Ecology, 87(1), 2006, pp. 5–14
© 2006 by the Ecological Society of America

NITROGEN UPTAKE, DISTRIBUTION, TURNOVER, AND EFFICIENCY OF USE IN A CO_2-ENRICHED SWEETGUM FOREST

RICHARD J. NORBY[1,3] AND COLLEEN M. IVERSEN[2]

[1]*Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-6422 USA*
[2]*Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Tennessee 37996 USA*

Abstract. The Progressive Nitrogen Limitation (PNL) hypothesis suggests that ecosystems in a CO_2-enriched atmosphere will sequester C and N in long-lived biomass and soil organic pools, thereby limiting available N and constraining the continued response of net primary productivity to elevated [CO_2]. Here, we present a six-year record of N dynamics of a sweetgum (*Liquidambar styraciflua*) stand exposed to elevated [CO_2] in the free-air CO_2 enrichment (FACE) experiment at Oak Ridge, Tennessee, USA. We also evaluate the concept of PNL for this ecosystem from the perspective of N uptake, content, distribution, and turnover, and N-use efficiency. Leaf N content was 11% lower on a leaf mass basis (N_M) and 7% lower on a leaf area basis (N_A) in CO_2-enriched trees. However, there was no effect of [CO_2] on total canopy N content. Resorption of N during senescence was not altered by [CO_2], so N_M of litter, but not total N content, was reduced. The N_M of fine roots was not affected, but the total amount of N required for fine-root production increased significantly, reflecting the large stimulation of fine-root production in this stand. Hence, total N requirement of the trees was higher in elevated [CO_2], and the increased requirement was met through an increase in N uptake rather than increased retranslocation of stored reserves. Increased N uptake was correlated with increased net primary productivity (NPP). N-use efficiency, however, did not change with CO_2 enrichment because increased N productivity was offset by lower mean residence time of N in the trees. None of the measured responses of plant N dynamics in this ecosystem indicated the occurrence of PNL, and the stimulation of NPP by elevated [CO_2] was sustained for the first six years of the experiment. Although there are some indications of developing changes in the N economy, the N supply in the soil at this site may be sufficient to meet an increasing demand for available N, especially as the roots of CO_2-enriched trees explore deeper in the soil profile.

Key words: carbon dioxide; FACE; free-air CO_2 enrichment; Liquidambar styraciflua; *net primary productivity; nitrogen uptake; nitrogen-use efficiency; Oak Ridge, Tennessee; plant N dynamics; progressive nitrogen limitation; retranslocation of reserves; sweetgum.*

INTRODUCTION

Forest response to CO_2 enrichment of the atmosphere has long been thought to be controlled by N availability (Kramer 1981, Strain and Bazzaz 1983, Oren et al. 2001, Hungate et al. 2003). Free-air CO_2 enrichment (FACE) experiments support the conclusions from smaller scale experiments that the N status of an ecosystem can influence the responses of C assimilation and plant production to elevated [CO_2] (Nowak et al. 2004). Conversely, widespread occurrence of increased net fractionation of ^{15}N between soil and leaf in FACE experiments provides evidence that elevated [CO_2] also has an important impact on N dynamics of diverse ecosystems (BassiriRad et al. 2003). Ecosystem models also indicate an important modifying role of N in CO_2 response through interactions involving litter quality and decomposition, litter quantity and soil N immobilization, and root production and N uptake or availability (McMurtrie et al. 2000).

Luo et al. (2004) have proposed the concept of progressive N limitation (PNL): the process by which available soil N becomes increasingly limited in CO_2-enriched ecosystems as C and N are allocated to long-lived biomass or soil organic matter (SOM) pools. Increased productivity and the corresponding increased demand for N are predicted to exacerbate any existing N limitation within the ecosystem and eventually limit the productivity response to elevated [CO_2]. Results of CO_2 enrichment experiments have been ambiguous with respect to the occurrence of PNL, and evaluation of forest responses has been particularly problematic. The vast majority of data on C–N interactions in CO_2-enriched trees comes from individual plants or expanding systems that are acquiring increasing amounts of N. Mature or non-expanding forests, however, recycle most of their N (Johnson et al. 2004), and this feature cannot be simulated in short-term studies with seedlings and saplings (Norby et al. 1999). FACE ex-

Manuscript received 23 December 2004; revised 31 March 2005; accepted 6 May 2005. Corresponding Editor: Y. Luo. For reprints of this Special Feature, see footnote 1, p. 3.

[3] E-mail: rjn@ornl.gov

periments in forests provide an opportunity to test the concepts of PNL over several years in realistic experimental systems in which the treatments provide minimal disturbance to the soil environment and the N cycle is in a recycling phase, as is the case with the closed-canopy deciduous forest in the Oak Ridge FACE experiment that we evaluate here. Observations sustained over many years are important to test the premise that CO_2 effects are transient (Luo and Reynolds 1999), although experiments to date still are short relative to the temporal scale of tree growth and forest development, and many models predict that it will take at least 10 years for potential declines in productivity response to develop (McMurtrie et al. 2000, Nowak et al. 2004). FACE experiments must be maintained as long as possible to predict the impacts of elevated [CO_2] as adjustments in C–N interactions occur, but the ecological concepts that are tested in the near term by FACE experiments (Nowak et al. 2004) can help to evaluate and refine hypotheses such as PNL.

We start our analysis with the premise that, although PNL is dependent on physical and microbial process in the soil controlling N availability, N limitation starts with plant physiology (N uptake, N-use efficiency, resorption) and ultimately affects ecosystem response through plant physiology (N deficiency, changes in growth and allocation). Hence, detailed and sustained observations on N dynamics in CO_2-enriched trees will inform the analysis of PNL. Here, we present a six-year record of N dynamics of a sweetgum (*Liquidambar styraciflua*) stand exposed to elevated [CO_2] in the FACE experiment at Oak Ridge, Tennessee, USA. Net primary productivity (NPP) in this stand has been significantly higher in CO_2-enriched plots, and the response has been sustained through time (Norby et al. 2002, 2004), thereby meeting one of the criteria for the development of PNL. The question we ask here is whether the sustained increase in NPP has altered the uptake and dynamics of N in this ecosystem in such a way that N availability will decline and create a negative feedback on continued stimulation of NPP. Understanding current N dynamics in this ecosystem is important for projecting trajectories of response into the future.

