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I am submitting herewith a dissertation written by Colleen Marie Iversen entitled "Forest responses to rising atmospheric CO_2: Causes and consequences of increased fine-root production in a CO_2-enriched sweetgum plantation." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

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Forest responses to rising atmospheric CO_2: Causes and consequences of increased fine-root production in a CO_2-enriched sweetgum plantation

A Dissertation
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Degree
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Colleen Marie Iversen
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Dedication

To my family and friends:

for their love and support, phone calls and care packages.

To my husband:

for his patience, sense of humor and home-cooked meals.

To my co-advisors:

for teaching me by example how to be a successful scientist and mentor, and for life lessons on the importance of dirt, good beer and Bob Dylan songs.

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Abstract

Increased forest growth in response to rising atmospheric concentrations of CO_2 may mitigate a portion of fossil fuel emissions, especially if carbon is sequestered in long-lived biomass or soil pools. Greater carbon uptake under elevated atmospheric [CO_2] in forested ecosystems may facilitate the production of small diameter (i.e. "fine") roots used for nutrient acquisition. Increased fine-root production in forested ecosystems may affect soil carbon storage and nitrogen cycling because fine roots live and die in the span of a year. My dissertation research took advantage of a long-term, on-going Free-Air CO_2-Enrichment experiment in a sweetgum (*Liquidambar styraciflua* L.) forest stand at Oak Ridge National Laboratory to investigate the *causes* and *consequences* of increased fine-root production under elevated [CO_2]. To examine the premise that N limitation was the *cause* of increased fine-root production in the CO_2-enriched sweetgum stand, I fertilized plots in an adjacent sweetgum plantation with 200 kg ha^{-1} of N as urea. The relative C flux to wood production that I observed in the fertilized treatment is consistent with the premise that increased root production in the adjacent FACE experiment is in response to N limitation. To examine the *consequences* of increased fine-root production under elevated [CO_2], I: (1) quantified fine-root biomass and N inputs at several soil depths using a long-term minirhizotron data set combined with continuous, root-specific measurements of root mass per unit length and [N], and (2) allowed fine roots grown under current and elevated [CO_2] to decompose in a common garden experiment by modifying existing litterbag methodology. I found that C and N inputs via root mortality were doubled under elevated [CO_2], and half of the inputs were below 30 cm soil depth. However, CO_2-enrichment had no effect on fine-root chemistry or decomposition rate, and therefore more root detritus may be incorporated into long-lived soil organic matter under elevated [CO_2]. Quantification of the effects of elevated CO_2 on the fate of a greater quantity of fine-root detritus, especially at depth in the soil, will provide critical information needed for predicting processes such as long-term soil C storage and N cycling in response to environmental change.

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Chapter 1
An introduction: Forest responses to rising atmospheric [CO_2]

Anthropogenic climate change and forest carbon storage

Atmospheric concentrations of CO_2 are rising and are projected to continue rising throughout this century, due in large part to increased fossil fuel emissions (IPCC, 2007). Rising atmospheric [CO_2] is predicted to increase plant production and ecosystem carbon (C) storage (Schimel, 1995), which could in turn mitigate a portion of fossil fuel emissions (IPCC, 2007). Forests are expected to play a large role in the mitigation of rising [CO_2] (Schimel *et al.*, 1994; IPCC, 2007) because they are currently responsible for the storage of nearly half of terrestrial C (Goodale *et al.*, 2002).

Soil nitrogen (N) availability often limits net primary production (NPP) in terrestrial, temperate ecosystems (Vitousek & Howarth, 1991), and N limitation may potentially constrain forest growth and C storage under elevated [CO_2] (Luo *et al.*, 2004). Increased forest growth and corresponding increases in N demand under elevated [CO_2] may exacerbate existing N limitation within an ecosystem (Oren *et al.*, 2001), and soil N availability will become increasingly limited as C and N are stored in long-lived (i.e., woody) biomass or soil organic matter (SOM) pools (Fig. 1.1; Oren *et al.*, 2001; Hungate *et al.*, 2003; Luo *et al.*, 2004; henceforth all figures and tables will be found in an appendix at the end of each chapter). Forest N dynamics were initially overlooked in terrestrial ecosystem models projecting substantial increases in forest C uptake and storage under elevated CO_2 (Hungate *et al.*, 2003); current models indicate much lower C storage when N cycling is incorporated (Rastetter *et al.*, 1997; McMurtrie *et al.*, 2000; McMurtrie *et al.*, 2001; Kirschbaum *et al.*, 2003).

Plants often compensate for limitations in external resource supply by allocating C to organs acquiring the most strongly limiting resources (Field *et al.*, 1992). For example, alleviation of C limitation under elevated [CO_2] may result in the greater production of small diameter (i.e., "fine") roots to facilitate the acquisition of limiting nutrients (i.e., Matamala & Schlesinger, 2000; Norby *et al.*, 2004). However, relative to wood production, C allocated to fine roots results in much less C fixed in biomass per unit N because roots are more costly to produce and maintain. For example, living fine

roots have an average C: N ratio of ~ 40: 1 (Jackson *et al*., 1997), compared to a C: N ratio of ~ 500: 1 in wood, (Hungate *et al.*, 2003; Norby & Iversen, 2006).

Changes in the partitioning of photosynthate to organs of differing N concentrations and longevity affect the rate at which C and N are cycled within an ecosystem (cf. Norby & Iversen, 2006). Unlike stem wood, which has a residence time of decades, fine roots turnover quickly in forested ecosystems (in 1 to 9 years, Gill & Jackson, 2000; Matamala *et al.*, 2003). Thus, annual inputs of C and N from decaying root litter constitute a substantial flux of nutrients to the soil system (Aerts *et al.*, 1992). A greater input of fine-root detritus under scenarios of elevated [CO_2] may result in increased C and N storage in the soil (i.e., Fig. 1.1), depending on the rate at which C and N are mineralized from decomposing roots (Gale *et al.*, 2000). Soils may be an important sink for rising [CO_2] as they account for two-thirds of the total C stored in terrestrial ecosystems and contain C fractions with long-residence times (Goodale *et al.*, 2002). However, more N is required per unit C stored in soil (soil C: N ~ 15, Cleveland & Liptzin, 2007) than in woody biomass (wood C: N ~ 500, Hungate *et al.*, 2003), which may further limit the N available for forest uptake and growth in response to rising atmospheric [CO_2] (Luo *et al.*, 2004).

My dissertation research takes advantage of a long-term, on-going Free-Air CO_2-Enrichment experiment at Oak Ridge National Laboratory (Norby *et al.*, 2001; Norby *et al.*, 2002; Norby & Iversen, 2006). Below I outline the experimental design and background for the CO_2-enriched sweetgum plantation where I conducted my research. I also present the rationale for the series of experiments I conducted to address the critical assumption that elevated [CO_2] will increase C and N storage in plant or soil pools.

Oak Ridge National Laboratory, Free-Air CO_2-Enrichment Experiment

Experimental Design

FACE (Free-Air CO_2 Enrichment) experiments in forests provide a unique opportunity to test the effects of elevated [CO_2] on N dynamics and C storage over time

in realistic experimental systems (Hendrey *et al.*, 1999). The Oak Ridge National Laboratory (ORNL) FACE experiment was initiated in 1998 in a 12-m tall, 10-year old, closed-canopy sweetgum stand (*Liquidambar styraciflua* L.) where the soil type is a moderately well-drained Aquic Hapludult with a silty clay loam texture (Norby *et al.*, 2002); the B soil horizon (which contains comparatively less organic matter than the surface O and A horizons) begins at 15 cm depth (Johnson *et al.*, 2004). ORNL FACE consists of five 25-m diameter plots, four of which have FACE apparatuses installed. Three plots have ambient, or "current", levels of [CO_2] while FACE rings in two plots maintain elevated daytime atmospheric [CO_2] during the growing season (Hendrey *et al.*, 1999; Norby *et al.*, 2001). Average daytime [CO_2] in the elevated plots is approximately 550 ppm, the atmospheric concentration projected to occur between the years 2050 and 2070 (IPCC, 2001, scenario A1B).

Background

CO_2-enrichment increased NPP in ORNL FACE, but after one year of increased stem production (Norby *et al.*, 2002) allocation of extra C and N has been mostly to fine roots (Norby *et al.*, 2004). Fine roots turn over in approximately one year at ORNL FACE (Matamala *et al.*, 2003); thus, elevated [CO_2] has not resulted in long-term C and N storage in tree biomass. Fine roots in the elevated [CO_2] plots have increased soil exploration throughout the soil profile (to depths up to 60 cm, Norby *et al.*, 2004).

Increased forest N uptake or immobilization in soil organic matter in response to elevated [CO_2] may decrease soil N availability and constrain future forest growth (Luo *et al.*, 2004). Norby & Iversen (2006) examined the effects of CO_2-enrichment on several aspects of the plant N cycle at ORNL FACE and found that N uptake, litter input, and green leaf and leaf litter C: N ratios in the fumigated plots have steadily increased since the onset of the experiment. Further, Jastrow *et al.* (2005) found evidence that elevated [CO_2] has increased C and N storage in soil organic matter in the top 5 cm of the soil. These ecosystem-level responses are predicted by Fig. 1.1 to lead to declining soil N availability. However, contrary to expectations, soil N availability has not yet limited

forest growth or N uptake under elevated [CO_2] at ORNL FACE or any other forested FACE experiment (Norby *et al.*, 2005; Finzi *et al.*, 2007).

Causes and consequences of increased fine-root production

In chapter 2, I investigated the *cause* of increased fine-root production at ORNL FACE. I proposed that increased fine-root production observed in the ORNL FACE sweetgum plantation in response to elevated [CO_2] was a physiological response to N limitation. To examine this premise I added annual additions of 200 kg ha^{-1} of N as urea to a sweetgum plantation adjacent to the ORNL FACE experiment to increase soil N availability. I hypothesized that N fertilization would increase sweetgum NPP and leaf [N], and increase the relative flux of C to wood production. A decreased fraction of NPP in fine-root production with N addition would be consistent with the premise that increased root production in the adjacent FACE experiment is in response to N limitation. This chapter is in press at the *Canadian Journal of Forest Research*.

In chapters 3 and 4, I examine the *consequences* of increased fine-root production at ORNL FACE. Greater root production under elevated [CO_2] may lead to increases in soil C storage and N cycling (Franklin, 2007). However, this depends on the longevity of the fine-root population (Norby *et al.*, 2000), the chemical characteristics of root detritus (Silver & Miya, 2001), and the soil depth at which roots are produced because soil properties such as oxygen availability, soil moisture, and temperature may stall the rate of microbial decomposition and the re-mineralization of C and N (Hunt, 1977; Baldock & Skjemstad, 2000). In chapter 3, I assessed the effect of elevated [CO_2] on root C and N inputs at several soil depths using a long-term minirhizotron data set combined with continuous, root-specific measurements of root mass per unit length and [N]. This chapter is in press at the *New Phytologist*.

Decomposition is the main process by which the organic N in plant material is recycled into an inorganic form available for plant uptake (Berg & Laskowski, 2006), and fine-root detritus is a particularly important source of organic matter because fine roots

have a high concentration of labile C and N (Berg, 1984; Guo *et al.*, 2004). However, most decomposition studies have omitted root decomposition because it is difficult to measure (Silver & Miya, 2001). Further, current decomposition methodology does not specifically test for an effect of increased litter quantity (Silver & Miya, 2001) though this is one of the main mechanisms whereby C and N are expected to be increasingly stored in soil organic matter (Diaz *et al.*, 1993; McMurtrie *et al.*, 2000; Luo *et al.*, 2004). In chapter 4, I examined whether fine roots produced under elevated [CO_2] decomposed more quickly than roots produced under current [CO_2] in a common garden experiment. To address the question of litter quantity, I modified existing litterbag methodology to examine how differences in the amount and diameter distribution of fine roots in a volume of soil would alter root decomposition rates.

Fine-root input and changes in soil C storage in response to elevated CO_2 are perhaps the most important, and least understood, processes being examined in the context of global climate change. My dissertation research focused on disentangling the feedbacks between the C and N cycles and a myriad of complex processes belowground, including fine-root production, turnover, decomposition and fate as soil organic matter. The research presented here will advance the understanding of terrestrial processes that regulate the C storage of important ecosystems, and as such, the results have the potential to inform policy decisions on future atmospheric change initiatives. To this end, the data will be used in the parameterization of ecosystem models (cf. McMurtrie *et al.*, 2000; McMurtrie *et al.*, 2001) and tree-growth models (cf. Franklin, 2007) to better predict how potential negative feedbacks of soil N availability may constrain long-term increases in forest productivity and C storage in response to rising atmospheric [CO_2].

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Appendix

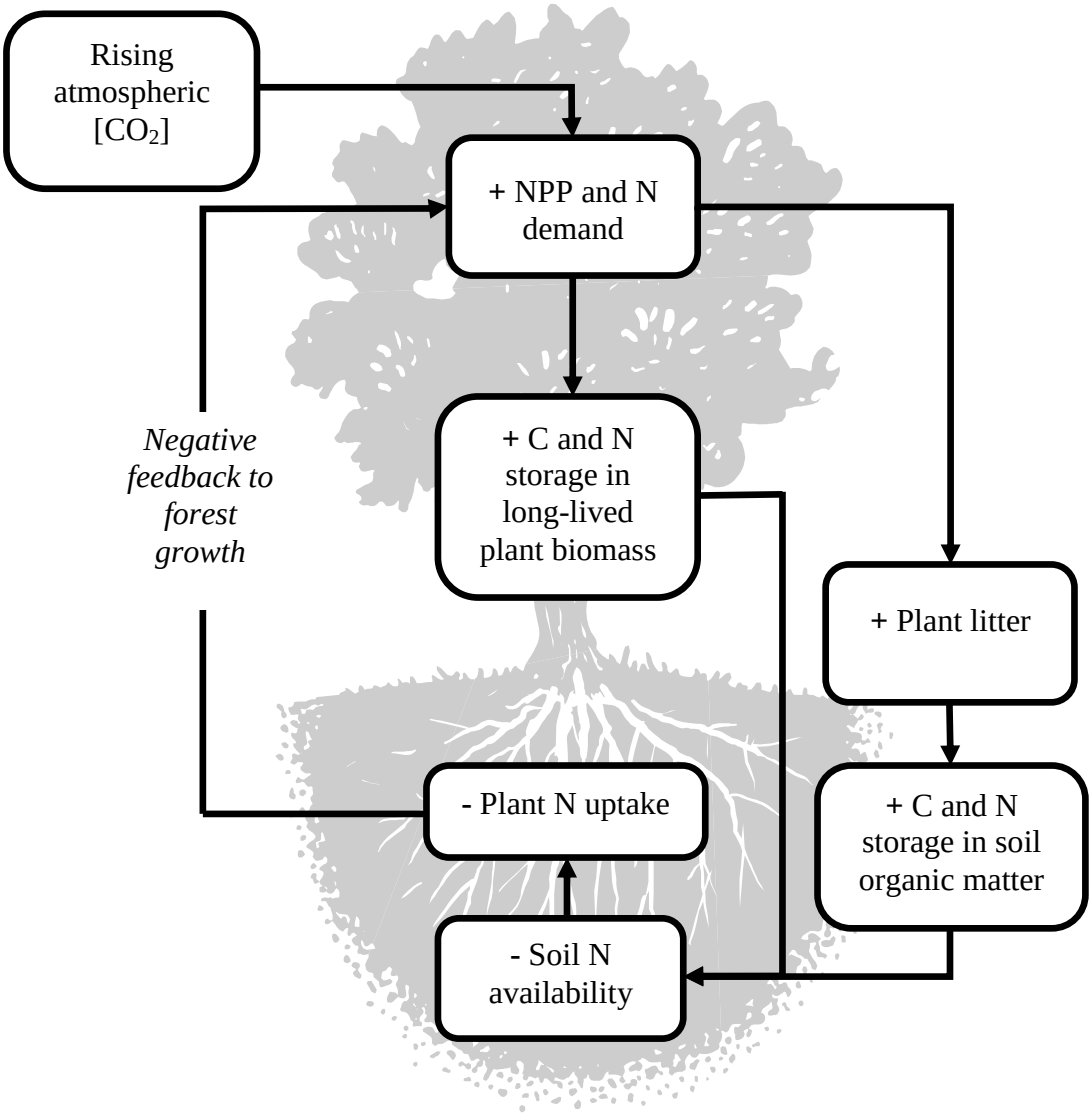

Figure 1.1: Conceptual model of C and N cycling under scenarios of elevated $[CO_2]$. Modified from Luo *et al*. (2004).

Chapter 2
Nitrogen limitation in a sweetgum plantation: Implications for carbon allocation and storage

My use of "we" in this chapter refers to my co-author and myself. This chapter is a lightly revised version of a paper of the same name by Colleen M. Iversen and Richard J. Norby *in press* at the *Canadian Journal of Forest Research*.

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 proportion 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.

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 & Howarth, 1991; Johnson, 2006). The C and N cycles in terrestrial ecosystems are closely linked because N-demanding processes such as photosynthesis (Field & 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 & 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 & McMurtrie, 1993; Pan *et al.*, 1998; McMurtrie *et al.*, 2000; McMurtrie *et al.*, 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 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 & 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 & 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 & Iversen, 2006) given that fine roots often turn over quickly in deciduous forests (Gill & 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 re-mineralized 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 & Iversen, 2006). These observations gave 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 proportion 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 & 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 & Steinbeck, 1998; Samuelson *et al.*, 2001; Chang, 2003; Scott *et al.*, 2004; Allen *et al.*, 2005; Williams & Gresham, 2006; *but see* Price & 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 re-distribute 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 & Tharp, 2007; Norby *et al*., 2007).

Materials and Methods

Experimental Design

We fertilized an 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.*, 2001a). 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* sp.

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 $year^{-1}$, Johnson *et al.*, 2004) but within the range of sweetgum plantation management practices (~100-400 kg N ha $year^{-1}$, 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 (Denver, Colorado, cf. Binkley, 1984). Upon removal, individual capsules were rinsed with de-ionized 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. 1a in Norby *et al.*, 2001b):

$$\text{Stemwood dry mass (kg)} = 0.355 \times \text{BA } (cm^2) - 2.24 \qquad \text{Eqn 2.1}$$

Data were corrected for changes in taper with stand age using a second allometric relationship (Fig. 1b in Norby *et al.*, 2001b) and 2004 – 2005 data collected from the ambient (i.e., "current") [CO_2] treatment in ORNL FACE to correct Eqn 2.1 (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 two years. Coarse root mass increment was also calculated based on allometric equations developed in FACE (Fig. 1c in Norby *et al.*, 2001b):

$$\text{Coarse root mass (kg)} = 0.049 \times \text{BA } (cm^2) + 4.91 \qquad \text{Eqn 2.2}$$

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 meter. 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 two 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 (i.e., 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 & 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 (g m^{-2} $year^{-1}$) using a mean turnover rate derived from the current [CO_2] rings in ORNL FACE (0.93 $year^{-1}$ in 2004 and 1.04 $year^{-1}$ in 2005, Norby *et al.*, 2004; Ledford *et al.*, 2007) along with measurements of the annual peak fine-root standing crop (cf. Gill & Jackson, 2000):

$$\text{Root production} = \text{Peak standing crop (g m}^{-2}) \times \text{Turnover (year}^{-1}) \qquad \text{Eqn 2.3}$$

We assumed that root metabolism (and thus root turnover, cf. Eissenstat *et al.*, 2000; 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 & Iversen, 2006). Wood N content was determined by the product of wood production (sum of Eqns 2.1 and 2.2) 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 Eqn 2.3. 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 in order to meet ANOVA criteria (cf. Gotelli & 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 × treatment interaction, the F statistic given in Table 2.1 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 ×

treatment effects were treated as random effects (Bennington & Thayne, 1994). We specified the "Kenward-Roger" option in the model statement to estimate the denominator degrees of freedom (Kenward & 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 covariate in the analysis of canopy production, and leaf area, mass and number. Differences were considered significant at $P < 0.1$. All responses reported in the results section are supported by the statistical analysis in Table 2.1.

