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Effects of elevated CO_2 on nutrient cycling in a sweetgum plantation

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Abstract. The effects of elevated CO_2 on nutrient cycling and selected belowground processes in the closed-canopy sweetgum plantation were assessed as part of a free-air CO_2 enrichment (FACE) experiment at Oak Ridge, Tennessee. We hypothesized that nitrogen (N) constraints to growth response to elevated CO_2 would be mitigated primarily by reduced tissue concentrations (resulting in increased biomass production per unit uptake) rather than increased uptake. Conversely, we hypothesized that the constraints of other nutrients to growth response to elevated CO_2 would be mitigated primarily by increased uptake because of adequate soil supplies. The first hypothesis was not supported: although elevated CO_2 caused reduced foliar N concentrations, it also resulted in increased uptake and requirement of N, primarily because of greater root turnover. The additional N uptake with elevated CO_2 constituted between 10 and 40% of the estimated soil mineralizeable N pool. The second hypothesis was largely supported: elevated CO_2 had no significant effects on tissue concentrations of P, K, Ca, or Mg and caused significantly increased uptake and requirement of K, Ca, and Mg. Soil exchangeable pools of these nutrients are large and should pose no constraint to continued growth responses. Elevated CO_2 also caused increased microbial biomass, reduced N leaching and increased P leaching from O horizons (measured by resin lysimeters), reduced soil solution NH_4^+, SO_4^{2-}, and Ca^{2+} concentrations, and increased soil solution pH. There were no statistically significant treatment effects on soil nutrient availability as measured by resin capsules, resin stakes, or *in situ* incubations. Despite significantly lower litterfall N concentrations in the elevated CO_2 treatment, there were no significant treatment effects on translocation or forest floor biomass or nutrient contents. There were also no significant treatment effects on the rate of decomposition of fine roots. In general, the effects of elevated CO_2 on nutrient cycling in this study were not large; future constraints on growth responses imposed by N limitations will depend on changes in N demand, atmospheric N deposition, and soil mineralization rates.

Introduction

The potential response of terrestrial ecosystems to elevated CO_2 may be constrained by the availability and cycling of nutrients. Many studies have shown that elevated CO_2 can cause reduced tissue nutrient concentrations and therefore cause

increased biomass production per unit uptake (Zak et al. 1993; McGuire et al. 1995; Bernston and Bazzaz 1996; Johnson et al. 1997, 2003; Curtis et al. 2000; Medlyn et al. 2000; Finzi et al. 2002). This finding has led to speculation that nutrient concentrations in litterfall and root detritus could also be reduced, causing reduced decomposition and N mineralization (Strain 1985). The weight of the evidence from numerous laboratory studies thus far shows little or no change in decomposition with elevated CO_2 in laboratory and open top chamber studies (Norby et al. 2001a,b), but this hypothesis remains to be tested in an ecosystem context. Some greenhouse and open top chamber studies have suggested that elevated CO_2 can also facilitate greater soil exploration by increasing root and mycorrhizal biomass (Norby et al. 1987; Rogers et al. 1992; Tingey et al. 1996; Pregitzer et al. 2000), increased N mineralization (e.g., Zak et al. 1993) or 'mining' of older soil N (Johnson et al. 2002a,b). Elevated CO_2 has been found to cause both increased (Körner and Arnone 1992) and decreased (Torbert et al. 1996) NO_3^- leaching, depending on the degree to which N mineralization and N uptake are affected.

Most of the studies cited above were conducted in greenhouse or open top chamber experiments, where feedbacks via biogeochemical cycles are either minimal or absent. These processes and the interactions among them cannot be adequately understood when they are removed from the system and studied in isolation. Free-air CO_2 enrichment (FACE) experiments, while they have been criticized for applying an unrealistic square wave treatment to a system with a 'memory' of low CO_2, are invaluable in providing some insights into ecosystem-level feedbacks among nutrient cycling processes. Oren et al. (2001) noted a large reduction in aboveground growth response to elevated CO_2 after 3 years treatment in the prototype FACE study at Duke, North Carolina. They attributed this decline in CO_2 response to progressive N deficiency, and this hypothesis was supported by increased growth response to CO_2 after the addition of N fertilizer. However, this response has not been repeated in the replicated experiment at the same site (Hamilton et al. 2001). Furthermore, Allen et al. (2000) did not find significant effects of elevated CO_2 on O horizon C:N ratio during the first year of treatment in the Duke FACE site, and thus the direct causes of the progressive N deficiency noted by Oren et al. (2001) apparently did not include changes in the litter quality – decomposition pathway. In a later study, Finzi et al. (2002) conducted a detailed analysis of the N cycle in response to elevated CO_2 at the Duke site. They found minimal additional N uptake during the 2 two years of treatment (because increases in biomass were offset by lower tissue N concentrations), but large increases in N uptake and requirement during the following 2 years. They found no treatment effects on soil N mineralization, however, and because of this they speculated that, while there was no evidence of any current decline in biomass response as of that date, an N constraint on continued growth response in the future was likely.

The purpose of this research is to investigate the effects of elevated CO_2 on nutrient cycling and selected belowground processes in the sweetgum FACE facility at Oak Ridge, Tennessee. Previous studies at this site have shown an average of 21% increase in net primary production with elevated CO_2 (Norby et al. 2002). During 2 years of intense monitoring, we made measurements of the parameters

needed to calculate uptake in this ecosystem, including nutrient fluxes in wood increment, litterfall, fine root production, foliar leaching, and soil leaching. We also measured fine root decomposition rates, microbial biomass, soil leaching, and soil nutrient availability using several methods. Our overall hypotheses was as follows:

Hypothesis 1: Nitrogen constraints to growth response to elevated CO_2 will be mitigated primarily by reduced tissue concentrations (resulting in increased biomass production per unit uptake) rather than by uptake.

Hypothesis 2: Constraints of other nutrients to growth response to elevated CO_2 will be mitigated primarily by increased uptake rather than by reduced tissue concentrations.

These hypotheses are based on the simple mathematical reality that nutrient limitations will constrain growth response to CO_2 unless elevated CO_2 facilitates (1) greater biomass production per unit nutrient uptake, (2) greater nutrient uptake, or (3) both. If elevated CO_2 causes increased nutrient uptake, additional nutrients must be obtained from the soil, atmospheric deposition, or, in the case of N, by N_2 fixation. If uptake is constrained by factors external to the tree, reduced tissue concentrations might counterbalance the increased production, resulting in no change in uptake. The latter scenario is especially relevant to N cycling. Nitrogen is unique among nutrients in that soil exchangeable/adsorbed pools are very small relative to annual uptake, requiring that they turn over many times per year to satisfy plant demands, and microbial competition for these mineral pools is intense (Johnson 1992). In contrast, soil exchangeable/adsorbed pools of the other macronutrients can be of sufficient size to satisfy uptake for an entire forest rotation (Cole and Rapp 1981). It is for that reason that we differentiate the hypotheses between N and the other nutrients in terms of their responses to elevated CO_2.

Site and methods

Site and treatments

The experimental site is a planted sweetgum (*Liquidambar styraciflua* L.) monoculture on the Oak Ridge National Environmental Research Park in Roane County, Tennessee (35°54′N, 84°20′W). Mean annual temperature (1962–1993) is 13.9 °C, and mean annual precipitation is 1371 mm, with a generally even distribution of precipitation throughout the year. The soil at the site, which is tentatively classified as an Aquic Hapludalf, developed in alluvium (Miegroet et al. 1995). Table 1 provides some soil chemical and physical data. With two exceptions (C in the 75–90 cm depths and extractable P in the 45–60 cm depths), there were no statistically significant pre-treatment differences in soil chemical properties.

