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FOREST LITTER PRODUCTION, CHEMISTRY, AND DECOMPOSITION FOLLOWING TWO YEARS OF FREE-AIR CO_2 ENRICHMENT

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Abstract. Increases in tree biomass may be an important sink for CO_2 as the atmospheric concentration continues to increase. Tree growth in temperate forests is often limited by the availability of soil nutrients. To assess whether soil nutrient limitation will constrain forest productivity under high atmospheric CO_2, we studied the changes in forest litter production and nutrient cycling in a maturing southern U.S. loblolly pine–hardwood forest during two years of free-air CO_2 enrichment. The objective of this paper is to present data on the chemistry of green leaves and leaf litter, nutrient-retranslocation efficiency, aboveground litter production, whole-system nutrient-use efficiency, decomposition, and N availability in response to forest growth under elevated CO_2.

The chemical composition of green leaves and leaf litter was largely unaffected by elevated CO_2. Green-leaf nitrogen (N) and phosphorus (P) concentrations were not significantly lower under elevated CO_2. N and P retranslocation from green leaves did not increase under elevated CO_2; therefore, leaf litter N and P concentrations were not significantly lower under elevated CO_2. The concentrations of carbon, lignin, and total nonstructural carbohydrates in litter were not significantly different under elevated CO_2.

Total aboveground litterfall increased significantly with CO_2 fumigation. The increase in litterfall was due to significant increases in loblolly pine leaf litter and bark production. The mass of leaves from deciduous species did not increase with CO_2 fumigation. Whole-system nutrient-use efficiency (aboveground litterfall/nutrient content of litterfall) did not increase as a consequence of forest growth under elevated CO_2, but N and P fluxes from vegetation to the forest floor increased significantly. During the second year of CO_2 fumigation, the flux of N and P to the forest floor in litterfall increased by 20% and 34%, respectively.

The rate of mass loss during one year of decomposition was unaffected by ''litter type'' (whether the litter was produced under ambient or elevated CO_2), nor by the ''site'' of decomposition (whether the litter was decomposed in the ambient or elevated CO_2 plots). N was immobilized in litter during decomposition, whereas P was mineralized. There was no consistent effect of litter type or site on nutrient dynamics in decomposing litter.

There was no significant effect of elevated CO_2 on the pool size of inorganic N (NH_4^+ and NO_3^-) in the top 7.5 cm of mineral soil. The rate of net N mineralization and nitrification in mineral soil was not significantly different between treatment and control plots.

Identifying the source of the nutrients lost in litterfall is critical to the long-term potential growth stimulation of forests under elevated CO_2. If the nutrients lost from biomass come from storage (e.g., the movement of nutrients from wood to leaves), then the increase in litter production should decrease over time as slowly replenished nutrient reserves are exhausted. If the nutrients lost in plant litter are replaced by uptake from soils, then it is possible (1) that trees acquire soil nutrients at a rate commensurate with growth stimulated by elevated CO_2; and (2) that forest productivity will be stimulated by elevated CO_2 in the long term.

Key words: carbon dioxide, effects of elevated levels; decomposition; FACE (free-air CO_2 enrichment); forest, southern United States; litter; nitrogen; nutrient cycling; nutrient-use efficiency; phosphorus; Pinus taeda; *retranslocation efficiency.*

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Introduction

The atmospheric concentration of CO_2 (herein abbreviated "$[CO_2]_a$") has increased from ~280 μL/L to ~360 μL/L during the past 200 yr (Keeling and Whorf 1994), largely due to global increases in fossil-fuel combustion and deforestation (Schlesinger 1997). Atmospheric CO_2 concentrations are projected to double during the next century (IPCC 1996). Plant biomass increases in direct response to growth at elevated CO_2 (e.g., Bazzaz and Miao 1993, Tissue et al. 1997), and increases in forest productivity are anticipated with the projected increase in $[CO_2]_a$ (Schimel et al. 1995), notably in mid-latitude ecosystems (e.g., Fan et al. 1998).

There may be a fundamental constraint on forest uptake of $[CO_2]_a$ as the concentration rises; forest productivity in most temperate ecosystems is limited by the availability of soil nutrients—notably nitrogen (N) and to a lesser extent phosphorus (P) (Mitchell and Chandler 1939, Vitousek and Howarth 1991, Reich et al. 1997, Fahey et al. 1998). Simulation experiments suggest that initial increases in forest growth and NPP in response to a doubling of $[CO_2]_a$ will exceed the rate of nutrient supply from soils, causing tree growth and NPP to decline to near original levels within a few years of the doubling of $[CO_2]_a$ (e.g., Comins and McMurtrie 1993, Luo and Reynolds 1999). Consequently, many simulation models suggest that tree biomass will likely be a relatively small sink for $[CO_2]_a$ (DeLucia et al. 1999).

The mechanistic basis for little or no effect of elevated CO_2 on forest productivity involves a negative feedback (Melillo 1983, Strain and Bazzaz 1983). The "negative-feedback" hypothesis (Fig. 1A) predicts that initial increases in plant growth and ecosystem productivity under elevated CO_2 are associated with an increase in nutrient-use efficiency (NUE). The increase in NUE, however, decreases litter nutrient concentrations and increases litter C concentrations (e.g., lignin; Cotrufo et al. 1998, Coûteaux et al. 1999). Because the initial rate of litter decomposition is negatively correlated with the concentration of C compounds and the C-to-nutrient ratio of litter, the mineralization rate and availability of soil nutrients for plant growth declines. Thus in the negative-feedback model there is little or no effect of elevated CO_2 on NPP because increases in NPP under elevated CO_2 are not matched by a concurrent increase in soil nutrient availability (Fig. 1A). In this conceptual model, productivity is stimulated only by an increase in the exogenous input of N (e.g., through enhanced atmospheric N deposition [Townsend et al. 1996, Holland et al. 1997] or N fixation).

The negative-feedback hypothesis contrasts with the view that an increase in the availability of one resource can be allocated to the acquisition of other limiting resources (cf. Tilman 1982). For example, fertilization with N and P increases the uptake of C by increasing the rate of photosynthesis relative to respiration (Brooks and Farquhar 1985, Pearcy et al. 1987, Drake et al. 1997). In contrast to the negative-feedback hypothesis, the "CO_2-fertilization" hypothesis (Fig. 1B) argues that soil nutrient limitations will not entirely offset the effects of elevated CO_2 on forest productivity because additional C is allocated belowground, stimulating soil nutrient availability and plant nutrient acquisition (Fig. 1B). As a modification to the hypothesis originally proposed by Zak et al. (1993), the CO_2-fertilization hypothesis argues that belowground C allocation increases fine-root production (Rogers et al. 1994), root nutrient-uptake capacity (BassiriRad et al. 1996), the number of mycorrhizal infections (Stulen and den Hertog 1993, Staddon et al. 1999), the amount of labile C deposited in the rhizosphere (Cheng 1999), and the production of extracellular enzymes and organic acids (DeLucia et al. 1997). Increases in labile C inputs to soils increase the size, turnover, and activity of microbes, thereby increasing the quantity of organic matter that is decomposed. Greater plant C allocation belowground increases the volume of soil explored by tree roots and the quantity of nutrients acquired by trees in competition with soil microbes (Johnson and Ball 1996). As the forest grows under elevated CO_2, the fluxes of N and P in vegetation, microbes, and soils increase and a positive feedback develops between elevated CO_2, NPP, and plant nutrient availability.

It is difficult to compare the evidence supporting the negative-feedback and CO_2-fertilization hypotheses in forest ecosystems, in part because there are few data on the response of entire forest ecosystems to elevated $[CO_2]_a$. A large body of literature on the growth responses of woody seedlings and saplings to elevated CO_2 in greenhouses, growth chambers, open-top chambers, and forests surrounding CO_2 springs shows large increases in the rate of photosynthesis and the accumulation of biomass in response to elevated CO_2 within one to several years of enrichment (Hattenschwiler et al. 1997, Curtis and Wang 1998). With regard to changes in tissue chemistry and plant nutrient availability, some studies support the negative-feedback hypothesis while other, very similar experiments support the CO_2-fertilization hypothesis (see reviews by O'Neill and Norby [1996], Cotrufo et al. [1998], Curtis and Wang [1998], Gahrooee [1998], Norby and Cotrufo [1998], and Couteaux et al. [1999]).

