Tree Physiology 25, 1501–1510

Effects of elevated concentrations of atmospheric CO_2 and tropospheric O_3 on decomposition of fine roots†

JACK A. CHAPMAN,[1,2] JOHN S. KING,[1] KURT S. PREGITZER[1] and DONALD R. ZAK[3]

[1] *School of Forest Resources and Environmental Science, Michigan Technological University, 1400 Townsend Dr., Houghton, MI 49931, USA*

[2] *Corresponding author (jchapman@mtu.edu)*

[3] *School of Natural Resources and Environment, University of Michigan, Ann Arbor, MI 48109, USA*

Received November 1, 2004; accepted March 5, 2005; published online September 1, 2005

Summary Rising atmospheric carbon dioxide (CO_2) concentration ($[CO_2]$) could alter terrestrial carbon (C) cycling by affecting plant growth, litter chemistry and decomposition. How the concurrent increase in tropospheric ozone (O_3) concentration ($[O_3]$) will interact with rising atmospheric $[CO_2]$ to affect C cycling is unknown. A major component of carbon cycling in forests is fine root production, mortality and decomposition. To better understand the effects of elevated $[CO_2]$ and $[O_3]$ on the dynamics of fine root C, we conducted a combined field and laboratory incubation experiment to monitor decomposition dynamics and changes in fine root litter chemistry. Free-air CO_2 enrichment (FACE) technology at the FACTS-II Aspen FACE project in Rhinelander, Wisconsin, elevated $[CO_2]$ (535 μl l^{-1}) and $[O_3]$ (53 nl l^{-1}) in intact stands of pure trembling aspen (*Populus tremuloides* Michx.) and in mixed stands of trembling aspen plus paper birch (*Betula papyrifera* Marsh.) and trembling aspen plus sugar maple (*Acer saccharum* Marsh.). We hypothesized that the trees would react to increased C availability (elevated $[CO_2]$) by increasing allocation to C-based secondary compounds (CBSCs), thereby decreasing rates of decomposition. Because of its lower growth potential, we reasoned this effect would be greatest in the aspen–maple community relative to the aspen and aspen–birch communities. As a result of decreased C availability, we expected elevated $[O_3]$ to counteract shifts in C allocation induced by elevated $[CO_2]$. Concentrations of CBSCs were rarely significantly affected by the CO_2 and O_3 treatments in decomposing fine roots. Rates of microbial respiration and mass loss from fine roots were unaffected by the treatments, although the production of dissolved organic C differed among communities. We conclude that elevated $[CO_2]$ and $[O_3]$ induce only small changes in fine root chemistry that are insufficient to significantly influence fine root decomposition. If changes in soil C cycling occur in the future, they will most likely be brought about by changes in litter production.

Keywords: carbon cycle, chemistry, dissolved organic carbon, FACE, field incubation, laboratory incubation, mass loss, microbial respiration.

Introduction

Since preindustrial times, atmospheric carbon dioxide (CO_2) concentration ($[CO_2]$) has risen nearly 30% and is predicted to double in the next 100–150 years (Stott et al. 2000, IPCC 2001). The steady rise in atmospheric $[CO_2]$ has been accompanied by an increase in tropospheric ozone (O_3) concentration ($[O_3]$) of 36% (IPCC 2001, Karnosky et al. 2003). These greenhouse gases may alter the terrestrial carbon (C) cycle by affecting forest productivity (Ceulemans and Mousseau 1994, Mooney and Koch 1994, Zak et al. 2000, Hamilton et al. 2002). Carbon dioxide is essential for photosynthesis and plant growth (Hoorens et al. 2003), whereas elevated $[O_3]$ is toxic to plants (Reich 1987). Research on the influence of elevated $[CO_2]$ and $[O_3]$ on forest ecosystems has been of major interest over the past decade because forests contain about 85% of the global plant C and 35% of the global soil C (Kirschbaum and Fischlin 1996, Gielen and Ceulemans 2001).

Of particular importance are how the growth and chemical composition of plant ephemeral tissues (leaves and fine roots) will be affected by elevated $[CO_2]$ and $[O_3]$ and the implications for long-term C storage in forest soils (King et al. 2001*a*). Studies of belowground production indicate that litter from fine roots is similar in magnitude to that from foliage (Persson 1980, McClaugherty et al. 1982, 1984, Harris et al. 1997, Pregitzer 2002). Thus, fine root decomposition processes are essential to the overall organic matter budgets of forest ecosystems (McClaugherty et al. 1984, Waring and Schlesinger 1985). It has been estimated that up to 50% of net primary production (NPP) is allocated belowground in temperate forest ecosystems (McClaugherty et al. 1982, Fogel and Hunt 1983, Berntson and Bazzaz 1996). Jackson et al. (1997) estimated that the production of fine roots accounts for as much as 33% of global annual net primary productivity.

The importance of trees in the global C cycle is due not only to their large standing biomass, but also to their interactions with other organisms, including soil microbial communities (Ceulemans and Mousseau 1994). In general, all plants contain the same classes of organic compounds, such as cellulose, hemicellulose, starches, proteins, lipids and polyphenols (phenolics, tannins and lignin), and each influences the de-

† *This paper was among those presented at the 18th North American Forest Biology Workshop, which was sponsored by the Society of American Foresters and the USDA Forest Service, and hosted by the Michigan Technological University, Houghton, MI, July 11–15, 2004.*

composition of plant residue by soil microorganisms (Martens 1999). Proportions of these compounds in plant tissues vary depending on species and maturity, but environmental factors, such as changes in atmospheric chemistry, also determine the production and allocation of these compounds for overall plant fitness (Martens 1999). Understanding how the concentrations of individual classes of plant organic compounds respond to environmental change is critical for predicting the amounts of these components in future soil organic inputs (Ehleringer et al. 2000).

Each class of compounds provides a unique quality and availability of C as a source of energy for microbial metabolism. Total nonstructural carbohydrates (TNC), including starches and sugars, and cellulose are readily available constituents and are decomposed quickly for energy and the synthesis of microbial biomass. Carbon-based secondary compounds (CBSCs) such as soluble phenolics, condensed tannins and lignin provide a low quality source of energy for microbes forming recalcitrant and long-lived components of C in soil (Cleve and Powers 1995). Changes in litter chemistry could alter microbial substrate availability, and as a result, the functioning of soil microbial communities, rates of decomposition and C and nitrogen (N) cycling (Geilen and Ceulemans 2001). Therefore, more research on the biochemical responses of fine roots to environmental change is needed. Current evidence reveals inconsistent effects of elevated [CO_2] on fine root litter chemistry and decomposition rates, and the effects of elevated [O_3] are even less well understood (King et al. 2001*c*). Studies aimed at determining how environmental change will affect the biochemistry of fine roots will further our understanding of nutrient and C cycling in forest soils (Waring and Schlesinger 1985, King et al. 2001*a*, Pregitzer 2002).

