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Research paper

Forest stand and canopy development unaltered by 12 years of CO₂ enrichment*

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Canopy structure—the size and distribution of tree crowns and the spatial and temporal distribution of leaves within them—exerts dominant control over primary productivity, transpiration and energy exchange. Stand structure—the spatial arrangement of trees in the forest (height, basal area and spacing)—has a strong influence on forest growth, allocation and resource use. Forest response to elevated atmospheric CO₂ is likely to be dependent on the canopy and stand structure. Here, we investigated elevated CO₂ effects on the forest structure of a *Liquidambar styraciflua* L. stand in a free-air CO₂ enrichment experiment, considering leaves, tree crowns, forest canopy and stand structure. During the 12-year experiment, the trees increased in height by 5 m and basal area increased by 37%. Basal area distribution among trees shifted from a relatively narrow distribution to a much broader one, but there was little evidence of a CO₂ effect on height growth or basal area distribution. The differentiation into crown classes over time led to an increase in the number of unproductive intermediate and suppressed trees and to a greater concentration of stand basal area in the largest trees. A whole-tree harvest at the end of the experiment permitted detailed analysis of canopy structure. There was little effect of CO₂ enrichment on the relative leaf area distribution within tree crowns and there was little change from 1998 to 2009. Leaf characteristics (leaf mass per unit area and nitrogen content) varied with crown depth; any effects of elevated CO₂ were much smaller than the variation within the crown and were consistent throughout the crown. In this young, even-aged, monoculture plantation forest, there was little evidence that elevated CO₂ accelerated tree and stand development, and there were remarkably small changes in canopy structure. Questions remain as to whether a more diverse, mixed species forest would respond similarly.

Keywords: canopy profile, elevated CO₂, FACE, *Liquidambar styraciflua*, leaf mass per area, leaf nitrogen, sweetgum.

Introduction

The productivity of forests and their contribution to the global carbon (C) cycle in future decades will depend in large measure on how the initial interaction between tree leaves and atmospheric CO₂ is manifested in tree growth and forest carbon cycling and modified by the interaction with other environmental

resources—light, water and nutrients. The capacity of forests to acquire resources from the environment is determined by forest structure (Enquist et al. 2009). Canopy structure—the size and distribution of tree crowns and the spatial and temporal distribution of leaves within them—integrates the influences of microclimate, light, nutrient availability and other factors and

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exerts dominant control over primary productivity, transpiration and energy exchange (Asner et al. 2003). The photosynthetic capacity of a plant canopy is strongly controlled by the dramatic gradient in light between the canopy top and bottom and its interaction with other environmental variables and the effects of environment on the structure and physiology of leaves throughout the canopy (Niinemets 2007, Forrester 2019). Stand structure—the spatial arrangement of trees in the forest (height, basal area and spacing)—has a strong influence on forest growth, allocation and resource use (Forrester 2019). Allocation, or the distribution of carbon and nutrients within trees, affects the nutrient uptake and the relationship between net primary productivity (NPP) and C turnover (Walker et al. 2019).

The interactions between elevated atmospheric CO₂ concentration (eCO₂) and other resources are bi-directional: even as the primary responses to eCO₂ (photosynthesis and stomatal conductance) are modified by resource supply, the availability and acquisition of light, water and nutrients can be altered as a secondary or indirect effect of growth in eCO₂ (Norby and Zak 2011). Resource availability can vary as the structure of a forest stand changes during successional development (Johnson 2006, Körner 2006). Hence, questions concerning eCO₂ and interactions with other resources in forests must be asked in the context of stand structure and stand development. As the balance of limiting resources shifts in response to eCO₂, it is possible that forest structure may respond and in turn alter the resource acquisition of the forest. We must understand whether forest structure is influenced by eCO₂ during successional development to understand resource use and its interaction with eCO₂ (Walker et al. 2019). If, for example, growth stimulation by eCO₂ early in plant succession leads to faster tree turnover, then early gains in biomass accumulation may not be realized over the longer term (Körner 2017). There is ample evidence for growth acceleration by eCO₂ of trees growing in isolation or in the establishment phase of a tree stand (e.g., Norby et al. 1992; Zak et al. 1993; Ceulemans et al. 1996; Rey and Jarvis 1997; Tissue et al. 1997), but this growth response cannot be sustained as a stand reaches canopy closure due to limitations in other resources (Norby et al. 1999). The long-term responses of a forest after canopy closure are less clear.

The free-air CO₂ enrichment (FACE) experiment at the Oak Ridge National Laboratory (ORNL) provided a framework to address questions about both CO₂ effects on stand structure and stand structure effects on CO₂ response. The forest stand in the experiment was an even-aged, monoculture plantation of sweetgum trees (*Liquidambar styraciflua* L.), which was established in 1988 with seedlings of uniform size and uniform spacing (2.3 × 1.2 m). These characteristics promoted a relatively fast stand development from a population of young seedlings to a closed-canopy forest. Casual observation and tree ring analysis indicated that the trees exhibited exponential growth until a transition to canopy closure and linear growth, which occurred several years prior to the

initiation of the FACE experiment in 1997 (Norby et al. 2001). Although important aspects of stand structure of native, uneven-aged, mixed species forests cannot be addressed, the more simplified forest in this experiment was ideal for testing hypotheses (Norby et al. 1999) and informing questions to be addressed in a new generation of FACE experiments (Norby et al. 2016). For example, variability within and between plots is much less in an even-aged, monoculture plantation forest, making detection of responses to eCO₂ more likely. Non-destructive measurements during the 12-year experiment provided data on stand structure, and a destructive harvest at the end of the experiment provided much more detailed analysis of canopy structure than was otherwise possible.

The experiment entailed a comprehensive set of plant and soil measurements, and consideration of the dynamic interactions between eCO₂, water and nitrogen were prominent (Norby et al. 2002). These interactions are likely to influence the development and structure of the canopy, which determines the interactions of the stand with light and is a key determinant of stand productivity. Elevated CO₂ had numerous effects on physiology and growth of trees, productivity of the forest stand and carbon and nutrient cycling processes during the 12-year experiment. An initial increase in aboveground growth did not persist; rather, after the first year, increases in NPP were accounted for by greater fine-root production (Norby et al. 2002). The NPP of the stand was 24% greater in eCO₂ for the first 5 years of the experiment, but NPP began to decline in the latter half of the experiment, and it declined faster in eCO₂ such that there was no longer any effect of CO₂ on NPP (Norby et al. 2010). The NPP increased with leaf area duration (LAD) in ambient CO₂ (aCO₂), but there was no significant relationship between NPP and LAD in eCO₂, and the declining response of NPP to eCO₂ cannot be attributed to leaf area (LA) dynamics (Norby et al. 2010). Rather, we interpreted the declining response of NPP to eCO₂ as resulting from a progressive nitrogen limitation in the stand. Leaf litter ¹⁵N content declined over the course of the experiment, indicating an increasingly tight N cycle, and it declined faster in eCO₂ (Garten et al. 2011). As N availability declined, so did canopy N content, leaf photosynthesis (Warren et al. 2015) and NPP (Norby et al. 2010). Given greater inputs of fine-root necromass to the soil (Iversen et al. 2008), soil carbon content increased in eCO₂, including in pools that are protected from rapid decomposition (Jastrow et al. 2005, Iversen et al. 2012).

Here, we ask whether the observed responses in forest productivity can be explained by changes in stand structure, including the distribution of aboveground biomass among individuals and the structure of the forest canopy, and whether stand development was affected by eCO₂. Our questions are informed by previous observations, models and conceptual analysis. An initial hypothesis for the ORNL FACE experiment was that eCO₂ would lower the light compensation point of leaves (e.g., Kubiske and Pregitzer 1996), thereby permitting

leaves to maintain a positive C balance deeper in the canopy (Norby et al. 1999). That hypothesis was initially rejected because the light compensation point was reduced by eCO₂ only in the upper canopy and not in leaves in the mid-to-lower canopy (Norby et al. 2003). The direct observations of canopy structure we report here allow us to address whether the canopy depth increased with eCO₂.

