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CO_2 enrichment and warming of the atmosphere enhance both productivity and mortality of maple tree fine roots

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Summary

• Fine roots are the key link for plant water and nutrient uptake, soil carbon (C) input and soil microbial activity in forest ecosystems, and play a critical role in regulating ecosystem C balance and its response to global change.

• Red maple (*Acer rubrum*) and sugar maple (*Acer saccharum*) seedlings were grown for four growing seasons in open-top chambers and exposed to ambient or elevated carbon dioxide concentration [CO_2] in combination with ambient or elevated temperature. Fine-root production and mortality were monitored using minirhizotrons, and root biomass was determined from soil cores.

• Both elevated [CO_2] and temperature significantly enhanced production and mortality of fine roots during spring and summer of 1996. At the end of the experiment in September 1997, fine root biomass was significantly lower in elevated temperature chambers, but there were no effects of elevated [CO_2] or the interactions between elevated [CO_2] and temperature.

• Deciduous trees have dynamic root systems, and their activity can be enhanced by CO_2 enrichment and climatic warming. Static measures of root response, such as soil core data, obscure the dynamic nature, which is critical for understanding the response of forest C cycling to global change.

Key words: red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), elevated CO_2, fine roots, root production, root mortality, temperature.

Introduction

Elevated atmospheric carbon dioxide concentration [CO_2] and rising global mean temperature are expected to influence ecosystem carbon (C) balance, which feeds back to global climate change. Given their importance in global C storage and exchange, forest ecosystems may exert significant impacts on global C budgets (Schlesinger, 1997). As the key link for plant water and nutrient uptake, soil C input, and soil microbial activity in forest ecosystems (Norby, 1994), fine roots play a critical role in regulating ecosystem C balance and sequestration of atmospheric CO_2 (Jackson *et al.*, 1997). In forest trees, although root biomass accounts for less than 20% of total biomass, more than 50% of the C acquired annually by plants can be allocated below ground (George & Marschner, 1996). Moreover, fine-root productivity and mortality will probably be sensitive to elevated atmospheric [CO_2] and rising temperature because fine roots have a high turnover rate (Hendrick & Pregitzer, 1992; Eissenstat *et al.*, 2000; Gill & Jackson, 2000).

Elevated [CO_2] has been shown to increase (Berntson & Bazzaz, 1997; Fitter *et al.*, 1999; Pregitzer *et al.*, 2000b) or decrease (Kandeler *et al.*, 1998) root productivity and biomass. The inconsistency could partly result from that the effect of elevated [CO_2] on fine roots varies with soil nitrogen (N) availability (Pregitzer *et al.*, 2000b) and species (Berntson & Bazzaz, 1996b). Both root growth and mortality are related to soil or air temperature (Kasper & Bland, 1992; Forbes *et al.*, 1997; McMichael & Burke, 1998; King *et al.*, 1999; Tierney *et al.*, 2003). Across the globe, turnover rates of fine roots increase exponentially with mean annual temperature in forests and grasslands (Gill & Jackson, 2000). However, our understanding of the interactive effects of elevated [CO_2] and rising temperature on tree fine roots is limited, partly because of very

few experiments with both [CO_2] and temperature treatments (Kandeler *et al.*, 1998).

As part of a comprehensive project on the responses of maple trees to elevated [CO_2] and temperature (Edwards & Norby, 1998; Norby *et al.*, 2000, 2003; Norby & Luo, 2004), this study was conducted to examine the responses of fine roots in two deciduous tree species, red maple (*Acer rubrum*) and sugar maple (*Acer saccharum*), to elevated [CO_2] and air temperature. The specific objectives were to determine how elevated [CO_2] and temperature affect root production, mortality, and biomass, root morphology and root tissue quality.

Materials and Methods

Research site and experimental design

Research was conducted in open-top chambers (OTCs) at the Oak Ridge National Laboratory's Global Change Field Research Facility on the National Environmental Research Park in Oak Ridge, TN, USA (35°54′ N; 84°20′ W). The mean annual temperature is 14.3°C, mean annual precipitation is 1378 mm and the mean length of growing season is 185 d. Twelve OTCs were constructed on soils classified as Captina silt loam (fine-silty, siliceous, mesic Typic Fragiudult) with moderate-to-medium granular structure and medium internal drainage. The chambers were 3.0 m in diameter and 2.4 m high. An additional 1.2-m panel was installed at the beginning of the third growing season (1996) to accommodate the height growth of the seedlings. A randomized complete block design was used with four treatments (the factorial combinations of two CO_2 concentrations with two temperature regimes) in each of three blocks. The chambers were modified to operate at either ambient or 4°C above ambient air temperature, in combination with ambient or elevated (+300 p.p.m.) atmospheric CO_2 concentration (Norby *et al.*, 1997). Fans continuously pushed air through double-walled polyvinyl chloride chamber panels and out the open chamber tops at 0.6 m^3 s^{-1}. The airstream was conditioned by evaporative coolers (to maintain ambient temperature) and voltage-regulated electrical resistance duct heaters. A proportional-integral-differential feedback control system regulated the cooling and heating systems to maintain air temperature inside the chambers at +0.4°C (± 0.3°C) or +4.0°C (± 0.3°C) relative to ambient air outside the chamber. Soil temperature at 10 cm depth was increased by 1.2°C by the warmer air in the elevated temperature chambers (Edwards & Norby, 1998). A constant flow of pure CO_2 was introduced into the airstream entering the chambers; the flow rate was manually adjusted to maintain a constant differential with ambient air. The temperature treatments were maintained year-round for 3.5 yr (April 1994 to September 1997), but the CO_2 treatments were suspended during the winter (November–March).

