	Leaf area loss	m <sup>2</sup> m <sup>-2</sup>
$L_{min}$	Annual minimum leaf area	m <sup>2</sup> m <sup>-2</sup>
$L_{peak}$	Annual maximum leaf area	m <sup>2</sup> m <sup>-2</sup>
$L_{prod}$	Leaf area production	m <sup>2</sup> m <sup>-2</sup>
$L_{rel}$	Leaf area relative to annual maximum	
$N$	Soil nitrogen	g m <sup>-2</sup>
RWC	Relative water content	
SLA	Specific leaf area	cm <sup>2</sup> g <sup>-1</sup>
$\theta$	Volumetric soil moisture	m <sup>3</sup> m <sup>-3</sup>

following year, until May of the year after that. For example, the production of 1998 would be calculated as the needle mass falling from May 1999 through April 2000.

A continuous trajectory of absolute leaf area gain was generated by

$$L_{\text{gain}(t)} = L_{\text{prod}} \times L_{\text{exp}(t)}, \quad (2)$$

where  $t$  is day of year and  $L_{\text{exp}}$  is relative leaf area expansion from needle elongation measurements. Interpolation of litterfall  $L$  between collection times produced a continuous trajectory of  $L_{\text{loss}}$ . The trajectories of  $L_{\text{exp}}$  and  $L_{\text{loss}}$  were combined at a daily time step to produce a continuous record of  $L$ :

$$L_{\text{p}(t)} = L_{\text{p}(t-1)} + L_{\text{gain}(t)} - L_{\text{loss}(t)}. \quad (3)$$

This approach to estimating  $L_{\text{prod}}$  [Eqn (3)] was successful over large periods of this study. However, the assumption that the timing of leaf production can be determined based on leaf loss is invalid when disturbance events cause premature loss of needles. Between 2001 and 2004 the site experienced four such perturbations: a moderate late summer drought in 2001, a severe spring to late summer drought in 2002, a severe ice storm in December 2002 and a moderate hurricane in September 2003. These events decoupled the timing of  $L_{\text{loss}}$  from  $L_{\text{prod}}$  because needles could be lost during the same year in which they were produced.

Most difficult to resolve was the partitioning of foliage lost during 2002, due to the effect of both the severe drought and ice storm. We dealt with the cumulative effects of these losses by using information about the age distribution of needles before current year needles were affected by the drought (day 193). Determining the proportion of  $L$  produced in 2001 and 2002 at that time allowed for the calculation of  $L_{\text{prod}}$  in 2002, with the balance of  $L$  representing needles remaining from 2001. The partition of  $L$  between 2001 and 2002 foliage was determined for each canopy third ( $i = 1-3$ ) by multiplying the fraction of 2002 needles ( $N_{f02}$ ) with the fraction of current year shoots ( $S_{fc}$ ) and pine leaf area found in that canopy third ( $L_f$ ):

$$L_{f02(i)} = N_{f02(i)} \times S_{fc(i)} \times L_{f(i)}, \quad (4)$$

where the fractions of 2002 needles and shoots were determined from needle and shoot counts, and  $L_f$  was determined from the vertical optical measurements.  $L_{\text{prod}}$  in 2002 was then calculated by summing  $L_{f02}$  from each canopy third and multiplying by pine  $L$  at day 193:

$$L_{\text{prod}} = L_{\text{p}(193)} \times L_{f02}. \quad (5)$$

We adjusted  $L_{\text{p}(193)}$  to reflect the  $L$  which would have been present had 2002 needles been fully expanded.

To calculate the amount of 2001 foliage lost during the 2001 drought, we assumed that the proportion of foliage lost prematurely was half of that lost prematurely during the drought of 2002. For the green leaf mass lost during Hurricane Isabel in September 2003, we assumed that the loss of leaf area from each cohort (2002 and 2003) was proportional to the quantity of each cohort in the canopy, reflecting an equal probability of mechanical dislodging.

*Hardwood.* Because there is no significant component of marcescent hardwood species (with dead leaves persisting on trees) at the site, annual foliage production was based on annual litterfall with no time lag. We did not quantify the temporal dynamics of leaf expansion, so hardwood leaf growth dynamics were calculated based on several parameters. Firstly, degree-day sums were used to determine the day at which leaf expansion began. Budbreak for hardwood species generally coincided with the beginning of pine diameter growth, for which 5 years of observations determined to begin at 426 degree-days (SD  $\pm$  21 degree-days). Degree-days represented the summation of mean daily temperature (minus a base temperature of 2 °C) beginning after the last period of 4 or more sequential days with mean daily temperature in each day of  $\leq 2$  °C. Secondly, based on a literature survey (Hunter & Lechowicz, 1992; Augspurger & Bartlett, 2003), 30 days was selected as the time necessary to complete leaf expansion, with relative leaf expansion ( $L_{\text{exp}}$ ) occurring exponentially as in Oren & Pataki (2001). This assumes that after the initial pulse of leaf expansion, indeterminate species produced relatively little additional foliage. The relative pattern of leaf loss was determined according to litterfall measurements. A small quantity of leaves occasionally remained attached long after senescence, resulting in a small amount of leaf fall through the winter. The date by which 95% of total litterfall, summed between two consecutive bud breaks, occurred was determined to be the date of full senescence; leaf fall occurring later was assigned to this date. At the daily time scale, relative  $L$  ( $L_{\text{rel}}$ ) was determined by

$$L_{\text{rel}} = L_{\text{exp}} - L_{\text{loss}}, \quad (7)$$

where  $L_{\text{loss}}$  is the relative loss of foliage. Finally,

$$L_{\text{h}(t)} = LM \times SLA \times L_{\text{rel}}, \quad (8)$$

where LM is annual hardwood leaf litter mass (g).

#### Soil water content

Within the FACE prototype and reference plots, volumetric soil water content ( $\theta$ ;  $\text{m}^3 \text{m}^{-3}$ ) was measured continuously from 2001 at eight locations, with four

probes at 30 cm and four probes at 10 cm (ThetaProbe ML1x or ML2x; Delta-T Devices, Cambridge, UK). Beginning in 1997, in each of the six replicated FACE plots,  $\theta$  of the upper 30 cm was measured continuously in four locations (CS615; Campbell Scientific, Logan, UT, USA). All sensors were sampled every 30 s, and 30-min averages were logged (21X or CR23X, Campbell Scientific, Logan, UT, USA). Growing season averaged relative water content (RWC) was calculated by dividing growing season averaged  $\theta$  by the field capacity  $\theta$  at the site (0.54; Oren *et al.*, 1998).

#### *N availability*

Annual net N mineralization rates ( $\text{g N m}^{-2} \text{yr}^{-1}$ ;  $N_{\text{min}}$ ) for 1998 from the six replicated FACE plots were taken from Finzi *et al.* (2002). The relationship between these mineralization rates and leaf N concentrations was applied to 1998 leaf N concentrations in the FACE prototype complex to estimate N mineralization rates in these plots. Available N was defined as

$$N_{\text{avl}} = N_{\text{min}} + N_{\text{dep}} + N_{\text{fix}} + N_{\text{fert}}, \quad (9)$$

where  $N_{\text{dep}}$  is N deposition (from Finzi *et al.*, 2006) and  $N_{\text{fix}}$  is N fixation (from Hofmockel & Schlesinger, 2007). These studies show that losses of N from this system are small. For  $N_{\text{fert}}$ , we assumed that half of the applied  $11.2 \text{ g N m}^{-2} \text{yr}^{-1}$  was available for plant uptake (Ducey & Allen, 2001).

