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EFFECTS OF FREE-AIR CO_2 ENRICHMENT (FACE) ON BELOWGROUND PROCESSES IN A *PINUS TAEDA* FOREST

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Abstract. Terrestrial vegetation and soils may act as important carbon sinks if rising atmospheric CO_2 stimulates plant production. We used free-air CO_2 enrichment (FACE) technology to expose three 30 m diameter plots of a loblolly pine (*Pinus taeda*) forest to elevated CO_2 at 200 μL/L above ambient levels, while three control plots were outfitted with FACE apparatus but were fumigated with ambient air. We quantified litterfall mass and chemistry, fine root biomass increment and turnover, CO_2 efflux from soils, $\delta^{13}C$ in soil CO_2, soil CO_2, soil microbial biomass C and N, and potential net N mineralization. After two growing seasons, elevated CO_2 caused significant increases in loblolly pine litterfall mass and fine root increment. Within the first year of FACE treatment, the concentration of CO_2 in soil had increased, and soil surface CO_2 efflux was generally higher at elevated CO_2, but this difference was not statistically significant. Loblolly pine litter C:N ratio, fine root turnover, microbial biomass C and N, and potential net N mineralization were not significantly affected by elevated CO_2. Our results suggest that elevated atmospheric CO_2 may accelerate inputs of organic matter to soil C pools in loblolly pine forests, but it may also accelerate losses of C from belowground by stimulating soil respiration.

Key words: atmospheric carbon dioxide; belowground processes and global change; fine roots; free-air CO_2 enrichment (FACE); litter quality; loblolly pine; microbial biomass; nitrogen cycling; pinus taeda; *soil respiration.*

INTRODUCTION

The amount of CO_2 in Earth's atmosphere is growing by ~3 Pg C per year, due largely to fossil fuel emissions and deforestation. The increase in atmospheric CO_2 may affect the biosphere directly through its effect on photosynthesis and indirectly through climate change (Houghton et al. 1996). When light, water, and soil nutrients are not limiting, elevated CO_2 increases productivity in most plant species by accelerating photosynthesis (Curtis 1996, Drake et al. 1997).

Soil organic matter contains ~1500 Pg C in the top meter of soil, which is about 2.5 times more than is contained in terrestrial vegetation (Schlesinger 1997). An additional 840 Pg C resides between 1 and 3 m depth (Jobbágy and Jackson 2000). Plant production creates ~60 Pg of organic C each year, while ~77 Pg C returns to the atmosphere as soil respiration reflecting the sum of root respiration and decomposition of organic matter (Raich and Potter 1995). Even a small net change in the flux of carbon from soils could dramatically affect the accumulation of atmospheric CO_2. Two critical questions limit our ability to predict feedbacks between the biosphere and atmospheric CO_2: (1). To what extent does carbon storage in vegetation and soils increase with elevated atmospheric CO_2? and (2). To what extent will soil nutrients support long-term increases in plant productivity with increased CO_2?

Increased net primary production (NPP) with elevated CO_2 will only cause significant soil C accumulation if a substantial proportion of the additional C fixed by plants with elevated CO_2 enters soil C pools that turn over slowly (Schlesinger 1990, Hungate et al. 1997*b*, Trumbore 2000). If the additional carbon is allocated exclusively to relatively labile pools such as nonstructural carbohydrates or root exudates, little net C storage may occur (Fig. 1; Ehleringer et al. 2000). Long-term stimulation of NPP with elevated CO_2 may require increased nitrogen uptake or higher N use efficiency. Elevated CO_2 may increase plant uptake of soil N by increasing root mass (Rogers et al. 1994). However, N availability could decline if the plant litter produced under elevated CO_2 decomposes slowly, reducing the rate of N mineralization relative to litter

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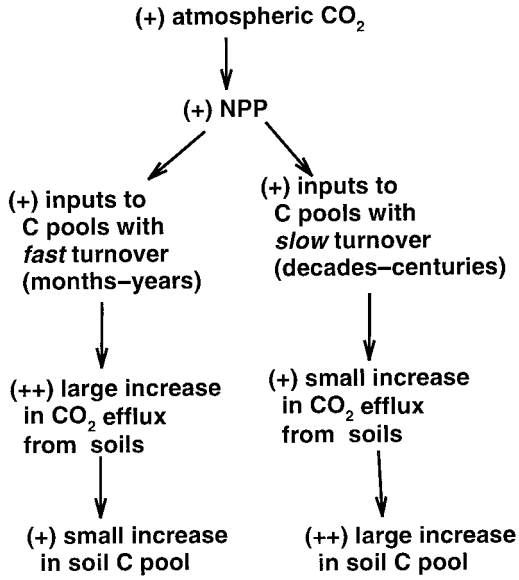

FIG. 1. Two hypothesized pathways through which elevated atmospheric CO_2 may affect soil C storage.

produced at ambient CO_2 (Cotrufo et al. 1994, Ball 1997).

Numerous plant species have been exposed to elevated CO_2, often in pot experiments using artificial soil (Curtis 1996). Although the results of these experiments have proven useful in exploring the physiological response of plants to elevated CO_2, they are less useful in testing the hypotheses we describe in Figs. 1 and 2, which involve ecosystem-level feedbacks between plants, soil microbes, and soil organic matter. In elevated CO_2 studies, pot size can affect plant growth (Thomas and Strain 1991), and the absence of soil organic matter and natural populations of decomposers alters nutrient availability relative to field soils. Open-top chambers have an advantage over pots because they can be placed over natural soils, but their size is usually limited to the height of tree saplings, and they may produce microclimatic artifacts by restricting air movement.