One-year-old red maple (*A. rubrum* L.) and sugar maple (*A. saccharum* Marsh.) seedlings were planted directly into the soil within the chambers in spring 1994. The seedlings were obtained from a commercial nursery in central Tennessee, which used a local, open-collected seed source. A few additional seedlings were planted in spring 1995 to replace those that did not survive after the first planting, for a total of 10 plants of each species per chamber. The sides and tops of the OTCs were covered with 73% shade cloth to avoid unnatural levels of light to these shade tolerant maple seedlings.

Production and mortality of fine roots

Four minirhizotron tubes, constructed of cellulose acetate butyrate (Bartz Technology Coporation, Santa Barbara, CA, USA) and measuring 185 cm long by 5 cm diameter, were inserted into the ground at the angle of 60° from vertical in each chamber in the summer of 1995; installation was completed by August. Tubes were wrapped above the soil surface with black foam insulation, and the upper ends were sealed with rubber stoppers. Video images were collected with a BTC-2 minirhizotron camera with a Smucker handle (Bartz Technology, Santa Barbara, CA, USA). Individual frames (12.4 × 18.0 mm) on the videotape were digitized using ROOTS software (Michigan State University, Lansing, MI, USA). The length and width of each root segment were measured and the incremental growth, death, or disappearance recorded. Fine roots were coded into six classifications: new, white, brown, dead, missing and visible but not measurable due to the poor quality of the picture.

Physical disturbance of soil and roots by the installation of minirhizotron tubes could affect the calculation and explanation of the root productivity and mortality (Eissenstat *et al.*, 2000). In our experiment, the first and second sets of video images of roots were not taken until November 1995 and February 1996, 3 months and 6 months after installation and during a period when root activity is low. More intensive observations at about 2- wk intervals were made from May to July 1996. Thus, there was time for the tree roots to adjust to the physical disturbance. The data used in this study consist of measurements collected on individual root segments of maple trees from 8 November 1995 to 16 July 1996. Subsequent images could not be processed because they were obscured by moisture and a clay film on the tubes.

Fine root production for a time-period was calculated for each chamber as the total length of living roots on the date ending the period minus the total length of those same roots on the date beginning the period. Fine root mortality for a time-period was calculated for each chamber as the total length of roots classified as dead or missing on the date ending a period, but not classified as dead or missing on the previous date. The total length of roots in 360 minirhizotron frames (four tubes with 90 223-mm^2 frames each) was expressed as millimeters of roots per square meter of viewing area.

Total length production and mortality of fine roots were analysed with a two-way mixed model analysis of data for

repeated measures design using the Mixed Procedure of the Statistical Analysis System (SAS Institute, Cary, NC, USA) software.

Biomass, specific root length (SRL), and root C and N concentrations

At the end of the experiment (September 1997), all the trees were cut at ground level. Leaves were removed from stems and leaves and stems were dried and weighed. Above-ground biomass during the first 3 yr of the experiment was estimated from a regression between stem basal area and dry mass. Above-ground biomass at the end of the second growing season (1995), when the minirhizotron measurements began, are given in Norby *et al.* (2000), and final above-ground biomass is presented in Norby & Luo (2004). After the stems had been removed from the plots, five soil cores (4.8 cm diameter and 20 cm deep) were collected from each chamber and immediately placed in ice boxes and taken back to the laboratory and stored in a freezer. The final analysis was conducted at Michigan Technological University.

Roots greater than 0.5 mm diameter were hand-picked from each core before the cores were processed through the root washer. These roots were added back to the pool of roots recovered by the root washer. Fine roots (< 0.5 mm diameter) were separated with a hydropneumatic root washer (Smucker *et al.*, 1982). Each root was then carefully hand washed in deionized water and sorted according to the following diameter classes: < 0.5 mm, 0.5–1.0 mm, 1.0–2.0 mm, 2.0–5.0 mm, and > 5.0 mm. After sorting, each size class was oven-dried (65°C) for 36 h and weighed. The > 2.0 mm size classes were not well represented because of the size and the number of cores; therefore, they were not included in any statistical analysis.

Specific root length (g m^{-1}) was estimated for fine roots. Fine roots were hand-excavated from the OTCs. These samples were collected by species and were kept completely frozen until analysis. Individually, fine-root samples were unfrozen in deionized water and 15 root segments were randomly dissected. These 15 fine-root segments were pressed under glass, video-taped and digitized using the ROOTS program for length measurement (Hendrick & Pregitzer, 1992). Length was calculated by using the diagonal distance of the grid on which fine-root segments were placed for videotaping. The 15 fine-root segments were carefully removed from the glass and oven dried (65°C) to determine mass.