#### *Statistical considerations*

The Duke Forest FACE experiment includes the six plots of the 'replicated' portion of the experiment ( $n = 3$ ), and the FACE prototype complex. The FACE prototype complex (Plots 7, 8 and auxiliary plots) included five ambient plots receiving no fertilization, which together with the three FACE plots gave a total of eight ambient replicates (Fig. 2). The complex also included five fertilized replicates. Given their concentration in the southern end of the site and smaller size, and in order to be conservative in interpreting the results, we treated the ambient plots of the complex collectively as one plot, resulting in four replicates each for ambient and elevated [CO<sub>2</sub>] conditions, and one each for fertilized and elevated [CO<sub>2</sub>] × fertilized conditions. Thus, reduced to  $n = 1$ , the fertilization treatments were used to generate information on the upper limits of leaf area under ambient and elevated [CO<sub>2</sub>] conditions. Statistical differences between the unreplicated (fertilized) and the replicated (unfertilized) treatments were assessed with one-sample *t*-tests (Sokal & Rohlf, 1995). The unfertilized treatments were first investigated as to whether the prototype complex

( $n = 1$ ) is a part of the 'replicated' population ( $n = 3$ ) for each variable. The smallest *P*-value was 0.136, and all other *P*-values were greater than 0.42, strongly rejecting the hypothesis that the prototype and 'replicated' plots are not from the same experiment. The effects of elevated [CO<sub>2</sub>] on peak *L*, minimum *L*, average *L*, *L* production, ratio of annual maximum to minimum *L*, and *L*-to-sapwood area ratio were analyzed through repeated measures ANOVA with plots blocked according to the pairing of plots established at the onset of the experiment ( $n = 4$  blocks). [CO<sub>2</sub>] effects during each year of the experiment were tested through linear contrasts within the repeated measures ANOVA. Effects of elevated [CO<sub>2</sub>] on leaf expansion and loss were also tested through repeated measures ANOVA on the parameters of sigmoidal regressions of the form:  $a / (1 + \exp^{-(x-x_0)/b})$ . The relationship between functional *L* and available N were evaluated using ordinary least-square regression. These statistical analyses were conducted in SAS (Version 8.0, Cary, NC, USA).

## Results

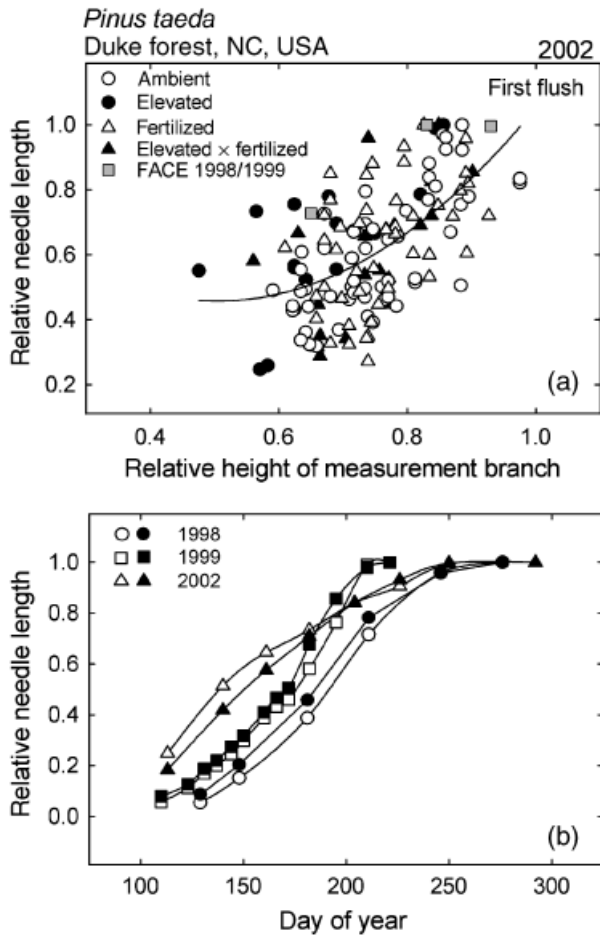
### *Foliage production and expansion*

In pines, maximum needle length varied through the canopy, with lower canopy needles reaching only ~50% of upper canopy needle length (Fig. 3a). Repeated measures analysis of the parameters of sigmoidal elongation trajectories – developed by weighting the trajectories of each canopy third by its leaf area – indicated that expansion patterns were not different between ambient and elevated [CO<sub>2</sub>] during any of the 3 years measured (Table 2). Effects of interannual variation in climate were more pronounced than [CO<sub>2</sub>] effects, with drought years showing a prolonged expansion period.

Combined for all years and both [CO<sub>2</sub>] treatments during the period 1998–2002 (thus excluding the post-ice storm year 2003), treatment-level  $L_{\text{prodP}}$  increased with increasing minimum *L* ( $L_{\text{minP}}$ ) – representing *L* at the start of the growing season – and treatment-level growing season average relative soil water content,  $\overline{\text{RWC}}$ . A multivariate least-square regression analysis (SAS Proc Reg and Proc Stepwise) revealed that the interaction of  $L_{\text{minP}} \times \overline{\text{RWC}}$  explained most of the variability in  $L_{\text{prodP}}$  under both [CO<sub>2</sub>] treatments (Table 3). Under ambient [CO<sub>2</sub>], the parameter for the effect of  $L_{\text{minP}}$  on  $L_{\text{prodP}}$  was significantly smaller, resulting in lower leaf area production for a given set of conditions.

### *Foliage loss*

Repeated measures ANOVA of regression parameters for the relative pattern of  $L_{\text{loss}}$  of the pine and hardwood



**Fig. 3** Relative needle length as a function of canopy position for pine under ambient CO<sub>2</sub>, elevated CO<sub>2</sub>, fertilization and elevated CO<sub>2</sub> with fertilization, during the 2002 growing season (a), and canopy averaged relative needle expansion ( $L_{exp}$ ) for pine over the course of the growing season for 1998, 1999 and 2002 growing seasons (b). The gray symbols in (a) show the average values for each canopy third measured in the FACE site in 1998 and 1999. Open symbols are used for ambient CO<sub>2</sub> and closed symbols are used for elevated CO<sub>2</sub>.

species indicated some differences between years, as well as treatments. For the pine year was a significant effect ( $P < 0.001$ ), but the pattern of needle loss was unaffected by [CO<sub>2</sub>] ( $P > 0.206$ ; Table 2). Considering the effect of high fertility (fertilization) through single observation vs. sample mean *t*-tests (Sokal & Rohlf, 1995) indicated a more rapid loss of foliage during drought under both ambient (2001 and 2002;  $P < 0.033$ ) and elevated [CO<sub>2</sub>] (2002 only;  $P = 0.031$ ; Fig. 4c). There was also year-to-year variation in loss patterns for hardwood leaves ( $P < 0.001$ ). Elevated [CO<sub>2</sub>] slowed  $L_{lossh}$  relative to ambient [CO<sub>2</sub>] from 1999 to 2003 (all  $P < 0.005$ ; Fig. 4d), also with indication of a more rapid loss in fertilized plots during drought years. ( $P < 0.05$  in two of four tests).

**Table 2** Significance (*P*-values) of repeated measures ANOVA factors on sigmoidal parameters for canopy averaged relative needle expansion ( $L_{exp}$ ) for pine over the course of the growing season for 1998, 1999 and 2002 growing seasons, and average annually relativized leaf area loss ( $L_{loss}$ ) for pines and hardwoods (1998–2002)

	Pine needle expansion		Pine needle loss		Hardwood leaf loss	
	<i>b</i>	<i>x0</i>	<i>b</i>	<i>x0</i>	<i>b</i>	<i>x0</i>
CO <sub>2</sub>	0.184	0.961	0.206	0.734	0.642	0.004
Year	0.165	0.016	<0.001	<0.001	<0.001	<0.001
CO <sub>2</sub> × year	0.198	0.250	0.755	0.088	0.653	0.009

**Table 3** Coefficients for response functions of 1998–2002 pine leaf area production ( $L_{prod}$ ) = minimum  $\bar{L}$  + (minimum  $\bar{L} \times \overline{RWC}$ ) and rate of loss of pine leaf area = intercept +  $L_{peakp} + \overline{RWC}$ , where  $\overline{RWC}$  is relative soil water content

Parameter	Ambient	Elevated
<i>Production</i>		
Intercept		1.06
$L_{minp}$	-0.08	
$L_{minp} \times \overline{RWC}$		1.01
$r^2$	0.55	0.98
<i>Rate of loss</i>		
Intercept		-0.27
$\overline{RWC}$		1.72
$r^2$	0.98	0.94