Results

N availability

Fertilization increased inorganic N availability (sum of NH_4-N and NO_3-N, Fig. 2.1), 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 × 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 m^{-2}) and fertilized plots (20.2 ± 2.5 cm^2 m^{-2}, $P > 0.1$). Annual stand BAI increased 30% in response to fertilization in 2004 and 50% in 2005 (Fig. 2.2). 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.*, 2001b), we found that BAI in the control

treatment was similar to that in the current [CO_2] treatment (~380 ppm) in ORNL FACE ($F_{(1,14)}$= 0.1, P > 0.1); there was no response of BAI to CO_2 enrichment (~550 ppm) in the adjacent FACE experiment in 2004 or 2005 ($F_{(1,6)}$= 0.2, P > 0.1, Fig. 2.2).

The canopy mass and area of individual trees felled in August 2004 increased in response to fertilization by 38 % (from 3.2 kg ± 0.3 SEM 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 $year^{-1}$) by ~ 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, ~14% in ORNL FACE, Norby *et al.*, 2003). Fertilization increased stem production efficiency (stem production per unit leaf area, Waring & Schlesinger, 1985) in 2004 (control, 256 ± 19 and fertilized, 308 ± 20 g m^{-2} $year^{-1}$) and in 2005 (control, 211 ± 21 and fertilized, 292 ± 25 g m^{-2} $year^{-1}$).

Canopy responses were driven not by increases in the number of leaves in the canopy, but by increases in the mean mass and area of individual leaves in response to fertilization (Table 2.2). 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 m^{-2} in the control plots and 91.0 ± 11.4 g m^{-2} in the fertilized plots in 2004. In 2005, peak mean fine-root standing crop was 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 2.3). 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. 2.3). 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 current [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 current [CO_2] treatment ($F_{(1,6)}$= 9.2, P = 0.02, Fig. 2.3).

LMA did not respond to fertilization in 2004 (data shown are from trees felled in 2004, Fig. 2.4a) 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. 2.4b) 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. 2.4b).

Stand production and N uptake

N fertilization increased annual stand NPP by 21% in 2004 and 32% in 2005 (Fig. 2.5a). Fertilization increased wood dry-matter increment (g m^{-2}, sum Eqns 2.1 and 2.2) 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. 2.5a).

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. 2.5b). 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. 2.5b). 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. 2.5b).

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. 2.2). Leaf [N] in the fertilized plots (Fig. 2.3), 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. 2.3). Declining leaf [N] is projected by stand-scale models to limit forest CO_2 responses (Comins & McMurtrie, 1993). The responses of BA growth and leaf [N] were strikingly similar between the control treatment in the fertilization experiment and the current [CO_2] treatment in ORNL FACE (Figs. 2.2 and 2.3), 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 & Mooney, 1983). Fertilization increased N_{area}, and we predicted that 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. 2.4a). 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. 2.4b). This was because leaf area and mass increased concurrently in response to fertilization in our study (Table 2.2). 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 & 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 & Allen, 1988). Forest leaf area index (LAI) has been shown to increase with site fertility (Vose & Allen, 1988; Samuelson *et al.*, 2001). This is attributed to increased leaf number, leaf size, or both (Linder & 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 individual trees. Leaf area production increased in the fertilized plots because leaves were larger (greater mass and area, Table 2.2), 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 & 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 & Howarth, 1991). However, fertilization experiments have generally focused on the aboveground components of NPP (Chapin *et al.*, 1986; Aber *et al.*, 1993; Nelson *et al.*, 1995; Vitousek & Farrington, 1997), because of inherent difficulties associated with sampling and quantifying fine-root production (as reviewed in Vogt *et al.*, 1998; Hendricks *et al.*, 2006). Research incorporating belowground responses to N fertilization has shown decreases, increases, and no change in forest fine-root production 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 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. 2.5a); 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 $N \cdot ha^{-1}$ compared with a control treatment of 560 kg $N \cdot ha^{-1}$) had no effect on sweetgum root production or mortality (Price & 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. 2.5a), 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, *pers. comm.*, 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, 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 & Chapin, 1993). Fertilization also increased the absolute amount of N resorbed by senescing leaves by up to 60% (Fig. 2.5b), because of increased leaf mass and N concentration (cf. Birk & 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 28% in 2004 and 2005, respectively). CO_2-enrichment also increased N uptake at ORNL FACE (Norby & 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 & 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 & 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; Norby *et al.*, 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 & 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; McMurtrie *et al.*, 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.

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Appendix

Table 2.1: ANOVA F statistics and corresponding probability values for measured responses to N-fertilization in 2004 and 2005.

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 ($P < 0.1$). Denominator degrees of freedom are as calculated by Kenward-Roger option. Values in boldface type indicate statistical significance at $P < 0.1$. ‡Indicates that the initial basal area of the tree was used as a covariate in the ANOVA. "Felled trees" refers to a one-time felling of one tree from each treatment plot in August, 2004.

Parameter	ANOVA statistics		
2004 only	***ddf***	***F***	***P***
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	***ddf***	***F***	***P***
Inorganic N availability (μmol cm^{-2} $month^{-1}$)	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} m^{-2} $year^{-1}$)	18	26.4	**<0.001**
Leaf area production (m^2 m^{-2} $year^{-1}$)	18	8.7	**0.009**
Fine-root standing crop (g m^{-2})	4	2.3	0.2
Stem production efficiency (g m^{-2} $year^{-1}$)	18	13.7	**0.002**
Total NPP (g m^{-2} $year^{-1}$)	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 m^{-2} $year^{-1}$)	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 m^{-2} $year^{-1}$)	10	92.1	**<0.001**
N requirement (2005, g N m^{-2} $year^{-1}$)	10	77.4	**<0.001**
N resorbed (g N m^{-2} $year^{-1}$)	4	86.8	**0.001**
Resorption efficiency (%)	4	9.1	**0.04**
Compartmental [N] (mg 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

Table 2.2: 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.

Year	Relative canopy height	Leaf mass (g leaf^{-1})		Leaf area (cm^2 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

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.

Table 2.3: Mean annual tissue N concentration (mg g^{-1}) in the control and fertilized plots.

Year	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 ± 0.48^{NS}
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 ± 0.51^{NS}

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

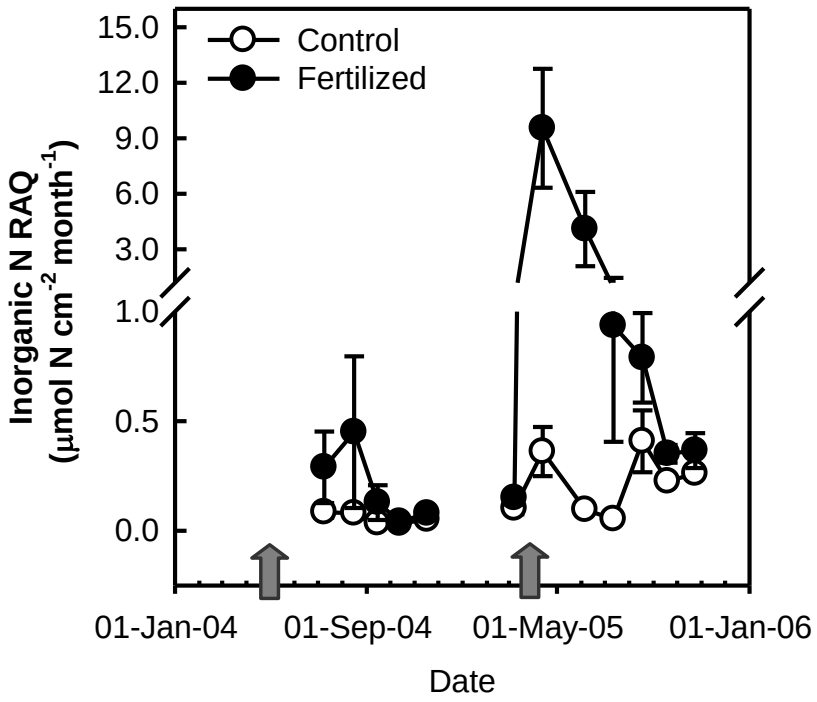


Figure 2.1: Total inorganic N availability (sum of NH_4-N and NO_3-N) assessed by resin capsules in the control and fertilized plots. Data are monthly (30 day) means ± 1 SEM (*n* = 6 for each treatment) expressed in units corresponding to resin adsorption quantity. RAQ, adsorption per cm^2 of resin surface. Gray arrows refer to fertilization events.

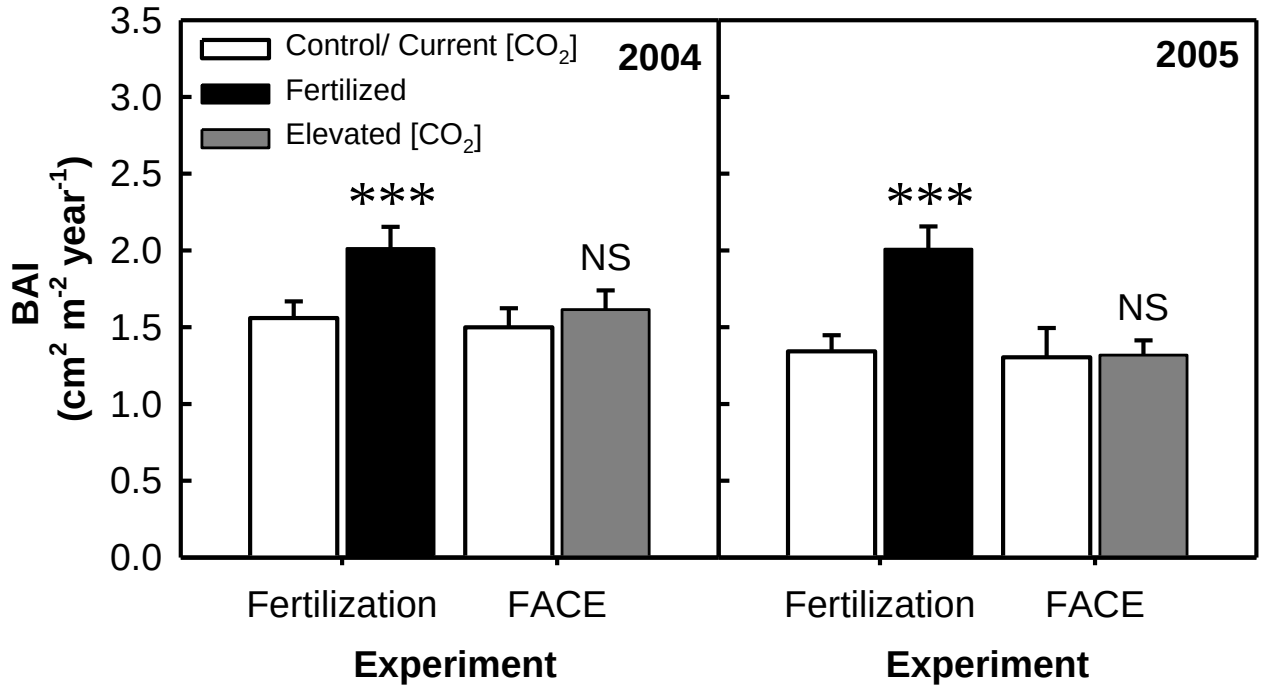


Figure 2.2: Annual mean stand BAI (cm^2 m^{-2} $year^{-1}$) ± 1 SEM in the fertilization experiment (n = 6 for each treatment) and adjacent ORNL FACE (n = 3 for current [CO_2] treatment and n = 2 for elevated [CO_2] treatment). FACE BAI data are from Norby and Tharp (2007). ***, $P \leq 0.001$; and NS, not significant ($P > 0.1$). P values correspond to differences between treatments within each experiment.

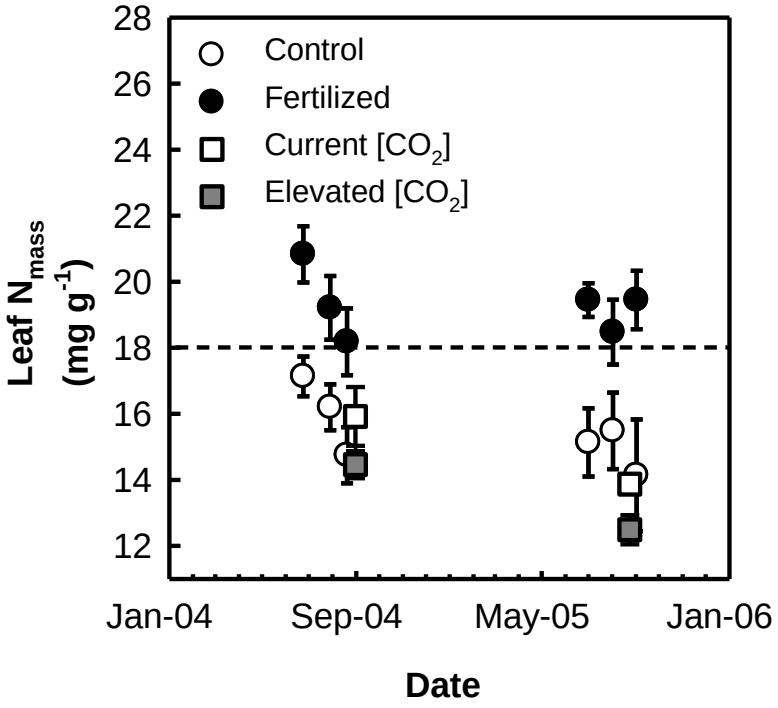


Figure 2.3: Leaf [N] sampled from tree canopies via a hydraulic lift in the fertilization experiment (n = 2 plots for each treatment) or from trees felled in August, 2004 (the last data point in 2004, n = 6 plots for each treatment). Leaf [N] was also sampled via hydraulic lift in the adjacent ORNL FACE experiment once in 2004 and 2005 (n = 3 plots for the current [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).

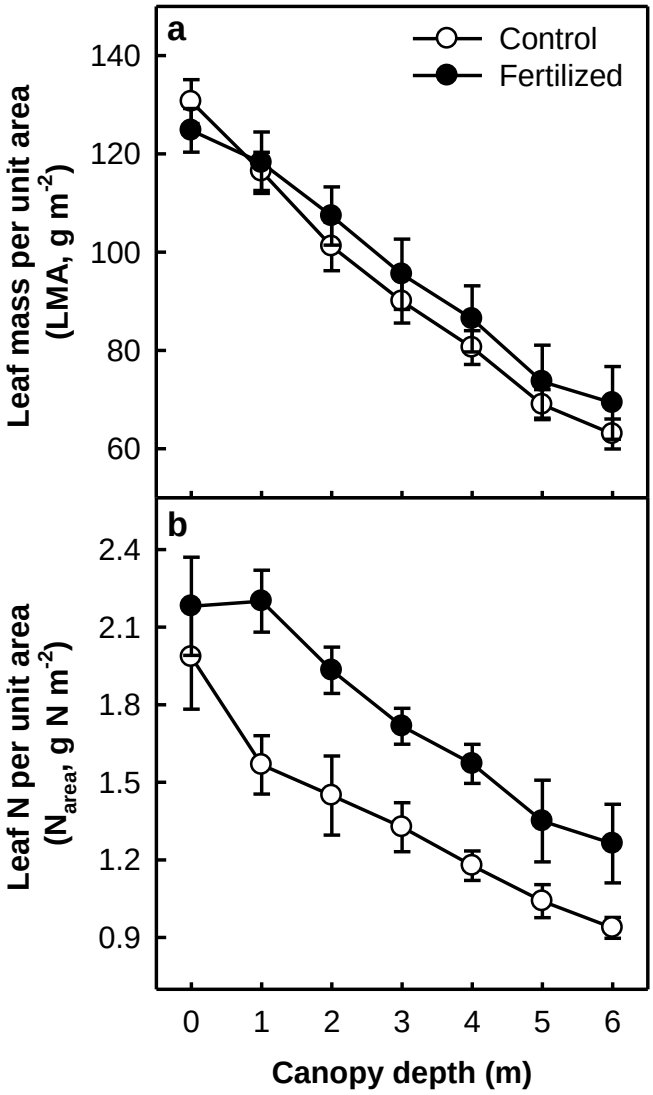
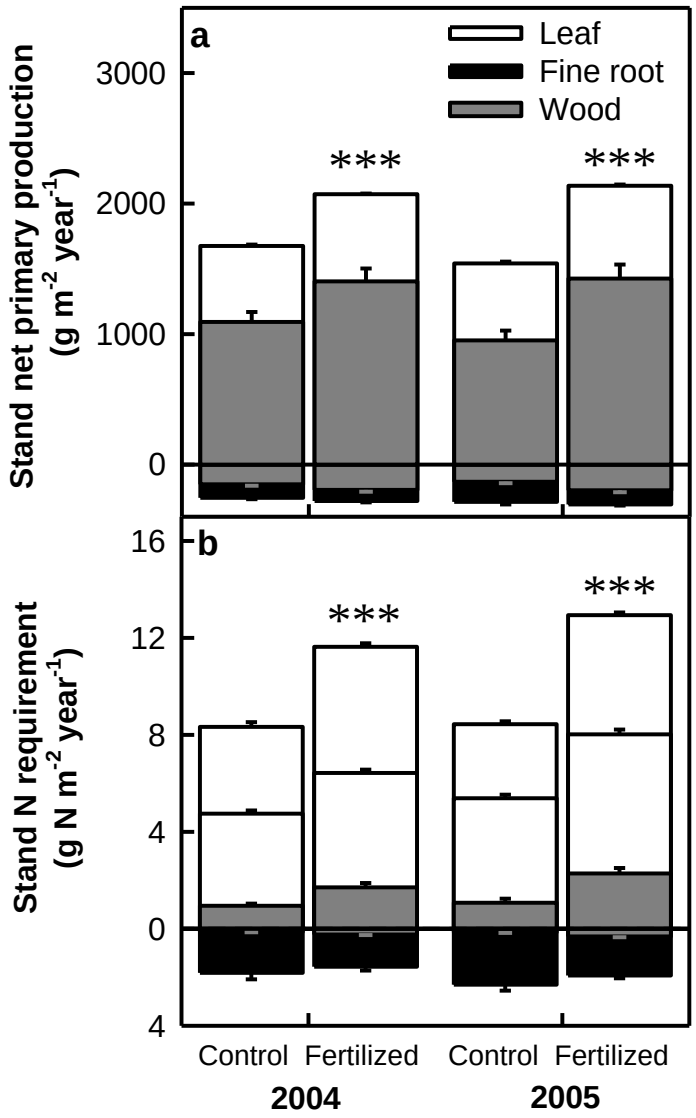


Figure 2.4: Changes in leaf mass and N per unit leaf area with canopy depth were measured on one tree felled from each treatment plot in August, 2004 (n = 6 plots per treatment). Data are (a) mean leaf mass per unit area (LMA) and (b) mean N per unit leaf area (Narea) ± 1 SEM.