The trees were planted in 1988 at a spacing of 2.3×1.2 m. Herbicide was used in 1989 and 1990 to control competition from weeds; no fertilizer has been added. Six 25-m diameter plots (including a 2.5 m wide buffer) were laid out in 1996, and FACE

Table 1. Soil nutrient concentrations in the ambient and elevated CO_2 treatment plots before the imposition of treatments. Data are means of two elevated or three ambient plots ± SE.

Horizon and depth (cm)	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient
	Carbon (%)		Nitrogen (%)		C:N ratio	
A1 (0–5)	1.60 ± 0.09	1.39 ± 0.09	0.13 ± 0.01	0.12 ± 0.01	12.5 ± 0.1	11.5 ± 0.7
Ap (0–15)	1.36 ± 0.07	1.21 ± 0.07	0.12 ± 0.01	0.11 ± 0.01	11.2 ± 0.1	11.0 ± 0.3
Bt1 (15–30)	0.95 ± 0.09	0.75 ± 0.06	0.09 ± 0.01	0.08 ± 0.01	10.6 ± 0.4	9.2 ± 0.2
Bt2 (30–45)	0.64 ± 0.14	0.40 ± 0.05	0.07 ± 0.01	0.05 ± 0.01	9.4 ± 0.5	7.8 ± 0.3
Bt3 (45–60)	0.43 ± 0.14	0.28 ± 0.03	0.05 ± 0.01	0.04 ± 0.01	8.2 ± 0.9	6.9 ± 0.6
Bt4 (60–75)	0.30 ± 0.05	0.22 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	7.3 ± 0.1**	6.1 ± 0.2
Bt4 (75–90)	0.28 ± 0.00*	0.22 ± 0.02	0.04 ± 0.00	0.03 ± 0.00	7.6 ± 0.0**	6.6 ± 0.3
	Phosphorus ($\mu g\, g^{-1}$)		Calcium ($cmol_c\, kg^{-1}$)		Potassium ($cmol_c\, kg^{-1}$)	
0–5	8.83 ± 1.00	8.64 ± 1.36	3.36 ± 0.14*	2.56 ± 0.43	0.16 ± 0.01*	0.13 ± 0.01
0–15	9.63 ± 0.63	11.41 ± 1.81	3.55 ± 0.82	2.54 ± 0.42	0.12 ± 0.02	0.12 ± 0.00
15–30	5.73 ± 0.56	6.13 ± 0.59	3.52 ± 0.38	2.94 ± 0.40	0.10 ± 0.00	0.12 ± 0.01
30–45	3.98 ± 1.31	4.74 ± 0.54	3.43 ± 0.77	2.92 ± 0.40	0.15 ± 0.05	0.11 ± 0.01
45–60	3.17 ± 0.67*	5.27 ± 0.82	3.26 ± 1.13	2.34 ± 0.28	0.10 ± 0.00	0.11 ± 0.01
60–75	3.17 ± 0.83	4.84 ± 0.20	2.22 ± 0.72	1.78 ± 0.23	0.11 ± 0.01	0.12 ± 0.00
75–90	3.00 ± 1.00	4.72 ± 0.23	1.82 ± 0.53	1.50 ± 0.27	0.11 ± 0.01	0.11 ± 0.01
	Magnesium ($cmol_c\, kg^{-1}$)		Cation exchange capacity ($cmol_c\, kg^{-1}$)		Base saturation (%)	
0–5	0.83 ± 0.10	0.72 ± 0.12	11.4 ± 0.1	10.4 ± 0.6	37 ± 2	32 ± 4
0–15	0.75 ± 0.15	0.54 ± 0.10	11.1 ± 0.7	9.7 ± 0.6	39 ± 6	33 ± 3
15–30	0.55 ± 0.08	0.51 ± 0.11	9.7 ± 0.3	9.4 ± 0.6	42 ± 3	38 ± 3
30–45	0.55 ± 0.08	0.53 ± 0.13	9.8 ± 0.7	8.8 ± 0.7	42 ± 5	41 ± 4
45–60	0.63 ± 0.10	0.54 ± 0.06	9.4 ± 0.8	8.2 ± 0.4	42 ± 9	36 ± 2
60–75	0.57 ± 0.03	0.60 ± 0.10	9.3 ± 0.3*	7.7 ± 0.6	32 ± 7	32 ± 2
75–90	0.60 ± 0.07	0.57 ± 0.08	8.7 ± 0.7	7.4 ± 0.7	30 ± 7	29 ± 3

*,**, and *** refer to statistically significant differences, $p < 0.10$, 0.05, and 0.01, respectively, students *t*-test.

apparatus (Hendrey et al. 1999) was assembled in four of them. Exposure to elevated [CO_2] commenced in two plots in April 1998, and has continued during the growing season (April–November) since then. The average daytime [CO_2] during the 1999–2000 growing seasons was 542 ppm in the two CO_2-enriched plots, excluding periods when the exposure system was not functioning. The site and experimental design was fully described by Norby et al. (2001a,b). In 1999 when monitoring of nutrient cycling began, there were approximately 91 trees per 314 m^2 measurement plot, with a total basal area of 35 $cm^2\,m^{-2}$ and average height of 14.6 m. Based on tree ring analysis, they have been in a linear growth phase since 1993, and the canopy has been closed since at least 1996 with a leaf area index of about 6.

During the early stages of the experiment, it became apparent that the FACE apparatus alone had no effect on any measurement, but that one of the two plots not instrumented with FACE apparatus was inherently different from the other five plots in terms of soil nutrient status. Thus, for the purposes of analyzing treatment effects, the two plots instrumented with FACE apparatus were combined with the one comparable plot not instrumented with FACE apparatus for the control treatment. This gave two replicates for elevated and three for ambient CO_2.

Vegetation sampling

Live foliage samples were collected in August of each year after canopy development was complete and prior to leaf senescence. Four leaves from each 1-m layer of the canopy were collected at random, with access provided by a hydraulic lift. Nitrogen concentration of each canopy layer was determined as described below, and a weighted average concentration for the entire canopy was calculated based on the relative distribution of leaves by layer (Norby et al. 2001a,b). Other elements were measured in a sample collected from mid-canopy, which in the case of N was representative of the entire canopy. The mass of live foliage was calculated from leaf litterfall, based on the assumption that foliage weight was 7% higher than total litter mass. Litterfall was collected in 0.19-m^2 baskets (seven per plot), usually within 1 week of when it fell. Litter and live foliage samples were oven-dried (70 °C) prior to weighing and analysis. Woody litter, which was primarily from dead trees, was not included. All litter collections for the year were combined for each basket, homogenized, and a subsample was finely ground in a ball mill for subsequent nutrient analysis. Annual wood increment (stem and coarse root) was estimated from allometric relationships with tree basal area increment, height growth, and taper (Norby et al. 2001a,b). It was not feasible to sample wood from within the experimental plots, and so wood concentrations were obtained by destructively sampling trees from areas adjacent to the study plots. Proportional wood samples were taken (either whole disks or pie sections) and analyzed for nutrients as described for foliage samples. Thus, it was assumed that weighted average wood concentrations were the same in all treatments. Fine roots ($<$1 mm diameter) were sampled from 10 points within each ring (0–15 cm depth) in June 1999. Fine roots of sweetgum were separated from other roots (predominantly Japanese honeysuckle [*Lonicera japonica*] and herbaceous plants) based upon color,

morphology, presence of mycorrhizae, and attachment to recognizable parent roots. Fine root production in 1999 and 2000 was derived by scaling up minirhizotron observations of root length density and specific root length (Johnson et al. 2001), as previously reported by Norby et al. (2002). We assume that fine root nutrient concentrations did not differ between years, and used the 1999 concentration values to estimate nutrient turnover in fine roots. Independent measurements of fine root N concentration showed little variation between 1999 and 2000.