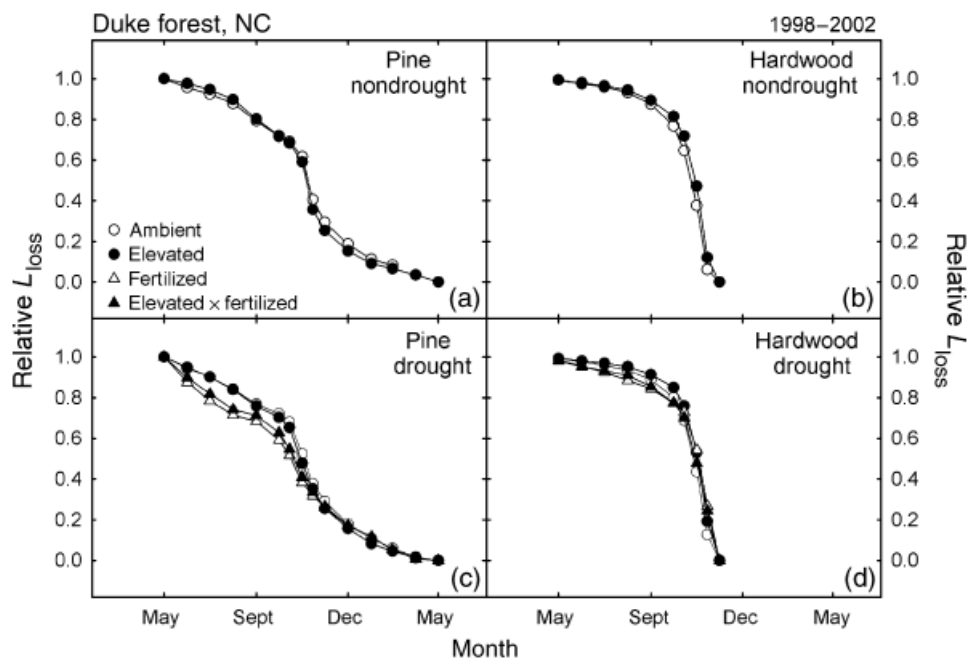
The model for pine leaf area production had an adjusted  $r^2 = 0.90$  with both [CO<sub>2</sub>] treatments included, and for pine leaf area loss, excluding data from 2001, the full model had an adjusted  $r^2 = 0.94$ . Only parameters that contribute significantly to a reduction in unexplained variation are included, and different values for the same parameter indicate difference at  $P < 0.05$ .

For the pine, the maximum rate of  $L_{lossp}$  (the derivative of the inflection point of fitted sigmoidal curves) was explained by  $\overline{RWC}$ . Excluding data from 2001, in which loss was enhanced by a drought beginning late in the growing season,  $\overline{RWC}$  explained 94% ( $P < 0.001$ ) of the variation in  $L_{lossp}$  with no significant difference in the relationship under elevated and ambient [CO<sub>2</sub>] (Table 3).

*Leaf area index*

Over the years after *L* reached a quasi-equilibrium (1999), pine *L* averaged  $2.51 (\pm 0.13) m^2 m^{-2}$  under ambient [CO<sub>2</sub>], and  $2.90 (\pm 0.14) m^2 m^{-2}$  under elevated [CO<sub>2</sub>]. During the same period, canopy





**Fig. 4** Average annually relativized leaf area loss ( $L_{loss}$ ) under ambient CO<sub>2</sub>, elevated CO<sub>2</sub>, fertilization and elevated CO<sub>2</sub> in combination with fertilization for pines and hardwoods under nondrought (1999, 2000) (a, b) and drought conditions (1998, 2001, 2002) (c, d).

$L$  averaged  $3.34 (\pm 0.17) \text{ m}^2 \text{ m}^{-2}$  and  $3.90 (\pm 0.19) \text{ m}^2 \text{ m}^{-2}$  under ambient and elevated [CO<sub>2</sub>], respectively. Overall, elevated [CO<sub>2</sub>] increased  $L_{peakp}$ ,  $L_{prodP}$ , and the annual mean  $L_p$ , ( $\bar{L}_p$ ) (Appendix A). Elevated [CO<sub>2</sub>] increased  $L_{peakp}$  and  $\bar{L}_p$  every year beginning in 1998 ( $P < 0.050$ ), and  $L_{prodP}$  during 5 of the 7 years since 1997 ( $P < 0.050$ ). While elevated [CO<sub>2</sub>] generally increased all measures of  $L$ , the absolute effect was dynamic from year to year (see Appendix A). The upper limits of leaf area response were assessed using a single observation vs. sample mean  $t$ -test (Sokal & Rohlf, 1995). According to this test, fertilization did not significantly increase annual  $\bar{L}_p$  (all  $P > 0.050$ ). Similarly, fertilization combined with elevated [CO<sub>2</sub>] did not produce higher annual  $\bar{L}_p$  than elevated [CO<sub>2</sub>] alone (all  $P > 0.050$ ).

For the hardwood, we found no significant effect of any treatments on  $L_{peakh}$ ,  $L_{prodh}$  or annual  $\bar{L}_h$  (all  $P > 0.050$ ). The lack of  $\bar{L}_h$  response, and the nonuniform distribution of hardwoods among plots, resulted in a slightly smaller effect of elevated [CO<sub>2</sub>] when annual mean canopy leaf area (pine + hardwood;  $\bar{L}_c$ ) was assessed in comparison with results for the pine  $\bar{L}_p$  (see Appendix B). Nevertheless, elevated [CO<sub>2</sub>] significantly increased annual  $\bar{L}_c$  in every year beginning in 1998 ( $P < 0.050$ ). Assessments of the upper limits of leaf area response at high fertility levels using a single observation vs. sample mean  $t$ -test (Sampson & Allen, 1995) showed that fertilization increased  $\bar{L}_c$  in 2001, but

fertilization under elevated [CO<sub>2</sub>] did not produce higher annual  $\bar{L}_c$  than elevated [CO<sub>2</sub>] alone ( $P > 0.050$ ).

The reconstructed  $L$  over the entire span of the study period (1994–2003) is shown in Fig. 4. The effects of several hurricanes (Hurricane Fran in 1996, Floyd in 1999; and Isabel in 2003) and a severe ice storm (December 2002) are detectable in the  $L$  time series.

#### Diagnosics

In order to assess the reliability of our estimated pine  $L$ , we considered three measures: (1) the relationship between  $L$  determined from the combination of litterfall, SLA and supporting measurements and the  $L$  determined optically, from LAI-2000, (2) the ratio of annual maximum  $L$  to winter minimum  $L$  and (3) the winter minimum leaf-to-sapwood area ratio. Firstly,  $L_c$ , composed only of  $L_p$  during the leafless period for hardwoods, was compared with that obtained from optical measurements, after correcting the optical measurements for clumping of pine foliage, and for woody surface area (Stenberg, 1996b; Pataki *et al.*, 1998b; Th  r  zien *et al.*, 2007). After these corrections, optical estimates of  $L$  had a more restricted range than the range generated based on our method; it was greater at the low end of  $L$  ( $+0.4 \text{ m}^2 \text{ m}^{-2}$ ) and lesser at the high end ( $-0.3 \text{ m}^2 \text{ m}^{-2}$ ). Thus, the average of the optical estimates over the entire  $L$  range was similar to that estimated based on our method ( $+0.06 \text{ m}^2 \text{ m}^{-2}$ ) regardless of the

treatment. This matches the bias reported in other comparisons performed in pine stands (Sampson & Allen, 1995).

Secondly, under ambient  $[\text{CO}_2]$  the mean ratio of maximum to winter minimum  $L_p$  for the entire study period is close to 1.8, with large excursions above and below this value occurring as a result of drastic climate-induced leaf area reductions and subsequent recoveries (Fig. 6a). A value of  $\sim 1.8$  is generated by needle longevity once a loblolly pine canopy reaches steady state (Kinerson *et al.*, 1974). The fertilized treatments showed a greater maximum/minimum amplitude during the severe drought year of 2002, reflecting greater sensitivity to drought (Linder, 1987; Raison *et al.*, 1992). Elevated  $[\text{CO}_2]$  resulted in a higher maximum-to-minimum  $L$  ratio in the first full year of the  $[\text{CO}_2]$  enrichment (1997;  $P = 0.021$ ), but otherwise did not affect this ratio ( $P = 0.104$ ).

