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Re-assessment of plant carbon dynamics at the Duke free-air CO_2 enrichment site: interactions of atmospheric [CO_2] with nitrogen and water availability over stand development

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Summary

- The potential for elevated [CO_2]-induced changes to plant carbon (C) storage, through modifications in plant production and allocation of C among plant pools, is an important source of uncertainty when predicting future forest function. Utilizing 10 yr of data from the Duke free-air CO_2 enrichment site, we evaluated the dynamics and distribution of plant C.
- Discrepancy between heights measured for this study and previously calculated heights required revision of earlier allometrically based biomass determinations, resulting in higher (up to 50%) estimates of standing biomass and net primary productivity than previous assessments.
- Generally, elevated [CO_2] caused sustained increases in plant biomass production and in standing C, but did not affect the partitioning of C among plant biomass pools. Spatial variation in net primary productivity and its [CO_2]-induced enhancement was controlled primarily by N availability, with the difference between precipitation and potential evapotranspiration explaining most interannual variability. Consequently, [CO_2]-induced net primary productivity enhancement ranged from 22 to 30% in different plots and years.
- Through quantifying the effects of nutrient and water availability on the forest productivity response to elevated [CO_2], we show that net primary productivity enhancement by elevated [CO_2] is not uniform, but rather highly dependent on the availability of other growth resources.

Introduction

Forested ecosystems are the dominant terrestrial sink for carbon (C) (Schimel *et al.*, 2000; Pacala *et al.*, 2001; Liski *et al.*, 2003). Therefore, determining current and future global C sinks necessitates accurate information on forest responses to increased atmospheric [CO_2] and climate change. Of particular interest is whether forest growth and C storage will increase under future conditions, possibly mitigating some of the human-induced rise in [CO_2]. Presently, there is growing recognition that plant growth responses to increased [CO_2] will not be as large as initially expected, and enhancements will not be uniform across the landscape, as a result of the uneven distribution of other growth resources, such as water and nutrients (e.g. Oren *et al.*, 2001; Gill *et al.*, 2002; Nowak *et al.*, 2004; Körner, 2006). However, there is inadequate quantification of how elevated [CO_2] may interact with the spatial and temporal heterogeneity of other growth resources, making it difficult to include such interactions in models that project future forest responses.

Net primary productivity (NPP) is generally expected to increase with increasing concentrations of atmospheric CO_2.

A recent synthesis of NPP across four temperate closed-canopy forest free-air CO_2 enrichment (FACE) sites found a surprisingly consistent enhancement, averaging 23% (Norby *et al.*, 2005). However, this uniform response of the average NPP did not take into account the considerable variability that occurred within each site (Norby *et al.*, 2005), possibly caused by variation in resource availability (e.g. Finzi *et al.*, 2002). Previous studies at the Duke FACE site demonstrated that the $[CO_2]$-induced enhancement of NPP was strongly related to nitrogen (N) availability through the positive effect of N availability on leaf area (McCarthy *et al.*, 2006a). Efforts to characterize how interactions between $[CO_2]$ and N availability affect NPP during the second rotation of *Populus* species in Italy (POP-EUROFACE) failed because previous usage as agricultural land rendered the site N unlimited (Liberloo *et al.*, 2006). Overall, few studies have examined C dynamics under elevated $[CO_2]$ within the context of stand development, or have explicitly taken advantage of natural variability in climate and resource availability to increase the relevance of FACE research for predicting future forest function (Osmond *et al.*, 2004).

Determining the effects of elevated $[CO_2]$ on forest ecosystem function and C storage requires not only knowledge of the degree to which elevated $[CO_2]$ will enhance tree biomass, but also where the additional C will be allocated. Carbon partitioning (i.e. the fraction of total production used by a given component; Litton *et al.*, 2007) affects C storage in two ways: first, partitioning to leaves or fine roots determines the ability of plants to capture additional resources; and, second, different plant parts turn over at different rates, so that partitioning to slow turnover parts (e.g. stems), or to parts that contribute to very slow-turning pools (e.g. soil C) would increase the residence time and C storage. The traditional view of allocation is that plants allocate their resources (C and nutrients) in such a way as to optimize their gain of further resources (including water), in response to their growth environment (e.g. Thornley, 1972; Dewar, 1993; McConnaughay & Coleman, 1999). According to these principles, an optimal allocation strategy would suggest that plants growing under elevated concentrations of atmospheric CO_2 should allocate proportionally more C to root formation, in order to exploit soil resources more fully (i.e. to increase water and nutrient uptake). Furthermore, the magnitude of this response should be driven by soil resource availability, with more nutrient or water-limited systems showing a greater increase in fine root allocation (e.g. Palmroth *et al.*, 2006; Litton *et al.*, 2007).

Free-air CO_2 enrichment sites allow empirical testing of these theoretical principles, by exposing an ecosystem to a new set of conditions and observing whether the theoretical behavior (or optimum) is achieved (Dewar *et al.*, 2009). Thus far, experimental results from the FACE sites at both Duke and the Oak Ridge National Laboratory (ORNL) have been used in such tests. At the Duke FACE site a majority of the extra NPP was allocated to wood production (DeLucia *et al.*, 2005). Similarly, in the sweetgum (*Liquidambar styraciflua* L.) plantation at the ORNL FACE site (stand age 10 yr at the initiation of $[CO_2]$ enrichment), a majority of the extra NPP was partitioned to wood production during the first year of $[CO_2]$ enhancement; however, after 3 yr, 75% of the $[CO_2]$-induced enhancement was partitioned to short-lived fine roots and foliage (Norby *et al.*, 2002, 2004). The disparity in allocation to fine roots occurred even though both Duke and ORNL FACE sites are considered to be N-limited sites (Finzi *et al.*, 2007). However, a recent application of a growth-optimization model correctly predicted these differing fine root allocation patterns at both sites based on changes in 'apparent' available soil N and differing root longevity (3 vs 0.53 yr; Franklin *et al.*, 2009). A recent synthesis of the largely accurate performances of several optimization models also suggests that models of this type may be useful for predicting global change effects on allocation patterns (Dewar *et al.*, 2009). However, additional data are needed to validate these models.

Studies of trees or ecosystems under elevated $[CO_2]$ have primarily focused on average responses, seldom proceeding further to assess how responses to changes in $[CO_2]$ may depend on other growth resources, such as water and N (c.f. Finzi *et al.*, 2002). Furthermore, most studies on the effect of elevated $[CO_2]$ on trees and forests are too brief to permit separating treatment effects on partitioning via the effect on the rate of development from the direct effects at comparable developmental stages (Körner, 2006). Our study, performed at the Duke FACE site, combines the results of 10 yr of studies on plant C pools and fluxes in order to assess the long-term effects of elevated $[CO_2]$ on stand development, as reflected in C production and partitioning. These data were collected during a time period with a broad range of climatic conditions and from a site with a variation of nearly 2.5-fold in natural N availability, allowing us to quantify how much productivity and partitioning are affected by water and N availability, and how the $[CO_2]$-induced response is affected by these two factors. The objective of this study was to examine the long-term effects of elevated $[CO_2]$ on stand C dynamics, focusing on the following questions. Does the elevated $[CO_2]$-induced enhancement of NPP change over time? Does elevated $[CO_2]$ change the proportion of C in different plant C pools, or the partitioning of new C, as the stand ages? And, how does elevated $[CO_2]$-induced enhancement of NPP vary with soil N and water availability?

Materials and Methods

Site description

The Duke FACE experiment is located within a loblolly pine (*Pinus taeda* L.) plantation on moderately low-fertility,

acidic clay-loam of the Enon Series, in the Blackwood Division of Duke University's Duke Forest, North Carolina (35°58′N, 79°06′W; elevation 130 m). The plantation was established in 1983. Broadleaf species present include *Liquidambar styraciflua*, *Acer rubrum*, *Ulmus alata* and *Cornus florida*. Summers are warm and humid, and winters are moderate. The average annual precipitation is 1145 mm, distributed fairly evenly throughout the year (in years receiving average or greater precipitation).