Nitrogen analyses were performed on two sets of root samples. First, N concentrations were determined for specific diameter classes from hand-picked roots to search for any differences in concentrations by diameter due to CO_2 or temperature treatments. Values were organized as chamber means (n = 2) with a treatment level (CO_2, temperature) and size classes. A second analysis was completed to determine N concentrations by species. The second analysis was performed on the fine roots from the hand-excavated samples for the SRL analysis. All roots were analysed for N concentrations with a Fison's CN elemental analyser. Three-way ANOVA was performed using SYSTAT.

Results

Both CO_2 and temperature main effects were statistically significant on total length production ($P < 0.01$ and $P < 0.05$, respectively), mortality ($P < 0.001$ and $P < 0.001$), and net production ($P < 0.01$ and $P < 0.05$) of fine roots during the period 8 November 1995 to 16 July 1996 (Fig. 1). Overall total length production of fine roots under elevated [CO_2] was 122% greater than that under ambient [CO_2]. Elevated temperature increased total length production by 265% compared with ambient temperature. Similar results were found in total length mortality. Overall total length mortality of fine roots under elevated [CO_2] was 137% higher than those under ambient CO_2. Elevated temperature enhanced total length mortality by 263% compared with ambient temperature. The effect of [CO_2] on total length production and mortality of fine roots was larger in elevated temperature, and the effect of temperature was larger in elevated [CO_2], but the CO_2 × temperature interaction was not statistically significant ($P > 0.05$).

Both fine root productivity and mortality generally followed the seasonal pattern of soil temperature, with much higher activity in spring and summer (Fig. 2). However, when there was a summer drought in June and July 1996, root productivity decreased substantially irrespective of soil temperature, whereas root mortality under elevated [CO_2] and temperature was much higher than in the other three treatments.

Both fine-root productivity and mortality exhibited exponential ($Y = a \times e^{bT}$) increases with soil temperature (Fig. 3). Similarly, Steele *et al.* (1997) also reported an exponential relationship between fine root production and soil temperature.

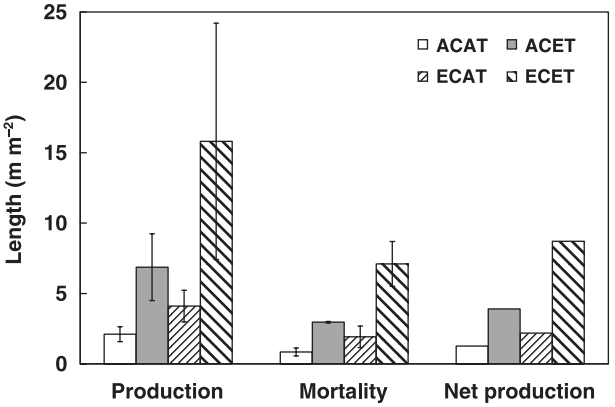

Fig. 1 Total length production and mortality (m m^{-2}; mean ± 1 SE) of fine roots (< 0.5 m diameter) from November 8 1995 to July 16 1996. ACAT, ambient [CO_2] and ambient temperature; ACET, ambient [CO_2] and elevated temperature; ECAT, elevated [CO_2] and ambient temperature; ECET, elevated [CO_2] and elevated temperature.

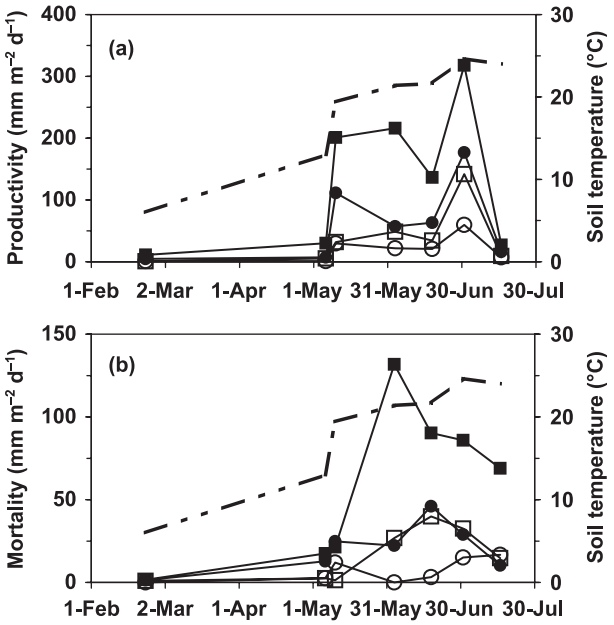

Fig. 2 Temporal variability of fine-root productivity (a) and mortality (b) during 1996 (mean ± 1 SE). Data are plotted at the end of each observation period; the first observation period began on 8 November of the preceding year. Open circles, ambient [CO_2] and ambient temperature; closed circles, ambient [CO_2] and elevated temperature; open squares, elevated [CO_2] and ambient temperature; closed squares, elevated [CO_2] and elevated temperature; dashed line, soil temperature.