Figure 2.5: 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 panel (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).


a
Leaf
Fine root
Wood
Stand net primary production (g m-2 year-1)
3000
2000
1000
0
***
***
b
Stand N requirement (g N m-2 year-1)
16
12
8
4
0
4
***
***
Control
Fertilized
Control
Fertilized
2004
2005

Chapter 3
CO_2-enrichment increases carbon and nitrogen input from fine roots in a deciduous forest

My use of "we" in this chapter refers to my co-authors and myself. This chapter is a lightly revised version of a paper of the same name by Colleen M. Iversen, Joanne Ledford, and Richard J. Norby *in press* at *New Phytologist*.

Abstract

The production of fine roots (less than 2 mm in diameter) is expected to increase under elevated atmospheric [CO_2], especially in N-limited forests where increased belowground C allocation may facilitate nitrogen N acquisition. Greater fine-root production under elevated [CO_2] may drive changes in soil C storage and N cycling because fine roots turnover quickly in forested ecosystems. However, the rate at which C and N are re-mineralized from fine-root detritus will depend on root population turnover and chemistry, and the soil depth at which the roots are produced. We assessed the effect of elevated [CO_2] on fine-root biomass and N inputs at several soil depths using a long-term minirhizotron data set combined with continuous, root-specific measurements of root mass per unit length and [N]. We conducted our research at the Oak Ridge National Laboratory (ORNL), Free-Air CO_2-Enrichment (FACE) experiment in a sweetgum (*Liquidambar styraciflua* L.) plantation in eastern Tennessee, USA where the sweetgum trees had been exposed to current or elevated atmospheric [CO_2] for 9 years. CO_2-enrichment had no effect on fine-root tissue density or [N] within a given diameter class. Root diameter explained 96% of the variation in fine-root mass per unit root length, and 65% of the variation in fine-root [N] across CO_2 treatments. Fine-root biomass production and peak standing crop doubled under elevated [CO_2]. Though fine-root population turnover was somewhat slower under elevated [CO_2], fine-root mortality was also nearly doubled under CO_2-enrichment. Over 9 years, fine-root mortality resulted in 681 g m^{-2} of extra C and 9 g m^{-2} of extra N input to the soil system under elevated [CO_2] relative to the current [CO_2] treatment. At least half of these inputs were below 30 cm soil depth where the microbial mineralization of C and N from fine-root detritus may be limited by soil temperature, oxygen availability, or moisture. Quantification of the effects of elevated CO_2 on fine-root detritus and its subsequent decomposition, especially at depth in the soil, will provide critical information needed for predicting processes such as long-term soil C storage and N cycling in response to environmental change.

Introduction

"Fine" roots (i.e., roots less than 2 mm in diameter that are the most active in water and nutrient uptake, Pregitzer, 2002; Pregitzer, 2003) comprise up to one-third of annual net primary production in terrestrial ecosystems (Jackson *et al.*, 1997). Further, the production of fine roots is expected to increase under elevated atmospheric [CO_2], especially in N-limited forests where increased belowground C allocation may facilitate N acquisition (Zak *et al.*, 1993; Eissenstat *et al.*, 2000; Norby & Jackson, 2000; BassiriRad *et al.*, 2001). Fine root populations turn over quickly, often within 1 to 9 years in forested ecosystems (Gill & Jackson, 2000; Norby & Jackson, 2000; Matamala *et al.*, 2003), and root detritus has a greater probability of being retained in the soil organic matter pool than surface litter because it is in intimate contact with the soil profile (Gale & Cambardella, 2000; Gale *et al.*, 2000). The stabilization of root-derived C in long-term soil pools (i.e. pools with longevities ranging from centuries to millennia, Schlesinger, 1997) may mitigate some portion of future atmospheric and climatic change. Forested ecosystems may be an especially important sink for rising [CO_2] given that forest soils currently store nearly half of belowground terrestrial C (Dixon *et al.*, 1994).

The annual dynamics of C and N input from root population growth and mortality (i.e., turnover) are important parameters in models projecting biosphere responses to atmospheric and climatic change in N-limited ecosystems (Aber *et al.*, 1997; Kirschbaum *et al.*, 2003; Franklin, 2007). Decaying fine roots of woody plants represent an important flux of labile C and N to the soil (Aerts *et al.*, 1992), because they turn over quickly (Eissenstat *et al.*, 2000; Norby & Jackson, 2000) and have relatively high concentrations of N and carbohydrates (Guo *et al.*, 2004). However, it is currently uncertain whether the input of labile root C and N to the soil profile will stimulate microbial degradation of organic matter and increase soil N availability (i.e., the "priming effect" cf. Kuzyakov *et al.*, 2000; Xiao *et al.*, 2007), allowing sustained forest responses to rising atmospheric [CO_2] (Zak *et al.*, 2000; Phillips, 2007). Alternatively, greater input of labile C could

increase microbial immobilization of available N and constrain forest production (de Graaff *et al.*, 2007).

Whether increased fine-root production will lead to changes in soil N availability and C storage depends largely on the turnover rate of the fine-root population (Norby & Jackson, 2000), the chemical characteristics of the root litter (Sollins *et al.*, 1996; Zak *et al.*, 2000; Silver & Miya, 2001), and the depth at which litter is input into the soil profile (Hunt, 1977; Fontaine *et al.*, 2007). Rising atmospheric [CO_2] is projected to decrease root turnover (as reviewed in Eissenstat *et al*., 2000) by altering a suite of fine-root characteristics that are linked to greater root longevity. For example, CO_2-enrichment has been shown to increase root diameter (cf. Pritchard & Strand, 2008b), decrease root N concentration (Cotrufo & Ineson, 1995), increase mycorrhizal infection (Pritchard *et al*., 2008a) and increase rooting depth (Norby *et al*., 2004; Pritchard *et al*., 2008b). Changes in root characteristics or rooting depth may increase root longevity (cf. Wells & Eissenstat, 2001; Baddeley & Watson, 2005; Joslin *et al*., 2006; Withington *et al*., 2006) by increasing nutrient gain per unit C cost (i.e., construction or maintenance respiration, cf. Eissenstat *et al*., 2000).

Fine-root turnover is hard to measure due to the "hidden" nature of belowground processes (Norby & Jackson, 2000; Matamala *et al.*, 2003; Pritchard & Strand, 2008; Strand *et al.*, 2008), and the fact that roots grow in a branched pattern, ranging from smaller diameter younger roots, to larger diameter older roots (Pregitzer, 2002; Pregitzer, 2003). Root production and mortality have been estimated with destructive soil sampling methods ranging from root ingrowth cores to sequential biomass cores (Vogt *et al.*, 1998; Majdi *et al.*, 2005), and whole-ecosystem C and N budgets have been used to constrain estimates of belowground inputs (Vogt *et al.*, 1998). Digitized images from minirhizotron cameras are now generally accepted to be a more accurate measure of root length production in ecosystems where production and mortality occur simultaneously (Johnson *et al.*, 2001; Tierney & Fahey, 2001), though long-term minirhizotron data sets are needed to adequately track the turnover rate of fine-root populations (Strand *et al.*,

2008). Quantitative relationships are necessary to derive root biomass and N content from minirhizotron measurements of root length and diameter (Vogt *et al.*, 1998; Tingey *et al.*, 2000; Johnson *et al.*, 2001), but this is complicated by the fact that root mass per unit length and root N concentration are both highly related to root diameter (Pregitzer *et al.*, 2002).

We quantified C and N input from fine-root mortality under ambient, i.e., "current", and elevated [CO_2] at several soil depths in a deciduous sweetgum forest in eastern North America by combining allometric relationships derived from samples of individual roots with a long-term minirhizotron data set. Fine-root production and mortality length estimates from minirhizotron images taken in ORNL FACE from 1998 to 2003 have been previously published (Norby *et al.*, 2004). Our research expanded these data by developing continuous relationships that enabled us to estimate root biomass and N content from root length and diameter. We used these relationships to calculate annual and cumulative root biomass and N input as a function of soil depth over the entire span of the experiment to-date (a period of 9 years ranging from 1998 to 2006). Our main questions were: do C and N inputs from root mortality increase in response to long-term CO_2-enrichment, and if so, what are the implications for soil C storage and N cycling?

Materials and Methods

Site Description

We conducted our research at the Oak Ridge National Laboratory (ORNL), Free-Air CO_2-Enrichment (FACE) experiment in a sweetgum (*Liquidambar styraciflua* L.) plantation in eastern Tennessee, USA. ORNL FACE has been described in detail elsewhere (Norby *et al.*, 2001; Norby *et al.*, 2002; Norby *et al.*, 2004), but briefly, the experiment consists of five 25-m diameter rings, of which four have a FACE apparatus installed. Two rings blow air enriched with CO_2 to achieve a concentration in the canopy of ~560 ppm, while two rings blow a current [CO_2] of ~380 ppm; the fifth ring serves as

a current [CO_2] treatment without a FACE apparatus. CO_2-enrichment was initiated in 1998 when the sweetgum trees were 10 years old and 12-m tall. The soil at the FACE site is described as an Aquic Hapludult, and consists of alluvium washed from upland soils (Norby *et al.*, 2001; Norby *et al.*, 2002).

Minirhizotron images

Cellulose acetate butyrate minirhizotron tubes (Bartz Technology, Santa Barbara, California) of 5.1 cm inner diameter were installed in each FACE ring in July, 1997 at a 60° angle from vertical to a depth of 60 cm (as described in Norby *et al.*, 2004). Measurements were made using five tubes per ring. Minirhizotron images were collected in each of 91 frames per tube (12.4 mm wide by 18 mm long) with a BTC-2 minirhizotron camera with a Smucker handle (Bartz Technology). Images were collected every 2 weeks during the growing season (April to October), and monthly during the winter (December to March, Table 3.1). Before 2004, we did not film during winter months because cold temperatures resulted in shrinkage of the minirhizotron tubes, but relatively milder winters since 2004 allowed us to film throughout the winter. For the purposes of this experiment, we considered a "year" to be the period between leaf-out in the spring (April) and leaf-out the next spring. Images were captured with the Targa+ video board (True Vision, Indianapolis, 1998 – 2002) or I-CAP system (Bartz Technology, 2003 – 2006), and digitized to obtain root diameter and length using ROOTS (Michigan State University, East Lansing, Michigan, 1998 – 2002) or RooTracker (Duke University, Durham, North Carolina, 2003 – 2006) software. The same analyst has processed all of the minirhizotron images captured since the inception of the experiment.

Root biomass and N content

To determine allometric relationships between root diameter, biomass and N content, we sampled 30-cm deep by 5-cm diameter cores from the current and elevated CO_2 treatments on 7 June, 8 July, and 27 September 2005 (15 – 30 cm only), and on 30

May, 24 July, and 14 September 2006 (in 2005, five subsamples per ring, and in 2006, six subsamples per ring). Individual cores were divided into depths of 0 – 15 cm and 15 – 30 cm in the field, and refrigerated at 4 °C until processed.

Roots were separated from the soil using a hydropneumatic elutriator with a 530 µm filter (Gillison Variety Fabrications, Benzonia, Michigan), and large pieces of organic matter were removed by hand with forceps. After washing, moist roots were refrigerated at 4 °C up to 1 month until they were scanned on a flatbed scanner (400 dpi, Epson Expression 1680, Epson, Long Beach, California). The high-resolution images obtained from the scanner were digitized using the *WinRhizo* root-scanning software program (Regent Instruments, Inc., Canada) to determine the total length, volume and surface area of root in diameter classes ranging from 0 to 4 mm in 0.1 mm increments. After the entire population of roots in each core was scanned, three small subsamples ranging from 2 to 20 individual roots (10 mg to 200 mg total mass depending on root size) were removed from each 0 – 15 cm core (five to six cores per ring), and from a composite of the 15 – 30 cm cores because they contained fewer roots (one composite per ring). Subsamples were selected by hand to encompass the range of diameter classes contained in the core or composite sample (i.e., roughly within 0 to 0.4 mm, 0.4 to 0.8 mm and 0.8 mm to 4 mm size classes). The small subsamples were re-scanned using the same *WinRhizo* parameters as above and oven-dried at 70 °C for at least 48 h to determine root mass per unit length (RML, mg cm^{-1}). After oven-drying, subsamples were ground on a Wig-L-Bug dental grinder (Crescent Dental Manufacturing Co., Chicago, Illinois), and total N content was determined on an elemental analyzer (Costech Analytical Technologies, Inc., Valencia, California).

Estimation of root biomass and N content from length measurements

We used the relationships between RML, root [N] and root diameter (see *Results*) to estimate root biomass from root length and diameter measurements derived from digitized root images from 1998 to 2006. Root production was calculated from the appearance and incremental growth of individual roots, while root mortality was

calculated from the disappearance of individual roots (without subsequent reappearance), from the minirhizotron frames as in Johnson *et al.* (2001). The biomass of individual roots was scaled to a volume of soil using the depth-of-field approach; we assumed that the depth of field in each minirhizotron window was 2 mm (cf. Johnson *et al.*, 2001). Annual mortality, production, and root N input (i.e., the N content of roots lost to mortality) were calculated based on the maximum diameter observed for each individual root within a given year, and standing crop was calculated based upon the maximum diameter observed on or before the date of peak standing crop. Root biomass and N content were summed within individual tubes for each of four depth increments. We assumed that roots did not resorb N prior to senescence (i.e., Nambiar & Fife, 1991; Aerts *et al.*, 1992; Gordon & Jackson, 2000), and that the N content of roots when they died was predicted by the relationship derived from live roots. We estimated production, mortality and N input over the winter when filming was infrequent (i.e., Table 3.1) by subtracting the standing crop in the last session in one year from that of the first session in the next year (using the associated diameters in the first and last filming sessions). Thus, our estimates of root production and mortality are net estimates, as roots probably formed and disappeared between filming intervals (cf. Johnson *et al.*, 2001). We calculated root turnover as in Gill & Jackson (2000), where turnover ($year^{-1}$) was equal to annual biomass mortality ($g\ m^{-2}\ year^{-1}$) divided by the peak standing crop ($g\ m^{-2}$).

Statistics

We used the "NLIN" procedure in SAS (Version 9.1, SAS Institute Inc., Cary, North Carolina) to fit a power function to the relationships between RML ($mg\ cm^{-1}$) and diameter and root [N] and diameter for the subsamples taken from cores in 2005 and 2006. We used the SAS "Mixed" procedure to test for differences in root biomass production, mortality, peak standing crop, N input and turnover under elevated [CO_2] from 1998 to 2006 using year as a repeated measure (ANOVA tables can be found in Table 3.2). Ring within each treatment or treatment × depth combination was the subject of the repeated measure (cf. Littell, 1996, n = 2 elevated [CO_2] plots, n = 3 current [CO_2]

plots); CO_2-treatment and soil depth were treated as fixed effects. We used the autoregressive (1) covariance structure in our model because it yielded the best goodness of fit (as determined by the Akaike's information criterion) when compared to other potential covariance structures (cf. Littell, 1996; Littell *et al.*, 1998). Non-normal data were log-transformed before analysis, and differences were considered significant at $P < 0.05$.

Results

All but 10 of the nearly 14,000 roots captured by minirhizotron filming between 1998 and 2006 were less than 2 mm in diameter; 99% of the roots were less than 1 mm in diameter. The distribution of root length lost to annual mortality among diameter classes did not significantly differ between the current and elevated [CO_2] treatment in any year ($P > 0.1$, Fig. 3.1). Across all years, the weighted average diameter of roots lost to mortality under elevated CO_2 tended to be only slightly larger on average (0.39 ± 0.01 mm under elevated CO_2 as compared with 0.36 ± 0.01 mm under current CO_2; $P > 0.08$, Table 3.3). There were no interactions among diameter, treatment or depth ($P > 0.3$).

The relationship between root diameter and RML followed a positive power function ($r^2 = 0.96$, $P < 0.0001$, Fig. 3.2a). RML did not differ between the current and elevated [CO_2] treatments ($P > 0.05$), or with soil depth ($P > 0.05$; data shown are pooled across depths). The relationship between root diameter and [N] followed a negative power function ($r^2 = 0.65$, $P < 0.0001$, Fig. 3.2b), and also did not differ between the current and elevated [CO_2] treatments or by soil depth ($P > 0.05$). Root [C] was not related to root diameter (i.e., the slope was not significantly different from 0, $P > 0.1$), and root [C] averaged 46.6 ± 0.2 % in the 0 – 15 cm soil depth, and 45.4 ± 0.1 % in the 15 – 30 cm soil depth (*data not shown*, overall depth effect, $P < 0.005$).

Elevated [CO_2] more than doubled fine-root biomass production ($P < 0.003$), and biomass production changed over time in both treatments ($P < 0.001$, Table 3.3). Production did not differ with soil depth ($P > 0.2$); there were no interactions among

treatment, depth or time ($P > 0.5$). Elevated [CO_2] approximately doubled peak standing crop across all years and soil depths ($P < 0.003$, Table 3.3). However, the depth dynamics of standing crop changed over time (soil depth × year interaction, $P < 0.05$). Combined across CO_2 treatments, peak standing crop was less below 45 cm soil depth than at either 0 – 15 or 15 – 30 cm in 1998 and 2000 ($P < 0.05$); in 2001 there was a large increase in standing crop, especially below 30 cm soil depth (Table 3.3). Thereafter, there was no difference in standing crop among depths ($P > 0.4$).

Elevated [CO_2] nearly doubled fine-root mortality ($P < 0.005$, Fig. 3.3). In contrast to production, the depth dynamics of mortality changed over time (soil depth × year interaction, $P < 0.05$); combined across CO_2 treatments, root mortality was much lower below 45 cm soil depth than at shallower depths (i.e. the 0 – 15 and 15 – 30 cm depth increments) from 1998 to 2000 ($P < 0.05$); in 2001 there was a large increase in root mortality input, especially at 45 – 60 cm soil depth. Thereafter, there was no difference in mortality among depths ($P > 0.1$). Elevated [CO_2] doubled the N input from fine-root mortality ($P < 0.01$), and the amount of N input from fine-root mortality changed over time in both treatments ($P < 0.0001$, Fig. 3.4). There were no differences in N input with soil depth ($P > 0.6$), and no interactions among treatment, depth or time ($P > 0.1$). Over 9 years, elevated [CO_2] nearly doubled the cumulative input of C ($P < 0.03$) and N ($P < 0.02$) from fine-root mortality (Figs. 3.3 and 3.4). There was no depth effect, or depth × treatment interactions on the cumulative input of C and N ($P > 0.5$).