Methods

Experimental site and treatments

The experimental site is a sweetgum (*Liquidambar styraciflua* L.) plantation that was established in 1988 on the Oak Ridge National Environmental Research Park in Roane County, Tennessee (35°54′ N, 84°20′ W). The soil at the site, which is classified as an Aquic Hapludult (Wolftever Series), has a silty clay loam texture and is moderately well drained. It is slightly acidic (water pH ~5.5–6.0) with high base saturation largely dominated by exchangeable Ca. Soil bulk density is 1.5 g/cm^3, C content is 74 Mg/ha, and N content is 11 Mg/ha. Mineralizable N in the entire soil profile was estimated to be ~200 kg/ha in 2001 (Johnson et al. 2004). 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 (Norby et al. 2001*b*).

Five plots 25 m in diameter were laid out in 1996, and FACE apparatus (Hendrey et al. 1999) was assembled in four of them; site disturbance was minimized during construction. When pretreatment measurements were made in 1997, the trees (~90/plot) were 12 m tall with average diameter of 11 cm and stand basal area of 28 cm^2/m^2. The trees were in a linear growth phase, leaf area index was 5.5 m^2/m^2, and the canopy was no longer expanding (Norby et al. 2003). 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 1998–2003 growing seasons was 544 ppm in the two CO_2-enriched plots, including periods when the exposure system was not functioning, and 391 ppm in ambient plots. The site and experimental design have been fully described elsewhere (Norby et al. 2001*b*).

Sample collection and analysis

Leaves (including petioles) were collected from the canopy (see Plate 1) during the growing season from a permanently installed hydraulic lift situated in the center of the plot. Four leaves were collected from each 1 m height increment of the canopy (measured from the ground). Usually the leaves came from four different trees, but occasionally (especially at the top and bottom of the canopy) different branches of the same tree were collected. Immature and senescent leaves were avoided. Collections were made on 4 August 1998; 26 May, 4 August, and 23 September 1999; 15 June and 16 August 2000; 15 May, 17 July, and 27 August 2001; 23 July, 30 August, and 30 September 2002; and 24 July 2003. Combined area of the four leaves was measured (LI-COR 3100; LI-COR, Lincoln, Nebraska, USA), and for some collections (1999–2002) leaf thickness was measured to enable calculation of volume and density. Leaves were then oven-dried and weighed. C and N concentrations of finely ground subsamples were determined on a Carlo Erba NA-1500 analyzer (Carlo Erba Elantech, Lakewood, New Jersey, USA), using atropine as a standard and orchard leaf NIST apple leaf (SRM1515; N concentration of 22.5 mg/g) as an internal quality check (National Institute of Standards and Technology, Gaithersburg, Maryland, USA).

The average N content per unit leaf mass (N_M, in milligrams per gram) of the canopy was calculated as a weighted average of N_M in each layer. The weighting factor was based on the previously determined relative distribution of leaf area with height of live canopy (Norby et al. 2001*b*). Foliar N content also was expressed on a leaf area basis (N_A, milligrams per square

PLATE 1. Leaves were sampled from throughout a sweetgum (*Liquidambar styraciflua*) canopy to determine their nitrogen content. The trees in 25-meter diameter plots were exposed to an elevated concentration of carbon dioxide by releasing CO_2-enriched air through vertical vent pipes seen in the background. Photo credit: R. J. Norby.

meter) by multiplying N_M by leaf mass per unit area (LM_A, grams per square meter) for each layer. Canopy height was converted to canopy depth such that the top of the canopy in a given year had a depth of zero. Peak canopy N content (N_{TOT}, kilograms per hectare) was calculated from the canopy-averaged N_M in July or August and peak canopy biomass of the 20 m diameter plot (Norby et al. 2003).

Leaf litter was collected approximately monthly during the spring and summer, and at least weekly during September and October from seven baskets (0.2 m^2 each) per plot (Norby et al. 2003). Each collection was oven-dried and weighed. At the end of the year, all of the collections for each basket were combined, thoroughly mixed, subsampled, and analyzed for C and N concentrations as previously described. N_M of fine roots (<1 mm diameter) was determined on samples collected for other purposes, including ingrowth cores (Norby et al. 2004), microbial (Sinsabaugh et al. 2003), and root respiration (George et al. 2003) measurements. Hence, the sampling methods (time of year, depth) varied in different years, but in all cases, at least two samples per plot were collected. The fine-root samples were washed from soil, oven-dried, ground, and analyzed for C and N concentrations as previously described. The total amount of N used in fine-root production was calculated as the product of annual fine-root productivity (Norby et al. 2004) and N_M.

Biomass and net primary production

Calculation of N budgets and N-use efficiency on a whole-stand basis required annual data on production and biomass of stand components and net primary production (NPP). These data sets and the methods by which they were assembled have been described previously. Briefly, stand-level wood increment was calculated from allometric equations using measurements of stem circumference, tree height and taper, and wood density (Norby et al. 2001*b*). Total annual leaf litter mass production was calculated from litter trap collections (Norby et al. 2001*b*), and peak canopy mass was determined from peak leaf area index and LM_A, as described by Norby et al. (2003). Annual fine-root production and peak fine-root standing crop were determined using minirhizotrons, camera, and digitizing (Bartz Technology Corporation, Santa Barbara, California, USA; see Norby et al. [2004] for details). Five minirhizotrons per plot were surveyed every two weeks, and the data on fine-root length production, mortality, and standing crop per tube were converted to mass per unit land area based on specific root length and scaling factors (Norby et al. 2004). Calculation of

NPP based on these data was described by Norby et al. (2002) and updated in Norby et al. (2004).

Calculation of uptake and N-use efficiency

Annual N requirement and uptake (in kilograms per hectare) were defined by Cole and Rapp (1981) and calculated as in Johnson et al. (2004). Requirement, which is the amount of N needed for production of new tissue, was defined as the N content of the canopy at peak mass plus N in fine-root production and woody increment. The N requirement of trees is met through new uptake and remobilization of stored N, and the amount available for remobilization is assumed to be the difference between peak canopy N mass and the amount of N lost in litterfall and net leaching. Hence, uptake was defined as the N content of litter, annual fine-root production, annual wood increment (bole, branches, and coarse roots), and throughfall minus deposition. This calculation of uptake assumes that fine-root N is not resorbed into perennial tissue prior to root death (Nambiar and Fife 1991). The N_M of wood was assumed to be a constant 1.86 mg/g based on analysis of samples of trees cut down during FACE site construction. Analyses of cores removed annually from trees in the experimental plots produced a similar value (1.89 ± 0.05 mg/g, mean ± SE; range 1.55–2.51 mg/g), but they were of insufficient resolution for more detailed calculations of N content of wood. Throughfall minus deposition was taken as a constant value of 6 kg/ha (Johnson et al. 2004).