This study was based on combined information from the FACE prototype and reference (plots 7 and 8, respectively) and the replicated FACE experiment (plots 1–6). Plots 7 (30 m in diameter) and 8 were established in 1993; plot 7 has received elevated concentrations of CO_2 (550 parts per million) during daylight hours of the growing season, according to the FACE protocol (Hendrey *et al.*, 1999), since 1994. Plots 1–6 were established near plots 7 and 8 at the end of August 1996. Three of these six 30-m-diameter plots receive elevated [CO_2], according to the FACE protocol (targeted at +200 ppm; Hendrey *et al.*, 1999). The average CO_2 concentration in these plots during 1996–2004 was 571 ppm, with 92% of 1-min [CO_2] averages within 20% of the target (average target = 573 ppm). In June 1998, plots 7 and 8 were both split in half by an impermeable barrier, and one half of each plot began receiving yearly N fertilization (11.2 g of N m^{-2} yr^{-1}); four additional reference plot pairs without FACE infrastructure (auxiliary plots; 10 × 10 m) were established nearby, and one member of each pair was fertilized (Oren *et al.*, 2001). A full description can be found at http://face.env.duke.edu.

Biometric variables

Net primary productivity for pines, hardwoods and the entire stand was calculated as the sum of the production of coarse wood (stems, branches, coarse roots), leaf litter (lagged for pines), fine roots and reproductive structures. Standing biomass was calculated as the sum of standing coarse wood, fine roots and foliage. Details are presented in the following sections.

Coarse wood pools The stem and branch biomass values of individual trees were calculated allometrically, based on tree diameters and heights. Annual diameter increments of 31–34 pines and 10–26 hardwoods per plot were determined from dendrometer bands, as described previously by Moore *et al.* (2006). From these measurements, relationships were developed between the individual tree basal area and the basal area increment, and were applied to unbanded trees. Relationships were developed separately for pines and hardwoods, and were plot- and year-specific. Bands were placed on all pines and hardwoods that were > 8 cm in 2004. The heights of banded pines were measured every 5 yr (1996, 2001 and 2006) using either a height pole or a survey laser (Criterion 400; Laser Technology Inc., Englewood, CO, USA). Plot-specific height–diameter relationships were created to calculate the height of all trees for the year they were developed, and height increments (between 1996 and 2001 and between 2001 and 2006) were calculated as the difference between consecutive height values. Height increments were distributed among years without height measurements, using the same relative proportions seen in diameter increments. Based on these measurements we concluded that the relationship of height vs diameter did not change under elevated [CO_2] (repeated-measures ANCOVA of the average height vs average diameter of dominant trees reported the [CO_2] and [CO_2] × year effect as $P > 0.650$; Fig. 1), although trees were taller under elevated [CO_2] (Fig. 2a; $P = 0.025$).

Previously at the Duke FACE site (e.g. DeLucia *et al.*, 1999; Hamilton *et al.*, 2002; Schäfer *et al.*, 2003; Finzi *et al.*, 2006; McCarthy *et al.*, 2006a), pine stem, branch and root standing biomasses were calculated as a function of diameter only, based on the site-specific equations of Naidu *et al.* (1998). Quantification of actual tree heights revealed that the fixed (i.e. invariant) height–diameter relationship of Naidu *et al.* (1998) represented measured values reasonably well at the beginning of the study (1996), but increasingly diverged from measured values, such that by 2006 most trees were underestimated in height by several meters (Fig. 2a,b). When an alternative approach (described below), which explicitly accounted for the effect of both diameter and height in determining standing biomass, was compared to the approach of Naidu *et al.* (1998), the two methods yielded similar standing biomass values at the beginning of the study, which then diverged over time because the equations of Naidu *et al.* (1998) progressively underestimated actual tree heights (Fig. 2c). This comparison suggests that the former equations worked reasonably well at the stand near the time they were developed, but were not flexible enough to describe the Duke FACE stand

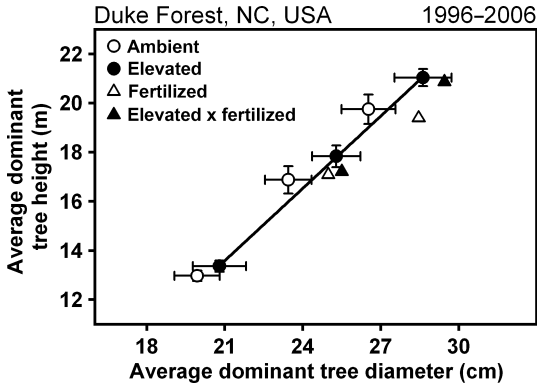

Fig. 1 Average height of dominant trees vs average dominant tree diameter under ambient [CO_2], elevated [CO_2], fertilization and elevated [CO_2] plus fertilization. Error bars indicate 1 SE.

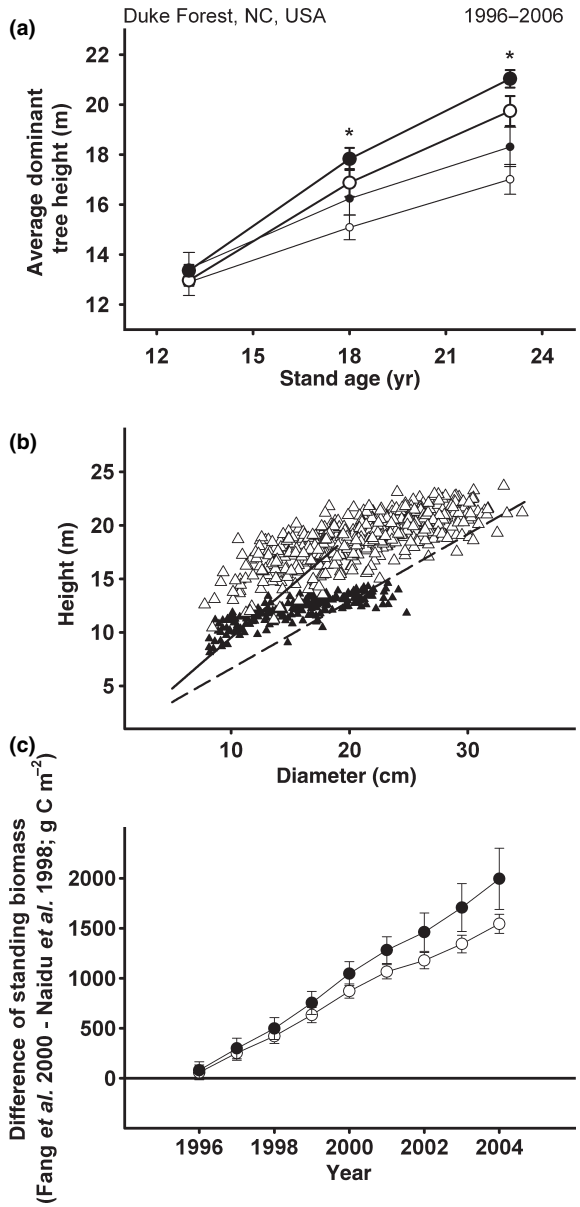

Fig. 2 (a) Average height of dominant trees vs stand age under ambient [CO_2] and elevated [CO_2], from measurements (thick lines; large open circles, ambient; large closed circles, elevated) and site-specific height–diameter equations (thin lines; Naidu *et al.*, 1998). (b) Comparison of measured height–diameter relationships (closed triangles, measured in 1996; open triangles, measured in 2006) with invariant height–diameter relationships calculated from Naidu *et al.* (1998) (solid line, suppressed trees; dashed line, dominant trees). Lines are not regressions to data point lines; they demonstrate the increasingly poor correspondence between measured and calculated tree heights. (c) Difference in standing stem biomass vs stand age using invariant height–diameter allometry (Naidu *et al.*, 1998) and the new variable height–diameter allometry (based on Fang *et al.*, 2000) for ambient (open circles) and elevated [CO_2] (closed circles). Error bars in panels a and c indicate 1 SE. Asterisks in (a) indicate time points with a statically significant ($P < 0.05$) difference between measured ambient and elevated values.

as it developed. Therefore, it was imperative to use an allometric approach which accounted for both height and diameter.