Averaged across all soil depths, turnover (calculated from root mortality and peak standing crop) was less under elevated [CO_2] ($P < 0.05$, Fig. 3.5). Turnover decreased over time in both treatments as the minirhizotron tubes were colonized ($P < 0.0001$), and stabilized in 2001 (i.e., turnover did not differ significantly among years after 2001, $P > 0.1$). We were unable to examine the effect of soil depth on turnover rate before 2001 because there were few roots below 30 cm (i.e., Table 3.3). However, we examined depth effects from 2001 onwards when root population turnover appeared stable. After 2001, the root population in both treatments turned over more slowly below 30 cm depth

(on average 1.1 ± 0.09 $year^{-1}$ in the current and 0.8 ± 0.06 $year^{-1}$in the elevated [CO_2] treatments) than closer to the soil surface (i.e., 0 – 15 cm, 1.3 ± 0.09 $year^{-1}$ in the current and 1.1 ± 0.10 $year^{-1}$in the elevated [CO_2] treatments, $P < 0.05$). There was no effect of year ($P > 0.2$), and there were no interactions among treatment, year or soil depth after 2001 ($P > 0.4$).

Discussion

Root biomass and N input

We quantified biomass and N input from fine-root mortality in a long-term CO_2 experiment in a sweetgum plantation by using continuous relationships to estimate the biomass and N content of individual roots from measurements of root length and diameter (i.e., Fig. 3.2a,b). Elevated [CO_2] nearly doubled the production of root biomass, and contrary to what we expected, root production was not greatest near the soil surface (i.e., was similar at all of the soil depths that we observed in both treatments, Table 3.3). In fact, it appeared that the largest increases in root production under elevated [CO_2] were deeper in the soil, though there was large variability associated with biomass estimates at depth (Table 3.3).

Overall, biomass and N input resulting from root mortality were twice as great in the elevated [CO_2] treatment relative to the current [CO_2] treatment (Figs. 3.3 and 3.4) even though the fine-root population turned over more slowly in the elevated relative to the current [CO_2] treatment (Fig. 3.5). Root mortality was greatest between November and March in both treatments (i.e., in the winter when filming was done at longer intervals, see Ledford et al., 2007 for raw data). Ignoring overwinter dynamics in our calculations of root mortality would have underestimated annual biomass inputs via root mortality by up to 65%, even in years when we filmed during winter months (i.e., 2004 – 2006). These findings highlight the importance of quantifying root growth and mortality after leaf senescence, and also of decreasing the interval between minirhizotron filming dates when feasible (cf. Johnson et al., 2001).

Root turnover

Root turnover is expected to decrease under elevated [CO_2] due to declining tissue [N] (cf. Cotrufo & Ineson, 1995; Cotrufo *et al.*, 1998; Curtis & Wang, 1998; Long *et al.*, 2004), and associated declines in construction or maintenance respiration (cf. Eissenstat *et al.*, 2000). However, elevated [CO_2] had no effect on sweetgum root [N] within a given diameter class (Fig. 3.2b). Instead, the decline in turnover rate we observed in response to CO_2-enrichment (i.e., Fig. 3.5) may be due to increased root proliferation deeper in the soil profile; we found that in both treatments, the root population turned over more slowly below 30 cm than closer to the soil surface (i.e., 0 – 15 cm). Lower N availability and cooler temperatures deeper in the soil profile may allow roots to live longer by reducing respiration costs (cf. Eissenstat *et al*., 2000; Burton *et al*., 2000; Baddeley & Watson, 2005; Joslin *et al*., 2006). Increased diameter has also been linked to greater root longevity in other ecosystems (Wells & Eissenstat, 2001), and the slight increase in root diameter that we observed under elevated [CO_2] (Table 3.3) may have also contributed to greater root longevity. Another mechanism for greater root longevity could be increased mycorrhizal infection (i.e., Eissenstat *et al*., 2000). This has been observed in a CO_2-enriched loblolly pine plantation (i.e., Pritchard *et al*., 2008a), but was beyond the scope of this investigation. Ultimately, a decline in root turnover under elevated [CO_2], or as roots grow deeper in the soil profile, may lead to delayed C and N input to the soil pool and result in increased storage of C and N in plant biomass.

Turnover estimated from production (*data not shown*) was initially greater, and more variable, than turnover estimated from root mortality (Fig. 3.5) indicating that the root population was not in equilibrium (cf. Burton *et al*., 2000). However, the long-term nature of this data set allowed us to observe the stabilization of root population turnover under current and elevated [CO_2] over time (Fig. 3.5, cf. Tierney & Fahey, 2002; Guo *et al.*, 2008; Pritchard & Strand, 2008; Strand *et al.*, 2008). Contrary to the widespread assumption that soil disturbance and increased root proliferation associated with installation of minirhizotron tubes do not affect estimates of root production or mortality

after 6 to 12 months (cf. Joslin & Wolfe, 1999; Johnson *et al.*, 2001), we found that turnover calculated from total mortality and peak standing crop did not stabilize until year 4 of the experiment (Fig. 3.5). The initial variability in turnover rate was likely due to soil disturbance and delayed colonization of the minirhizotron tubes; we have not identified any environmental signals (e.g. temperature or precipitation) that would explain the result. Our data indicate that short-term minirhizotron data sets should be viewed with caution (cf. Stark *et al*., 2008).

Root characteristics

Several authors have advocated the use of root order (i.e., the ontological order of a root's connection within a network of roots, Pregitzer, 2002; Pregitzer *et al.*, 2002; Guo *et al*., 2008) over root diameter to describe root physiology and morphology. However, our results demonstrate that diameter is an excellent proxy for root mass and [N], especially given the difficulty of determining root order from minirhizotron images. Diameter explained 96% of the variation in root mass per length, and 65% of the variation in root [N] (Fig. 3.2a,b). These relationships did not differ between 0 and 30 cm soil depth, and are linear in a log-log space as predicted by allometric analysis (as reviewed in Enquist *et al.*, 2007; cf. Andersen *et al.*, in press). The relationship between root diameter and [N] may differ deeper in the soil profile, but we were unable to obtain enough biomass samples below 30 cm depth to develop robust relationships similar to those in Fig. 3.2. Continuous, root-specific relationships enabled us to avoid potentially confounding effects associated with differences between the diameter distribution of the minirhizotron data and the data used to quantify root biomass (cf. Johnson *et al.*, 2001; Majdi *et al*., 2005; Tingey *et al*., 2005). For example a root mass per length of 0.65 mg $cm^{-1}$was used previously to estimate the biomass of roots less than 0.5 mm in diameter in ORNL FACE (Norby *et al.*, 2004). This root mass per length corresponds to a root diameter of approximately 0.47 mm (Fig. 3.1), and overestimates the mass of a length of root in one of the most prominent diameter classes (0.2 to 0.3 mm, Fig. 3.1), by 150 to 450%.

Implications for long-term soil C and N storage

Elevated [CO_2] has increased annual N uptake in the ORNL FACE sweetgum stand by approximately 20%, but since 2000, nearly all the extra N taken up by the sweetgum trees under elevated [CO_2] was allocated to support fine-root growth (methods as described in Norby & Iversen, 2006, additional unpublished data from R. Norby). Increased N uptake is necessary to support fine-root proliferation under elevated [CO_2] because small first-order roots have a high [N] (Pregitzer *et al*., 2002, Fig. 3.2b), and we found no decline in individual root C: N under elevated [CO_2] (Fig. 3.2b).

Over 9 years, approximately 681 g m^{-2} of the extra C, and 9 g m^{-2} of the extra N taken up by the sweetgum stand under elevated [CO_2] were returned to the soil via root mortality. Note that this calculation assumes that N is not resorbed from senescing roots (i.e., Nambier & Fife, 1991; Aerts, 1992; Gordon & Jackson, 2000). The average C: N of fine root detritus was ~ 75, which is somewhat greater than the global average C: N of living fine roots (~ 40, Jackson *et al.,* 1997). Up to half of C and N input to the soil from root mortality was below 30 cm soil depth (Figs. 3.3, 3.4), where soil properties such as oxygen availability, soil moisture, and temperature may stall the rate of microbial decomposition and the re-mineralization of N (Hunt 1977; Baldock & Skjemstad, 2000). If all of the "extra" N input from greater fine-root mortality under elevated [CO_2] were sequestered in soil organic matter, this would support ~135 g m^{-2} of extra soil C storage over all soil depths if C continues to be stored in soil organic matter pools with a C: N of ~ 15 (cf. Jastrow *et al*., 2005).

Jastrow *et al.* (2005) have previously shown C accrual in the top 5 cm of soil at ORNL FACE attributable to elevated [CO_2] to be ~ 44 g m^{-2} $year^{-1}$ (over the period of 1997 to 2002). Our data on root C input over 9 years cannot account for all of this accrual; we find only 8 g C m^{-2} $year^{-1}$ additional input from fine-root mortality under elevated [CO_2] in the top 15 cm of soil (39 g C m^{-2} $year^{-1}$ in elevated [CO_2] compared to 31 g C m^{-2} $year^{-1}$ in the current [CO_2] treatment.) We suspect this discrepancy may be explained in part by the minirhizotron system missing root growth in the top 5 cm of the

soil (cf. Heeraman & Juma, 1993). Root biomass did not decline in a linear or exponential fashion with soil depth in ORNL FACE as has been observed in other forested ecosystems (Jackson *et al.*, 1996; Matamala & Schlesinger, 2000, Table 3.3). Missed growth at the top of the soil profile could increase the additional root biomass input in the elevated [CO_2] treatment to ~20 g C m^{-2} $year^{-1}$ (~ 80 g m^{-2} $year^{-1}$ in elevated [CO_2] compared to ~ 60 g m^{-2} $year^{-1}$ under current [CO_2]) given that up to 50% of root biomass in the top 15-cm at ORNL FACE is found from 0 to 5 cm depth (J. Jastrow, *personal communication*). Further, we have shown that overwinter mortality (between October and March) can contribute up to two thirds of annual biomass input, and we may have missed C and N inputs before overwinter filming began in 2004. Lastly, leaf litter inputs at the top of the soil may contribute to soil C accrual; annual leaf litter inputs were approximately 225 g m^{-2} under elevated [CO_2] compared to 210 g m^{-2} under current [CO_2] (methods as in Norby *et al*., 2002, additional unpublished data from R. Norby).

Greater C and N sequestration in long-term soil pools under elevated [CO_2] is projected to decrease soil N availability and ultimately, forest production (Luo *et al*., 2004). However, limited soil N availability has not constrained forest production or stand N uptake in response to elevated [CO_2] thus far in any of the forested FACE experiments (Norby *et al*., 2005; Finzi *et al.*, 2007; Iversen & Norby, 2008). Increased soil exploration by fine roots has facilitated greater N acquisition under elevated [CO_2] in forested ecosystems (Norby *et al.*, 2004; Norby & Iversen, 2006; Finzi *et al*., 2007; Pritchard *et al*., 2008b), and root proliferation could further stimulate soil N availability throughout the soil profile by supplying a "fresh" source of organic matter and energy to the microbial community (cf. Fontaine *et al*., 2007). However, it remains difficult to project future forest responses to global change because the relationship between forest N uptake and soil N availability is not well-represented in ecosystem models (cf. Finzi *et al*., 2007). Further, the soil organic matter dynamics that determine N mineralization in ecosystem models like CENTURY are only simulated in the first 20 cm of the soil profile (Parton *et al.*, 1988; Ma & Shaffer, 2001). Continued data-model integration is an important goal in advancing our understanding of belowground processes and their

impact on ecosystem responses to global change (Classen & Langley, 2005; Jackson *et al.*, 2000).

Conclusion

Dynamic C and N cycling in soils are key components of ecosystem responses to atmospheric and climatic change and their feedbacks to the atmospheric CO_2 and the global C cycle. Here, we have shown that in a forest growing in an elevated concentration of atmospheric CO_2, the flux of C and N into the soil nearly doubled due to stimulated root production and mortality. Moreover, much of the C and N input occurred relatively deep in the soil profile where the dynamics of root decomposition and C and N mineralization are likely to be different from what is commonly observed and modeled in the upper profile. Contrary to expectations, root [N] did not decline under elevated [CO_2]; other mechanisms including increased root diameter or rooting depth may be responsible for the decline in root turnover we observed in response to CO_2-enrichment. Continued progress in understanding the interface between root detritus and soil C and N cycling, especially at depth in the soil, will improve our ability to predict ecosystem responses to atmospheric and climatic change.

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Appendix

Table 3.1: Minirhizotron sampling scheme.

Year	First sampling date	Last sampling date	Number of sessions	Date of peak standing crop
1998	19 Feb 1998	30 Oct 1998	17	27 Jul
1999	18 Mar 1999	26 Oct 1999	15	21 Jun
2000	7 Mar 2000	30 Oct 2000	18	26 Jun
2001	14 Mar 2001	9 Oct 2001	16	27 Aug
2002	15 Mar 2002	22 Oct 2002	17	16 Jul
2003	11 Mar 2003	8 Dec 2003	23	30 Sep
2004	17 Mar 2004	30 Mar 2005	22	14 Oct
2005	15 Apr 2005	15 Mar 2006	21	7 Sep
2006	29 Mar 2006	7 Mar 2007	22	10 Nov

Table 3.2: ANOVA table of repeated-measures analyses for main response variables.

Parameter	Source	ANOVA statistics			
		ndf	***ddf***	***F***	***P***
Weighted average diameter (mm)	Treatment	1	12	3.5	0.09
	Depth	3	12	1.1	0.39
	Treatment × Depth	3	12	0.8	0.54
	Year	8	95	5.7	**<0.0001**
	Treatment × Year	8	95	0.8	0.62
	Depth × Year	24	95	1.1	0.32
	Treatment × Depth × Year	24	95	0.8	0.77
Production* (g m^{-2} year)	Treatment	1	12	14.8	**0.002**
	Depth	3	12	1.6	0.24
	Treatment × Depth	3	12	0.8	0.52
	Year	8	96	4.0	**0.0004**
	Treatment × Year	8	96	0.9	0.51
	Depth × Year	24	96	0.9	0.67
	Treatment × Depth × Year	24	96	0.4	0.99
Peak standing crop* (g m^{-2})	Treatment	1	12	14.8	**0.002**
	Depth	3	12	0.9	0.47
	Treatment × Depth	3	12	1.1	0.39
	Year	8	96	14.6	**<0.0001**
	Treatment × Year	8	96	1.7	0.10
	Depth × Year	24	96	1.9	**0.02**
	Treatment × Depth × Year	24	96	0.8	0.70

Table 3.2: Continued.

Parameter	Source	ANOVA statistics			
		ndf	***ddf***	**F**	***P***
Mortality* (g m^{-2} year)	Treatment	1	12	14.0	**0.003**
	Depth	3	12	2.8	0.08
	Treatment × Depth	3	12	0.8	0.54
	Year	8	96	8.3	**<0.0001**
	Treatment × Year	8	96	0.7	0.67
	Depth × Year	24	96	1.8	**0.03**
	Treatment × Depth × Year	24	96	0.7	0.80
N input (g N m^{-2} year)	Treatment	1	12	10.7	**0.007**
	Depth	3	12	0.5	0.67
	Treatment × Depth	3	12	0.6	0.65
	Year	8	96	7.7	**<0.0001**
	Treatment × Year	8	96	1.3	0.27
	Depth × Year	24	96	1.4	0.12
	Treatment × Depth × Year	24	96	0.7	0.89
Turnover* ($year^{-1}$)	Treatment	1	12	7.4	**0.02**
	Depth	3	12	3.3	0.06
	Treatment × Depth	3	12	2.9	0.08
	Year	8	93	7.3	**<0.0001**
	Treatment × Year	8	93	1.9	0.08
	Depth × Year	24	93	0.8	0.73
	Treatment × Depth × Year	24	93	1.1	0.39

Table 3.3: Weighted average root diameter, and annual biomass production and peak standing crop estimates.

Data are means ± 1 SE of the mean (n = 3 in the current [CO_2] treatment, and n = 2 in the elevated [CO_2] treatment) at four soil depths, and also for the entire soil profile (0 – 60 cm). The weighted average diameter is of the population of roots lost annually to mortality. The length of root of a given diameter was used to proportionally weight diameter estimates. Thus, relatively rare roots with a large diameter did not bias the estimated diameter of the root population. Peak standing crop was determined to be when root biomass was greatest in at least 3 of the 5 rings.

Year	Soil depth	Weighted average diameter (mm)		Production ($g\ m^{-2}\ year^{-1}$)		Peak standing crop ($g\ m^{-2}$)	
		Current $[CO_2]$	Elevated $[CO_2]$	Current $[CO_2]$	Elevated $[CO_2]$	Current $[CO_2]$	Elevated $[CO_2]$
1998	0-15 cm	0.37 ± 0.00	0.35 ± 0.01	87 ± 29	57 ± 21	34 ± 14	43 ± 30
	15-30 cm	0.37 ± 0.03	0.46 ± 0.09	46 ± 17	66 ± 2	38 ± 11	32 ± 9
	30-45 cm	0.40 ± 0.07	0.38 ± 0.07	15 ± 7	26 ± 10	9 ± 4	23 ± 13
	45-60 cm	0.36 ± 0.03	0.44 ± 0.05	9 ± 6	23 ± 13	4 ± 2	10 ± 5
	Total profile	0.38 ± 0.01	0.40 ± 0.01	157 ± 52	171 ± 4	85 ± 28	107 ± 4
1999	0-15 cm	0.41 ± 0.02	0.43 ± 0.00	129 ± 18	118 ± 1	66 ± 14	54 ± 25
	15-30 cm	0.41 ± 0.06	0.43 ± 0.07	79 ± 58	98 ± 45	64 ± 42	53 ± 18
	30-45 cm	0.34 ± 0.02	0.36 ± 0.04	11 ± 3	34 ± 17	5 ± 1	23 ± 8
	45-60 cm	0.43 ± 0.03	0.46 ± 0.04	21 ± 11	36 ± 20	21 ± 9	30 ± 16
	Total profile	0.42 ± 0.03	0.43 ± 0.02	239 ± 79	286 ± 9	157 ± 61	160 ± 22
2000	0-15 cm	0.44 ± 0.01	0.42 ± 0.06	86 ± 11	177 ± 35	28 ± 3	46 ± 5
	15-30 cm	0.44 ± 0.04	0.49 ± 0.01	68 ± 33	110 ± 65	35 ± 15	52 ± 27
	30-45 cm	0.44 ± 0.05	0.45 ± 0.03	69 ± 55	144 ± 59	6 ± 3	49 ± 7
	45-60 cm	0.44 ± 0.04	0.41 ± 0.05	47 ± 46	105 ± 45	1 ± 1	17 ± 9
	Total profile	0.45 ± 0.02	0.45 ± 0.02	270 ± 104	536 ± 165	69 ± 15	164 ± 36

Table 3.3: Continued.