The distribution of leaves within tree crowns affects the light penetration through the canopy and the distribution of foliar nitrogen (N) content and leaf mass per area (LMA), which has direct implications for canopy photosynthesis. Because of the large N cost of chlorophyll and chlorophyll-binding proteins, within-canopy variation in light interception efficiency depends on variation in N investments in light harvesting. The structural and physiological modifications in leaves through the vertical canopy profile and across the within-canopy light gradient collectively improve light-harvesting capacity in low light and improve the photosynthetic capacity in high light (Niinemets 2007). In previous analysis at the ORNL FACE site, we concluded that increased NPP was related more to increased light-use efficiency than to increased light absorption (Norby et al. 2010). However, the structure of the canopy—the vertical distribution of leaves and variation in leaf morphology and nitrogen content along the vertical distribution—also can influence canopy photosynthesis and gross primary productivity (GPP). A long-established theoretical result states that, for a given total canopy N content, canopy photosynthesis is maximized when the within-canopy gradient in leaf N per unit LA (N_{area}) is equal to the light gradient (Field 1983, Hirose and Werger 1987, Buckley et al. 2013). However, it is widely observed that N_{area} declines less rapidly than light in real plant canopies, which can be explained as an optimal adjustment of the within-canopy N profile so as to maximize canopy C export, subject to a lower-bound constraint on LMA (Dewar et al. 2012). Optimality concepts applied to leaf-level coordination of photosynthetic parameters are being incorporated into terrestrial biosphere models (e.g., Ali et al. 2016, Haverd et al. 2018), but many questions remain as to which processes and variables to optimize and over what time scale (Dewar et al. 2009). The canopy can be represented in models by multiple layers with exponentially decreasing N content, and the fraction of photosynthetic N allocated to chlorophyll increases with canopy depth, thereby increasing light harvesting efficiency at the lower light levels, while simultaneously reducing excess N investments in the carbon fixation (Rubisco and electron transport) components of photosynthesis (Caldararu et al. 2020). By increasing the availability of CO₂ for initial fixation by Rubisco, eCO₂ would be expected to both increase N use efficiency and further reduce N demand and investments by these latter components. Does growth in eCO₂ thereby alter optimum or realized N distribution? In a modeling study, doubling CO₂ did not affect the optimal N distribution irrespective of how N is partitioned among photosynthetic components (Hikosaka and Hirose 1998), and

preliminary modeling of the ORNL FACE canopy did not clearly indicate that eCO₂ would lead to a change in the distribution of nitrogen (A.W. King, personal communication). Here, we test whether eCO₂ altered N distribution in a real forest canopy.

Key advances in the modeling of forest production responses to eCO₂ will come from understanding how stand development influences the interaction of CO₂ with water, N and other resources to regulate NPP and wood allocation and the subsequent stand-level impacts on mortality and self-thinning (Walker et al. 2019). Our goal for the current analysis is to quantify and analyze the changes in forest structure through time and in response to eCO₂. We will consider two aspects of forest structure: (i) canopy structure, including leaves, individual tree crowns and crown characteristics at the stand-level; and (ii) stand structure and its differentiation into dominant, intermediate, suppressed and dead trees. We will evaluate the hypothesis that eCO₂ will accelerate the tree and stand development without causing a fundamental alteration of structure. Thus, we predict that the differentiation of trees into different crown classes will be accelerated by eCO₂, while the canopy structure remains unaltered.

Materials and methods

The ORNL FACE experiment was initiated in 1997 in a 1.3-ha *L. styraciflua* plantation that had been established 9 years earlier on the Oak Ridge National Environmental Research Park in Tennessee, USA (35°54'N, 84°20'W). Five 25-m diameter plots were laid out, and FACE apparatus for the delivery of air enriched with added CO₂ (or ambient air as a control) was constructed in four of them (the fifth plot with no FACE apparatus was also considered a control.) During the pre-treatment year, 12 trees were cut down and were used for establishing allometric relationships, including canopy structure (Norby et al. 2001). Elevated CO₂ treatments started in May 1998, and they were continued during the growing season (April through October) for 12 years. The average daytime [CO₂] near the top of the canopy over the duration of the experiment was 547 ppm in the eCO₂ plots and was 395 ppm in aCO₂ plots. Within-plot coefficient of variation was 3% in aCO₂ and 11% in eCO₂. Details of the experimental design, CO₂ exposure dynamics, stand structure and site conditions have been provided elsewhere (Norby et al. 2001; <https://richnorby.org/home/face-home/>), and data on meteorological conditions and biological responses are publicly available (<https://data.eess-divide.lbl.gov/>).

Basal area growth increment (BAI) was monitored on all trees throughout the experiment based on the change in circumference of dendrobands (Norby et al. 2001). Daily leaf area index (LAI) from 1998 to 2008 was calculated based on the measurements of photosynthetically active radiation (PAR) above and below the canopy, dry mass of litter and leaf mass per unit area (LMA), as previously described (Norby et al. 2003).

In July 2009, during the 12th and final years of CO₂ exposure, 16 trees were cut and measured, 4 from each of the two eCO₂ plots and 2 or 3 from each of the three ambient plots. Harvesting occurred after LA development was complete but before any senescence had occurred. Because of a number of constraints on which trees could be harvested, they were not chosen randomly, but they were representative of the full range of tree size across the plots. The trees were felled into a prepared lay-down area outside the experimental plots to minimize canopy damage during felling and so that all parts could be recovered without any mixing with other trees or debris (see Figure S1 available as Supplementary data at *Tree Physiology* Online). Total tree height was measured, and the bole was cut into sections and weighed for determination of allometric relationships. At the same time, the tree crown was processed in 1-m sections that were marked off starting from the top of the tree (Figure S1 available as Supplementary data at *Tree Physiology* Online). From each section, 20 mature leaves were randomly collected as a subsample, and these were processed as described below. Additional subsamples were collected for other researchers or for a sample archive; the dry mass of these leaves was accounted for by measuring their fresh mass and by applying the dry mass-to-fresh mass ratio determined on the 20-leaf subsample. Leaves were kept cool and in sealed plastic bags from the time they were picked until they were weighed, or they were flash-frozen in liquid nitrogen in pre-weighed foil packets and were weighed later while frozen. All remaining leaves within each 1-m crown section were put into paper bags, dried in a forced-draft oven at 70 °C and weighed.

The 20-leaf subsamples were processed within 2 h after the tree was felled. Total fresh weight was first determined and then the LA was measured with a LI-COR LI-3100C area meter. The thickness of five of the leaves, chosen randomly, was measured with digital calipers at three interveinal locations. The subsamples were then oven-dried, weighed and ground in a ball mill for additional analysis and archiving. Concentrations of C and N were determined on 10-mg samples of the dried, ground and homogenized leaves on a Costech C-N Analyzer (as in Norby and Iversen 2006). All analyses were based on whole leaves (blades and petioles).

The 20-leaf subsamples were used to calculate the fresh mass-to-dry mass ratio, LMA, leaf volume (area × thickness), leaf tissue density (mass per unit volume), average leaf size and N content per unit leaf mass. These ratios were then applied to the total dry mass of leaves per section (including the measured or calculated dry mass of the various subsamples) to calculate the total LA, leaf number and N content. We calculate that, from the 16 trees, we processed ~77,000 leaves comprising 489 m² and 130 kg (fresh mass). The N content per tree was converted to a ground area basis by dividing by ground area per tree, which was calculated as LA per harvested tree divided by plot-level LAI; LAI of the plot was measured as in Norby et al. (2003).

To calculate the stand-level canopy structure, the crown structure of each tree in the plot must be determined. Total LA of each tree was calculated based on a regression between LA and basal area increment (BAI) for the year which were established on the harvested trees: $LA = 9.89 \exp(0.262 \times BAI)$, $R^2 = 0.86$ (see Figure S2 available as Supplementary data at *Tree Physiology* Online). The BAI was chosen as the regressor because the regression using BAI was stronger than one based on basal area (or diameter). The calculated LA of each tree in 2009 was distributed vertically over the tree's crown based on the proportional distribution of LA with the crown depth determined on the harvested trees. Summing these individual distributions into a stand-level canopy LA distribution required data on each tree's height. The height of a constant subset of 25 trees per plot (all trees in three rows, thereby capturing a full and unbiased range of heights and crown classes) was measured trigonometrically each year with a Haglöl ultrasonic hypsometer (Längele, Sweden) as described by Norby et al. (2001). For the trees that had not been measured for height, plot-specific regressions of the form: $H = a + b \times \ln(BA)$ were used to estimate height in 2009 from the measured basal area (BA) of each tree (see Figure S3 available as Supplementary data at *Tree Physiology* Online). Tree heights were rounded to the nearest meter, and the tallest tree within a plot defined the top of the canopy. The canopy depth of each 1-m layer of each tree was then equal to its crown depth plus the difference between the tree height and canopy height. The LA by canopy depth was summed across all trees within a plot. A similar calculation was made for canopy structure in 1998 by using the LA distribution determined from harvested trees in 1997. The calculation of LA distribution at the stand level is illustrated in Table S1 (available as Supplementary data at *Tree Physiology* Online).