Stem biomass of individual trees was derived from stem volume multiplied by wood density. Inside-bark stem volume was calculated from measurements (or estimates) of individual tree heights and diameters by integrating a taper equation based on more than 1000 trees in the coastal plain and Piedmont regions of the southeastern USA (Fang *et al.*, 2000). The relationship between bark volume (as a fraction of total stem volume) and relative height on stems, presented in Schultz (1997), was scaled to the site-measured bark fraction at breast height and was used to calculate absolute bark volume. Wood and bark volumes were converted to biomass using a specific gravity of 0.427 g cm^{-3} for wood and a specific gravity of 0.279 g cm^{-3} for bark (Naidu *et al.*, 1998). In December 2002, a severe ice storm caused substantial tree breakage (23% of pines lost tops; McCarthy *et al.*, 2006b). After this event, subsequent standing biomass estimates of broken pines were corrected by subtracting the biomass associated with broken tops (measured after the storm; McCarthy *et al.*, 2006b), and subtracting biomass increment overestimates that resulted from the changed allometry. Lacking better information, individual tree branch biomass was calculated from stem biomass using the same proportionality between these components as presented in Naidu *et al.* (1998). Plot-level coarse root biomass (> 2 mm) was calculated as a function of aboveground biomass (Johnsen *et al.*, 2004).

Biomass increments were calculated by subtracting consecutive standing biomass pools. Woody biomass increments of *P. taeda* from fertilized plots were reduced by 8% to account for fertilization-induced reductions in specific gravity (Oren *et al.*, 2001). Conversions from biomass to C were made using C contents of 0.48 for stems and branches and 0.44 for roots (Oren *et al.*, 2001).

For hardwoods, individual tree stem and branch biomass estimates were calculated directly, based on measured or calculated diameters. Species-specific equations were available for *A. rubrum, L. styraciflua*, *Liriodendron tulipifera*, *Quercus alba*, *Quercus falcata*, *Ulmus* spp. and *Carya* spp. (Clark *et al.*, 1986). For species without specific equations, the equation of the most similar species was used. Plot-level hardwood coarse roots were calculated from aboveground biomass, using the same proportion observed in pines. As with pines, biomass increments were calculated by subtracting consecutive standing biomass estimates. Conversions from biomass to C were made using C contents of 0.48 for stems and branches and C contents of 0.44 for roots (Schäfer *et al.*, 2003).

Foliage According to standard procedures (e.g. Ryan, 1991), foliage production for *P. taeda* was determined by

lagging collected leaf litterfall masses by 2 yr in order to account for foliage longevity, correcting when necessary for the effects of droughts and storms (see McCarthy *et al.*, 2007); for hardwood species, foliage production was based on that year's litterfall mass (McCarthy *et al.*, 2007). Leaf litter was collected twice-monthly during peak litterfall (September to December) and monthly otherwise from either 12, 0.16-m^2 baskets per plot (Finzi *et al.*, 2002, 2006) or four, 0.5-m^2 litter baskets (plots 7, 8; starting in 2001). A period of parallel sampling, positioning both collectors in the same plots, found no difference in the sampled litterfall between the two different types of collectors (McCarthy *et al.*, 2007). Samples were dried (65°C, 4 d), separated into pine needles or hardwood leaves, and weighed. Standing leaf biomass was calculated at its maximum (mid-September) by dividing leaf area index (McCarthy *et al.*, 2007) with litter specific leaf area (SLA) for hardwood or green needle SLA for pines (DeLucia *et al.*, 2002). Litter-specific values were used for hardwoods, as repeated measurements at this site (Finzi *et al.*, 2001; McCarthy *et al.*, 2007) have shown no differences between litter and green leaf SLA. Calculations for standing biomass of pine foliage use canopy averaged green needle SLA in order to determine leaf biomass at the maximum leaf area (September) when both current year and previous year foliage is present. Foliage biomass was converted to C using a C content of 0.48 for pine foliage, and a C content of 0.46 for hardwood foliage (Schäfer *et al.*, 2003).

Fine roots The fine root standing biomass (diameter < 2 mm) for 1997–1998 was taken from a previous publication (Matamala & Schlesinger, 2000). The fine root biomass in 2002–2005 was determined from four soil cores per plot, taken at near-maximum fine root biomass (Jackson *et al.*, 2009). At each measurement time point, the organic litter layer (O horizon) was collected first, and then the top 30 cm of the mineral soil (A horizon) was sampled using a 5-cm-diameter corer (AMS Inc., American Falls, ID, USA). Coring depths of 30 cm were chosen based on early profile measurements, finding no fine roots below 25 cm (Matamala & Schlesinger, 2000), and subsequent measurements showing few roots between 15 and 30 cm. Live roots were manually extracted from the O and A horizon samples and cleaned in deionized water. Fine roots were removed and dried to constant weight at 55°C. Fine root production (g m^{-2} yr^{-1}) was estimated by determining the proportion of average annual root length standing crop (assuming that this is equal to mass turnover rate) that was produced during a given year (using 12 mini-rhizotrons per plot in plots 1–6) and multiplying this value by the average annual standing crop quantified using soil cores (Matamala & Schlesinger, 2000; Jackson *et al.*, 2009), as described in Pritchard *et al.* (2001, 2008a). Fine root biomasses were corrected for soil rock volume by multiplying the estimates from coring with the nonrock fraction of soil, and were then converted to C using a C content of 0.41 (Matamala & Schlesinger, 2000). Fine roots were not separated between pine and hardwood. For the purpose of calculating total pine and hardwood NPP and standing biomass, fine roots were partitioned based on the ratio of foliage biomass of each species to the stand foliage biomass.

Reproductive organs The production of reproductive organs was determined using two different methods. First, the production of pine and hardwood reproductive materials (seeds, cones and fruits) was determined from the same litterfall traps used to collect foliage. Reproductive organ biomass was converted to C using a C content of 0.5. Additionally, for pine, estimates of seed and cone production were also made using cone and seed counts. Cones on all *P. taeda* trees within plots 1–6 were counted from above-canopy towers using binoculars, starting in September 1999 (LaDeau & Clark, 2001, 2006). The average number of seeds per cone was determined from cones collected in September 2000 (LaDeau & Clark, 2006). Combining the total number of seeds and cones per ring with the average mass (0.021 g and 21 g) and C fraction (56% and 49%) of seeds and cones yielded estimates of production of seeds and cones for each year (Way *et al.*, 2009; S. L. LaDeau, unpublished).

Atmosphere and soil variables

Atmospheric measurements Vapor pressure deficit (VPD) was calculated according to Buck (1981), from upper canopy level air temperature and relative humidity (HMP35C and HMP45C; Vaisala, Helsinki, Finland), which were monitored continuously in each plot, starting in 1997. Above-canopy precipitation (P; tipping bucket TI; Texas Instruments, Austin, TX, USA) was measured in plots 4 and 7. Photosynthetically active radiation was measured in plot 1 (LI190SB; Li-Cor, Lincoln, NE, USA). Sensors were sampled every 30 s, and 30-min averages were logged (21X or CR23X; Campbell Scientific, Logan, UT, USA). Monthly averages of air temperature and VPD, and monthly totals of photosynthetically active radiation and precipitation, are shown in Fig. 3. Growing season potential evapotranspiration (PET) was derived from adjusted pan evaporation values (Kohler *et al.*, 1955), from a station approx. 7 km from the study site. These data were obtained from the National Climatic Data Center (http://www.ncdc.noaa.gov).