Year	Soil depth	Weighted average diameter (mm)		Production ($g\ m^{-2}\ year^{-1}$)		Peak standing crop ($g\ m^{-2}$)	
		Current $[CO_2]$	Elevated $[CO_2]$	Current $[CO_2]$	Elevated $[CO_2]$	Current $[CO_2]$	Elevated $[CO_2]$
2001	0-15 cm	0.40 ± 0.03	0.36 ± 0.00	105 ± 36	118 ± 36	59 ± 25	125 ± 14
	15-30 cm	0.39 ± 0.05	0.42 ± 0.06	118 ± 65	188 ± 109	94 ± 40	199 ± 130
	30-45 cm	0.41 ± 0.06	0.42 ± 0.00	85 ± 57	166 ± 81	81 ± 59	145 ± 70
	45-60 cm	0.49 ± 0.09	0.50 ± 0.07	105 ± 90	381 ± 206	76 ± 68	300 ± 155
	Total profile	0.43 ± 0.05	0.43 ± 0.04	414 ± 206	853 ± 530	311 ± 161	768 ± 418
2002	0-15 cm	0.35 ± 0.03	0.39 ± 0.02	52 ± 17	129 ± 15	45 ± 28	91 ± 17
	15-30 cm	0.42 ± 0.01	0.41 ± 0.08	79 ± 7	123 ± 48	57 ± 22	108 ± 62
	30-45 cm	0.38 ± 0.03	0.41 ± 0.02	70 ± 34	221 ± 31	48 ± 36	152 ± 58
	45-60 cm	0.38 ± 0.04	0.47 ± 0.01	45 ± 27	329 ± 116	24 ± 16	251 ± 113
	Total profile	0.39 ± 0.02	0.42 ± 0.03	245 ± 61	802 ± 258	175 ± 75	603 ± 265
2003	0-15 cm	0.27 ± 0.01	0.28 ± 0.01	61 ± 24	78 ± 14	39 ± 17	73 ± 7
	15-30 cm	0.31 ± 0.01	0.36 ± 0.04	29 ± 11	72 ± 17	40 ± 7	64 ± 15
	30-45 cm	0.33 ± 0.05	0.33 ± 0.02	34 ± 23	74 ± 7	30 ± 16	133 ± 1
	45-60 cm	0.36 ± 0.03	0.35 ± 0.03	26 ± 5	92 ± 42	30 ± 16	138 ± 31
	Total profile	0.31 ± 0.02	0.32 ± 0.03	149 ± 54	316 ± 40	139 ± 46	407 ± 46

Table 3.3: Continued.

Year	Soil depth	Weighted average diameter (mm)		Production (g m^{-2} $year^{-1}$)		Peak standing crop (g m^{-2})	
		Current [CO_2]	Elevated [CO_2]	Current [CO_2]	Elevated [CO_2]	Current [CO_2]	Elevated [CO_2]
2004	0-15 cm	0.42 ± 0.06	0.30 ± 0.05	70 ± 23	89 ± 40	87 ± 19	94 ± 26
	15-30 cm	0.28 ± 0.02	0.32 ± 0.01	40 ± 16	73 ± 11	43 ± 14	86 ± 5
	30-45 cm	0.33 ± 0.03	0.35 ± 0.01	70 ± 53	83 ± 23	81 ± 62	160 ± 28
	45-60 cm	0.28 ± 0.04	0.38 ± 0.01	29 ± 20	127 ± 22	45 ± 31	185 ± 37
	Total profile	0.36 ± 0.01	0.33 ± 0.02	209 ± 79	372 ± 118	255 ± 87	525 ± 106
2005	0-15 cm	0.28 ± 0.03	0.35 ± 0.08	19 ± 5	75 ± 19	15 ± 5	70 ± 19
	15-30 cm	0.30 ± 0.03	0.43 ± 0.11	29 ± 18	105 ± 41	31 ± 16	107 ± 41
	30-45 cm	0.32 ± 0.02	0.29 ± 0.02	26 ± 20	85 ± 38	35 ± 23	93 ± 8
	45-60 cm	0.29 ± 0.02	0.34 ± 0.07	22 ± 16	77 ± 31	25 ± 19	88 ± 20
	Total profile	0.30 ± 0.01	0.35 ± 0.07	96 ± 56	341 ± 158	107 ± 61	358 ± 108
2006	0-15 cm	0.28 ± 0.06	0.34 ± 0.01	28 ± 8	87 ± 6	19 ± 4	58 ± 13
	15-30 cm	0.29 ± 0.07	0.40 ± 0.11	32 ± 17	67 ± 15	27 ± 13	44 ± 2
	30-45 cm	0.25 ± 0.01	0.29 ± 0.05	18 ± 16	67 ± 2	12 ± 9	111 ± 40
	45-60 cm	0.26 ± 0.08	0.43 ± 0.10	23 ± 18	32 ± 1	24 ± 20	34 ± 2
	Total profile	0.30 ± 0.03	0.37 ± 0.06	102 ± 49	254 ± 29	82 ± 44	247 ± 59

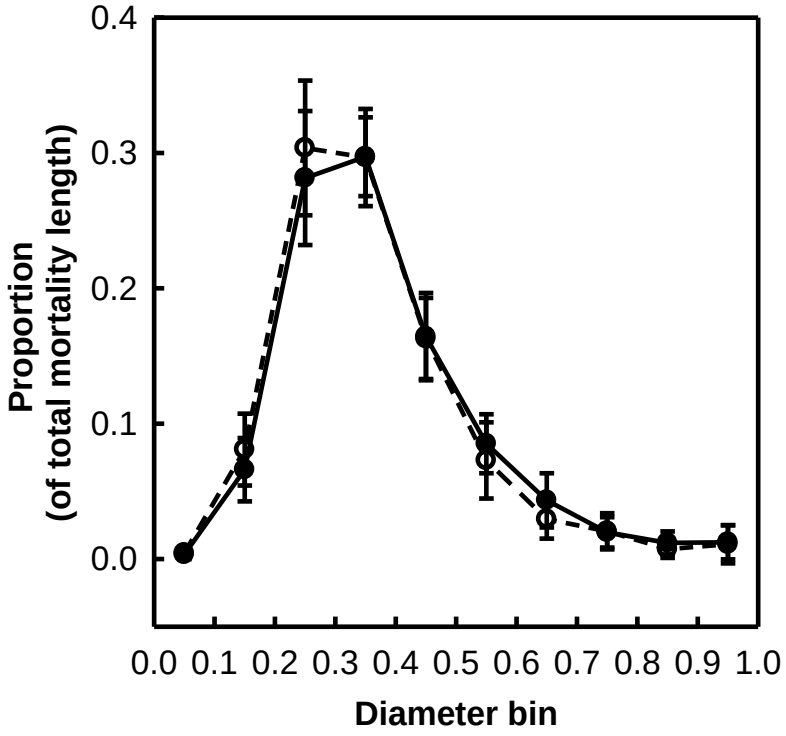
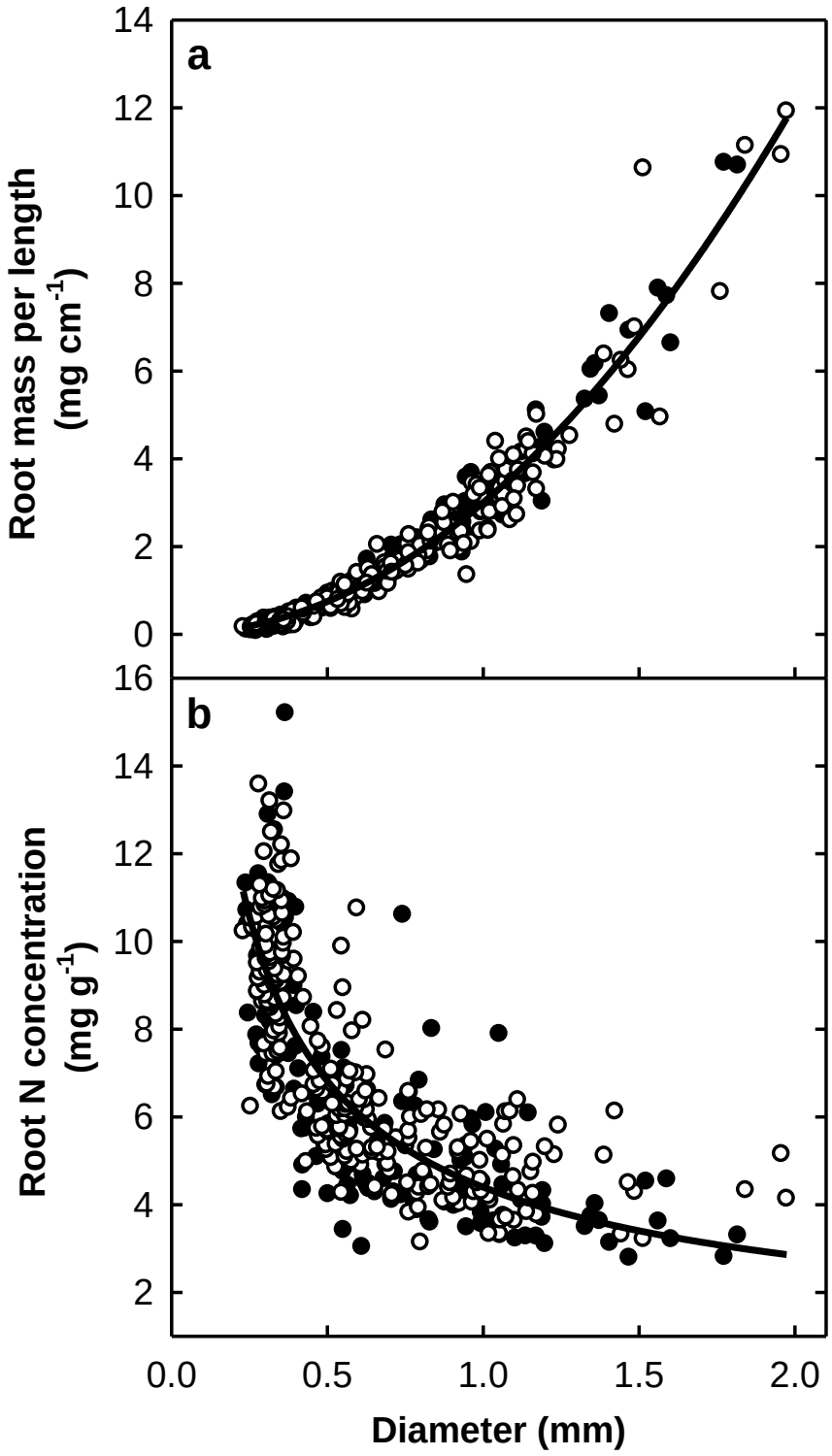


Figure 3.1: Distribution of fine-root mortality length in diameter classes (i.e., bins) of 0.1 mm. The length of root in diameter classes greater than 1 mm was included in the analysis, but the proportion was minimal and was not included in the figure. Data are averaged over all years of the experiment (1998 to 2006); the error shown is ± 1 pooled standard error (SE). $n = 3$ in the current [CO_2] treatment (open circles) and $n = 2$ in the elevated [CO_2] treatment (closed circles).

Figure 3.2: The relationship between root diameter and root mass per unit length (RML, a) or root N concentration (b). In panel (a), the relationship between root diameter and root mass per unit length follows a positive power function: RML = 3.00 × Diameter $^{2.01}$ that does not differ between the current and elevated [CO_2] treatments or with soil depth. n = 292 in the current [CO_2] treatment (open circles) and n = 195 in the elevated [CO_2] treatment (closed circles). Data are pooled across collection dates in 2005 and 2006 and across depths. In panel (b), the relationship between root diameter and [N] follows a negative power function that does not differ between treatments or with soil depth. The relationship is: [N] = 4.40 × Diameter $^{-0.63}$, where n = 243 in the current [CO_2] treatment (open circles) and n = 190 in the elevated [CO_2] treatment (closed circles). Data are pooled across collection dates in 2005 and 2006 and across depths. Some data points are missing from the initial subsample collections because samples weights were too small for combustion to determine root [N].


a
b
Root mass per length
(mg cm-1)
Root N concentration
(mg g-1)
Diameter (mm)
0
2
4
6
8
10
12
14
16
0.0
0.5
1.0
1.5
2.0

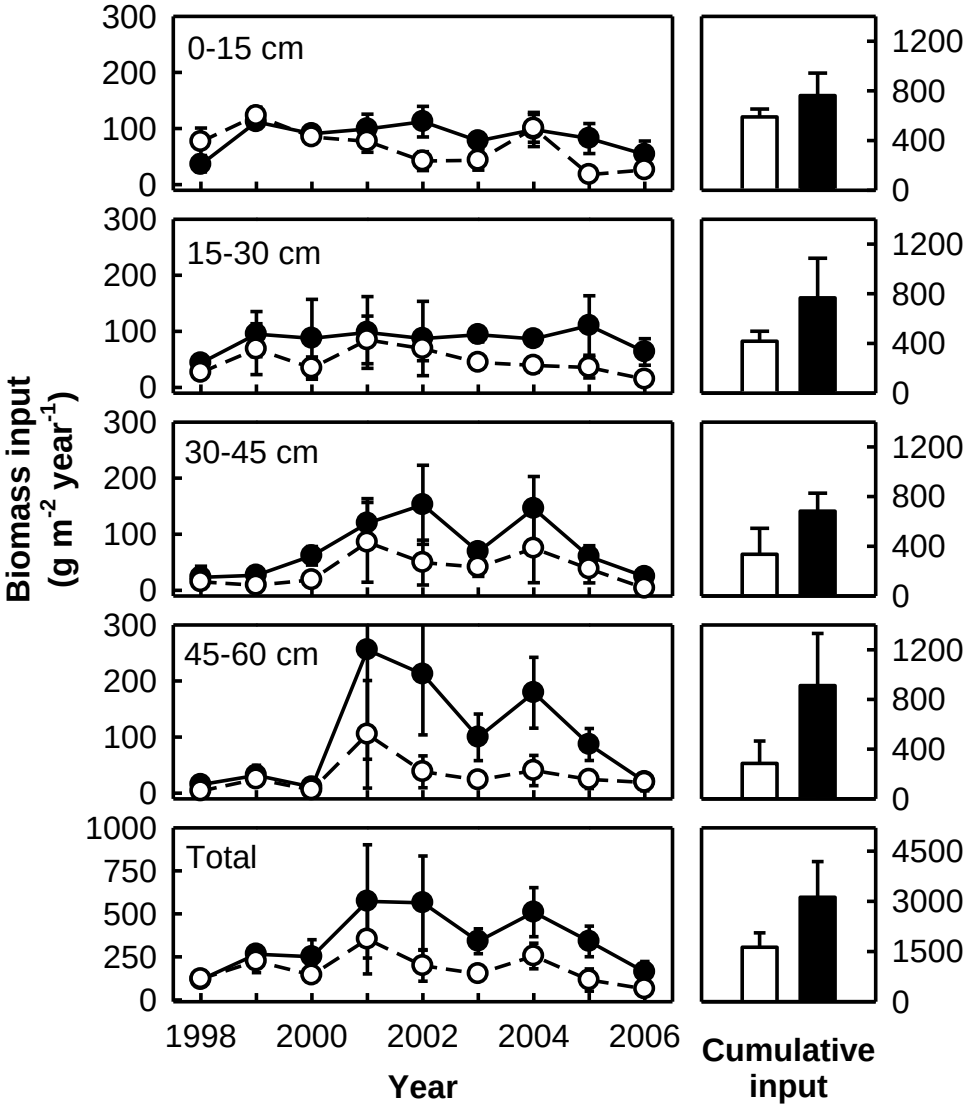


Figure 3.3: Average annual root biomass input ± 1 SE by soil depth (in 15-cm increments) and for the total soil profile (0 – 60 cm) as calculated from the relationship derived in Fig. 3.2a. Within each year, n = 3 in the current [CO_2] treatment (open circles), and n = 2 in the elevated [CO_2] treatment (closed circles). Cumulative C and N input over the experiment to-date (1998-2006) are shown as bars for each soil depth and for the total soil profile, where n = 3 in the current [CO_2] treatment (open bars), and n = 2 in the elevated [CO_2] treatment (closed bars).

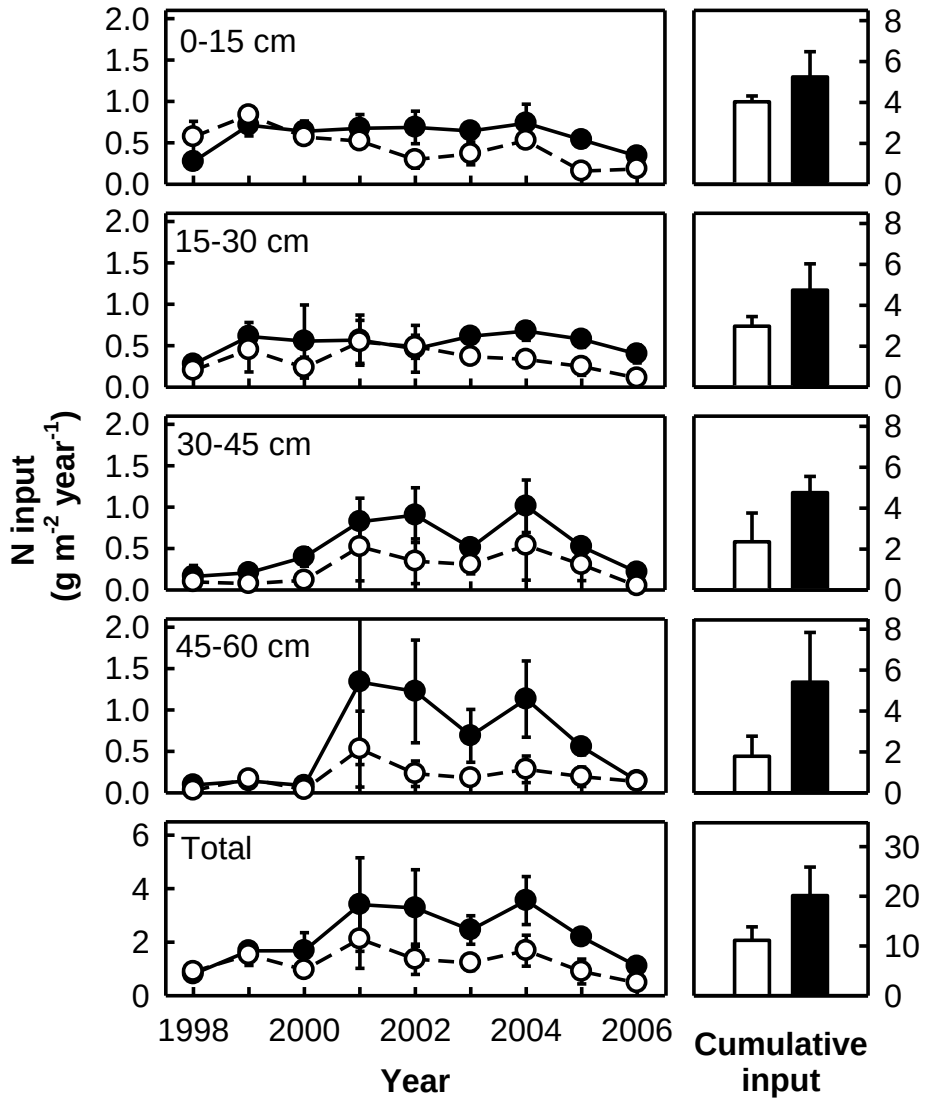


Figure 3.4: Average annual N input from root mortality ± 1 SE by soil depth (in 15-cm increments) and for the total soil profile (0 – 60 cm) as calculated from the relationship derived in Fig. 3.2b. Within each year, n = 3 in the current [CO_2] treatment (open circles), and n = 2 in the elevated [CO_2] treatment (closed circles). Cumulative C and N input over the experiment to-date (1998-2006) are shown as bars for each soil depth and for the total soil profile, where n = 3 in the current [CO_2] treatment (open bars), and n = 2 in the elevated [CO_2] treatment (closed bars).