All of the trees in the plots were classified into four crown classes in the surveys conducted in 1999 (year 2), 2001 (year 4) and 2006 (year 9), following the definitions of Helms (1998) for even-aged stands. Dominant trees had crowns extending above the crowns of immediate neighbors; co-dominant trees formed the main canopy with immediate neighbors; intermediate trees had crowns extending into the lower portion of the main canopy and the crowns of suppressed trees were completely overtopped by the crowns of one or more neighboring trees. Dominant trees received full light to the canopy top and partial light to the mid-canopy; co-dominant trees received full light to the canopy top but relatively little to mid-canopy; intermediate trees received little direct light to canopy top only; suppressed trees rarely received direct light.

Results

Stand development and structure

Peak LAI in 1998–2008 averaged 5.4 ± 0.3 (SE) in aCO₂ and 5.5 ± 0.3 in eCO₂ (Figure 1). Effects of eCO₂ on LAI varied depending primarily on the occurrence of seasonal droughts

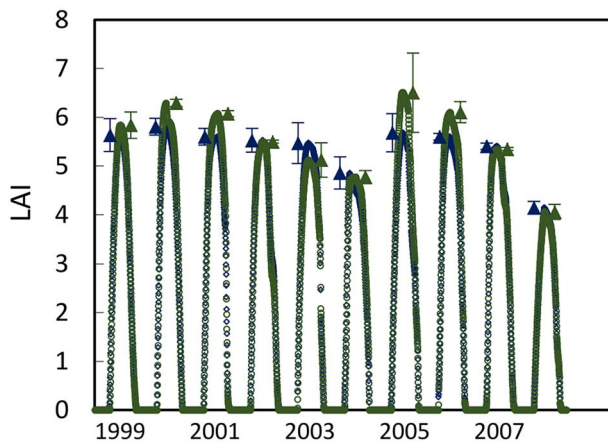


Figure 1. Daily LAI over 10 growing seasons. Data are the mean of three plots in aCO₂ (blue square symbols) and two plots in eCO₂ (green triangle symbols). Error bars are the SE of the means of three aCO₂ plots or two eCO₂ plots on the day of maximum annual LAI.

(Warren et al. 2011). Peak LAI was 8–9% greater in eCO₂ during mid-summer in non-drought years but declined in eCO₂ relative to the ambient plots during late-season droughts in 2002 and 2007 (Warren et al. 2011). The integration of LAI over the growing season, or LAD, varied year to year without any clear trend over time and with no effect of eCO₂ (Norby et al. 2010). There were no effects of eCO₂ on leaf phenology. The average time of 50% leaf fall over 12 years was day-of-year 283 ± 2.4 in both ambient and eCO₂ (Norby 2021). In 2 years (2004 and 2008), abscission occurred 5–6 days later in eCO₂, and in 2002, leaf abscission was earlier in eCO₂ in response to a late-season drought (Warren et al. 2011).

During the 12-year experiment, the trees grew from an initial average height of 12.7 m to a final height of 18.2 m. Height growth was well described by an asymptotic model (Figure 2). The small, non-significant pretreatment difference in average height between treatments was maintained during the experiment, and there was no difference in height growth between ambient and eCO₂ plots. Stand structure changed substantially over the course of the experiment. Basal area distribution shifted from a relatively narrow distribution to a much broader one, and the modes of the distributions increased by 39% (Figure 3A). Initial tree size (basal area) in 1997 accounted for 55–60% of the variation in BAI from 1997 to 2009, and the eCO₂ effect on BAI increased with initial size (see Figure S4 available as Supplementary data at *Tree Physiology* Online). As is also apparent in the density distribution (Figure 3A), initial basal area was slightly greater in aCO₂, but cumulative BAI was greater in eCO₂ (Figure S4 available as Supplementary data at *Tree Physiology* Online), suggesting small but barely detectable changes in stand structure. The fraction of stand basal area that was accounted for by the largest quartile of trees increased substantially from 1998 (36%) to 2009 (51%), but there was little evidence of a CO₂ effect on basal area distribution (Figure 3B).

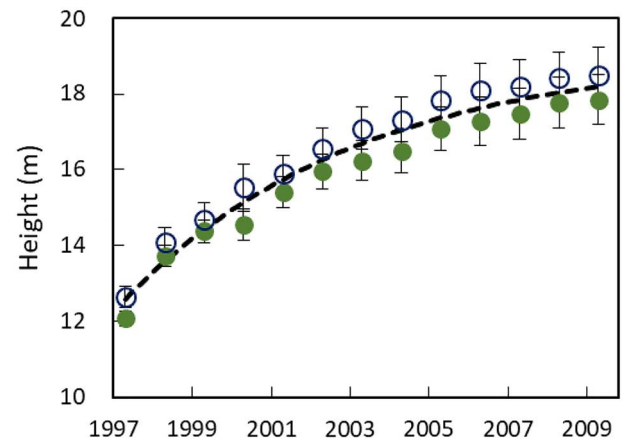


Figure 2. Average height \pm SE in three ambient plots (open blue symbols) and two elevated CO₂ plots (closed green symbols). Average tree height in each plot was generated from measurements of 25 trees. Regression is: $H = 19.07 - 6.48 \times 0.846^{\text{yearnum}}$; yearnum is calendar year–1997.

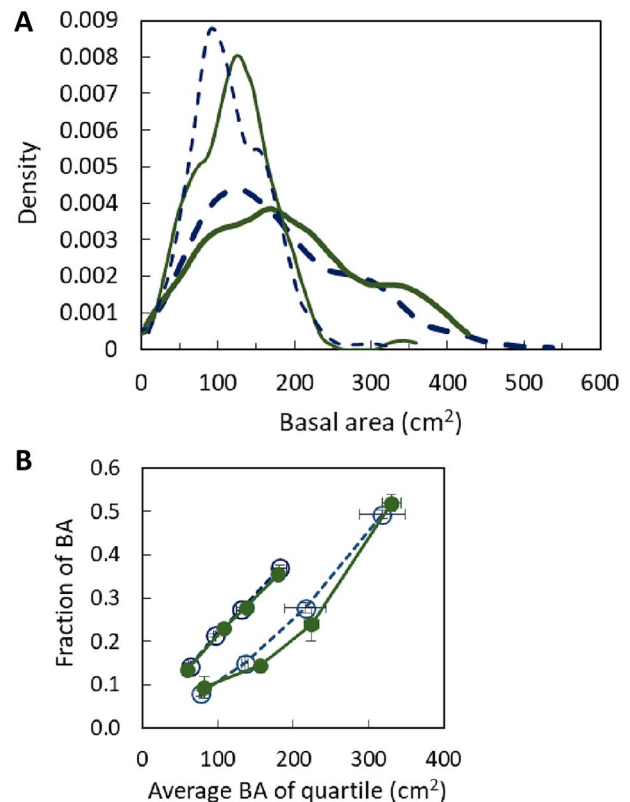


Figure 3. Basal area distribution. (A) Probability density function of all trees in three ambient plots (blue dashed lines) and two elevated CO₂ plots (green solid lines) in 1998 (thin lines, left skewed) and 2009 (thick lines, right skewed). The function was generated in Stata 16.1 (College Station, Texas) using the Epanechnikov kernel function with a bandwidth of 32. (B) The fraction of total basal area in 1998 (thin lines) and 2009 (thick lines) represented by each quartile plotted against the average basal area of the quartile. Data are the mean of three plots in aCO₂ (blue symbols, dashed line) and two plots in elevated CO₂ (green symbols, solid line) \pm SE.