To understand if the changing composition of the atmosphere will alter forest fine root litter decomposition, we performed a paired laboratory and field incubation experiment on fine roots produced at the concentrations of atmospheric CO_2 and tropospheric O_3 predicted for the year 2050. During the laboratory incubation, we analyzed the biochemical characteristics of decomposing roots and monitored two products of decomposition: microbial respiration and dissolved organic C (DOC). The field incubation was performed to compare in situ decomposition dynamics to the laboratory incubation. The incubations were performed with fine roots and soil from the FACTS-II Aspen Free-air CO_2 enrichment (FACE) Project in Rhinelander, Wisconsin (Dickson et al. 2000). This experiment includes forest stands dominated by trembling aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marsh.) and sugar maple (*Acer saccharum* Marsh.). We hypothesized that: (1) increased C availability (elevated [CO_2]) will increase C allocation to C-based secondary compounds (CBSCs) in trees, thereby decreasing rates of decomposition; (2) because of the slower growth characteristics of maple, the accumulation of CBSCs will be greatest in the aspen–maple community relative to the aspen and aspen–birch communities; and (3) decreases in C availability caused by elevated [O_3] will counteract shifts in C allocation induced by elevated [CO_2].

Methods

FACTS-II: the Aspen FACE project

The Forest–Atmosphere Carbon Transfer and Storage (FACTS-II) Aspen FACE research project is located on the USDA Forest Service, Harshaw Experimental Farm, near Rhinelander, Wisconsin (45.6° W, 89.5° N). The 32-ha site includes twelve 30-m-diameter FACE plots arranged in a randomized complete block design. There are three replicates (blocks) of four treatments: control (ambient air), elevated [CO_2], elevated [O_3] and a combination of elevated [CO_2] and [O_3]. The target value for the elevated [CO_2] treatment was 560 μl l^{-1}. Ozone target concentrations were set similar to those in urban areas in the southwestern Great Lakes Region. Because O_3 production is influenced by light and temperature, sunny days received 90–100 nl l^{-1}, cloudy days received 50–60 nl l^{-1} and cool or wet days (< 15 °C) received no O_3 fumigation. The replicated treatments were blocked to accommodate a soil fertility gradient across the site. Trees were fumigated during daylight hours for the growing seasons of 1998 through 2001 (time of root harvest for this study). For more detailed information about the Aspen FACE project, see Dickson et al. (2000).

Seedlings or cuttings were planted at 1 × 1 m spacing in June 1997. Trees had been subjected to the experimental treatments for 3 years at the time fine roots were sampled for this experiment. Each plot is divided into three forest community types (referred to as "community" for the remainder of the paper). The east half of each plot is a trembling aspen community comprising five clones of varying sensitivity to CO_2 and O_3, and early or late leaf phenology. The northwest quarter of each plot is occupied by a sugar maple and trembling aspen community consisting of maple trees and aspen clones (Clone 216) in a 2:1 ratio. The southwest quarter of each plot is occupied by a paper birch and trembling aspen community consisting of birch trees and aspen clones in a 2:1 ratio.

Sampling

Fine roots (0.5–1.0 mm in diameter) were sampled in early November 2001. Ten soil cores, 15 cm diameter × 25 cm deep, were collected from each community in all plots, totaling 360 cores. A coarse mesh sieve (4 mm) removed roots from the soil. Soil adhering to the fine roots was washed off with deionized water. Roots were sorted into size classes, immediately frozen in liquid N, and then freeze-dried and stored at –20 °C. Soil sampling was repeated in the fall of 2002 as described above, but with a 2.54-cm-diameter corer to a depth of 10 cm. These samples provided the microbial inoculum for the laboratory incubations. Soil was stored in plastic bags at 4 °C until the beginning of the incubation.

Laboratory incubation

Fine roots were incubated for 240 days in the dark at 20 °C. Fine root material (300 mg) was homogenized and placed in the upper portion of microlysimeters made from modified Falcon filtration units (Falcon Filter Unit #7102, Becton Dickonson, Cockeysville, MD) (Zak et al. 1993). The microlysimeters were used in the quantification of microbial respira-

tion, soluble decomposition products (dissolved organic carbon (DOC)) and chemical composition of soil and litter during the incubation (Zak et al. 1993, Randlett et al. 1996, King et al. 2001*b*). Soil (10 g) from each community and plot from the field site was placed under fine roots separated by two overlapping fiberglass mesh screens (1 mm). Visible organic matter in the samples was removed by hand before placing soil in the microlysimeters. A glass-fiber filter between two fine-mesh stainless steel screens divided the upper portion of each microlysimeter from the lower portion. The four outlets of each microlysimeter were sealed with rubber septa and all seams were sealed with silicone to create an airtight system. The incubation was started on February 28, 2003 by adding 10 ml of deionized water to the microlysimeters, flushing the units with CO_2-free air, and placing them in an incubator.

The design of the laboratory fine root incubation duplicated the field design of the Aspen FACE Project. The incubation included five sets of microlysimeters, each set with 36 units corresponding to each of the community split plots within each FACE plot. Four additional microlysimeters per set containing only soil were included as blanks. A set of microlysimeters was destructively harvested on Days 15, 30, 60, 120 and 240. At each harvest, one microlysimeter corresponding to each community and plot, and the four blanks corresponding to each treatment, were sampled for microbially respired CO_2, DOC and decomposed fine roots. Microbially respired CO_2 was sampled by withdrawing a 40-µl gas sample from the head space with an air-tight syringe. Dissolved organic C was sampled by flushing the upper portion of each unit with 50 ml of deionized water under vacuum and withdrawing the solution from the bottom half of the unit with a 60-ml syringe. The solution was filtered (0.45 µm), acidified to pH 2–3 with HCl, and stored at 4 °C. Microbially respired CO_2 was quantified by injecting gas samples into a CP-3800 gas chromatograph (Varian, Palo Alto, CA) equipped with a TCD and Poropak Q column (Supelco, Bellefonte, PA). Dissolved organic C accumulation was determined with a SSM-5000A total organic C analyzer (Shimadzu Scientific Instruments, Columbia, MD).

At each of the five harvests, fine root material was removed by separating roots from the screen and microlysimeter with tweezers. Soil under the root mass was collected with a spatula and stored in a plastic bag at 4 °C. Any remaining root material was rinsed free from the screens and microlysimeters with deionized water onto a 40-mesh soil sieve and finally rinsed free of all soil with deionized water. Fine root material was then wrapped in aluminum foil, frozen in liquid N, freeze-dried and stored at –20 °C.

Non-harvested microlysimeters were flushed with CO_2-free air during scheduled harvests and on a biweekly basis until Day 70. At Day 70, most of the highly labile C had been consumed (Schmidt 2000). This process avoided CO_2 saturation in the units. Soil water content was maintained at field capacity in the units by flushing with 50 ml of deionized water during harvests.