In this study, we use a fully replicated field study to understand the relationship between forest productivity and nutrient cycling in a maturing southern United States loblolly pine–hardwood forest exposed to two years of free-air CO_2 enrichment ("FACE"; Hendrey et al. 1999). The primary objective of this paper is to present data on system-level responses to elevated CO_2. These include changes in foliar chemistry, nutrient-retranslocation and nutrient-use efficiency, aboveground litter production, decomposition, and N availability. We focus on aboveground litter dynamics because litterfall represents the largest pathway of nutri-

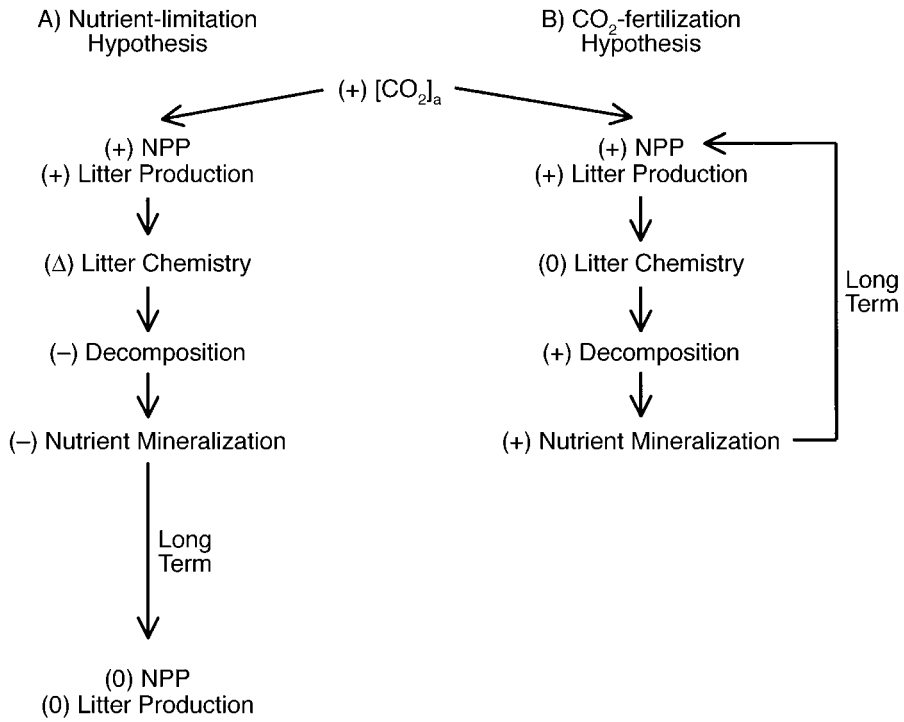

FIG. 1. A diagrammatic representation of the effect of CO_2 fertilization on net primary production (NPP) and nutrient cycling. (A) The nutrient-limitation hypothesis. (B) The CO_2-fertilization hypothesis. See *Introduction* for details. The ''+'' symbol indicates a positive effect, the ''△'' indicates a change, the ''0'' symbol indicates no change, and the ''−'' symbol indicates a decrease; $[CO_2]_a$ = atmospheric concentration of CO_2.

ent transfer between vegetation, microbes, and soils in this ecosystem at this point in time (DeLucia et al. 1999). Changes in any of these parameters in response to forest production under elevated CO_2 will likely affect long-term patterns of nutrient cycling and thus the response of this forest ecosystem to increased $[CO_2]_a$ (Fig. 1). A secondary objective of this study is to examine how different plant species respond to elevated CO_2 in terms of foliar chemistry, nutrient-retranslocation efficiency, and decomposition.

METHODS AND MATERIALS

Site, vegetation, and soils

Each study plot at the FACE (free-air CO_2 enrichment) facility is surrounded by a 34-m-diameter plenum for distributing air to 16 aluminum towers forming a 30-m-diameter circle inside the plenum. Each tower, which can be extended as the forest grows in height, supports two vertical, PVC (polyvinyl chloride) vent pipes with outlets from the ground to the canopy at ≥50-cm intervals. In the three CO_2-enriched plots, CO_2 is maintained at 200 μL/L above ambient (360 μL/L). The three control plots receive the same volume of air (without added CO_2) to replicate any potential micrometeorological effects of free-air operation on the forest ecosystem. Over most of the forest volume, the observed concentration is within 10% of the nominal +200 μL/L enrichment (Hendrey et al. 1999). CO_2 fertilization began on 27 August 1996.

The site is recovering from abandonment in 1983, when a previous forest was cut, tree boles removed, and branches burned. The loblolly pine canopy is currently 15 m tall. The overstory is dominated by loblolly pine (*Pinus taeda*) comprising 98% of tree basal area, and, to a much lesser extent, sweet gum (*Liquidambar styraciflua*). The understory sapling layer is dominated by red maple (*Acer rubrum*), red bud (*Cercis canadensis*), and dogwood (*Cornus florida*). The seedling and herbaceous layer has >50 species. Structurally, the forest is entering the ''thinning phase'' of stand development, when competition for light and soil resources is considered intense (Peet 1992).

The topography of the 90-ha site in which the six FACE plots are located is relatively flat. There is a 15-m elevational gradient between the highest and lowest plots. The soils are classified as Ultic Alfisols of the Enon Series. The soils are very deep (>15m), highly weathered, and derived from igneous parent materials. Soils are moderately acidic (pH $CaCl_2$=5.0).

Green-leaf chemistry

We measured the concentration of N, P, total nonstructural carbohydrates (TNC), and specific-leaf mass on fully expanded, green leaves. We made these mea-

surements on loblolly pine and four understory hardwood species—red bud, red maple, sweet gum, and dogwood. We chose these hardwood species because they are among the most abundant and they are representative of the mixture of deciduous litter collected in the litter baskets. Each species occurs in each of the six FACE plots with the exception of red bud, which is absent in one of the ambient-CO_2 plots.

The longevity of loblolly pine foliage in the Piedmont of North Carolina (USA) is 19 mo (Zhang and Allen 1996) so that at any time there are needles of two different ages on a single branch (i.e., two cohorts). We collected foliage samples from both needle cohorts—those produced in the current year and those produced in the previous year—at three heights within the canopy—the bottom 25%, the middle 50%, and the top 25% of the crown. Our samples were collected above a randomly selected location on each arm of a boardwalk that extends through each FACE ring to the north, south, east, and west. At each of these locations and heights we sampled a single branch on each of four trees and collected 5–8 fascicles from each flush of each cohort along a primary branch. Foliage samples were collected in October 1996, September 1997, and September 1998. For simplicity, we refer to each cohort of foliage as ''new'' or ''old'' depending upon whether the cohort was produced during the current calendar year (new) or in the previous calendar year (old). We calculated the average concentration of N and P in each cohort by taking the average of the N and P concentration in the bottom, middle, and top of the canopy.

We collected green foliage samples for the hardwood species in late July 1997 and early August 1998. At those points in the growing season, leaves were fully expanded and mature. We measured green chemistry at leaf maturity to avoid unusually high nutrient concentrations in rapidly expanding, young leaves (Chapin and Kedrowski 1983). We sampled approximately three leaves from 10–20 different trees of each species within each ring. The leaves of a given species that were collected within a ring were placed into a single bag, oven dried at 65°C for 4 d, ground, and analyzed for chemical constituents.

We measured the N and P concentration of green leaves in a sulfuric–salicylic acid Kjeldahl digestion (Lowther 1980) followed by colorimetric analysis on an automated ion analyzer (TRAACS 800 Autoanalyzer [Bran + Leubbe, Buffalo Grove, Illinois, USA]).

We measured the concentration of TNC (starch, glucose, sucrose, and fructose) in green leaves and leaf litter colorimetrically using a modification of the Dubois et al. (1956) method. In brief, ground samples were extracted three times with a methanol:chloroform:water (12:5:3 volume to volume) solution. We then added perchloric acid and determined the quantity of solubilized sugars and starches colorimetrically using the phenol–sulfuric acid method.

