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# Fine root chemistry and decomposition in model communities of north-temperate tree species show little response to elevated atmospheric $CO_2$ and varying soil resource availability

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**Abstract** Rising atmospheric [$CO_2$] has the potential to alter soil carbon (C) cycling by increasing the content of recalcitrant constituents in plant litter, thereby decreasing rates of decomposition. Because fine root turnover constitutes a large fraction of annual NPP, changes in fine root decomposition are especially important. These responses will likely be affected by soil resource availability and the life history characteristics of the dominant tree species. We evaluated the effects of elevated atmospheric [$CO_2$] and soil resource availability on the production and chemistry, mycorrhizal colonization, and decomposition of fine roots in an early- and late-successional tree species that are economically and ecologically important in north temperate forests. Open-top chambers were used to expose young trembling aspen (*Populus tremuloides*) and sugar maple (*Acer saccharum*) trees to ambient (36 Pa) and elevated (56 Pa) atmospheric $CO_2$. Soil resource availability was composed of two treatments that bracketed the range found in the Upper Lake States, USA. After 2.5 years of growth, sugar maple had greater fine root standing crop due to relatively greater allocation to fine roots (30% of total root biomass) relative to aspen (7% total root biomass). Relative to the low soil resources treatment, aspen fine root biomass increased 76% with increased soil resource availability, but only under elevated [$CO_2$]. Sugar maple fine root biomass increased 26% with increased soil resource availability (relative to the low soil resources treatment), and showed little response to elevated [$CO_2$]. Concentrations of N and soluble phenolics, and C/N ratio in roots were similar for the two species, but aspen had slightly higher lignin and lower condensed tannins contents compared to sugar maple. As predicted by source-sink models of carbon allocation, pooled constituents (C/N ratio, soluble phenolics) increased in response to increased relative carbon availability (elevated [$CO_2$]/low soil resource availability), however, biosynthetically distinct compounds (lignin, starch, condensed tannins) did not always respond as predicted. We found that mycorrhizal colonization of fine roots was not strongly affected by atmospheric [$CO_2$] or soil resource availability, as indicated by root ergosterol contents. Overall, absolute changes in root chemical composition in response to increases in C and soil resource availability were small and had no effect on soil fungal biomass or specific rates of fine root decomposition. We conclude that root contributions to soil carbon cycling will mainly be influenced by fine root production and turnover responses to rising atmospheric [$CO_2$], rather than changes in substrate chemistry.

**Keywords** Trembling aspen · Sugar maple · Carbon-based secondary compounds · Soil C cycling

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## Introduction

Fine roots of trees are an important part of the terrestrial carbon (C) cycle because they comprise a large fraction of annual net primary production that, as

ephemeral tissues, are returned to the soil on relatively short time scales (Vogt et al. 1986). Due to the difficulty of observing fine roots in situ, our knowledge of fine root biology is still in its infancy (Nadelhoffer 2000; Pregitzer 2002). We have a rudimentary understanding of fine root longevity, contributions to soil $CO_2$ efflux, root-microbial interactions, responses to competition, herbivory and disease, and how any of these change as a function of resource availability, seasonality, and environment (King et al. 2002). These knowledge gaps limit our ability to predict how human-caused changes to the environment will affect the cycling of C in forest ecosystems through changes in fine root dynamics. Rapidly rising atmospheric [$CO_2$] and nitrogen (N) deposition are two environmental factors that have strong potential to directly affect fine root dynamics. Because terrestrial vegetation and soils contain approximately 2,060 pg C compared to the 750 pg in the atmosphere (Schlesinger 1997), a small fractional change in C storage on land could have significant impacts on atmospheric $CO_2$ accumulation.

Plants allocate assimilated C between growth, respiration, and chemical defense as influenced by their life history characteristics (Herms and Mattson 1992). Changes in resource availability such as light, nitrogen (N), or atmospheric [$CO_2$] can affect "growth-dominated" species differently than "differentiation-dominated" species. Growth-dominated species tend to allocate "extra" C (relative to N) to growth, whereas differentiation-dominated species would be expected to allocate it to the production of carbon-based secondary defense compounds (Loomis 1932; Herms and Mattson 1992; Koricheva et al. 1998). In addition, to gain broad insight into how forests will be impacted by human-caused environmental change, experiments are needed that compare responses of species from multiple functional groups (Hättenschwiler 2001; King et al. 2001a, 2001b).

The fate of organic matter in the soil is influenced by both the quantity produced and its biochemical composition. Fine root (and leaf litter) biochemistry is particularly important because it may directly affect the metabolic functioning of soil microbial communities that control the rate and extent of organic matter degradation. Biochemical changes in foliage induced by elevated [$CO_2$], such as increased production of condensed tannins and phenolics, have been shown to affect the performance of insect herbivores (McDonald et al. 1999; Agrell et al. 2000; Percy et al. 2002; Kopper and Lindroth 2003). Similarly, increased C assimilation under elevated $CO_2$ may lead to increased mycorrhizal colonization of roots (Smith and Read 1997), which could increase the content of recalcitrant fungal materials in fine roots, such as chitin. We need to determine if these types of biochemical changes will affect the functioning of decomposer communities in soil as has been shown for insect herbivores. Chemically altered organic inputs could lead to changes in genetic induction of extra-cellular microbial enzymes or microbial community composition that fundamentally alter soil C cycling (Larson et al. 2002; Phillips et al. 2002). Therefore, an immediate research challenge is to elucidate the roles of quantitative changes in production and qualitative changes in chemistry of plant-derived inputs in eliciting the observed changes in microbial community composition and metabolism.