Chemical analyses were performed on fine roots from the laboratory incubation. Total nitrogen (%N) and carbon (%C) concentrations in roots were determined with an NA-1500 Series II elemental analyzer (CE Elantech, Lakewood, NJ) run with National Institute of Standards and Technology pine needle and peach leaf standards as a quality control. Soluble sugars and starch were extracted from samples with methanol:chloroform:water. Starch in the remaining pellet was hydrolyzed enzymatically (R-Biopharm, Marshell, MI). Reducing sugars were determined colorimetrically (490 nm) by the phenol-sulfuric acid method (Dubois et al. 1956). A standard curve was constructed with D-glucose. Lipids were determined directly by weighing the residue after evaporating the chloroform fraction to dryness under a stream of compressed air (Poorter and Villar 1997). Soluble phenolics were assayed according to Booker et al. (1996) with Folin Ciocalteu reagent and $NaCO_3$. Absorbance was measured at 724 nm and compared with standard curves prepared with catechin. Extractable condensed tannins were determined by the acid-butanol method (Porter et al. 1986, Hagerman and Butler 1989). Samples were extracted in 70% acetone in 10 mM ascorbic acid at 4 °C, and the extracts treated with *n*-butanol activated with HCl and ferric ammonium sulfate, and then heated to 90 °C for 50 min. Absorbance of extracts was determined at 550 nm and compared with condensed tannin standards prepared from each species following the method of Booker et al. (1996). All colorimetric assays were performed with a Beckman DU 640 spectrophotometer (Beckman Coulter, Fullerton, CA). Lignin was determined directly as the acid-insoluble residue after samples (50 mg) had been extracted with phenol:acetic acid:water:dilute H_2SO_4 (5%), followed by digestion in concentrated (72%) H_2SO_4 (Booker et al. 1996). Hemicellulose was determined by extracting the pellet used for tannins with 10% KOH at 30 °C for 24 h; the extract was mixed with ice-cold ethanol in 4 M acetic acid and cooled to –20 °C for 24 h to precipitate hemicellulose (Dickson 1979). Cellulose was then calculated as the remaining cell wall material after removal of other constituents. Ash content was determined by combustion of subsamples in a muffle furnace at 500 °C.

Field incubation

Uniform lengths of several intact freeze-dried root samples with a mean diameter of 1 mm were placed in mesh bags (total mass of about 0.135 g) and buried at the forest floor–mineral soil interface in the respective split-plots from which they were harvested. The mesh bags were constructed with a stainless steel bottom (250 µm mesh size) and fiberglass top (1000 µm mesh size). Bags were labeled and tethered to small PVC posts to facilitate recovery. A complete set of bags (36) was recovered from all split-plot sections after 61, 188, 414, 517 and 737 days incubation. Recovered bags were dried to constant mass at 65 °C and the decomposed roots carefully separated by hand from soil, fungal hyphae and root ingrowth, weighed and ashed to correct for mineral content.

Statistical analysis

A fixed-effects model in an analysis of variance (ANOVA) for a randomized complete block design was used to evaluate effects of treatment and community on the chemistry of decomposing fine roots and decomposition products (King et al.

2001*a*). Data were tested for normality by inspecting residuals and normal probability plots, and when necessary, were normalized by log transformation. Treatment effects were considered significant if $P < 0.05$.

Treatment and community effects on microbial respiration and DOC were evaluated by an estimated coefficients approach, whereby coefficients of model parameters describing decomposition over time are compared across treatments. Decomposition processes are typically modeled as exponential decay functions (Olsen 1963, McClaugherty et al. 1984). We chose a two-parameter exponential decay function to model the time-dependent behavior of the response of microbially respired CO_2 and the production of DOC:

$$Y = b_0 e^{-b_1 t} \quad (1)$$

where: Y = response variable (i.e., respired CO_2 and DOC); b_0 = the initial value of the response variable (at time 0); b_1 = decay rate constant (k value); and t = time (in days).

To test for differences in the response curves across treatments, we used the parameter estimates as data in the randomized complete block ANOVA.

Results

Fine root litter chemistry during decomposition

Concentrations of soluble sugars, starch and lipids declined during the incubation and were not greatly affected by elevated $[CO_2]$ or $[O_3]$. Community ($P = 0.002$) and time ($P < 0.001$) had significant effects on soluble sugar concentrations which, averaged across treatments and species, decreased from 16.4 to 7.2 mg g^{-1} over the incubation period. Community type had significant effects ($P = 0.031$) on root starch concentrations (Table 1). Root starch concentrations declined rapidly from 16.6 mg g^{-1} on Day 15 of the incubation to 2.3 mg g^{-1} on Day 60. Mean lipid concentrations across species and treatments decreased from 60.7 to 51.5 mg g^{-1} from Day 15 to Day 240 of the incubation, respectively. The pattern of loss was similar across communities; however, the aspen–maple litter produced in elevated $[CO_2]$ resulted in the lowest lipid concentrations after Day 120, resulting in a significant $CO_2 \times$ time interaction (Table 1).

Fine root tissue N concentrations and C/N ratios responded to treatments uniquely in each community and were subject to complex interactions between CO_2, O_3, species and time (Table 1). Nitrogen concentrations increased for all treatments and communities from 1.1 to 1.9 mg g^{-1} during the 240-day incubation (Figure 1, Table 1). The C/N ratios steadily declined throughout the incubation period from 44.1 to 28.4 mg g^{-1} (Figure 1). For both aspen and aspen–maple communities, the highest C/N ratios were observed in elevated $[CO_2] + [O_3]$ for the entire incubation. From Day 225 to Day 240 of incubation, litter in the elevated $[CO_2]$ aspen–birch plots maintained the highest C/N ratio of all treatments and communities.

Concentrations of soluble phenolics were unresponsive to elevated $[CO_2]$ or $[O_3]$; however, community and time had significant effects (Table 1). On Day 15 of the incubation, aspen litter had lower concentrations of soluble phenolics (11.4 mg g^{-1}), averaged over treatments, than aspen–birch and aspen–maple litters (21.9 and 16.3 mg g^{-1}, respectively). By the end of the incubation, soluble phenolic concentrations for aspen, aspen–birch and aspen–maple communities averaged across treatments had decreased to 6.6, 10.6 and 8.4 mg g^{-1}, respectively. Concentrations of condensed tannins differed significantly among communities ($P < 0.001$) and there was a significant $CO_2 \times$ time interaction ($P = 0.008$) (Table 1, Figure 2). Averaged across treatments and species, condensed tannins concentrations rapidly decreased from 18.5 to 0.9 mg g^{-1} during the incubation. The aspen community had the lowest con-

Table 1. The P values for treatment and community effects on constituents in decomposing fine root litter produced under the experimental treatments at the Aspen FACE project. Abbreviations: N = nitrogen; C/N = carbon/nitrogen ratio; Com = community; T = time; and ns = not significant ($P > 0.05$).