Nitrogen-use efficiency (NUE, in megagrams of dry mass per kilogram of N) was calculated using the approach described by Berendse and Aerts (1987) by dividing annual NPP (in megagrams of dry mass per hectare) of each plot by annual N uptake (kilograms of N per hectare). There are two components to NUE: N productivity (A_N, NPP divided by plant N content), and mean residence time (MRT) of N in the plant (Berendse and Aerts 1987), which are related as

$$\mathrm{NUE}\left(\frac{\text{g NPP/yr}}{\text{g NPP uptake/yr}}\right) = A_N\left(\frac{\text{g NPP/yr}}{\text{g N content}}\right) \times \mathrm{MRT}\left(\frac{\text{g N content}}{\text{g N uptake/yr}}\right).$$

Plant N content was taken as the sum of N in wood increment (i.e., not including N in old wood), leaves at peak canopy mass, and fine roots at peak fine-root standing crop. MRT was calculated as peak N content divided by uptake; it is the reciprocal of N turnover rate.

Data were analyzed by ANOVA for a completely random design with the plot as the experimental unit (n = 3 plots for ambient [CO_2] and n = 2 plots for elevated [CO_2]). Interactions between treatment and canopy depth or year were tested when appropriate.

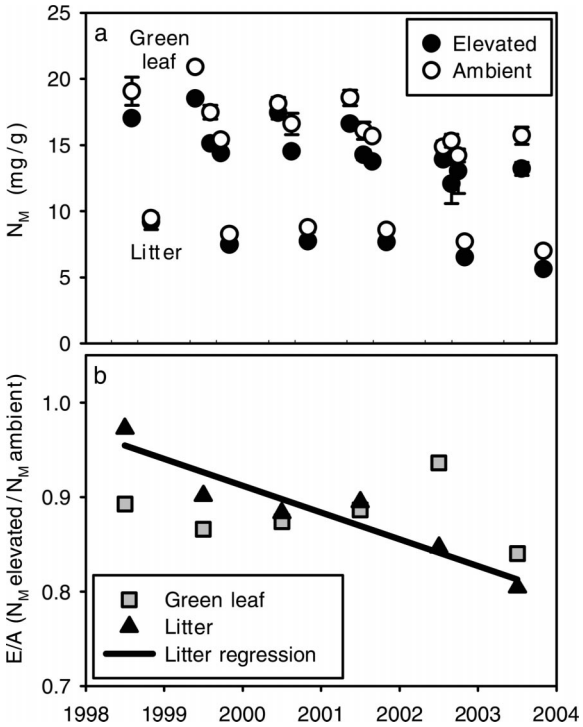

FIG. 1. (a) N_M (N content, on a leaf mass basis) of green leaves and fresh leaf litter representing whole-canopy averages. Data are the means ± SE of three plots in ambient (A) levels of CO_2 or two plots in elevated (E) levels of CO_2; if SE bars are not visible, they are smaller than the symbol. (b) The relative response to CO_2 (E/A) of N_M of leaves in July or August and N_M of litter. The regression equation for litter is: E/A = −0.0283 × year + 57.5; R^2 = 0.88, $P < 0.006$.

RESULTS

Whole-canopy nitrogen concentration (N_M) varied seasonally, with higher values in May and June when the canopy was still developing and little change after canopy development was complete in mid-July (Fig. 1a). N_M always was lower in CO_2-enriched plots, and except in the first year of treatment (1998), the difference in canopy N_M was also observed in leaf litter. The average of all measurements of canopy N_M was 16.8 mg/g in ambient [CO_2] and 14.9 mg/g in elevated [CO_2], a difference of 11% ($P < 0.001$). Leaf litter N_M over six years of observation was 8.5 mg/g and 7.7 mg/g in ambient and elevated [CO_2], a 10% difference ($P < 0.001$). The relative effect of CO_2 enrichment on midsummer, whole-canopy N_M was fairly constant year to year (range 6.4–13.4%), but the effect on litter N_M increased in magnitude from 2.8% in 1998 to 19.6% in 2003 (Fig. 1b). The linear regression of the relative CO_2 response (E/A) for litter N_M vs. time was statistically significant (R^2 = 0.88, $P < 0.006$).

The whole-canopy N concentrations in Fig. 1a are the weighted averages of concentrations measured at 1-m intervals throughout the vertical extent of the canopy. In ambient [CO_2] N_M (averaged over the six years

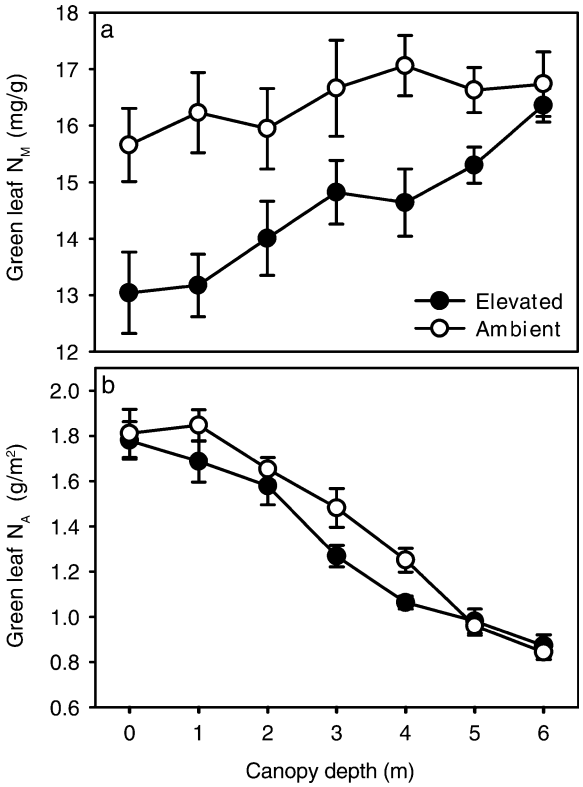

FIG. 2. Nitrogen distribution in the canopy. (a) N_M in the canopy in July or August as a function of canopy depth. (b) N_A (N content, on a leaf area basis) as a function of canopy depth. Data are the means ± SE of three plots in ambient [CO_2] or two plots in elevated [CO_2] averaged over six years.

of observation) was constant with canopy depth (Fig. 2a). When expressed on a leaf area basis (N_A), foliar N content declined with depth in canopy (Fig. 2b), reflecting the similar decline in LM_A (Fig. 3a). Canopies in elevated [CO_2] showed similar patterns, but with some significant departures. N_M was lower in elevated [CO_2] than in ambient plots at every depth, but the CO_2 effect was greater toward the top of the canopy (CO_2 × depth: $P < 0.04$). The CO_2 effect on N_M at the top of the canopy can be attributed to a dilution effect: LM_A was higher in elevated [CO_2] only at the top of the canopy (CO_2 × depth: $P < 0.002$). This increase in LM_A in elevated [CO_2] was associated primarily with increased leaf density (Fig. 3b), which was probably related, in part, to higher content of nonstructural carbohydrates (Sholtis et al. 2004). There was no effect of [CO_2] on leaf thickness (Fig. 3c). In addition to the dilution of N with more dry matter per unit leaf, there also was a direct effect of [CO_2] on N content per unit leaf area: N_A was 8% lower in elevated [CO_2] ($P < 0.001$). If the canopy is characterized as comprising three layers, there was a significant ($P < 0.041$) effect of CO_2 on distribution of N. There was no difference in N_A at the top of the canopy, but elevated [CO_2] reduced N_A in the middle layer (Fig. 2b).