Soil water content Beginning in 1997, volumetric soil water content (θ; m^3 m^{-3}) of the upper 30 cm was measured continuously in four locations (CS615; Campbell Scientific) in plots 1–6. Within plots 7 and 8, θ at 30-cm depth was measured continuously at two locations over the entire study period; an additional two probes at 30 cm and four

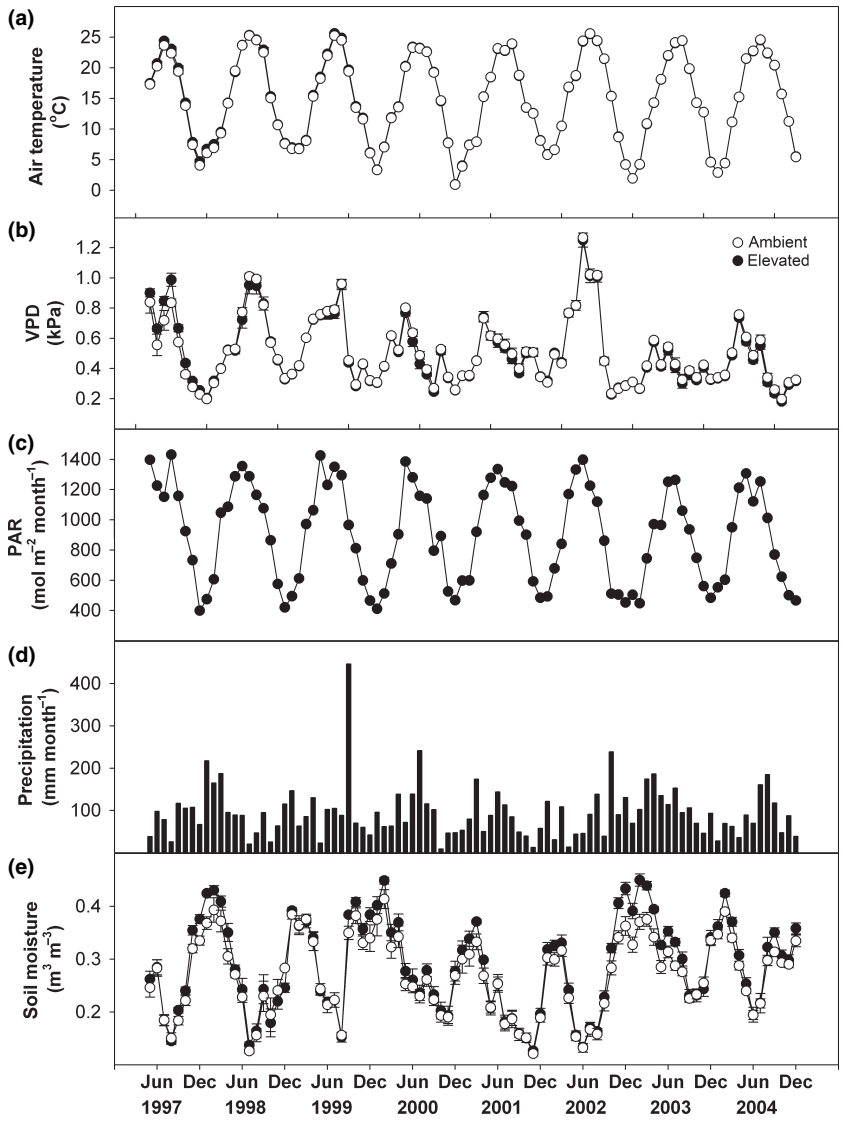

Fig. 3 Monthly average air temperature (a), vapor pressure deficit (VPD) (b) and soil moisture (e) under ambient [CO_2] (open circles) and elevated [CO_2] (closed circles). Monthly total photosynthetically active radiation (PAR) (c) and precipitation (d) is shown for the entire site. Error bars in panels a, b and e indicate 1 SE.

probes at 10 cm were added in 2001 (ThetaProbe ML1x or ML2x; Delta-T Devices, Cambridge, UK). Sensors were sampled every 30 s, and 30-min averages were logged. Moisture values were corrected for soil rock volume by multiplying the sensor outputs with the nonrock fraction of soil. Monthly averages of soil moisture are shown in Fig. 3e.

Nitrogen availability Annual net N-mineralization rates (g of N m^{-2} yr^{-1}; N_{min}) were measured during 3 yr: 1998, 2003 and 2005 (Finzi *et al.*, 2006; A. Gallet-Budynek, unpublished). According to Finzi *et al.* (2006), rates of annual N mineralization were measured in the top 15 cm using the buried bag technique (Eno, 1960), wherein soil cores were analyzed for initial NH_4 and NO_3 concentrations, field incubated and then remeasured for NH_4 and NO_3 concentrations. In 1998, 15 cores (4.78 cm diameter × 15 cm depth) were taken per plot per sample period; in 2003 and 2005 four cores were taken per plot per sample period. The cores were extracted and placed in polyethylene bags. Initial concentrations of NH_4 and NO_3 were determined on 20-g subsamples of soil from each polyethylene bag. Samples were incubated in the field for 1 month, and then removed to the laboratory to determine the NH_4 and NO_3 concentrations. At this time a new set of cores was collected for incubation during the following month. The annual rate of net mineralization was then calculated by summing the differences between the concentration of inorganic N in incubated and initial samples across the 12 months. The concentrations of inorganic N were measured using an autoanalyzer (Lachat QuickChem FIA+ 8000 Series; Zellweger Analytics, Milwaukee, WI, USA). Nitrogen mineralization values were corrected for soil rock volume by multiplying the estimates from cores with the nonrock fraction of soil.

Table 1 Rock volume (%) by plot

Ring	Rock volume (%)	SE
1	5.56	3.02
2	4.50	0.99
3	4.22	1.22
4	6.96	0.76
5	14.71	3.73
6	17.83	5.66
7	8.26	1.06
8	11.87	4.55

Plots with elevated [CO_2] are underlined.

Mineralization rates were pooled across years to estimate an average mineralization rate for each plot. For plots 7 and 8, N-mineralization rates were measured only in 2005; therefore the average rates were calculated based on the relationship of 2005 N mineralization with the average mineralization derived from plots 1–6. Considering that losses of N from this system have been shown to be negligible (Phillips *et al.*, 2001; Finzi *et al.*, 2002, 2006), available N was defined as the sum of N mineralization, N deposition (from Sparks *et al.*, 2008) and N fixation (from Hofmockel & Schlesinger, 2007).

Soil rock fraction The average rock fraction of the soil in plots 1–8 was determined in 2005 by excavating two pits (0.3 × 0.3 × 0.3 m) within each plot and two pits (0.5 × 0.5 × 0.3 m) directly adjacent to the plots. The volume of rocks extracted (estimated using water displacement) was divided by the volume of the pit (estimated by metering fine sand to refill each pit). The rock fraction varied from 4 to 18% between the plots, with an average of 9% (Table 1).

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 and 8, and four auxiliary plots) includes five ambient plots receiving no fertilization and five fertilized replicates. Given the small size of the auxiliary plots, we treated the ambient plots of the complex collectively as one plot, resulting in four replicates each for ambient and elevated [CO_2] conditions, and one each for fertilized and elevated [CO_2] × fertilized conditions. The fertilization treatments were used only to generate information on the upper limits of responses under ambient and elevated [CO_2] conditions, through one-sample t-tests (Sokal & Rohlf, 1995). The effects of elevated [CO_2] on tree height, plant biomass pools and fluxes and C partitioning 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). The effects of [CO_2] during each year of the experiment were tested through linear contrasts within the repeated-measures ANOVA. The relationships of wood production and NPP with available N were evaluated using ordinary least-squares regression. The relationship of the residuals of stand NPP vs available N, against growing season precipitation minus potential evapotranspiration (P-PET) was evaluated using ordinary least-squares regression within each treatment. As treatment with elevated [CO_2] did not begin in plots 1–6 until nearly the end of the growing season in 1996, data from 1996 are considered pretreatment. All statistical analyses were conducted in SAS (Version 8.0; SAS Institute Inc., Cary, NC, USA).