Source	Sugars	Starch	Lipids	N	C/N	Total phenolics	Tannins	Lignin	Cellulose	Hemicellulose
CO_2	ns	ns	ns	ns	0.053	ns	ns	ns	ns	ns
O_3	ns	ns	ns	ns	ns	ns	ns	0.034	ns	0.019
$CO_2 \times O_3$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Com	0.002	0.031	ns	0.010	0.001	< 0.001	< 0.001	0.031	0.003	ns
$CO_2 \times$ Com	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$O_3 \times$ Com	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$CO_2 \times O_3 \times$ Com	ns	ns	ns	0.004	0.007	ns	ns	ns	ns	ns
T	< 0.001	ns	0.018	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001	0.005
$CO_2 \times T$	ns	ns	< 0.001	ns	ns	ns	0.008	ns	ns	ns
$O_3 \times T$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$CO_2 \times O_3 \times T$	ns	ns	ns	0.005	0.012	ns	ns	ns	0.024	ns
Com $\times T$	ns	ns	ns	ns	0.006	ns	ns	ns	ns	ns
$CO_2 \times$ Com $\times T$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
$O_3 \times$ Com $\times T$	ns	ns	ns	0.010	ns	ns	ns	ns	ns	ns
$CO_2 \times O_3 \times$ Com $\times T$	ns	ns	ns	< 0.001	ns	ns	ns	ns	ns	ns

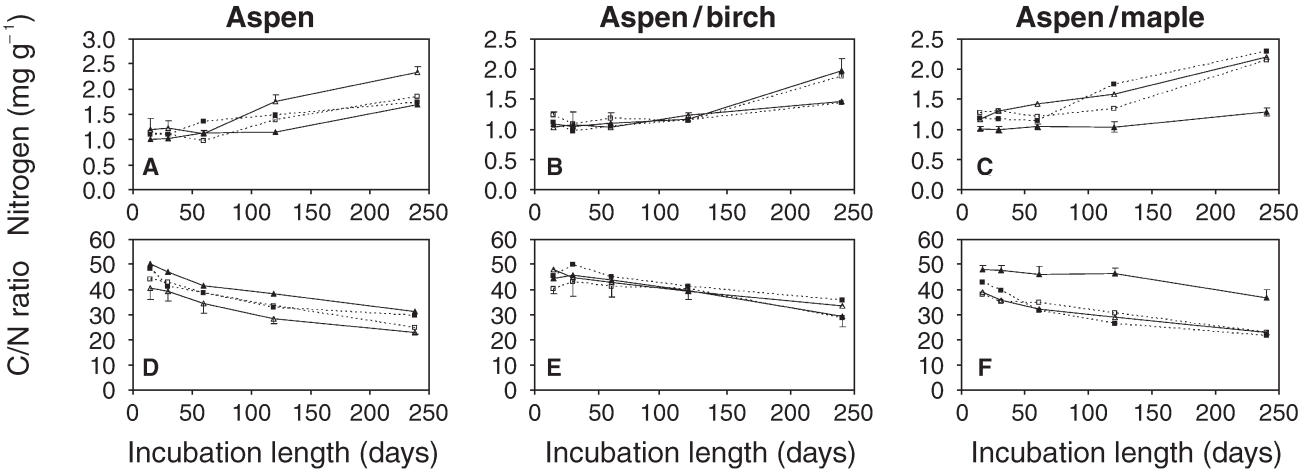

Figure 1. Mean concentration of nitrogen and C/N ratio of decomposing fine root litter (0.5–1.0 mm) produced in ambient air (control; □), elevated [CO_2] (■), elevated [O_3] (△), or elevated [CO_2] + [O_3] (▲) at the Aspen FACE project. Bars represent standard errors of the mean (shown on one treatment only for clarity); $n = 3$.

centrations of condensed tannins early in the incubation, and they were undetectable by Day 120. Concentrations of condensed tannins in aspen–maple and aspen–birch communities were near zero between Days 120 and 240.

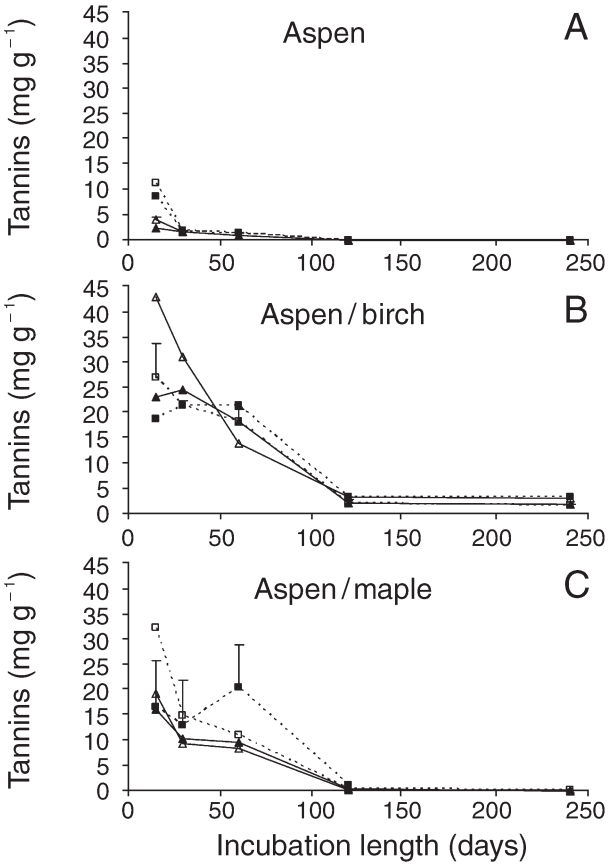

Figure 2. Mean concentrations of condensed tannins in decomposing fine root litter (0.5–1.0 mm) produced in ambient air (control; □), elevated [CO_2] (■), elevated [O_3] (△), or elevated [CO_2] + [O_3] (▲) at the Aspen FACE project. Bars represent standard errors of the mean (shown on one treatment only for clarity); $n = 3$.

In all communities, lignin concentration increased during the incubation (Figures 3A–3C). Averaged over treatments and species, lignin concentrations increased from 171.8 to 527.7 mg g^{-1} by Day 120 and declined to 498.1 mg g^{-1} by Day 240. Elevated [O_3] caused a significant reduction in lignin concentration (Table 1) compared with the corresponding controls in all communities that persisted until the end of the incubation.

Cellulose concentrations declined from 610.6 to 422.1 mg g^{-1} during the incubation (Figures 3D–3F). A significant $CO_2 \times O_3 \times$ time interaction occurred because of the lower cellulose concentrations in the elevated [CO_2] + [O_3] treatment toward the end of the incubation compared with the control. Hemicellulose concentrations were significantly affected by O_3 and time (Table 1). The decrease in hemicellulose concentrations was significantly slower in elevated [O_3], especially in the aspen–maple community (Figures 3G–3I). In the aspen community, hemicellulose concentrations decreased during the first 60 days from 111.3 to 94.9 mg g^{-1}, then rapidly increased to 146.2 mg g^{-1} by Day 120 and declined thereafter to 99.8 mg g^{-1} by the end of the incubation. A similar trend was observed in the aspen–birch and aspen–maple communities, with hemicellulose concentrations declining after Day 120.