The reduced concentration of N in CO_2-enriched canopies was offset by increased leaf mass production (Norby et al. 2003) such that there was no effect on peak canopy N mass (Fig. 4a). N mass in litter also was unaffected by [CO_2], although there was an increasing deficit of N returned in elevated plots compared to that in ambient plots (Fig. 4b). The linear regression of E/A for litter N_{TOT} vs. time was highly significant ($R^2 = 0.95$, $P < 0.001$). The relative difference between peak canopy N_{TOT} and litter N_{TOT}, which is the fraction resorbed during senescence, averaged 51% and was not affected by CO_2 treatment.

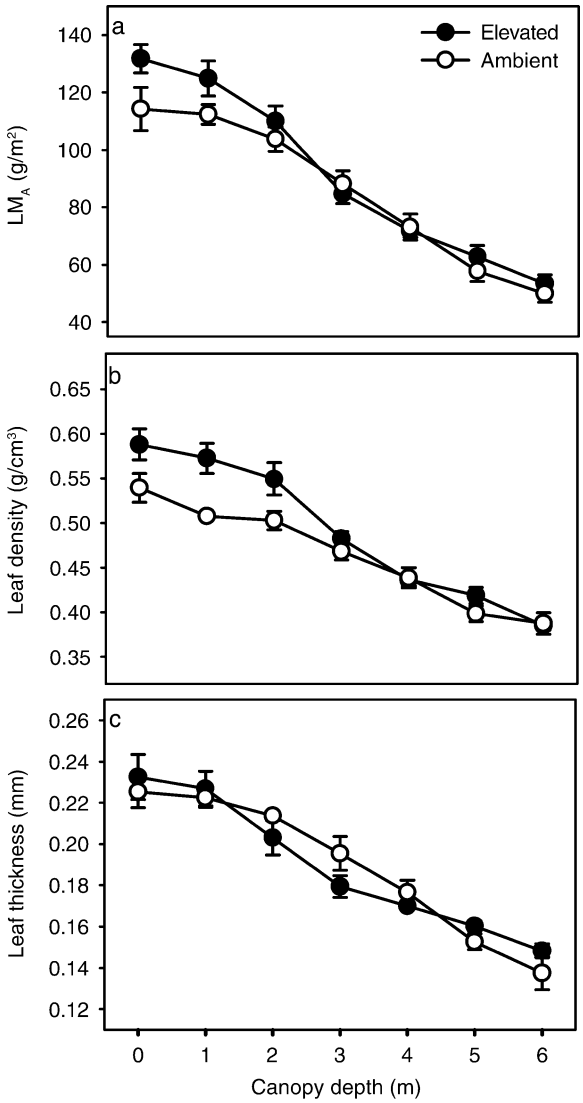

FIG. 3. Leaf morphological characteristics: (a) leaf dry mass per unit area (LM_A); (b) leaf density (dry mass per unit leaf volume); and (c) leaf thickness. Data are the means ± SE of three plots in ambient [CO_2] or two plots in elevated [CO_2] averaged over six years (for LM_A) or four years (for density and thickness). Error bars may be obscured by the data symbol.

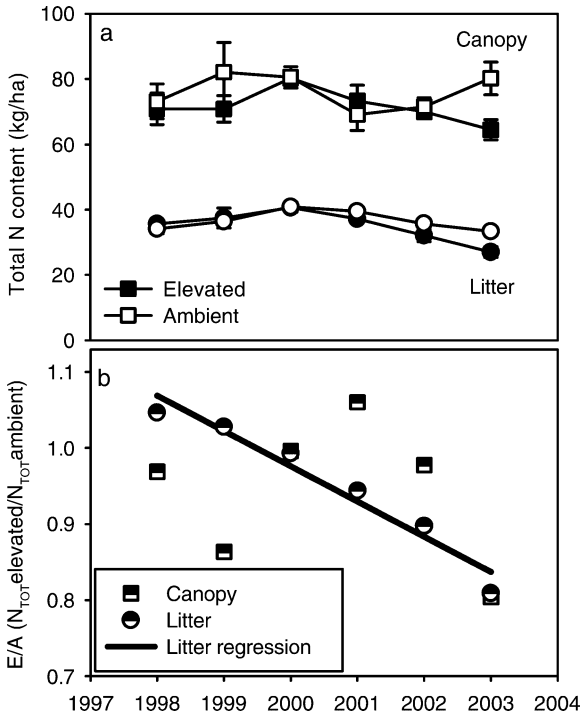

FIG. 4. (a) Nitrogen content (N_{TOT}) per unit land area of the canopy at peak biomass and total N returned to the soil in leaf litter. Data are the means ± SE of three plots in ambient [CO_2] or two plots in elevated [CO_2]. (b) The relative response (E/A, elevated/ambient) of N_{TOT} to elevated [CO_2]. The regression equation for litter is: E/A = −0.0465 × year + 93.9; $R^2 = 0.95$, $P < 0.001$.

Fine-root N_M was more variable than leaf N_M, both within and between years (Fig. 5a). There was a significant effect of year ($P < 0.004$), but no effect of CO_2 or the CO_2 × year interaction. Assuming that N_M in fine roots did not vary during the year (data are insufficient to test this assumption), the total amount of N used in fine-root production (Fig. 5b) reflected the large effect of [CO_2] on root production beginning in 2000 (Norby et al. 2004). Total fine-root N (per unit land area) varied with year ($P < 0.09$) and increased significantly with [CO_2] ($P < 0.011$). These data represent primarily roots less than 0.5 mm in diameter. Larger diameter roots (up to 2 mm) had lower N_M, but minirhizotron analysis indicated that 85% of fine-root production was in roots smaller than 0.5 mm (Norby et al. 2004).