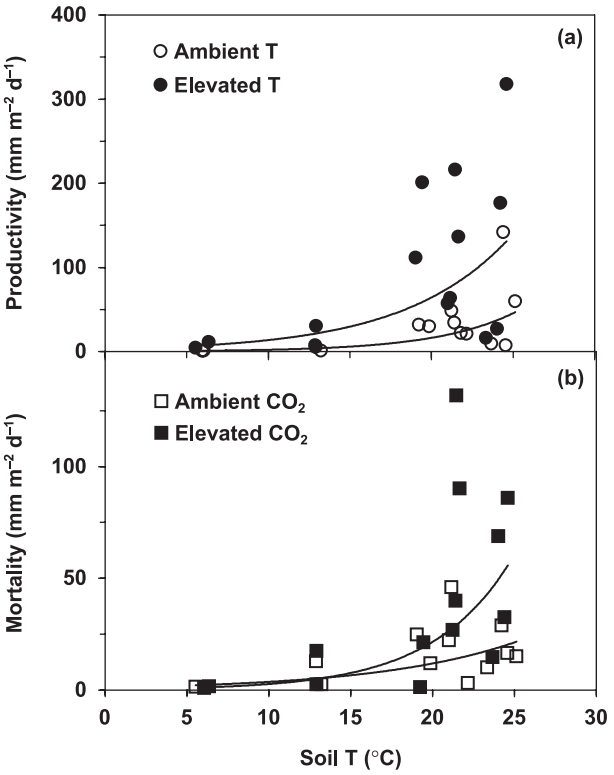

Fig. 3 Exponential relations of fine-root productivity (R_{prod}) and mortality (R_{mort}) with soil temperature (T). Each data point was the average values for the two levels of temperature or [CO_2] treatments during each observing period. Open circles, ambient temperature: $R_{prod} = 0.0269e^{0.205T}$, $R^2 = 0.6963$, $P < 0.001$. Closed circles, elevated temperature: $R_{prod} = 2.8869e^{0.155T}$, $R^2 = 0.525$, $P < 0.001$. Open squares, ambient [CO_2]: $R_{mort} = 1.154e^{0.117T}$, $R^2 = 0.422$, $P < 0.001$. Closed squares, elevated [CO_2]: $R_{mort} = 0.346e^{0.207T}$, $R^2 = 0.6097$, $P < 0.001$.

A literature review has shown that root turnover across different biomes in the world increased exponentially with mean annual temperature (Gill & Jackson, 2000). Based on the parameter of **b** in the exponential functions, we calculated temperature sensitivity ($Q_{10} = e^{10b}$) of fine-root productivity and mortality.

Both CO_2 and temperature differentially affected Q_{10} of fine-root productivity and mortality. It was found that CO_2 treatment had no effect on Q_{10} of fine-root productivity, therefore we pooled together the two CO_2 concentrations and only examined the temperature effect. By contrast, temperature treatment did not affect the Q_{10} of fine-root mortality, so we pooled together the two temperature levels for each CO_2 treatment. Temperature sensitivity of fine-root productivity decreased from 7.79 under ambient temperature to 4.72 under elevated temperature. Elevated [CO_2] increased Q_{10} of fine-root mortality from 3.21 to 7.90.

Across the seven sampling periods, fine-root mortality showed a positive linear correlation ($R^2 = 0.50$, $P < 0.05$) with fine-root productivity for the four treatments, which was consistent with the results of Berntson & Bazzaz (1996), suggesting proportional changes in fine-root mortality with productivity (i.e. large root systems have greater root mortality).

By the end of the experiment (September 1997), root biomass under elevated [CO_2] was not different from that under ambient [CO_2] across all size classes ($P > 0.05$). However, elevated temperature significantly decreased root biomass by 34%, 53% and 43% for size class < 0.5 mm ($P < 0.05$), 1.0–2.0 mm ($P < 0.05$), and < 2.0 mm (total roots, $P < 0.05$), respectively, but had no effects on size class 0.5–1.0 mm ($P > 0.05$) (Fig. 4). There were no interactive effects of CO_2 and temperature on root biomass ($P > 0.05$). Elevated [CO_2] compensated for the negative effects of elevated temperature on root biomass. Overall, the temperature-induced reduction in root biomass was 27% under elevated [CO_2], which is much less than 60% under ambient [CO_2].

Elevated temperature significantly decreased the ratio of fine-root biomass to aboveground not primary product (ANPP) by 36% ($P < 0.05$) and the ratio of fine-root biomass to leaf biomass by 45% ($P < 0.05$), marginally reduced the ratio of fine-root biomass to annual stem increment (29%, $P = 0.0922$), and had no significant effect on the ratio of fine-root biomass to above-ground biomass ($P > 0.10$) (Fig. 5). There were no significant effects of CO_2 or CO_2 × temperature interaction on these ratios.

Results of three-way ANOVA showed that species ($P < 0.05$), CO_2 ($P < 0.05$), and the interactions of CO_2 and temperature