Leaf area was measured on a leaf-area meter (LI-COR model 3100, LI-COR, Lincoln, Nebraska). The area of each leaf was determined from the average of three replicate measurements. Each leaf was dried at 60°C for 5 d and weighed. Specific leaf mass was calculated as the ratio of leaf mass and leaf area (in grams per square centimeter).

Leaf litter production and chemistry

We collected aboveground litterfall from 5 June 1996 through 31 December 1998 by placing 12 replicate 40 $\times$ 40 cm baskets in each plot. Litterfall was collected once per month between January and August and twice per month between September and December to avoid leaching leaf litter during the period of peak litterfall. The samples were brought back to the laboratory, dried at 65°C for 4 d, and weighed. After each sample was weighed, the contents of each of the 12 bags taken from within a single ring were composited into a single, larger sample. The composited sample was sorted into seven categories: pine needles, deciduous leaves, pine branches, deciduous branches, reproductive structures, bark, and other. The ''other'' category consisted of small, difficult-to-identify fragments of aboveground litterfall and frass. We then weighed each of the seven components separately.

Between 15 October and 15 November of each year we collected recently abscised leaves from the top of the forest floor once a week. We distinguished freshly fallen litter from older litter based on foliage color (brightly or lightly colored litter was considered fresh) and friability (friable litter was considered old). This litter was used in the decomposition experiment and was only air dried (see *Decomposition*, below).

We measured the concentration of C in litter by combustion in an elemental analyzer (NA1500 Series 1 [Carlo Erba Instruments, Milan, Italy]). The concentrations of N, P, and TNC in litter were measured with the same protocols as outlined for green-leaf chemistry.

We measured the ratio of $^{13}C/^{12}C$ (referred to as ''$\delta^{13}C$'') of loblolly pine needles and deciduous leaf litter on a SIRA series II isotope ratio mass spectrometer (Micromass, Manchester, UK) operated in automatic-trapping mode after combustion (DUMAS combustion) of a sample in an element analyzer (NA1500 Series 1 [Carlo Erba Instruments, Milan, Italy]). The reference CO_2, calibrated against standard Pee Dee belemnite, (Pee Dee Formation, South Carolina, USA) was obtained from Oztech (Dallas, Texas, USA). The accepted accuracy of ^{13}C with this analysis procedure is $\pm$0.1‰.

We measured the lignin concentration in litter using a modified version of an acetyl-bromide extraction procedure (Ilyama and Wallis 1990). We placed 15 mg of each ground sample into a 20 $\times$ 150 mm test tube. To each test tube we added 10 mL of distilled water and then heated the test tubes for 1 h at 65°C, stirring each 10 min. We then filtered each sample through a Whatman GF/A glass fiber filter (Whatman Incorporated,

Clifton, New Jersey) and rinsed each filter 3 times with 5 mL of cold water, 5 mL of ethanol, 5 mL of acetone, and 5 mL of diethyl ether, in that order. We placed each filter disc in a 20-mL glass scintillation vial (uncapped) and placed the scintillation vials in a drying oven at 70°C, overnight. The following morning, we added 2.5 mL of 25% (mass to mass) acetyl bromide in acetic acid and 100 μL of perchloric acid to each glass scintillation vial. We capped the vials and placed them in an oven for 30 min at 70°C, swirling every 10 min. We cooled the samples in a refrigerator for 1 h, and then transferred the solutions and the plant material on the filter disc to 50-mL volumetric flasks by rinsing with acetic acid. To each 50-mL volumetric flask, we added 10 mL of 2 moL/L sodium hydroxide (NaOH) and brought each flask to volume with acetic acid. The volumetric flasks were capped with glass stoppers and allowed to settle overnight. The following morning, we measured sample absorbance at 280 nm on a spectrometer (Hitachi U-2000 [Hitachi Instruments, San Jose, California, USA]). We used a National Bureau of Standards Pine sample as our reference material.

We calculated retranslocation efficiency on a mass basis as

$$\text{N or P retranslocation efficiency(\%)} = \frac{(\text{Green leaf[N, P]} - (\text{Leaf litter[N, P]})}{\text{Green leaf[N, P]}}. \quad (1)$$

We calculated nutrient-use efficiency according to Vitousek (1982):

$$\text{Nutrient-use efficiency} = \frac{\text{Litterfall mass } (\text{g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1})}{\text{N or P content in litterfall } (\text{g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1})}. \quad (2)$$

Decomposition

For each species, we composited the air-dried leaf litter from the three treatment and control plots into two larger samples based on whether the litter was produced under ambient CO_2 or elevated CO_2. This material was used to fill litterbags with a 2.0 ± 0.1 g sample of loblolly pine, sweet gum, flowering dogwood, red maple, or red bud litter. The litterbags were 10 × 10 cm with 1-mm mesh openings on top and 0.1-mm openings on the bottom.

Decomposition was followed for 1 yr from November 1997 to November 1998, in a reciprocal-transplant field study. We randomly allocated the litterbags of each species and litter type to each CO_2 treatment. Thus, for each species the litter produced under ambient CO_2 was decomposed in the ambient and elevated CO_2 plots, and the same for litter produced under elevated CO_2. We placed the litterbags beneath the freshly fallen litter. We collected litterbags from the field following 4, 8, and 12 mo of decomposition. This procedure generated a total of 360 litterbags (2 litter types × 2 sites of decomposition × 3 replicate plots per CO_2 treatment × 5 species × 3 harvest dates × 2 replicates per harvest).

At each sampling period we measured the remaining mass and the N and P content of litter. The handling of the litter and its chemical analysis was similar to that described in the previous section on foliar chemistry. We calculated the rate of mass loss as the difference between the mass of litter initially present in the litterbag and the mass of the remaining litter on a given harvest date divided by the amount of litter initially present. We used the full year of decomposition data to calculate the exponential decay coefficient k as outlined in Olson (1963). Decay coefficients were calculated according to:

$$X_t = X_0 e^{-kt} \quad (3)$$

where X_t is the mass of litter remaining at time, t, X_0 is the amount of litter initially present, and k is the exponential decay coefficient.

Soil nitrogen

We collected 24 cores per plot (7.5 cm depth and 2 cm diameter) in June and October 1997 and in April, June, August, and October 1998. In June 1997 we created four composite samples per plot with 6 cores in each composite, whereas on subsequent dates we created a single, larger composite sample made up of all 24 cores from each plot. We placed samples in an ice chest immediately after coring, and then stored them in a refrigerator (~4°C) overnight before processing. Before beginning analytical procedures we removed stones and roots with forceps. In June 1997 we also added de-ionized 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 of each composite by measuring concentrations of NO_3^- and NH_4^+ in 2 mol KCl/L extracts (TRAACS 800 [Bran + Leubbe]) obtained before and after a 30-d aerobic laboratory incubation at 22°C (Binkley and Hart 1989).

Data Analysis

We used four different statistical tests to analyze our data. These were: (1) split-plot ANOVA and one-way ANOVA for green-leaf chemistry, leaf-litter chemistry, and nutrient-retranslocation efficiency; (2) analysis-of-covariance (ANCOVA) for litter production, nutrient fluxes in litter, and nutrient-use efficiency; and (3) repeated-measures ANOVA for decomposition and N availability. Below we outline the rationale for each of the different statistical approaches.

Foliar chemistry

We analyzed our data on foliar chemistry (i.e., green-leaf chemistry, leaf-litter chemistry, and retransloca-

tion efficiency) using split-plot ANOVA (Underwood 1997). Both CO_2 treatment (two levels) and Species identity (five levels) are independent, fixed effects. The six replicate plots are a randomly sampled factor nested in each CO_2 treatment because the three replicate plots were randomly assigned a CO_2 treatment. However, the plots nested in each level of CO_2 are independent of Species because each level of Species is present in each plot. There are a total of 30 degrees of freedom to allocate in this statistical model (2 CO_2 levels $\times$ 3 replicates $\times$ 5 species). For green tissue chemistry we assumed that the two different loblolly pine needle cohorts were independent samples analogous to different species and thus there was a total of 36 df.