Annual N uptake (Fig. 6) was significantly higher in elevated [CO_2] ($P < 0.006$), with an average increase over the six years of 29%. Most of the difference was in the fine-root component, but this assumes that N_M of wood was not affected by CO_2 enrichment. Wood N_M was very low (<2 mg/g) and small differences were very difficult to detect; however, the entire range of observed values of wood N_M alters the calculated N uptake by only −4% to +8%. Uptake accounted for 74% of annual N requirement in ambient CO_2 and 79% in elevated [CO_2] (significantly different at $P < 0.02$), with retranslocation accounting for the remaining fraction of requirement.

N uptake and NPP were strongly correlated: NPP of each plot increased as N uptake increased (Fig. 7). The NUE isopleths indicate that NUE declined as N uptake increased; CO_2-enriched plots generally had higher N uptake so they were shifted toward lower NUE (Fig. 7). NUE tended to be lower in elevated [CO_2] in all but the first year, but was not significantly different. NUE calculated alternatively as NPP divided by N requirement (e.g., Finzi et al. 2002) also did not differ between treatments. NUE was unresponsive to [CO_2] because of the offsetting responses of the two components of NUE: A_N was higher in elevated [CO_2] ($P < 0.058$), whereas MRT was significantly lower ($P < 0.014$). This pattern was reversed in 2001, a year with an especially large response of fine roots to CO_2 enrichment and, hence, relatively less N in leaves to support C assimilation.

Discussion

The concept of PNL (progressive nitrogen limitation) derives from the principle that formation of organic matter requires N and other nutrients in relatively fixed proportions with C. If elevated [CO_2] causes accumulation of plant biomass or SOM, then N will be sequestered along with C, and N availability will pro-

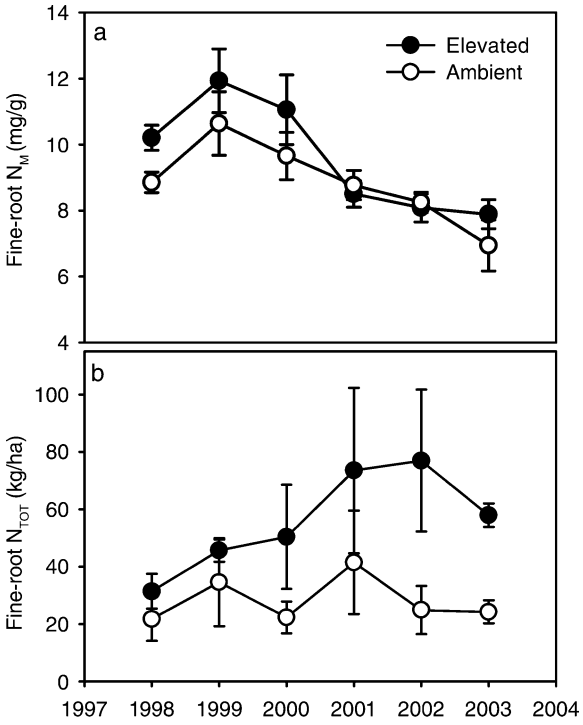

FIG. 5. (a) N_M in fine roots and (b) total amount of N used in annual fine-root production per unit land area. Data are the means ± SE of three plots in ambient [CO_2] or two plots in elevated [CO_2].

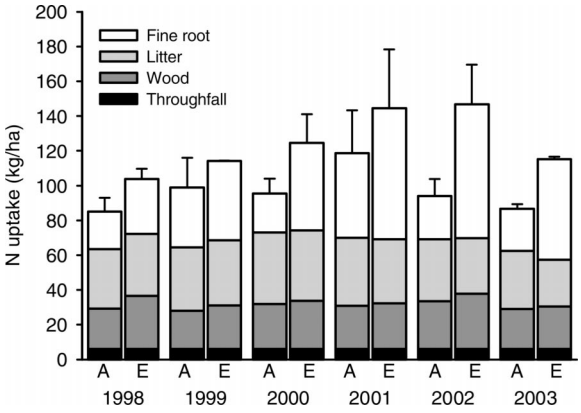

FIG. 6. Annual nitrogen uptake per unit land area in ambient (A) or elevated (E) [CO_2]. The components of N uptake are throughfall minus deposition, wood increment, leaf litter, and fine-root production. Data are the means ± SE of three plots in ambient [CO_2] or two plots in elevated [CO_2] of total uptake.

gressively decline unless compensated by additional N supplies or reduced losses. The progressive decline in N availability constrains the long-term responses of NPP and C storage (Luo et al. 2004). To evaluate whether PNL is likely to occur in the sweetgum stand, we must first ask whether stimulation of NPP in elevated [CO_2] is causing additional sequestration of C and N in long-lived (woody) plant biomass or SOM pools. If that is the case, we can then consider whether PNL already is developing by evaluating the effects of elevated [CO_2] on plant N pools and fluxes. Luo et al. (2004) list four parameters that can be sensitive, albeit partial, indicators of PNL, and here we have presented six-year records on three of them: (1) canopy N amount, (2) whole-plant N amount, and (3) annual N uptake. A fourth indicator, declining N mineralization rate in the soil, can also be evaluated.

C and N sequestration

In the Oak Ridge FACE experiment, elevated [CO_2] caused a sustained increase in NPP, but the additional C was allocated to fast-turnover pools and did not accumulate in plant biomass after the first year of treatment (Norby et al. 2002, 2004). Unless there was a large, undetected, and unprecedented increase in N_M of wood (and most available evidence indicates that elevated CO_2 causes no change or small reductions in N_M of wood; Cotrufo and Ineson 2000, Kaakinen et al. 2004), there is no evidence that significantly more N was sequestered in long-lived woody biomass in the CO_2-enriched trees. Nevertheless, elevated [CO_2] stimulated the input of C and N to soil pools through fine-root turnover, and if some fraction of the C and N in dead fine roots is protected from microbial decomposition, enhanced sequestration into long-lived SOM pools may result. Jastrow et al. (2005) documented increased soil C in the top 5 cm of soil in the sweetgum plantation in response to elevated [CO_2], amounting to an average accrual rate of 44 ± 9 g $C \cdot m^{-2} \cdot yr^{-1}$. A portion of accrued soil C was stabilized by association with soil minerals. The C:N ratio of SOM is substantially lower than that of woody biomass, so SOM accumulation in response to CO_2 enrichment would more likely lead to N sequestration and PNL than woody biomass accumulation.