Trees store large amounts of C in woody biomass that has a relatively long residence time, and forest ecosystems make up 80% of biomass and 60% of NPP on land (Houghton and Skole 1990). In order to predict the potential for carbon sequestration by terrestrial vegetation, we must learn how large-statured, intact forests respond to elevated CO_2. FACE (free-air CO_2 enrichment) technology can expose large plots of intact forest to elevated CO_2 without microclimatic artifacts or soil disturbance. In this paper, we summarize results from several studies in the literature that have examined effects of elevated CO_2 on belowground C and N cycling, and we report first- and second-year results from a FACE experiment in a maturing loblolly pine forest that may provide added insights into the functioning of forests under elevated atmospheric CO_2.

Belowground carbon inputs, losses, and storage

Leaf litterfall and fine root turnover contribute important inputs to soil carbon pools (Raich and Nadelhoffer 1989, Schlesinger 1997, Jackson et al. 2000), and these inputs may increase as the atmospheric CO_2 rises. Double-ambient CO_2 caused large increases in tree leaf biomass in various open-top chamber studies (Norby et al. 1995, Johnson et al. 1997, Tissue et al. 1997), suggesting elevated CO_2 will accelerate the flux of organic matter to soil via litterfall. In a review of over 160 studies, Rogers et al. (1994) found that root dry mass also exhibited large increases with elevated CO_2. Pregitzer et al. (1995) found that elevated CO_2 nearly doubled C inputs to soil from *Populus* root turnover. Elevated CO_2 also increased the amount of C added directly to the belowground system by root exudation in *Pinus echinata* (Norby et al. 1987).

Changes in the rate at which carbon is lost from the soil could play a key role in determining the extent to which ecosystems can sequester atmospheric CO_2 (Hungate et al. 1997*b*). In order for elevated CO_2 to substantially increase C storage in soils over the time scales relevant to humans (i.e., decades to centuries), elevated CO_2 must cause increases in the rate of inputs to relatively large, active pools of soil organic matter that turn over on a similar time scale (Harrison et al. 1993). Large increases in soil respiration due to elevated CO_2 that parallel increases in NPP may indicate increased carbon allocation to small pools that turn over rapidly (i.e., root metabolites and root exudates),

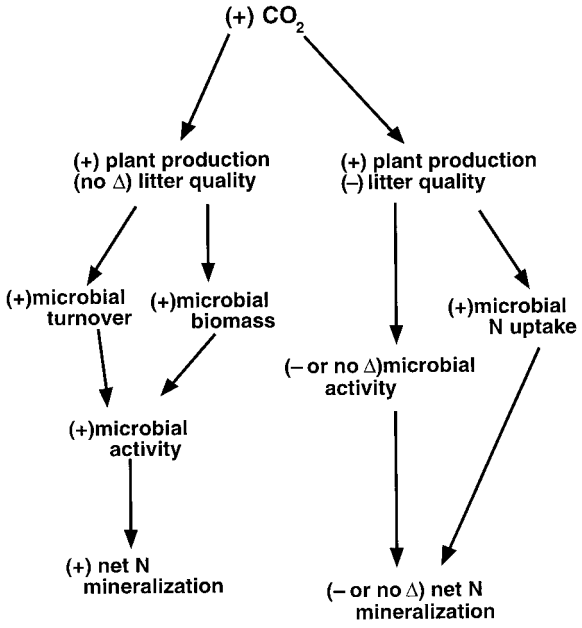

FIG. 2. Two hypothesized pathways through which elevated atmospheric CO_2 may affect soil N availability.

and if elevated CO_2 affects only these pools, C storage belowground may not increase substantially (Hungate et al. 1997*b*).

A few studies on a variety of plant growth forms have examined the effect of high atmospheric CO_2 on soil respiration under field conditions. In an open-top chamber experiment with ponderosa pine seedlings, soil CO_2 efflux increased significantly at 525 μL/L CO_2 (Vose et al. 1995). Luo et al. (1996) found that belowground respiration increased in grassland plots exposed to 720 μL/L CO_2 in open-top chambers, and soil respiration increased under cotton exposed to 550 μL/L CO_2 in a FACE experiment (Nakayama et al. 1994). Johnson et al. (1994) found that root biomass, but not soil microbial respiration, best correlated with higher soil partial pressure CO_2 (pCO_2) under ponderosa pine seedlings grown at elevated CO_2. In a grassland ecosystem exposed to elevated CO_2, Hungate et al. (1997*b*) found increases in soil respiration that paralleled increases in NPP, suggesting that elevated CO_2 caused little C accumulation in large soil organic matter pools. Instead, elevated CO_2 accelerated soil C cycling by stimulating root metabolism and inputs to relatively small, labile C pools such as root exudates (Hungate et al. 1997*b*).

Nutrient cycling and nutrient availability

Soil nutrient availability currently limits plant productivity in many ecosystems (Vitousek and Howarth 1991), and some researchers have suggested that nutrient limitations will prevent a long-term CO_2 fertilization effect on NPP (e.g., Díaz et al. 1993). In a meta-analysis, Curtis and Wang (1998) found that low nutrient conditions reduced the effect of elevated CO_2 on plant biomass. However, increased C resources in plants exposed to elevated CO_2 may increase the ability of plants to capture soil nutrients if fine root production increases (Baker et al. 1990, Idso et al. 1991, Idso and Kimball 1992, Rogers et al. 1992, Rogers et al. 1994, Berntson and Bazzaz 1997). Increased carbon supply to roots with elevated CO_2 may increase root uptake capacity for some nutrients (BassiriRad et al. 1996). Elevated CO_2 may also increase the potential for plant nutrient acquisition by stimulating mycorrhizal colonization of roots (Díaz et al. 1993, Stulen and den Hertog 1993, O'Neill 1994) and root exudation of phosphorus-mobilizing carbon compounds such as phosphatases and organic acids (DeLucia et al. 1997). In some ecosystems, increased symbiotic N fixation may act to ameliorate N limitation to elevated-CO_2 response (Vogel et al. 1997).