Carbon and nitrogen concentrations on all vegetation samples were determined with a NA1500 nitrogen analyzer (CE Instruments, Milan, Italy), with atropine as a standard and NIST apple leaf (SRM1515; N concentration of 22.5 $mg\,g^{-1}$) as an internal quality check (National Institute of Standards and Technology, Gaithersburg, MD). The other nutrients were analyzed at and the Oregon State University (OSU) Soil and Plant Testing Lab. At OSU, plant samples were dry-ashed at 550 °C for 4 h, dissolved in 5% (v/v) HNO_3, and analyzed by inductively coupled plasma emission spectroscopy (ICP).

Solution fluxes

Bulk atmospheric deposition, throughfall, and soil solution fluxes are measured with both conventional water collectors (throughfall funnels and tension lysimeters) and with resin throughfall lysimeter collectors (Susfalk and Johnson 2002). The conventional throughfall samples (eight replicates per plot) were collected monthly (replacing collection bottles each time), bulked by ring, and analyzed for pH, conductivity, and HCO_3^- (titration to pH 5.0) Ca^{2+}, K^+, Mg^{2+}, and Na^+ (atomic absorption spectrophotometry), SO_4^{2-} Cl^- (ion chromatograph) NH_4^+, NO_3^-, and ortho-P (automated colorimetric analysis) at the Water Analysis Lab, Desert Research Institute, Reno, Nevada (DRI). Soil solutions were sampled on a monthly basis during the wet season using a Prenart® tension lysimeter system (three replicate lysimeters per ring at 20 cm depth). Soil solution samples were analyzed individually for NO_3^- (because of the importance of this ion as an indicator of N status) and bulked by ring for analysis of pH, conductivity and other ions as in the case of throughfall samples. Soil solution NO_3^- concentrations in Ring 4 (ambient) were anomalously high after the initial installation, and for that reason, we installed three extra lysimeters in that ring in February of 2000. Subsequent collections from the new lysimeters showed NO_3^- concentrations closer to those in the other ambient treatment rings; thus, it was concluded that the initial lysimeters were placed in a 'hotspot'. The analyses in this paper include values from the original Ring 4 installation as well as those excluding Ring 4 data. Soil leaching fluxes from the lysimeter data were calculated by multiplying the weighted average concentrations by an estimate of soil water flux derived from the Cl^- balance calculations, as has been done in the past for the nearby Walker Branch Watershed (Johnson and Todd 1990). The resin collectors were removed and replaced annually. The resins were extracted with 2 M KCl and analyzed for NH_4^+, NO_3^-, and ortho-P by automated colorimetric analysis by at DRI.

Soil nutrient availability

In 1999, soil N and P availability were assessed in July using Plant Root Simulator Probes (PRS^{TM}), which consist of anion or cation exchange membranes imbedded in plastic stakes (Western Ag Innovations, Inc., Saskatoon, Canada). The stakes were pounded into the soil and allowed to remain in contact with it for 1 month (nine replicates per ring). After removal, the stakes were extracted with 1 M HCl and the extract analyzed for NH_4^+, NO_3^-, and ortho-P. N mineralization in the field was measured in July 1999 using the sequential coring technique. Nine cores per ring were taken to a depth of 20 cm with a 4 cm id pvc pipe placed in a cooler with blue ice, and sent to DRI for extraction for NH_4^+ and NO_3^- (initial values). At the same date as this sampling, another set of cores was installed, capped, and left in place for 1 month. These samples were then retrieved and analyzed for NH_4^+ and NO_3^- (final). The difference in $NH_4^+ + NO_3^-$ between the two samplings is interpreted as net N mineralization rate in the field. In 2001, we changed techniques to the WECSA® Soil Access System (Warrington Ecological Analysis, Ft. Collins, CO). This system employs a mixed-bed cation/anion exchange resin capsule (Unibest PST-1; Yang and Skogley 1992; Dobermann et al. 1994) fitted to the end of a permanently-installed pvc pipe. The capsules were allowed to remain in contact with the soil for a 1 month period after which they were extracted with 2 M KCl, and the extracts analyzed for NH_4^+, NO_3^-, and ortho-P.

Soil microbial biomass

Soil microbial biomass C was analyzed using the fumigation–extraction method described by Vance et al. (1987) with some minor modifications. Twenty grams of fresh, homogenized subsamples were fumigated in a 125-ml Erlenmeyer flask with purified $CHCl^3$ for 48 h. After removal of residual $CHCl^3$, 50 ml of 0.5 M K_2SO_4 solution was added and the sample was shaken for 1 h before filtration of the mixture and freezing for later analysis. Organic C of each extract was analyzed on a Shimadzu 5050A Analyzer and microbial biomass C was calculated as the difference between fumigated and non-fumigated samples adjusted by proportionality coefficients for C ($K_{en} = 0.33$). Soil samples taken for microbial biomass are analyzed for NH_4^+, NO_3^-, and ortho-P (2 M KCl for NH_4^+ and NO_3^-, anion membranes for ortho-P) at DRI.

Soil CO_2 efflux

Soil surface CO_2 efflux rates were measured using a method described in Cheng et al. (2000). Carbon dioxide evolved from an area of 400 cm^2 was continuously trapped for 24 h using a closed-circulation system. There were two sampling areas in each FACE plot (ring) and the average of the two sampling areas was used to calculate the mean and standard error for both the ambient and the elevated CO_2 treatments.

Root decomposition

Roots for field and laboratory incubations were sorted by size class using digital calipers and snips. Non-root organic material, dead and decaying roots, and adhering soil were removed, and samples were composited by plot and air-dried for 6 h under approximately 50% relative humidity and [24 °C] to a moisture content of approximately 59%. Prior to incubation, three subsamples were oven-dried, ground and analyzed for C, N, P, Ca, Mg, P, Fe, Cu, Mn, Zn, B, and lignin at the OSU Soil and Plant Testing Laboratory, as described above. Lignin was determined by Acid-Detergent Fiber (ADF) extraction, followed by sulfuric acid digestion (Rowland and Roberts 1994). Air-dried root samples were weighed out and placed into laboratory incubation mini-chambers and into field decomposition root litter bags (approximately 1 g oven-dried material per bag, based on calculations from percent moisture). Root litter bags were constructed from very fine mesh nylon stocking material. At the time of placement into the field (July 1999), roots from litter bags were dipped in a slurry of the previously collected and sieved rhizosphere soil to create a fine coating of soil on the roots and assure reinoculation with native soil microbes. Immediately prior to burying the bags across transects in the treatment plots (10 bags per plot) at a depth of approximately 10 cm, additional rhizosphere soil was added to each bag. Root composites removed from a given plot were replaced in the same plot. Control bags (2 per plot), containing only rhizosphere soil, were also installed and used to determine the amount, if any, of 'unintended' root material in the bag at the conclusion of the incubation. Fine root litter bags were collected in July 2000 (after 12 months of exposure). Roots were carefully cleaned, and the oven-dry weight of the remaining root material determined. Subsamples of these partially decayed roots were analyzed for the above-described nutrient and lignin concentrations and ratios.