Thirdly, we calculated winter  $L_{\text{minp}}$ -to-sapwood area ratio. Except in the last 2 years of the experiment, when drought and the ice storm reduced the ratio,  $L_{\text{minp}}/A_{\text{sp}}$  under ambient  $[\text{CO}_2]$  was similar to a locally generated allometric value (Pataki *et al.*, 1998b; Fig. 5b). Elevated  $[\text{CO}_2]$  had no effect on this ratio ( $P = 0.590$ , all yearly contrasts  $P > 0.05$ ). Compared with elevated  $[\text{CO}_2]$  alone, adding fertilization resulted in a significantly higher  $L_{\text{minp}}/A_{\text{sp}}$  during 2002–2003 ( $P < 0.050$ ), but showed greater sensitivity to drought through a more severe reduction in  $L_{\text{minp}}/A_{\text{sp}}$  during the last 2 years of the study. Excluding the post-ice storm year 2003, fertilization increased the annual  $L_{\text{minp}}/A_{\text{sp}}$  by 18%, similar to another study with loblolly pine (Ewers *et al.*, 2000).

The peak  $L_p$  for the ambient plots – both fertilized and unfertilized – based on the comprehensive method used here, although high, is within the range reported for loblolly pine (Vose & Allen, 1988; Hennessey *et al.*, 2004; Sword Sayer *et al.*, 2004).

#### Leaf area enhancement

In order to account for the differential displays of pine and hardwood  $L$  in the canopy  $L$  (i.e. hardwoods are leafless part of the year), we evaluated the  $[\text{CO}_2]$ -induced enhancement of  $\bar{L}$  during the period in which foliage is active, representing the functional  $\bar{L}$ . Functional  $\bar{L}$ , expressed as leaf area duration, is the  $L$  averaged over months with mean temperature  $> 9^\circ\text{C}$  for pine, and over foliated months for hardwoods, multiplied by the fraction of the year these months represent. The  $[\text{CO}_2]$ -induced enhancement ratio of the functional  $\bar{L}_c$  was generated by summing the functional  $\bar{L}$  of pine and hardwood. Using the pairing of plots established at the onset of the FACE experiment, enhancement ratios for each pair of plots were generated, and normalized (divided) by their initial (pretreatment) ratio.

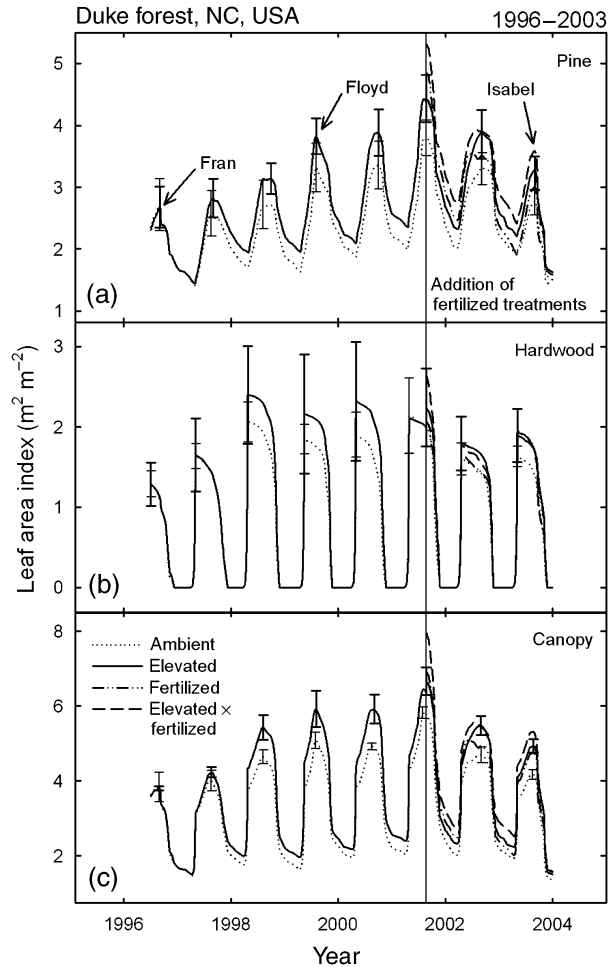


Fig. 5 Leaf area index from 1996–2003 for pine (a), hardwood (b) and canopy (pine + hardwood) (c) with ambient  $\text{CO}_2$ , elevated  $\text{CO}_2$ , fertilization and elevated  $\text{CO}_2$  with fertilization. Bars indicate 1 SE, and are placed at the peak  $L$  of each year. Hurricanes and changes in experimental configuration are noted.

The  $[\text{CO}_2]$ -induced enhancement ratio of the functional  $\bar{L}_p$  was significantly greater than zero beginning in 1998, and averaged 16% ( $\pm 1\%$ ; Fig. 7a). The elevated  $[\text{CO}_2]$  enhancement of functional  $\bar{L}_c$ , averaging 14% ( $\pm 1\%$ ), was significant 5 of the last 6 years; it was statistically insignificant in 2002 due to both decreasing differences and increasing variability (Fig. 7b). Although the effect cannot be tested directly, data from the fertilized elevated  $[\text{CO}_2]$  plot suggests that an upper limit for  $[\text{CO}_2]$  enhancement of functional  $\bar{L}_p$  and  $\bar{L}_c$  is  $\sim 30\text{--}40\%$  and  $\sim 30\text{--}50\%$ , respectively.

#### Vertical leaf area distribution

The dates of vertical leaf area profile measurements represent near peak and near minimum leaf area for pines. The only point at which there was a significant

effect of [CO<sub>2</sub>] was on day 320, 2002 (Fig. 8a), where the level in which leaf area density was highest was shifted upwards in the canopy under elevated [CO<sub>2</sub>] compared with the canopy under ambient [CO<sub>2</sub>] ( $P < 0.001$ ). However, there were some changes in the profiles between the time of maximum  $L_p$  (Fig. 8a and b) and minimum  $L_p$  (Fig. 8c and d) for the treatments for which measurements were available at both times. Compared with the profile at minimum  $L_p$ , the profile at near maximum  $L_p$  had a narrower relative canopy distribution, and the relative position of the highest density of  $L$  was shifted upwards in the profile under both ambient [CO<sub>2</sub>] and elevated [CO<sub>2</sub>].

On an absolute basis, canopy length measurements show that [CO<sub>2</sub>] lengthened tree crowns (after accounting for stand density) by  $\sim 0.3$  m ( $P = 0.004$ ). Fertilization did not have a detectable effect on crown length ( $P = 0.184$ ).

#### Nitrogen effects on leaf area enhancement

Although the proportion of pine in the canopy at  $L_{peakc}$  decreased with decreasing N availability using, as an index, the N availability estimates from 1998 (not shown;  $r^2 = 0.761$ ,  $P = 0.023$ ), some compensation was made through the response of hardwood  $L$  to [CO<sub>2</sub>] (Fig. 9a vs. 9b). Here, we present the functional  $\bar{L}$ , but all other expressions of  $L$  depicted similar patterns. In 1996, the first year of [CO<sub>2</sub>]-enrichment in the fully replicated FACE experiment and before [CO<sub>2</sub>] induced an impact on  $L$ , available N explained 89% of the variability in functional  $\bar{L}_p$  and 76% in  $\bar{L}_c$  ( $P = 0.005$  and  $0.023$ , respectively), and there were no differences between [CO<sub>2</sub>] treatments in the relationships of  $\bar{L}_p$  or  $\bar{L}_c$  to N ( $P = 0.695$  and  $0.073$ , respectively; Fig. 9a and b).