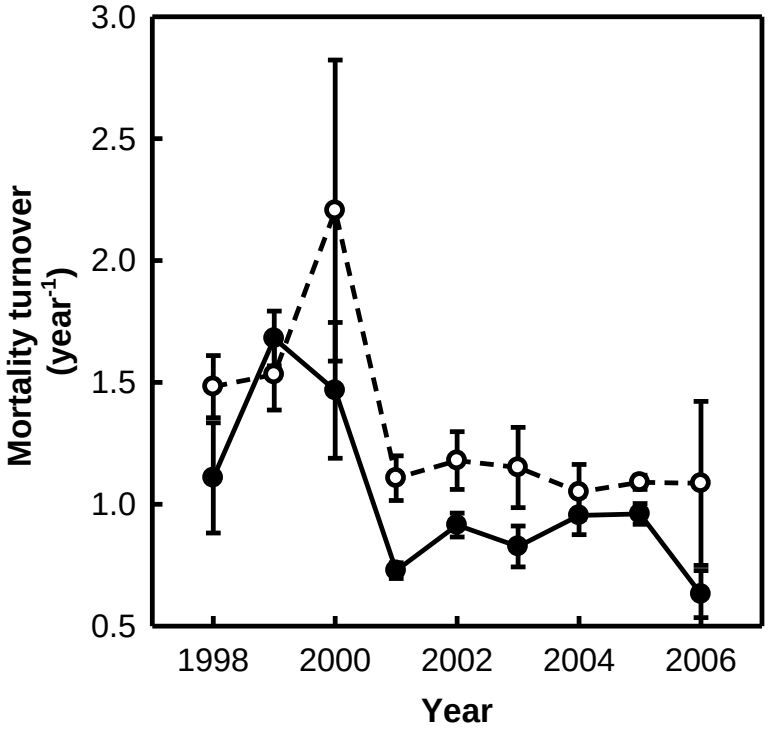


Figure 3.5: Root turnover ± 1 SE calculated as in Gill & Jackson (2000): Turnover (year^{-1}) = Root mortality (g m^{-2} year^{-1}) / Peak standing crop (g m^{-2}). Data are cumulative turnover rates across all soil depths. Within each year, n = 3 in the current [CO_2] treatment (open circles), and n = 2 in the elevated [CO_2] treatment (closed circles).

Chapter 4
Root quantity not quality drives differences in root mass loss in a CO_2-enriched sweetgum plantation

My use of "we" in this chapter refers to my co-authors and myself. This chapter is a lightly revised version of a paper of the same name by Colleen M. Iversen, Richard J. Norby, and Aimée T. Classen that will be submitted for publication.

Abstract

The increased production of small diameter (i.e., "fine") roots under elevated [CO_2] may increase soil C storage, but this depends on how quickly C is mineralized from decomposing roots. We harvested sweetgum (*Liquidambar styraciflua* L.) roots from the current and elevated [CO_2] treatments at the Oak Ridge National Laboratory, Free-Air CO_2 Enrichment experiment in June and July, 2005. CO_2-enrichment had doubled fine-root production at this site. Our objective was to determine whether roots grown under elevated [CO_2] would decompose more quickly than roots grown under current [CO_2] and to modify existing litterbag methodology. We dispersed sweetgum roots of representative length and diameter in a fine bead matrix, and wrapped the matrix in a water-permeable membrane. We placed the cores in the soil of a common garden area within the sweetgum plantation and measured root mass loss over a period of 10 months. Overall, sweetgum roots decomposed quickly (k = 0.80 year^{-1}). Further, the initial volume of roots in each decomposition core was more important in determining the decomposition rate constant than the treatment in which the roots were initially grown, because of high spatial and temporal variability in root standing crop. More root biomass remained after 10 months in the elevated [CO_2] cores which initially had a greater volume, suggesting that the remaining detritus may potentially be incorporated into long-lived soil organic matter. Our results highlight the importance of incorporating the effect of initial variability in root population morphology and quantity when predicting root decomposition rates.

Introduction

Up to one-third of global annual net primary production is allocated belowground to fine roots (< 2 mm diameter, Jackson *et al.*, 1997), and fine-root production is expected to increase as forests respond to elevated [CO_2], especially in N-limited ecosystems (Matamala *et al.*, 2003; Norby *et al.*, 2004; Chapter 3). Root population turnover represents a key input of C and nutrients belowground (Scheffer & Aerts, 2000; Guo *et al*., 2004; Rasse *et al.*, 2005; Parton *et al.*, 2007, Chapter 3), and the storage of root-derived C in long-lived soil organic matter pools may be an important mechanism in mitigating rising fossil fuel emissions (Dixon *et al*., 1994). However, the incorporation of detritus into soil organic matter depends in large part on soil microbial activity, which may be stimulated by the input of labile C input from increased fine-root production under elevated [CO_2] (Zak *et el*., 2000). The question of whether root detritus will be incorporated into long-term soil organic matter pools or quickly mineralized and returned to the atmosphere is difficult to assess given the methodological limitations associated with examining root decomposition (cf. Dornbush *et al.*, 2002).

The turnover of fine roots of a single tree species may result in detritus as chemically and physically complex as a diverse assemblage of leaf litter. This is because the branched root form, ranging from smaller diameter root tips to larger diameter, woody roots (Pregitzer *et al.*, 1997; Pregitzer, 2002; Pregitzer, 2003), is highly related to root function and chemistry (cf. Pregitzer *et al.*, 1997; Chapter 3). For example, the difference in N concentration among sweetgum *(Liquidambar styraciflua* L.*)* roots decreasing in diameter from 2 mm to 0.2 mm (i.e., ~ 2 mg N g^{-1} to ~16 mg N g^{-1}, Chapter 3) nearly spans the global range in leaf [N] (cf. Reich & Oleksyn, 2004). Because of the close association between root chemistry and morphology, rates of root decomposition often vary with root diameter (Berg, 1984; King *et al.*, 1997; Silver & Miya, 2001; Personeni & Loiseau, 2004).

Root input and soil organic matter accrual are often correlated (Allard *et al.*, 2005; Jastrow *et al.*, 2005; Rasse *et al.*, 2005). However, few studies have specifically

examined the role of initial differences in root quantity on root decomposition rates (Personeni & Loiseau, 2004). Litterbags are the most commonly used technique to examine *in situ* leaf and fine-root litter decomposition in terrestrial ecosystems (Gholz *et al.*, 2000; Robertson & Paul, 2000; Silver & Miya, 2001; Berg & Laskowski, 2006). However, litterbags typically measure the proportional mass loss of a standard mass of air-dried plant substrate (usually over a period of months or years in flat mesh bags; cf. Bocock, 1957; Shanks & Olson, 1961; Berg & Laskowski, 2006). Thus, litterbags do not specifically test for initial differences in root quantity though this is one of the main mechanisms whereby C and N are expected to be increasingly stored in soil organic matter under elevated [CO_2] (Diaz *et al.*, 1993; McMurtrie *et al.*, 2000; Luo *et al.*, 2004). Assumptions inherent in the litterbag method that may be valid for leaf litter (as reviewed in Robertson & Paul, 2000) may be less valid for the decomposition of complex fine-root litter that decays within the soil profile (i.e. Dornbush *et al.*, 2002).

Our objective in this experiment was to test whether roots grown under elevated [CO_2] would decompose at a different rate than roots grown under ambient, or "current" [CO_2] by modifying current litterbag methodology to examine whether initial length and diameter distribution of fine roots would affect the rate at which roots decompose. We modified existing litterbag methodology by: (1) measuring the decomposition of a complex assemblage of fine roots in an experimental design incorporating root quantity and diameter distribution, (2) using fresh roots to avoid the artifact of air-drying, (3) placing roots in a glass bead matrix (600 µm) to mimic the structural separation of roots in a soil environment (we did not use soil to avoid confounding our measurements of root decay with roots that we were unable to completely remove from soil matrix), and (4) wrapping the root-bead mixture in a fine-mesh (3 µm) to prevent the in-growth of roots from the surrounding soil and to prevent the loss of fragmented material. The root decomposition bags were deployed in a common garden to isolate the effects of root morphology and chemistry on root decay from potential differences in soil climate, chemistry and microbial community between treatments. We hypothesized that root detritus in cores collected from the elevated [CO_2] treatment would decompose more

quickly because there would be more labile C available for microbial biosynthesis and growth. Further, we predicted that initial root morphological characteristics in both the current and elevated [CO_2] treatments (e.g., diameter distribution and total root length) would be important in determining the rate at which fine-roots decomposed.

Materials and Methods

Site description

Our experiment was conducted at the Oak Ridge National Laboratory (ORNL) Free-Air CO_2-Enrichment (FACE) experiment in a sweetgum (*Liquidambar styraciflua* L.) plantation in eastern Tennessee. The sweetgum plantation was established in 1988. The soil is described as moderately well-drained Aquic Hapludult with a silty clay loam texture. The experimental design has been well-described elsewhere (Norby *et al.*, 2001; Norby *et al.*, 2002; Norby *et al.*, 2004), but briefly, the experiment consists of five 25-m diameter rings, of which four have a FACE apparatus installed. Two rings blow air enriched with CO_2 to achieve a concentration in the canopy of ~550 ppm, while two rings blow an ambient, or current, [CO_2] of ~380 ppm; the fifth ring serves as a current [CO_2] treatment without a FACE apparatus. CO_2-enrichment was initiated in 1998 when the sweetgum trees were 10 years old and 12-m tall. The mean temperature above the canopy in 2006 was 16.0°C (minimum –5.9°C and maximum 39.0°C), the mean soil temperature in 2006 was 14.7°C (minimum 0.7°C and maximum 26.9°C); total precipitation was 1184 mm (Riggs *et al.*, 2007).

Experimental design

Soil cores (5 cm × 15 cm) were collected from the ORNL FACE sweetgum plantation in June and July, 2005, and refrigerated (4 °C) until processed (within 30 days) to limit further decomposition while avoiding potential cell wall breakdown associated with freezing. Cores were collected from experimental plots exposed to either current [CO_2] (n = 3) or elevated [CO_2] (n = 2; 5 cores per plot × 5 plots × 2 months = 50 cores total). We used the variation in root length and diameter distribution found among the

cores from different treatment plots and sampling dates as part of our experimental design (Table 4.1) because we were interested in the effects of the extensive spatial and temporal variability in root standing crop within our site on decomposition.

Roots were washed from the soil using a hydropneumatic elutriator root separation system with a 530 µm filter (Gillison's Variety Fabrication, Benzonia, Michigan). Large pieces of organic matter were removed and the moist roots were refrigerated at 4 °C. *WinRhizo* root scanning software (400 dpi, Regent Instruments, Inc., Canada) and a high-resolution scanner (Epson Expression 1680, Epson, Long Beach, California) were used to determine the total root length, surface area, and volume of the root assemblage washed from each soil core. Root characteristics were quantified in diameter classes ranging from 0 mm to 4 mm in 0.1 mm increments with a filter set to 4:1 (length: width) as a cutoff. This enabled us to differentiate linear root matter from relatively round organic matter. Root surface area and volume were calculated within individual diameter classes and summed over the entire core. Thus, our morphological data were based on the actual area of each pixel (*WinRhizo*, 2005).

After the initial scan on *WinRhizo*, three small subsamples ranging from 2 to 20 individual roots (10 mg to 200 mg total mass depending on root size, ~15 % of total root length) were removed from each core for [N] analysis (as in Chapter 3). Subsamples were selected to encompass the range of diameter classes contained in each core (i.e., roughly within 0 to 0.4 mm, 0.4 to 0.8 mm and 0.8 mm to 4 mm size classes), and were re-scanned using the same *WinRhizo* parameters as above to determine an average diameter for each subsample. Subsamples were then oven-dried at 70 °C and ground on a Wig-L-Bug dental grinder (Crescent Dental Manufacturing Co., Chicago, Illinois). Root [N] was determined on an elemental analyzer (Costech Analytical Technologies, Inc., Valencia, California).

Additional root samples taken in 2005 and 2006 were also scanned, and then oven-dried at 70°C to a constant weight to determine predictive volume-to-length ratios. This allowed us to account for the small portion of organic matter that was left in each

root core in order to include the smallest diameter roots in the samples. Because the organic matter weighed much less per unit volume than a root, and the relationships developed for individual roots (as in Chapter 3) over-predicted the mass of roots in an entire core. Thus, the initial mass of fine roots in each core used in the decomposition experiment was determined based on the relationship between root volume (cm^3) and mass (mg) of the additional samples ($r^2 = 0.95$, $n = 47$, $P < 0.0001$):

$$\text{Root mass} = 203.3 \times \text{Root volume} - 6.7 \qquad \text{Eqn 4.1}$$

The initial [N] of root detritus was determined by fitting a relationship between root [N] (mg g^{-1}) and diameter (mm) to the average diameter for the root population in each decomposition core (*see* Results section, Fig. 4.1). Though the diameter – [N] relationship was initially determined from small subsamples, the average diameter of the total assemblage of roots in each core was weighted by the length of root in each 0.1 mm diameter class, and thus takes into account the average diameter of individual roots. We assumed that small pieces of organic matter remaining in the cores would not significantly affect this relationship due to their small mass; this is demonstrated by the fit of entire root assemblages sampled from 15 cm deep × 5 cm diameter soil cores in September, 2005 to the relationship in Fig. 4.1.

The root assemblage sampled from each soil core (minus small subsamples taken for [N] analysis) was re-scanned in December, 2005 in case mass was lost during refrigeration before being placed in decomposition cores. At this point, woody roots in which the average diameter was larger than 1 mm were removed from the cores, but many cores still contained roots in which at least part of the root was greater than 1 mm in diameter. The assemblage of fresh fine roots was distributed in a fine glass bead substrate (600 μm diameter) by combining in the field, and wrapped with a water-permeable membrane (Versapor, Pall Gelman Sciences, Ann Arbor, MI, 3 μm pore size). We used live fine roots for three reasons: (1) dead or senesced roots are rarely observed in our study site, (2) it is difficult to determine when roots have died, and (3) N is probably not resorbed from senescing roots (Aerts, 1990; Nambiar & Fife, 1991), and

thus fresh and senesced root [N] does not differ (as reviewed in Gordon & Jackson, 2000).

The membrane-wrapped cores (final core size 5 cm diameter × 15 cm depth) were fit snugly into pre-made 5-cm diameter × 15-cm deep holes in a common garden area of the ORNL FACE sweetgum plantation in December, 2005 (outside of the treatment rings). We retrieved 10 cores representative of each treatment (current and elevated [CO_2]) and sampling date (June and July, 2005) five times over a period of 10 months (at 2, 3, 4, 6, and 10 months). Upon retrieval, the roots were washed by hand from the bead matrix, re-scanned on an Epson scanner, and re-analyzed using *WinRhizo* to assess a suite of litter characteristics including final volume, surface area, length, and diameter distribution. Root litter was then oven-dried at 70°C to a constant weight, and subsamples were ashed by combustion in a muffle furnace at 550°C for 6 hours; all mass data are expressed on an ash-free dry mass (AFDM) basis. The [N] of the root detritus remaining in each core was determined on a Costech elemental analyzer.

Statistical Analysis

We use the SAS "Mixed" procedure (SAS Institute, Inc., Version 9.1, Cary, North Carolina) to compare the initial characteristics of the fine-root assemblage in the cores collected in the current and elevated [CO_2] treatments, in June and July, 2005 using ring as the statistical replicate (n = 3 rings in the current [CO_2] treatment and n = 2 rings in the elevated [CO_2] treatment). We used the SAS "NLIN" procedure to fit a single-exponential decay model (cf. Wieder & Lang, 1982) developed by Jenny *et al.* (1949) and Olson (1963) to the relative amount of AFDM remaining in each core at each of five collection dates for each month and treatment combination:

$$Y_t = Y_0 \times e^{-kt} \qquad \text{Eqn 4.2}$$

where Y_t is the percent of initial AFDM mass remaining at time t (years), Y_0 is equal to 100%, and k is the root decomposition rate constant (year^{-1}). We assessed the importance

of initial root population characteristics in determining the amount of mass lost in each core by fitting the residuals from Eqn 4.2 with a multiple regression model including root morphological measurements as predictors using the SAS "All Possible Regressions" procedure. We set alpha *a priori* to equal 0.1, and considered differences to be significant at $P < 0.1$.

Results

Root length in the decomposition cores was directly correlated to root length in the soil cores originally sampled from the treatments ($r = 0.96$, *data not shown*) even though small subsamples were removed from the initial soil cores for [N] analysis. Using ring as the statistical replicate, the initial average diameter of the root assemblage in the cores was significantly greater for the roots grown under elevated [CO_2] ($P < 0.002$), but average diameter did not differ by month ($P > 0.3$) and there were no month × treatment interactions ($P > 0.7$, Table 4.1). The initial length of roots in each core did not differ between treatments, but was less in cores collected in June than in July ($P < 0.05$). The initial surface area and volume of roots were greater in the elevated [CO_2] treatment ($P < 0.08$), and were greater in the July collection than the June collection in both treatments ($P < 0.05$). There were no month × treatment interactions for initial length, surface area or volume ($P > 0.4$, Table 4.1). Also, the relationship between root diameter and [N] did not differ between CO_2 treatments ($P > 0.5$, Fig. 4.1).

We used individual cores as the statistical unit when we examined root decomposition rates. There was no difference in decay rate between the current and elevated [CO_2] treatments ($P > 0.05$, Fig. 4.2), or between roots collected in June and July ($P > 0.05$, *data not shown*), and no interactions between treatment and collection date ($P > 0.05$). Thus, we pooled the data across treatments to determine an overall decomposition rate constant (k). A single exponential model (Eqn 4.2) yielded a decay rate (k) of 0.97 ± 0.06 year^{-1} (all values presented in the text are mean ± 1 standard error of the mean unless otherwise specified, Fig. 4.2, solid line, $P < 0.0001$, $n = 49$, $r^2 = 0.62$).

The residual error of each core from the overall exponential decay function changed over time. The residual average was significantly less than zero at the 2-month ($t_{(1,9)}$= -3.0, P = 0.01), and 3-month collections ($t_{(1,9)}$ = -2.7, P = 0.02). Negative residuals may indicate that the Y-intercept was not equal to the assumed 100% (Berg & Laskowski, 2006). An alternative calculation of the exponential decay model as a two-parameter model (cf. Chen *et al.*, 2002) with both the Y-intercept and slope as parameters (instead of assuming Y_0 = 100 % as in Eqn 4.2) yielded a better fit to the data. The Y-intercept parameter was estimated as 92.8 ± 2.5 % (P < 0.0001), and the overall decay rate (k) was 0.80 ± 0.08 year^{-1} (Fig. 4.2, P < 0.0001, n = 49, r^2 = 0.66).