For the laboratory incubation study, the air-dried fine roots from the same composited plot subsamples described above were weighed and placed in 25-ml glass jar containers (eight sub-samples per plot). Ten grams dry weight of previously stored 'rhizosphere' soil and 1 gram dry wet of roots were added to each container. Ten grams dry weight of soil were added to two additional 'soil only' containers per plot. Dry weights were calculated from previously determined moisture contents for roots and soil. Water was added to each container in amounts necessary to bring the soil moisture to 25%. The containers were placed in growth chambers at a constant temperature (22 °C) and relative humidity (90%). Lids were placed loosely on the containers to allow for air and moisture exchange. Moisture levels were checked each week by weighing each container and its contents and adding enough water to bring each container back to its original weight. Five-minute measurements of CO_2 evolution (LiCor 6259) from each of the 50 containers were taken weekly during the first month and later approximately monthly. Measurements were converted to micromoles of CO_2 per minute per oven-dry gram of fine root material. Corrections were made for respiration rates from control 'soil only' containers. These measurements were in turn used to calculate percent mass

loss over time from this fine root material over a 360 day period. We assumed 0.614 mg of root material decomposed per mg CO_2 evolved.

Calculations

Uptake, requirement, and translocation of each nutrient were calculated according to the following equation (Cole and Rapp 1981):

$$U = \mathrm{LF} + \mathrm{FL} + \mathrm{RT} + \mathrm{WI} \tag{1}$$

$$\mathrm{R} = \mathrm{NF} + \mathrm{NR} + \mathrm{WI} \tag{2}$$

$$T = R - U \tag{3}$$

$$T = \mathrm{NF} + \mathrm{NR} - \mathrm{LF} - \mathrm{FL} - \mathrm{RT} \tag{4}$$

where U = uptake, LF = litterfall, FL = foliar leaching, RT = root turnover, WI = increment in woody tissues, R = requirement (defined as nutrients needed for construction of new tissues), NF = new foliage, NR = new roots, and T = translocation.

In most cases, root requirement is assumed equal to root turnover (no translocation; Nambiar and Fife 1991); thus, NR = RT, and Equation (4) reduces to

$$T = \mathrm{NF} - \mathrm{LF} - \mathrm{FL} \tag{5}$$

$T = \mathrm{NF} - \mathrm{LF} - \mathrm{FL}$There is often confusion about the estimation of translocation. As used in this model, translocation is expressed in kg ha^{-1}, and thus has components of both concentration and mass; thus, it is possible, for instance, to have no change in nutrient concentration from live foliage to litterfall yet have positive translocation because of reduced leaf mass compared to live foliage mass. Also, translocation in the above model accounts for foliage leaching, and thus the simple observation that nutrient concentrations in senesced leaves is lower than in live leaves does not necessarily imply that translocation is positive (i.e., the lost nutrients could have leached away).

Statistical analyses

Statistical analyses were performed using student's t-tests for treatment effects in Microsoft Excel® for those ecosystem components that were not measured more than once (e.g., soil nutrient pools). For multiple measurements over time, General Linear Model (GLM) in DataDesk® software (Velleman 1997) was used to detect the effects of treatment and time. A plot was considered a replicate in all cases. In cases where there were replicate samples within a ring (e.g., resin lysimeters), statistical analyses were run both on plot averages and using a nested design (plot nested within treatment).

Results

Nutrient uptake, requirement, and translocation

The average ecosystem contents of C, N, P, K, Ca and Mg for 1999 are given in Table 2 and the average fluxes for these nutrients for the 1999 and 2000 growing seasons are given in Table 3. In Table 3, 'requirement' and 'uptake' for C are calculated in much the same way as for the other nutrients: requirement = new foliage + new roots + wood increment; uptake = litterfall + new roots + wood increment (foliar C leaching was not measured). Although there is no definitive 'requirement' for C and total uptake of C as CO_2 (including that lost in plant respiration) would be much greater than that shown in Table 2, expression of the C cycle in this way facilitates easy comparison with the nutrient cycles and represents the net C accumulation in vegetation.

Norby et al. (2002) previously noted that elevated CO_2 caused significantly greater net primary productivity in this study for the first 3 years of treatment (averaging 21% greater than in ambient CO_2 for 1998, 1999, and 2000). In the dataset used for this analysis, C 'requirement' and 'uptake' were significantly greater with elevated CO_2 by 16–17%. This increase in net C 'uptake' was accompanied by statistically significant increases in the uptake of N, K, and Mg, and significant increases in the requirement of N, K, Ca, and Mg (Table 3). Foliage and litterfall N concentrations were significantly lower with elevated CO_2, but this was offset by the increases in foliage biomass and litterfall mass such that foliage N content and litterfall N flux were unaffected by treatment (Tables 2–4). Nitrogen concentrations in fine roots did not differ significantly with treatment, and thus there was a large (but statistically non-significant) increase in root N turnover. The effects of treatment on biomass-weighted (proportional sample) wood concentrations are not known, but no significant treatment effects on new wood N concentrations were noted in core samples taken in 1999 (R.J. Norby, unpublished data).

Throughfall fluxes of N and P measured by resin collectors were substantially greater than those measured in normal water collectors (Table 3). We suspect that the resin collectors are more accurate, as the waters could have experienced changes between collection periods in the field, especially during the summer. The resin collectors showed a positive net canopy exchange (NCE, which equals throughfall minus precipitation), indicating that foliar leaching took place. In contrast, the water collectors showed negative NCE values, indicating that foliar uptake occurred. In both cases, the absolute value of NCE is added to total uptake (the assumption being that positive NCE, or foliage N leaching, arises from soil N uptake and that negative NCE indicates foliar uptake; Johnson et al. 1991). Thus, despite the differences in NCE between resin and water collectors, total N and P uptake values did not differ significantly by collector type. NCE values were relatively small compared to other components of N and P uptake in any event. In the cases of K, Ca, and Mg no resin data were collected; however, we are not concerned with spoilage between collections for those nutrients. There were no treatment effects on throughfall fluxes of K, Ca, or Mg.

Table 2. Ecosystem carbon and nutrient contents in ambient and elevated CO_2 plots in 1999.

Component	Carbon (kg ha^{-1})		Nitrogen (kg ha^{-1})		Phosphorus (kg ha^{-1})		Potassium (kg ha^{-1})		Calcium (kg ha^{-1})		Magnesium (kg ha^{-1})	
	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient
Vegetation												
Tree												
Foliage	2.5	2.2***	79	76	6.6	5.8	39	36	68	55***	18	17
Woody*	66.1	66.5	153	164	24	23	212	214	554	557	83	84
Fine roots	2.1	1.2	32	18	3.1	2.1	18	11	28	18	9	5
Total tree	70.7	69.9	264	258	33.7	30.9	269	261	650	630	110	106
Understory	0.5	0.4	24	20	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Total veg.	71.2	70.3	288	278	33.7	30.9	269	261	650	630	110	106
Forest floor	1.8	1.7	43	40	4.4	4.1	6	5	68	63	8	8
Soil	94.2	72.8	9794	8561	69.2	90.6	670	660	8750	6920	1100	1000
Total ecosystem	167.2	144.8	10125	8879	107.3	125.6	945	926	9468	7613	1218	1114

Includes branch, bole, and stump.