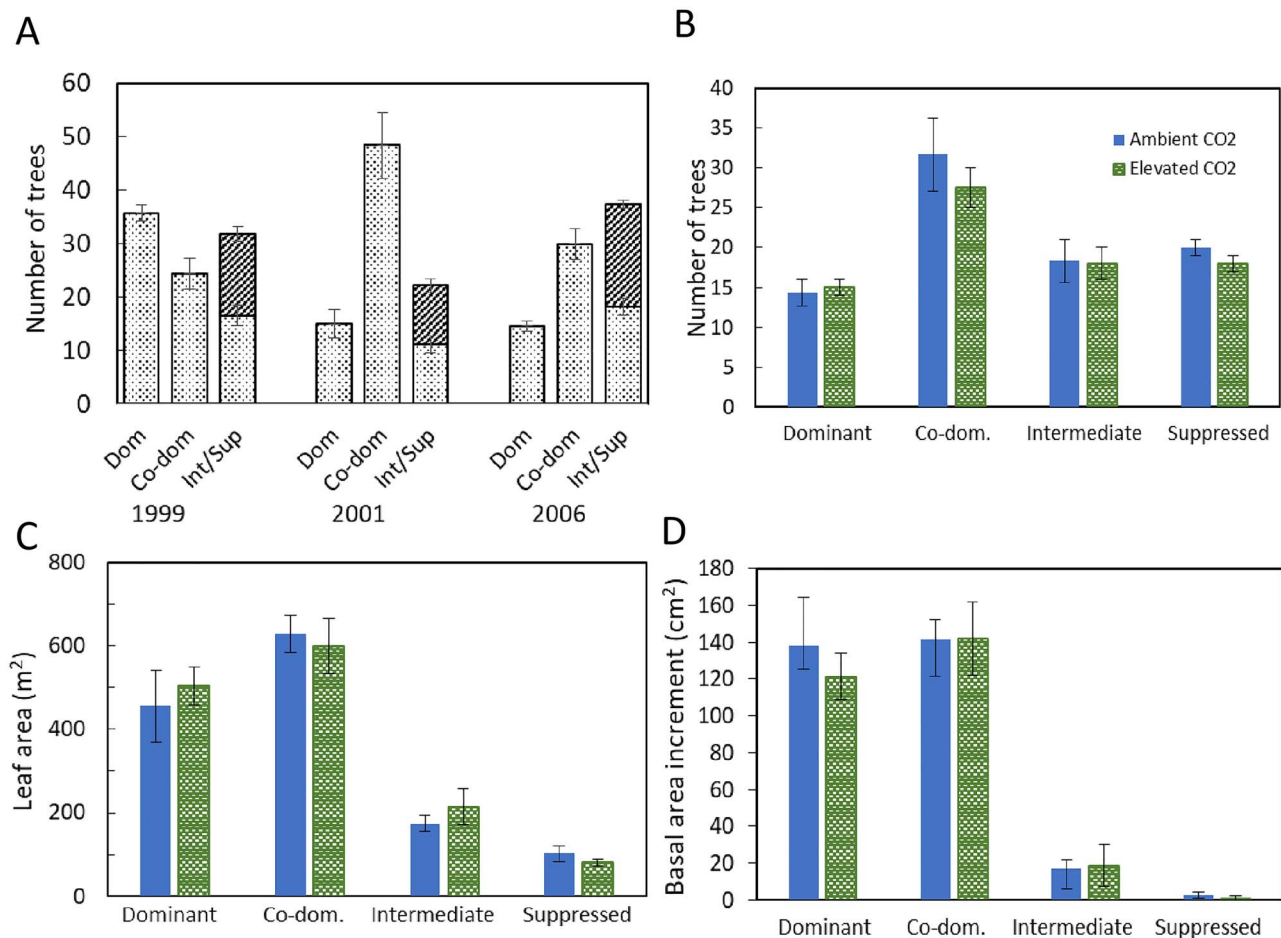


Figure 4. Stand structure by dominance class. (A) Number of trees in different canopy dominance classes (dominant, co-dominant, intermediate and suppressed), averaged across all plots (\pm SE). Horizontal line in the bars for intermediate + suppressed class indicated the separation between the two classes. (B) Number of trees in the different dominance classes in 2006 in ambient and elevated CO₂ plots. (C) LA and (D) BAI in 2006 by dominance class.

The change in stand structure was also reflected in a differentiation into crown classes (Figure 4). Early in the experiment (1999), shortly after canopy closure had occurred, the majority of the trees were classified as dominant, but during the next 2 years, the number of dominant trees decreased and co-dominant trees increased, indicating increased competition for light. Five years later, the number of co-dominant trees (37% of the population) had declined, and the fraction of trees classified as intermediate (22%) or suppressed (23%) increased; the fraction of dominant trees (18%) remained about constant, and there was no effect of eCO₂ (Figure 4A and B). In 2006, LA and BAI were disproportionately much greater for dominant trees, and intermediate and suppressed trees contributed very little to stand growth (Figure 4C and D). There was no effect of eCO₂ on crown class differentiation or the contribution of different crown classes to LA or BAI (Figure 4C and D). Across all plots, 6–15% of individuals were lost through mortality over the 12 years of the experiment (0–4 trees per plot per year; assessed as standing dead). There

was no effect of eCO₂ on mortality (see Figure S5 available as Supplementary data at *Tree Physiology Online*). All of the mortality occurred in suppressed trees; hence, there was little effect of mortality on stand basal area (or aboveground dry matter) increment.

Leaf-level responses

Leaf characteristics varied with crown depth; any effects of eCO₂ were much smaller than the variation within the crown and were consistent throughout the crown. The LMA averaged 10% greater in eCO₂ ($P < 0.02$, Figure 5A) and decreased with crown depth. LMA can be decomposed as the product of leaf tissue density (Figure 5B) and thickness (Figure 5C); both components contributed to the trends with crown depth but were not significantly affected by eCO₂. Leaves in eCO₂ were 14% ($P < 0.03$) smaller than those in aCO₂; leaf size generally declined with crown depth except for increased size in the top 2 m in eCO₂ (Figure 5D).

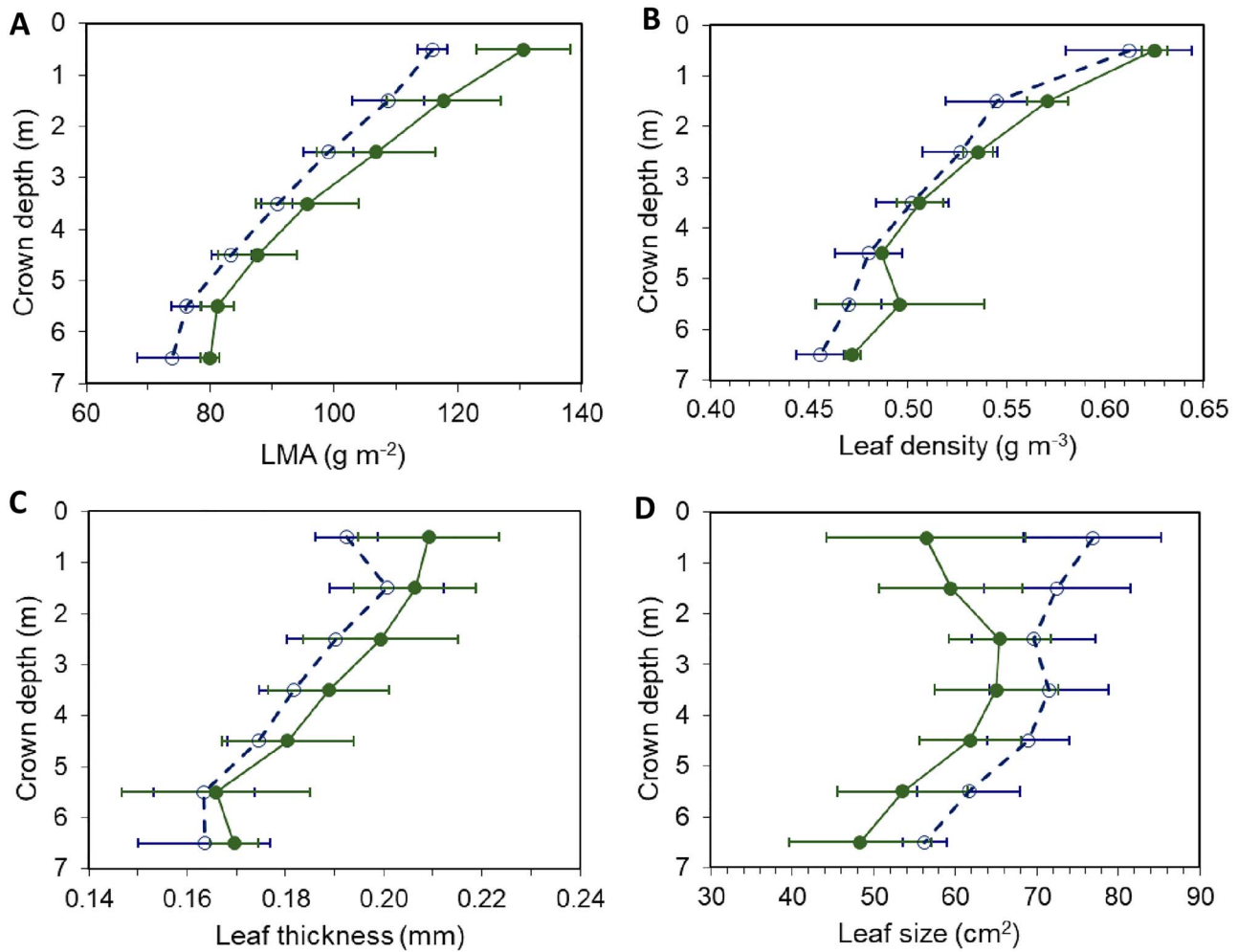


Figure 5. Leaf morphology as a function of depth in the crown (crown depth of 0 is the top of the tree). (A) LMA; (B) leaf tissue density (mass per unit leaf volume); (C) leaf thickness and (D) leaf size. Leaf measurements were made on 20 leaves from each 1-m section of the crown profile of two to four harvested trees per plot at the end of the experiment (2009). Data are the means (\pm SE) of three plots in aCO₂ (open blue symbols) and two plots in eCO₂ (closed green symbols).