To test the hypothesis that elevated atmospheric [$CO_2$] and soil resource availability will alter fine root chemistry and microbial metabolism, we grew the shade-intolerant trembling aspen and shade-tolerant sugar maple in an open top chamber experiment for 2.5 years. At the end of the experiment, a complete harvest was performed in which we quantified fine root biomass. Fine roots were analyzed for changes in biochemistry, including mycorrhizal colonization, and were incubated in the lab to observe the effects of root tissue chemistry on soil fungal microbial biomass and metabolism. We hypothesized that under increased C availability (elevated [$CO_2$] and/or low soil resource availability) the growth dominated aspen would increase fine root production with minor changes in chemistry. We hypothesized that sugar maple fine root biomass would be less responsive to increased C availability, but production of carbon based secondary compounds (CBSC) would be stimulated. We expected the effects of the treatments to be additive, in that changes in growth and chemistry would be greatest under conditions of highest C availability (elevated $CO_2$, low soil resources) and least at low C availability (ambient $CO_2$, high soil resources). We also reasoned that fungal biomass and microbial metabolism would decrease on substrates that had higher concentrations of CBSC, resulting in decreased fine root decomposition.

## Materials and methods

### Field site

In the spring of 1997, a randomized complete block ($N = 5$) design of atmospheric $CO_2$ and soil resource treatments was established in an open-top chamber facility at the University of Michigan Biological Station near Pellston, MI. There were 20 3-m diameter open-top chambers (10 ambient, 10 elevated $CO_2$), commonly used in air pollution research. The $CO_2$ fumigation system has been previously described (Curtis et al. 2000). It consisted of a centrally located ambient sampling line used to monitor background $CO_2$ concentration of the field site, and sampling and $CO_2$ dispensing lines distributed to each elevated $CO_2$ chamber. Sampling lines were switched to a Li-Cor 6262 infra-red gas analyzer by a computer control system that recorded 2 min averages over a 24-h period. Carbon dioxide dispensing lines ran to each elevated $CO_2$ chamber from a common manifold connected to a 6-ton liquid $CO_2$ reservoir. Individual volume flow regulators were ad-

justed manually to maintain the $CO_2$ treatment differential at +20 Pa (±10%) in each elevated $CO_2$ chamber. Due to poor growth, one block of treatments was removed for an $N$ of 4 in the study.

In the spring of 1997, 12 seedlings of each species were planted in separate sections of the open-top chambers from seed (sugar maple) or root sprouts (aspen) that had been propagated that winter. The maple seed was from a wild source in western Upper Peninsula of Michigan (Baraga County). The aspen was from clones that occur several miles from the research site in the northern Lower Peninsula of Michigan (Emmet County). Plants were watered by natural precipitation only.

Chambers were fumigated continuously from mid-April to mid-October in 1997 and 1998, and from mid-April to mid-July in 1999. Mean (standard deviation) daytime ambient and elevated $CO_2$ concentrations for the period of study were 36.2 (12.0) Pa and 56.2 (5.2) Pa, respectively. Chambers were installed on open-bottom root boxes filled with one of two soil mixes with wooden dividers separating species (Pregitzer et al. 1995). The high soil resource availability soil was composed of a homogenized A-horizon of Kalkaska series soil (sandy, mixed, frigid, Entic Haplorthod) and the low soil resource availability soil consisted of a 4:1 mix of Rubicon series C-horizon (sandy, mixed, frigid Entic Haplorthod) with A-horizon of the Kalkaska series soil. These soil mixes have been shown to have initial net N-mineralization rates of 45–89 ng $g^{-1}$ $d^{-1}$ and 319–345 ng $g^{-1}$ $d^{-1}$, for the low and high soil resource availability treatments, respectively (Pregitzer et al. 1995; Kubiske et al. 1998; Zak et al. 2000a, 2000b). The low- and high- soil resource availability soil mixes also varied in the following properties, respectively: texture (93 and 72% sand, 2.5 and 10.1% clay), available water content (0.014 and 0.053 MPa), total C (3,559 and 12,489 mg $kg^{-1}$ soil), and total N (260 and 996 mg $kg^{-1}$ soil) (Curtis et al. 2000). Minor differences occurred in C:N (13.7 and 12.5), pH (6.74 and 6.08), and extractable P (13.7 and 12.5 mg P $kg^{-1}$ soil) between the low and high soil resource availability treatments, respectively. Soil physical and chemical properties of both soil mixes were well within the range of natural variation experienced by trembling aspen and sugar maple on the glacially derived soils of the region (Zak et al. 2000b).

In July of 1999, a complete above- and below-ground harvest was performed. Before the whole tree harvest, fine roots were sampled by collecting ten soil cores in each of the aspen and maple sections of the chambers. Fine roots were defined as ≤ 0.5 mm diameter. The 5-cm diameter cores traversed the entire depth of the experimental soil mixtures (45 cm deep). The ten cores per section were aggregated and fine roots separated from the soil by hand. The data presented in this study are for live roots only, which were separated from dead roots based on color (light tan to brown) and consistency (e.g., intact, succulent cortex). Once removed from the soil, live fine roots were rinsed clean with de-ionized water, flash frozen with dry ice, and lyophilized. Estimates of root biomass are expressed scaled to the volume of soil in each species chamber section (e.g., g dry weight/chamber section).