In 2001, the year in which  $L$  reached its maximum (Fig. 5), the spatial variation in functional  $\bar{L}_p$  was highly correlated to N ( $r^2 = 0.94$  and  $0.99$  for ambient and elevated [CO<sub>2</sub>];  $P = 0.161$  and  $0.051$ , respectively). More importantly, the slope of the response of  $\bar{L}_p$  to N was steeper under elevated [CO<sub>2</sub>] ( $P = 0.103$  for Fig. 9a), leading to an enhancement of  $\bar{L}_p$  which increases with N. At low N, there was no difference in  $\bar{L}_p$  between the [CO<sub>2</sub>] treatments. Despite the sensitivity of functional  $\bar{L}_p$  to available N, functional  $\bar{L}_c$  under ambient and elevated [CO<sub>2</sub>] was insensitive to N over the range of N availability investigated ( $P = 0.661$  and  $0.841$ , respectively), but higher under elevated [CO<sub>2</sub>] ( $P = 0.092$ ). This insensitivity to N leads to a constant enhancement of functional  $\bar{L}_c$  across the native N gradient (Fig. 9b).

N availability in the prototype complex was estimated differently from that in the other six plots. Nevertheless, adding the data from the unfertilized plots of the prototype-complex to the data from the

other three plots in each treatment did not change the relationship between functional  $\bar{L}_p$  and available N under either treatment ( $P = 0.998$  and  $0.800$  for ambient and elevated [CO<sub>2</sub>], respectively; Fig. 9c). With more statistical power ( $n = 4$  vs.  $n = 3$ ) the relationship under elevated [CO<sub>2</sub>] was more clearly different from that under ambient conditions ( $P = 0.061$ ). Similarly, the relationships for functional  $\bar{L}_c$  were not unaltered by the addition of the unfertilized plots of the prototype complex (Fig. 9d).

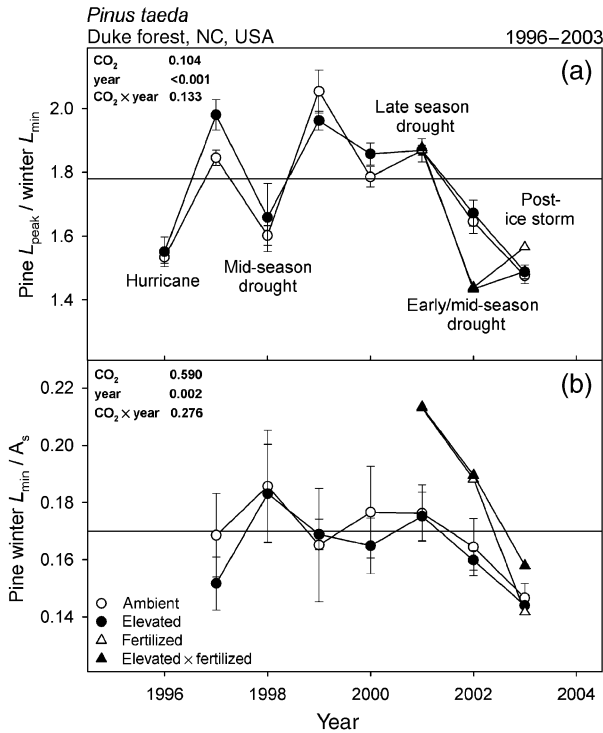
#### Discussion

Canopy leaf area index affects the recruitment and growth of subcanopy individuals, and ecosystem processes such as water, energy and carbon fluxes. We evaluated the effects of elevated [CO<sub>2</sub>] on the magnitude and dynamics of  $L$  in the canopy of a pine forest with a variable hardwood component. Previous publications from the Duke FACE site have suggested that [CO<sub>2</sub>]-induced increases in  $L$  have been minimal (Lichter *et al.*, 2000; DeLucia *et al.*, 2002). These assessments were based on optical measurements or on a small subset of the variables used in this study to reconstruct the leaf area of pine, and thus could be subject to methodological errors (optical measurements – Gower & Norman, 1991; Sampson & Allen, 1995; Stenberg, 1996b; Law *et al.*, 2001) or errors associated with unconstrained leaf area dynamics and changes in allometry. The diagnostic assessment of our  $L$  reconstruction demonstrated that the results discussed below are well constrained.

We show that [CO<sub>2</sub>] had a significant impact on the absolute values of the pine component of  $L$ ,  $L_p$  (Fig. 5), yet did not affect the seasonal dynamics of  $L_p$  (Figs 3, 4, and 6). Elevated [CO<sub>2</sub>] induced a 16% increase in functional  $\bar{L}_p$ , and a similar enhancement for the entire canopy,  $\bar{L}_c$  (14%; Fig. 7). However, the variation in the response among plots within a treatment was not random – much of the variation was determined by the spatial variation in N availability and the associated change in the proportion of the canopy  $L$  comprised of hardwood species. Below we discuss the effects of [CO<sub>2</sub>] enrichment on the temporal dynamics and the spatial *average* and *distribution* of  $L$ .

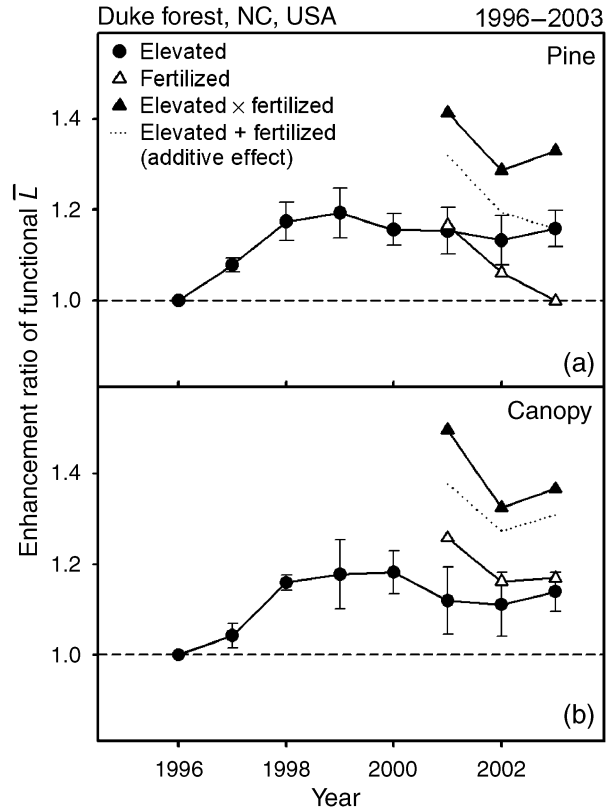
#### Treatment-average foliage expansion and loss

Evaluating the impact of elevated [CO<sub>2</sub>] on  $L$  requires consideration of both leaf production and foliage development and loss, because these dynamics determine how long foliage is displayed and functions. While we detected no systematic differences in the intra-annual dynamics of pine  $L$  with elevated [CO<sub>2</sub>], either in terms of development or loss, elevated [CO<sub>2</sub>] slowed leaf loss



**Fig. 6** 1996–2003 ratio of peak pine leaf area ( $L_{peak}$ ) to winter minimum pine leaf area ( $L_{minp}$ ) (a) and pine winter minimum leaf area-to-sapwood area  $L_{minp}/A_{sp}$  (b) for ambient  $CO_2$ , elevated  $CO_2$ , fertilization and  $CO_2$  in combination with fertilization. Bars indicate 1 SE. Events likely to have contributed to the observed pattern are noted. Reference line in (a) is the average ratio across all years and reference line in (b) is at 0.17, an independently established allometric value for loblolly pine (Pataki *et al.*, 1998b).

for the composite of many hardwood species (Figs 3 and 4, Table 2). Other studies on the effect of  $[CO_2]$  on  $L$  dynamics have generated mixed results. While leaf phenology did respond to elevated  $[CO_2]$  in certain species (*Pinus sylvestris*, *Quercus myrtifolia*, *Populus* spp.; Jach & Ceulmans, 1999; Li *et al.*, 2000; Sigurdsson, 2001; Tricker *et al.*, 2004), no consistent pattern has emerged yet, either within or among species or forest types, reflecting perhaps the length of the time series available. For example, elevated  $[CO_2]$  affected neither emergence nor abscission date of either sun or shade leaves of canopy sweetgum at our site (Herrick & Thomas, 2003), but in another sweetgum forest it caused a significant variation in canopy duration (Norby *et al.*, 2003). Because the effect was inconsistent among the 4 study years, the authors of the latter study concluded that there was no effect on leaf dynamics. This study shows that  $[CO_2]$ -enrichment can decrease the rate of late season hardwood leaf loss (Fig. 4d), perhaps reflecting a better leaf carbon balance and a lesser need for water than plants under ambient  $[CO_2]$



**Fig. 7** Treatment level enhancement ratios of average functional leaf area ( $\bar{L}$ ) for elevated  $CO_2$ , fertilization and elevated  $CO_2$  in combination with fertilization, for pine (a) and canopy (pine + hardwood) (b). The additive effect (elevated  $CO_2$  + fertilization) is indicated with a dotted line. Bars indicate 1 SE. Functional leaf area for pine is derived from months when monthly average temperature is  $>9^\circ C$ , and canopy leaf area is derived from the average functional pine leaf area plus the average leaf area of hardwoods during their foliated period. Enhancement ratios have been corrected for pretreatment differences, such that the enhancement ratios should represent only treatment-induced enhancements.

