Nitrogen limitation in a sweetgum plantation: implications for carbon allocation and storage

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Abstract: The N status of temperate forests is closely linked to their C fluxes, and altered C or N availability may affect ecosystem C storage through changes in forest production and C allocation. We proposed that increased fine-root production previously observed in a sweetgum (*Liquidambar styraciflua* L.) forest in response to elevated [CO_2] was a physiological response to N limitation. To examine this premise, we fertilized plots in the sweetgum plantation adjacent to the Oak Ridge National Laboratory free-air CO_2-enrichment (FACE) experiment. We hypothesized that N fertilization would increase sweetgum net primary production, leaf [N], and the relative flux of C to wood production. Annual additions of 200 kg·ha^{-1} of N as urea increased soil N availability, which increased stand net primary production, stand N uptake, and N requirement by about one-third. Increased leaf [N] and leaf area production in the fertilized plots increased stem production and shifted relative flux of C to wood production. We conclude that sweetgum production on this site is limited by soil N availability and a decreased fraction of net primary production in fine-root production with N addition is consistent with the premise that increased fine-root production in the adjacent FACE experiment is in response to N limitation.

Résumé : Le statut en N des forêts tempérées est étroitement lié au flux de C et une modification de la disponibilité en C ou N peut affecter l'immobilisation de C dans l'écosystème par le biais de changements dans la productivité de la forêt et de l'allocation en C. Nous croyons que l'augmentation de la production de racines fines déjà observée dans une forêt de copalme d'Amérique (*Liquidambar styraciflua* L.) à la suite d'une augmentation de la concentration en CO_2 constitue une réaction physiologique à une limitation en N. Pour vérifier cette prémisse, nous avons fertilisé des parcelles d'une plantation de copalme d'Amérique adjacente au dispositif expérimental de Oak Ridge sur l'enrichissement en CO_2 à l'air libre (FACE). Nous avons posé l'hypothèse que la fertilisation en N augmenterait la production primaire nette et la concentration foliaire en N du copalme d'Amérique et augmenterait la proportion du flux de C allouée à la production de bois. Des amendements annuels de 200 kg·ha^{-1} de N sous forme d'urée ont augmenté la disponibilité en N du sol, ce qui a augmenté d'environ 33 % la production primaire nette du peuplement, le prélèvement en N du peuplement et les besoins en N. La hausse de la concentration foliaire en N et de la production en surface foliaire observée dans les parcelles fertilisées a augmenté la production des troncs et a changé la proportion du flux de C allouée à la production de bois. Nous concluons que la productivité du copalme d'Amérique sur cette station est limitée par la disponibilité du sol en N et qu'une diminution de la proportion de production primaire nette allouée à la production racinaire à la suite d'un amendement en N est conforme à la prémisse que l'augmentation de la production racinaire observée dans l'expérience FACE adjacente est causée par une déficience en N.

[Traduit par la Rédaction]

Introduction

An understanding of the current nutrient status of an ecosystem and how nutrient status is reflected in plant response is important to our ability to predict how ecosystem processes will change over time in response to changing environmental conditions (Vitousek and Howarth 1991; Johnson 2006). The C and N cycles in terrestrial ecosystems are closely linked because N-demanding processes such as photosynthesis (Field and Mooney 1983) and the biosynthesis and decomposition of organic matter (McGuire et al. 1995) draw upon a relatively small soil mineral N pool (Johnson 2006). Soil N availability often limits net primary production (NPP) in terrestrial temperate ecosystems (Vitousek

and Howarth 1991), and over time N limitation may restrict increases in forest production and C storage in response to rising [CO_2] (McGuire et al. 1995; Luo et al. 2004; Johnson 2006). Thus, the links among forest production, N uptake, and soil N availability are important components of models that project forest responses to changing environmental conditions, including increased temperature and N deposition or elevated [CO_2] (Comins and McMurtrie 1993; Pan et al. 1998; McMurtrie et al. 2000, 2001; Kirschbaum et al. 2003).

Plant responses to N limitation are best understood in the context of external inputs and internal recycling that control the resources available for plant uptake and use. Plants often compensate for changes in external resource supply with increased C flux to organs acquiring the most strongly limiting

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resources (Field et al. 1992). For example, alleviation of C limitation under elevated [CO_2] may result in greater fine-root production to satisfy greater demands for nutrients (i.e., Matamala and Schlesinger 2000; Norby et al. 2004), while relative increases in aboveground production, especially of perennial woody tissue, are expected when N limitation is alleviated (Chapin et al. 1986; Field et al. 1992, as reviewed in Litton et al. 2007). Changes in the partitioning of photosynthate to organs of differing N concentrations and longevity affect the rate at which C and N are recycled internally within an ecosystem (i.e., Norby and Iversen 2006). Increased wood production results in greater C fixed in biomass per unit N and increased residence time of C and N in plant biomass, while increased fine-root production results in faster rates of C and N cycling through the ecosystem (Norby and Iversen 2006), given that fine roots often turn over quickly in deciduous forests (Gill and Jackson 2000). Changes in the amount of N required per unit biomass production, the rate and amount of C storage, and the amount of N remineralized and available for plant use have long-term and ecosystem-wide implications for our global forests and climate (Chapin et al. 1986; Rastetter et al. 1997; Hungate et al. 2003; Kirschbaum et al. 2003; Luo et al. 2004).

N limitation may be important in shaping the responses observed in the Oak Ridge National Laboratory (ORNL) free-air CO_2-enrichment (FACE) experiment, in which sweetgum (*Liquidambar styraciflua* L.) trees have been exposed to elevated [CO_2] since 1998. Alleviation of C limitation under elevated [CO_2] resulted in increased wood production (a summation of stemwood and coarse root growth increments) and NPP in the first year of the experiment (Norby et al. 2002), but in subsequent years, increased NPP was accounted for by increased fine-root production (Norby et al. 2004). Instead of facilitating C storage, CO_2 enrichment has increased C and N cycling rates within the sweetgum plantation at ORNL FACE because fine roots have a lower C:N ratio and mean residence time than wood (Norby and Iversen 2006). These observations give rise to two questions: (*i*) Was increased fine-root production due to limited soil N supply? (*ii*) If so, will additional N gained from soil mining increase the fraction of NPP in wood increment and result in long-term C storage in plant biomass?