We tested the main effect of elevated CO_2 on foliar chemistry with the plots-nested-with-CO_2-treatment term as the error term. We tested for differences among Species in foliar chemistry using the species $\times$ plot-nested-with-CO_2 treatment as the error term. We tested for a CO_2 $\times$ Species interaction with the species $\times$ plot-nested-with-CO_2 treatment as the denominator in the F statistic (Underwood 1997). When the CO_2 $\times$ Species interaction term was significant, we performed one-way ANOVA to determine which species responded significantly to elevated CO_2.

Litter production, nutrient fluxes, and nutrient-use efficiency

In our preliminary analysis of the 1996 data we found considerable ring-to-ring variation in litterfall mass, N and P concentrations, and N and P fluxes in litterfall. This underlying between-ring variation often masked the effects of elevated CO_2 on litter production and chemistry following CO_2 fumigation in 1997 and 1998. We therefore used analysis of covariance (ANCOVA) using the 1996 pretreatment data as the covariate in our tests for the effects of elevated CO_2 on litter production and chemistry (Underwood 1997). In the models with homogeneous slopes, we reported the mean differences among treatments using the least-squares adjusted means and the pooled standard error of the mean. Least-squares means are advantageous because they take into account the preexisting variation among experimental units and are therefore the best unbiased estimate of the treatment effect (Underwood 1997).

We considered the 1996 litterfall data pretreatment data despite the start of CO_2 fumigation on 27 August 1996. We reasoned that at this point in the growing season the elevated CO_2 was unlikely to have affected litter production or chemistry in the 1996 calendar year. We verified this assumption using a $\delta^{13}C$ tracer. The CO_2 in the fumigated rings is depleted in ^{13}C with a $\delta^{13}C$ ratio of $\sim -20.5‰$. The $\delta^{13}C$ values in the loblolly pine and deciduous leaf litter from the ambient and elevated CO_2 plots in 1996 were not significantly different from one another (Table 1) supporting the idea that the leaves that fell in 1996 assimilated little or no new C after 27 August 1996. In contrast, the $\delta^{13}C$ values for litter produced under elevated CO_2 in 1997 and 1998 were significantly depleted in ^{13}C ($-33‰$ to $-41‰$), demonstrating that plants assimilated the CO_2 during the two full years following the onset of CO_2 fumigation (Table 1).

TABLE 1. The $\delta^{13}C$ content of loblolly pine needles and deciduous leaves under ambient and elevated CO_2.

Species	Year	$\delta^{13}C$ (‰) Ambient	$\delta^{13}C$ (‰) Elevated	$F_{1,4}$	P
Loblolly	1996	−29.67 (0.21)	−29.87 (0.07)	0.72	NS
	1997	−29.11 (0.44)	−33.03 (0.21)	64.04	<0.01
	1998	−28.99 (0.26)	−41.42 (0.44)	592.93	<0.0001
Deciduous	1996	−30.50 (0.10)	−31.04 (0.56)	0.94	NS
	1997	−29.67 (0.11)	−41.66 (0.58)	419.94	<0.0001
	1998	−30.19 (0.35)	−41.71 (0.31)	614.49	<0.0001

Note: The data are means with 1 SE in parentheses below (n = 3, ambient and elevated).

Decomposition and soil nitrogen

The decomposition study was designed as a reciprocal-transplant study. We tested for differences in mass loss and N and P dynamics as a function of (1) litter type—whether the litter was produced under ambient or elevated CO_2; (2) the site of decomposition—whether the litter was decomposed in the plots receiving ambient or elevated CO_2; and (3) the interaction between litter type and site of decomposition.

We analyzed the data using repeated-measures ANOVA (SAS Institute 1990). First we looked for broad patterns in mass loss and N and P dynamics by averaging across all species. Each data point in each plot at each harvest in this analysis was the average of 10 litter decomposition bags (5 species $\times$ 2 replicates per harvest). Second we examined species responses individually. Each data point in each plot was the average of the two replicate litter decomposition bags (2 replicate bags per species per harvest).

The data on extractable NH_4^+ and NO_3^- and the rate of net mineralization and nitrification were averaged across the sampling dates within a given calendar year. We tested for significant differences between ambient and elevated CO_2 with one-way ANOVA (SAS Institute 1990).

RESULTS

Foliar chemistry and nutrient retranslocation efficiency

Species differed significantly from one another in green-leaf TNC (total nonstructural carbohydrates) concentrations, specific-leaf mass (SLM), and N and P concentrations (Appendix A). In contrast, growth under

TABLE 2. The nitrogen and phosphorus concentration of leaves and the nutrient retranslocation efficiency (mass basis) of individual species under ambient (A) or elevated (E) CO_2.

	Green leaves				Leaf litter				Retranslocation efficiency (%)			
	[N] (mg/g)		[P] (mg/g)		[N] (mg/g)		[P] (mg/g)		Nitrogen		Phosphorus	
Species	A	E	A	E	A	E	A	E	A	E	A	E
1997												
Red bud	23.6	20.9	1.30	1.08	10.1	10.0	0.50	0.66	56.7	52.0	**61.7**	**38.9***
	(2.9)	(0.5)	(0.22)	(0.01)	(0.5)	(1.2)	(0.10)	(0.06)	(3.1)	(7.0)	(1.2)	(5.6)
Dogwood	15.1	14.0	1.07	0.97	9.0	6.7	0.65	0.63	40.2	52.4	39.5	37.3
	(0.7)	(0.2)	(0.12)	(0.11)	(0.2)	(0.4)	(0.11)	(0.17)	(4.0)	(3.1)	(5.1)	(10.8)
Red maple	NA		NA		7.3	6.6	0.59	0.57	NA		NA	
					(0.3)	(0.3)	(0.14)	(0.12)				
Sweet gum	NA		NA		6.7	6.3	0.54	0.66	NA		NA	
					(0.3)	(0.4)	(0.09)	(0.09)				
Loblolly pine												
New	11.5	11.1	1.00	1.00	…	…	…	…				
	(0.4)	(0.1)	(0.07)	(0.06)								
Old	9.3	8.7	0.77	0.83	4.7	4.9	0.36	0.39	55.1	57.3	65.0	64.1
	(0.2)	(0.1)	(0.13)	(0.11)	(0.1)	(0.1)	(0.05)	(0.06)	(3.5)	(2.5)	(3.5)	(5.8)
1998												
Red bud	26.6	22.2	1.44	1.25	11.3	11.4	0.50	0.72	**57.5**	**48.0***	65.8	43.3
	(0.7)	(1.7)	(0.06)	(0.06)	(0.1)	(1.5)	(0.08)	(0.13)	(1.6)	(7.8)	(3.8)	(8.8)
Dogwood	15.9	16.3	1.10	1.14	9.6	7.1	0.56	0.64	40.0	56.7	47.7	45.3
	(0.5)	(0.2)	(0.17)	(0.13)	(1.4)	(0.4)	(0.06)	(0.16)	(7.7)	(2.4)	(4.7)	(8.4)
Red maple	15.8	15.4	1.20	1.27	7.4	7.0	0.38	0.59	53.0	54.8	69.2	55.0
	(0.6)	(0.6)	(0.11)	(0.12)	(0.5)	(0.6)	(0.12)	(0.15)	(2.5)	(2.6)	(6.9)	(7.9)
Sweet gum	16.1	14.0	1.27	1.13	7.1	6.4	0.53	0.61	56.0	54.0	58.2	46.8
	(1.3)	(1.0)	(0.12)	(0.05)	(0.5)	(0.3)	(0.09)	(0.12)	(0.6)	(1.7)	(7.0)	(10.0)
Loblolly pine												
New	10.5	9.4	1.12	1.11	…	…	…	…				
	(0.4)	(0.8)	(0.09)	(0.04)								
Old	8.5	8.3	0.81	0.91	4.9	5.0	0.31	0.38	56.9	55.0	69.0	62.6
	(0.6)	(0.7)	(0.11)	(0.11)	(0.3)	(0.1)	(0.05)	(0.07)	(3.3)	(1.0)	(2.6)	(5.1)

Notes: Data are means with 1 SE in parentheses below. Within each litter-chemistry category and for each species, significant differences between ambient and elevated CO_2 are indicated by boldface type, with the level of significance indicated by a superscript symbol.

* $P < 0.05$.

elevated CO_2 had no significant effect on these leaf properties (Table 2, Appendix A).