Fine root decomposition

In all community types, microbial respiration gradually decreased during the incubation (Figure 4A). Dissolved organic C production was greatest early in the incubation and then decreased rapidly until Day 60 (Figure 4B). Elevated [CO_2] and [O_3] had no significant effects on microbial respiration rates in any community type, although there were consistent trends throughout the incubation. Dissolved organic C production differed significantly between communities, but was unaffected by the elevated [CO_2] and [O_3] treatments (Table 2). Mass loss of decomposing roots in the field incubation was similar across communities. Averaged over communities, nearly 50%

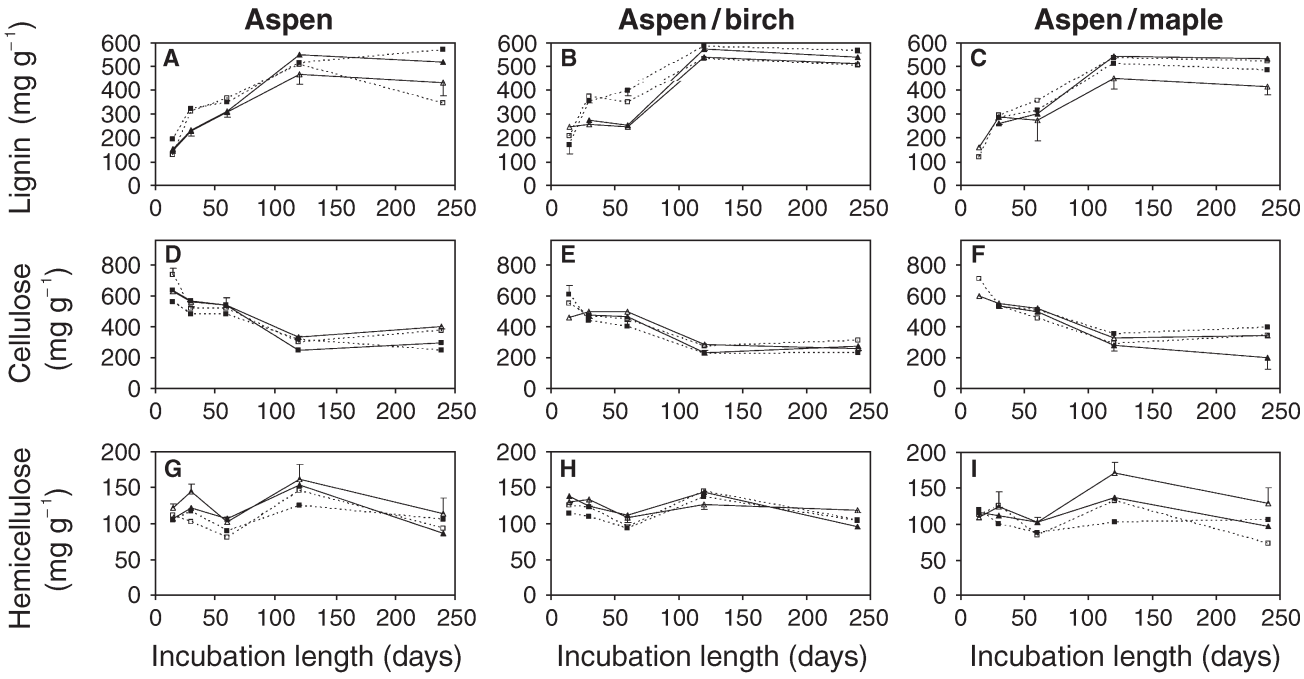

Figure 3. Mean concentrations of lignin, cellulose and hemicellulose in decomposing fine root litter (0.5–1.0 mm) produced in ambient air (control; □), elevated [CO_2] (■), elevated [O_3] (△), or elevated [CO_2] + [O_3] (▲) at the Aspen FACE project. Bars represent standard errors of the mean (shown on one treatment only for clarity); $n = 3$.

of fine root mass was lost by the end of the incubation (Figure 4C), and elevated [CO_2] reduced decomposition by 6.5% over the incubation period ($P = 0.07$).

Discussion

We predicted species-specific responses to elevated [CO_2], specifically that elevated [CO_2] stimulates the production of CBSCs in maple fine root litter to a greater degree than in aspen and birch fine root litter. Because of the recalcitrance of CBSCs, we predicted a reduction in fine root decomposition in proportion to the concentration of CBSCs. Further, we predicted that elevated [O_3] would reduce the C source strength in trees, causing decreases in concentrations of recalcitrant compounds and increases in decomposition rates in all communities. In general, we found that fine root litter produced in elevated [CO_2] and [O_3] exhibited minor changes in chemical composition in all communities, and that these changes did not significantly affect rates of decomposition.

Fine root litter chemistry during decomposition

Overall, only minor effects were observed in fine root chemical composition in response to the influence of either elevated [CO_2] or [O_3]. Concentrations of soluble sugars declined steadily, reaching the lowest concentrations on Day 120, whereas starch concentrations decreased rapidly from 16.6 to 2.3 mg g^{-1} by Day 60. This agrees with the findings of King et al. (2001*a*), that starch concentrations of trembling aspen litter approach zero by Day 55. After Day 120 of our study, concentrations of soluble sugars began to increase for the remainder of the incubation. This may be explained as a result of the coincident decomposition of polysaccharides, such as hemicellulose and cellulose (Berg and McClaugherty 2003).

Increases in N concentration and associated decreases in C/N ratios are well-known phenomena during the decomposition process, being caused largely by N immobilization by soil microorganisms (Kainulainen and Holopainen 2002, Berg and McClaugherty 2003). Dilution of N concentration and increased C/N ratios in response to elevated [CO_2] have been reported in many studies (Cotrufo et al. 1998, Curtis and Wang 1998), whereas others have shown no such effect (Norby et al. 1999). In our study, N concentration increased during the incubation period, but no significant reduction in N concentration occurred in elevated [CO_2] alone. In both aspen and aspen–maple communities, elevated [O_3] tended to increase foliar N concentration relative to controls throughout the incubation. Other elevated [O_3] studies have found positive effects, negative effects and no effects on foliar N concentrations, with the most common response being no effect (Koricheva et al. 1998). In our study, the elevated [CO_2] + [O_3] treatment resulted in the lowest N concentration in the aspen–maple community, and this effect persisted throughout the incubation. As a result of the reduced N concentration in response to elevated [CO_2] + [O_3], C/N ratios increased in the fine roots of the aspen–maple community. Furthermore, the aspen–maple community was affected the most by elevated [CO_2] + [O_3], having the highest C/N ratios compared with all other treatments during decomposition. This relatively high C/N ratio also persisted throughout the entire incubation period, suggesting that the interaction of elevated [CO_2] and [O_3] can have long-lasting effects on fine root litter quality during de-