## Laboratory incubations

To assess whether growth under the atmospheric $CO_2$ and soil resource availability treatments affected soil fungal biomass and microbial metabolism, a 98-day laboratory incubation was performed using the harvested fine root material as the substrate. The incubation was started with five complete sets of microlysimeters (37 units per set), each set corresponding to a harvest date over the course of the incubation. The statistical design of the incubation duplicated that of the field experiment, with a microlysimeter corresponding to both species in each open-top chamber (two sp.×16 chambers, $N = 4$), plus five blanks containing the soil mixture but no root material. The microlysimeters consisted of modified filtration units (Falcon filter 7102, Becton Dickinson, Cockeysville, MD; Zak et al. 1993). Each unit contained an air-tight upper and lower compartment, separated by a perforated platform accessed through butyl rubber septa. Approximately 0.135 g root and 13.5 g of experimental soil mix were placed in the upper compartment on glass-fiber filter circles. The soil mix consisted of 99% (w/w) homogenized beach sand (Misery Bay, MI) and 1% Kalkaska A-horizon from a forest adjacent to the open-top chamber facility. The incubation was started by flushing the units with $CO_2$-free air, adding 10 ml deionized water, and placing them in an incubator in the dark at 25°C.

At each harvest, microbially respired $CO_2$ was sampled with a gas-tight syringe from the head space of the microlysimeters and immediately analyzed on a Tracor 540 gas chromatograph (San Jose, CA) equipped with a thermal conductivity detector. Standard gas (2.01% $CO_2$ in air) traceable to National Institute of Standards and Technology (NIST) was used to develop standard curves and check standards were run every ten samples. The soil in each unit was then extracted with 50 ml 0.01 M $CaCl_2$ solution which was collected in the bottom half of the units, passed through 0.45 µm filters, acidified to pH 2.0–3.0 with HCl, and analyzed for dissolved organic carbon (DOC) on a Shimadzu TOC5000A total organic carbon analyzer (Wooddale, IL). Potassium hydrogen phthalate (KHP) was used to develop a standard curve and run check standards every ten samples. All measurements of microbially evolved $CO_2$ and DOC were adjusted by subtracting amounts derived from the soil-only blanks. Remaining root material was separated from the soil by hand and lyophilized. A sub-sample of remaining fine root biomass from each microlysimeter was combusted in a muffle furnace (450°C for 8 h) to correct for mineral soil contamination and ash content.

### Fine root chemistry and soil ergosterol

Biochemical composition was determined on fine roots harvested from the field to assess biochemical quality at the beginning of the laboratory incubation. Lyophilized root samples were analyzed for non-structural carbohydrates (starch and soluble sugars), soluble phenolics, condensed tannins, and lignin. Concentrations of ergosterol, N, and C/N ratios were also determined. Samples (25 mg) were extracted for soluble sugars with methanol:chloroform:water. Starch in the pellet was then hydrolyzed by perchloric acid digestion (Tissue and Wright 1995). All resulting reducing sugars were determined colorimetrically using the phenol-sulfuric acid method (Dubois et al. 1956) with absorbance at 490 nm and a standard curve constructed from d-glucose. Soluble phenolics were assayed according to Booker et al. (1996). After reacting samples with Folin–Ciocalteu reagent and $NaCO_3$, absorbance was measured at 724 nm, and quantified using a standard curve prepared from catechin. Condensed tannins were determined using the acid-butanol assay (Porter et al. 1986; Hagerman and Butler 1989; Lindroth 1996 personal communication). Samples (35 mg) were extracted in acetone–ascorbic acid, and the extracts reacted with *N*-butanol activated with HCl and ferric ammonium sulfate, and then heated at 90°C for 50 min. Absorbance of extracts was read at 550 nm and compared to standards prepared from roots of each species following the method of Booker et al. (1996). All colorimetric methods were run on a Beckman DU 640 spectrophotometer (Fullerton, CA). Lignin was determined directly as the acid-insoluble residue after samples (50 mg) were extracted with phenol:acetic acid:water and dilute $H_2SO_4$ (5%) to remove confounding low molecular weight phenolics, followed by digestion in concentrated (72%) $H_2SO_4$ (Booker et al. 1996). To assess the fungal content of roots and soil, ergosterol was determined by HPLC following the methodology of Ekblad et al. (1998). N and C concentrations were determined on samples (3–5 mg) using a Carlo Erba NA 1500 Series II elemental analyzer (Beverly, MA).

### Statistical analyses

Effects of atmospheric $CO_2$ and soil resource availability on fine root biomass, tissue chemistry, and soil ergosterol were tested using two-way ANOVA for split-plot randomized complete block design (Proc GLM, Statistical Analysis System, version 6.12, SAS Institute, Cary, NC). The atmospheric $CO_2$ and soil resource availability treatments were treated as main effects and species was considered a split-plot factor. Inspection of residuals and normal probability plots indicated the need to log transform data before analysis to normalize variance across treatments (Sokal and Rolhf 1995). Effects of the treatments on measures of decomposition (microbial respiration, DOC) were evaluated by ANOVA of response variables over the course of the incubation (Steel and Torrie 1980; Schmidt 2000). Treatment effects were considered significant if $P \leq 0.05$, and marginally significant if $0.05 < P < 0.10$.

## Results

### Fine root biomass

Sugar maple fine root biomass (< 0.5 mm diameter) was much greater than that of aspen ($P = 0.002$), averaging 128.2 (10.5) g per chamber (mean (SE)) compared to 88.2 (6.7) g per chamber, respectively, across the atmospheric $CO_2$ and soil resource availability treatments (Fig. 1). This is noteworthy in that the aspen trees were much larger by the end of the experiment, with total root biomass averaging 1264.6 g (101.9) in aspen chamber sections compared to 439.7 g (34.1) in maple chamber sections (data not shown). The allocation of biomass to fine roots was much greater in the young sugar maple trees compared to aspen ($P < 0.001$). Averaged over $CO_2$ and soil resource treatments, sugar maple allocated 30.7% (2.1) of total root biomass to fine roots compared to 7.2% (0.2) in trembling aspen. This pattern of allocation was insensitive to the atmospheric $CO_2$ and soil resource treatments ($P > 0.05$) in sugar maple (Fig. 1). In aspen, a significant $CO_2$×N interaction ($P = 0.021$) was caused by a 62% reduction in biomass allocation to fine