Canopy N amount

Canopy N amount can be used to indicate whether soil N availability is limiting C production (Luo et al. 2004). There was no effect of elevated [CO_2] on the N_{TOT} of the sweetgum canopy, but whole-canopy N concentration (N_M) was 11% lower in elevated [CO_2]. This effect was a result both of lower N content (N_A) of mid-canopy leaves and the dilution effect of increased LM_A at the top of the canopy. Averaged over the whole canopy and all six years, the CO_2 effect on N_A (8% decline) was insufficient to translate into a consistent effect on whole-canopy N content. The relative responses of N_M and N_A in this experiment were consistent with 30–40 observations on younger, field-grown angiosperm trees of 16 species, which averaged 14% and 6% less in elevated [CO_2] (Norby et al. 1999). However, that analysis indicated that the response diminished with duration of exposure, whereas the current experiment shows no such trend. The response of the sweetgum canopy also is strikingly similar to that of 16 other species in four other FACE experiments: 12% decline in N_M and 7% decline in N_A (Ellsworth et al. 2004).

The maintenance of canopy N in CO_2-enriched trees is important for sustaining enhanced photosynthetic uptake and C production, which was observed in this experiment at the leaf level as increased PNUE (Sholtis

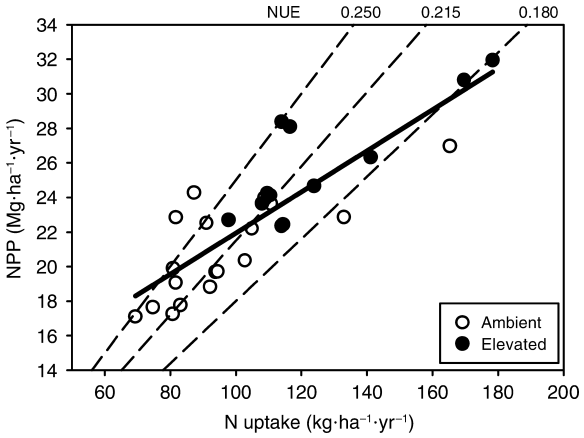

FIG. 7. The relationship between net primary productivity (NPP) and annual N uptake. Each point represents one plot and one year. The dotted lines represent isopleths of nitrogen-use efficiency expressed as Mg NPP per kg N. The solid line is a regression line: NPP = 0.119 × N uptake + 10.1 ; R^2 = 0.72, $P < 0.001$.

et al. 2004) and at the ecosystem level as increased GPP (DeLucia et al. 2005). In other FACE studies, the effects of CO_2 enrichment on photosynthesis and carboxylation capacity occurred primarily through changes in leaf N rather than through a fundamental change in the relationship between the photosynthetic machinery and N (Ellsworth et al. 2004).

The distribution of N in the canopy also is an important link between N supply and C production. N content (N_A) declined with canopy depth, as has been observed in other sweetgum canopies (Kuers and Steinbeck 1998). The gradient of N in the canopy helps to optimize N use with respect to maximization of photosynthesis (Takeuchi et al. 2001). CO_2 enrichment had only minor effects on N distribution, consistent with theoretical analysis (Hikosaka and Hirose 1998).

Nevertheless, N_M may have been less than optimal for tree growth. Whole-canopy N_M in the FACE experiment usually was less than the critical N_M (18 mg/g) reported as necessary for achieving 90% of maximum stem growth in sweetgum plantations (Scott et al. 2004*a*), and the deficit was greater in elevated [CO_2]. If soil N availability were larger, such that canopy N amount was increased, N_M would be closer to the critical value and stem production would be expected to increase.

Plant N content

Plant N content is a sensitive indicator of PNL because changes in the plant N pool are easier to detect than changes in soil N pools. Luo et al. (2004) state that an increase in plant N indicates that soil N supply also has increased, but an initial increase in plant N followed by a decline over time could indicate PNL. Here, plant N content was significantly higher (17%) in elevated [CO_2], and there was no trend through time that would indicate PNL. The increased N content, however, does not indicate increased N sequestration in biomass, which would represent a primary mechanism underlying PNL. Most of the increase in peak N content was in fine roots, which have a short residence time (Norby et al. 2004). The effect of elevated [CO_2] on N content of woody tissue was difficult to determine accurately, but it is unlikely that there was an important effect of [CO_2] on N sequestration in biomass because (1) woody dry matter increment was not significantly increased by CO_2 enrichment after the first year, (2) wood increment accounted for only 5% of peak N content, and (3) there was no indication of substantial changes in N_M of wood. Other observations of the response of wood N_M to elevated CO_2 have shown either no effect (Kaakinen et al. 2004) or small decreases (Cotrufo and Ineson 2000). Consistent with our results, Finzi et al. (2002) reported that in the loblolly pine (*Pinus taeda*) FACE experiment, the fine-root N increment was the only component that increased significantly in elevated [CO_2], and there was no increase in the concentration of N in wood.

Annual N uptake

The third indicator of PNL suggested by Luo et al. (2004) is annual N uptake. If elevated [CO_2] initially stimulates uptake but the response declines with time, PNL is indicated. In the sweetgum stand, however, N uptake was consistently higher in elevated [CO_2], and there was no indication of a declining response over the six years of observation. Hence, this third plant indicator also tells us that PNL was not occurring in the sweetgum stand. The increase in plant N uptake was strongly correlated with the CO_2 effect on NPP. This is not to suggest that increased NPP was *caused* by increased N uptake; it is also legitimate to suggest that increased N uptake was caused by the enhancement of NPP. Indeed, the increase in NPP was associated with more root production, and N uptake was positively correlated with root-length duration (Norby et al. 2004).

NPP could increase in elevated [CO_2] without increased N uptake if N-use efficiency also were increased, as was observed in the pine FACE experiment in two of four years (Finzi et al. 2002). Increased NUE is a mechanism whereby PNL is avoided, at least temporarily (Luo et al. 2004). However, NUE did not increase in the sweetgum stand because gains in A_N were offset by lower MRT. In this sweetgum stand, higher A_N in elevated [CO_2] can be explained by the effect on PNUE, which is primarily a function of the increased substrate (CO_2) supply for photosynthesis rather than a change in N metabolism (Sholtis et al. 2004). MRT is controlled, in part, by the resorption of N from organs prior to abscission. Resorption efficiency from leaves (51%) was similar to the expected value for resorption efficiency in sweetgum once foliar demand reaches a steady state (Kuers and Steinbeck 1998, Scott et al. 2004*b*). However, foliar resorption efficiency was not affected by CO_2 enrichment, as also reported in sweetgum by Herrick and Thomas (2003). The lower MRT in elevated [CO_2] was associated with the large fraction of N content in fine roots, organs that have a relatively short life and from which N apparently is not retranslocated prior to abscission (Nambiar and Fife 1991). As a result of the shorter MRT in elevated [CO_2], relatively less of the annual N requirement was met by retranslocation, and the additional N requirement instead was associated with increased uptake, as was also observed in the loblolly pine FACE experiment (Finzi et al. 2002).