Crown-level responses

The LA distribution of trees in ambient and eCO₂ was similar (CO₂ \times depth not significant), although trees grown in eCO₂ tended to have relatively more LA higher in the crown and relatively less deep in the crown (Figure 6A). The distributions were well described by third-order polynomials, which indicated maximum LA fraction, 3.3 m from top of the crown in aCO₂ and 2.8 m in eCO₂. In 1997, prior to onset of CO₂ treatments, maximum LA fraction was at 2.3-m crown depth. Foliar N distribution in the crowns (Figure 6B) followed a similar pattern to that of LA fraction, although there was insufficient material for replicated N analysis of the leaves deepest in the crown. Total N content of the crowns per unit ground area in 2009 was 5.42 g m⁻² in aCO₂ and 4.76 g m⁻² (12% less, $P = 0.09$) in eCO₂.

The difference in total crown N content occurred primarily lower in the crown. Comparing N content from crown top to 4 m depth with N content at 4–8 m, there was a marginal

CO₂ \times position interaction, $P = 0.10$. N content per unit leaf mass (N_{mass}), averaged over the entire crown was significantly reduced by eCO₂ (13.6 mg g⁻¹ in aCO₂ vs. 11.1 mg g⁻¹ in eCO₂, $P < 0.01$, Figure 7A). The N_{mass} was generally similar throughout the crowns (depth effect not significant), but it tended to increase with crown depth in eCO₂; hence, the effect of eCO₂ on N_{mass} was greater near the top of the crown (19.1% less in eCO₂, $P = 0.05$). The N content per unit LA (N_{area}) decreased with canopy depth in both aCO₂ and eCO₂ and was 6.2% less in eCO₂ ($P < 0.01$, Figure 7B).

Canopy responses

Combining the data on LA distribution within individual tree crowns with the distribution of tree heights across the plots (see Table S1 available as Supplementary data at *Tree Physiology Online*) provides an integrated estimate of the LA distribution of the forest stand canopy in 1998 and 2009 (Figure 8A and B). Since trees of different heights had the densest part of the

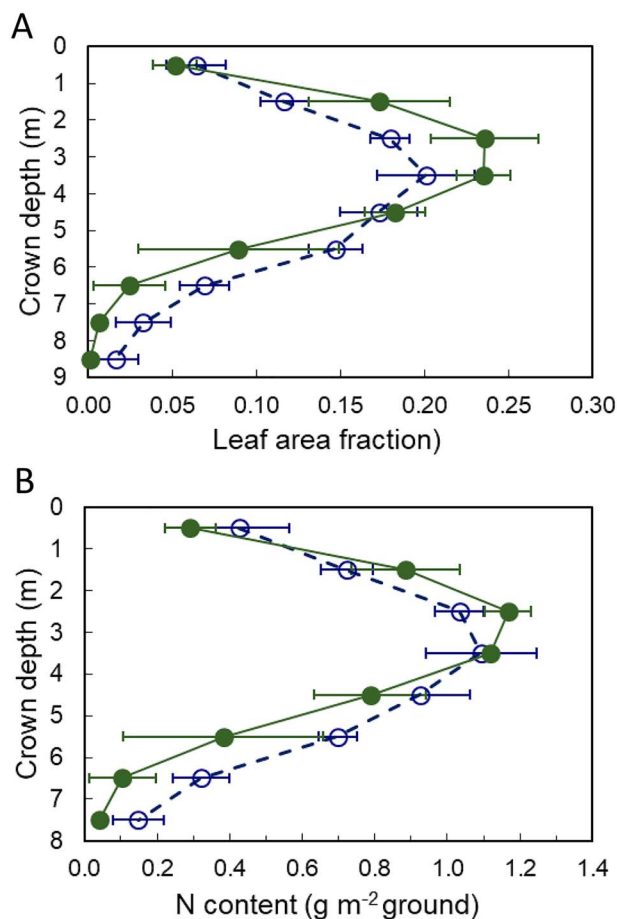


Figure 6. Crown profiles of (A) fraction of total LA and (B) nitrogen content per unit ground area in each 1-m section of the crown. Data are the means (\pm SE) of three plots in aCO₂ (open blue symbols) or two plots in elevated CO₂ (closed green symbols) based on measurements of two to four harvested trees per plot at the end of the experiment (2009).

crown at different positions in the canopy, and the densest crown position of an individual tree occurs lower in the canopy if the tree is shorter than the maximum canopy height, the LA distribution of the canopy was somewhat broader than that of the average tree, and the maximum density occurred deeper in the canopy (4–5 m) than that calculated for the average tree. There was little effect of CO₂ enrichment on LA distribution, and little change from 1998 to 2009, even as the top of the canopy (the height of the tallest tree in the plot) increased 17.6 m above ground to 22.6 m.

Discussion

Stand structure

We predicted that eCO₂ would accelerate the tree and stand development, such as the differentiation of the stand into different crown classes, without causing a fundamental change in canopy structure. There were noticeable shifts in the structure

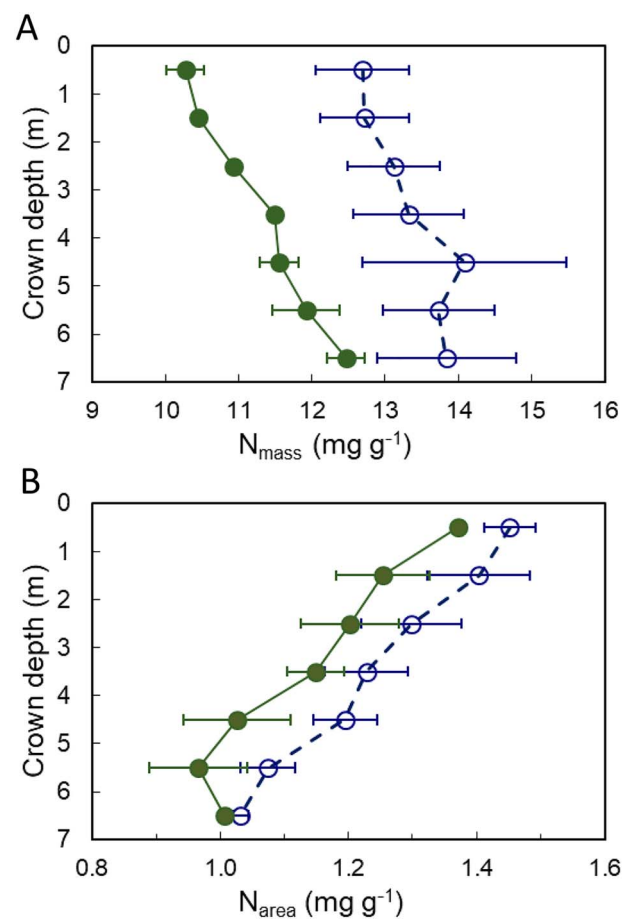


Figure 7. Foliar nitrogen as a function of depth in the crown and CO₂ treatment. (A) N content on a leaf mass basis and (B) on a LA basis. Data are the means (\pm SE) of three plots in aCO₂ (open blue symbols) or two plots in elevated CO₂ (closed green symbols) based on analysis of 20 leaves per 1-m section from two to four harvested trees per plot at the end of the experiment (2009).

of the sweetgum stand during the course of the experiment: more trees became suppressed, some of them died and the largest quartile of trees accounted for an increased fraction of stand basal area (Figure 3B). Intermediate and suppressed crown classes contributed very little to stand BAI (Figure 4D). However, there is little evidence that stand structure or its temporal development was altered by eCO₂. Tree ontogeny can be accelerated by eCO₂ when there is a capacity for an expanding LA and the exponential growth that entails, and exponential growth can lead to large effects of eCO₂ on aboveground biomass (Norby 1996). However, the ORNL FACE experiment was initiated after canopy closure had occurred, and these potential mechanisms of accelerated aboveground stand development were precluded. (Belowground, eCO₂ increased the rate at which roots occupied the soil volume; Iversen et al. 2008.) By contrast, in the Rhinelander FACE experiment in a planted northern hardwood stand, the eCO₂ treatments started when the trees were young and less than 25 cm tall. In that