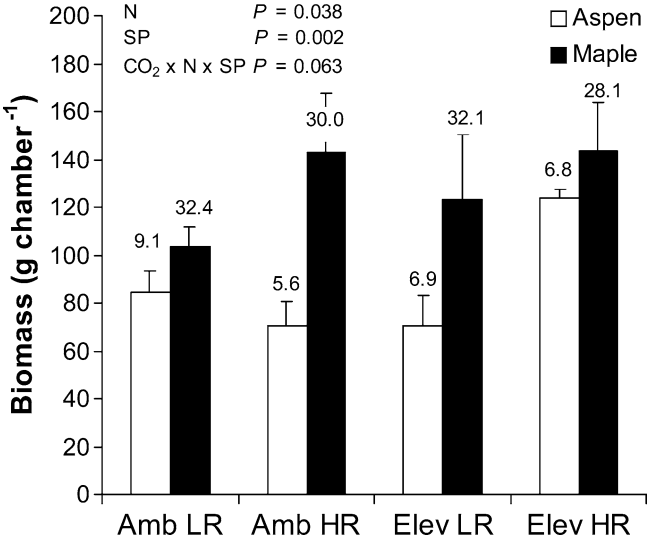


**Fig. 1** Fine root biomass (< 0.5 mm diameter) of trembling aspen and sugar maple produced after 2.5 growing seasons under varying atmospheric $CO_2$ and soil resource availability at the University of Michigan Biological Station. Values are means of dry weight scaled to the volume of soil in each chamber section ($N = 4$) and error bars are one standard error. Values over bars are the fraction (%) of total root biomass allocated to fine roots. "*Amb*", "*Elev*" denote ambient and elevated $CO_2$ treatments, respectively; "*LR*", "*HR*" denote the low and high soil resource availability treatments, respectively

**Table** 1 Means (standard error) and statistical significance (*P*-values) of chemical parameters of trembling aspen and sugar maple fine roots after 2.5 growing seasons under varying atmospheric $CO_2$ and soil resource availability at the University of Michigan Biological Station. Units are mg $g^{-1}$ except for C/N which is dimensionless, and ergosterol which is μg $g^{-1}$

| | N | C/N | Soluble sugars | Starch | Soluble phenolics | Cond. tannins | Lignin | Root ergosterol |
|---|---|---|---|---|---|---|---|---|
| *Treatment* | | | | | | | | |
| Amb LR Aspen | 13.82 | 27.66 | 38.95 | 29.22 | 64.44 | 7.05 | 325.60 | 81.76 |
| | (0.57) | (0.99) | (4.55) | (0.32) | (7.92) | (0.61) | (44.80) | (19.48) |
| Amb HR Aspen | 16.22 | 22.56 | 26.12 | 28.20 | 53.61 | 4.32 | 365.87 | 163.71 |
| | (0.79) | (0.59) | (3.33) | (0.81) | (5.96) | (0.99) | (34.45) | (16.91) |
| Elev LR Aspen | 13.26 | 28.48 | 38.75 | 35.00 | 70.99 | 7.03 | 337.75 | 134.53 |
| | (0.59) | (1.00) | (2.91) | (1.50) | (5.63) | (2.11) | (20.45) | (12.95) |
| Elev HR Aspen | 16.78 | 23.92 | 28.10 | 27.37 | 55.50 | 5.33 | 346.82 | 120.89 |
| | (0.34) | (0.29) | (1.95) | (3.05) | (7.23) | (1.32 | (33.79) | (26.69) |
| Amb LR Maple | 14.06 | 28.46 | 41.10 | 25.00 | 63.78 | 13.92 | 322.10 | 53.09 |
| | (0.47) | (1.48) | (3.9) | (1.82) | (11.78) | (3.60) | (24.62) | (14.82) |
| Amb HR Maple | 16.57 | 23.42 | 39.12 | 28.15 | 64.23 | 13.27 | 345.57 | 90.57 |
| | (0.70) | (0.57) | (6.60) | (0.46) | (0.61) | (2.45) | (26.32) | (25.04) |
| Elev LR Maple | 13.72 | 28.22 | 45.70 | 26.47 | 82.31 | 17.11 | 278.12 | 68.84 |
| | (0.70) | (1.70) | (2.21) | (0.92) | (6.68) | (2.93) | (38.00) | (7.57) |
| Elev HR Maple | 15.42 | 25.30 | 31.77 | 26.32 | 70.80 | 14.95 | 281.05 | 88.31 |
| | (0.51) | (1.00) | (4.39) | (2.92) | (4.62) | (3.08) | (23.50) | (15.98) |
| *Source* | | | | | | | | |
| $CO_2$ | 0.044 | 0.099 | ns | ns | 0.013 | ns | 0.063 | ns |
| SR | 0.000 | 0.002 | 0.092 | ns | 0.012 | 0.088 | ns | 0.075 |
| $CO_2$×SR | ns | ns | ns | 0.070 | ns | ns | ns | 0.093 |
| SP | ns | ns | 0.014 | 0.027 | ns | 0.000 | 0.008 | 0.014 |
| $CO_2$×SP | ns | ns | ns | ns | ns | ns | 0.052 | ns |
| SR×SP | ns | ns | ns | 0.057 | ns | ns | ns | ns |
| $CO_2$×SR×SP | ns | ns | ns | ns | ns | ns | ns | ns |

ns—not statistically significant ($P > 0.05$). "$CO_2$", "SR", and "SP" denote the atmospheric $CO_2$, soil resource availability, and species experimental factors, respectively.

roots in response to increased soil resource availability at ambient, but not elevated [$CO_2$].