A multiple regression approach to fit all of the residuals from the two-parameter decay function identified a significant model (P < 0.0001, n = 49) explaining 20% of the variation in the residuals (Table 4.2). On average, there was more biomass and N remaining in the cores sampled from the CO_2-enriched treatment than the current [CO_2] treatment after 10 months (P < 0.05, Figs. 4.3a, b). Across all cores, root tissue density declined linearly as mass was lost from the roots (Fig. 4.4, P < 0.0001, r^2=0.69, n = 49).

Discussion

Decomposition rate and mass remaining

We examined whether roots grown under elevated [CO_2] would decompose more quickly than roots grown under current [CO_2] by modifying existing litterbag methodology to test for effects of root quantity and quality. This was done because we have previously shown that the largest effects of elevated [CO_2] in our experiment are on root production and mortality (Chapter 3). Averaged over both sampling dates, soil cores sampled from the elevated [CO_2] treatment initially contained a greater surface area and volume of roots, and roots were on average larger in diameter when compared to those sampled from the current [CO_2] treatment (Table 4.1). The quantity of roots was greater in July than in June in both treatments because production during the summer months

was greater than mortality; peak fine-root standing crop in 2005 did not occur until 7 September (i.e., Chapter 3).

Contrary to what we hypothesized, there was no effect of CO_2-enrichment on root decomposition rate; roots grown under current and elevated [CO_2] decomposed at a rate of approximately 0.80 $year^{-1}$ in our decomposition cores (Fig. 4.2, dashed line). A lack of treatment effect may have been due to the high spatial and temporal variability in fine-root standing crop; the coefficient of variation for initial root volume within a treatment ring ranged from 15 to 50 %, and the coefficient of variation for the difference in root volume between June and July was approximately 50 % (Table 4.1). Our decomposition core design encompassed this spatial and temporal variability, which is in contrast to the approach used in standard litterbag methodology of artificially assigning a standard mass of air-dried roots to a litterbag based on broad diameter classes (i.e., < 1 mm, < 5 mm, < 10 mm, cf. Berg, 1984; Silver & Miya, 2001).

Root chemistry (i.e., [N]) has been shown previously to affect root decomposition rate (Berg, 1984, Silver & Miya, 2001). Thus, a similar decomposition rate for roots collected from the current and elevated [CO_2] treatments may also be related to the fact that elevated [CO_2] had no effect on root [N] within a given diameter class (Fig. 4.1, Chapter 3). The average [N] of the root assemblage was initially less in the cores collected from the CO_2-enriched treatment (Fig. 4.3a), but this was because the average diameter of the root standing crop sampled from the elevated [CO_2] treatment was initially greater than the current [CO_2] treatment (Table 4.1). However, the N content of the root detritus from the elevated [CO_2] treatment was generally greater because there was more root mass in each core (Fig. 4.3a). We did not observe N immobilization in any of the root decomposition cores (i.e., there was no initial increase in the N content of the detritus as microbes took up N from the surrounding soil); the N content of root detritus tracked root mass loss (Fig. 4.3b). This supports the findings of Parton *et al.* (2007) that fine root litter does not immobilize N due to relatively low root C: N.

Though roots sampled from the current and elevated [CO_2] treatments decomposed at the same rate, more root biomass remained in the elevated cores after 10 months because there was initially a greater amount of root biomass on average in the soil cores sampled under elevated [CO_2] (Fig 4.3b). This may mean that more root detritus, and therefore C and N (Fig. 4.3a), may be incorporated into long-lived soil pools under elevated [CO_2] (cf. Gale *et al*., 2000). A greater quantity of root litter under elevated [CO_2] may also mean that proportionally more N is mineralized from decaying roots, providing a source of N for tree uptake. We focused solely on litter characteristics in this experimental design, so it is possible that changes in microbial community composition or activity under elevated [CO_2] may alter the relationships we found here (Zak *et al*., 2000). Further, we artificially killed all of the roots within a volume of soil at the same time, but decomposition will depend on the timing of root death, which is highly related to root diameter (Wells & Eissenstat, 2001; Pregitzer, 2003). Larger diameter roots tend to live longer, but when these roots die, all of the lower-order roots that depend on the larger diameter root for photosynthates also die (Eissenstat & Volder, 2005). In this case, the input of detritus containing a diversity of root orders with different chemical and morphological characteristics (Fig. 4.1) may increase decomposition in a manner similar to that observed in leaf litter diversity experiments (i.e. Hättenschwiler et al. 2005).

Relative importance of root characteristics

Few studies have quantified the effect of natural variation within a single population of fine roots on root decomposition rates (but see Dornbush *et al.*, 2002). We quantified which factors were important in determining root decomposition rate by regressing morphological predictors against the residual error of each core from the overall decomposition rate (Fig. 4.2, dashed line). We did not include mass or N content in the model, because these parameters were highly correlated to root volume (Eqn 4.1) and diameter (Fig. 4.1), respectively, and morphological parameters are more easily determined using non-destructive methods, such as minirhizotron sampling (Chapter 3).

A multiple regression approach to fit all of the residuals from the decay function identified a significant model explaining 20% of the variation in the residuals (Table 4.2). Initial diameter (mm) and initial root volume (cm^3), which is in integration of cross-sectional area and length and highly related to initial root mass, were significant predictors of the overall variation in core mass loss over the course of the experiment. Together, they explained a small but significant portion of the variation (partial r^2 = 0.12). In support of our hypothesis, initial root volume (cm^3) was negatively related to the residuals, indicating that a greater initial quantity of roots decomposed more quickly. Our data also support traditional litterbag experiments that show larger diameter root classes decompose more slowly than fine roots (i.e., roots larger than 5 mm in diameter, as reviewed in Silver & Miya, 2001). We find that this is true even within a narrow range of root diameters in a single tree stand.

The portion of a year that the samples were incubated also explained a portion of the variation (partial r^2 = 0.08), probably indicating that different processes were operating throughout the course of the decomposition experiment (i.e., initial leaching or a lag time in microbial colonization). The fact that the two-parameter decay model was a better fit to our data than a model assuming a Y-intercept of 100 % indicates that decomposition proceeded more quickly than predicted by the decay function at the onset of the experiment (cf. Chen *et al.*, 2002). We attribute this to the leaching of cellular components from the roots as they were broken down internally (Yavitt & Fahey, 1986; Berg, 2000; Chen *et al.*, 2002). Leaching loss was also indicated by a linear decline in root tissue density as mass was lost from the roots (Fig. 4.4). The initial relationship between root volume and mass (Eqn 4.1) changed over time as decomposition proceeded (i.e., mass decreased more rapidly than root volume). Leaching loss may explain why diameter was an important predictor of decomposition rate and positively related to the residuals (i.e., slower decomposition); larger diameter roots may have been less susceptible to leaching due to a smaller surface area relative to volume (i.e. Gunnarsson *et al.*, 1988; King *et al.*, 1997), or because the ratio of structural to non-structural material is higher in larger diameter roots (Guo *et al*., 2004).

Abiotic fragmentation of litter is a key factor in decomposition and is necessary for microbial colonization and litter breakdown (Gunnarsson *et al.*, 1988; Maraun & Scheu, 1995), but it has rarely been demonstrated experimentally, especially belowground. We observed an unexpected initial increase in root volume in the decomposition cores (Fig 4.5). Our modified litterbag methodology allowed us to be certain that the roots were not contamination from soil or root in-growth into the bags; thus, we attributed the increase in volume to root fragmentation. Roots in the larger diameter classes initially fragmented along the long axis and were categorized by the scanning program as smaller diameter roots. Thus, the volume of roots in the smaller diameter classes exhibited a net gain as the volume of roots in larger diameter classes decreased (Fig. 4.5). Though fragmentation probably occurred throughout the decomposition process, the volume transfer to smaller diameter classes was most easily seen early on before decomposition resulted in a net loss from all diameter classes.

Roots have been found to decompose more quickly in intact soil than in litterbags because of maintained rhizosphere interactions (10-25% faster in Dornbush *et al.*, 2002, *and see* Personeni & Loiseau, 2004). We chose to modify existing litterbag methodology to better mimic intact soil core conditions instead of assessing root decomposition in intact cores. The substantial variation associated with estimates of initial root population in intact cores would have prevented accurate quantification of root morphological characteristics important in predicting root decomposition rate (cf. Dornbush *et al.*, 2002). Even so, the root decay rate that we observed in our decomposition cores was on average 50% faster than the root decay found in a traditional litterbag experiment at the ORNL FACE experiment in 2000 in which sweetgum roots < 1 mm diameter were air-dried, placed in bags made of fine nylon mesh stocking, and buried at a depth of 10 cm for 1 year (~0.54 $year^{-1}$, Johnson *et al.*, 2004). The decay rate in our experiment also exceeded that of other litterbag experiments on a similar soil type (~0.5 $year^{-1}$, as reviewed in Silver & Miya, 2001).

The magnitude of the decomposition rate is surprising in our study given the mesh size we used to prevent the in-growth of roots from the surrounding soil (cf. Berg, 1984; Fahey *et al.*, 1988; Gholz *et al.*, 2000), and also to prevent the loss of fragmented material (Robertson & Paul, 2000). While large enough to allow microbial community colonization (average bacterial and fungal diameter 1 µm, Frey *et al.*, 1999; Berg & Laskowski, 2006), the mesh opening was three orders of magnitude smaller than that used in traditional litterbag experiments (1 mm average opening, Silver & Miya, 2001) and excluded macrofauna. However, it has been shown previously that a mesh size of 20 µm increased the moisture content of litter by approximately 50% over a mesh size of 500 µm after 10 months (Lenz & Eisenbeis, 1998), and increased moisture may have facilitated microbial activity. Further, it is probable that the bead matrix used we used to mimic the structural separation of roots in the soil exhibited thermal and moisture characteristics that differed from the surrounding soil matrix, which perhaps contributed to the fragmentation process. Thus, the relatively rapid decomposition rate we observed may be a result of the modified litterbag methodology. However, this methodology retains the critical aspects of soil that are missing from litterbags, and the relative differences between treatments should not be affected.

Conclusion

Our data indicated that spatial and temporal variation in root diameter distribution and volume within a volume of soil may be as important in determining root decomposition rate as elevated [CO_2], especially given that CO_2-enrichment had no effect on initial root [N]. Thus, increased the input of root-derived C and N under elevated [CO_2] may mean that roots will decompose more quickly, but could also mean that more detritus may remain and become incorporated into the soil profile depending on the spatial and temporal variation in root mortality. Our results highlight the importance of incorporating the effect of initial variability in root population morphology and quantity on mass loss over time. This modification of existing litterbag methodology will allow us to better address new questions regarding the importance of changing morphological

characteristics of the root population over time and space to root decay. Future research should concentrate on the interactions among root quantity, decomposition rate and soil depth.

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Appendix

Table 4.1: Initial characteristics of the roots placed in the decomposition cores.

Treatment and collection date	Average diameter (mm)	Length (cm)	Surface area (cm^2)	Volume (cm^3)
Current [CO_2]				
June, 2005	0.39 ± 0.01	1201 ± 156	120 ± 12	1.7 ± 0.2
Range	0.30 – 0.47	859 – 2388	88 – 228	1.1 – 3.3
July, 2005	0.38 ± 0.01	1731 ± 183	174 ± 18	2.3 ± 0.3
Range	0.35 – 0.44	983 – 2485	99 – 238	1.2 – 3.7
Elevated [CO_2]				
June, 2005	0.42 ± 0.01	1425 ± 98	158 ± 12	2.3 ± 0.2
Range	0.38 – 0.50	985 – 1653	101 – 212	1.3 – 3.6
July, 2005	0.43 ± 0.01	2039 ± 414	227 ± 40	3.4 ± 0.6
Range	0.39 – 0.49	928 – 3434	95 – 373	1.1 – 5.4

Data are the average each morphological characteristic ± 1 SEM (n = 3 rings in the current [CO_2] treatment and n = 2 rings in the elevated [CO_2] treatment). The range of values for all cores used in the decomposition experiment is given below the average (note that the range encompasses all cores within a treatment/month combination, where 5 subsamples were taken in each ring for a total of 10 or 15 cores per treatment for each sampling date). Diameter data are calculated as a weighted average of the length of root in each of 16 diameter classes (0 to 1.2 mm by 0.1 mm increments, and 2 to 4 mm by 1 mm increments).

Table 4.2: Overall multiple-regression model predicting the variation in the residuals of the two-parameter exponential decay function.

Variable	Parameter estimate	Partial r^2	*F*-statistic	*P*-value	VIF
Intercept	-39.4			0.02	0
Incubation time (years)	12.8	0.08	4.0	0.03	1.0
Initial volume (cm^3) [*log*]	-20.9	0.09	5.0	0.03	1.3
Initial diameter (mm)	97.9	0.03	1.6	0.03	1.3

Variable selection was based on the lowest AIC values in SAS "all possible" regression function. Partial r^2 and *P*-values were derived from SAS "stepwise" regression function applied to selected model. Incubation time corresponds to the portion of a year that the cores were incubated *in situ* and initial volume was log-transformed to satisfy normality criteria before inclusion in the model. VIF corresponds to the variance inflation factor (lower numbers indicate that multi-colinearity between variables is low, and VIF = 1 indicates that there is no correlation with other variables).

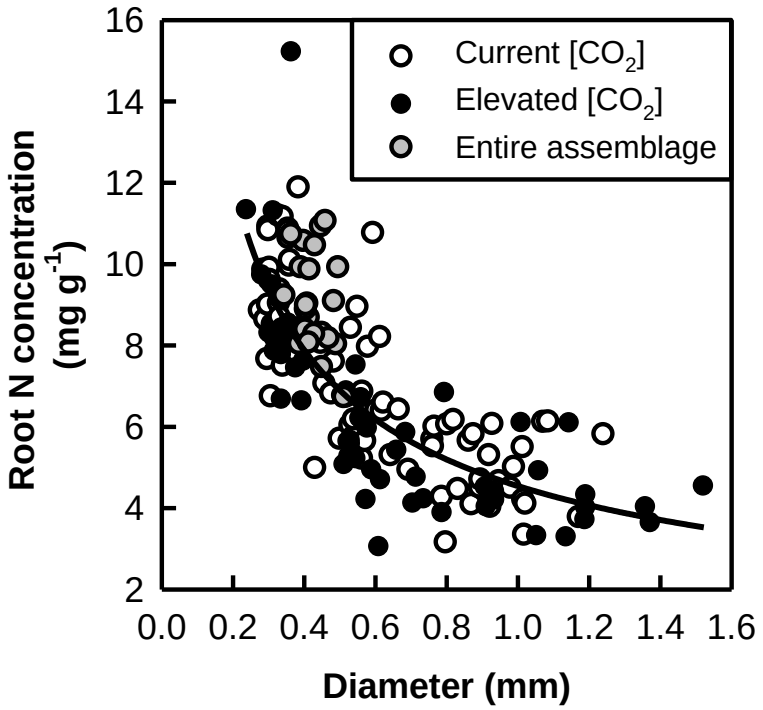

Figure 4.1: The relationship between the diameter and [N] of small root subsamples taken from 0 – 15 cm deep soil cores in the current (n = 80) and elevated [CO_2] treatments (n = 58). The variation is best explained by a negative power function: Root [N] = 4.54 × Diameter$^{-0.60}$ (r^2 = 0.64, P < 0.0001). The relationship between the average diameter and [N] of an entire assemblage of roots from cores sampled in September, 2005 is overlain in grey symbols.

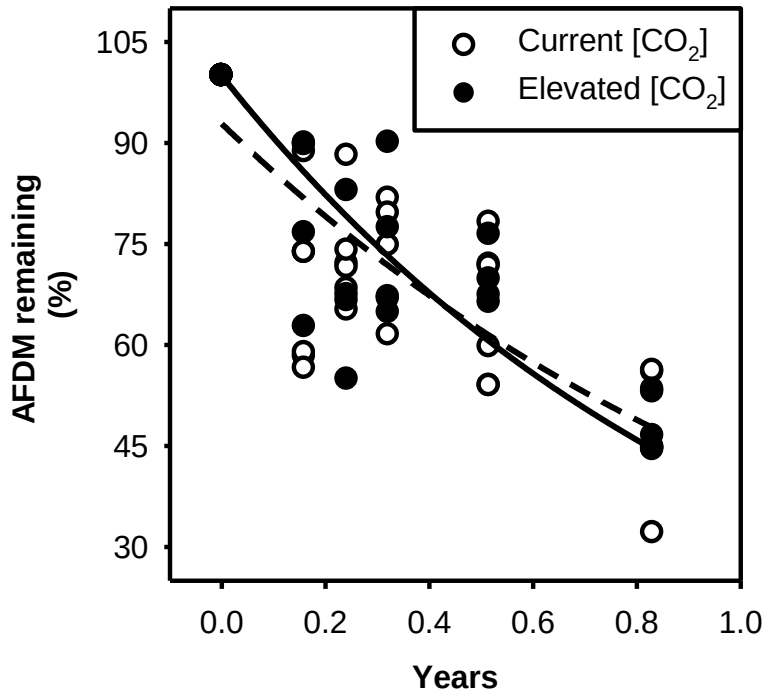

Figure 4.2: Exponential decay functions for roots collected from the current and elevated [CO_2] treatments. Solid line corresponds to decay rate of 0.97 ± 0.06 $year^{-1}$, where the Y-intercept was specified as 100 %. Dashed line corresponds to a decay rate of 0.80 ± 0.08 $year^{-1}$, where the intercept was included as a model parameter, and estimated as 92.8 ± 2.5 %. Each data point corresponds to one core; points below the line indicate faster decomposition than predicted by the model.