The classic test of plant nutrient limitation is whether there is an increase in NPP with the addition of a limiting nutrient (Chapin et al. 1986; Vitousek and Howarth 1991). Sweetgum response to N addition has been shown to be site specific (cf. Chang 2003 and references therein; Scott et al. 2004), and previous studies have focused on aboveground responses (Nelson et al. 1995; Kuers and Steinbeck 1998; Samuelson et al. 2001; Chang 2003; Scott et al. 2004; Allen et al. 2005; Williams and Gresham 2006; but see Price and Hendrick 1998; Rieckermann et al. 1999). Ideally, the occurrence of and sweetgum response to N limitation in ORNL FACE would be tested by adding N to the FACE plots, but doing so would compromise the ongoing experiment. Instead, we fertilized a separate part of the same sweetgum plantation on the Oak Ridge National Environmental Research Park (NERP) to gain a more fundamental understanding of N limitation at this site. This enabled us to assess whether soil N availability is limiting sweetgum production and to determine how sweetgum trees respond to changing N availability. We hypothesized that increased N availability would (*i*) increase stand NPP, (*ii*) increase leaf [N] above a concentration critical for maximum stem production and redistribute N lower in the canopy, and (*iii*) increase the proportion of NPP accounted for by wood production. To better support our hypotheses, we compare our experimental results with unpublished data collected at the same time in ORNL FACE (i.e., Ledford et al. 2007; Norby and Tharp 2007; Norby et al. 2007).

Methods

Experimental design

We fertilized a 85 m × 50 m sweetgum plantation on the Oak Ridge NERP in eastern Tennessee, USA. The plantation is part of the ORNL FACE sweetgum plantation and the sweetgum were planted in the same cohort, but are separated from ORNL FACE by a small stand of sycamore (*Platanus occidentalis* L.). One-year-old bare-root sweetgum saplings were hand planted at a spacing of 2.2 m × 1.7 m (compared with 2.4 m × 1.3 m spacing in ORNL FACE) in the spring of 1988 (Norby et al. 2001). The canopy at both sites has been closed since 1996. Initial soil N content was higher in the fertilization experiment (16 Mg·ha⁻¹) than in ORNL FACE (11 Mg·ha⁻¹). Soil in both sections of the plantation was classified as an Aquic Hapludult with a silty clay loam texture. Dominant understory species included the invasive C_4 annual grass *Microstegium vimineum* (Trin.) Camus and the invasive vine *Lonicera japonica* Thunb., with scattered *Rubus* spp.

We initiated the fertilization experiment in 2004 when the trees were 17 years old and approximately 19 m tall. The sweetgum plantation was fertilized in March 2004 and March 2005, in a generalized randomized block design (Addelman 1969). Experimental plots were arranged in three blocks, each comprising four 16 m × 12 m plots. Each block contained two untreated controls; two amended plots received 200 kg·ha⁻¹ of N as urea (46% N) by hand spreading before the first leaf-out of sweetgum and the onset of understory growth. We added enough N to alleviate potential N limitation (cf. Chapin et al. 1986), and as such, this amount was much greater than local N deposition (~10–15 kg N·ha⁻¹·year⁻¹, Johnson et al. 2004) but within the range of sweetgum plantation management practices (~100–400 kg N·ha⁻¹·year⁻¹, Nelson et al. 1995). Treatment replicates ($N = 2$) within a block allowed us to test for interaction between block and treatment (Addelman 1969; Newman et al. 1997). Within each replicate plot, sweetgum NPP, N content, and soil N availability were measured throughout the 2004 and 2005 growing seasons in a 13 m × 9 m area inside of a surrounding 1.5 m buffer. Sweetgum basal area (BA) measurements within the buffer indicated that there was no cross-contamination across adjacent treatments. A hydraulic lift at the intersection of the plots in the first block provided access to at least one tree in each plot for canopy leaf collection.

Soil N availability

Changes in soil N availability were assessed monthly over the growing season (March–November) with mixed-bed resin capsules and associated access systems (Warrington

Ecological Systems Analysis, Denver, Colorado, cf. Binkley 1984). Upon removal, individual capsules were rinsed with deionized water to remove soil particles, air-dried, and then extracted three times with 20 mL of 2 mol·L^{-1} KCl (for a total of 60 mL). Extracts were filtered with Whatman number 1 filter paper and frozen until analyzed for NH_4-N and NO_3-N (in 2004 on a Bran + Luebbe AutoAnalyzer 3, Bishop International, Akron, Ohio; in 2005 samples were sent to the stable isotope / soil biology laboratory of the University of Georgia Institute of Ecology or the Colorado Plateau analytical laboratory of Northern Arizona University).

Woody response

The basal area increment (BAI, cm^2) of each tree was assessed by measuring the change in stem circumference at 1.3 m height between April (prior to leaf out) and November (after leaf fall). Stand BAI (cm^2·m^{-2}) was calculated by summing tree BAI over the plot area. Stemwood production (dry mass increment) was estimated for each tree as the difference between initial and final BA of individual trees (cm^2) using allometric equations developed in ORNL FACE (Fig. 1*a* in Norby et al. 2001):

$$[1] \quad \text{Stemwood dry mass (kg)} = 0.355 \ \text{BA(cm}^2) - 2.24$$

Data were corrected for changes in taper with stand age using a second allometric relationship (Fig. 1*b* in Norby et al. 2001) and 2004–2005 data collected from the ambient [CO$_2$] treatment in ORNL FACE to correct eq. 1 (2004 correction factor = 1.986, 2005 correction factor = 2.013). We assumed that fertilization had a relatively small effect on taper and tree height over a period of 2 years. Coarse root mass increment was also calculated based on allometric equations developed in FACE (Fig. 1*c* in Norby et al. 2001):

$$[2] \quad \text{Coarse root mass (kg)} = 0.049 \ \text{BA(cm}^2) + 4.91$$

Coarse roots have a structure and chemistry similar to that of stemwood (Gifford 2000), and annual wood production was the summation of stem dry matter increment and coarse root increment. Wood production (stemwood + coarse root increment) of each tree was summed over the 117 m^2 plot area within the buffer and expressed as grams per square metre. Stemwood [N] was assessed on 4 mm diameter cores taken from two trees per plot in November 2005 using an increment corer. The cores were frozen until analysis and separated into growth increments corresponding to 2004 and 2005. After oven-drying at 70 °C, entire annual growth increments (~ 30 mg) were combusted on a Costech elemental analyzer (Costech Analytical Technologies, Inc., Valencia, California), to determine wood [N]. We assumed that coarse root [N] was equal to measurements of stemwood [N] because of the similarity between coarse root and stemwood chemistry (Gifford 2000), and we applied the [N] measurements to the total estimate of wood (coarse root + stemwood) incremental production.