Fine root biomass responded to the elevated $CO_2$ and soil resource availability treatments differently for each species, as indicated by the marginally significant $CO_2$×N×species (SP) interaction (Fig. 1). At ambient [$CO_2$], fine root biomass in the aspen chamber sections decreased (−16%) at high soil resource availability, averaging 84.5 and 70.6 g in the low and high soil resources availability treatments respectively. A post hoc comparison of means test indicated this difference was not statistically significant. At elevated [$CO_2$], aspen fine root biomass increased with increased soil resource availability, averaging 70.2 and 124.0 g at low and high soil resource availability, respectively (+76%). Maple fine root biomass responded consistently to the experimental main effects with no apparent interaction. Root biomass averaged 113.2 g at low soil resources availability, and increased 26% to 143.3 g at high soil resources availability. At ambient [$CO_2$], maple fine root biomass averaged 123.2 and increased 8% to 133.4 g under elevated [$CO_2$].

## Fine root chemistry

Fine root tissue N concentration ranged from 13.2 to 16.7 mg $g^{-1}$, and was similar for both species (Table 1). Soil resource availability had the greatest impact on root N concentrations (main effect $P = 0.000$), which decreased an average of 18% in the high soil resources treatment compared to low soil resources. Elevated $CO_2$ caused a significant (main effect $P = 0.041$), but minor (1.4%) decrease in root N concentration. The C/N ratio was consistent between species and was influenced mainly by soil resource availability (main effect $P = 0.002$). Root C/N ratio averaged 28.2 at low soil resource availability and decreased 15% to 23.8 at high soil resource availability. Soluble sugars ranged from 26.1 to 45.7 mg $g^{-1}$ and were most strongly affected by species (main effect $P = 0.014$), with sugar maple having on average 22% higher concentration than aspen for a given $CO_2$ and N treatment. Soil resource availability had a marginally significant effect on soluble sugars (main effect $P = 0.092$), with concentrations decreasing at high soil resource availability. Root starch concentrations ranged from 25.0 to 35.0 mg $g^{-1}$ . On average, root starch concentrations were slightly higher for aspen compared to maple ($P = 0.027$), and subject to both $CO_2$×N and N×SP interactions (Table 1). In aspen, root starch concentrations decreased at high soil resource availability under elevated [$CO_2$], but remained constant across soil resource availability treatments at ambient [$CO_2$]. In maple, root starch concentrations were more or less uniform across levels of atmospheric [$CO_2$] and soil resource availability. Concentrations of soluble phenolics ranged from 53.6 to 82.3 mg $g^{-1}$ and were similar for the two species. Interestingly, elevated [$CO_2$]

caused an average 13% increase in soluble phenolics ($P = 0.013$), whereas increased soil resource availability caused a 13% decrease ($P = 0.012$). Condensed tannins concentrations were lower in aspen compared to maple, averaging 5.9 and 14.8 mg $g^{-1}$, respectively. There appeared to be a trend towards reduced condensed tannins in aspen at high soil resource availability ($P = 0.088$), and the effect of the atmospheric $CO_2$ treatment was not statistically significant. Lignin concentrations ranged from 278.1 to 365.8 mg $g^{-1}$, and were on average 13% higher in aspen compared to maple across $CO_2$ and soil resource availability treatments ($P = 0.008$). The significant $CO_2$×SP interaction was due to 1 and 16% reductions in lignin concentration at elevated [$CO_2$] in aspen and maple roots, respectively. Finally, root ergosterol concentrations were most strongly influenced by species ($P = 0.014$), averaging 125.2 μg $g^{-1}$ in aspen and 75.2 μg $g^{-1}$ in maple. Soil resource availability and atmospheric [$CO_2$] marginally influenced root ergosterol, but responses were inconsistent.

## Microbial respiration and soil solution DOC

During the first 14-day of the incubation microbial respiration resulted in the accumulation of approximately 2.67 mg C in the headspace of the microlysimeters (Fig. 2a). Microbial respiration declined steadily over the 98-day incubation, reaching a level of 0.50 mg C evolved per 14-day period by the end of the experiment, or 18% of initial rates. Repeated measures analysis revealed that neither species nor the $CO_2$ and soil resource availability treatments had statistically significant effects on rates of microbial respiration over the course of the incubation (Schmidt 2000). Similarly, DOC production was relatively high early in the incubation with average concentrations of 4.9 mg $l^{-1}$, which declined to 0.16 mg $l^{-1}$ by the end of the experiment (Fig. 2b). As with microbial respiration, neither species nor the $CO_2$ and soil resource availability treatments had statistically significant effects on rates of DOC production during the course of the incubation (Schmidt 2000).

## Soil ergosterol

Concentrations of ergosterol in the experimental soil mix, which was largely composed of sand, were low, averaging 0.19 μg $g^{-1}$ (Fig. 3). Concentrations declined over the 98-day incubation to approximately 0.075 μg $g^{-1}$, close to the limit of detection. Ergosterol in soil associated with decomposing aspen fine roots was