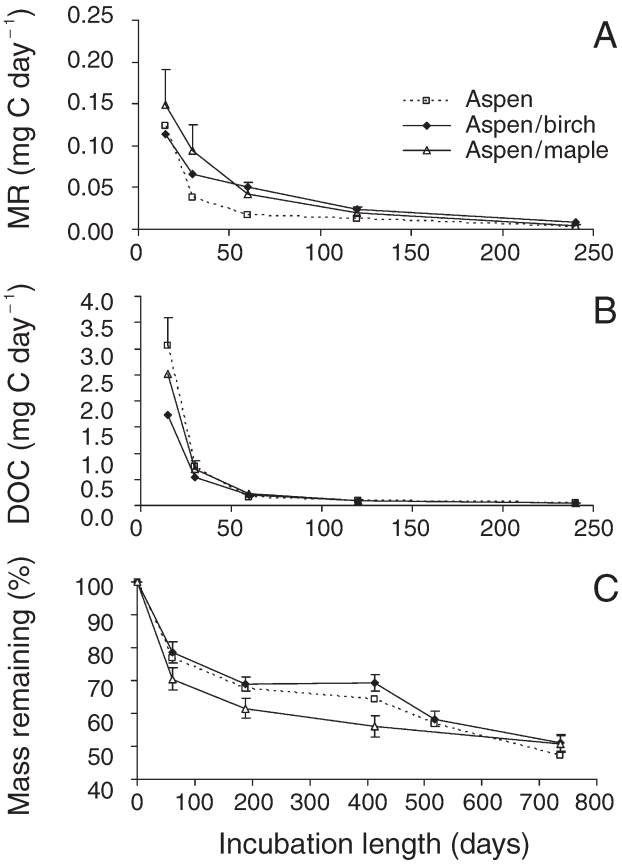

Figure 4. Decomposition of fine root litter (0.5–1.0 mm) produced in ambient air (control), elevated [CO_2], elevated [O_3], or elevated [CO_2] + [O_3] at the Aspen FACE project. Values representing each community are averaged across CO_2 and O_3 treatments. Decomposition is quantified as CO_2 produced by microbial respiration (MR), the production of dissolved organic carbon (DOC) and mass loss over time. Bars represent standard errors of the mean (shown on one or two treatments only for clarity); $n = 3$.

Table 2. The *P* values for constituents in decomposing fine root litter produced under the experimental treatments at the Aspen FACE project. Abbreviations: MR = microbial respiration; DOC = dissolved organic carbon; ML = mass loss; ns = not significant ($P > 0.05$); and na = not applicable.

Source	MR	DOC	ML
CO_2	ns	ns	0.069
O_3	ns	ns	ns
$CO_2 \times O_3$	ns	ns	ns
Community	ns	0.019	0.002
$CO_2 \times$ community	ns	ns	ns
$O_3 \times$ community	ns	ns	ns
$CO_2 \times O_3 \times$ community	ns	ns	ns
Time	na	na	< 0.001
$CO_2 \times$ time	na	na	ns
$O_3 \times$ time	na	na	ns
$CO_2 \times O_3 \times$ time	na	na	ns
Community × time	na	na	0.045
$CO_2 \times$ community × time	na	na	ns
$O_3 \times$ community × time	na	na	ns
$CO_2 \times O_3 \times$ community × time	na	na	ns

composition. King et al. (2001*c*) also recorded a high C/N ratio in aspen leaf litter produced in elevated [CO_2] that persisted over the entire incubation period.

Because of the recalcitrance of soluble phenolics, a trend of increased concentrations during decomposition has been reported (Peñuelas and Estiarte 1998). In our study, elevated [CO_2] and [O_3] did not have significant effects on concentrations of soluble phenolics, which decreased by 52% during decomposition. Condensed tannins are also believed to have decomposition patterns similar to those of soluble phenolics (Kraus et al. 2003), and condensed tannin concentrations have been reported to increase as a result of elevated [CO_2] (Berg and McClaugherty 2003). Compared with primary metabolites, like sugars and starch, the decomposition of secondary metabolites, such as phenolics and tannins, is slow (Kainulainen et al. 2003). Our finding of a higher concentration of soluble phenolics at the end of the incubation supports this; however, condensed tannin concentrations decreased in a manner similar to primary metabolites (starch and sugar). Concentrations of condensed tannins decomposed fastest in the aspen community, approaching zero by Day 60, whereas in the aspen–birch and aspen–maple communities, concentrations fell to 2.8 and 0.6 mg g^{-1}, respectively, by Day 120. In a similar incubation study, King et al. (2001*c*) found that condensed tannin concentrations in aspen leaf litter declined more than 90% after 2 weeks. Because tannins are a CBSC, decomposition of this compound should be relatively slow (Waring and Schlesinger 1985); however, our results indicate the opposite. King et al. (2001*b*) reported that condensed tannins decompose quickly and suggested these compounds may provide a greater energy source to decomposers than previously thought (but see Martin and Haider 1980), a conclusion supported by the results of our study.

The recalcitrance of lignin results in a much lower rate of decomposition relative to other organic compounds, causing its concentration to increase with time. The increase in lignin concentrations until Day 120 in our experiment was in response to the rapid early decomposition of starch, condensed tannins and cellulose. After Day 120, lignin began to decompose. Microorganisms responsible for the decomposition of lignin are incapable of using lignin as a primary energy source and must derive energy from other C sources (Berg 1986). The decomposition of hemicellulose and increases in sugar concentrations after Day 120 likely provided a supply of labile compounds available to lignin decomposers. In agreement with our hypothesis, elevated [O_3] significantly reduced lignin concentrations in all community types, and this reduction persisted throughout decomposition in the aspen–maple community. This response agrees with predictions that slow-growing species will have the greatest biochemical response to atmospheric pollutants compared with rapidly growing species. Lignin concentrations were not significantly affected by elevated [CO_2], which is consistent with other FACE studies

(Finzi and Schlesinger 2002, Parsons et al. 2004). Peñuelas and Estiarte (1998) reviewed the effects of elevated [CO_2] on secondary metabolism and also found evidence that concentrations of lignin and polysaccharides did not increase.

The lack of significant responses of hemicellulose and cellulose to elevated [CO_2] in our study is consistent with the conclusions of Peñuelas and Estiarte (1998). Ozone, however, caused a significant increase in hemicellulose concentrations. Most O_3 research has focused on the functioning and physiology of plants, whereas the effects of elevated [O_3] on the chemical composition of fine root tissues and fine root decomposition have received little attention.