Leaf and leaf litter responses

Fertilization effects on leaf [N] and morphology were measured on leaves sampled from the hydraulic lift at three relative canopy heights two times in 2004 and three times in 2005, and also on leaves sampled from trees felled within each experimental plot in August 2004 (for a total of three measurements per year). Leaves were stripped from the canopy of felled trees in 1 m increments, oven-dried at 70 °C, and weighed to determine total canopy mass per tree. Subsamples of 20 leaves from each canopy height were scanned on a Li-3100 leaf area meter (LI-COR, Lincoln, Nebraska), oven-dried, and weighed to determine leaf mass per unit area (LMA, g·m^{-2}) before being ground and combusted in a Costech elemental analyzer to determine leaf [N]. Leaves subsampled from the hydraulic lift throughout the growing season in both years were treated in a similar manner.

Leaf litter was collected weekly or every 2 weeks from four 0.2 m^2 litter baskets per replicate plot. Litter collections were timed to minimize the possibility of N leaching loss due to rain. After collection, litter was oven-dried at 70 °C and weighed to determine annual leaf production. Canopy mass per unit ground area was calculated from leaf litter mass by assuming a 7% dry mass loss with resorption (Norby et al. 2000), and a weighted average of LMA was used to determine canopy leaf area production per unit ground area. Litter was combined by date into five groups to determine litter [N] (representing changes in [N] from early litterfall to late litterfall). Litter [N] analyses were performed on a Carlo Erba (Milan, Italy; 2004 samples) or Costech (2005 samples) elemental analyzer.

Fine-root response

Fine-root (<1 mm diameter) peak standing crop was measured from 0–30 cm soil depth in mid-July 2004 and 2005. Each year, two (2005) or three (2004) 5 cm diameter by 30 cm deep soil cores were taken in each replicate plot, and roots were separated from the soil by washing in a hydropneumatic root washer (Gillison's Variety Fabrication, Benzonia, Michigan). Roots were oven-dried (70 °C) and weighed to determine biomass before being ground for N analysis. The date of peak standing crop was estimated from minirhizotron data collected previously at ORNL FACE (Norby et al. 2004).

Newly produced roots were collected for [N] analysis from root ingrowth into 15 cm deep cores filled with root-free soil and incubated in situ from May to October in 2004 and 2005. Specifically, four soil cores (6 cm diameter) were taken in each plot, and after the soil was removed, the holes were refilled with control or fertilized soil that had been sieved through a 1 mm mesh to remove existing root matter. An inner-core (5 cm diameter) was removed at the end of the growing season and roots were separated from the soil as described above, oven-dried (70 °C), and ground. The fine-root [N] of both the standing crop biomass and newly produced roots was determined with a Carlo Erba (2004 samples) or Costech (2005 samples) elemental analyzer.

We refrained from using the ingrowth cores to estimate fine-root production at this site because root ingrowth into root-free soil is a net estimate of production that could underestimate true production rates by up to 50% (Fahey and Hughes 1994; Hendricks et al. 2006). This is especially true in ecosystems where root growth, and thus colonization of the ingrowth cores, is relatively slow (Vogt et al. 1998). Instead, we estimated fine-root production using a mean turnover rate derived from the ambient [CO$_2$] rings in

ORNL FACE ($0.93 \cdot \text{year}^{-1}$ in 2004 and $1.04 \cdot \text{year}^{-1}$ in 2005, Norby et al. 2004; Ledford et al. 2007) along with measurements of the annual peak fine-root standing crop (cf. Gill and Jackson 2000):

$$[3] \quad \text{Root production } (g \cdot m^{-2} \cdot \text{year}^{-1})$$
$$= \text{Peak standing crop } (g \cdot m^{-2})$$
$$\times \text{Turnover } (\cdot \text{year}^{-1})$$

We assumed that root metabolism (and thus root turnover, cf. Eissenstat et al. 2000 and Withington et al. 2006) was unaffected by N fertilization because the [N] of newly produced fine roots did not increase in response to fertilization (see Results section).

Stand production and N uptake

Total stand production and N uptake were calculated as the sum of wood (stemwood + coarse root) increment, litter, and fine-root production and the N content of each compartment, respectively (cf. Norby and Iversen 2006). Wood N content was determined by the product of wood production (sum of eqs. 1 and 2) and annual stemwood N concentration. Litter N content was determined using weighted estimates of litter production and litter N concentration. Fine-root N content was the product of [N] of new roots collected from ingrowth cores and fine-root production estimated in eq. 3. Resorption was calculated as the difference between predicted total canopy N content (using weighted estimates of litter mass plus 7% and leaf [N]) and calculated leaf litter N content, while resorption efficiency was the fraction of green canopy N resorbed. Stand N requirement was calculated as total stand N uptake plus the total amount of N resorbed from the canopy.