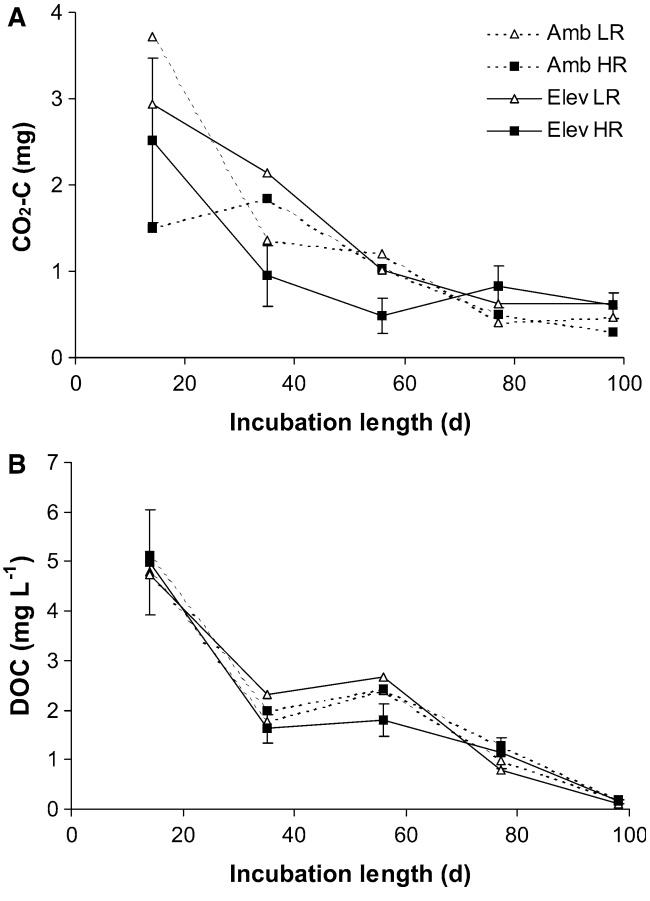


**Fig. 2** Microbially respired $CO_2$-C (**a**) and dissolved organic carbon (**b**) from the 98-day incubation of fine roots from trembling aspen and sugar maple produced after 2.5 growing seasons under varying atmospheric $CO_2$ and soil resource availability at the University of Michigan Biological Station. Values were averaged over the non-significant factor species ($N = 8$), and *error bars* are one standard error (shown on one treatment only for clarity). Treatment legend is the same as Fig. 1. Data adapted from Schmidt (2000)

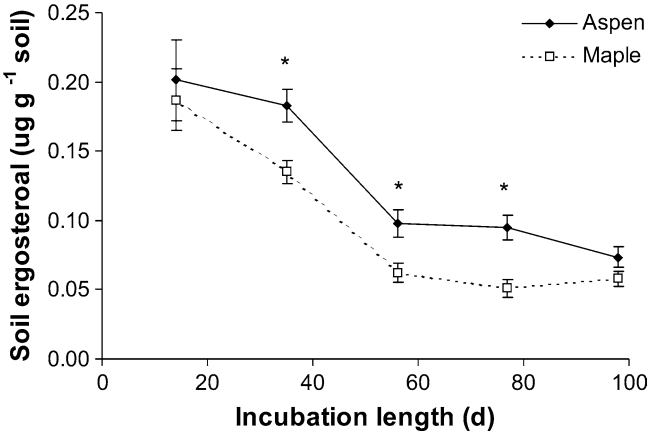


**Fig. 3** Soil ergosterol content in the experimental soil mix from the 98-day laboratory incubation of trembling aspen and sugar maple fine roots produced after 2.5 growing seasons under varying atmospheric $CO_2$ and soil resource availability at the University of Michigan Biological Station. Values are averaged over the non-significant factors of atmospheric $CO_2$ and soil resource availability ($N = 16$) and *error bars* are one standard error (shown on one treatment only for clarity). *Denotes significant at $P \leq 0.05$. Data adapted from Schmidt (2000)

consistently higher than in maple and this difference was statistically significant on three of the five harvest dates. The relative abundance of soil ergosterol between the two species was consistent with differences in live root ergosterol concentrations, and the amount of microbially respired $CO_2$. The atmospheric $CO_2$ and soil resource availability treatments did not affect soil ergosterol for the duration of the incubation (Schmidt 2000).

## Discussion

We hypothesized that the growth-dominated trembling aspen would respond to increased C availability (elevated [$CO_2$] and/or low soil resources) primarily by increasing root growth, whereas the differentiation-dominated sugar maple would respond through changes in root biochemistry (e.g., increased production of C-based secondary compounds). A second hypothesis was that changes in root biochemistry (C/N, soluble phenolics, tannins, lignin) would alter the functioning of soil microbial communities.

### Root growth

Due to different biomass allocation patterns of the two species, the standing crop of fine roots was much greater for sugar maple compared to trembling aspen, even though the aspen trees were much larger by the end of the experiment. King et al. (1999a, 1999b) demonstrated that coarse root biomass (all roots $>1$ mm diameter including the tap or heart root) scales well with shoot biomass in young (trembling aspen) and mature (loblolly pine) trees. Hence, the increased partitioning to fine roots relative to coarse roots in sugar maple observed here also represents greater allocation to fine roots relative to shoots. The long-lived, very shade-tolerant sugar maple exhibits slow aboveground growth in favor of forming well-established root system that enables it to tolerate herbivory while in the forest understory, and meet a relatively high demand for soil resources (Godman et al. 1990). The short-lived, highly shade-intolerant trembling aspen exhibits rapid above-ground growth in response to competition for light at the expense of maintaining a large fine root system (Perala 1990).

Fine root growth responses of trembling aspen and sugar maple to experimentally altered C and N availability also support their characterization as growth-dominated and differentiation-dominated species, respectively. Trembling aspen fine root biomass increased 76% in response to elevated [$CO_2$], but only under conditions of high soil resource availability. Plants were C-limited and did not increase growth in response to greater soil resource-availability unless C availability increased. These results are consistent with previous studies that used similar methodology; that is, open-top chambers and open-bottom root boxes filled with similar experimental soil mixes. Pregitzer et al. (1995) exposed five clones of *Populus*× *euramericana* to varying soil resource availability and elevated [$CO_2$] for 158-day and reported a small non-significant, decrease in fine root biomass at high soil resource availability. Consistent with the current study, they reported a 70% increase in fine root biomass due to elevated [$CO_2$] at high soil resources. Kubiske et al. (1998) exposed the same trembling aspen clones as used in our study to elevated [$CO_2$] and varying soil resource availability for 150-day. Fine root length standing crop increased approximately 45% due to elevated [$CO_2$] at high soil resource availability, but did not respond to elevated [$CO_2$] at low soil resource availability (Kubiske et al. 1998).