In addition to changing the ability of plants to acquire soil nutrients, elevated CO_2 may change the rate at which nutrients become available (Zak et al. 1993), although researchers do not yet agree on the magnitude, or even the direction, of these projected changes (Díaz et al. 1993, Zak et al. 1993). For example, if elevated CO_2 increases plant C inputs to soil but does not decrease decomposition rates, nitrogen availability may increase due to greater activity of C-limited soil microbes that mineralize nitrogen (Fig. 2; Zak et al. 1993, McGuire et al. 1995). Alternatively, elevated CO_2 may decrease rates of organic matter decomposition and N mineralization if it increases the C:N or lignin:N ratio of litter (decreased ''litter quality''; Strain and Bazzaz 1983, Cotrufo et al. 1994, Ball 1997). Most field studies have not shown consistent declines in litter decomposition under elevated CO_2 (Hirschel et al. 1997, Norby and Cotrufo 1998), and the effect of elevated CO_2 on litter decomposition rate may vary among plant species in both magnitude and direction (Franck et al. 1997). Increased inputs of litter with a high C:N ratio could also cause increases in biomass of C-limited soil microbes, shifting the balance of plant–microbe competition for nutrients in favor of microbes (Fig. 2; Díaz et al. 1993). Some experiments have found no effect or even negative effects of elevated CO_2 on microbial biomass (Hungate et al. 1996, Berntson and Bazzaz 1998, Lussenhop et al. 1998), while increased microbial biomass under elevated CO_2 has been observed in soil under tallgrass prairie (Rice et al. 1994), tall herb and acidic grassland communities (Díaz et al. 1993), and poplar saplings (Zak et al. 1993).

Whether elevated CO_2 increases or decreases nitrogen availability may depend on the ecosystem of interest, and mechanisms for these changes remain poorly understood. Zak et al. (1993) observed increased net N mineralization under aspen hybrids exposed to elevated CO_2. Gross N mineralization increased in two annual grasslands exposed to elevated CO_2, apparently due to increased soil moisture (Hungate et al. 1997*a*). Hungate et al. (1996) observed accelerated NH_4^+ production with elevated CO_2 in soil under three introduced grass species in California, but they found small effects among three native herbaceous species. Berntson and Bazzaz (1998) found decreased NH_4^+ production in temperate forest mesocosms grown under elevated CO_2.

Objectives

In this paper, we discuss the effects of free-air CO_2 enrichment on belowground C cycling and nutrient availability in a loblolly pine forest in North Carolina after 28 mo of elevated CO_2 treatment. We are testing the following hypotheses in this forest ecosystem:

(1) Elevated CO_2 will increase C inputs to soil through increased leaf and root production (Fig. 1). (2). Elevated CO_2 will increase losses of C from soils through increased soil respiration (Fig. 1). (3). Elevated CO_2 will decrease litter decomposability and slow net N mineralization (Fig. 2).

METHODS

FACE (Free-Air CO_2 Enrichment)

Few published studies have investigated the effects of elevated CO_2 on C cycling and nutrient availability

in intact ecosystems (Owensby et al. 1993, Niklaus and Körner 1996, Hungate et al. 1997*a*), and only one has examined the effects of elevated CO_2 on these processes in a forest ecosystem with mature trees (Hättenschwiler et al. 1997). FACE is used to expose large, replicated plots of intact forest to elevated CO_2 with little effect on microclimatic conditions (Hendrey et al. 1999). The FACE facility in the Duke Forest (Orange County, North Carolina) consists of six experimental plots, each of 30-m diameter (Fig. 3). Beginning on 27 August 1996, three of these plots received a continuous supplement of CO_2 (24 h/d, throughout the year) with a target CO_2 enrichment of 200 μL/L above ambient. Actual mean enrichments at the center of FACE plots ranged from 199 to 203 μL/L during this study. Air enriched with CO_2 enters each plot through a circular array of 32 perforated pipes that extend from the forest floor to the top of the canopy. Three other plots are fully instrumented controls that receive no CO_2 supplement.

The forest at this site is an even-aged stand of loblolly pine (*Pinus taeda* L.), planted in 1983 after the harvest of similar vegetation. The pines were ~14 m tall at the beginning of our study and comprise 98% of the total basal area. Because the plantation has not been managed since the planting of this pine cohort, a diversity of deciduous species has invaded, and a few of these individuals also reach the canopy. Topography of the 90-ha site is relatively flat, with an elevational gradient of 15 m between the highest and lowest plots. Soils are Ultic Alfisols of the Enon Series, a deep, highly weathered profile developed from igneous parent materials. Soils are slightly acid (pH 5.0) and poorly drained from late fall through early spring.

Litterfall

We collected plant litterfall during June through December of 1996 and throughout 1997 and 1998 by placing 12 replicate 40 × 40 cm baskets in each plot. Litterfall was collected once per month throughout the year and twice per month between September and December, the period of peak litterfall in this forest. Subsamples were dried for 4 d at 65°C and ground to pass a no. 10 mesh. We measured the N concentration of loblolly pine litter using a Kjeldahl digestion procedure (Lowther 1980) followed by colorimetric analysis (TRAACS 800 Autoanalyzer, Bran Leubbe, Elmsford, New York, USA). We measured C in pine litter from the largest single collection of the year in fall 1996 (approximately one-third to one-half of annual pine litterfall appeared in this collection), and throughout the year in 1998. Litter C was determined by combustion in an elemental analyzer (NA1500 Series 1, Carlo Erba Instrumentazione, Milan, Italy). New loblolly pine needles typically appear in March and last 18 mo, so the first cohort of needles that grew entirely under elevated CO_2 appeared in March 1997, and most of these fell during autumn 1998.