On average, 52% of the N and 53% of the P present in green leaves was retranslocated to overwintering tissues prior to leaf senescence. With the exception of N in dogwood in 1998 and P in red bud in 1997, there was no significant effect of elevated CO_2 on the efficiency with which N or P was retranslocated from green leaves prior to senescence in either year of CO_2 fumigation (Table 2, Appendix B). Significantly less N and P was retranslocated from the leaves of dogwood and red bud, respectively, in response to elevated CO_2. Although not statistically significant, P-retranslocation efficiency was lower in all species in response to growth under elevated CO_2 (Table 2). Consequently, leaf-litter P concentrations were generally higher in leaf litter produced under elevated CO_2 (Table 2).

There were no significant main effects of elevated CO_2 on the chemistry of leaf litter including the concentrations of C, TNC, N, and P (Tables 2 and 3). With the exception of sweet gum in 1998, elevated CO_2 had no effect on leaf litter lignin concentrations (Table 3). There were, however, large statistically significant differences among the five species in leaf-litter chemistry (Appendix B).

Litter production, nutrient fluxes, and nutrient-use efficiency

Total aboveground litterfall mass increased during the two years of CO_2 fumigation (Table 4). During the first year of CO_2 fumigation, total aboveground litterfall mass was 9% higher under elevated CO_2 than ambient CO_2. During the second year of CO_2 fumigation, aboveground litterfall was 26% higher under elevated CO_2 than ambient CO_2 (Table 4). The increase in litterfall mass under elevated CO_2 was statistically significant in 1998 only (Table 4). We observed a similar pattern in the fluxes of N and P in litterfall (defined as litterfall mass $\times$ litterfall N and P concentration). Nutrient fluxes in litterfall increased in both years of CO_2 fumigation (Table 4). The fluxes were not significantly different in the first year of CO_2 treatment, but in the second year the N and P fluxes in litterfall increased significantly by 20% and 34%, respectively (Table 4).

Nitrogen-use efficiency (NUE) increased slightly but not statistically significantly during the two years of CO_2 fumigation (Table 4). During the first year of CO_2 fumigation phosphorus-use efficiency (PUE) under elevated CO_2 decreased by 5%, and during the second

TABLE 3. The concentration of carbon, lignin, and total nonstructural carbohydrates (TNC) in the leaf litter of individual species under ambient (A) or elevated (E) CO_2.

	% Carbon		% Lignin		% TNC	
Species	A	E	A	E	A	E
1997						
Red bud	47.7	45.6	17.7	16.9	12.2	11.3
	(0.7)	(0.8)	(1.2)	(0.6)	(0.1)	(0.1)
Dogwood	45.4	44.8	8.9	7.8	10.8	12.4
	(1.6)	(0.6)	(0.6)	(0.9)	(1.3)	(1.2)
Red maple	46.4	46.5	13.8	12.9	9.2	10.9
	(0.3)	(0.9)	(1.3)	(0.7)	(1.1)	(0.6)
Sweet gum	45.6	45.4	13.7	14.7	12.4	12.8
	(0.5)	(0.4)	(1.0)	(0.2)	(0.6)	(0.3)
Loblolly	50.2	50.6	20.9	21.3	7.4	9.3
pine	(0.2)	(0.2)	(0.2)	(0.5)	(0.7)	(0.7)
1998						
Red bud	45.6	45.1	22.3	20.8	10.3	10.1
	(0.4)	(0.2)	(0.8)	(0.8)	(0.2)	(1.3)
Dogwood	42.5	42.8	10.8	8.1	10.9	13.0
	(0.4)	(0.8)	(1.1)	(0.3)	(0.8)	(1.0)
Red maple	46.0	46.1	15.3	15.5	8.6	9.9
	(0.2)	(0.3)	(0.5)	(0.1)	(0.3)	(0.7)
Sweet gum	44.5	45.3	**19.3**	**21.0***	11.2	11.8
	(1.0)	(0.6)	(0.1)	(0.6)	(0.9)	(0.8)
Loblolly	49.7	49.5	23.6	22.5	11.6	12.9
pine	(0.2)	(0.4)	(0.7)	(0.4)	(0.4)	(0.1)

Notes: Data are means with 1 SE in parentheses below. Significant differences between ambient and elevated CO_2 within a category are indicated by boldface type, with the level of significance indicated by a superscript symbol.
* $P < 0.05$.

year of CO_2 fumigation, PUE decreased by 11%. In neither year was the decrease in PUE statistically significant (Table 4).

Of the seven components into which aboveground litterfall was sorted, only loblolly pine needles and loblolly pine bark increased significantly during the two years of CO_2 fumigation (Fig. 2). Notably, the mass of deciduous leaves produced under elevated CO_2 did not increase relative to control plots even after two years of CO_2 fumigation (Fig. 2). The mass of branches and reproductive structures was higher under elevated CO_2 following two years of fumigation, but neither increase was statistically significant because of large variation among the rings (Fig. 2).

The overstory loblolly pine affected leaf-litter production by the deciduous understory in response to CO_2 fumigation (Fig. 3). In the pretreatment year, there was a negative correlation between the mass of loblolly pine litterfall and the mass of deciduous leaf litterfall (Fig. 3). The slope of the negative correlation did not differ between the ambient- and elevated-CO_2 plots (Table 3). In 1997 and 1998 the negative correlation between the loblolly pine litterfall mass and deciduous leaf litterfall was significantly affected by CO_2 treatment, and the slope of the negative correlation differed between the ambient- and elevated-CO_2 plots (Fig. 3). When loblolly pine litterfall mass was >350 g/m^2 there was little difference in the mass of deciduous leaf litterfall in the ambient- and the elevated-CO_2 plots—i.e., the distance between the two regression lines was small (Fig. 3). In contrast, when the litterfall of loblolly pine was <300 g/m^2 deciduous litterfall was much higher in the elevated-CO_2 plots than in the ambient-CO_2 plots—i.e., the distance between the two regression lines was large (Fig. 3).

Decomposition

Across all species, neither litter type nor site of decomposition had any effect on the rate of mass loss (Fig. 4, Appendix C). Leaf litter decomposing in the ambient-CO_2 plots immobilized slightly but significantly more N (116% of initial N remaining) than leaf litter decomposing in the elevated-CO_2 plots (108% of

TABLE 4. The average of total aboveground litterfall, the flux of N and P in litterfall, and N- and P-use efficiency in the ambient (A) and elevated (E) CO_2 plots during the first two years of CO_2 fumigation (1997 and 1998).

	Fluxes			Nutrient-use efficiency		
	CO_2			CO_2		
Year	A	E	% CO_2 effect	A	E	% CO_2 effect
Litterfall (g·m^{-2}·yr^{-1})						
1997	509.3	552.6^{NS}	+8.5			
	(11.3)	(11.3)				
1998	597.0	754.6*	+26.4			
	(23.6)	(23.6)				
Nitrogen (g·m^{-2}·yr^{-1})						
1997	3.27	3.33^{NS}	+2.5	158.4	164.1^{NS}	+3.6
	(0.07)	(0.07)		(3.4)	(5.8)	
1998	3.42	4.10**	+20.2	176.1	184.8^{NS}	+4.9
	(0.06)	(0.06)		(8.0)	(8.0)	
Phosphorus (mg·m^{-2}·yr^{-1})						
1997	229.1	249.4^{NS}	+10.2	2358	2246^{NS}	−4.7
	(10.1)	(10.1)		(333)	(402)	
1998	225.9	302.6*	+34.0	2864	2563^{NS}	−10.5
	(17.4)	(17.4)		(547)	(431)	

Notes: Each value is the least-squares mean with the pooled standard error of the mean (1 SE) in parentheses below.
* $P < 0.05$; ** $P < 0.01$; NS, not significant.