N mineralization

A fourth indicator of PNL discussed by Luo et al. (2004), declining N mineralization rate, was not evaluated here, but previous analysis (Zak et al. 2003) revealed no effect of elevated [CO_2] on microbial N cycling. One factor that might alter this in the future is a CO_2 effect on litter quality. A meta-analysis of N_M of naturally senesced litter from field experiments in-

dicated only small effects of [CO_2], especially under realistic field conditions (Norby et al. 2001*a*), despite prior predictions that a CO_2 effect on litter quality would be an important pathway regulating ecosystem response (Strain and Bazzaz 1983). In this sweetgum stand, N_M of leaf litter has been consistently (except for the first year) and significantly lower (by 10%) in elevated [CO_2]. We cannot yet say whether this effect on litter quality has or will translate into an effect on N mineralization. Leaf litter does not accumulate in this forest, probably because of high earthworm activity (Johnson et al. 2004). There also was no effect of [CO_2] on total N return in leaf litter; however, a deficit in N return was becoming increasingly large each year, and this may be an advanced indicator of developing PNL that will bear close observation in the future. The recycling of fine-root N may also affect N mineralization and availability. Fine-root N_M was not affected by CO_2 enrichment (this study), nor was fine-root decomposition rate (Johnson et al. 2004), but more detailed analyses are needed to determine if these responses vary with depth in the soil profile.

Conclusions and future trajectories

None of the measured responses of plant N dynamics in this system indicated the occurrence of PNL as discussed by Luo et al. (2004), and the stimulation of NPP by elevated [CO_2] was sustained over the first six years of the experiment. N cycled through the trees faster in elevated [CO_2], as did C, but only minor changes in the distribution or use of N were noted. The effects of [CO_2] that were apparent occurred at the interface between C and N cycles: N_M at the top of the canopy was lower because of increased LM_A, and A_N was increased because of enhanced PNUE. These observations are especially significant because this is the longest observation period for N dynamics in a non-expanding deciduous forest exposed to elevated [CO_2]. We cannot, however, say that PNL will not start developing in this experimental forest sometime in the future. Two indications that might suggest a change in the N economy are the increasing deficit of N return in leaf litter and the greater reliance of CO_2-enriched trees on uptake rather than retranslocation to meet their N requirement. However, the N supply in the soil may be sufficient to meet an increasing demand for available N (Johnson et al. 2004), especially as the roots of CO_2-enriched trees explore deeper in the soil profile (Norby et al. 2004).

We can propose different scenarios or trajectories of response into the future. To date, the additional C taken up from the CO_2-enriched atmosphere was allocated primarily to fine-root production, which enabled increased access to available N pools in the soil. The increased N that was taken up, however, was used primarily to support the additional fine-root production in what appears to be a futile cycle (Norby et al. 2004). One possible outcome could be that once the more extensive and deeper root system is established, the C allocated to root production and the additional N taken up by those roots will be allocated to aboveground processes, and woody increment will be enhanced by elevated [CO_2]. Over the longer term, increased wood production will increase the retention time of N in the plant, thereby further increasing the reliance on uptake to meet the N requirement and pushing the system toward PNL. Another possible response trajectory might be that the enhanced cycling of C and N through the fine-root system continues in response to CO_2 enrichment, leading to protection of some fraction of that C and associated N in SOM pools, lowering N availability, and also pushing the system toward PNL. These scenarios would change if there were new exogenous sources of available N to the ecosystem, such as increased atmospheric deposition or the introduction of N_2 fixers. It also is possible that the system will continue functioning as it is described here: increased NPP but no additional accumulation of woody biomass, no apparent N limitation, and sufficient soil N reserves to support sequestration into SOM without reducing N availability and creating a PNL. However, CO_2 enrichment experiments in this sweetgum plantation and other forests may never be of sufficient duration to resolve these long-term adjustments between the C and N cycles.

Acknowledgments

This work would not have been possible without the help of many project participants who assisted with the collection and analysis of plant samples, including Johnna Sholtis, Sara Jawdy, Carolyn Reilly, Joanne Ledford, Caroline DeVan, Don Todd, and Kenneth Lowe. Research was sponsored by the U.S. Department of Energy, Office of Science, Biological and Environmental Research. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract DE-AC05-00OR22725.

Literature Cited

BassiriRad, H., J. V. H. Constable, J. Lussenhop, B. A. Kimball, R. J. Norby, W. C. Oechel, P. B. Reich, W. H. Schlesinger, S. Zitzer, H. L. Sehtiya, and S. Silim. 2003. Widespread foliage δ ^{15}N depletion under elevated CO_2: inferences for the nitrogen cycle. Global Change Biology **9**: 1582–1590.

Berendse, F., and R. Aerts. 1987. Nitrogen-use efficiency: a biologically meaningful definition? Functional Ecology **1**: 293–296.

Cole, D. W., and M. Rapp. 1981. Elemental cycling in forest ecosystems. Pages 341–409 *in* D. E. Reichle, editor. Dynamic properties of forest ecosystems. Cambridge University Press, London, UK.

Cotrufo, M. F., and P. Ineson. 2000. Does elevated atmospheric CO_2 concentration affect wood decomposition? Plant and Soil **224**:51–57.

DeLucia, E. H., D. J. Moore, and R. J. Norby. 2005. Contrasting responses of forest ecosystems to rising atmospheric CO_2: implications for the global C cycle. Global Biogeochemical Cycles **19**:GB3006.

Ellsworth, D. S., P. B. Reich, E. S. Naumburg, G. W. Koch, M. E. Kubiske, and S. D. Smith. 2004. Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment exper-

iments in forest, grassland and desert. Global Change Biology **10**:2121–2138.

Finzi, A. C., E. H. DeLucia, J. G. Hamilton, D. D. Richter, and W. H. Schlesinger. 2002. The nitrogen budget of a pine forest under free air CO_2 enrichment. Oecologia **132**:567–578.

George, K., R. J. Norby, J. G. Hamilton, and E. H. DeLucia. 2003. Fine-root respiration in a loblolly pine and sweetgum forest growing in elevated CO_2. New Phytologist **160**:511–522.

Hendrey, G. R., D. S. Ellsworth, K. F. Lewin, and J. Nagy. 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. Global Change Biology **5**:293–309.