Results

Plant carbon pools and annual net primary productivity fluxes

The NPP of pine before treatment (1996) was not significantly different between the elevated and ambient [CO_2] treatments (Table 2; P = 0.293; n = 3). Elevated [CO_2] increased pine biomass production, starting in 1997 and continuing every year thereafter (Fig. 4a; maximum P < 0.017). There was no significant interaction effect of elevated [CO_2] and time on NPP ([CO_2] × year; P = 0.626), indicating that the [CO_2]-induced enhancement remained fairly consistent as the stand developed. Elevated [CO_2] increased stand (pine plus all other species) biomass production every year from 1997 onwards (Fig. 4b; maximum P < 0.003), with no trend over time ([CO_2] × year; P = 0.713). The average enhancement was 277 g of C m^{-2} yr^{-1} or 28% greater stand level NPP. Relative to ambient [CO_2] without fertilization, fertilizing significantly enhanced pine NPP under ambient [CO_2] in 1999, 2000 and 2003, and under elevated [CO_2] in all years except 2002 (P < 0.05). Fertilization effects on stand NPP were similar to those for pine alone (Fig. 4b).

Although elevated and ambient treatments had similar standing biomass at the start of treatment (1996 in Table 2; P = 0.536 and P = 0.383 for the pine and the entire stand, respectively), by 2000, plots under elevated [CO_2] had accumulated significantly more standing biomass (P = 0.023 for stand) and by 2002 plots under elevated [CO_2] had accumulated significantly more pine biomass (P = 0.039). The mean rate of change in pine and stand biomass under elevated [CO_2] was greater than under ambient [CO_2] (Fig. 4c,d; [CO_2] × year P = 0.002 for the pine and P = 0.004 for the entire stand). Notably, the variation in plant biomass among plots was reduced when considering the entire stand rather than pine alone, reflecting a somewhat compensating effect of higher hardwood biomass in plots with the lowest pine biomass. Standing biomass was calculated using the annual maximum value for foliage and September values for fine roots. Using minimum values

Table 2 Net primary productivity (NPP) and standing biomass for 1996 (pretreatment) to 2004

	Ambient	SE	Elevated	SE	Fertilized	Elevated × fertilized
Pine NPP (g of C m^{-2})						
1996	953	80	1026	131		
1997	964	91	1210	153		
1998	820	86	1063	96		
1999	996	20	1261	68	1136	1302
2000	1093	21	1338	91	1328	1454
2001	886	59	1127	96	1050	1264
2002	628	58	820	120	821	905
2003	833	23	1089	94	985	1107
2004	928	40	1216	123	1067	1150
Pine biomass (g of C m^{-2})						
1996	4717	670	5045	827		
1997	5465	712	6020	958		
1998	5936	560	6590	771	5550	5404
1999	6679	563	7547	821	6419	6395
2000	7518	554	8586	894	7445	7538
2001	8165	579	9390	992	8186	8472
2002	8448	572	9810	1088	8484	8908
2003	8722	625	10 417	1119	8810	9630
2004	9349	631	11 249	1221	9140	10 365
Stand NPP (g of C m^{-2})						
1996						
1997	1045	87	1325	120		
1998	926	77	1203	76		
1999	1100	13	1397	46	1243	1435
2000	1186	16	1470	68	1435	1577
2001	979	58	1252	76	1143	1391
2002	693	56	906	111	888	982
2003	936	26	1219	73	1102	1222
2004	1030	43	1339	101	1163	1257
Stand biomass (g of C m^{-2})						
1996	5103	596	5584	705		
1997	5888	639	6622	817		
1998	6418	504	7304	654	6061	6071
1999	7198	504	8308	697	6958	7103
2000	8076	493	9421	753	8031	8307
2001	8774	517	10 283	840	8807	9305
2002	9067	516	10 726	931	9100	9742
2003	9397	565	11 413	949	9479	10 346
2004	10 076	573	12 292	1054	9839	10 987

n = 4 for ambient and elevated; n = 1 for fertilized and elevated × fertilized.

would have reduced the standing biomass by approx. 4%. More than half of the standing biomass present in 2004 was produced in the 8 yr since [CO_2] enrichment began (57% under ambient [CO_2] and 62% under elevated [CO_2]). Lower initial biomass in fertilized plots under ambient and elevated [CO_2], combined with low statistical power, precluded detection of any significant effects of these treatments on the standing biomass of the pine or the entire stand ($P > 0.05$).

Plant carbon partitioning

Repeated-measures analysis on the partitioning of NPP (into pine stems, branches, coarse roots, foliage; hardwood stems and branches, coarse roots, foliage; and combined fine roots) revealed a significant change in partitioning over the course of the experiment (maximum $P = 0.002$), but no effect of elevated [CO_2] (minimum $P > 0.262$; Fig. 5a–d). In particular, a greater fraction of NPP was partitioned to foliage, and a lesser fraction to stems, as the stand aged. However, the actual magnitude of the temporal change was small, with allocation to stems decreasing from 50 to 46% of NPP and to foliage increasing from 18 to 23% (excluding the severe drought year of 2002). Partitioning to reproductive structures was measured, but not included in this analysis, as it comprises < 1% of the total stand NPP. For more information on the distribution of NPP within specific plots see Supporting Information Tables S1,S2.

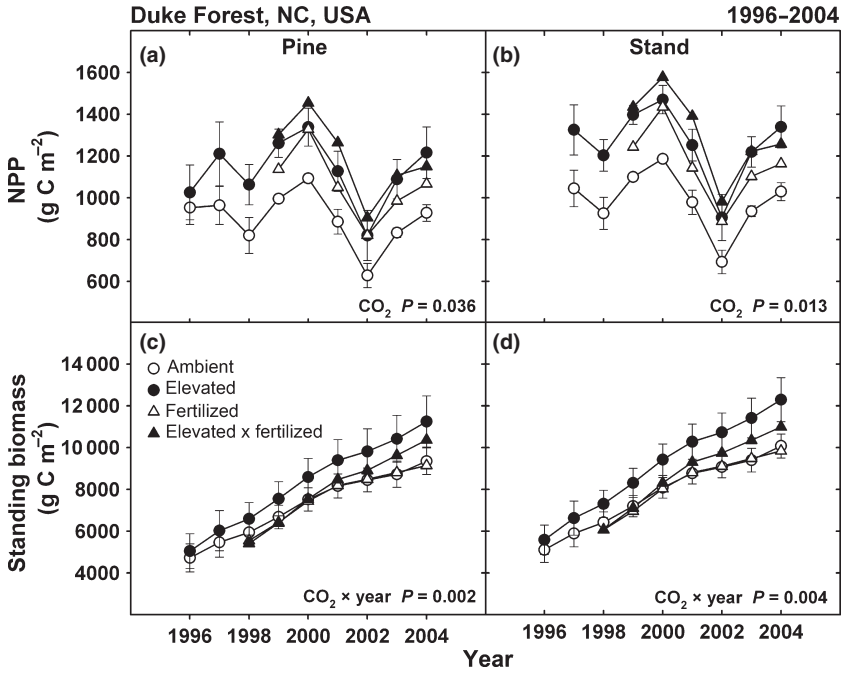

Fig. 4 Time series of pine net primary productivity (NPP) (a), stand NPP (b), standing pine biomass (c) and standing stand biomass (d) under ambient [CO_2], elevated [CO_2], fertilization and elevated [CO_2] plus fertilization. Error bars in all panels indicate 1 SE; for ambient [CO_2], fertilization treatments (n = 1) are the average of five plots and for elevated [CO_2] are half of one plot.