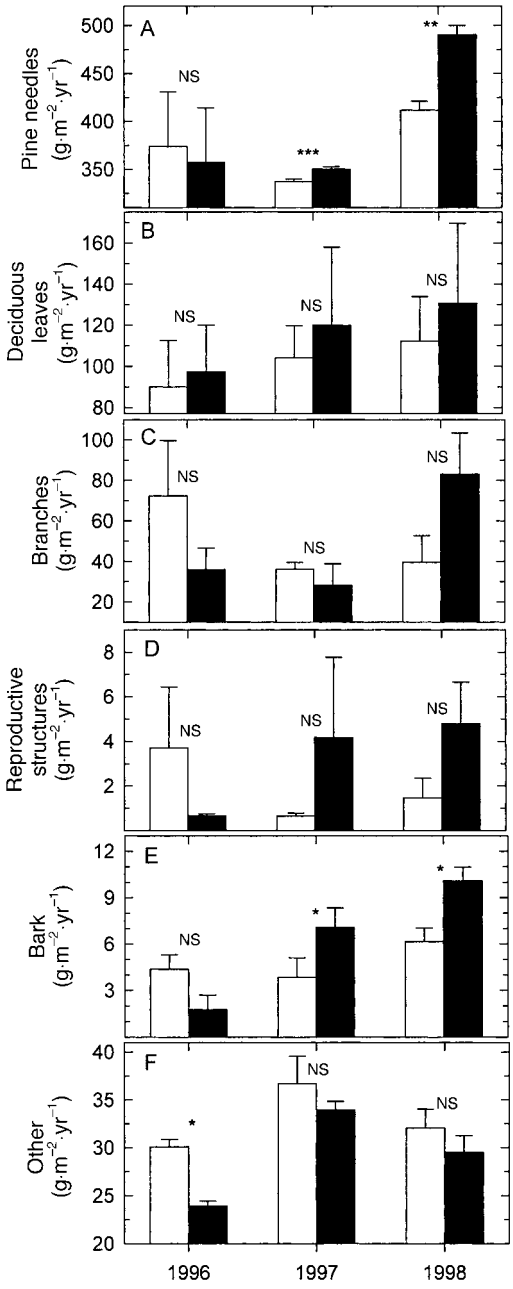

FIG. 2. The flux of aboveground litterfall divided into six different components. The open bars depict the treatment means for the ambient-CO_2 plots. The solid bars depict the treatment means for the elevated-CO_2 plots. The sample size is $n = 3$ for each treatment; 1996 is the year prior to the onset of CO_2 fumigation. Litter in 1996 was collected starting 1 June; 1997 and 1998 are the first and second years of CO_2 fumigation, respectively. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS, not significant.

initial N remaining, Appendix C). Leaf litter produced under elevated CO_2 mineralized significantly more P during decomposition (73% of initial P remaining) than leaf litter produced under ambient CO_2 (81% remaining), although this effect was also relatively small (Appendix C).

Species differed significantly ($P < 0.001$) from one another in the rate of mass loss (Fig. 4) and N and P immobilization during decomposition (Table 5). In red maple, there was significantly higher mass remaining in the litter decomposing in the ambient-CO_2 plots (63% of initial mass) than in the elevated-CO_2 plots (60% of initial mass). Loblolly pine litter decomposing in the ambient-CO_2 plots immobilized more N than did litter decomposing in the elevated-CO_2 plots (Table 5). Both sweet gum and red bud litter produced under elevated CO_2 mineralized more P during decomposition than litter of the same species produced under ambient CO_2 (Table 5).

Soil nitrogen

The quantity of extractable NH_4^+ and NO_3^- varied significantly with time being highest in June and lowest in October (data not shown). Ammonium (NH_4^+) dominated the pool of extractable, inorganic N (Table 6). Inorganic N concentrations were not significantly different between the elevated- and ambient-CO_2 plots (Table 6). The rate of potential N mineralization also varied with time but was not consistently higher or lower during any period of the year

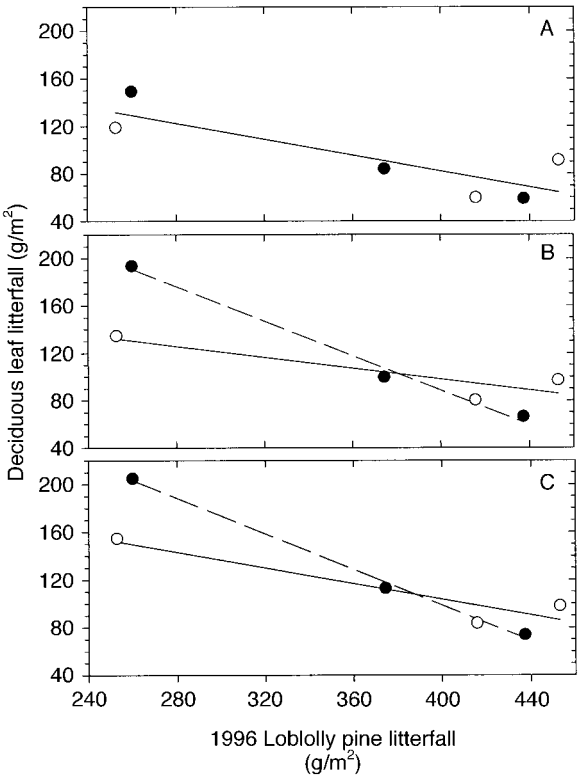

FIG. 3. The correlation between loblolly pine needle litterfall in 1996 (pretreatment) and deciduous leaf litterfall in (A) 1996, (B) 1997, and (C) 1998; ○ = ambient-CO_2 plots, and ● = elevated-CO_2 plots.

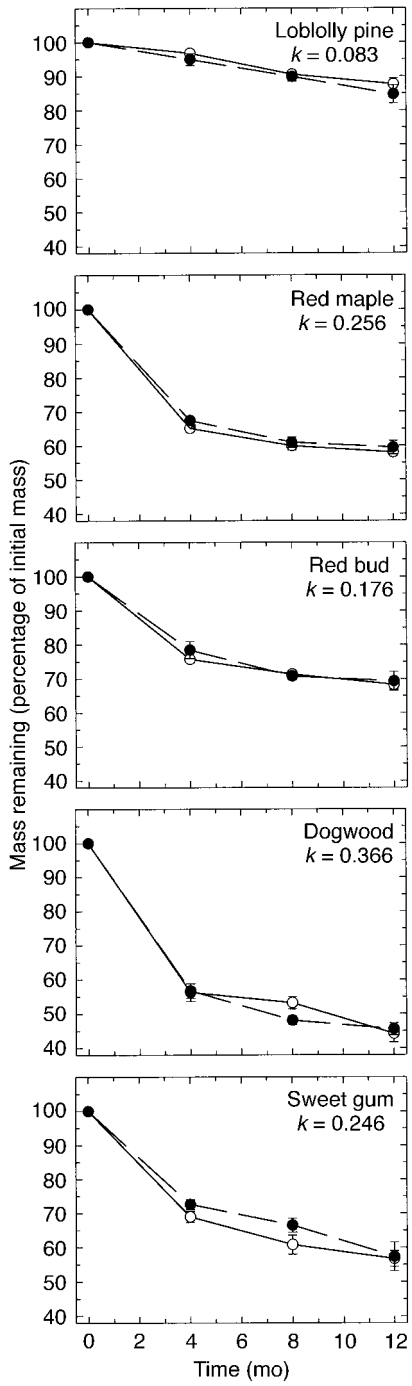

FIG. 4. Species-specific rates of mass loss for litter produced under ambient CO_2 (○) and elevated CO_2 (●) following 4, 8, and 12 mo of decomposition in the field (''*k*'' refers to the annual mass rate loss constant given in Eq. 3).

(data not shown). Rates of nitrification were lower than rates of net mineralization. We did not detect any significant differences in the rate of net N mineralization or net nitrification between the elevated- and ambient-CO_2 plots during either year of CO_2 fumigation (Table 6).

DISCUSSION

Foliar chemistry and retranslocation efficiency

Reductions in leaf nutrient concentrations could affect long-term C gain in plants exposed to elevated CO_2 because of the important relationship between the rate of C assimilation and the concentration of N and P in leaves (Peterson et al. 1999). Growth under elevated CO_2 often reduces the N concentration of green leaves (Cotrufo et al. 1998, Staddon et al. 1999). Cotrufo et al. (1998) summarized 182 reports of green-leaf N concentrations and found a statistically significant, average reduction of 16%. Across all species at the Duke free-air CO_2-enrichment (FACE) site, green-leaf N and P concentrations decreased by an average of 8% and 2%, respectively, although neither reduction was statistically significant (Table 2, Appendix B). Ellsworth (1999) Namburg and Ellsworth (2000) measured a large enhancement of photosynthesis and an increase in photosynthetic nutrient-use efficiency (NUE) in loblolly pine and several understory hardwood species in response to elevated CO_2. The increase in photosynthetic NUE in this ecosystem was therefore due to an up-regulation of photosynthesis rather than a decrease in tissue N concentrations.