(Schäfer *et al.*, 2002, 2003). Regardless of treatment, some of the interannual variation in the  $L$  dynamics was caused by extreme events such as the ice storm of December 2002, and hurricanes (Fig. 5). Yet, most of the interannual variation was caused by drought (Fig. 6) and, for the pine component, to the degree of canopy closure. Since 1998,  $L_{prodP}$  was positively related to the minimum  $L_p$  – occurring at the beginning of the growing season – and soil moisture during foliage expansion. The rate of  $L_{lossP}$  was also affected by soil moisture availability during the growing season. Low soil moisture depressed the maximum rate of  $L_{lossP}$  by increasing litterfall during nonpeak times (Fig. 4c). Overall, environmental drivers had the greatest impact on the dynamics of leaf area index, whereas elevated  $[CO_2]$  had little impact.

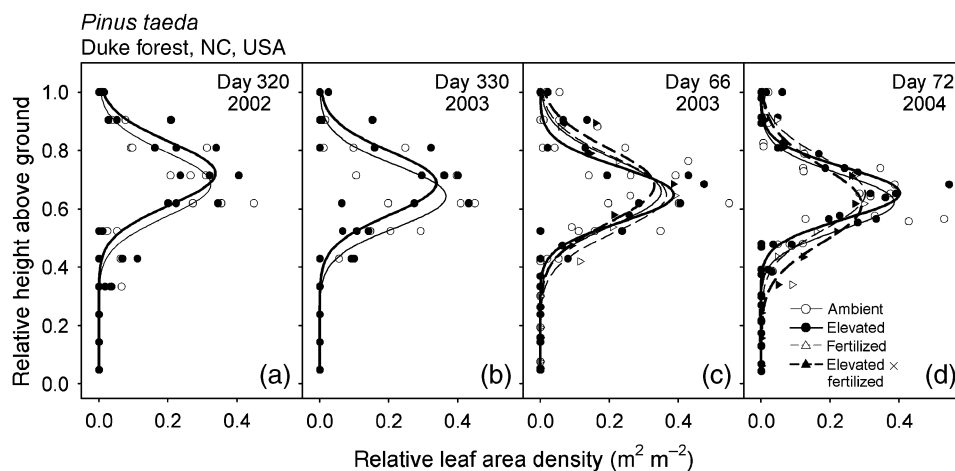


Fig. 8 Relative leaf area density (LAD) of pine as a function of relative height above the ground under ambient CO<sub>2</sub>, elevated CO<sub>2</sub>, fertilization and elevated CO<sub>2</sub> plus fertilization at four measurement times (a) day 320, 2002, (b) day 330, 2003, (c) day 66, 2003, (d) day 72, 2004. The fertilized treatments were measured only at day 66, 2003 and day 72, 2004.

#### Treatment-average leaf area index

To describe the treatment effect on leaf area index we focused on some of its most relevant metrics, such as  $L_{\text{peak}}$ ,  $L_{\text{min}}$ ,  $L_{\text{prod}}$  (see Appendices A and B) and the functional  $\bar{L}$ . In contrast to other studies in closed canopy forests (Hättenschwiler *et al.*, 1997; Gielen *et al.*, 2003; Norby *et al.*, 2003), we found that the enhancement of leaf area by elevated [CO<sub>2</sub>] was sustained, albeit at a lower than initial level, even after the canopy closed in 1999. Functional  $\bar{L}_p$  enhancement under elevated [CO<sub>2</sub>] stabilized at ~16% ( $\pm 1\%$ ), with climate events introducing some variability (Fig. 7a).

We observed very little impact of elevated [CO<sub>2</sub>] through direct assessment of hardwood  $L$ . In addition, environmental variables were not successful in describing annual variations of hardwood  $L$ . A major factor likely preventing us from detecting differences in  $L_{\text{prod}}$  was the uneven distribution of hardwoods across plots, the effect of which was not overcome even with appropriate pairing of plots for statistical testing. Combining  $\bar{L}_h$  with  $\bar{L}_p$  into functional  $\bar{L}_c$  (Figs 5c and 7b) shows similar patterns to those observed with  $\bar{L}_p$  alone, as expected since pine dominated in most plots. Thus, after 1999 (the period after the canopy reached quasi-equilibrium) functional  $\bar{L}_c$  showed a [CO<sub>2</sub>]-induced enhancement of 14% ( $\pm 1\%$ ).

#### Spatial variability of leaf area and [CO<sub>2</sub>]-induced enhancement

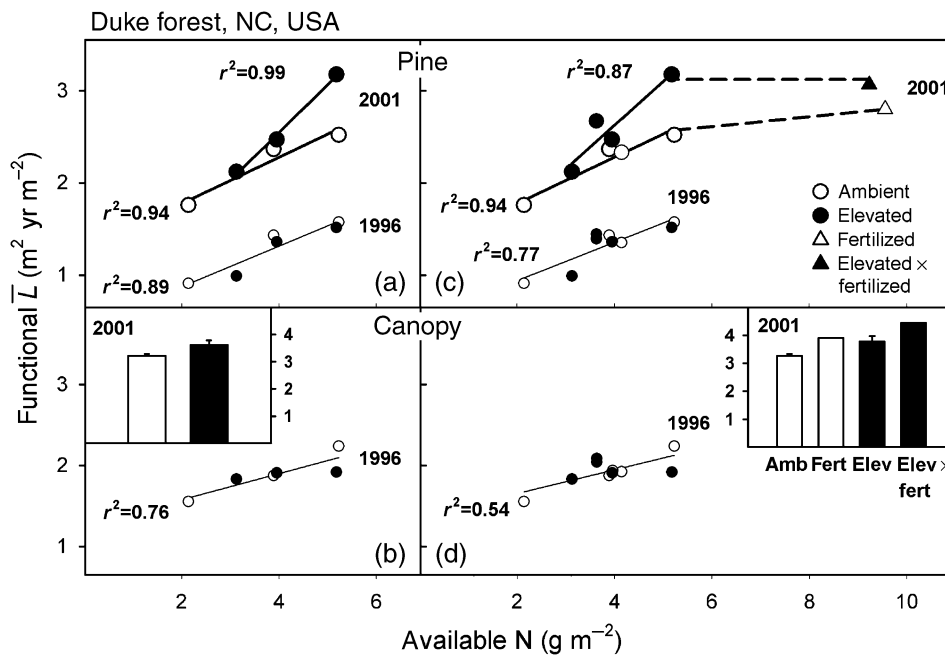
We evaluated the effect of [CO<sub>2</sub>] on the spatial distribution of leaf area. We assessed the effect on the vertical distribution of  $L$  through the canopy, and on the hor-

izontal distribution of  $L$  over the site using plot-specific information on N availability.

How leaf area is distributed vertically within a forest canopy is important in determining the light environment within and below the canopy (Stenberg *et al.*, 1994; Larsen & Kershaw, 1996). Elevated [CO<sub>2</sub>] did not change the vertical distribution of  $L$  in three poplar species (*Populus alba*, *P. nigra* and *P. × euramericana*; Gielen *et al.*, 2003). Similarly, in our study [CO<sub>2</sub>] did not strongly affect vertical distribution of  $L$ , although the pine leaf area tended to move upward relative to ambient plots, and individual crown length increased by ~6%.