N.D. = Not determined.

*,***, and *** refer to statistically significant differences, $p < 0.10$, 0.05, and 0.01, respectively, students *t*-test.

Table 3. Average ecosystem carbon and nutrient fluxes in ambient and elevated CO_2 plots for 1999–2000. For nitrogen and phosphorus, fluxes in atmospheric deposition, throughfall, and soil leaching were measured using both resin collectors and traditional water collectors (funnels for deposition and throughfall, tension lysimters for soil solution) and calculations for uptake and translocation are given using both measurements. O horizon leaching was collected only with resin collectors.

Component	Carbon ($mg\,ha^{-1}$)		Nitrogen ($kg\,ha^{-1}$)		Phosphorus ($kg\,ha^{-1}$)		Potassium ($kg\,ha^{-1}$)		Calcium ($kg\,ha^{-1}$)		Magnesium ($kg\,ha^{-1}$)	
	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient
Atmos. dep.												
Water collectors	N.D.	N.D.	15	15	1.5	1.5	6	6	7	7	3.4	3.4
Resin collectors	N.D.	N.D.	12	12	1.7	1.7						
Throughfall												
Water collectors	N.D.	N.D.	6	7	0.6	0.8	15	15	14	17	2.9	3.2
Resin collectors	N.D.	N.D.	15	14	2.2	2.6						
Net canopy exch.												
Water collectors	N.D.	N.D.	−9.1	−7.7	−0.9	−0.7	25	22 **	78	76	16.5	16.8
Resin collectors	N.D.	N.D.	3	1	0.7	1.1						
Litterfall	2.5	2.1**	38	39	4.0	4.4	9	9	7	7	−0.5	−0.2
Root turnover	2.1	1.2	32	18	3.1	2.1	21	19	44	40	7.4	6.8
Wood increment	6.7	6.1	18	16	3.0	2.7	18	11	28	18	8.7	5.5
Uptake												
Water collectors	11.1	9.4	97	79 *	10.7	9.2	73	60 **	158	143	34.3	29.9*
Resin collectors	11.1	9.4	93	76 *	10.5	9.5						

Requirement	11.1	9.5	130	113 *	12.8	10.9	80	66*	144	114**	35.4	29.3*
Translocation												
Water collectors	0.1	0.1	33	34	2.0	1.6						
Resin collectors	0.1	0.1	37	37	2.4	1.3						
Leaching												
O horizon (resin)	N.D.	N.D.	8	10 *	4.5	3.4*						
A horizon (resin)	N.D.	N.D.	6	8	0.7	0.8						
A horizon (water)	N.D.	N.D.	0.6	1.5	0.2	0.2						

Includes branch, bole, and stump.

N.D. = Not determined.

*, and *** refer to statistically significant differences, $p < 0.10$, 0.05, and 0.01, respectively, students *t*-test.

Both foliar and litterfall N concentrations were significantly lower with elevated CO_2, but this was offset by increase in biomass such that foliage N content and litterfall N flux were not significantly different between treatments (Tables 3 and 4). Litterfall K concentrations and fluxes were significantly greater with elevated CO_2, but there were no significant treatment effects on litterfall concentrations or fluxes of P, Ca, or Mg. Among the micronutrients, the only treatment effects on tissue concentrations were in the cases of Fe in foliage (significantly lower with elevated CO_2), B in litterfall (lower with elevated CO_2) Cu in fine roots (lower with elevated CO_2) and Zn in fine roots (higher with elevated CO_2) (Table 4).

The differences in concentrations between live foliage and litterfall are the result of the processes of translocation (either into or out of foliage between the time of sampling and senescence), foliar leaching, foliar uptake, and the weight loss of leaves between the time of foliage sampling (mid summer) and senescence. Nitrogen, P, K, Mn, and Zn all showed decreases in concentration between live foliage and litterfall, indicating that translocation back into the tree plus foliar leaching offset foliar uptake and weight loss (which averaged 7%) (Table 4). Calcium, Fe, Cu, and B showed increases in concentration between live foliage and litterfall in excess of what could be accounted for by the weight loss, indicating that translocation into foliage and foliar uptake offset foliar leaching. Their slight increases in Mg concentration between live foliage and litterfall could be accounted for the weight loss alone. There were no statistically significant treatment effects on the percentage change in concentrations from live foliage to litterfall except in the case of Fe, where there was a greater net increase with elevated CO_2 (not shown). In the case of N, the percent declines in concentrations between live foliage and litterfall were nearly identical in ambient ($49 \pm 2\%$) and elevated ($48 \pm 1\%$) CO_2.

There were slight but statistically significant treatment effects on C:K (lower with elevated CO_2) and C:Ca (higher with elevated CO_2) ratios in uptake (Table 5). There were no statistically significant treatment effects on C:nutrient ratios in uptake for N, P, or Mg or for any nutrient in requirement (Table 5).

Soil solution concentrations and leaching

Throughfall and soil solution concentrations were monitored from April 1999, through March 2001. There were no statistically significant treatment effects on throughfall concentrations (Table 6). There were very large temporal variations in soil solution concentrations, especially in the case of NO_3^- (Figure 1). We also saw evidence of 'hotspots' with high NO_3^- in one control plot – details of this phenomenon will be discussed in a later paper. Despite the large variations, there were statistically significant treatment effects on soil solution pH (higher under elevated CO_2), NH_4^+, SO_4^{2-}, and Ca^{2+} (all lower under elevated CO_2) (Figure 1 and Table 6). There were no statistically significant treatment effects on leaching in either the O or A horizons as measured by resin lysimeters when statistical tests were performed using all replicates within each ring in a nested design (rings nested within treatment). When plot averages only were used, however, NO_3^- leaching was

Table 4. Nutrient concentrations in foliage and litterfall. Data are means of concentrations measured in 1999 and 2000 in two elevated or three ambient plots ± SE.

	mg g^{-1}					μg g^{-1}				
	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn	Fe	Cu	B	Zn
Foliage										
Ambient	16.6 ± 0.5	1.3 ± 0.01	7.6 ± 0.3	12.0 ± 0.8	3.6 ± 0.2	512 ± 29	16 ± 7	4.2 ± 0.5	24 ± 1	151 ± 6
Elevated	14.7 ± 0.3*	1.2 ± 0.00	7.6 ± 1.0	13.3 ± 1.1	3.6 ± 0.2	474 ± 29	13 ± 7***	3.8 ± 0.3	27 ± 2	152 ± 13
Litterfall										
Ambient	8.5 ± 0.2	0.81 ± 0.05	4.5 ± 0.4	16.7 ± 0.5	3.5 ± 0.1	456 ± 25	48 ± 8	5.3 ± 0.6	32 ± 1	91 ± 22
Elevated	7.6 ± 0.1**	0.75 ± 0.04	4.9 ± 0.5**	15.3 ± 0.4	3.3 ± 0.1	435 ± 24	31 ± 1	4.5 ± 0.3	28 ± 1*	69 ± 35
Fine roots										
Ambient	7.3 ± 2.8	0.81 ± 0.05	4.3 ± 1.1	6.8 ± 0.2	2.1 ± 0.1	291 ± 6	1079 ± 47	23.9 ± 1.6	19 ± 1	23 ± 1
Elevated	7.3 ± 3.4	0.75 ± 0.04	4.1 ± 1.7	6.5 ± 0.3	2.0 ± 0.1	311 ± 23	965 ± 54	19.8 ± 0.9*	18 ± 1	26 ± 2**

*,***, and *** refer to statistically significant differences, $p < 0.10$, 0.05, and 0.01, respectively, students *t*-test.