Statistical analysis

The effect of N fertilization on inorganic soil N availability, annual wood, leaf litter, and fine-root production, and compartmental changes in N concentration and content were analyzed using the SAS "Mixed" procedure (SAS Institute Inc., Cary, North Carolina). Log- or reciprocal transformations were used on non-normal data to meet ANOVA criteria (cf. Gotelli and Ellison 2004). We initially included year as an effect in the overall model, but analyzed treatment years (2004 and 2005) separately where there were significant interactions between treatment and year or when methods differed slightly between years. When there is no significant year $\times$ treatment interaction, the F statistic given in Table 1 is that of treatment alone and refers to a mean response over 2004 and 2005. Fertilization was treated as a fixed effect, while block and block $\times$ treatment effects were treated as random effects (Bennington and Thayne 1994). We specified the "Kenward–Roger" option in the model statement to estimate the denominator degrees of freedom (Kenward and Roger 1997) for tests of the fixed fertilization effect. A repeated-measures framework was used to assess fertilization effects on inorganic N availability and leaf collections from the hydraulic lift over time. When two trees were sampled from the same plot via the hydraulic lift, a mean value was used so that each plot was a statistical replicate ($N = 2$ for each treatment) with both treatment and height as fixed factors. Initial stem BA was used as a cova-

riate in the analysis of canopy production, and leaf area, mass, and number. To avoid type II errors, differences were considered significant at $P < 0.1$. All responses reported in the results section are supported by the statistical analysis in Table 1.

Results

N availability

Fertilization increased inorganic N availability (sum of NH_4-N and NO_3-N; Fig. 1), which varied throughout 2004 and 2005 (repeated measures, $F_{[11,118]} = 27.33$, $P < 0.001$). The magnitude of the fertilization effect depended on the time of year (treatment $\times$ date interaction, $F_{[11,118]} = 2.51$, $P < 0.01$). Fertilization increased N availability immediately after fertilization and again after leaf senescence; the greatest differences between treatments were after fertilization when soil moisture was high in April and June 2005. Patterns were not as clear in 2004, because the resin access system was not installed until June 2004, 3 months after fertilizer was applied.

Compartmental production

Mean stand BA did not initially differ between the control (26.8 ± 2.6 cm$^2 \cdot$m^{-2}) and fertilized plots (20.2 ± 2.5 cm$^2 \cdot$m^{-2}, $P > 0.1$). Annual stand BAI increased 30% in response to fertilization in 2004 and 50% in 2005 (Fig. 2). In comparing the response of the fertilized sweetgum stand with the response of sweetgum in the adjacent ORNL FACE site (BAI calculated as in Norby et al. 2001), we found that BAI in the control treatment was similar to that in the ambient [CO_2] treatment ($\sim$380 ppm) in ORNL FACE ($F_{[1,14]} = 0.1$, $P > 0.1$); there was no response of BAI to CO_2 enrichment ($\sim$550 ppm) in the adjacent FACE experiment in 2004 or 2005 ($F_{[1,6]} = 0.2$, $P > 0.1$, Fig. 2).

The canopy mass and area of individual trees felled in August 2004 increased in response to fertilization by 38% (from 3.2 ± 0.3 (1 SEM) kg in the control to 4.5 ± 0.9 kg in the fertilized plots) and 27% (from 37.3 ± 3.6 m^2 in the control to 47.5 ± 8.0 m^2 in the fertilized plots), respectively. Increases in individual tree canopy mass were limited to larger trees (BA covariate, $F_{[1,9]} = 39.5$, $P < 0.001$), as were increases in tree canopy area (BA covariate, $F_{[1,9]} = 30.2$, $P < 0.001$). Fertilization did not statistically increase the number of leaves in the canopy (control, 4942 ± 441 and fertilized, 5852 ± 990).

Fertilization increased stand leaf area production (m$^2 \cdot$m^{-2} ground area$\cdot$year^{-1}) by $\sim$7% in 2004 (from 4.9 ± 0.1 in the control to 5.2 ± 0.1 in the fertilized plots) and 2005 (from 5.3 ± 0.2 in the control to 5.6 ± 0.1 in the fertilized plots). The magnitude of the fertilization effect was less at the stand level than in individual tree canopies because the stand-level response includes the gap fraction (i.e., the fraction of the forest canopy that does not consist of leaves, $\sim$14% in ORNL FACE, Norby et al. 2003). Fertilization increased stem production efficiency (stem production per unit leaf area, Waring and Schlesinger 1985) in 2004 (control, 256 ± 19 and fertilized, 308 ± 20 g$\cdot$m$^{-2} \cdot$year^{-1}) and in 2005 (control, 211 ± 21 and fertilized, 292 ± 25 g$\cdot$m$^{-2} \cdot$year^{-1}).

Canopy responses were driven not by increases in the

Table 1. ANOVA F statistics (denominator degrees of freedom are as calculated by Kenward–Roger option) and corresponding probability values for measured responses to N fertilization in 2004 and 2005.

Parameter	ANOVA statistics		
	ddf	F	P
2004 only			
Felled trees*			
Canopy mass (kg)[†]	9	5.4	**0.05**
Canopy area (m^2)[†]	9	3.5	**0.096**
Leaf number[†]	4	1.5	0.3
LMA	4	0.2	0.7
N_{area}	4	7.8	**0.05**
2004 and 2005			
Inorganic N availability ($\mu mol \cdot cm^{-2} \cdot month^{-1}$)	118	48.2	**<0.001**
Leaf morphology			
Leaf mass (g)	30	9.15	**0.005**
Leaf area (cm^2)	30	11.85	**0.002**
Stand dynamics			
BAI ($cm^2 \cdot m^{-2} year^{-1}$)	18	26.4	**<0.001**
Leaf area production ($m^2 \cdot m^{-2} \cdot year^{-1}$)	18	8.7	**0.009**
Fine-root standing crop ($g \cdot m^{-2}$)	4	2.3	0.2
Stem production efficiency ($g \cdot m^{-2} \cdot year^{-1}$)	18	13.7	**0.002**
Total NPP ($g \cdot m^{-2} \cdot year^{-1}$)	18	32.9	**<0.001**
Wood production	18	26.3	**<0.001**
Leaf litter production	18	25.8	**<0.001**
Fine-root production	4	2.3	0.2
C flux (% NPP)			
Wood mass fraction	2	8.7	**0.099**
Leaf litter mass fraction	18	6.9	**0.02**
Fine-root mass fraction	4	4.9	**0.09**
Stand N uptake (g $N \cdot m^{-2} \cdot year^{-1}$)	4	25.8	**0.007**
Wood N content	18	36.5	**<0.001**
Leaf litter N content (2004)	4	5.1	**0.09**
Leaf litter N content (2005)	4	17.1	**0.01**
Fine-root N content	2	1.8	0.3
N requirement (2004, g $N \cdot m^{-2} \cdot year^{-1}$)	10	92.1	**<0.001**
N requirement (2005, g $N \cdot m^{-2} \cdot year^{-1}$)	10	77.4	**<0.001**
N resorbed (g $N \cdot m^{-2} \cdot year^{-1}$)	4	86.8	**0.001**
Resorption efficiency (%)	4	9.1	**0.04**
Compartmental [N] ($mg \cdot g^{-1}$)			
Stemwood [N]	18	28.5	**<0.001**
Leaf [N]	30	101.49	**<0.001**
Leaf litter [N]	4	21.8	**0.01**
Peak standing crop root [N]	20	12.9	**0.002**
New fine root [N]	18	0.3	0.6