A recent synthesis of results from four FACE sites evaluated the response of  $L$  to elevated [CO<sub>2</sub>] after canopy closure (defined as  $L > 90\%$  of the maximum reached in natural stands of the same species in the region; Norby *et al.*, 2005). The [CO<sub>2</sub>]-induced  $L$  enhancement in hardwood canopies was relatively large for canopies composed of species with inherently low  $L_{\text{peak}}$  and decreased for species with high  $L_{\text{peak}}$ . The Duke FACE pine fell in with the general pattern formed by the hardwood species, on average showing a moderate response to [CO<sub>2</sub>], in line with its relatively low value of native  $L_{\text{peak}}$ . However, our results demonstrate that spatial variation in the response of  $L$  to [CO<sub>2</sub>] can be caused not only when the control on maximum  $L$  is the species composition, but also when the availability of resources (e.g. of N) limits  $L$ .

This study was performed in a  $\sim 0.5 \times 1$  km stand, uniformly planted on a relatively homogenous site with level topography, yet there was readily discernable horizontal variability in  $L$  (Oren *et al.*, 2006). We found that the CV of functional  $\bar{L}_p$  in ambient [CO<sub>2</sub>] ranged 15–27% among years. Nitrogen availability also ranged



**Fig. 9** Average functional leaf area ( $\bar{L}$ ) as a function of the available N for ambient and elevated  $[\text{CO}_2]$  in 1996 (pretreatment) and 2001 (year of maximum leaf area index), for pine (a, c) and canopy (pine + hardwood) (b, d). Regressions in (a, b) include only data from the replicated FACE, while regressions in (c, d) also include data from the prototype complex. Average values from fertilized treatments are included in (c, d) to suggest upper bounds on the response of  $\bar{L}$  to elevated  $[\text{CO}_2]$ . Canopy functional leaf area for 2001 is represented as treatment averages due to the lack of a significant relationship with available N.

considerably (Finzi *et al.*, 2002), and already at the beginning of the study significantly explained the variation in  $L$  (Fig. 9a–d). With time, even more of the spatial variation in  $\bar{L}_p$  was explained by N availability (Fig. 9a and c). Moreover, where N was low, the limited development of pine canopy under ambient  $[\text{CO}_2]$  provided a greater opportunity for hardwoods to establish and develop crowns.

Following the commencement of  $[\text{CO}_2]$  enrichment, the increase in functional  $\bar{L}_p$  was also controlled by N availability, which explained much of the variability among the  $[\text{CO}_2]$  enriched plots (Fig. 9a and c). We used the data from fertilized plots to assess whether the  $[\text{CO}_2]$ -induced responses observed under native N are likely increase with further increase in N availability (Fig. 9c). The data suggested that in contrast to the forest under ambient  $[\text{CO}_2]$ , the maximum site  $\bar{L}_p$  under elevated  $[\text{CO}_2]$  was achieved near the maximum native available soil N ( $\sim 5 \text{ g N m}^{-2} \text{ yr}^{-1}$ ). Thus, no further increase in functional  $\bar{L}_p$  is expected with further increase in N through fertilization. Taken together, the  $[\text{CO}_2]$ -induced  $\bar{L}_p$  enhancement ratio would increase from zero over the range of native fertility, and decrease thereafter as greater availability of N allowed  $\bar{L}_p$  under ambient  $[\text{CO}_2]$  to approach the site maximum (Fig. 9c).

When hardwoods and pine were considered together, there was little spatial variation in  $\bar{L}_c$ , and it was not

controlled by N availability (Fig. 9b and d). Under both ambient and elevated  $[\text{CO}_2]$ , the response of hardwood  $L$  (mostly due to canopy individuals) at very low N compensated for low pine  $L$ , and brought  $\bar{L}_c$  to the site maximum across the entire range in N. This response is different from that observed in the pine, and resulted in a constant  $[\text{CO}_2]$ -induced enhancement ratio of  $\bar{L}_c$  throughout the entire range of native N. These results suggest that on nutrient poor sites the pine may not be able to respond to elevated  $[\text{CO}_2]$ , as was also shown for wood production (Oren *et al.*, 2001), but that certain hardwood species may be able to respond. We note, however, that lack of data disallows the evaluation of the potential response of hardwood species to  $[\text{CO}_2]$  under more limiting N availability.

Given the rarity of FACE experiments, application of their results to a meaningful scale must be done through model extrapolation to regions and larger areas. For these extrapolations, it is essential to quantify interaction effects such as these described above. In summary, this study demonstrates that the variation in the leaf area of the pine at this site is not random, but is strongly affected by plant available nitrogen and climate. We show that, *spatially*, the response of pine leaf area to elevated  $[\text{CO}_2]$  is correlated with nitrogen availability, but that this pattern disappears when total canopy leaf area is evaluated because hardwood leaf

area compensates for low pine leaf area in low fertility sections of the site (Fig. 9). That availability of different resources interacts in affecting many growth processes is not new. Allowing for the possibility of such interactions would facilitate a greater understanding of the mechanisms driving the responses of forests to elevated [CO<sub>2</sub>]. The commencement of split-plot fertilization within the replicated FACE experiment will permit a formal analysis of some of these interactions.

### Acknowledgements

We thank J. S. Pippen, A. Melvin, S. Gach, J. Janssen, J. Monfort, and J. Sibley for assistance with litter sorting and LAI-2000 measurements. We also thank A. C. Oishi, and Drs S. Palmroth, R. H. Waring, and P. C. Stoy for useful comments. This study was supported by the Department of Energy through the Office of Biological and Environmental Research and its National Institute for Global Environmental Change, Southeast Regional Center at the University of Alabama, and by the US Forest Service through both the Southern Global Climate Change Program and the Southern Research Station.

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## Appendix A

**Table A1** Treatment means for peak ( $L_{\text{peakp}}$ ) and minimum leaf area ( $L_{\text{minp}}$ ), leaf area production ( $L_{\text{prodp}}$ ) and annual average leaf area ( $\bar{L}_p$ ) for pine