Table 5. Carbon production per unit nutrient uptake and requirement and ANOVA tests for treatment and year effects. C/NR = carbon/nitrogen using resin throughfall data, C/PR carbon/phosphorus using resin throughfall data. Average of values for 1999 and 2000.

Ratio	C:Nutrient in uptake ($kg\,kg^{-1}$)		C:Nutrient in requirement ($kg\,kg^{-1}$)	
	Ambient	Elevated	Ambient	Elevated
C:N*				
Water collectors	119	116	84	87
Resin collectors	124	121	84	87
C:P*				
Water collectors	1031	1036	887	888
Resin collectors	991	1085	887	888
C:K	157	153*	144	141
C:Ca	66	71*	83	79
C:Mg	315	326	325	320

significantly lower with elevated CO_2 and ortho-P was significantly higher with elevated CO_2 (Table 3).

Soil nutrient availability

In addition to standard soil analyses, we have measured treatment effects on soil available N and P by various methods, including *in situ* incubations, Plant Root Simulator (c) probes (commercially available resin membranes imbedded in plastic stakes), and, during this last year, with resin capsules using the WECSA© system. None of these measurements revealed any treatment effects on soil N or P availability (Table 7).

Soil microbial biomass C and surface CO_2 efflux

Soil microbial biomass C as measured by the fumigation–extraction method increased to some degree under elevated CO_2 compared to the ambient CO_2 treatment across all four sampling dates, even though only the June 1999 sampling date showed a statistically significant ($P<0.05$) difference (Figure 2). Percent enhancement by the CO_2 enrichment ranged from 5 to 23%. These results indicated a weak trend of an increased soil microbial activity and possibly an increased microbial immobilization of some mineral nutrients such as N. The amount of N immobilized into this enhanced microbial biomass in the elevated CO_2 treatment represented approximately 4–22 $kg\,N\,ha^{-1}$ in the 0–20 cm top soil layer (assuming a bulk density of 1.2 and a microbial biomass C:N ratio equal to 7). This amount is similar in magnitude as N needed for net woody tissue increment.

Table 6. Probability values for repeated measures ANOVA tests on the effects of treatment and time effects on soil solution concentrations. (EC = electrical conductivity, ANC = acid neutralizing capacity.)

	pH	EC	ANC	SO_4^{2-}	Cl^-	NO_3^-	NH_4^+	NH_4^+	Mg^{2+}	K^+	Na^+	Ortho-P
Throughfall												
Treatment	0.87	0.94	I.S.	1.00	0.64	0.28	0.43	0.68	0.90	0.86	0.88	0.42
Time	0.01	0.15	I.S.	0.35	0.09	<0.01	0.05	0.34	0.33	0.27	0.01	0.01
*Treat * Time*	0.34	0.81	I.S.	0.91	0.81	0.68	0.47	0.97	0.94	0.55	0.91	0.78
Soil solution												
Treatment	0.01	0.49	0.79	0.01	0.98	0.12	0.07	<0.01	0.48	0.87	0.36	0.77
Time	0.61	0.31	<0.01	0.84	<0.01	0.08	0.01	<0.01	0.18	0.48	<0.01	0.30
Treat * Time	0.80	0.54	0.94	0.97	0.57	0.61	0.17	0.79	0.97	0.79	1.00	0.97

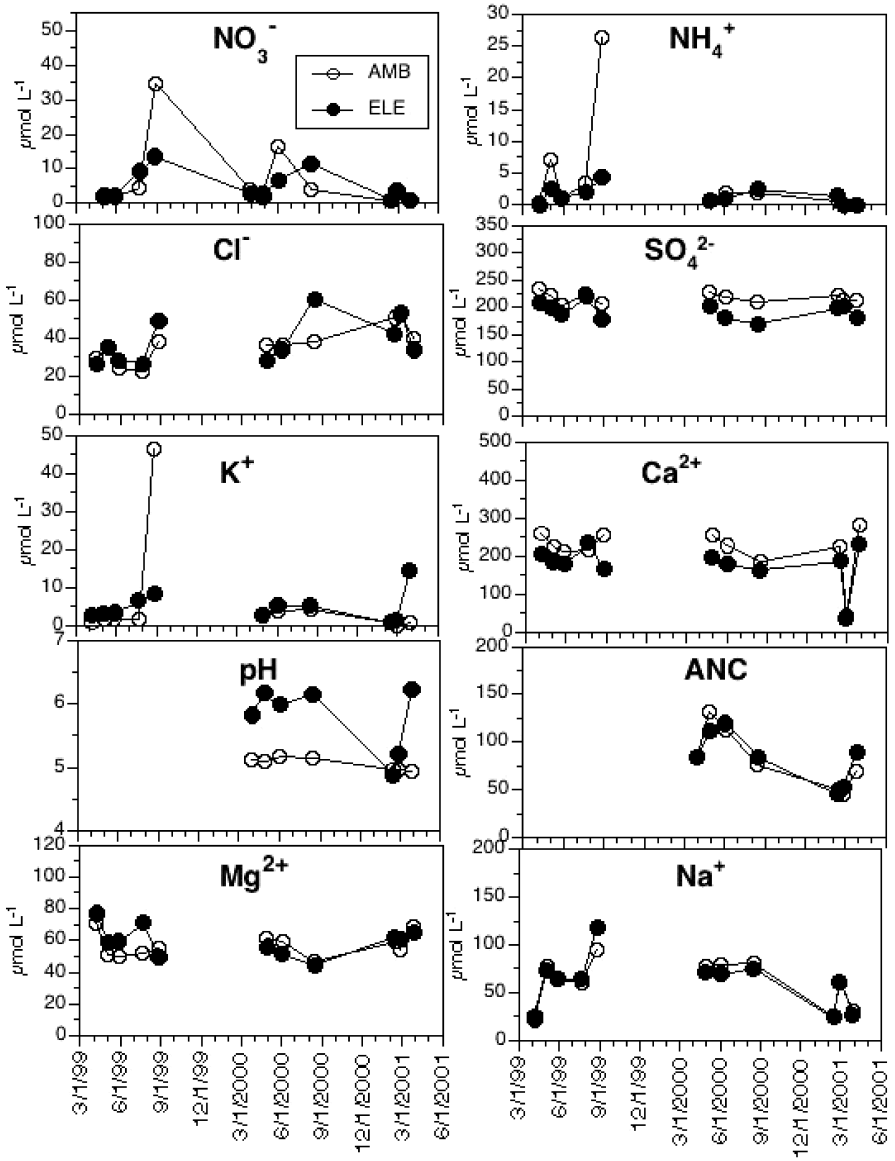

Figure 1. Soil solution concentrations under ambient and elevated CO_2. See Table 6 for statistical analyses.

Fine root decomposition and nutrition

Results from both the field and laboratory experiments showed no treatment effects on fine root decomposition. Results of the laboratory incubations are shown in Figure 3. Both summed CO_2 evolution and calculated root mass loss were virtually

Table 7. Soil N availability as measured by *in situ* incubation, Plant Root Simulator Probes®, and resin capsules.