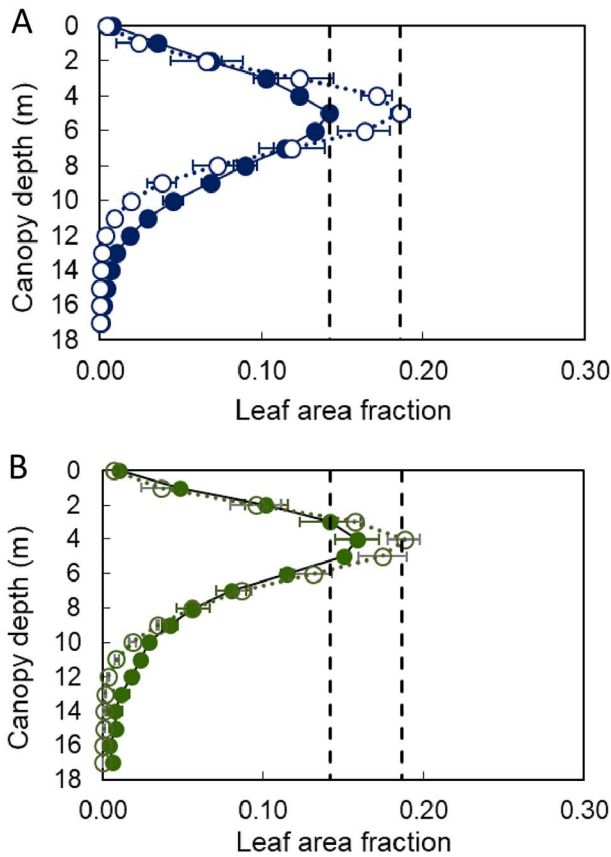


Figure 8. Canopy profiles in 1998 (open symbols, dotted lines) and 2009 (solid symbols and lines), with the top of the canopy determined by the tallest tree in the plot. Data are the fraction of total plot LA and are the mean (\pm SE) of three ambient plots or two elevated plots in each 1-m layer of the plot's canopy in (A) aCO₂ and (B) eCO₂. Vertical dashed lines indicate peak fraction in aCO₂ to facilitate comparison with eCO₂.

study, eCO₂ increased LA and NPP (Walker et al. 2019), but this initial response was transient: as the canopy approached closure, the relative effect of eCO₂ on NPP declined from 68 to 25% (Talhelm et al. 2014).

Canopy structure

Forest canopy structure is a function of both stand structure (e.g., the height distribution of trees within the plot) and LA distribution within crowns of individual trees. While tree height in all plots increased by 5 m during the 12-year experiment, and basal area increased 37%, there was remarkably little change in the canopy structure through time or in response to eCO₂. Maximum LA density of the crowns of individual trees occurred 2.8–3.3 m deep in 2009 (Figure 6A) compared to 2.3 m in 1997. The base of the crown of harvested trees also increased ~5 m from a height of 7 m in 1997 to 11.5 m in aCO₂ in 2009 and 12.3 m in eCO₂. Hence, as the trees grew in height, lower leaves and branches were shed, new leaves were formed at the tops of the crowns and the tree crown maintained a similar profile. Co-dominant trees comprised a larger fraction of plot LA than the taller dominant trees, so maximum LA density at the

canopy scale was deeper (4–5 m) than that of the average crown. Maximum LA density of crowns of trees grown in eCO₂ was slightly (0.5 m) higher than that of trees in aCO₂, but this apparent difference was not statistically significant. We had previously rejected the hypothesis that eCO₂ would lower the light compensation point of leaves and thereby promote the increased retention of leaves deeper in the canopy (Norby et al. 2003). The current structural analysis also supports rejection of that hypothesis—eCO₂ had no significant effect on the depth of tree crowns or the forest canopy. Similarly, there was no strong effect of eCO₂ on vertical distribution of LA of the loblolly pine (*Pinus taeda* L) forest in the Duke FACE experiment, although pine LA tended to move upward relative to trees in aCO₂ and individual crown length increased ~6% (McCarthy et al. 2007). The height–diameter relationship of the pines (but not sweetgum) also changed over time (Kim et al. 2020). The capacity for more substantial effects of eCO₂ on the LAI and canopy structure may be greater in stands with low LAI (Norby and Zak 2011), although there was no response of LAI in the EucFACE experiment with a LAI of less than 2.2 (Duursma et al. 2016).

Although eCO₂ had no effect on crown or canopy structure, there were effects on leaf morphology within crowns. The LMA was greater in eCO₂ and decreased with depth in the crown as has been frequently observed in many studies (Poorter et al. 2009). The relative effect of eCO₂ on LMA and other leaf morphological traits did not vary much with depth. Increases in leaf tissue density (mass per unit leaf volume) and leaf thickness both contributed to the effect of eCO₂ on LMA, but the contribution of thickness was greater, especially at the top of the canopy. These observations coincide with the general conclusion of Poorter et al. (2009), who discussed LMA as the leaf-level cost of light interception, a key trait in plant growth, and an indicator of plant strategy. Their synthesis concluded that increased leaf thickness reflects larger mesophyll cells in eCO₂ rather than an increase in the number of layers of mesophyll cells, and larger mesophyll cells is related to the number and volume of chloroplasts and photosynthetic capacity in high light. Increased leaf tissue density is strongly related to starch accumulation (Poorter et al. 2009), and starch accumulation in eCO₂ foliage has been associated with a negative feedback on photosynthetic capacity (Thomas and Strain 1991). These observations have been used to confront terrestrial biosphere model assumptions that LMA is constant for a given plant functional type (De Kauwe et al. 2014). This assumption is an acknowledged source of uncertainty in model representation of canopy processes in eCO₂ and an area requiring model improvements (Medlyn et al. 2015).

Nitrogen distribution

As generally assumed in models, N_{area} declined with canopy depth in the sweetgum stand (Figure 7B). For example, the QUINCY model keeps LMA constant so a fractional change in

total leaf N results in equivalent changes in N_{mass} and N_{area} (Caldararu et al. 2020). However, it is widely observed that LMA declines with depth in forest canopies (Coble et al. 2017), as was observed here (Figure 5A). Hence, N_{mass} was relatively constant with depth in $a\text{CO}_2$ and increased with depth in $e\text{CO}_2$. The LMA appears to be approaching a minimum value at the bottom of the crown, which is consistent with Dewar's explanation for the distribution of N being different from a theoretical optimum (Dewar et al. 2009). In the sweetgum stand, N_{mass} , and to a lesser extent N_{area} , was reduced in $e\text{CO}_2$, but there was no effect of $e\text{CO}_2$ on the pattern of N_{area} with crown depth. In the Rhinelander FACE experiment, $e\text{CO}_2$ also reduced leaf N on mass basis but not area basis (Takeuchi et al. 2001). The fractional distribution of N with depth was not altered by $e\text{CO}_2$ in the sweetgum stand and closely matched the fractional distribution of LA. That is, $e\text{CO}_2$ apparently had no effect on optimization of N distribution, as was also the case in Rhinelander FACE (Takeuchi et al. 2001). If this non-responsiveness of N distribution to $e\text{CO}_2$ holds generally, one potential source of uncertainty in models depending on N distribution is removed. In woody species, the structural adjustments in LMA in relation to light gradients are responsible for most of the variation in the determinants of photosynthetic capacity; alterations in N partitioning play a secondary role (Niinemets 2007). Hence, improvements in model representation of LMA should be an important objective (Medlyn et al. 2015).

Implications

Our hypothesis surmised that $e\text{CO}_2$ would accelerate the tree and stand development without causing a fundamental alteration of the stand structure. We predicted that the differentiation of trees into different crown classes would be accelerated while the canopy structure remained unaltered. The second part of this hypothesis was supported: there was no significant difference between ambient and $e\text{CO}_2$ plots in crown structure, canopy structure or the distribution of stem biomass among trees. However, there also was little evidence that $e\text{CO}_2$ accelerated tree and stand development above ground. While $e\text{CO}_2$ did not alter the stand development of structure, stand structure likely affected the response of the forest to $e\text{CO}_2$. The differentiation into crown classes over time led to an increase in the number of unproductive intermediate and suppressed trees but a greater concentration of stand basal area in the largest trees, which contributed most to the $e\text{CO}_2$ response. We also note that there were substantial responses of the root system to $e\text{CO}_2$ (Iversen et al. 2008), suggesting that belowground adjustments to $e\text{CO}_2$ were the dominant response mode in this forest.

Our observations emphasize the likely value of including more detailed descriptions of canopy structure in models. There has been a progression from 'big leaf' models (Sellers et al. 1992, Thornton and Zimmermann 2007) to multi-layer

canopy models (Bonan et al. 2018, Caldararu et al. 2020) and demographically structured approaches (Medvigy et al. 2009, Fisher et al. 2018), but LMA, which is necessary for translating mass of C allocated to leaf production to LA and photosynthetic surface, is often treated as a constant for a given plant functional type. Our data, and observations from many other studies, show that LMA follows predictable trends through the tree crown and in response to $e\text{CO}_2$. Additionally, models that include demographic processes (e.g., mortality and self-thinning) and fine-scale horizontal and vertical heterogeneity in stand structure will better represent processes that govern carbon fluxes and their responses to environmental change (Medvigy and Moorcroft 2012, Walker et al. 2019, Holm et al. 2020). The detailed canopy structural analysis we have presented required a destructive harvest that could be accomplished only on a subset of the trees at the end of the experiment. New nondestructive approaches using terrestrial laser scanning provide opportunities for more expansive and robust analyses (Disney 2019).