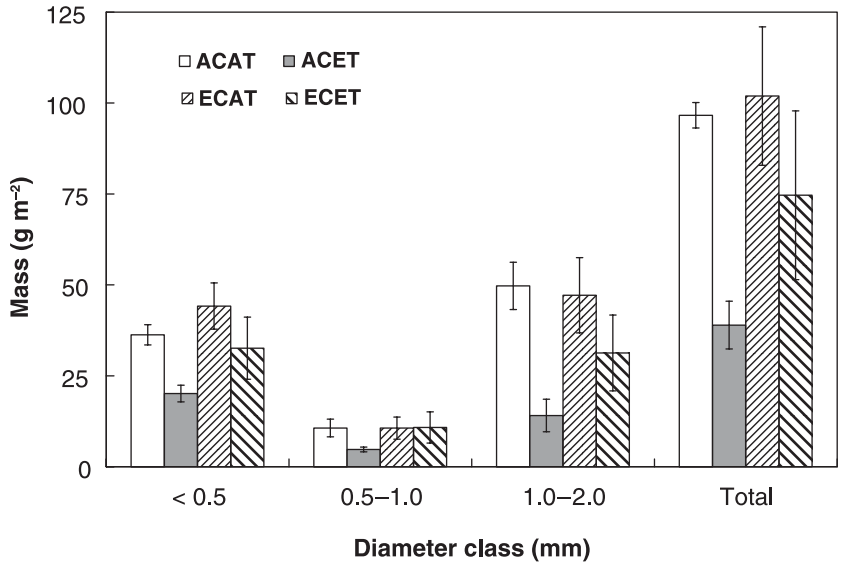

Fig. 4 Root biomass (g m^{-2}; mean ± 1 SE) for different diameter classes at the end of the fourth growing season (1997). ACAT, ambient [CO_2] and ambient temperature; ACET, ambient [CO_2] and elevated temperature; ECAT, elevated [CO_2] and ambient temperature; ECET, elevated [CO_2] and elevated temperature.

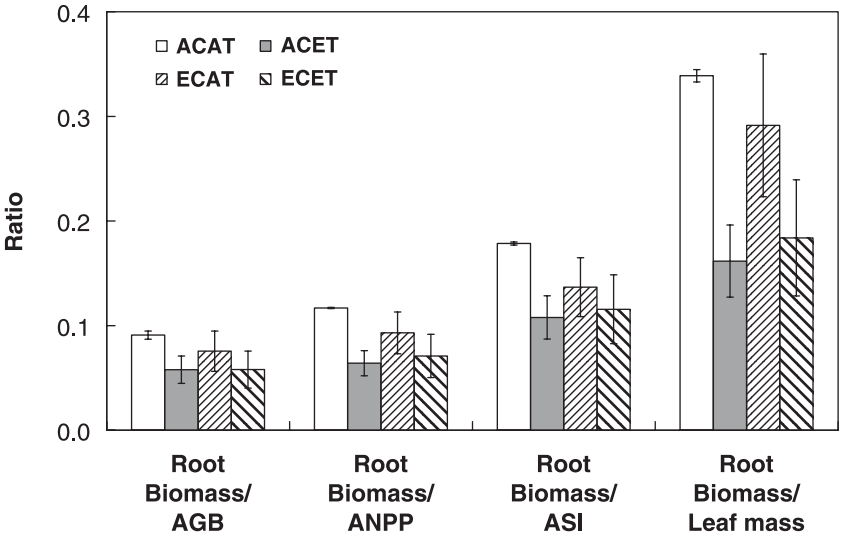

Fig. 5 The ratios (mean ± 1 SE) of root biomass (< 2.0 mm) to above-ground biomass (AGB), net primary production (NPP), annual stem increment (ASI), and leaf mass. ACAT, ambient [CO_2] and ambient temperature; ACET, ambient [CO_2] and elevated temperature; ECAT, elevated [CO_2] and ambient temperature; ECET, elevated [CO_2] and elevated temperature.

($P < 0.05$) had significant effects on specific root length (SRL) of fine roots, whereas elevated temperature did not affect SRL ($P > 0.05$, Fig. 6). On average, SRL of red maple (57.4 m g^{-1}) was 7.6% lower than that of sugar maple (53.4 m g^{-1}). Elevated [CO_2], on average, decreased SRL of fine roots by 9.7% and 6.4% for red maple and sugar maple, respectively. The interactions of elevated [CO_2] and CO_2 × temperature had significant effects of SRL. Elevated [CO_2] reduced SRL of red maple 16.1% and 3.9% at ambient and elevated temperature, respectively. Elevated [CO_2] reduced SRL of sugar maple by 17.6% at ambient temperature but increased it by 7% at elevated temperature.

Nitrogen concentration in fine roots (Fig. 7) was approximately 12 g kg^{-1}, except in red maple roots in ambient CO_2 and elevated temperature (ACET) which had a significantly higher concentration (15.6 g kg^{-1}). Hence, the effects of CO_2, temperature, and CO_2 × temperature were statistically significant ($P < 0.01$, 0.001, and 0.05, respectively), and that of species was marginally significant ($P < 0.10$).

Discussion

Effect of elevated [CO_2] on fine root production and mortality

The magnitude (122%) of the increase in fine-root production under elevated [CO_2] observed in our study was intermediate within the range (60–240%) reported for other tree species (Idso & Kimball, 1992; Norby *et al.*, 1995; Zak *et al.*, 1993; Pregitzer *et al.*, 1995; Rey & Jarvis, 1997; Crookshanks *et al.*,