Mineral N and N mineralization in soils (July 1999) ($mg\,kg^{-1}$)			
Initial			
Ambient	2.30 ± 0.34	13.05 ± 1.39	15.35 ± 1.51
Elevated	2.96 ± 0.44	13.05 ± 0.77	16.00 ± 0.83
Final			
Ambient	9.45 ± 1.70	15.67 ± 2.11	25.12 ± 3.52
Elevated	9.30 ± 2.60	13.78 ± 1.22	23.07 ± 3.56
Net mineralization			
Ambient	7.15 ± 1.69	2.62 ± 2.67	9.77 ± 4.01
Elevated	6.34 ± 2.65	0.73 ± 1.18	7.07 ± 3.63
PRS probes (July 1999) ($\mu mol\,cm^{-2}$)			
Ambient	0.033 ± 0.006	0.043 ± 0.002	0.076 ± 0.006
Elevated	0.039 ± 0.008	0.040 ± 0.003	0.079 ± 0.009
Resin capsules (July 2001) ($mmol\,cm^{-2}$)			
Ambient	0.21 ± 0.06	0.73 ± 0.05	0.93 ± 0.09
Elevated	0.25 ± 0.07	0.74 ± 0.09	1.00 ± 0.14

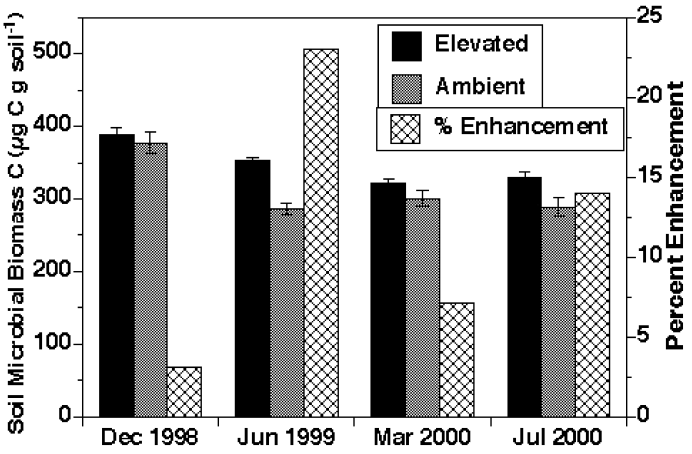

Figure 2. Soil microbial biomass C in elevated (filled bar) and ambient (open bar) CO_2 treatments in the top soil layer (0–20 cm), and percent enhancement (crossed bar) by the elevated CO_2 treatment over the ambient treatment using values from the ambient treatment as 100%.

equal across treatments, with approximately 35% mass lost over the 360-day incubation period. As in the laboratory experiment, fine root decomposition rates were nearly identical between treatments in the field experiment (42% loss under elevated CO_2 and 41% in the ambient treatment). Despite the fact that laboratory roots were kept constantly moist and at a constantly higher temperature (22 °C) than field roots (soil temperatures in the field ranged from approximately 2 to

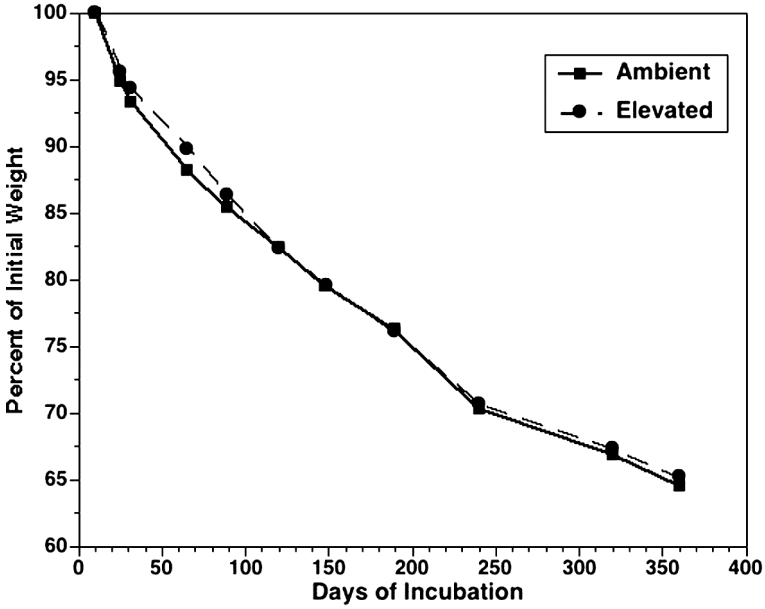

Figure 3. Cumulative rate of root mass loss as calculated from rates of root decomposition by treatment as measured by CO_2 evolution during laboratory incubations. Mass loss expressed as percentage of initial mass.

20 °C), roots incubated in soil actually decayed at a slightly faster average rate (0.112% per day v.s. 0.097% per day).

Following the 12-month field incubations, the partially decomposed roots were again analyzed for nutrients. The only statistically significant treatment difference was for Ca, which was significantly higher (11%) in roots grown and decomposing under elevated CO_2 ($p = 0.02$). Pre-incubation treatment differences in concentrations of Zn and Cu disappeared following a year of decomposition. For 7 of the 10 elements (P, K, Mg, Mn, B, Zn, Fe) total nutrient content in the roots declined substantially in both treatments, with losses ranging from 38 to 69% (data not shown). Copper and Ca contents declined very little under elevated CO_2 (0 and 10%, respectively) and only slightly in the ambient (17 and 22%, respectively). Although the C:N ratio declined in both treatments during a year of field decomposition (from 61 to 40) there were no treatment differences, and N was not effectively immobilized by decomposing fine roots. A small percentage of the N content was lost from roots in both treatments (13–16%).

Discussion

Hypothesis 1 (N constraints to growth response to elevated CO_2 will be mitigated primarily by increased biomass production per unit uptake rather than by increased uptake) was not supported by the results of this study: both uptake and requirement

of N were significantly greater with elevated CO_2. The reductions in foliar and litterfall N concentrations were almost exactly offset by the increases in foliar and litter biomass such that foliage N content ($kg\,ha^{-1}$) and litterfall N flux ($kg\,ha^{-1}\,year^{-1}$) were unaffected by elevated CO_2. The increased uptake and requirement were due largely to the greater fine root production and N turnover; fine root N concentrations were unaffected by elevated CO_2. Elevated CO_2 had no effect on percent translocation, and thus there was a potential for reduced litter quality and decomposition rates (Strain 1982). In this stand, however, such effects were minimal: the soil had been invaded by earthworms and they had accelerated decomposition to the point where forest floor pools were very small.

The observation that N uptake increased with elevated CO_2 implies that either the soil N pool was tapped or that N_2 fixation increased in order to meet the increased demand. This is similar to findings in other studies of elevated CO_2 (Johnson et al. 1997; 2000a, 2003; Finzi et al. 2002). The increased uptake with elevated CO_2 (17–18 $kg\,ha^{-1}\,year^{-1}$) constitutes 22% of total uptake under ambient CO_2 and only a small fraction (<0.02%) of total soil N pools in the site. Mineralizeable N, as assessed by the in situ incubations (Table 7), constituted about 50 $kg\,N\,ha^{-1}$, or about 2% of the total N pool in the Ap horizon (0–15 cm). If the same ratio were applied to the entire soil profile, mineralizeable N would total about 200 $kg\,ha^{-1}$. Thus, it would seem that the added 17–18 $kg\,N\,ha^{-1}$ for increased N uptake with elevated CO_2 could have been obtained from the mineralizeable N pool – although it should be added that no treatment effects on the mineralizeable N pool were observed as of 1999.