Are the results we report here informative about stand structure responses that will occur in a mature, native deciduous forest? The sweetgum stand in the ORNL FACE experiment was a young, even-aged and monoculture plantation with a high density of stems. Nevertheless, it had many of the characteristics of a mature forest, although the definition of 'mature' is vague and cannot be given an exact age or preclude secondary succession and biomass accumulation (Martin et al. 2016). The sweetgum forest had attained canopy closure prior to the initiation of the experiment, the trees had transitioned from exponential growth and were in a linear growth phase and fine-root standing crop was not increasing in $a\text{CO}_2$. However, stand structure is interdependent with species composition and function (McElhinny et al. 2005), and an older, uneven-aged, mixed-species forest is more complex in its structure than our sweetgum stand. In a more complex forest stand, a shift in community composition that favors more shade-tolerant species would counteract the within-species optimization responses (Dybzinski et al. 2013). Differences among species in their growth responses to $e\text{CO}_2$ or their N requirements could lead to changes in canopy structure which were not apparent in our study. Reproductive growth in a mature forest alters allocation patterns in a manner that was absent from the sweetgum plantation. (The sweetgum trees were physiologically capable of reproduction based on observations of trees in the plantation that had greater exposure to light or N, but no flowers or fruit were produced in the experimental plots.)

Conclusions

An important objective for the next generation of FACE experiments is to address questions regarding how mature and diverse forests will respond to $e\text{CO}_2$, including how changes in canopy

structure interact with water relations and drought stress (Norby et al. 2016). Canopy structural changes have been difficult to incorporate into experimental designs of past experiments investigating forest responses to eCO₂ due to limitations to destructive sampling in long-term experiments. Terrestrial laser scanning provides the opportunity for tracking changes in canopy structure without the need for a destructive harvest, so more detailed time series analyses will be possible that were not available for the ORNL FACE experiment. Nevertheless, the ORNL FACE experiment provided a useful experimental system for considering whether structural development alters response to eCO₂ and whether eCO₂ alters structural development. Our results showed remarkably small changes in canopy structure.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Authors' contributions

R.J.N. wrote the paper with input from J.M.W., C.M.I. and A.P.W. R.J.N., J.M.W., C.M.I., J.C. and S.S.J. measured the trees throughout the experiment and conducted the final harvest.

Data availability

Data supporting analysis in this paper can be downloaded from the US Department of Energy ESS-Dive data repository (<https://data.ess-dive.lbl.gov/>). Basal area data: doi: 10.15485/1463816; leaf area data: doi: 10.15485/1463810; tree height: doi: 10.15485/1798175; final harvest data: doi:10.15485/1779883.

Conflict of interest

None declared.

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Fig. S1



Fig. S1. 16 trees were felled into a cleared area, 1-m sections were marked off, 20 leaves per section were subsampled for morphological and chemical analyses, and the remaining leaves were harvested.

Fig. S2

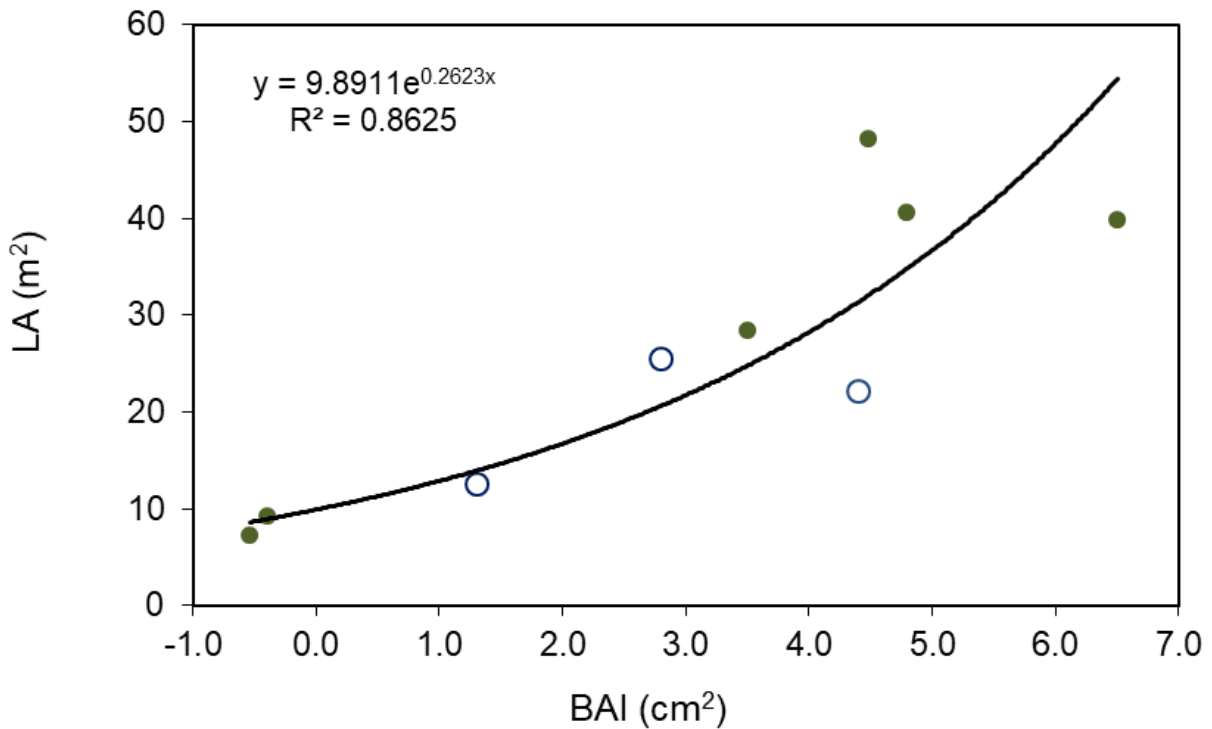
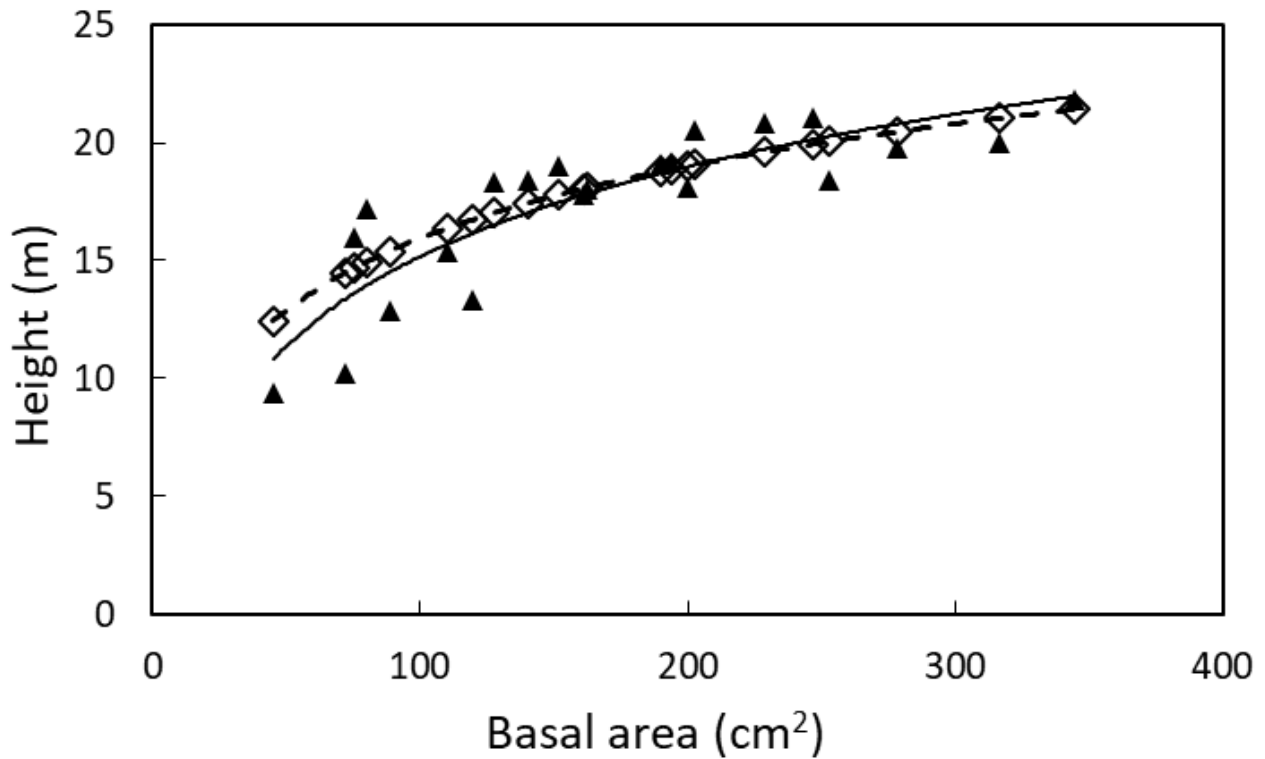