Note: For data collected in both 2004 and 2005, the F statistic given is that of the mean treatment effect over both years, except for leaf litter N content and annual N requirement, for which there was a significant treatment × year interaction ($P < 0.1$). Values in boldface type indicate statistical significance at $P < 0.1$.

*"Felled trees" refers to a one-time felling of one tree from each treatment plot in August 2004.

[†]Initial BA of the tree was used as a covariate in the ANOVA.

number of leaves in the canopy but by increases in the mean mass and area of individual leaves in response to fertilization (Table 2). Leaf mass changed throughout the growing season (repeated measures, $F_{[4,45]} = 4.8$, $P < 0.01$), while leaf area did not ($F_{[4,45]} = 1.1$, $P > 0.1$), but the effects of N fertilization on leaf mass and area were not affected by date (i.e., no date × treatment interaction for leaf mass, $F_{[4,45]} = 0.03$, $P > 0.1$, or area, $F_{[4,45]} = 0.2$, $P > 0.1$). Leaf mass and leaf area were greater in the fertilized plots throughout the

canopy in both 2004 and 2005 (no depth × treatment interactions for leaf mass, $F_{[2,18]} = 1.66$, $P > 0.1$, or area, $F_{[2,18]} = 1.58$, $P > 0.1$), and both declined significantly from the top to the bottom of the canopy (depth effect, mass, $F_{[2,18]} = 39.58$, $P < 0.001$, and area, $F_{[2,18]} = 7.31$, $P < 0.01$).

Fertilization did not significantly affect peak mean fine-root standing crop, which was 110.7 ± 11.8 $g \cdot m^{-2}$ in the control plots and 91.0 ± 11.4 $g \cdot m^{-2}$ in the fertilized plots in 2004. In 2005, peak mean fine-root standing crop was

Fig. 1. Total inorganic N availability (sum of NH_4-N and NO_3-N) assessed by resin capsules in the control and fertilized plots. Data are monthly (30 day) means ± 1 SEM ($N = 6$ for each treatment) expressed in units corresponding to resin adsorption quantity. RAQ, adsorption per cm^2 of resin surface. Gray arrows refer to fertilization events.
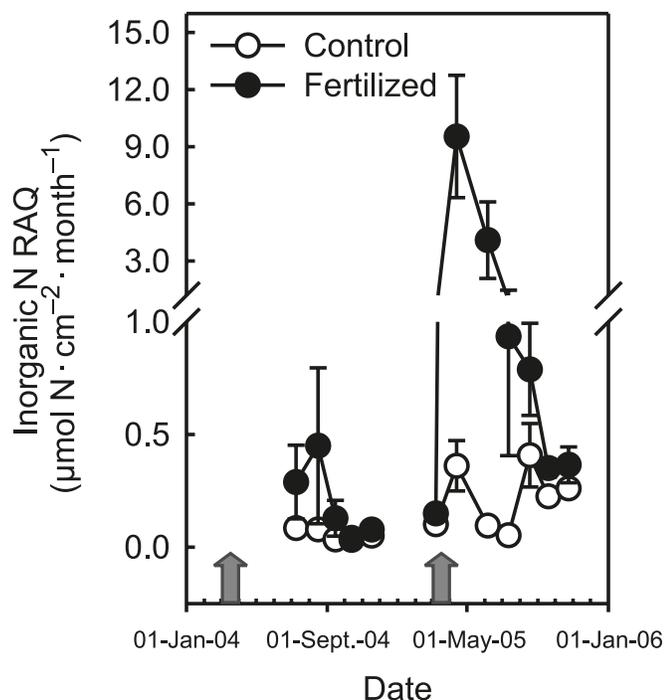
$148.5 ± 17.9$ g·m^{-2} in the control plots and $103.6 ± 8.5$ g·m^{-2} in the fertilized plots.

Compartmental N content

Fertilization increased the N concentration (N_{mass}, mg·g^{-1}) of stemwood, leaf litter, and fine-root peak standing crop, but did not affect the N_{mass} of newly produced roots (Table 3). Leaf N_{mass} differed by date ($F_{[4,45]} = 5.3$, $P <$ 0.01), but across all dates, N fertilization increased leaf N_{mass} by ∼20%–40% (Fig. 3). Leaf N_{mass} did not differ by canopy depth ($F_{[2,45]} = 0.1$, $P > 0.1$). Leaf N_{mass} was similar between the control treatment in the fertilization experiment and the ambient [CO_2] treatment in ORNL FACE (note that we compared similar collection dates within each year, $F_{[1,10]} = 0.98$, $P > 0.1$). In contrast with the effects of N fertilization, leaf N_{mass} declined by ∼13% under elevated [CO_2] as compared to the ambient [CO_2] treatment ($F_{[1,6]} =$ 9.2, $P = 0.02$, Fig. 3).

LMA did not respond to fertilization in 2004 (data shown are from trees felled in 2004, Fig. 4a) or 2005 (data not shown), but declined with canopy depth in each treatment (depth effect, $F_{[6,65]} = 48.73$, $P < 0.001$). Fertilization increased leaf N content per unit leaf area (N_{area}, g N·m^{-2}) in 2004 (data shown are from trees felled in 2004; Fig. 4b) and 2005 (data not shown). N_{area} mirrored LMA and declined with canopy depth in each treatment (depth effect, $F_{[5,65]} =$ 24.60, $P < 0.001$, Fig. 4b).