In the current study, the 62% decrease in biomass partitioning to aspen fine roots under high soil resources at ambient [$CO_2$] is consistent with the growth-dominated life history of the species. Under conditions of low C availability, biomass was allocated preferentially to shoots to increase C assimilation relative to N acquisition. These results are consistent with Pregitzer et al. (1995), who reported decreased partitioning to fine roots in *Populus* relative to foliage with increased soil resource availability. In contrast to trembling aspen, the differentiation-dominated sugar maple exhibited smaller growth responses to increased resource availability, and greater response to soil resource availability rather than C availability. In response to higher soil resource availability, fine root biomass increased by 26% with no shifts in partitioning (e.g., the plants simply grew larger), and only increased modestly ($+8$ %) under elevated [$CO_2$].

### Root chemistry

We predicted that conditions of enhanced C availability (relative to N) would stimulate production of non-structural carbohydrates and subsequently carbon-based secondary defensive compounds (CBSCs), especially in differentiation-dominated species (Herms and Mattson 1992; Peñuelas et al. 2002). Hence, in our experiment, we expected to observe significant shifts in sugar maple fine root biochemical composition, and little or no change in that of trembling aspen. Consistent with a recent review of plant secondary metabolism (Koricheva et al. 1998) we found good correspondence between model predictions and plant responses for some, but not all, constituents and the two species did not always respond as expected.

In the current study, highest C/N ratio occurred under conditions of highest relative C availability (elevated [$CO_2$], low soil resources), whereas lowest C/N ratio occurred at lowest relative C availability (ambient [$CO_2$], high soil resources). This pattern of response to the experimental treatments is consistent with that of leaf litter of the same plants (King et al. 2001a, 2001b), so

plant biochemical responses (at least C/N ratio) to altered resource availability appear to be uniform within the plant. Nitrogen concentrations and C/N ratio were similar between the two species, as were responses to C and soil resource availability. Relative to leaf litter of these same plants, fine root C/N ratio was much lower than in sugar maple, which ranged from 44 to 87 (King et al. 2001b), and similar to aspen, which ranged from 21 to 23 (King et al. 2001a). It was noted that the low C/N ratio of the aspen leaf litter in that study could have been an artifact of growth within the open-top chambers, as C/N ratio of wild aspen litter has been reported to range from 54 to 78 (King et al. 2001a). Although the evidence is limited, nutrient retranslocation appears to be minimal in senescing fine roots (Nambiar 1987; Gordon and Jackson 2000), so litter derived from fine root turnover appears to be of much higher "quality" as a substrate for microbial metabolism compared to leaf litter.

Fine root non-structural carbohydrates responded as predicted to altered soil resource availability, but were less responsive, in general, to elevated atmospheric [$CO_2$]. Soluble sugar concentrations were higher in sugar maple fine roots compared to aspen, and generally declined with greater N availability, as expected. Starch and soluble sugars concentrations were low and generally unresponsive to elevated [$CO_2$], making correction for non-structural carbohydrates less of an issue with fine roots than it is for foliage (Peñuelas et al. 2002). Starch concentrations were similar between species, and declined in response to greater soil resource availability at elevated [$CO_2$] in aspen, but not sugar maple. This is consistent with the growth response in that under increased C availability aspen was able to produce more root biomass at high soil resources, which decreased storage carbohydrate (e.g., starch). In sugar maple, starch concentrations appeared to be uncorrelated to growth responses.

Soluble phenolics, condensed tannins, and lignin are all synthesized from simple carbohydrates via the shikimic acid pathway and are therefore directly linked to C assimilation and metabolism of the plant (Taiz and Zeiger 1991). Accordingly, we would expect concentrations of these compounds to increase with increased relative C availability, especially in differentiation-dominated species. Further, these compounds are thought to play important roles in defense from insect herbivores (Lindroth 1996), have anti-fungal and anti-bacterial properties (Taiz and Zeiger 1991), and control organic matter decomposition (Berg 1984; Mellillo et al. 1989). Concentrations of soluble phenolics were similar for both species, and responded as hypothesized, increasing in response to elevated [$CO_2$] and decreasing at high soil resource availability. Concentrations of condensed tannins were much higher in sugar maple fine roots compared to trembling aspen, and decreased in response to high soil resource availability, but were unresponsive to elevated [$CO_2$]. In general, concentrations of soluble phenolics and condensed tannins in fine roots were very low and differences between treatments were small in absolute magnitude. The effect of elevated [$CO_2$] was less consistent and often depended on soil resource availability. Interestingly, lignin concentrations were similar in both species, averaging 340 mg $g^{-1}$, except that there was a 16% decrease in sugar maple roots under elevated [$CO_2$].

There are few published reports on fine root biochemical responses to elevated [$CO_2$]. Consistent with our study, Parsons et al. (2003) reported decreased N concentration (increased C/N ratio) in roots $<2$ mm diameter of *Betula papyrifera* and *Acer saccharum* grown at elevated [$CO_2$] (70 Pa). Condensed tannins increased slightly in birch under elevated [$CO_2$], but not in maple (Parsons et al. 2003). In contrast to our study, Parsons et al. (2003) found no change in lignin concentration due to elevated [$CO_2$] in either species. Blaschke et al. (2002) reported increased lignin concentration in leaves and roots of *Fagus sylvatica* seedlings grown at low soil resource availability under elevated [$CO_2$] (70 Pa) for three to four growing seasons. However, these authors found that at high soil resource availability lignin concentration was unaffected or decreased with elevated [$CO_2$]. Runion et al. (1999) also reported decreased lignin concentration in fine roots of *Pinus palustris* under elevated [$CO_2$]. Taken together, these results support our hypothesis that elevated atmospheric [$CO_2$] may alter fine root chemistry, but soil resource (N) availability will have dominant influence on fine root tissue secondary chemistry (Lambers 1993).