Fine root production

We quantified fine root production between November 1997 and November 1998 using a modification of the ''compartment-flow'' method of Santantonio and Grace (1987), described by the following equation: root production = $I + M + D$ where I is the increment in live root biomass between November 1997 and November 1998; M is the sum of positive increments in dead root biomass during the six, 2-mo time intervals between November 1997 and November 1998; and D is the sum of estimates of root decomposition during the six, 2-mo time intervals. We calculated I for each plot by regressing live fine root biomass against time over the six time intervals. Decomposition constants were estimated throughout the year by litter bag studies and the application of a simple model (Santantonio and Grace 1987) to account for fluctuations in mean monthly soil temperature, which was measured at 15-cm depth. Estimates of decomposition were combined with measurements of the biomass of dead roots and used to calculate the fluxes in and out of the dead-root pool.

We collected cores for fine root measurement every other month between November 1997 and November 1998. At each sampling time, we collected five soil cores (5 cm diameter and 20-cm depth) from randomly selected points in each plot. Samples were stored on ice in the field; then they were transported to the laboratory and stored at 4°C until processed. Shortly after collection, roots were separated from soil by careful hand picking and then sorted by diameter; fine roots were defined as those with diameters ≤1 mm. Live and dead roots were separated based on color of the vascular tissue, strength, and flexibility. Roots that were difficult to pull apart and had white or yellow vascular tissue were considered alive. Sorted roots were washed with tap water, dried at 60°C for 4 d, and weighed. An average root biomass per plot was calculated for live and dead fine roots.

Two root decomposition studies were carried out, one during the winter of 1997–1998 and another during the summer of 1998 For the winter decomposition experiment, live fine roots (<1 mm diameter) from the June 1997 collection were dried and pooled together within a plot. Subsamples of ~0.16-g dry mass were placed in 5 × 10 cm litter bags constructed using 0.2-mm fiberglass mesh. In September 1997, we installed at least four root litter bags in each plot by sliding the litter bag between two sharpened spatulas that had been pushed into the soil to a depth of 10 cm and carefully pried apart. Root litter bags were collected in March 1998 after 6 mo in the field. Roots were washed with tap water and oven dried for 3 d at 65°C. Root mass remaining was calculated as the percentage of mass present in March vs. the initial mass of the roots. For

FIG. 3. FACE (free-air CO_2 enrichment) site in loblolly pine forest, Durham, North Carolina. Each experimental plot is 30 m in diameter. Six plots are used in the replicated experiment, while the seventh plot (at upper left) is a prototype used to refine FACE technology. (Photo by Will Owens.)

the summer decomposition experiment, live fine roots collected on March 1998 were treated as above and placed in the field during May 1998 and collected during November 1998.

Soil CO_2 efflux

We measured soil respiration as soil surface CO_2 efflux using a soda lime method (Edwards 1982, Raich et al. 1990) on nine dates during 1997 (Fig. 4). PVC rings of 30.5 cm diameter and 15 cm in height were inserted through leaf litter and pressed 3 cm into soil during April 1996. These rings were left in place for the duration of the experiment. For each measurement, ~45 g of 8-mesh soda lime was dried at 100°C and weighed in a glass jar. The jars were capped tightly until placement inside a PVC ring, which was immediately covered with a reflective Plexiglas lid and sealed against an O-ring with Apiezon grease (M and I Materials Limited, Manchester, UK). The jars were retrieved ~24 h later and then dried and reweighed to determine the quantity of carbonate formed. These mass gains were then multiplied by 1.41 to correct for loss of water during the reaction of CO_2 with soda lime (Grogan 1998).

$\delta^{13}C$ and CO_2 in soil pore space

The CO_2 used to fumigate the FACE plots is derived from the combustion of natural gas. It carries a $\delta^{13}C$ of −44 ‰, so the ambient CO_2 in the fumigated plots has a ^{13}C isotopic ratio of −21‰, derived from 360 μL/L of ambient CO_2 with $\delta^{13}C$ of −8‰ and 200 μL/L of added CO_2 with $\delta^{13}C$ of −44‰. Photosynthesis discriminates against ^{13}C, producing organic matter with a mean $\delta^{13}C$ of −39.3 in FACE-treated leaves (Ellsworth 1999). We used this label to trace FACE derived C into CO_2 respired in the soil by microbes and roots. Soil gas-sampling ''wells'' were installed in each of the FACE plots at 30-cm depth. Each gas-sampling well consisted of a 5 cm diameter and 20 cm long PVC pipe buried vertically in the soil, open at the bottom and sealed at the top with a two-holed rubber stopper. Two 0.6 cm diameter Kynar plastic tubes (Elf Atochem North America, Incorporated, Philadelphia, Pennsylvania, USA) extended from the stopper to the soil surface, and the tops of the tubes were sealed with Kynar caps attached with stainless-steel Swagelok tube connectors (Swagelok, Solon, Ohio, USA) with nylon ferrules. Gas samples taken from the soil gas wells were collected in 75-cm^3 Whitey stainless-steel gas cylinders (Whitey Company, Highland Heights, Ohio, USA) that were sealed with Nupro bellows valves equipped with Kel-F stem tips (Nupro Company, Willoughby, Ohio, USA). The sample cylinders were pre-evacuated in the laboratory. When sampling the gas wells, the sample gas was first pulled through a portable stainless-steel manifold that was evacuated in the field and purged