Stand production and N uptake

N fertilization increased annual stand NPP by 21% in

2004 and 32% in 2005 (Fig. 5a). Fertilization increased wood dry-matter increment (g·m^{-2}, sum eqs. 1 and 2) by 28% and 50% in 2004 and 2005, while leaf production (g litterfall·m^{-2}) increased by 10% and 12% in 2004 and 2005, respectively. Fine-root production did not respond to fertilization. Fertilization increased the wood mass fraction of total NPP, and decreased the leaf and fine-root mass fractions (Fig. 5a).

Stand N uptake (i.e., the sum of leaf, new wood, and new root compartmental N content) increased with fertilization by 16% in 2004 and 28% in 2005 (Fig. 5b). Wood N content increased 81%–113% in response to fertilization. Fertilization increased litter N content by 14% in 2004 and 30% in 2005. Fertilization did not significantly affect fine-root N content in either year.

Fertilization increased the total amount of N required for biomass production by 29% in 2004, and 37% in 2005 (Fig. 5b). Canopy resorption efficiency increased from 46 ± 2% in the control to 54 ± 1% in the fertilized plots in 2004, and from 42 ± 1% in the control to 48 ± 1% in the fertilized plots in 2005. The total amount of N resorbed in the fertilized plots was 56% greater than the control in 2004 and 61% greater than the control in 2005 (Fig. 5b).

Discussion

The degree to which northern temperate forests respond to changing environmental conditions will depend on the current nutrient status of each ecosystem (Luo et al. 2004). We examined the premise that increased fine-root production in response to CO_2 enrichment at ORNL FACE (Norby et al. 2004) was a physiological response to N limitation. To avoid compromising the ongoing ORNL FACE experiment, we fertilized a separate portion of the same sweetgum plantation to test whether sweetgum production on the Oak Ridge NERP was limited by N availability. We hypothesized that alleviation of N limitation would result in an increased proportion of NPP in wood production.

We found evidence to support our hypothesis that soil N availability limits sweetgum production on the Oak Ridge NERP. Fertilization increased stand NPP by 21%–32%, largely because of increased stem production (annual BAI was up to 50% greater in the fertilized plots than in the control, Fig. 2). Leaf [N] in the fertilized plots (Fig. 3), which regulates the photosynthetic process of C gain (Evans 1989), often exceeded the critical [N] of ∼18 mg·g^{-1} required for maximum sweetgum stem production (Scott et al. 2004). In contrast, leaf [N] declined significantly below this threshold with CO_2 enrichment at ORNL FACE (Fig. 3). Declining leaf [N] is projected by stand-scale models to limit forest CO_2 responses (Comins and McMurtrie 1993). The responses of BA growth and leaf [N] were strikingly similar between the control treatment in the fertilization experiment and the ambient [CO_2] treatment in ORNL FACE (Figs. 2 and 3), though soil N was initially greater in the fertilized stand (16 Mg·ha^{-1} compared with 11 Mg·ha^{-1} at ORNL FACE).

Whole-canopy photosynthesis is theoretically maximized when leaf N is distributed so that leaves receiving the highest irradiance have the highest [N] (Field and Mooney 1983). Fertilization increased N_{area}, and we predicted that

Fig. 2. Annual mean stand BAI ($cm^2 \cdot m^{-2} \cdot year^{-1}$) ±1 SEM in the fertilization experiment ($N = 6$ for each treatment) and adjacent ORNL FACE ($N = 3$ for ambient [CO_2] treatment and $N = 2$ for elevated [CO_2] treatment). FACE BAI data are from Norby and Tharp (2007). ***, $P \leq 0.001$; NS, not significant ($P > 0.1$). P values correspond to differences between treatments within each experiment.
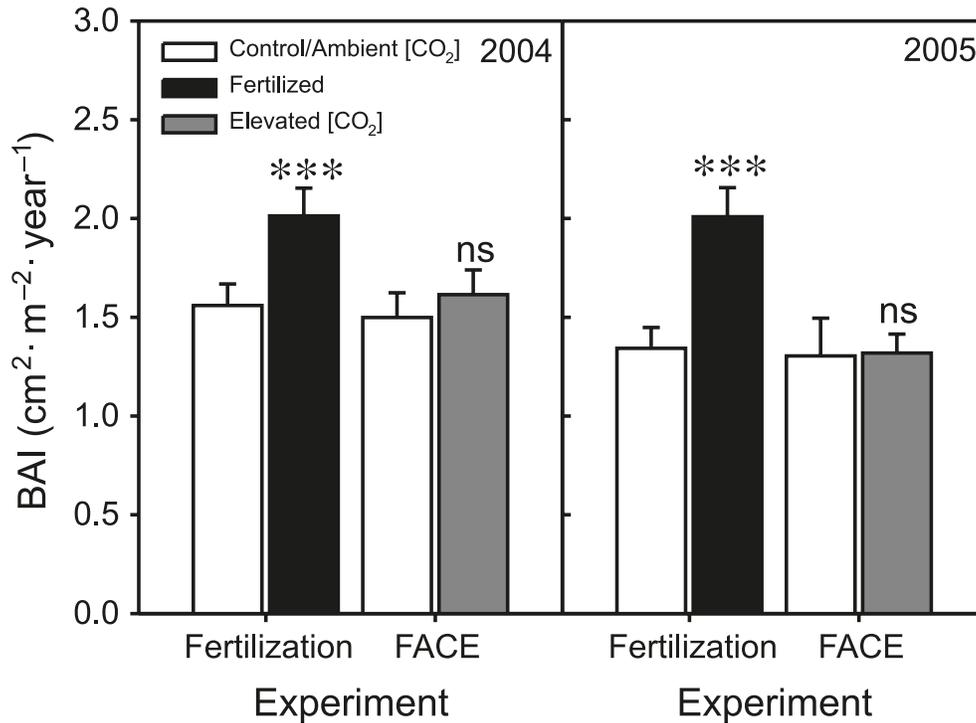
Table 2. Mean individual leaf mass and area measurements of leaves sampled at three canopy heights from the hydraulic lift in block one in late June and July 2004 and early June, July, and August 2005.