Fine root litter decomposition

Altered patterns of C assimilation and allocation in response to elevated [CO_2] and [O_3] were hypothesized to change fine root chemistry and decomposition. We monitored decomposition by measuring C loss as soil microbial respiration (respired CO_2) and DOC from decomposing fine root litter. These measurements generally represent two stages of decomposition. Microbial respiration reflects rapid turnover of labile forms of C (Hungate et al. 1997), and DOC reflects the turnover of more recalcitrant forms but can also contain labile C fractions like carbohydrates (Herbert and Bertsch 1995, Ibrahima et al. 1995, Vestgarden 2001). Elevated [CO_2] and [O_3] did not significantly influence either measurement of decomposition in our study. Decomposition of fine root litter differed by community type, although high variation or low replication ($n = 3$), or both, may have limited our ability to detect treatment effects. However, unaltered decomposition rates of litter produced at elevated [CO_2] have been reported in previous studies (Randlett et al. 1996, King et al. 2001*a*, 2001*b*, Norby et al. 2001). Unaltered microbial respiration rates in our study may reflect minimal changes in chemistry of fine roots produced at elevated [CO_2] or [O_3] or both, compared with ambient air; in which case, sources of microbial substrate would not have been significantly altered. The results of the laboratory incubation agree with the field incubation that showed that the elevated [CO_2] and [O_3] treatments had only minor effects on rates of mass loss.

The hypothesis that elevated [O_3] would offset elevated [CO_2] effects on decomposition could not be evaluated because there were no [CO_2] effects. This suggests that microbial communities are able to compensate for minor alterations in litter chemistry early in the decomposition process, and decomposition rates of litter produced in elevated [CO_2] will not be changed by elevated [O_3]. Elevated [O_3] and [CO_2] + [O_3] resulted in a significantly greater loss of C through leaching in the aspen community and to a lesser extent in the aspen–maple community, only during the first 15 days of decomposition. In all community types, DOC production after 2 months of decomposition was almost identical regardless of treatment. The DOC represents decomposition of recalcitrant forms of C, suggesting that, in the long term, effects on littler chemistry initiated by either elevated [CO_2] or [O_3] will not affect long-lasting pools of C derived from plant litter.

We conclude that fine root biochemical responses were not significantly affected by elevated [CO_2] and [O_3]. The realization that the laboratory incubation was highly controlled and removed the influence of changes in water, temperature and macro-fauna associated with litter decomposition on the forest floor should be noted. However, when mass loss data from the field incubation was pooled for all communities, only a marginal reduction in decomposition was detected in response to elevated [CO_2] and no effect in response to elevated [O_3] was found. As in the laboratory incubation, we found little evidence from the field incubations suggesting that chemical changes in fine root litter induced by elevated [CO_2] and [O_3] will influence fine root litter decomposition in the future. Therefore, the predicted elevated [CO_2] and [O_3] mediated changes in fine root biomass production are likely to have a greater influence on forest C cycling than changes in fine root chemistry.

Acknowledgments

This research was supported by the USDA NRI Competitive Grants Program No. 2001-35107-11262 and additional support from the USDA Forest Service Northern Global Change Program.

References

Berg, B. 1986. Nutrient release from litter and humus in coniferous forest soil—a mini review. Scand. J. For. Res. 1:359–369.

Berg, B. and C. McClaugherty. 2003. Plant litter: decomposition, humus formation, carbon sequestration. Springer-Verlag, Berlin, pp 1–279.

Berntson, G.M. and F.A. Bazzaz. 1996. The allometry of root production and loss in seedlings of *Acer rubrum* (Aceraceae) and *Betula papyrifera* (Betulaceae): implications for root dynamics in elevated CO_2. Am. J. Bot. 83:608–616.

Booker, F.L., S. Anttonen and A.S. Heagle. 1996. Catechin, proanthocyanidin and lignin contents of loblolly pine (*Pinus taeda*) needles after chronic exposure to ozone. New Phytol. 132:483–492.

Cleve, K.V. and R.F. Powers. 1995. Soil carbon, soil formation and ecosystem development. *In* Carbon Forms and Functions in Forest Soils. Eds. W.W. Mcfee and J.M. Kelly. Soil Science Society of America, Madison, WI, pp 155–200.

Cotrufo, M.F., P. Ineson and A. Scott. 1998. Elevated CO_2 reduces the nitrogen content of tissues. Global Change Biol. 4:43–54.

Ceulemans, R. and M. Mousseau. 1994. Effects of elevated atmospheric CO_2 on woody plants. New Phytol. 127:425–446.

Curtis, P.S. and X. Wang. 1998. A meta-analysis of elevated CO_2 effects on woody plant mass, form and physiology. Oecologia 113: 299–313.

Dickson, R.E. 1979. Analytical procedures for the sequential extraction of ^{14}C-labelled constituents from leaves, bark and wood of cottonwood plants. Physiol. Plant 45:480–488.

Dickson, R.E., K.F. Lewin, M.D. Isebrands et al. 2000. Forest atmosphere carbon transfer and storage (FACTS-II)—the aspen free-air CO_2 and O_3 enrichment (FACE) project: an overview. USDA Tech. Rep. NC-214. USDA, Forest Service, North Central Forest Exp. Sta., St. Paul, MN, 68p.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350–356.

Ehleringer, J.R., N. Buchmann and L.B. Flanagan. 2000. Carbon isotope ratios in belowground carbon cycling processes. Ecol. Appl. 10:412–422.

Finzi, A.C. and W.H Schlesinger. 2002. Species control variation in litter decomposition in a pine forest exposed to elevated CO_2. Glob. Change Biol. 8:1217–1229.

Fogel, R. and G. Hunt. 1983. Contribution of mycorrhizae and soil fungi to nutrient cycling in a Douglas-fir ecosystem. Can. J. For. Res. 12:219–232.

Gielen, B. and R. Ceulemans. 2001. The likely impact of rising atmospheric CO_2 on natural and managed *Populus*: a literature review. Environ. Pollut. 115:335–358.

Hagerman, A.E. and L.G. Butler. 1989. Choosing appropriate methods and standards for assaying tannin. J. Chem. Ecol. 15:1795–1810.

Hamilton, J.G., E.H. DeLucia, K. George, S.L. Naidu, A.C. Finzi and W.H. Schlesinger. 2002. Forest carbon balance under elevated CO_2. Oecologia 131:250–260.

Harris, W.F., R.S. Kinerson, Jr. and N.T. Edwards. 1997. Comparison of belowground biomass of natural deciduous forest and loblolly pine plantations. Pedobiologia 17:369–381.

Herbert, B.E. and P.M. Bertsch. 1995. Characterization of dissolved and colloidal organic matter in soil solution: a review. *In* Carbon Forms and Functions in Forest Soils. Eds. W.W. McFee and J.M. Kelly. Soil Science Society of America, Madison, WI, pp 63–88.

Hoorens, B., R. Aerts and M. Stroetenga. 2003. Is there a trade-off between the plant's growth response to elevated CO_2 and subsequent litter decomposability? Oikos 103:17–30.

Hungate, B.A., E.A. Holland, R.B. Jackson, F.S. Chapin, III, H.A. Mooney and C.B. Field. 1997. The fate of carbon in grasslands under carbon dioxide enrichment. Nature 388:576–579.