Increases in nutrient retranslocation prior to senescence could increase the residence time of existing nutrient capital within plant biomass and contribute to a long-term enhancement of growth under elevated CO_2. Despite the importance of nutrient retranslocation in maintaining plant nutrient balance and ecosystem productivity (Switzer and Nelson 1972, Pugnaire and Chapin 1993), there are few direct measurements of nutrient-retranslocation efficiency in studies of plant responses to high CO_2. Norby and Cotrufo (1998) suggested that nutrient-retranslocation efficiency was lower under elevated CO_2 because the proportional reduction in green-leaf N concentration is usually greater than the proportional reduction in litter N concentration (e.g., Cotrufo et al. 1998). However it is difficult to argue from these data that retranslocation efficiency per se changed because green-leaf N concentrations were initially lower in the leaves growing under elevated CO_2. In our current study, leaf-litter N concentrations were largely unaffected by elevated CO_2 while P concentrations increased slightly (Table 2). Consequently, we found that N-retranslocation efficiency remained largely unchanged while P retranslocation decreased slightly (Table 2). These results are consistent with Norby et al. (2000) who found no change in N-retranslocation efficiency in two maple species growing under elevated CO_2 and with other studies that have found little variation in nutrient-retranslocation efficiency in response to resource manipulation (Birk and Vitousek 1986, Aerts 1996). In this ecosystem, higher

TABLE 5. The fraction of initial N and P remaining in leaf litter produced under ambient (A) and elevated (E) CO_2 (''litter type'') and in litter decomposed in the ambient- and elevated-CO_2 plots (''site'') after 12 mo of decomposition in the field.

	Litter type				Site			
	Initial N remaining (%)		Initial P remaining (%)		Initial N remaining (%)		Initial P remaining (%)	
Species	A	E	A	E	A	E	A	E
Red bud	119.1 (10.9)	97.3 (6.6)	**99.7** (4.2)	**74.8*** (4.8)	112.8 (8.2)	100.5 (3.7)	85.7 (6.4)	86.6 (4.1)
Dogwood	83.1 (4.2)	104.5 (4.8)	70.1 (3.3)	63.0 (4.8)	93.9 (5.63)	93.8 (5.0)	68.0 (3.3)	65.0 (3.3)
Red maple	103.7 (2.6)	109.0 (4.5)	66.9 (3.7)	70.9 (2.5)	105.6 (3.6)	106.6 (2.0)	70.5 (2.4)	67.0 (2.6)
Sweet gum	116.6 (8.1)	112.3 (9.0)	**81.2** (4.0)	**67.1**** (4.2)	117.1 (9.2)	111.8 (3.5)	71.6 (3.8)	76.7 (3.9)
Loblolly	131.4 (14.3)	126.5 (9.2)	78.7 (12.6)	91.2 (3.7)	**133.6** (12.7)	**114.2*** (6.4)	104.8 (8.6)	69.4 (6.2)

Notes: Data are means with 1 SE in parentheses below. Significant differences between ambient and elevated CO_2 within a category are bold, with the level of significance indicated by a superscript symbol.

* $P < 0.05$; ** $P < 0.01$.

retranslocation efficiency does not seem to be an important mechanism for nutrient retention in plant biomass in response to elevated CO_2.

Litter production, nutrient fluxes, and nutrient-use efficiency

Total aboveground litterfall increased by 9% in the first year of CO_2 fumigation (1997) and 26% in the second year of CO_2 fumigation (1998) (Table 4). The increase in litterfall was largely due to increases in loblolly pine leaf litterfall and to a lesser extent loblolly pine bark production (Fig. 2). The increase in litterfall mass in 1997 was smaller than the 24% increase in loblolly pine biomass in the same year (DeLucia et al. 1999) because loblolly pines retain their needles for 19 mo (Zhang and Allen 1996). The 26% greater litterfall in 1998 largely reflected the CO_2 stimulation of loblolly pine production in 1997 (DeLucia et al. 1999). Based on the 25% stimulation of loblolly pine production in 1998, we expect a similar increase in aboveground litterfall in 1999.

The other components of aboveground litterfall—deciduous leaves, branches, and reproductive structures—were not statistically significantly affected by elevated CO_2 (Fig. 2). While the forest canopy is increasing in height, it may take several years before the increase in height growth leads to increases in branchfall. Similarly, most trees in this forest ecosystem are either too young to be reproductive (e.g., the understory hardwoods) or are just entering reproductive maturity (e.g., the overstory loblolly pines). If growth under elevated CO_2 increases C allocation to reproduction (cf. Jackson et al. 1994, Roy et al. 1996, Wayne et al. 1999), then there should be a gradual increase in the mass of reproductive structures in the elevated-CO_2 plots relative to the control plots over time.

Deciduous leaf litterfall did not increase in response to CO_2 fumigation in either year (Fig. 3). Most deciduous trees are growing in the shaded understory, below the loblolly pine canopy. Even though the deciduous trees are shaded, the mass of deciduous leaves produced under elevated CO_2 could have increased relative to the ambient plots because elevated CO_2 decreases the photosynthetic light compensation point of most C_3 species. Shade-grown individuals under high CO_2 almost always have higher biomass than shade-grown individuals of

TABLE 6. The mean concentration of inorganic N and the net rate of N mineralization and nitrification in aerobic, laboratory soil incubations in 1997 and 1998.

	NH_4^+-N (μg/g soil)		NO_3^--N (μg/g soil)		Net mineralization (μg·g soil^{-1}·30 d^{-1}		Net nitrification (μg·g soil^{-1}·30 d^{-1}	
Year	A	E	A	E	A	E	A	E
1997	1.46 (0.23)	1.60 (0.34)	0.063 (0.005)	0.066 (0.002)	3.51 (0.86)	4.25 (1.38)	1.07 (0.46)	2.24 (1.31)
1998	2.37 (0.44)	2.26 (0.31)	0.171 (0.010)	0.206 (0.021)	1.40 (0.76)	2.07 (1.99)	0.49 (0.36)	0.82 (0.66)

Notes: The soils were sampled in June and October of 1997, and in April, June, August, and October of 1998. Data are means under ambient (A) or elevated (E) CO_2 with 1 SE in parentheses below. There were no significant ($P < 0.05$) differences in inorganic N concentrations, mineralization rates, or nitrification rates.

the same species under ambient CO_2 (e.g., Bazzaz and Miao 1993). Overstory–understory competition for light may explain why deciduous-leaf litterfall did not increase in response to elevated CO_2 (Fig. 2). There was a negative correlation between the abundance of deciduous-leaf litter and loblolly pine litter that, in 1997 and 1998, differed between ambient- and elevated-CO_2 plots (Fig. 3). When loblolly pine abundance was relatively high there was little increase in the mass of deciduous-leaf litter collected in the elevated-CO_2 plots relative to ambient CO_2. When loblolly pine abundance was relatively low deciduous litterfall was much higher in the elevated-CO_2 plots (Fig. 3). Given that elevated CO_2 stimulated loblolly pine production (DeLucia et al. 1999), the increase in loblolly pine foliage biomass under elevated CO_2 may have decreased light availability in the understory to the point where the increase in C gain due to a higher rate of photosynthesis under high CO_2 was offset by a proportional reduction in light availability. Supporting this line of argumentation, DeLucia et al. (1999) found no stimulation of subcanopy hardwood biomass production in either year of CO_2 treatment in this forest ecosystem.