If the soil N pool was indeed tapped for the additional N needed for uptake, it would imply that roots somehow out-competed microbes for available N in the soil. This implication runs contrary to previously-held views of soil N cycling (Paul and Clark 1989), but is implicit in other studies of elevated CO_2 where N uptake is increased with no apparent source other than the soil (e.g., Johnson et al. 1997, 2000a; Finzi et al. 2002). Further confounding this issue is the fact that we found that microbial activity and microbial N uptake were enhanced with elevated CO_2. Thus, as in other studies, the exact source of and mechanisms facilitating the additional N for uptake by both roots and microbes remains unclear.

Hypothesis 2 was largely supported by the results of this study: elevated CO_2 did not cause lower tissue concentrations of P, K, Ca, or Mg (although it did cause greater litterfall K concentrations). With the exception of the very slight (8%) increase in C:Ca ratio in uptake, there elevated CO_2 did not cause greater on C:nutrient ratios in either uptake or requirement for these nutrients. Elevated CO_2 caused significantly greater requirement and uptake of K, Ca, and Mg but not P. However, the magnitudes of the differences in P uptake between the ambient and elevated CO_2 treatments (9 and 17% as calculated from water and resin data, respectively) were not substantially different from those in Ca (10%) or Mg (15%); thus, the statistical analyses may indicate more of a difference between the response of P and that of the other nutrients than is real.

A major caveat in all uptake and requirement calculations is the fact that no woody nutrient concentration data are available from the actual treatment plots, as

noted in the Methods section. Thus, the data summarized in Tables 2, 3, and 5 represent samples taken from outside the plots and woody concentrations are assumed to be equal among all plots and treatments. Limited analyses of wood samples taken with shallow cores in some trees within the treatment plots confirmed that N concentrations did not vary among treatments, but there was insufficient sample for other nutrient analyses.

Soil exchangeable pools of P, K, Ca, and Mg were very large and there would seem to be no possibility that a deficiency in these nutrients will develop any time soon under either ambient or elevated CO_2. As to the possibility of an N deficiency, previous research on an adjacent stand of sycamore (*Platanus occidentalis*) at a much earlier age (1–3 years old) revealed a positive growth response to N fertilization (Miegroet et al. 1995). However, we do not know if this 'deficiency' applies to the sweetgum stand studied here at this stage of stand development. The N increments in these stands (16–18 $kg\,ha^{-1}\,year^{-1}$) approximately equal atmospheric N deposition (12–15 $kg\,ha^{-1}\,year^{-1}$), as is the case in nearby Walker Branch Watershed (Johnson et al. 1982). (We note that atmospheric N deposition rates measured here were somewhat higher than the value of 10 $kg\,ha^{-1}\,year^{-1}$ measured with much more sophistication at a nearby *Pinus taeda* L. stand during 1985–1988; Johnson and Lindberg 1992).

The reduction in O horizon N leaching, while very small compared to uptake, supports the conjecture that N is still limiting in this stand. Greater uptake with elevated CO_2 was probably also a factor in the observed lower soil solution NH_4^+ concentration. The reasons for the greater O horizon P leaching with elevated CO_2 are unclear; they may reflect pre-treatment differences due to site factors (as might the differences in N leaching); however, the fact that soil extractable P pools were initially larger in the ambient than in the elevated CO_2 plots does not support this conjecture. Perhaps the greater pH in soil solutions with elevated CO_2 caused P desorption from soils; however, how this became reflected in litter P fluxes is not immediately clear.

Many of the differences in soil solution concentrations with elevated CO_2 are difficult to explain. The greater soil solution pH and nearly identical ANC values with elevated CO_2 suggests that soil pCO_2 was lower in the elevated CO_2 treatment – a result contrary to the surface CO_2 efflux data obtained here and in previous studies (Johnson et al. 1995; Andrews and Schlesinger 2001). Differences in uptake could account for the lower soil solution Ca^{2+} concentrations and leaching with elevated CO_2 (Table 3); it is more probable, however, the differences in soil solution Ca^{2+} were a simple result of charge balance considerations resulting from the lower soil solution SO_4^{2-} (i.e., reduced mineral acid anion concentrations must be accompanied by reduced cation concentrations in order to maintain charge balance). Reasons for the reduced soil solution SO_4^{2-} with elevated CO_2 are not clear. Although we do not have uptake data, but we do not feel that uptake was a major factor, based on previous research which has shown that S uptake is generally much lower than leaching in forests of this area (Johnson et al. 1982). Soil solution pH differences do not explain the differences in soil solution SO_4^{2-}, either: the greater soil solution pH with elevated CO_2 would be expected to cause higher soil

solution SO_4^{2-} (and perhaps also ortho-P) concentrations because of desorption from soils. Dilution effects due to lower water flux with elevated CO_2 were also considered but discarded as a possible explanation for the lower soil solution SO_4^{2-} concentrations. Wullschleger and Norby (2001) found 13% lower sap velocity in trees under elevated CO_2 at this site, but concluded that the effects on stand-level annual transpiration would be minimal. This conclusion is supported by the fact that Cl^- fluxes were unaffected by treatment. Thus, dilution by greater water flux under elevated CO_2 does not seem to be a likely explanation for the observed lower soil solution SO_4^{2-} concentrations.

Summary and conclusions

The first hypothesis of this study (N constraints to growth response to elevated CO_2 will be mitigated primarily by increased biomass production per unit uptake rather than by increased uptake) was not supported: elevated CO_2 caused increased N uptake and requirement, mostly because of increased fine root turnover. The observed reductions in foliar and litterfall N concentrations with elevated CO_2 were largely offset by increases in production. The mineralizeable N pool in the soil seemed to be adequate to have supplied the increased N uptake with elevated CO_2.

The second hypothesis (constraints of P, K, Ca, and Mg to growth response to elevated CO_2 will be mitigated primarily by increased uptake rather than by increased biomass production per unit uptake) was largely supported by the results of this study: elevated CO_2 caused significantly greater uptake and requirement of K, Ca, and Mg, did not cause significantly lower tissue concentrations of P, K, Ca, or Mg, and caused only a slight increase in C:Ca ratios in uptake. Elevated CO_2 had no significant effects on fine root C:nutrient ratios or on fine root decomposition rate.

Elevated CO_2 had no significant effect on any measure of soil nutrient availability. There were significant treatment effects on soil leaching and soil solution concentrations of N that could be explained by differences in uptake, but other differences in leaching and concentrations of ortho-P, SO_4^{2-}, Ca^{2+}, and pH were difficult to explain.

In general, the effects of elevated CO_2 on nutrient cycling in this study were not large. The additional uptake of P, K, Ca, and Mg with elevated CO_2 constituted small fractions of the soil exchangeable pools of these nutrients and should present no constraints to continued responses. The additional uptake of N constituted a very small portion of the total N reserves and between 10 and 40% of the estimated mineralizeable N pools in the soil. The potential negative feedbacks via the litterfall N cycle were moot, given the very high turnover rate of the forest floor due to the presence of earthworms. At this stage, N limitations do not seem to impose a firm constraint on continued growth response to elevated CO_2 in this stand. Potential constraints by N limitations in the future will depend on growth rates, N demand, changes in atmospheric N deposition, and changes in the many factors affecting N mineralization in the soil.

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