As with NPP partitioning, the distribution of C in plant biomass pools depended strongly on time (maximum $P < 0.006$), but not on [CO_2] ([CO_2] effect minimum $P = 0.138$ and [CO_2] × year effect minimum $P = 0.055$; Fig. 5e–g). The exception to this was a minimal, but significant, decrease in the proportion of standing fine root biomass under elevated [CO_2] (0.97% vs 1.08% by 2004; [CO_2] × year effect $P = 0.016$). Additionally, linear contrasts indicated that the proportion of standing pine coarse roots was significantly lesser under elevated [CO_2] during 1997–2002 and the proportion of standing pine foliage was significantly lesser in 1998–1999. However, the actual magnitude of these changes was small (< 1%), well within the measurement or estimate uncertainty. The vast majority of stand C was in pine biomass (> 90%), particularly in stems (> 60%). Although the [CO_2] treatment did not affect the distribution of C in live biomass pools, there was a significant temporal shift in the distribution of C in several of the pools as the stand developed. From 1997 to 2004 the fraction of standing C in pine stems increased by 4% ($P = 0.001$), while the fraction of C in short-lived tissues (pine and hardwood foliage and fine roots) decreased ($P < 0.001$, $P = 0.018$, and $P < 0.001$, respectively). Mycorrhizal C was not measured until late in the experiment, but its omission earlier in the study is not expected to have much of an impact on the assessment of standing biomass, as mycorrhizal C represented < 1% of the live biomass at the end of the study period (K. K. Treseder, unpublished). For more information on the distribution of standing biomass within specific plots see Tables S3,S4.

Annual net primary productivity fluxes – interaction effects of [CO_2] with nitrogen and water availabilities

Thus far, the results have been presented as treatment-level averages. However, even before the onset of [CO_2] treatment, there was large variation in plant biomass among plots, reflecting differences in site resources. Although other factors may also vary between plots, most of the difference in initial (1996) standing biomass was related to our index of available N (Fig. 6; $R^2 = 0.94$, $P = 0.001$). Subsequently, variation of temporally averaged stand NPP among plots under treatment with ambient and elevated [CO_2] was related to N (Fig. 7a; $R^2 = 0.63$ and $R^2 = 0.74$ for ambient and elevated [CO_2] respectively), with a significant interaction of [CO_2] and N ($P = 0.003$). Similar results were obtained for pine NPP, and for pine and stand wood production (data not shown). Accounting for spatial variation in productivity among plots did not address the appreciable interannual variability in NPP. After testing a number of environmental factors (mean growing season VPD, growing season length, mean growing season temperature, mean growing season θ, growing season precipitation, growing season PET and P-PET), P-PET was found to have the most explanatory power ($R^2 = 0.48$, $P < 0.001$). When the residual variation in stand NPP (after accounting for N) was related to P-PET (Fig. 7b), the data from 2 yr (2002 and 2003) were found to fall well below the rest of the data. Because NPP in these years represents the carryover effects of the previous year's disturbance (as reflected in substantially reduced leaf area index; McCarthy *et al.*, 2007), rather than the effect of weather conditions during the year of

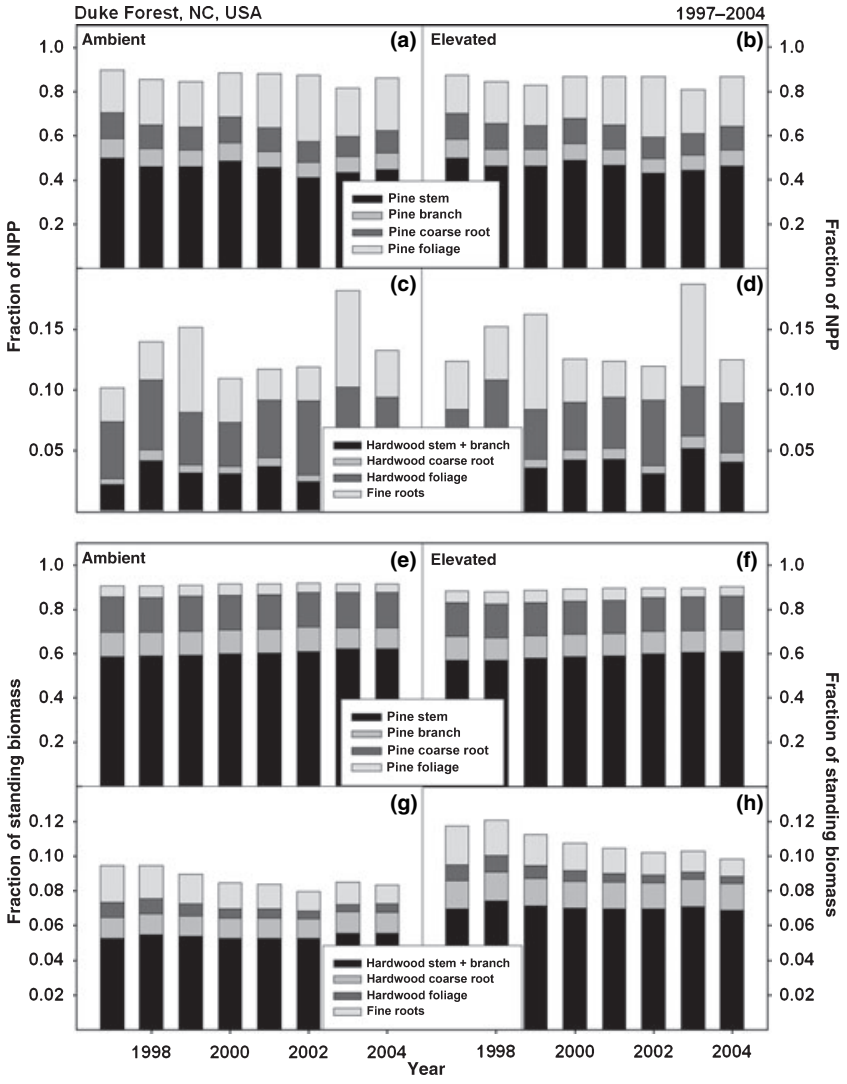

Fig. 5 Proportion of net primary productivity (NPP) (a–d) and standing biomass (e–h) allocated to various plant components for each year of the study under ambient and elevated [CO_2] conditions.

observation, analyses were made excluding these 2 yr (Fig. 7b, thick line). Elevated [CO_2] did not affect the relationship between the residuals of the NPP vs N relationships and P-PET (P = 0.637). Similar results were obtained for pine NPP, and for pine and stand wood production (data not shown).

Multivariate regression models accounting for the combined effects of N and water availabilities on NPP (for ambient and elevated [CO_2] treatments separately) were used to generate a continuous surface of absolute and relative [CO_2]-induced enhancement, demonstrating the dependence of the [CO_2]-induced enhancement of stand NPP on available N and P-PET; (Fig. 8). The regression models, having a significant N-availability index (P = 0.014 and P < 0.001 for ambient and elevated [CO_2], respectively) and P-PET terms (P = 0.009 and P = 0.013 for ambient and elevated [CO_2], respectively) captured much, but not all, of the variability in NPP (R^2 = 0.48 and 0.58 for ambient and elevated [CO_2], respectively; P < 0.001 for both). The interaction of N × P-PET was not significant in either treatment (P = 0.170 and 0.907 for ambient and elevated [CO_2], respectively). From the surface, the absolute [CO_2]-induced enhancement of stand NPP increased with both increasing N and P-PET (Fig. 8a). However, it was evident that the [CO_2] interaction is stronger with N than with P-PET, as the enhancement changed more over the N gradient (approx. 130 g of C m^{-2} from the lowest N to the highest N) than the P-PET gradient (approx. 30 g of C m^{-2} from the lowest P-PET to the highest P-PET), even though the ranges in P and PET over the study period encompassed much of their respective ranges in the longest record available in this area. This pattern was also confirmed by the multivariate regression of all data combined, testing for [CO_2], N and P-PET effects and their potential interactions. Stepwise regression revealed only three significant effects: N, P-PET and N × [CO_2] (P = 0.003, P < 0.001, P < 0.001, respectively). The interaction terms of P-PET × [CO_2], P-PET × N and the three-way

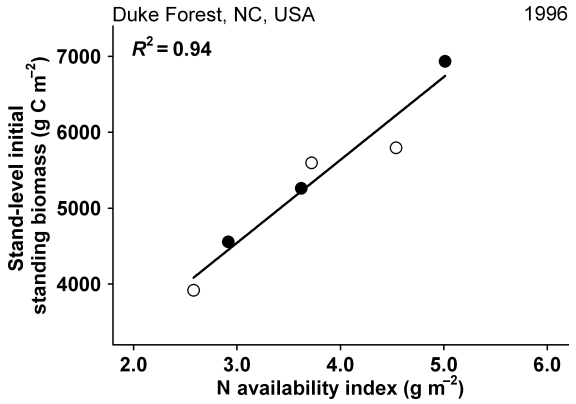

Fig. 6 Stand-level initial (pretreatment) standing biomass (1996) as a function of the soil nitrogen (N)-availability index under ambient [CO_2] (open circles) and elevated [CO_2] (closed circles).