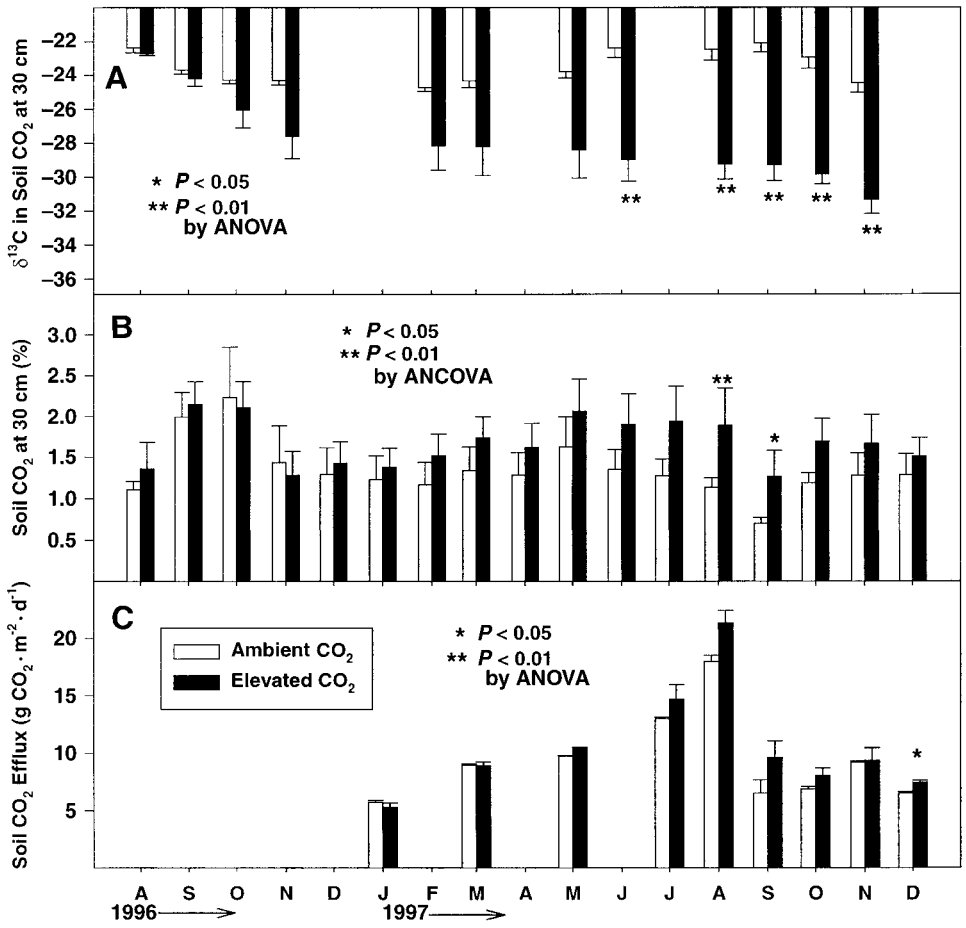

FIG. 4. Soil pore space CO_2 and $\delta^{13}C$ and soil surface CO_2 efflux in the three FACE plots (filled bars) and three control plots (open bars) in a loblolly pine forest. Data are means, and error bars show 1 SE ($n = 3$ plots per CO_2 treatment).

with two volumes of sample gas using a hand pump. Carbon dioxide was concentrated in the samples via cryogenic purification and vacuum distillation (Boutton 1991), and the $^{13}C/^{12}C$ ratio was determined by stable isotope ratio mass spectrometry (VGISOGAS series 2) at the Duke University Phytotron. All values are expressed as parts per thousand (‰) in delta notation (Craig 1953) where

$$\delta^{13}C(‰) = \left[\left(\frac{^{13}C/^{12}C \text{ sample}}{^{13}C/^{12}C \text{ standard}}\right) - 1\right] \times 1000.$$

The accepted standard is the Pee Dee Belemnite carbonate (Craig 1957).

At separate times, the concentration of CO_2 in the soil gas wells was measured using a field-portable infrared gas analyzer (IRGA, EGM-1, PP Systems Incorporated, Haverhill, Massachusetts, USA). The IRGA was placed in-line with the soil gas well and pumped gas from the well through a magnesium perchlorate water trap to remove water vapor before analysis for CO_2.

Potential net N mineralization and microbial biomass N

In late June and early July of 1996, we collected eight cores per plot to obtain baseline measurements of microbial biomass C and N. To quantify microbial biomass C and N during the FACE experiment, we collected 24 cores per plot (7.5 cm depth and 2 cm diameter) in June 1997, October 1997, April 1998, and June 1998. In June 1997, we created four composite samples per plot with six cores in each composite, whereas on subsequent dates we created a single, larger composite sample made up of 24 cores from each plot to accommodate soil requests from other investigators. We placed samples in an ice chest immediately after coring, and then stored them in a refrigerator ($\simeq$4°C) for up to two 2 d before processing. Before beginning analytical procedures, we removed stones and roots with forceps. In June 1997, we also added deionized water with a spray bottle to increase gravimetric soil moisture to ~30%. This treatment appeared to produce a heterogeneous distribution of soil moisture within

samples, and on subsequent dates, samples were left at field moisture. We estimated potential net N mineralization in duplicate subsamples from each composite by measuring concentrations of NO_3^- and NH_4^+ in 2 mol/L KCl extracts (TRAACS 800, Bran-Leubbe) obtained before and after a 30-d aerobic laboratory incubation at 22°C (Binkley and Hart 1989). We measured microbial biomass C and N using chloroform fumigation-extraction (Brookes et al. 1985, Vance et al. 1987, Gallardo and Schlesinger 1990). We converted chloroform-flush data to microbial biomass values by dividing N flushes by 0.54 (Joergensen and Mueller 1996) and C flushes by 0.45 (Joergensen 1996).