Relative canopy height	Leaf mass ($g \cdot leaf^{-1}$)		Leaf area ($cm^2 \cdot leaf^{-1}$)	
	Control	Fertilized	Control	Fertilized
2004				
Upper	5.1±1.2	5.6±0.7	488±104	522±60
Middle	3.1±0.1	4.4±0.7	389±42	513±71
Lower	2.2±0.2	2.5±0.7	367±16	384±93
2005				
Upper	5.4±0.3	7.0±0.4	476±25	614±26
Middle	4.0±0.3	5.7±0.7	426±37	609±65
Lower	2.6±0.1	2.7±0.7	381±8	400±77

Note: Mean morphology of leaves sampled from a hydraulic lift at three relative canopy heights ± 1 SEM. Data are averaged across two or three sampling dates in 2004 and 2005, respectively; $N = 2$ for each treatment.

fertilization would lower the distribution of N_{area} within the canopy to better optimize photosynthetic return (Hirose 1987). LMA, which is often the primary driver of N distribution within a canopy (Rosati et al. 2000), decreased with canopy depth and light penetration (Fig. 4a). However, in contrast with what we hypothesized, there were no changes in N optimization with additional N (i.e., no interaction between depth and fertilization effect on N_{area}, Fig. 4b). This was because leaf area and mass increased concurrently in response to fertilization in our study (Table 2). The effect of N availability on canopy N optimization may have been small because of a tradeoff between light-use efficiency and N-use efficiency at the leaf level (cf. Hirose and Bazzaz 1998).

Stand leaf area determines the amount of photosynthetically active radiation (PAR, Cannell et al. 1987; Norby et al. 2003) intercepted, and the efficiency with which the intercepted PAR is converted to C gain determines stemwood growth (Vose and Allen 1988). Forest leaf area index (LAI) has been shown to increase with site fertility (Vose and Allen 1988; Samuelson et al. 2001). This is attributed to increased leaf number, leaf size, or both (Linder and Rook 1984). N fertilization increased sweetgum canopy leaf area production (m^2 leaf·m^{-2} ground, which as calculated is approximately 5% greater than peak LAI, owing to a small amount of leaf turnover throughout the growing season, i.e., Norby et al. 2003) as well as the canopy mass and area of

Table 3. Mean annual tissue N concentration (mg·g^{-1}) in the control and fertilized plots.

Treatment	Stemwood	Leaf litter	Fine-root peak standing crop	New fine roots
2004				
Control	0.86±0.04	8.86±0.19	10.11±0.21	16.10±1.13
Fertilized	1.21±0.06***	10.12±0.19*	11.57±0.44**	15.79±0.48ns
2005				
Control	1.13±0.13	9.26±0.18	8.41±0.42	14.05±0.50
Fertilized	1.59±0.06***	10.75±0.25*	9.98±0.55**	15.09±0.51ns

Note: Each value is averaged over six replicate plots within each treatment ±1 SEM. ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; ns, not significant ($P > 0.1$).

Fig. 3. Leaf [N] sampled from tree canopies via a hydraulic lift in the fertilization experiment ($N = 2$ plots for each treatment) or from trees felled in August 2004 (the last data point in 2004, $N = 6$ plots for each treatment). Leaf [N] was also sampled via hydraulic lift in the adjacent ORNL FACE experiment once in 2004 and 2005 ($N = 3$ plots for the ambient [CO_2] treatment, and $N = 2$ plots for the elevated [CO_2] treatment) ±1 SEM. The critical threshold of 18 mg·g^{-1} necessary for 90% of potential stem production is represented by a dashed line. FACE leaf [N] data are from Norby et al. (2007).

Fig. 4. Changes in leaf mass and N per unit leaf area with canopy depth were measured on one tree felled from each treatment plot in August 2004 ($N = 6$ plots per treatment). Data are (*a*) mean leaf mass per unit area (LMA, g·m^{-2}) and (*b*) mean N per unit leaf area (N_{area}, g N·m^{-2}) ±1 SEM.
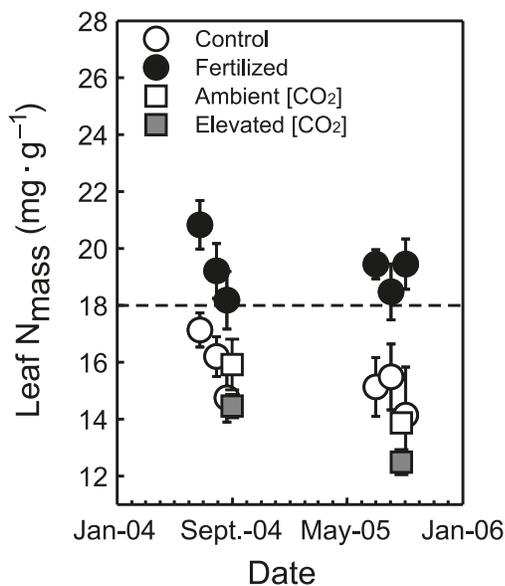
individual trees. Leaf area production increased in the fertilized plots because leaves were larger (greater mass and area, Table 2), not because there were more of them. There was a tendency for the fertilized trees to have more leaves, and a lag in response may be expected if fertilization affects the formation of leaf primordia in overwintering buds, especially as leaf number was assessed only in the first year of the experiment. Stem production per unit leaf area, (i.e., stem growth efficiency; Waring and Schlesinger 1985) also increased 20%–40% in response to fertilization, probably because of increased leaf [N] (Chang 2003; Allen et al. 2005).