	Ambient	SE	Elevated	SE	Fertilized	Elevated × fertilized	Effect	P-value
$L_{\text{peakp}}$								
1996	2.72 <sup>a</sup>	0.42	2.67 <sup>a</sup>	0.33				
1997	2.59 <sup>a</sup>	0.37	2.82 <sup>a</sup>	0.31				
1998	2.74 <sup>a</sup>	0.41	3.18 <sup>b</sup>	0.25				
1999	3.32 <sup>a</sup>	0.40	3.83 <sup>b</sup>	0.29				
2000	3.36 <sup>a</sup>	0.38	3.89 <sup>b</sup>	0.37				
2001	3.82 <sup>a</sup>	0.29	4.47 <sup>b</sup>	0.39	4.89 <sup>ab</sup>	5.35 <sup>b</sup>	CO <sub>2</sub>	<b>0.029</b>
2002	3.28 <sup>a</sup>	0.26	3.88 <sup>b</sup>	0.40	3.55 <sup>a</sup>	3.93 <sup>ab</sup>	Year	<b>&lt;0.001</b>
2003	2.78 <sup>a</sup>	0.20	3.28 <sup>b</sup>	0.28	3.00 <sup>ab</sup>	3.59 <sup>b</sup>	Year × CO <sub>2</sub>	0.391
$L_{\text{minp}}$								
1996	1.77 <sup>a</sup>	0.28	1.73 <sup>a</sup>	0.22				
1997	1.41 <sup>a</sup>	0.22	1.43 <sup>a</sup>	0.19				
1998	1.71 <sup>a</sup>	0.24	1.94 <sup>a</sup>	0.23				
1999	1.63 <sup>a</sup>	0.24	1.95 <sup>b</sup>	0.16				
2000	1.87 <sup>a</sup>	0.19	2.09 <sup>a</sup>	0.17				
2001	2.05 <sup>a</sup>	0.16	2.39 <sup>b</sup>	0.21	2.61 <sup>ab</sup>	2.85 <sup>ab</sup>	CO <sub>2</sub>	<b>0.045</b>
2002	2.01 <sup>a</sup>	0.15	2.32 <sup>b</sup>	0.22	2.46 <sup>ab</sup>	2.75 <sup>b</sup>	Year	<b>&lt;0.001</b>
2003	1.89 <sup>a</sup>	0.16	2.20 <sup>b</sup>	0.18	1.91 <sup>ab</sup>	2.41 <sup>ab</sup>	Year × CO <sub>2</sub>	0.093
$L_{\text{prodp}}$								
1996	1.57 <sup>a</sup>	0.25	1.56 <sup>a</sup>	0.23				
1997	1.72 <sup>a</sup>	0.23	1.95 <sup>a</sup>	0.23				
1998	1.69 <sup>a</sup>	0.19	1.94 <sup>b</sup>	0.16				
1999	1.89 <sup>a</sup>	0.21	2.10 <sup>a</sup>	0.17				
2000	1.97 <sup>a</sup>	0.15	2.34 <sup>b</sup>	0.29				
2001	1.99 <sup>a</sup>	0.15	2.32 <sup>b</sup>	0.21	2.60 <sup>ab</sup>	2.85 <sup>b</sup>	CO <sub>2</sub>	<b>0.032</b>
2002	1.70 <sup>a</sup>	0.12	2.05 <sup>b</sup>	0.20	2.13 <sup>ab</sup>	2.17 <sup>ab</sup>	Year	<b>&lt;0.001</b>
2003	1.29 <sup>a</sup>	0.07	1.55 <sup>b</sup>	0.15	1.62 <sup>ab</sup>	1.69 <sup>b</sup>	Year × CO <sub>2</sub>	0.517
$\bar{L}_p$								
1996	2.11 <sup>a</sup>	0.32	2.08 <sup>a</sup>	0.25				
1997	2.00 <sup>a</sup>	0.29	2.13 <sup>a</sup>	0.25				
1998	2.20 <sup>a</sup>	0.32	2.52 <sup>b</sup>	0.24				
1999	2.38 <sup>a</sup>	0.30	2.76 <sup>b</sup>	0.21				
2000	2.53 <sup>a</sup>	0.28	2.88 <sup>b</sup>	0.27				
2001	2.80 <sup>a</sup>	0.21	3.25 <sup>b</sup>	0.27	3.50 <sup>ab</sup>	3.83 <sup>ab</sup>	CO <sub>2</sub>	<b>0.031</b>
2002	2.75 <sup>a</sup>	0.21	3.15 <sup>b</sup>	0.31	3.11 <sup>ab</sup>	3.44 <sup>ab</sup>	Year	<b>&lt;0.001</b>
2003	2.11 <sup>a</sup>	0.16	2.48 <sup>b</sup>	0.22	2.23 <sup>ab</sup>	2.67 <sup>ab</sup>	Year × CO <sub>2</sub>	0.142

All values are  $\text{m}^2 \text{m}^{-2}$ . Superscript letters indicate statistical differences between treatments ( $P < 0.05$ ).

Bold values indicate  $< 0.05$ .

Appendix B

**Table B1** Treatment means for peak leaf area ( $L_{\text{peakh}}$ ) for hardwoods, and peak leaf area ( $L_{\text{peakc}}$ ), leaf area production ( $L_{\text{prodc}}$ ) and annual average leaf area ( $\bar{L}_c$ ) for the canopy

	Ambient	SE	Elevated	SE	Fertilized	Elevated × fertilized	Effect	P-value
<i>Hardwood</i>								
$L_{\text{peakh}}$								
1996	1.29 <sup>a</sup>	0.16	1.28 <sup>a</sup>	0.27				
1997	1.63 <sup>a</sup>	0.15	1.65 <sup>a</sup>	0.46				
1998	2.06 <sup>a</sup>	0.25	2.40 <sup>a</sup>	0.61				
1999	1.84 <sup>a</sup>	0.18	2.16 <sup>a</sup>	0.74				
2000	1.90 <sup>a</sup>	0.28	2.32 <sup>a</sup>	0.74				
2001	2.16 <sup>a</sup>	0.33	2.38 <sup>a</sup>	0.52	2.35 <sup>a</sup>	3.01 <sup>a</sup>	CO <sub>2</sub>	0.347
2002	1.59 <sup>a</sup>	0.20	1.79 <sup>a</sup>	0.33	1.65 <sup>a</sup>	1.77 <sup>a</sup>	Year	<b>0.001</b>
2003	1.63 <sup>a</sup>	0.13	1.89 <sup>a</sup>	0.33	1.96 <sup>a</sup>	1.93 <sup>a</sup>	Year × CO <sub>2</sub>	0.224
<i>Canopy</i>								
$L_{\text{peakc}}$								
1996	3.84 <sup>a</sup>	0.40	3.81 <sup>a</sup>	0.05				
1997	4.01 <sup>a</sup>	0.27	4.23 <sup>a</sup>	0.13				
1998	4.65 <sup>a</sup>	0.18	5.43 <sup>b</sup>	0.33				
1999	5.08 <sup>a</sup>	0.21	5.92 <sup>b</sup>	0.48				
2000	4.95 <sup>a</sup>	0.09	5.92 <sup>b</sup>	0.39				
2001	5.87 <sup>a</sup>	0.12	6.75 <sup>b</sup>	0.38	7.03 <sup>b</sup>	8.11 <sup>b</sup>	CO <sub>2</sub>	0.0641
2002	4.71 <sup>a</sup>	0.21	5.49 <sup>b</sup>	0.25	5.12 <sup>ab</sup>	5.58 <sup>ab</sup>	Year	<b>&lt;0.001</b>
2003	4.18 <sup>a</sup>	0.13	4.94 <sup>b</sup>	0.18	4.74 <sup>ab</sup>	5.31 <sup>b</sup>	Year × CO <sub>2</sub>	0.266
$L_{\text{prodc}}$								
1996	2.89 <sup>a</sup>	0.28	2.89 <sup>a</sup>	0.07				
1997	3.36 <sup>a</sup>	0.17	3.62 <sup>a</sup>	0.22				
1998	3.69 <sup>a</sup>	0.06	4.36 <sup>b</sup>	0.47				
1999	3.76 <sup>a</sup>	0.06	4.29 <sup>a</sup>	0.58				
2000	3.88 <sup>a</sup>	0.10	4.67 <sup>b</sup>	0.50				
2001	4.17 <sup>a</sup>	0.20	4.72 <sup>a</sup>	0.40	5.01 <sup>a</sup>	5.93 <sup>b</sup>	CO <sub>2</sub>	0.116
2002	3.36 <sup>a</sup>	0.16	3.86 <sup>a</sup>	0.21	3.86 <sup>ab</sup>	3.96 <sup>ab</sup>	Year	<b>&lt;0.001</b>
2003	2.93 <sup>a</sup>	0.09	3.46 <sup>a</sup>	0.23	3.60 <sup>b</sup>	3.64 <sup>b</sup>	Year × CO <sub>2</sub>	0.145
$\bar{L}_c$								
1997	2.74 <sup>a</sup>	0.25	2.88 <sup>a</sup>	0.06				
1998	3.22 <sup>a</sup>	0.20	3.75 <sup>b</sup>	0.08				
1999	3.19 <sup>a</sup>	0.21	3.74 <sup>b</sup>	0.18				
2000	3.38 <sup>a</sup>	0.15	3.98 <sup>b</sup>	0.13				
2001	3.80 <sup>a</sup>	0.10	4.41 <sup>b</sup>	0.22	4.60 <sup>b</sup>	5.21 <sup>b</sup>	CO <sub>2</sub>	<b>0.041</b>
2002	3.56 <sup>a</sup>	0.15	4.08 <sup>b</sup>	0.19	3.98 <sup>ab</sup>	4.37 <sup>b</sup>	Year	<b>&lt;0.001</b>
2003	2.80 <sup>a</sup>	0.12	3.29 <sup>b</sup>	0.11	3.10 <sup>ab</sup>	3.51 <sup>b</sup>	Year × CO <sub>2</sub>	0.148

All values are m<sup>2</sup> m<sup>-2</sup>. Superscript letters indicate differences at  $P < 0.05$ .

Bold values indicate  $< 0.05$ .