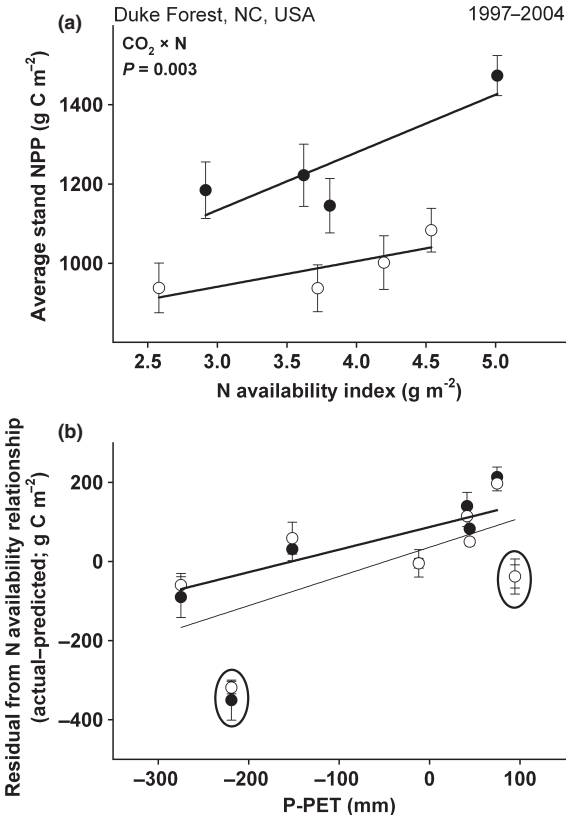

Fig. 7 (a) Stand-level net primary productivity (NPP), averaged over the study, as a function of an index of soil nitrogen (N) availability, under ambient [CO_2] (open circles) and elevated [CO_2] (closed circles). (b) Treatment averaged residuals (actual–predicted) from the relationship of stand NPP vs the index of soil N availability as a function of growing season precipitation minus potential evapotranspiration (P-PET) for ambient [CO_2] and elevated [CO_2]. Error bars in all panels represent 1 SE. In (b), circled points indicate data representing 'carryover' effects of previous disturbance; thin lines represent fits to all data, and thick lines are the fits only to data without carryover effects.

interaction of P-PET × N × [CO_2] were not significant (minimum P = 0.227). The relative [CO_2]-induced enhancement was nearly constant across the P-PET gradient, except at very high concentrations of N, where the relative enhancement increased with decreasing P-PET (Fig. 8b). The average enhancement (using the average N-availability index of the plots and the average P-PET observed during the study) was 271 g of C m^{-2} or 26% (Fig. 8a,b). This is similar to the treatment-level enhancement, of 277 g of C m^{-2} (or 28%), calculated as the average ratio of elevated NPP/ambient NPP.

Discussion

Elevated [CO_2] has the potential to change many aspects of forest ecosystem development and function. We found that elevated [CO_2] resulted in a sustained increase in plant biomass production, the magnitude of which was determined by water and, particularly, N availability. The more available these resources were, the greater was the [CO_2]-induced enhancement in NPP. Notably, on a relative basis, [CO_2]-induced enhancement was mostly invariant with changing P-PET. Thus, the anticipated increase in the relative [CO_2]-induced enhancement with increasing moisture limitation (i.e. the amelioration of drought effects by increased [CO_2] (e.g. Strain & Bazzaz, 1983) was observed only where N availability was very high. Elevated [CO_2] led to increases in NPP (averaging 277 g of C m^{-2} yr^{-1}) and in standing plant C (averaging 217 g of C m^{-2} yr^{-1} greater rate of accumulation), yet did not change the distribution of C among biomass pools or fluxes.

Plant carbon pools and average annual net primary productivity fluxes

The majority of living plant biomass currently in the study ecosystem has been produced since the onset of the experiment (averaging 57% in plots under ambient [CO_2] and 62% in plots under elevated [CO_2]; Fig. 4d). This means that the vast majority of actively functioning plant tissue (where older wood is nonfunctional or less functional than newer wood) was formed since the commencement of the experiment. Qualitatively, our findings regarding the effects of elevated [CO_2] on standing plant biomass and NPP are not fundamentally different from previous assessments at this site (e.g. Hamilton *et al.*, 2002; Schäfer *et al.*, 2003; Finzi *et al.*, 2006). At the treatment level, elevated [CO_2] significantly increased NPP and standing plant C, and the enhancement of NPP was maintained over time. However, correction of the systematic underestimation of tree height, and the consequently substantial underestimates of standing biomass and NPP (e.g. DeLucia *et al.*, 1999, 2002; Hamilton *et al.*, 2002; Schäfer *et al.*, 2003; McCarthy *et al.*, 2006a), bring the stand in line with observations of NPP and standing biomass in similar stands in the North

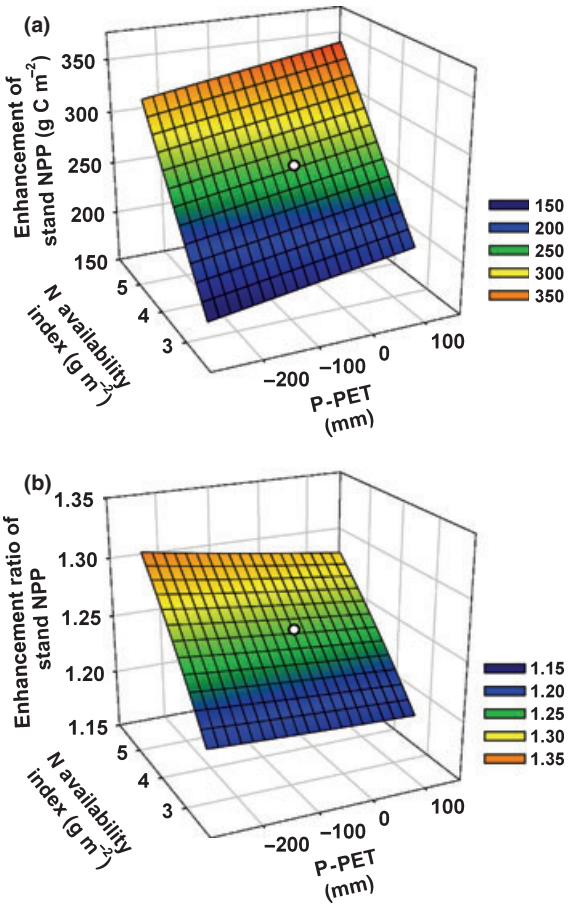

Fig. 8 Absolute (a) and relative (b) [CO_2]-induced enhancement surfaces as a function of an index of nitrogen (N) availability and precipitation minus growing season potential evapotranspiration (P-PET). The open circle in both panels indicates the enhancement at the average N-availability index and the P-PET value observed in the study.

Carolina Piedmont region (e.g. Kinerson *et al.*, 1977), as well as moderating what was believed to be a sharp decline in NPP with stand age (Finzi *et al.*, 2006). Furthermore, accurate quantities of plant C pools and fluxes are critical for closing ecosystem C and N budgets in both [CO_2] treatments, and are essential for testing and constraining models intended to predict the effect of elevated [CO_2] on forest ecosystems.