One consequence of enhanced primary production in a nutrient-limited ecosystem at elevated CO_2 could be an increase in NUE and a decrease in litter nutrient concentrations with important feedback effects on nutrient cycling (Fig. 1). In this study, neither nitrogen-use efficiency nor phosphorus-use efficiency changed significantly as a consequence of forest growth under elevated CO_2 (Table 4). To the contrary, by the end of the second growing season under elevated CO_2, the amount of N returned to the forest floor in litterfall increased by 20% and the quantity of P increased by 34% (Table 4). The increased flux of N and P from vegetation to soils could significantly accelerate rates of nutrient cycling in this ecosystem if decomposition rates stay the same or increase.

Decomposition

In this study there was no significant effect of litter type or site of decomposition on the rate at which mass was lost from litterbags (Fig. 4, Appendix C). These results are consistent with our litter-chemistry data. During initial decomposition, mass loss is correlated with the ratio of C or certain C-based compounds and nutrients in litter (Taylor et al. 1989). While C:N and lignin:N ratios were highly correlated with the rate of mass loss ($P < 0.01$), there were no consistent effects of elevated CO_2 on litter chemistry (Table 3, Appendix B). Coûteaux et al. (1999) reviewed the literature on the decomposition of litter produced under ambient and elevated CO_2. Among 60 published observations, they found that litter produced under elevated CO_2 decomposed more slowly in 17 cases, more rapidly in 15 cases, and was not significantly different in 28 cases. Collectively, there is little evidence to support a generalization of slower decomposition rates for litter produced under elevated CO_2.

The rate of mass loss was not affected by the site of decomposition in our study, implying that forest growth under elevated CO_2 had little effect on the activity of decomposers in the forest floor. If increases in aboveground litterfall increase the input of labile C substrates to the forest floor then microbial activity, including decomposition, could increase over time (the so called ''priming effect''; Jenkinson 1971). In our study, a higher rate of primary production is likely to have increased the flux of labile C to the forest floor in the form of total nonstructural carbohydrates (TNC) in litter and dissolved organic C in throughfall (Lichter et al. 2000). However, 1998 was the first year in which a large increase in C inputs to the forest floor occurred in response to CO_2 fumigation (DeLucia et al. 1999, Allen et al. 2000). Thus, we cannot rule out future changes in the rate of litter decomposition in the plots under elevated CO_2 because insufficient time may have elapsed to detect this effect in litterbag studies.

There was a small but significant effect of the site of decomposition on N immobilization in leaf litter (Appendix C). This trend was largely driven by the litter of loblolly pine that immobilized less N during decomposition in the elevated plots (Table 5). Speculatively, the difference in N immobilization may reflect a change in the structure of the microbial community. Bacteria typically have a C:N ratio between 3:1 and 5:1 whereas fungi have C:N ratios up to 15:1 (Paul and Clark 1986). Less N would be immobilized per unit microbial biomass if fungi increasingly dominated the microbial biomass during exposure to elevated CO_2. The pattern of N immobilization was unrelated to any of the measured litter chemistry parameters.

There was a significant effect of litter type on P mineralization during decomposition (Appendix C, Table 5). On average more P was mineralized from litter produced under elevated CO_2 than under ambient CO_2. Using stepwise linear regression (SAS Institute 1990), we found that the quantity of P mineralized from litter by the end of 1 yr was significantly correlated with the initial C:N ratio of litter (parital $R^2 = 0.43$, partial $F = 13.8$, $P < 0.01$) and the initial P concentration of litter (partial $R^2 = 0.26$, partial $F = 14.0$, $P < 0.01$). The simplest explanation for the rapid mineralization of P is that the slightly higher initial concentration of P in litter produced under elevated CO_2 (Table 2) led to an initially higher rate of P leaching from litter.

Soil nitrogen

During the first two years of this study, forest growth under elevated CO_2 did not affect the pool size of inorganic N ($NH_4^+ + NO_3^-$) or the rate of net N mineralization or nitrification in 30-d laboratory soil incubations under constant temperature and field moisture (Table 6). These results are consistent with Rice et al. (1994) who found no significant effect of elevated

CO_2 on net N mineralization in tallgrass prairie. Our results contrast with Johnson et al. (1996) who found a decrease in potentially mineralizable N in soils under *Pinus ponderosa* growing under elevated CO_2, and with Zak et al. (1993) who found a significant increase in potentially mineralizable N in soils under *Populous* clones growing under elevated CO_2.

Several mechanisms have been proposed to explain increases or decreases in plant available N as a function of microbial activity in response to elevated CO_2 (see Diaz et al. 1993, Zak et al. 1993, Kampichler et al. 1998, Cheng 1999). In brief, the size of microbial biomass is typically limited by C availability (Zak et al. 1994). If enhanced plant production under elevated CO_2 increases C availability to microbes, then the biomass of microbes will increase, immobilize N, and decrease plant N availability (Woods et al. 1987, Diaz et al. 1993). Microbial biomass has not increased in response to CO_2 fumigation at this site (Allen et al. 2000). In contrast, if plant C allocation belowground alters the trophic interactions of soil communities and increases the grazing rate on soil microbes (e.g., Zak et al. 1993) or alters the activity or composition of the microbial community favoring gross mineralization over gross immobilization, then plant available N could increase. M. P. Osgood and R. L. Sinsabaugh (*unpublished manuscript*) measured an increase in the activity of enzymes produced by microbes, associated with the mineralization of relatively labile C compounds (e.g., cellobiohydrolase, beta-glucosidase) and N compounds (N-acetylglucosaminidase, leucine aminopeptidase). At this point in time we cannot exclude the possibility of future changes in net N mineralization as C inputs to the forest floor and mineral soil are increasing yearly (DeLucia et al. 1999).

Summary

Identifying the source of the N and P lost in aboveground litterfall will be critical to assessing whether the increases in forest productivity and nutrient fluxes we measured in 1998 will be sustained in the long term. If the nutrients required for the enhanced productivity of this forest ecosystem under elevated CO_2 are met by the retranslocation of N and P from storage (e.g., wood), then we predict forest productivity and nutrient fluxes will decrease over time as reserves of N and P are eventually exhausted. In contrast, if the demand for nutrients is met by increases in uptake from soils, then forest productivity and nutrient fluxes may be stimulated in response to growth under elevated CO_2.

Although decomposition rates have not changed as a consequence of elevated CO_2, the forest floor is increasing in mass due to the increase in aboveground litterfall. Thus, N and P mineralization from the aggrading forest floor should provide at least a fraction of the enhanced plant demand for N and P under elevated CO_2. However, the mineralization of N and P from the forest floor alone cannot meet the enhanced productivity demand for nutrients in this ecosystem within a given year. The nutrient release from a given litter cohort in a given year is smaller than the measured uptake of N and P by vegetation.

The degree to which increases in atmospheric CO_2 will stimulate N and P uptake from soils may be fundamentally constrained. The atmosphere contains a virtually unlimited pool of biologically reactive C in the form of CO_2. CO_2 can be assimilated directly into plant biomass, and uptake rates are only limited by the diffusion of CO_2 inside the leaf and leaf biochemistry. In contrast, soils contain a very large pool of chemically and physically bound N and P. The transformation of N and P to plant-available forms (NH_4^+, NO_3^-, PO_4^{3-}, low-molecular-mass amino acids) is required prior to plant uptake, and these transformations in soils are governed by many biotic and abiotic processes. Because plant production does not affect all the processes that govern nutrient mineralization, increases in C supply belowground alone may not be sufficient to stimulate rates of nutrient mineralization from forest soils.

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APPENDIX A

A table presenting results of a split-plot ANOVA test for the effect of elevated CO_2, species, and their interaction on percentage green-leaf total nonstructural carbohydrates (%TNC), N and P concentrations per unit mass, and specific-leaf mass (SLM) is available in ESA's Electronic Data Archive: *Ecological Archives* E082-005-A1.

APPENDIX B

A table presenting results of a split-plot ANOVA test for the effect of elevated CO_2, species, and their interaction on nutrient-retranslocation efficiency and leaf-litter chemistry in the first 2 yr of CO_2 fumigation (1997 and 1998) is available in ESA's Electronic Data Archive: *Ecological Archives* E082-005-A2.

APPENDIX C

A table presenting results of a repeated-measures ANOVA on the effect of litter type, site of decomposition, and harvest data on mass loss and the quantity of N and P remaining in litter following 1 yr of decomposition in the field is available in ESA's Electronic Data Archive: *Ecological Archives* E082-005-A3.