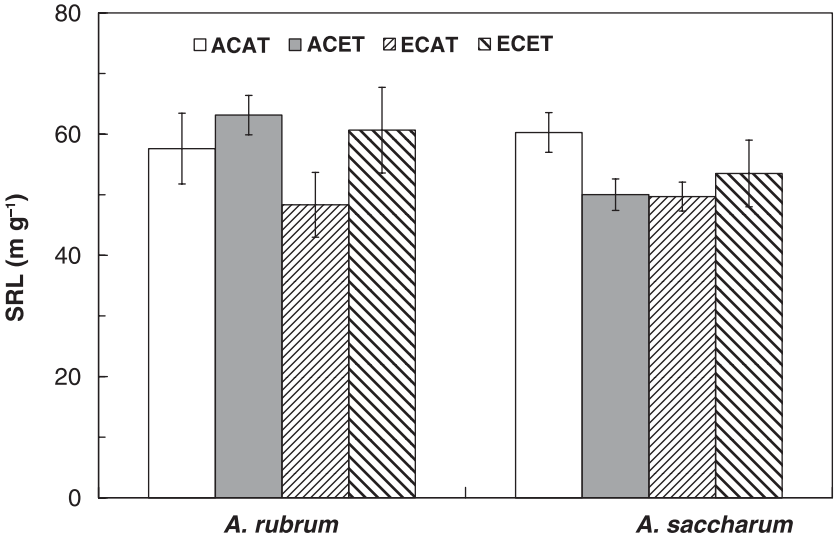

Fig. 6 Specific root length (SRL; mean ± 1 SE) of fine roots in *Acer rubrum*) and *Acer saccharum*. ACAT, ambient [CO_2] and ambient temperature; ACET, ambient [CO_2] and elevated temperature; ECAT, elevated [CO_2] and ambient temperature; ECET, elevated [CO_2] and elevated temperature.

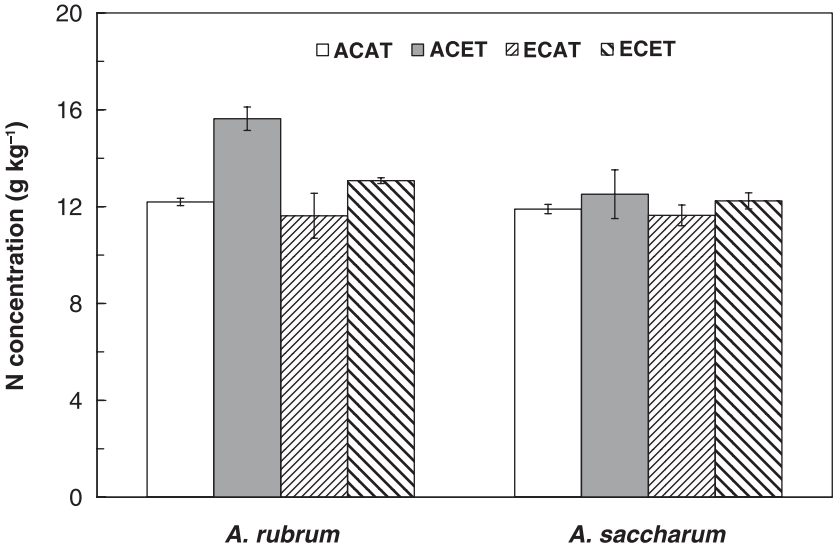

Fig. 7 Fine-root nitrogen (N) concentration (mean ± 1 SE) in *Acer rubrum* and *Acer saccharum*. ACAT, ambient [CO_2] and ambient temperature; ACET, ambient [CO_2] and elevated temperature; ECAT, elevated [CO_2] and ambient temperature; ECET, elevated [CO_2] and elevated temperature.

1998). The effects of elevated [CO_2] on fine-root mortality have been reported to be more variable than the effects on productivity. Increase (Berntson & Bazzaz, 1996; Fitter *et al.*, 1996, 1997), no change (Berntson & Bazzaz, 1996), and decrease (Day *et al.*, 1996) in root mortality under elevated [CO_2] have been observed. The inconsistency could be attributable to differences in plant species (Berntson & Bazzaz, 1996), soil N availability (Pregitzer *et al.*, 1995; King *et al.*, 2002), plant developmental patterns (Norby *et al.*, 2000) and vegetation types (Fitter *et al.*, 1996, 1997).

Effect of elevated temperature on fine root production and mortality

Our results showed that elevated temperature stimulated both length production and mortality of fine roots, which was consistent with those reported in other studies (Hendrick & Pregitzer, 1993, 1997; Forbes *et al.*, 1997; Fitter *et al.*, 1999; King *et al.*, 1999; Tierney *et al.*, 2003). Several potential mechanisms could explain the increase in fine-root production and mortality with higher temperature. First, maintenance respiration of plant roots increases exponentially with temperature (Johnson, 1990; Atkin *et al.*, 2000), resulting in greater root mortality (Gill & Jackson, 2000). Second, stimulated microbial activity at higher temperature could lead to increased net N mineralization and availability (Piatek & Allen, 1999; Zak *et al.*, 1999; Rustad *et al.*, 2001). The increased net N mineralization and availability can also lead to increased fine-root N concentration (Pregitzer *et al.*, 1998, 2002; King *et al.*, 2002), length extension, production and mortality (Pregitzer *et al.*, 1995; King *et al.*, 1999, 2002; Nadelhoffer, 2000; Pregitzer *et al.*, 2000a). Third, higher soil temperature could interact

with the concomitant changes in water availability (Kuhns *et al.*, 1985; Kramer & Boyer, 1995; Piatek & Allen, 1999). Fine-root mortality might be accelerated by decreases in soil moisture (Pregitzer *et al.*, 1993). Finally, pathogen and herbivore load could also increase in warmer soils, leading to higher root mortality (Eissenstat & Yanai, 1997).