Herrick, J. D., and R. B. Thomas. 2003. Leaf senescence and late-season net photosynthesis of sun and shade leaves of overstory sweetgum (*Liquidambar styraciflua*) grown in elevated and ambient carbon dioxide concentrations. Tree Physiology **23**:109–118.

Hikosaka, K., and T. Hirose. 1998. Leaf and canopy photosynthesis of C3 plants at elevated CO_2 in relation to optimal partitioning of nitrogen among photosynthetic components: theoretical prediction. Ecological Modelling **106**: 247–259.

Hungate, B. A., J. D. Dukes, M. R. Shaw, Y. Luo, and C. B. Field. 2003. Nitrogen and climate change. Science **302**: 1512–1513.

Jastrow, J. D., R. M. Miller, R. Matamala, R. J. Norby, T. W. Boutton, C. W. Rice, and C. E. Owensby. 2005. Elevated atmospheric CO_2 increases soil carbon. Global Change Biology, *in press*.

Johnson, D. W., W. Cheng, J. D. Joslin, R. J. Norby, N. T. Edwards, and D. E. Todd, Jr. 2004. Effects of elevated CO_2 on nutrient cycling in a sweetgum plantation. Biogeochemistry **69**:379–403.

Kaakinen, S., K. Kostiainen, F. Ek, P. Saranpää, M. E. Kubiske, J. Sober, D. F. Karnosky, and E. Vapaavuori. 2004. Stem wood properties of *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* saplings after 3 years of treatments to elevated carbon dioxide and ozone. Global Change Biology **10**:1513–1525.

Kramer, P. J. 1981. Carbon-dioxide concentration, photosynthesis, and dry-matter production. BioScience **31**:29–33.

Kuers, K., and K. Steinbeck. 1998. Leaf area dynamics in *Liquidambar styraciflua* saplings: responses to nitrogen fertilization. Canadian Journal of Forest Research **28**:1660–1670.

Luo, Y., and J. F. Reynolds. 1999. Validity of extrapolating field CO_2 experiments to predict carbon sequestration in natural ecosystems. Ecology **80**:1568–1583.

Luo, Y., et al. 2004. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. BioScience **54**:731–739.

McMurtrie, R. E., R. C. Dewar, B. E. Medlyn, and M. P. Jeffreys. 2000. Effects of elevated [CO_2] on forest growth and carbon storage: a modeling analysis of the consequences of changes in litter quality/quantity and root exudation. Plant and Soil **224**:135–152.

Nambiar, E. K. S., and D. N. Fife. 1991. Nutrient retranslocation in temperate conifers. Tree Physiology **9**:185–207.

Norby, R. J., M. F. Cotrufo, P. Ineson, E. G. O'Neill, and J. G. Canadell. 2001*a*. Elevated CO_2, litter chemistry, and decomposition: a synthesis. Oecologia **12**:153–165.

Norby, R. J., P. J. Hanson, E. G. O'Neill, T. J. Tschaplinski, J. F. Weltzin, R. A. Hansen, W. Cheng, S. D. Wullschleger, C. A. Gunderson, N. T. Edwards, and D. W. Johnson. 2002. Net primary productivity of a CO_2-enriched deciduous forest and the implications for carbon storage. Ecological Applications **121**:261–1266.

Norby, R. J., J. Ledford, C. D. Reilly, N. E. Miller, and E. G. O'Neill. 2004. Fine-root production dominates response of a deciduous forest to atmospheric CO_2 enrichment. Proceedings of the National Academy of Sciences (USA) **101**: 9689–9693.

Norby, R. J., J. D. Sholtis, C. A. Gunderson, and S. S. Jawdy. 2003. Leaf dynamics of a deciduous forest canopy: no response to elevated CO_2. Oecologia **136**:574–584.

Norby, R. J., D. E. Todd, J. Fults, and D. W. Johnson. 2001*b*. Allometric determination of tree growth in a CO_2-enriched sweetgum stand. New Phytologist **150**:477–487.

Norby, R. J., S. D. Wullschleger, C. A. Gunderson, D. W. Johnson, and R. Ceulemans. 1999. Tree responses to rising CO_2: implications for the future forest. Plant, Cell and Environment **22**:683–714.

Nowak, R. S., D. S. Ellsworth, and S. D. Smith. 2004. Functional responses of plants to elevated atmospheric CO_2: do photosynthetic and productivity data from FACE experiments support early predictions? New Phytologist **162**: 253–280.

Oren, R., D. S. Ellsworth, K. H. Johnsen, N. Phillips, B. E. Ewers, C. Maier, K. V. R. Schafer, H. McCarthy, G. Hendrey, S. G. McNulty, and G. G. Katul. 2001. Soil fertility limits carbon sequestration by forest ecosytems in a CO_2-enriched atmosphere. Nature **411**:469–472.

Scott, D. A., J. A. Burger, D. J. Kaczmarek, and M. B. Kane. 2004*a*. Growth and nutrition response of young sweetgum plantations to repeated nitrogen fertilization on two site types. Biomass and Bioenergy **27**:313–325.

Scott, D. A., J. A. Burger, D. J. Kaczmarek, and M. B. Kane. 2004*b*. Nitrogen supply and demand in short-rotation sweetgum plantations. Forest Ecology and Management **189**:331–343.

Sholtis, J. D., C. A. Gunderson, R. J. Norby, and D. T. Tissue. 2004. Persistent stimulation of photosynthesis by elevated CO_2 in a sweetgum (*Liquidambar styraciflua*) forest stand. New Phytologist **162**:343–354.

Sinsabaugh, R. L., K. Saiya-Cork, T. Long, M. P. Osgood, D. A. Neher, D. R. Zak, and R. J. Norby. 2003. Soil microbial activity in a *Liquidambar* plantation unresponsive to CO_2-driven increases in primary production. Applied Soil Ecology **24**:263–271.

Strain, B. R., and F. A. Bazzaz. 1983. Terrestrial plant communities. Pages 177–222 *in* E. R. Lemon, editor. CO_2 and plants. Westview Press, Boulder, Colorado, USA.

Takeuchi, Y., M. E. Kubiske, J. G. Isebrands, K. S. Pregtizer, G. Hendrey, and D. F. Karnosky. 2001. Photosynthesis, light and nitrogen relationships in a young deciduous forest canopy under open-air CO_2 enrichment. Plant, Cell and Environment **24**:1257–1268.

Zak, D. R., W. E. Holmes, A. C. Finzi, R. J. Norby, and W. H. Schlesinger. 2003. Soil nitrogen cycling under elevated CO_2: a synthesis of forest FACE experiments. Ecological Applications **13**:1508–1514.