Statistical analyses

In all analyses, we averaged the multiple measurements made in each FACE or control plot so that $n = 3$ (three plots per CO_2 treatment). We used one-way analyses of variance (ANOVA) or repeated-measures ANOVA to test for the significance of differences among elevated and ambient CO_2 treatments (SAS Institute 1995). We used analysis of covariance (ANCOVA; SAS Institute 1995) to refine our tests for CO_2 effects on parameters for which suitable pretreatment or early-treatment data exist. Pretreatment measurements were conducted in late June and early July 1996 for microbial biomass C and N, and in early August 1996 for soil CO_2. Pretreatment and early-treatment measurements were made during June through December of 1996 for loblolly pine litterfall mass and during November 1996 for loblolly pine litterfall C:N ratio. Litter collected during late 1996, after FACE turn-on (27 August 1996), is an acceptable covariate because it grew for ~14 mo of its 18-mo life-span under ambient CO_2, and its mass and chemistry were unlikely to have been affected by CO_2 treatment. In fact, mass spectrometric analyses of 1996 litter show that $\sigma^{13}C$ in litter from FACE plots had not changed significantly to reflect the isotope signature of added CO_2 (A. C. Finzi, *unpublished data*), suggesting that nearly all C in these needles was fixed prior to elevated CO_2 treatment. In any case, use of a covariate that has been affected by the CO_2 treatment should yield a conservative test for a CO_2 effect with ANCOVA, since some portion of the variability in the response that would have been explained by CO_2 will instead be explained by the covariate. To meet a key assumption of ANCOVA, we tested the significance of the interaction between a covariate and CO_2 in each analysis before performing ANCOVA. This test was significant at $P < 0.05$ in only one case–the 4 November 1996 measurement of soil CO_2 when the interaction P value was 0.042 (Fig. 4).

Results and Discussion

Belowground carbon inputs, losses, and storage

We observed statistically significant increases in loblolly pine litterfall mass with elevated CO_2 during 1997 and 1998 (ANCOVA, $P = 0.042$ and 0.010, respectively, Fig. 5, Table 1). In 1997, loblolly pine litterfall mass increased only slightly, but by 1998, the first year in which most needles falling had originated under elevated CO_2, FACE treatment had increased loblolly pine litterfall mass by ~64 $g \cdot m^{-2} \cdot yr^{-1}$ (Table 1), which represents a 15% increase over the mean value of loblolly pine litterfall in ambient CO_2 plots. These results are consistent with increases in leaf-level photosynthesis observed in elevated CO_2 plots at this site (Ellsworth 1999). DeLucia et al. (1999) report 26% greater forest NPP under FACE conditions during 1998. Our results are also consistent with the increases in leaf mass observed in studies of tree saplings exposed to elevated CO_2 for three to four years (Norby et al. 1995, Johnson et al. 1997, Tissue et al. 1997).

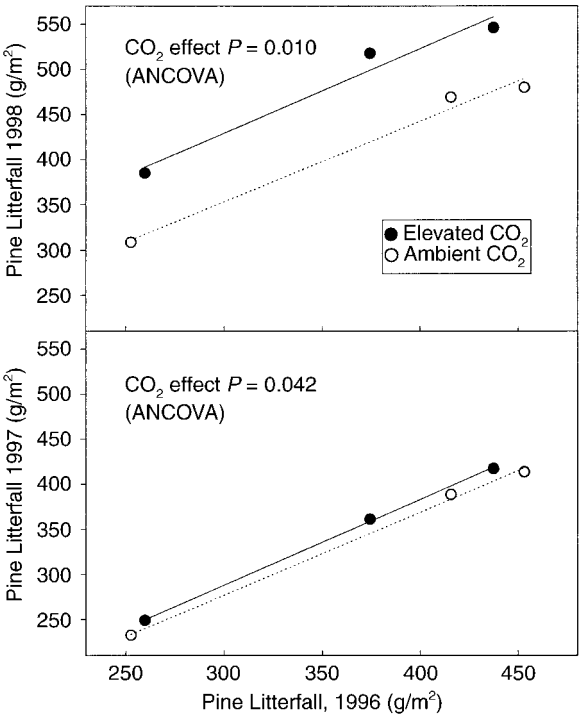

FIG. 5. Loblolly pine litterfall mass in FACE plots (filled circles) and ambient plots (open circles). Each point is derived from the mean of data from twelve 40 × 40 cm litterfall baskets in each plot. Each line is a simple linear regression fit to the three points in a CO_2 treatment. The P value given by ANCOVA tests the significance of the difference between the y intercepts of two parallel lines, with one line fit through each CO_2 treatment.

Matamala and Schlesinger (2000) found that fine root biomass increment increased significantly with elevated CO_2 (ANOVA, $P = 0.017$, Table 1). We found that fine root turnover (M + D) between November 1997 and November 1998 was also higher in FACE plots than in controls, but this difference was not statistically significant (ANOVA, $P = 0.223$, Table 1). These results are consistent with the increased fine root production found by Pregitzer et al. (1995).

TABLE 1. Changes in a loblolly pine forest exposed to free-air CO_2 enrichment (elevated plots) or ambient CO_2.