Ibrahima, A., R. Joffre and D. Gilon. 1995. Changes in litter during the initial leaching phase: an experiment on the leaf litter of Mediterranean species. Soil Biol. Biochem. 27:931–939.

IPCC. 2001. A report of working group I of the Intergovernmental Panel on Climate Change. http://www.ipcc.ch/.

Jackson, R.B., H.A. Mooney and E.-D. Schulze. 1997. A global budget for fine root biomass, surface area and nutrient contents. Proc. Natl. Acad. Sci. USA 94:7362–7366.

Kainulainen, P. and J.K. Holopainen. 2002. Concentrations of secondary compounds in Scots pine needles at different stages of decomposition. Soil Biol. Biochem. 34:37–42.

Kainulainen, P., T. Holopainen and J.K. Holopainen. 2003. Decomposition of secondary compounds from needle litter of Scots pine grown under elevated CO_2 and O_3. Glob. Change Biol. 9:295–304.

Karnosky, D.F., D.R. Zak, K.S. Pregitzer et al. 2003. Tropospheric O_3 moderates responses of temperate hardwood forests to elevated CO_2: a synthesis of molecular to ecosystem results from the Aspen FACE project. Funct. Ecol. 17:289–304.

King, J.S., K.S. Pregitzer, D.R. Zak, J. Sober, J.G. Isebrands, R.E. Dickson, G.R. Hendry and D.F. Karnosky. 2001*a*. Fine root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO_2 and tropospheric O_3. Oecologia 128:237–250.

King, J.S., K.S. Pregitzer, D.R. Zak, M.E. Kubiske and W.E. Holmes. 2001*b*. Correlation of foliage and litter chemistry of sugar maple, *Acer saccharum*, as affected by elevated CO_2 and varying N availability, and effects on decomposition. Oikos 94:403–416.

King, J.S., K.S. Pregitzer, D.R. Zak, M.E. Kubiske, J.A. Ashby and W.E. Holmes. 2001*c*. Chemistry and decomposition of litter from *Populus tremuloides* Mich. grown at elevated atmospheric CO_2 and varying N availability. Glob. Change Biol. 7:65–74.

Kirschbaum, M. and A. Fischlin. 1996. Climate change impacts on forests. *In* Climate Change 1995—Impacts, Adaptations and Mitigation of Climate Change: Scientific-Technical Analysis. Contribution of Working Group II to the Second Assessment Report of the Intergovernmental Panel of Climate Change (IPCC). Eds. R. Watson, M.C. Zinyowera and R.H. Moss. Cambridge University Press, Cambridge, pp 95–129.

Koricheva, J., S. Larsson, E. Haukioja and M. Keinanen. 1998. Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. Oikos 83: 212–226.

Kraus, T.E.C., R.A. Dahlgren and R.J. Zasoki. 2003. Tannins in nutrient dynamics of forest ecosystems: a review. Plant Soil 256:41–66.

Martin, J.P. and K. Haider. 1980. Microbial degradation and stabilization of ^{14}C labeled lignins, phenolics and phenolic polymers in relation to soil humus formation. *In* Lignin Biodegradation: Microbiology, Chemistry and Potential Applications. Vol. 2. Eds. T.K. Kirk, T. Higuchi and H.M. Chang. CRC Press, West Palm Beach, FL, pp 78–100.

Martens, D.A. 1999. Plant residue biochemistry regulates soil carbon cycling and carbon sequestration. Soil Biol. Biochem. 32:361–369.

McClaugherty, C.A., J.D. Aber and J.M. Melillo. 1982. The role of fine roots in the organic matter and nitrogen budgets of two forested ecosystems. Ecology 63:1481–1490.

McClaugherty, C.A., J.D. Aber and J.M. Melillo. 1984. Decomposition dynamics of fine roots in forested ecosystems. Oikos 42: 378–386.

Mooney, H.A. and G.W. Koch. 1994. The impact of rising CO_2 concentrations on the terrestrial biosphere. Ambio 23:74–76.

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

Norby, R.J., M.F. Cotrufo and P. Ineson. 2001. Elevated CO_2, litter chemistry and decomposition: a synthesis. Oecologia 127: 153–165.

Olson, J.S. 1963. Energy storage and the balance of producers and decomposers in ecological systems. Ecology 44:322–331.

Parsons, W.F.J., R.L. Lindroth and J.G. Bockheim. 2004. Decomposition of *Betula papyrifera* leaf litter under the independent and interactive effects of elevated CO_2 and O_3. Glob. Change Biol. 10: 1666–1677.

Peñuelas, J. and M. Estiarte. 1998. Can elevated CO_2 affect secondary metabolism and ecosystem function? Trees 13:20–24.

Persson, H. 1980. Spatial distribution of fine-root growth, mortality and decomposition in a young Scots pine stand in Central Sweden. Oikos 34:77–87.

Poorter, H. and R. Villar. 1997. The fate of acquired carbon in plants: chemical and construction costs. *In* Plant Resource Allocation. Eds. F.A. Bazzaz and J. Grace. Academic Press, San Diego, CA, pp 39–72.

Porter, L.J. 1986. Number and weight-average molecular weights for some proanthocyanidin polymers (condensed tannins). Aust. J. Chem. 39:557–562.

Pregitzer, K.S. 2002. Fine roots: a new perspective. New Phytol. 154: 267–273.

Randlett, D.L., D.R. Zak, K.S. Pregitzer and P.S. Curtis. 1996. Elevated atmospheric carbon dioxide and leaf litter chemistry: influences on microbial respiration and net nitrogen mineralization. Soil Sci. Soc. Am. J. 60:1571–1577.

Reich, P.B. 1987. Quantifying plant responses to ozone: a unifying theory. Tree Physiol. 3:63–91.

Schmidt, K. 2000. Effects of elevated CO_2 and soil fertility on fungal biomass and root litter in decomposing fine roots of trembling aspen (*Populus tremuliodes* Michx.) and sugar maple (*Acer saccharum*

Marsh.). M.Sc. Thesis, Michigan Technological Univ., Houghton, MI, 64p.

Stott, P.A., S.F.B. Tett, G.S. Jones, M.R. Allen, J.F.B. Mitchell and G.J. Jenkins. 2000. External control of 20th century temperature by natural and anthropogenic forgings. Science 290:2133–1268.

Vestgarden, L.S. 2001. Carbon and nitrogen turnover in the early stages of Scots pine (*Pinus sylvestris* L.) needle litter decomposition: effects of internal and external nitrogen. Soil Biol. Biochem. 33:465–474.

Waring, R.H. and W.H. Schlesinger. 1985. Forest ecosystems: concepts and management. Academic Press, San Diego, CA, 340 p.

Zak, D.R., K.S. Pregitzer, P.S. Curtis, J.A. Teeri, R. Fogel and D.L. Randlett. 1993. Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. Plant Soil 151:105–117.

Zak, D.R., K.S. Pregitzer, J.S. King and W.E. Holmes. 2000. Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. New Phytol. 147:201–222.