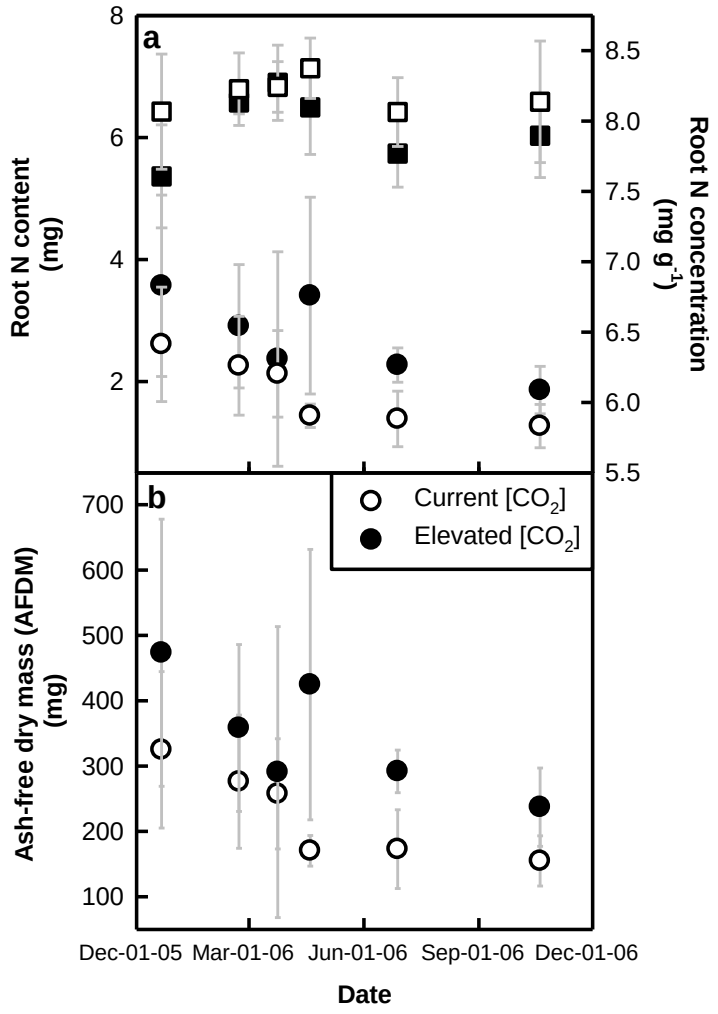

Figure 4.3: (a) Initial and final [N] and N content, and (b) initial and final ash-free dry mass of fine roots decomposed in a common garden over nearly 1 year. Circles in panel (a) correspond to N content (left axis), while squares correspond to [N] concentration (right axis). Error bars (in grey) are ± 1 standard deviation because samples in December, 2005 represent initial values of all remaining samples (n = 30 cores in the current [CO_2] treatment and n = 20 cores in the elevated [CO_2] treatment). At each collection date thereafter, n = 6 cores in the current [CO_2] treatment and n = 4 cores in the elevated [CO_2] treatment.

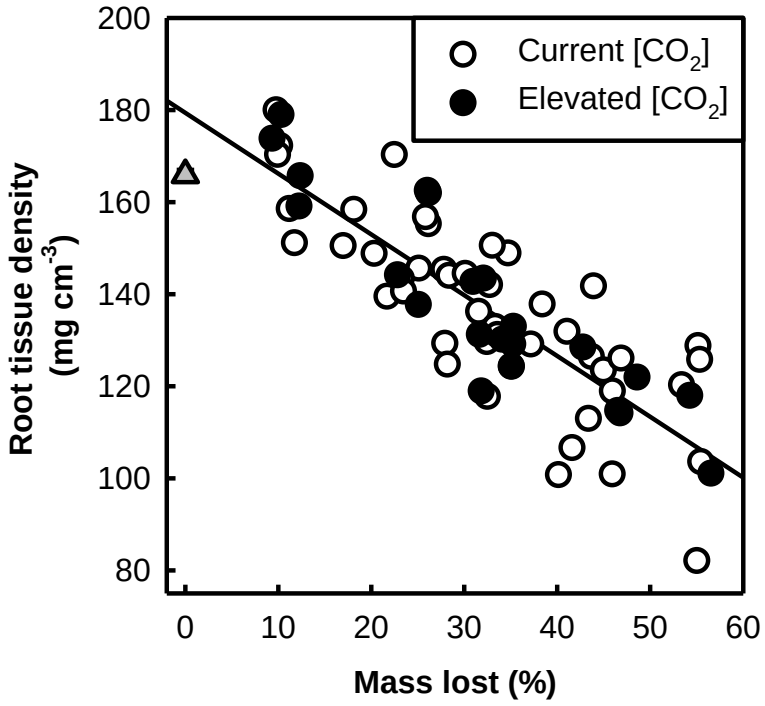

Figure 4.4: Root tissue density was inversely related to percent mass loss in the root decomposition cores. Tissue density = -1.3 × Mass lost – 179.4. The triangle (grey) symbol indicates the initial average density of all root cores (± 1 standard deviation, bars are too small to be seen).

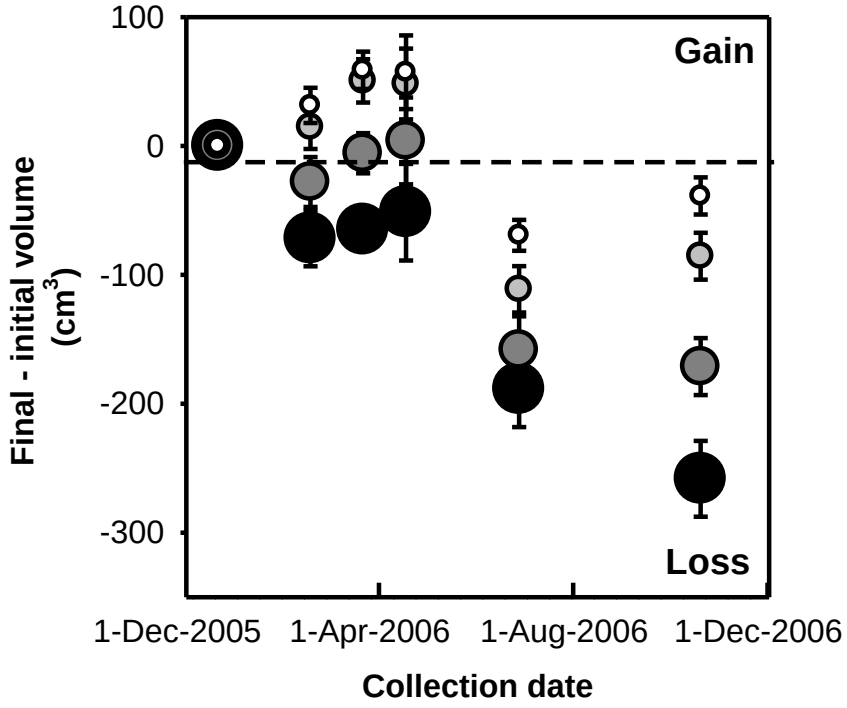

Figure 4.5: Larger diameter roots initially fragmented along the long axis and were quantified by the *WinRhizo* program as smaller diameter roots. Root volume was summed within each core in size classes are as follows: ○, 0 < diameter ≤ 0.4 mm; ○, 0.4 < diameter ≤ 0.8 mm; ○, 0.8 < diameter ≤ 1.2 mm; ●, 1.2 < diameter ≤ 4 mm. The volume shown for each size class is the net result of volume loss due to fragmentation and volume gain of fragmented roots from larger diameter classes. Data are mean ± 1 SE for each size class (n = 10 cores collected at each date).

Chapter 5
Conclusions: Forest responses to rising atmospheric CO_2

Belowground processes and ecosystem function

The research I have presented here addresses an important question in the field of ecosystem ecology: *How do belowground processes mediate ecosystem function?* Belowground processes often receive less attention than their aboveground counterparts because they are less easily observed and measured. Nevertheless, belowground processes such as decomposition and nutrient cycling have long been known to be an important component of ecosystem function (Tansley, 1935; Lindeman, 1942; Teal, 1962) and are also recognized as an important component of ecosystem responses to changing environmental conditions (Kramer, 1981; Mooney *et al*., 1991; Curtis *et al*., 1994; Berntson & Bazzaz, 1996). Progress has been made in recent years in bringing belowground research to the forefront of ecological thinking, but the integration of belowground processes with plant production is still a major challenge (cf. Nilsson *et al*., 2008). This is especially true in forested ecosystems where long experimental time frames and large-scale manipulations are necessary (Likens *et al*., 1970; Johnson, 2006).

Recent technological advances have allowed us to examine belowground processes in more detail (cf. Pendall *et al*., 2004; Hendricks *et al*., 2006). For example, minirhizotron systems, soil isotopic labeling, and soil organic matter fractionation into different functional pools are all relatively recent innovations (Davidson *et al*., 1991; Johnson *et al*., 2001; Six *et al*., 2002a) In the field of atmospheric change research, experimental designs like the free-air CO_2 enrichment system in forests (i.e., FACE, Hendrey *et al*., 1999) have allowed us to test our hypotheses regarding the effects of CO_2-enrichment on belowground processes such as root production, soil C storage and soil N availability in intact, functioning, forested ecosystems (Norby *et al*., 1999). Previous studies had been limited to small tree seedlings and saplings in pots or chambers (Kramer, 1981). Further, hypotheses that are tested in FACE experiments are not narrowly constrained to the effects of atmospheric change on ecosystem function. CO_2 experiments can be used to address questions regarding disturbance, nutrient limitation or succession (Johnson, 2006). Also, the CO_2 applied to the elevated treatments is often

labeled with a depleted isotopic signature (i.e., relatively more ^{12}C than ^{13}C) because of a combination of a fossil plant signature and the combustion process used to derive the CO_2 source (Hendrey *et al*., 1999). The incorporation of this label into plant tissue and soil organic matter (SOM) allows the differentiation of different pools of C that would not be possible under normal conditions (i.e., Matamala *et al*., 2003; Jastrow *et al*., 2005).

Enhanced C storage in forested ecosystems depends on N availability

The overarching question of my dissertation research was: *Will enhanced C storage in forested ecosystems slow the increase of atmospheric CO_2 concentrations?* Terrestrial ecosystems are expected to be an important sink for rising atmospheric CO_2 because of a fertilization effect of CO_2 on plant photosynthesis (Stitt, 1991). However, there is a great deal of uncertainty in modeled CO_2 effects on terrestrial ecosystems; projected stimulation in terrestrial NPP from CO_2-fertlization range from 6 to 33 % (IPCC, 2007). This is in part because models projecting future ecosystem responses are often focused on only one aspect of terrestrial ecosystem function: the C cycle (Hungate *et al*., 2003; IPCC, 2007). Forests are an important component of terrestrial C fluxes as they currently store nearly half of the terrestrial C pool in biomass and soil pools with long residence times (Goodale *et al*., 2002). However, it has long been known that the availability of soil N for plant uptake limits forest NPP in temperate ecosystems (Vitousek & Howarth, 1991). The question of whether N availability will limit forest responses to rising [CO_2] has long been discussed (Kramer, 1981; Diaz *et al*., 1993; Zak *et al*., 1993; Berntson & Bazzaz, 1996), and has recently gained renewed attention (Luo *et al*., 2004).

Norby *et al*. (2005) found that across a broad range of forest production, elevated [CO_2] increased forest NPP by 23 ± 2%. Greater C fixed via photosynthesis, and subsequently used in the production of forest biomass, are important steps in removing CO_2 from the atmosphere (Kramer, 1981). However, depending on where C is allocated within the ecosystem, increased NPP will not necessarily translate to long-term C storage (Curtis *et al*., 1994). For example, the percentage of NPP allocated to long-lived woody

biomass in the four CO_2-enriched forests examined in Norby *et al*. (2005) was highly variable, and ranged from 11 to 93 %. C allocated to ephemeral leaf or fine-root tissue will turnover quickly (i.e., within a span of 1 to 3 years, Norby *et al*., 2002; Matamala *et al.*, 2003), and microbial degradation may quickly release the extra C fixed back to the atmosphere. Ultimately, processes that facilitate a positive net ecosystem carbon balance (cf. Chapin *et al*., 2006) are important over long time periods in slowing the increase in atmospheric [CO_2]. A net gain in ecosystem C over long time periods can only occur if C is not lost to plant respiration, heterotrophic respiration (including microbial decomposition), or leaching (Chapin *et al*., 2006). My dissertation research indicates that linkages between the C and N cycles in forested ecosystems might have global consequences for long-term ecosystem C storage.

Implications for ecosystem responses to rising atmospheric CO_2

C allocation

I found that increased fine-root production in a CO_2-enriched sweetgum plantation at Oak Ridge National Laboratory was most likely a mechanism for greater N acquisition in response to N limitation within the stand. Ecologists currently have a poor mechanistic understanding of the way in which C is allocated among biomass pools (Litton *et al*., 2007), especially C allocated belowground for nutrient acquisition (Hendricks *et al*., 1993; Pregitzer *et al*., 1995; Eissenstat *et al*., 2000). This is in part because it is difficult to measure the interactions among root turnover and soil nutrient availability at a relevant scale (i.e., the scale of an individual root or patch of soil, cf. Robinson, 2005). Cost-benefit analyses have been used to model expected root lifespan (Eissenstat *et al*., 2000), but we have a poor understanding of the relative gain in N per unit C invested in fine-root production because of the patchiness of soil resources and the non-uniform distribution of root systems (Robinson, 2005). One current modeling approach that has incorporated data from my N fertilization experiment estimates soil N availability from measurements of root production rather than soil N mineralization by

assuming that root production represents a plants C investment in N acquisition (Franklin *et al*., in review).

Fine-root turnover

Fine root populations turn over quickly in forested ecosystems (cf. Gill & Jackson, 2000). Therefore, C and N allocated to fine roots are not stored in long-lived tree biomass, and C and N may cycle more quickly through the ecosystem (Norby & Iversen, 2006). I found that greater root mortality under elevated [CO_2] doubled the input of fine-root detritus, and therefore C and N, to the soil profile. Greater inputs of organic matter to the soil are predicted to affect soil N availability, but the direction of the response is idiosyncratic and difficult to predict based on current data (Zak *et al*., 2003). Greater detritus may either decrease N availability through greater N immobilization in decaying root residue, or increase N availability through the stimulation of microbial activity and N mineralization (Berntson & Bazzaz, 1996). This dichotomy was an early controversy in atmospheric change research (Diaz *et al*., 1993; Zak *et al*., 1993), and has proven to be difficult to resolve from empirical data (Zak *et al*., 2003). This may be because the methods that we currently use to measure soil N availability are too broad, both spatially and temporally, to address these questions (Schimel & Bennett, 2004).

A question that often remains unaddressed in both ecosystem ecology and CO_2 research is how soil N availability may change in response to root proliferation into previously unexplored soil (cf. Fontaine *et al*., 2007). My dissertation research indicates that interactions between root turnover and soil N availability are important to examine throughout the soil profile (and not just the top 10 to 30 cm as is done currently, e.g. Zak *et al*., 2003). An experiment linking rates of N cycling with root turnover at several soil depths using isotopic labeling may help to resolve this question, and increase our understanding of the sustainability of forest responses to rising atmospheric [CO_2].

C stored in SOM

Soils may be an important sink for the additional C fixed in response to rising atmospheric [CO_2] because they account for two-thirds of the total C stored in terrestrial ecosystems, and contain C fractions with long-residence times (Schimel *et al*., 1994). I found that the decomposition rate of roots produced under elevated [CO_2] did not differ from that of roots produced under current [CO_2]. Thus, a greater amount of root detritus has the potential to be incorporated into SOM under elevated [CO_2]. However, the amount of root detritus that will be stored long-term in SOM pools is difficult to project because SOM is complex; it consists of a continuum of decomposing plant detritus of differing physical and chemical properties ranging from easily decomposed to highly resistant (Paustian *et al*., 1997; Baldock & Skjemstad, 2000; Berg, 2000). Decomposing root residue can act as a nucleus for the formation of relatively long-lived soil microaggregates (Gale *et al*., 2000) that protect SOM from decomposition through a series of complex processes involving physicochemical protection and litter recalcitrance (Six *et al*., 2002b; Six *et al*., 2004). However, studies that examine microaggregate formation rarely directly link root characteristics with SOM accumulation (Six *et al*., 2001). The link between root quality and quantity and C and N protection in SOM at ORNL FACE could be examined by tracking the ^{13}C signature of decomposing fine roots into different fractions of the soil pool over time using recently developed soil fractionation procedures (i.e., Six *et al*., 2002a; Jastrow *et al*., 2005). Such an experiment would substantially advance our understanding of belowground processes.

Data-model synthesis

The link between empirical data and ecosystem modeling is an important focus of both ecosystem ecology and atmospheric and climatic change research (cf. Classen & Langley, 2005) because a good understanding of current ecosystem processes is necessary to accurately project future ecosystem responses to changing environmental conditions. At least some of the data collected from the ORNL FACE experiment have been used as a benchmark in the current generation of IPCC models (i.e., Norby *et al*.,

2005) upon which policy decisions are based. However, projections of future ecosystem responses to atmospheric and climatic change are uncertain in part because: (1) data may not exist to correctly parameterize or test current models, or (2) the conceptual framework of current models may not be configured in a way that correctly represents ecosystem function under changing environmental conditions. For example, large-scale models like those used in the most recent IPCC report focus solely on the C cycle because data on soil nutrient feedbacks are limited (IPCC, 2007; Matthews, 2007).

The empirical data generated by my dissertation research addressed critical gaps in our current understanding of forest responses to rising [CO_2], and will inform the next generation of ecosystem models (i.e., Franklin *et al*., in review) and experiments. For example, I found that C and N input from fine-root mortality at depth in the soil (i.e., below 30 cm) may be an important component of future forest responses to rising atmospheric [CO_2]. Currently, the soil organic matter dynamics that determine N mineralization in ecosystem models like CENTURY are only simulated in the first 20 cm of the soil profile (Ma & Shaffer, 2001), and therefore there is no mechanism in the models whereby greater fine-root proliferation at depth could result in increased in N uptake. Thus, empirical results indicating that forest N uptake under elevated [CO_2] will increase, especially in N-limited ecosystems, were not predicted by models (cf. Norby & Iversen, 2006; Finzi *et al*., 2007).

Final thoughts

The CO_2-fertilization effect on vegetation production is an important component of future climate change predictions because enhanced storage of C in pools with long residence times may mitigate a portion of fossil fuel burning by slowing the increase of atmospheric [CO_2] (IPCC, 2007). However, the CO_2-fertilization effect is one of the most uncertain components of the global C cycle (e.g., Matthews, 2007). Accurate projections of future climate change are an especially urgent need given that feedbacks from the global C cycle are leading to climate forcing that is more intense and rapid than previously expected (Canadell *et al*., 2007). I have shown here that ecosystem N

limitation may play an important role in mediating forest responses to rising [CO_2], and that soil N cycling, especially at depth, should be better integrated into existing model framework. Continued progress in understanding and modeling the continuum from C fixed in plant biomass to C stored in long-lived organic matter pools (i.e. Chapin *et al*., 2006), and the interactions between soil C storage and N cycling, will require creativity in methods development and a smaller-scale focus (for example, at the scale of an individual root or patch of soil). A related need is for a framework to relate these new, smaller-scale investigations to whole-ecosystem (or larger) responses. The ultimate goal of my current and future research program is to improve our ability to predict ecosystem responses to environmental change and thus better inform policy decisions (e.g. IPCC, 2007).

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Vita

Colleen Marie Iversen grew up in Dansville, Michigan. She received her Bachelor of Science degree from Hope College in 2001, and her Master of Science degree from the University of Notre Dame in 2004. She is an ecosystem ecologist who uses a variety of field and laboratory techniques to understand and predict how ecosystems are shaped by climatic change. Specifically, her work investigates how global change alters carbon, nitrogen, or phosphorus cycling in ecosystems, and how the feedbacks between nutrient limitation and plant production will affect ecosystem processes. Her future research program will continue to be broadly focused on: (1) using large manipulative experiments to test ecosystem responses to global climate change, (2) using cutting-edge techniques, such as soil fractionation and isotope pool dilution, to examine soil carbon and nutrient cycling, and (3) developing new techniques to examine belowground processes.