Fig. S2. Relationship between leaf area (LA) per tree and basal area increment (BAI) determined on nine trees harvested in 2009 from ambient (open blue symbols) and elevated CO₂ (solid green symbols). BAI data were not available for seven harvested trees, and those trees are not included in this analysis.



$$H = a + b \times (\text{year}-1997) + c \times \ln(\text{BA})$$

Plot	Intercept (a)	Year number coefficient (b)	Ln(BA) coefficient (c)	R ²
1	- 7.3098	4.4210	0.2412	0.78
2	-11.1718	5.2286	0.2575	0.84
3	-3.6109	3.5237	0.4276	0.83
4	-8.3702	4.771	0.2337	0.78
5	-4.5164	3.9158	0.2554	0.78

Fig. S3. Relationship between basal area and height of trees in plot 1 in 2009. Solid triangles are the measured height; open diamonds are the estimated height using the equation and coefficients for plot 1 in the table below. Solid line is the regression line through the measured points; dashed line is the regression line using the coefficients in the table.

Fig. S4

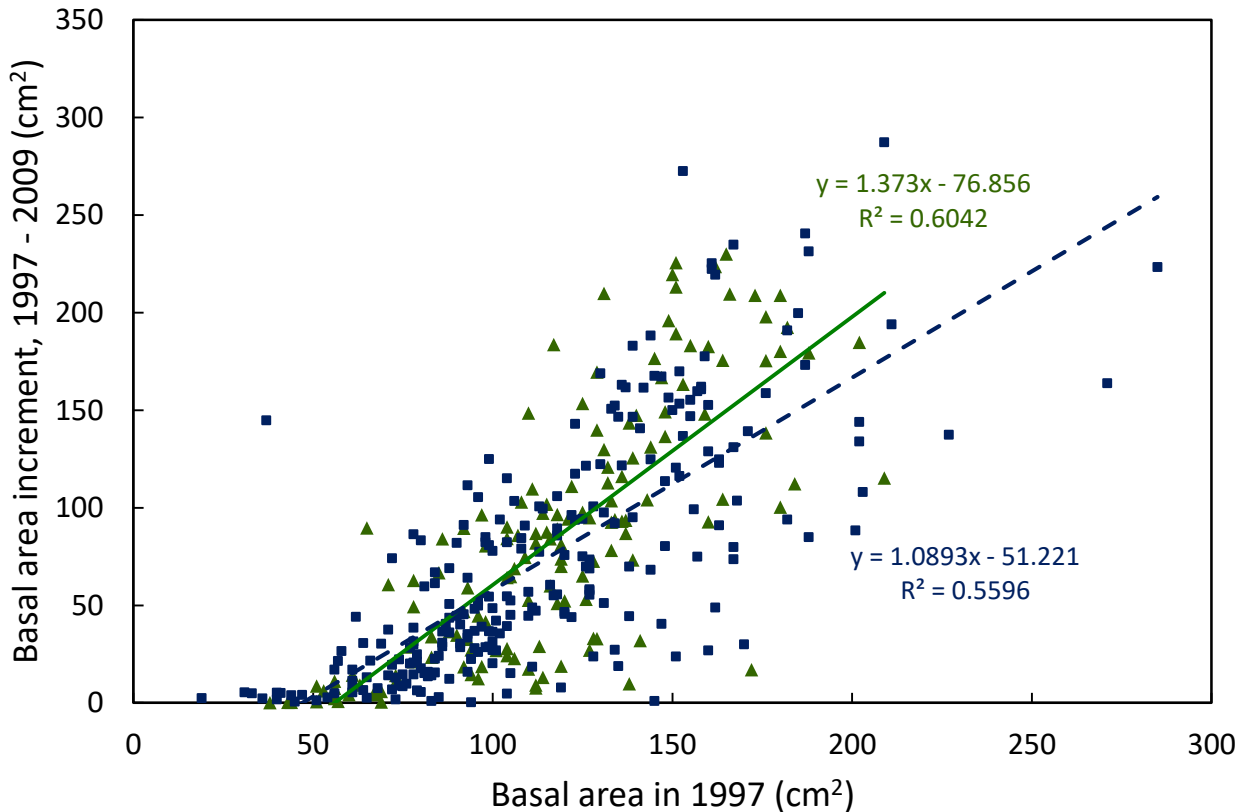


Fig. S4. Relationship between basal area increment over the 12-year experiment and the initial basal area in the pre-treatment year. Each symbol is an individual tree in ambient CO₂ (blue squares) or elevated CO₂ (green triangles). The slopes are significantly different ($t = 12.18$, $P < 0.001$).

Fig. S5

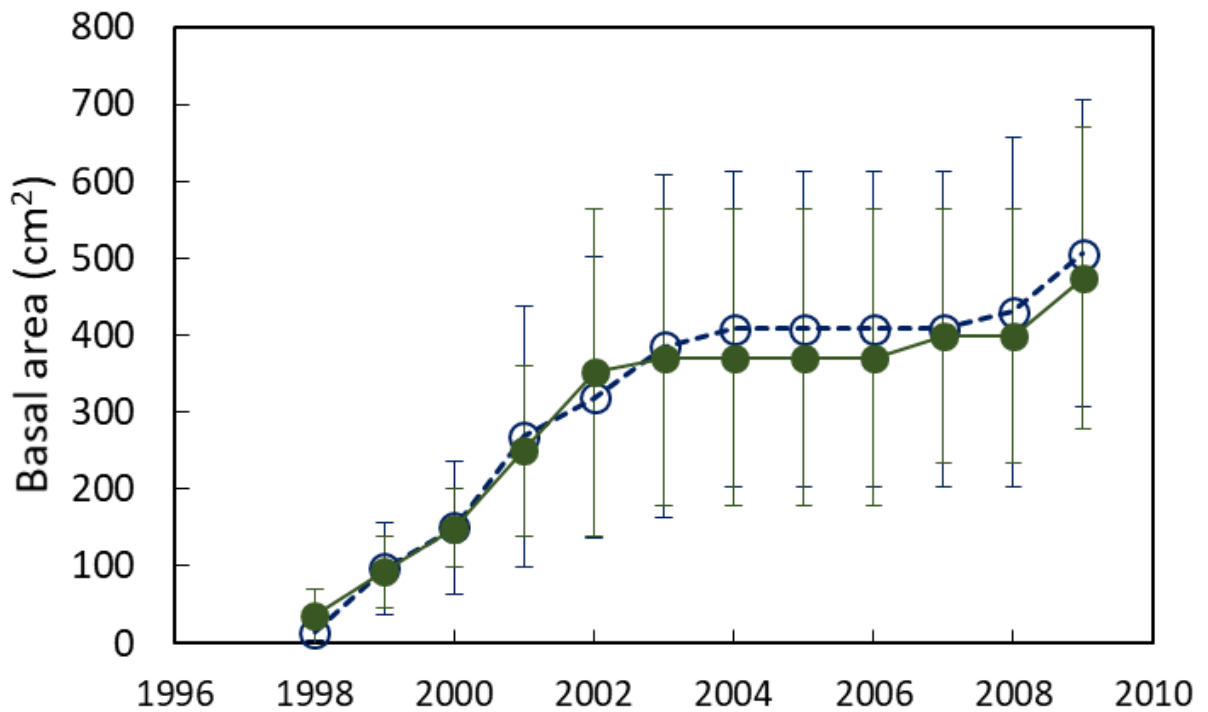


Fig. S5. Cumulative basal area of trees that died in ambient (open blue symbols) and elevated CO₂ (solid green symbols). Data are the total per plot, averaged over CO₂ treatment (\pm SE).

Supplemental Table S1.

Calculation of plot-level leaf area distribution by depth in canopy based on height and leaf area of each tree, which are calculated by regression with basal area and basal area increment.

Details of data sources and calculations are shown as comments in individual cells.

This file is for plot 2 in 2009; similar calculations were done for all plots in 1998 and 2009.

[Excel file Table S1.xlsx](#)