Variable	Date	Ambient CO_2 Mean (1 SE)	Elevated CO_2 Mean (1 SE)	*P* (ANOVA)	*P* (ANCOVA)
Pine litterfall mass (g/m^2)	Jun–Dec 1996	374 (62)	357 (52)	0.846	···
	1997 (whole year)	345 (57)	342 (49)	0.976	0.042
	1998 (whole year)	419 (55)	483 (50)	0.439	0.010
Loblolly pine litterfall C:N†	1996 (November)	142 (4)	153 (7)	0.243	···
	1998 (entire year)	106 (3)	96 (6)	0.202	0.186
Fine root production ($g \cdot m^{-2} \cdot yr^{-1}$)‡	Nov 1997–Nov 1998	238 (31)	325 (26)	0.099	···
Fine root biomass increment ($g \cdot m^{-2} \cdot yr^{-1}$)‡‖	Nov 1997–Nov 1998	42.8 (7.5)	80.0 (5.7)	0.017	···
Fine root turnover ($g \cdot m^{-1} \cdot yr^{-1}$)‡	Nov 1997–Nov 1998	195 (27)	245 (21)	0.223	···
Microbial biomass N (mg N/kg soil)§	(pre-FACE)	88.2 (14.3)	91.6 (4.4)	0.830	···
	Jun 1997	68.0 (7.0)	72.1 (6.6)	0.689	0.737
	Oct 1997	77.1 (8.4)	84.8 (9.2)	0.572	0.579
	Apr 1998	74.1 (11.5)	77.7 (6.2)	0.799	0.906
	Jun 1998	88.3 (9.9)	87.0 (5.8)	0.920	0.766
Microbial biomass C (mg N/kg soil)§	(pre-FACE)	571 (83)	621 (31)	0.606	···
	Jun 1997	517 (57)	502 (27)	0.834	0.764
	Oct 1997	523 (59)	592 (88)	0.552	0.740
	Apr 1998	601 (116)	611 (83)	0.946	0.690
	Jun 1998	620 (37)	572 (49)	0.486	0.554

Notes: Each observation is the mean of three plots with the standard error of the mean in parentheses. *P* values are from one-way ANOVA or ANCOVA with $n = 3$. ANCOVAs were conducted using 1996 pre-FACE data for microbial biomass, and 1996 pre- and early-treatment data for litterfall.

† Loblolly pine litter N was determined by Kjeldahl digestion. Litter C was determined by combustion in an elemental analyzer.

‡ Fine roots were those <1 mm diameter. Production was determined using a modification of the ''compartment-flow'' model of Santantonio and Grace (1987). Increment was determined by regressing biomass against time in each plot.

§ Fumigation-extraction method. Chloroform-labile N and C were divided by $K_{EN} = 0.54$ or $K_{EC} = 0.45$ to determine microbial biomass pools.

‖From Matamala and Schlesinger (2000).

After 14 mo of FACE treatment, the $\delta^{13}C$ of CO_2 in soil pore space had dropped to −31‰ in FACE plots (Fig. 4), showing that a substantial quantity of $\delta^{13}C$-depleted FACE CO_2 that was fixed during the experimental period had been transported belowground and appeared as CO_2 due to root or microbial respiration. This decline in $\delta^{13}C$ values cannot be explained by diffusion of FACE CO_2 downward into soil because CO_2 in aboveground air in FACE rings has a $\delta^{13}C$ of ~−21‰. The $\delta^{13}C$ values appear to diverge within 1 mo of beginning FACE treatment, and repeated-measures ANOVA shows a highly significant $CO_2 \times$ date interaction ($P < 0.0001$), although that trend does not produce statistically significant comparisons between elevated and ambient CO_2 plots until June 1997 (ANOVA, $P = 0.009$, Fig. 4A).

Soil CO_2 at 30-cm depth was significantly higher under FACE in August and September 1997 (ANCOVA, $P = 0.007$ and 0.016, respectively, Fig. 4B), and a repeated-measures ANOVA using all dates after the FACE treatment began shows a significant $CO_2 \times$ date interaction ($P = 0.041$). This difference cannot be explained by diffusion of FACE CO_2 downward through the soil profile, since the 200 μL/L CO_2 elevation in high-CO_2 plots is insignificant relative to the typical concentrations of 20 000 μL/L (2%) seen at 30-cm depth. Soil CO_2 efflux was significantly higher in the FACE plots than in the ambient CO_2 plots only during December 1997 (ANOVA, $P = 0.044$, Fig. 4C). Repeated-measures ANOVA suggests there was no $CO_2 \times$ date interaction, but there was a marginally significant main effect of CO_2 on soil CO_2 efflux ($P = 0.084$).

Our observation of increased soil CO_2 and the trend toward higher soil CO_2 efflux may indicate an increase in carbon allocation to belowground pools that turn over quickly. Such an effect would be consistent with the results of Hungate et al. (1997*b*), who found that increased plant production in open-top chambers was accompanied by a large increase in soil respiration in annual grasslands. Measurements of soil C fluxes in their open-top chambers showed that elevated CO_2 increased root respiration and inputs to soil pools with short residence times, suggesting that elevated CO_2 may have increased rates of soil C cycling with little effect on soil C storage (Hungate et al. 1997*b*).

Increases in soil CO_2 in this FACE study during 1997 are likely due to changes in the rate of C flux to the belowground system via roots. The increase in loblolly pine litterfall observed during 1997 was small, and little of this litter was likely to have decomposed during 1997, because the peak loblolly pine litterfall period occurs in late October and early November. Accelerated CO_2 production belowground may have been the result of increased root metabolism, increased C use by mycorrhizae, or increased microbial decomposition of fine roots, root cortical cells, or root exudates. We expect

further increases in soil CO_2 efflux when the bulk of 1998 litter decomposes.