Interaction effects of [CO_2] with nitrogen and water availabilities

In the absence of additional information, initial standing biomass has sometimes been used as a covariate to account for the impact of initial conditions on later effects of elevated [CO_2]; however this is likely to be less appropriate for long-term studies in which trees are no longer experiencing exponential growth. It is more desirable to identify the causes of spatial differences and to employ those to interpret the effects of [CO_2]. Thus, by extending earlier work linking NPP to net N mineralization (Finzi *et al.*, 2002), this study shows that the N-availability index – statistically interchangeable with initial plant biomass (Fig. 6) – explains much of the spatial variation in NPP at this site and strongly determines the response of NPP to elevated [CO_2] (Fig. 7a). The positive interaction with elevated [CO_2] results in progressively smaller absolute enhancements of stand NPP as available N decreases. In the extreme, very low availability of nutrients (e.g. in *P. taeda* on sandy soils and *Picea abies* on sandy glacial till), have resulted in no observable response to elevated [CO_2] (Oren *et al.*, 2001; Ward *et al.*, 2008). In both of these studies, significant [CO_2]-induced responses were observed in fertilized trees.

Unsurprisingly we also found that water availability, here represented as growing season P-PET, explained much of the remaining interannual variability in productivity within each treatment (Fig. 7b). This is consistent with previous analysis of factors driving inter-annual variation in pine basal area increment (BAI), as well as previous multivariate analyses showing significant, positive effects of precipitation on NPP under ambient and elevated [CO_2] at this site (Finzi *et al.*, 2006; Moore *et al.*, 2006). When we combined the responses of NPP to N and P-PET to assess the effect of varying both N and water availability on the elevated [CO_2]-induced enhancement of stand NPP (Fig. 7), we found on an absolute basis, [CO_2]-induced enhancement increased with increasing N and P-PET, while on a relative basis, [CO_2]-induced enhancement was mostly invariant with changing P-PET. This relative response contrasts with the finding that [CO_2]-induced relative enhancement of BAI was strongly related to growing season P, mean temperature and VPD (Moore *et al.*, 2006), the latter two variables positively related to PET. Basal area increments are just one component of stand level NPP, so the disparity in results between BAI and NPP could be caused by the nonlinear increase in tree biomass with increasing tree diameter, and the nonstem biomass components, which are likely to have differential sensitivity to environmental drivers. Two years of our study (2002 and 2003) exhibited NPP that was seemingly decoupled from the current year's water availability (P-PET). While we have no measure of nonstructural carbohydrates, it seems likely that depletion of these reserves (through drought and ice storm) could have resulted in the below-average NPP observed in these years.

Early conceptual models predicted that relative [CO_2]-induced enhancement would increase with increasing nutrient availability and decreasing water availability, such that ecosystems with high water availability and low nutrient availability should be virtually unresponsive to elevated [CO_2], and ecosystems with low water availability and high nutrient availability should have the greatest response (e.g. Strain & Bazzaz, 1983). In forests, subsequent data has supported the predictions regarding N availability (Oren *et al.*,

2001; Nowak *et al.*, 2004). By contrast, the projections of greater relative enhancement with decreasing water availability have largely not been borne out by data, with enhancement in forests actually increasing with precipitation (Nowak *et al.*, 2004). Our results add to the very few that quantify the important role that both nutrient and water availability are expected to have in facilitating forest productivity response to elevated [CO_2].

Plant carbon partitioning

Our findings of pine standing biomass distribution of 67% to stem, 10% to branches, 18% to roots and 4% to foliage (at age 21 yr), closely match the expected proportions for loblolly pine of ≥ 15 yr, of 65–70% to stem, 10% to branches, 15–20% to roots and 3–5% to foliage (Schultz, 1997). Unlike many assessments of ecosystem C pools and fluxes, this study also captured partitioning to reproductive organs. Although reproductive structures currently represent less than 1% of the total annual C partitioned at the Duke FACE site Table S2), reproduction in loblolly pine was significantly enhanced in plots under high [CO_2] and may indicate long-term ecosystem consequences for forest composition (e.g. LaDeau & Clark, 2001, 2006). Similarly, although allocation to mycorrhizae has been estimated to be only approx. 5% of NPP in this forest (K. K. Treseder, unpublished data), significant increases in ectomycorrhizal colonization under elevated [CO_2] (Garcia *et al.*, 2008; Pritchard *et al.*, 2008b) are likely to be an important factor contributing to increased NPP under elevated [CO_2].

The results from other FACE studies have been mixed with regard to whether elevated [CO_2] causes substantial shifts in the proportion of C in various plant pools and whether there is a shift in the partitioning patterns of new C. The *L. styraciflua* plantation at the ORNL FACE site showed a distinct shift, from up to 80% of the extra NPP induced by [CO_2] partitioned to slow pools (wood) during the early phase of the experiment, to only 25% after 3 yr (with the remainder partitioned to fine roots; Norby *et al.*, 2002). Similarly, in the last year of the first rotation in the POP-EUROFACE experiment on *Populus* species, root pools were increased relatively more under elevated [CO_2] than were aboveground woody components (Gielen *et al.*, 2005). However, the root-to-shoot ratio was unchanged, and the fraction of NPP allocated to woody aboveground biomass was high, ranging (among species) from 53 to 67% (Calfapietra *et al.*, 2003; Gielen *et al.*, 2005). Unlike the first rotation, in which the relative accumulation of biomass in stems and roots did not change, during the second (coppice) rotation of *Populus* species at POP-EUROFACE, elevated [CO_2] resulted in greater C accumulation in branches and less accumulation in stems as compared to trees under ambient [CO_2]; the ratio of aboveground and belowground biomass remained the same (Liberloo *et al.*, 2006). In contrast, when grow from the start under elevated [CO_2], neither the fraction of standing biomass in various pools, nor the partitioning of NPP of *Populus tremuloides* was changed by elevated [CO_2] (King *et al.*, 2005). These differing outcomes suggest that elevated [CO_2] does not have a uniform effect on allocation, but rather must be considered in conjunction with other site factors, as reflected, for example, in leaf area index (Palmroth *et al.*, 2006). Additionally, practical approaches to estimating biomass may also constrain the ability to detect some changes in partitioning: in our present study, we can only identify resource-induced changes in partitioning to branches and coarse roots when they are reflected in changes in height or diameter.

Plant C partitioning is the outcome of many processes that are influenced by both internal and external factors (Dewar, 1993; Cannell & Dewar, 1994), and is frequently poorly resolved, even under ambient [CO_2] conditions (Litton *et al.*, 2007). However, modeling and empirical advances have been made in some aspects of ecosystem C partitioning. A recent resurgence in optimization models has shown that these models can reproduce measured allocation patterns in current and global change settings (Dewar *et al.*, 2009; Franklin *et al.*, 2009). These studies accurately predicted the quantities of fine root allocation at Duke and ORNL FACE sites, capturing their different partitioning patterns (Franklin *et al.*, 2009). Furthermore, a recent analysis of total belowground C allocation (TBCA) across these four forest FACE sites demonstrated significant, inverse relationships between TBCA and leaf area index and between TBCA and NPP (Palmroth *et al.*, 2006), with a higher fraction of the [CO_2]-enhanced NPP allocated belowground where leaf area and NPP are low.

A recent review of plant responses to elevated [CO_2] calls for forest FACE experiments to be analyzed with respect to factors driving variation in intrasite growth signals, over as many years as possible, with the recognition that broad averages of elevated [CO_2] effects on the C cycle are dependent primarily on the frequency of representation of particular conditions in the literature (Körner, 2006). Here we show that elevated [CO_2] may significantly increase the rate of biomass production and the rate of ecosystem-level C storage. However, we also show that NPP enhancement is highly dependent on the availability of other growth resources. Thus, we add our findings to a growing body of literature suggesting that the rate at which extra C will be sequestered with increasing atmospheric [CO_2] would greatly depend on the spatial and temporal distributions of other growth resources.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Pine biomass production 1996 (pretreatment) – 2004

Table S2 Stand biomass production 1997–2004

Table S3 Pine standing biomass 1996 (pretreatment) – 2004

Table S4 Stand-level standing biomass 1996 (pretreatment) – 2004

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