A long-standing hypothesis has been that increased C availability due to elevated atmospheric [$CO_2$] could stimulate mycorrhizal colonization of fine roots (O'Neill et al. 1987; Lewis et al. 1994; Hodge 1996). This has important implications not only for nutrient acquisition of the plant, but also for fine root litter inputs to soil. Mycorrhizal colonization has been shown to dramatically increase fine root longevity, decreasing turnover (King et al. 2002), and mycorrhizal root litter will contain chitin and other constituents of the fungal symbiont. Both factors likely affect microbial metabolism of fine root litter. We quantified ergosterol in fine roots as an index of mycorrhizal colonization. This membrane sterol is specific to fungi and is an indicator of live fungal biomass (Frey et al. 1994; Djajakirana et al. 1996; Stahl and Parkin 1996; Ekblad et al. 1998). Although the reason for the apparent difference in mycorrhizal colonization between aspen and sugar maple in this study is not known (it may be related to the VAM vs ectomycorrhizal nature of the symbiosis for aspen and maple, respectively), the higher ergosterol concentrations in aspen suggest that aspen ecosystems may experience greater inputs of root-fungal detritus to soil. Consistent with earlier studies (Lewis et al. 1994; Sanders et al. 1998; but see Lukac et al. 2003), we found no stimulation of colonization of fine roots by mycorrhizae under elevated [$CO_2$], as estimated by the ergosterol analysis. This suggests that fungal-root inputs to soil are primarily controlled by fine root turnover responses to al-

tered resource availability. A continuing research challenge is to characterize how elevated atmospheric [$CO_2$] affects turnover of mycorrhizal compared to non-mycorrhizal roots.

### Root decomposition

Although concentrations of root tissue constituents that affect early (N, total non-structural carbohydrates), mid (soluble phenolics, condensed tannins), and late (lignin) stages of decomposition dynamics changed in response to elevated $CO_2$ and soil resource availability, we detected no change in the functioning of soil microbial communities during the 98-day fine root incubation. We attribute this to the generally small absolute changes in root chemistry under the experimental treatments, supporting the conclusion of Heyworth et al. (1998), that ecosystem processes that are affected by secondary metabolites will be little altered under elevated [$CO_2$]. Our results are consistent with leaf litter decomposition experiments from the same plants (King et al. 2001a, 2001b), where there were small changes in some biochemical constituents but no change in rates of microbial respiration or production of DOC due to elevated [$CO_2$] over the course of 111-day incubations. Our results also agree with a meta-analysis of the elevated $CO_2$-decomposition literature (Norby et al. 2001) which concluded that litter produced under elevated [$CO_2$] generally had slightly lower concentrations of N and slightly higher concentrations of lignin, but these changes in chemistry had no effect on microbial respiration or rates of mass loss. No change in DOC concentrations in soil solution from northern hardwood ecosystems developing under elevated atmospheric [$CO_2$] were observed after 2 years in a FACE experiment (King et al. 2001c) or a 4-year open-top chamber study of spruce and beech model communities (Hagedorn et al. 2002).

Recent reports indicate that the activity of soil microbial extra-cellular enzymes that degrade cellulose (cellobiohydrolase) and chitin (*N*-acetylglucosaminidase) has increased in early- but not late-successional north temperate forest communities developing under elevated [$CO_2$] (Larson et al. 2002; Phillips et al. 2002). Although we did not quantify cellulose in the current study, the lack of a change in specific rates of microbial respiration ($CO_2$ evolved $g^{-1}$ root litter) or in mycorrhizal colonization (root ergosterol content) under elevated [$CO_2$] suggests the observed changes in extracellular enzyme activity are due to quantitative changes in root litter inputs rather than changes in biochemistry. Therefore, changes in root litter inputs in response to altered environmental conditions (Pregitzer et al. 1995, 2000) can be expected to be proportionately greater in more dynamic systems. Further, the content of ergosterol in soil was consistently higher in microlysimeters with aspen fine roots as the substrate compared to sugar maple, and was not affected by the atmospheric $CO_2$ or soil resource availability treatments. This indicates that the saprophytic fungal communities decomposing the roots differed in biomass between species, but this difference was not great enough to influence microbial metabolism of the substrate, nor was microbial metabolism affected by the rather small changes in chemical composition of roots produced at varying levels of C and N availability.

## Conclusions

It should be kept in mind that the current study was conducted with young trees under highly controlled conditions. However, trembling aspen and sugar maple often establish and compete on disturbed sites in the Upper Great Lakes region, so our model communities provided reasonable analogs to natural situations in terms of soil resources, tree density, species composition, soil microbial communities, etc. We conclude that growth responses of trembling aspen and sugar maple to altered resource availability are consistent with characterization of these species as growth-dominated and differentiation-dominated, respectively. Sugar maple fine root biomass increased in response to greater soil resource availability, but not greater C availability (elevated $CO_2$), whereas aspen showed increased root growth only under conditions of higher C availability. Soil resource availability had a stronger influence on root biochemistry than did atmospheric $CO_2$, and was inversely related to non-structural carbohydrates and CBSC concentrations. Absolute changes in fine root chemistry in response to altered resource availability were small and did not influence specific rates of microbial metabolism. Therefore, soil C cycling will most likely be influenced by fine root production and turnover responses to the changing environment rather than changes in root chemistry in trembling aspen and sugar maple ecosystems.

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