The accepted definition of nutrient limitation is an increase in NPP with the addition of a limiting nutrient (Chapin et al. 1986; Vitousek and Howarth 1991). However, fertilization experiments have generally focused on the aboveground component of NPP (Chapin et al. 1986; Aber

et al. 1993; Nelson et al. 1995; Vitousek and Farrington 1997), because of inherent difficulties associated with sampling and quantifying fine-root production (as reviewed in Vogt et al. 1998 and Hendricks et al. 2006). Research incorporating belowground responses to N fertilization has shown decreases, increases, and no change in forest fine-root pro-

Fig. 5. Mean annual stand production and N requirement ±1 SEM of each biomass compartment; N = 6 for each treatment. Data are: (a) Annual total NPP (g·m^{-2}·year^{-1}) sub-divided into different biomass compartments: leaf production, wood production, which is the summation of stemwood (above the "0" line) and coarse root (below the "0" line) increments, and fine-root production, which was measured at 0–30 cm depth. (b) Annual total N requirement (g·m^{-2}·year^{-1}) divided into biomass compartments (as above). ***, $P \leq 0.001$. P values correspond to total NPP and total N requirement in (a) and (b), respectively. The leaf compartment in (b) is divided into the fraction of canopy N supplied by new N uptake (below the line) and the fraction provided by remobilization of internal N (above the dotted line).

and mortality of fine roots with a higher [N] (Hendricks et al. 1993; Eissenstat et al. 2000; Withington et al. 2006).

We based our premise that greater N demands in the ORNL FACE site led to greater fine-root production on allocation theory, which suggests that plants maximize growth by adjusting the relative flux of C towards the most limiting resource (Field et al. 1992; Friedlingstein et al. 1999). Our hypothesis that alleviation of N limitation in the fertilization experiment would decrease the proportion of total NPP in fine-root production was supported by data from our fertilization experiment (Fig. 5a); the constant allocation hypothesis was not supported. N fertilization did not significantly affect the peak standing crop or production of fine roots in 2004 or 2005, despite an increase in total NPP. N fertilization did not increase the tissue [N] of newly produced fine roots, and thus, root metabolism (and root turnover cf. Eissenstat et al. 2000; Withington et al. 2006) was unaffected. This observation supports our assumption in the estimation of fine-root production that turnover was similar in control and fertilized plots. Previous work has shown that very high levels of fertilizer (1120 kg N·ha^{-1} compared with a control treatment of 560 kg N·ha^{-1}) had no effect on sweetgum root production or mortality (Price and Hendrick 1998).

Total stand NPP increased in the fertilized plots because wood (stemwood + coarse root) production increased and fine-root production did not significantly change. Thus, increased soil N availability resulted in relatively more C flux to wood at the expense of ephemeral fine-root and leaf tissue (Fig. 5a), resulting in greater C storage in sweetgum biomass. Though root production was a small percentage of total NPP at our site, it is within the range of findings in other temperate forests (as reviewed in Vogt et al. 1996). Simultaneous work at this site found decreased soil C efflux in the fertilized plots in 2005 (K. Sides and E. Felker-Quinn, personal communication, 2005), which supports the premise of less C flux to fine roots in response to N fertilization. Conversely, alleviation of C limitation at ORNL FACE resulted in increased C flux belowground to fine-root production at the expense of the wood and leaf fractions (unpublished data, as calculated in Norby et al. 2004).

Plant dynamics are controlled by the total amount of N taken up from the soil (Aerts 1999), and changes in the balance between nutrient retention and loss may affect ecosystem nutrient cycling (Aerts 1999). We found that N fertilization increased N resorption efficiency (on a mass basis) by 10%–15%. Though this is counterintuitive, relationships between nutrient resorption and site nutrient availability are often weaker than expected (Aerts 1996), and increased N resorption efficiency under scenarios of greater N availability may be related to changes in the ratio of soluble and insoluble N with fertilization (cf. Pugnaire and Chapin 1993). Fertilization also increased the absolute amount of N resorbed by senescing leaves by up to 60% (Fig. 5b), because of increased leaf mass and N concentration (cf. Birk and Vitousek 1986).

Thus, sweetgum in the fertilized plots are proportionally more dependent on internal N cycling because the annual N requirement of the sweetgum plantation fulfilled by resorption increased, although the absolute amounts of N taken up and returned to the soil via litterfall were still greater than in the unfertilized plots (stand N uptake increased by 16% and

duction in response to gradients of N availability (reviewed in Hendricks et al. 1993; Ostertag 2001). Within a species and ecosystem, theory suggests that N fertilization could result in either (i) decreased relative C flux to fine-root production (i.e., differential allocation) or (ii) unchanged C flux to fine-root production (i.e., constant allocation), but increased root turnover rates due to increased metabolic cost

28% in 2004 and 2005, respectively). CO_2 enrichment also increased N uptake at ORNL FACE (Norby and Iversen 2006), but N cycling at ORNL FACE is increasingly dominated by external cycling through the soil system, because increased fine-root partitioning has resulted in a relatively fast return of N to the soil (Norby and Iversen 2006). The source of the additional N in the FACE experiment is uncertain and is the subject of continuing research.

Implications for forest responses to changing environmental conditions

Soil N availability in temperate ecosystems has long been proposed to limit plant production and C storage in response to changing environmental conditions (Kramer 1981; Berntson and Bazzaz 1996). We demonstrated that sweetgum production on the Oak Ridge NERP was limited by soil N availability and that alleviation of N limitation increased the relative flux of C to perennial woody tissue. Both of these findings have implications for the response of the adjacent ORNL FACE sweetgum plantation to increased C availability under elevated $[CO_2]$. N fertilization decreased the proportion of NPP in fine-root production in our experiment, which supports our premise that increased fine-root proliferation in ORNL FACE was a physiological response to N limitation. Annual increases in NPP under elevated $[CO_2]$ in ORNL FACE demonstrate that N limitation does not necessarily preclude increased production in response to CO_2 enrichment (i.e., Norby et al. 2002, 2004). Instead, multiple limitations (e.g., N and C availability) may affect the partitioning of photosynthate to tissue with different mean residence times and affect long-term C and N storage within an ecosystem (Luo et al. 2004; Norby and Iversen 2006). Disentangling the linkages between the C and N cycles will allow us to better project long-term temperate forest responses to global climate and environmental change (McMurtrie et al. 2000, 2001; Reich et al. 2006), and future scenarios will depend on the size of the available soil N pool and the ability of the ecosystem to meet increased forest demand for N in response to changing environmental factors.

Acknowledgements

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