Fine-root biomass

By the end of experiment (September 1997), elevated [CO_2] had no significant effect ($P > 0.05$) on fine-root biomass, which was not consistent with previous studies (Rogers *et al.*, 1994; Pregitzer *et al.*, 1995; Day *et al.*, 1996; Berntson & Bazzaz, 1997; Curtis & Wang, 1998; Pregitzer *et al.*, 2000b). It may be that elevated [CO_2] increases the rate at which root systems occupy the soil in a developing stand without increasing root mass in fully occupied soil. Elevated temperature, on the other hand, caused significant reductions in root biomass, which is similar to those found in other studies (Bassow *et al.*, 1994; Soussana *et al.*, 1996; Forbes *et al.*, 1997; Kandeler *et al.*, 1998). The response pattern of root biomass observed in this study was also supported by results of soil respiration (Edwards & Norby, 1998) and above-ground biomass production (Norby *et al.*, 2000; Norby & Luo, 2004) (i.e. lower soil respiration and above-ground biomass production under elevated temperature).

Elevated [CO_2] compensated for the negative effects of increased temperature on above-ground production and biomass observed in the same experiment (Norby *et al.*, 2000; Norby & Luo, 2004). In this study, reductions in root biomass due to elevated temperature were, on average, 27% under elevated [CO_2], and much less than that under ambient [CO_2] (60%, Fig. 4). A similar phenomenon was observed in *Betula populifolia*, *Betula alleghaniensis*, and *Acer pensylvanicum* (Bassow *et al.*, 1994). Elevated [CO_2] could (1) reduce evapotranspiration and increase soil moisture, leading to less water stress, (2) increase the competitive inhibition of oxygenation such that the relative stimulation of assimilation by elevated [CO_2] increases with temperature (Long, 1991), and (3) increase the temperature optimum for photosynthesis and release the heat stress at high temperature range (Long, 1991).

Comparison between root production observed with minirhizotrons and root biomass measured with soil cores

The final harvest data in 1997 appear to conflict with the with the minirhizotron observations in 1996, which showed net productivity increasing more in elevated [CO_2] and elevated temperature chambers than in ambient [CO_2] and temperature. After accounting for the initial root length in November 1995, the standing crop of root length was higher in elevated [CO_2] and elevated temperature in July 1996, whereas the 1997 harvest data indicated no effect of [CO_2] and a significant negative impact of temperature on root mass. A possibility is that the indirect estimate from minirhizotrons was measuring a different population of roots or otherwise in error. To compare the data sets, we estimated root mass in grams per square meter from the minirhizotron data by assuming a soil volume based on a depth of viewing field of 2 mm and dividing root length by SRL. Although there are a number of tenuous assumptions in this approach, the overall estimate of root biomass in July 1996 of 36 g m^{-2} was similar to the soil core data for roots < 1 mm diameter. Hence, we should assume the data sets are comparable and look for biological or environmental explanations for the apparent discrepancy in response to [CO_2] and temperature increases. There are several possible explanations.

First, summer droughts are probably responsible for the reductions of root biomass under elevated temperature. Meteorological data showed that there were summer drought periods in 1995 (1 July–30 July and 9 August–12 September), 1996 (13 June–12 July), and 1997 (21 August–22 September). Total precipitation during these periods (14.1, 25.8, 17.1 and 20.7 mm) were 89, 75, 86 and 78% lower than the long-term (50 yr) averages (133.0, 103.1, 124.7 and 92.6 mm) during the same periods, whereas average air temperatures (26.8, 26.9, 25.6 and 23.3°C) were 1.7, 3.0, 1.5 and 0.5°C higher than the long-term averages (25.1, 23.9, 24.1 and 22.8°C).

During these drought periods, heat and water stress could have interacted to negatively affect plant photosynthesis (C. Gunderson, unpubl. data) and growth, leading to reduced above- and below-ground biomass (Hendrick & Pregitzer, 1993; Norby *et al.*, 2000; Norby & Luo, 2004). As shown in Fig. 2, there was no difference in root productivity among the four treatments during the period 1–16 July (the later part of the drought period in 1996), whereas the root mortality under elevated [CO_2] and temperature was still higher. The drought-induced differential responses of root productivity and mortality in middle and late summer could offset the enhanced net production (production minus mortality) during the earlier period of the growing season, leading to the reduced root biomass at the end of the experiment. The interannual variability in the above-ground biomass production supported this argument. In 1994, when there was no obvious drought period, elevated temperature increased above-ground biomass production. However, the above-ground biomass production observed in 1995, 1996 and 1997 was lower under elevated temperature than under ambient temperature.

Second, fine-root productivity and mortality in temperate forests is highly seasonal (Pregitzer *et al.*, 2000a). Fine-root production is usually greater than mortality earlier in the growing season, whereas fine-root mortality is usually greater than productivity later in the growing season. The minirhizotron data in this study are available only for the first half of the growing season and provide a partial picture of root production and mortality dynamics. Elevated [CO_2] and temperature stimulated root production more than mortality in the

first half of the growing season, but their relative effects on production and mortality might have reversed later in the growing season (as was the case with respect to elevated temperature at the last observation period in July), resulting in a loss of earlier gains in fine root standing crop. Although the minirhizotron data provide an incomplete picture, they do clearly show that the fine root population in this system is dynamic and responsive to elevated [CO_2] and temperature, whereas the static measure of response provided by the soil core data provide no indication of the dynamics.