Nutrient cycling and nutrient availability

Loblolly pine leaf litter C:N ratio was unchanged by elevated CO_2 (ANCOVA, $P = 0.186$, Table 1) during 1998. The absence of a treatment effect on litter C:N in this loblolly pine forest is consistent with the results of open-top chamber studies with unrestricted soil volumes that have found few significant effects of elevated CO_2 on litter chemistry (e.g., O'Neill and Norby 1996). Effects of elevated CO_2 on litter chemistry vary with plant species (Franck et al. 1997). In some studies, elevated CO_2 has increased the C:N and lignin:N ratios of litter and decreased the rate of litter decomposition (Cotrufo et al. 1994), while in other cases, elevated CO_2 has had no effect on litter chemistry or decomposition (O'Neill and Norby 1996).

At the Duke Forest FACE site, elevated CO_2 caused statistically significant increases in loblolly pine litterfall mass (Table 1) and in aboveground NPP (DeLucia et al. 1999), but it caused only small changes in fine root production (i.e., the sum of fine root biomass increment, mortality, and decomposition; Table 1). However, Matamala and Schlesinger (2000) found increased fine root biomass increment in elevated CO_2 plots by November 1998. Their result is consistent with that of Johnson et al. (1996), who found increased total biomass of *Pinus ponderosa* seedlings grown in open-top chambers at high CO_2, even though nitrogen appeared to limit plant growth. Johnson et al. (1996) suggested that increased root biomass under elevated CO_2 allowed greater exploration for soil nutrients.

In this FACE experiment, microbial biomass C and N did not differ between CO_2 treatments on any sampling date (ANCOVA, $P > 0.5$ in all cases, Table 1). Repeated-measures ANOVA corroborates the results of the ANCOVAs and shows no main effect of elevated CO_2 on microbial biomass C or N or an interaction with time ($P > 0.5$). Potential net N mineralization was also unaffected by elevated CO_2 (Fig. 6, and repeated measures ANOVA $P > 0.5$). Results from other experiments suggest that effects of elevated CO_2 on N availability may vary among species and ecosystems (Zak et al, 1993 Berntson and Bazzaz 1998). Our lack of a CO_2 effect on microbial biomass and net N mineralization is not consistent with the results of Díaz et al. (1993), who found >80% increases in microbial biomass with elevated CO_2, or with the results of Zak et al. (1993), who found both increased microbial biomass and increased net N mineralization under elevated CO_2. Our results for these parameters, which extend through the first 21 mo of FACE treatment, are short-term relative to the dynamics of pine needle growth, senescence, and litterfall, but they are long term relative to the studies by Díaz et al. (1993, up to 112 d) and Zak et al. (1993, 152 d).

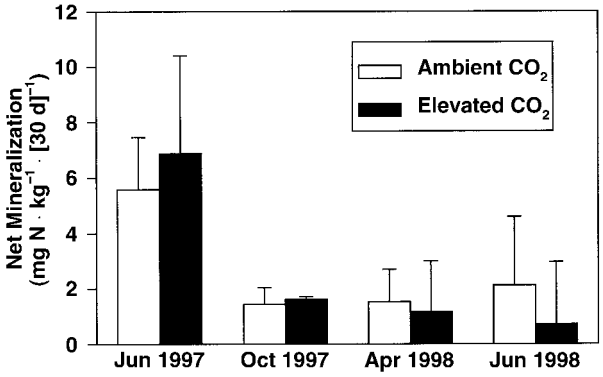

FIG. 6. Potential net N mineralization in FACE plots (filled bars) and control plots (open bars). Error bars show 1 SE ($n = 3$ plots per CO_2 treatment). Samples were incubated in the laboratory for 30 d at ~22°C. Soil moisture was adjusted to ~30% gravimetric water content in June 1997 and was left at field moisture on other dates. On each date, we collected 24 cores (2 cm diameter and 7.5 cm depth) per plot. In June, we incubated subsamples from four six-core composites, while on other dates we incubated subsamples from a single 24-core composite sample.

In the present experiment, the majority of loblolly pine needles that originated under elevated CO_2 did not fall until autumn 1998, and little of the additional litterfall mass we observed under elevated CO_2 could have become available to soil decomposers prior to our measurements of microbial biomass and net N mineralization. Because microbial biomass and net N mineralization were not significantly affected by elevated CO_2, we suspect that elevated CO_2 did not increase the availability of easily decomposable organic matter derived from fine roots (Fig. 2). The increased soil CO_2 we observed was probably due to increased respiration by roots and microbes closely associated with roots. At this time, we cannot rule out the possibility of increased soil C storage, which will be examined at 5 yr intervals during this FACE experiment.

CONCLUSIONS

The Duke Forest FACE study reveals significant increases in loblolly pine leaf litterfall mass and fine root biomass increment with elevated CO_2. These changes are consistent with increased photosynthetic rates and greater stem growth observed at this site. We did not detect significant changes in litterfall chemistry or fine root turnover. Soil CO_2 at 30-cm depth increased on some sampling dates, and soil CO_2 efflux showed marginally significant increases with elevated CO_2. These data, combined with the rapid decline in $\delta^{13}CO_2$ in FACE plots, suggest that plants allocated large quantities of C to root metabolism and to the metabolism of root-associated microbes such as mycorrhizae, and that these fluxes increased with elevated CO_2.

Elevated CO_2 did not significantly alter microbial biomass or potential net N mineralization. This result suggests that elevated CO_2 had little effect on inputs

of labile organic matter by roots. However, our data do not preclude the possibility that elevated CO_2 stimulated fluxes into larger organic matter pools that turn over slowly.

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