Finally, elevated temperature has been found to stimulate soil N mineralization and availability (Rustad *et al.*, 2001; Melillo *et al.*, 2002). Increased soil N availability has been associated with reduced fine-root biomass but increased fine-root production (i.e. higher root turnover rate) (Nadelhoffer, 2000).

Our understanding of the interactive effects of elevated [CO_2] and temperature on tree roots is limited, largely because there are only a few experiments with both [CO_2] and temperature treatments (King *et al.*, 1996, 1997; Soussana *et al.*, 1996; Kandeler *et al.*, 1998). Our results did not show any CO_2 and temperature interactive effects on fine-root productivity, mortality, and biomass even though the main effects of elevated [CO_2] and elevated temperature on fine-root productivity and mortality were significant, which was consistent with those of BassiriRad *et al.* (1993). In coniferous trees, King *et al.*, 1997 found that the interaction of elevated [CO_2] and temperature significantly increased fine-root biomass of *Pinus taeda*, but had no effect on that of *P. ponderosa*.

Below-ground to above-ground biomass ratio

It is proposed that elevated [CO_2] initially increases C assimilation, with a greater proportion allocated to the roots to maintain a balance of resources, resulting in increased root-to-shoot and root-to-whole-plant mass ratios (Norby *et al.*, 1986, 1995; Rogers *et al.*, 1994). For example, Norby *et al.* (1987) and Rattray *et al.* (1995) reported that the proportion of ^{14}C translocated below-ground increased in elevated [CO_2] compared with ambient [CO_2]. However, results from many other studies did not show any clear evidence that elevated [CO_2] substantially changed the proportion of C allocated to root mass in tree species (Taylor *et al.*, 1994; Wullschleger *et al.*, 1995; Berntson & Bazzaz, 1996; Curtis & Wang, 1998; Tingey *et al.*, 2000). We could not make any conclusion whether elevated [CO_2] affected the below-ground C allocation based only on the data of the ratio of below-ground to above-ground biomass.

Our results showed significant decreases in the ratios of root biomass to above-ground biomass or net primary productivity under elevated temperature. Gunn & Farrar (1999) also found decreased dry mass of roots relative to shoot in *Dactylis glomerata* under high temperature. The underlying mechanisms for the reduced root : shoot ratios are not clear and need further research.

Specific root length

Results from this study showed that elevated [CO_2] caused significant reductions in SRL of fine roots (0.5 mm) in maple trees grown in open-top chambers. Similarly, two short-term studies on *P. taeda* (Larigauderie *et al.*, 1994; King *et al.*, 1997) showed that SRL of secondary roots decreased in elevated [CO_2]. Crookshanks *et al.* (1998) also reported a transitory reduction in SRL of fine roots in *Pinus sylvestris* exposed to elevated [CO_2] after 3 months, but no effect after 6 months. However, increase (Larigauderie *et al.*, 1994) and no changes (Berntson & Bazzaz, 1997; King *et al.*, 1997; Janssens *et al.*, 1998; Pregitzer *et al.* 2000b) in SRL of fine roots were also reported under elevated [CO_2]. Changes in SRL could affect the efficiency of water and nutrient uptake, root longevity, and root respiration rate in plants (Reich *et al.*, 1998; Eissenstat *et al.*, 2000; Gill & Jackson, 2000).

Root N concentration

Reduced fine-root N concentration under elevated [CO_2] observed in our study and previous studies (Berntson & Bazzaz, 1997; Cotrufo *et al.*, 1998; Pregitzer *et al.*, 2000b) were largely attributable to the stimulated plant growth and biomass accumulation. The magnitude of reduced N concentration in fine roots under elevated [CO_2] is typically 10–25% (Rogers *et al.*, 1999). Our results that elevated temperature increased root N concentration were consistent with that of Kandeler *et al.* (1998). Higher root N concentration could have resulted from enhanced N diffusion in the soil and plant N uptake due to greater N mineralization and availability under high temperatures (BassiriRad *et al.*, 1993; BassiriRad, 2000; Rustad *et al.*, 2001). Higher N concentration could lead to greater fine-root mortality (Pregitzer *et al.*, 1998, 2002; King *et al.*, 2002) and alteration of soil N cycling such as microbial N immobilization (Zak *et al.*, 2000).

Conclusions

Both elevated [CO_2] and temperature increased fine-root productivity and mortality in two maple tree species with a dynamic fine-root population. Stimulation of fine-root production and mortality under global change conditions provides a mechanism for increased flux of C to soil and possible sequestration in soil organic matter pools (Matamala *et al.*, 2003). Soil cores indicated that elevated temperature caused significant reductions in fine-root biomass, whereas elevated [CO_2] had no effect. The soil core data, which provide no information about the dynamic nature of the root system, are not necessarily inconsistent with the minirhizotron data from the previous year. The effect of droughts, the probable increase in root mortality late in the growing season, and temperature-induced changes in soil N status could have contributed to the different responses of fine-root productivity and root biomass.

Insufficient data are available on the interactive effects of elevated [CO_2] and temperature on fine-root productivity and mortality, biomass, and morphology to seek general response patterns.

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