Chemistry and Long-Term Decomposition of Roots of Douglas-Fir Grown under Elevated Atmospheric Carbon Dioxide and Warming Conditions

H. Chen* University of Illinois at Springfield
P. T. Rygiewicz and M. G. Johnson USEPA
M. E. Harmon Oregon State University
H. Tian Auburn University
J. W. Tang Chinese Academy of Sciences

Elevated atmospheric CO_2 concentrations and warming may affect the quality of litters of forest plants and their subsequent decomposition in ecosystems, thereby potentially affecting the global carbon cycle. However, few data on root tissues are available to test this feedback to the atmosphere. In this study, we used fine (diameter ≤ 2 mm) and small (2–10 mm) roots of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings that were grown for 4 yr in a 2 × 2 factorial experiment: ambient or elevated (+ 180 ppm) atmospheric CO_2 concentrations, and ambient or elevated (+3.8°C) atmospheric temperature. Exposure to elevated CO_2 significantly increased water-soluble extractives concentration (%WSE), but had little effect on the concentration of N, cellulose, and lignin of roots. Elevated temperature had no effect on substrate quality except for increasing %WSE and decreasing the %lignin content of fine roots. No significant interaction was found between CO_2 and temperature treatments on substrate quality, except for %WSE of the fine roots. Short-term (≤ 9 mo) root decomposition in the field indicated that the roots from the ambient CO_2 and ambient temperature treatment had the slowest rate. However, over a longer period of incubation (9–36 mo) the influence of initial substrate quality on root decomposition diminished. Instead, the location of the field incubation sites exhibited significant control on decomposition. Roots at the warmer, low elevation site decomposed significantly faster than the ones at the cooler, high elevation site. This study indicates that short-term decomposition and long-term responses are not similar. It also suggests that increasing atmospheric CO_2 had little effect on the carbon storage of Douglas-fir old-growth forests of the Pacific Northwest.

Elevated atmospheric CO_2 has been generally shown to decrease the substrate quality of living plant tissues mainly by increasing the C/N ratio or lignin/N ratio via decreasing nitrogen (N) concentration (Strain and Bazzaz, 1983; Mooney et al., 1991; Norby and Cotrufo, 1998; Chen et al., 2001; Graaff et al., 2004). Early hypotheses stated that CO_2–induced reductions in plant tissue quality would yield reduced litter quality, which would slow litter decomposition and litter-mediated nutrient recycling in the soil (e.g., Strain and Bazzaz, 1983). An assumption of this hypothesis is that CO_2–induced reductions in litter quality would be sufficiently large to result in altered decomposition. Although many studies indicate that rising atmospheric CO_2 concentrations nearly always result in increased living plant tissue C/N ratios, this does not necessarily lead to large changes in C/N ratios of plant detrital material (e.g., Franck et al., 1997; Hirschel et al., 1997; Hu et al., 1999). Chen et al. (2001) conducted a metadata analysis and found that N concentrations of tree litters including senescent leaves and green leaves produced in elevated CO_2 environments were about 20% lower on average, with a range of 1.1 to 51.5% lower than the control treatment values. However, according to a metadata analysis by Norby et al. (2001) naturally senesced leaves in field experiments showed that the N concentration was 7.1% lower in elevated CO_2 concentration treatments than in ambient CO_2 concentration treatments. Even CO_2–induced increases in litter C/N ratio have been observed (Ball and Drake, 1997; Hirschel et al., 1997; Robinson et al., 1997; Parsons et al., 2003, 2004; Cotrufo et al., 2005). The impact of litter quality on decomposition rates varies from a decrease (Angelis et al., 2000; Parsons et al., 2004) to no change (Couteaux et al., 1999; Johnson et al., 2000; Lutze et al., 2000; Matamala and Schlesinger, 2000; Dilustro et al., 2001; King

Published in J. Environ. Qual. 37:1327–1336 (2008).
doi:10.2134/jeq2007.0266
Received 25 May 2007.
*Corresponding author (hchen40@uis.edu).

677 S. Segoe Rd., Madison, WI 53711 USA

H. Chen, Biology Dep., Univ. of Illinois at Springfield, One University Plaza, Springfield, IL 62703. P.T. Rygiewicz and M.G. Johnson, U.S. Environmental Protection Agency, National Health and Environmental Effects Research Lab., Western Ecology Div., 200 SW 35th St., Corvallis, OR 97333. M.E. Harmon, Dep. of Forest Science, Oregon State Univ., 321 Richardson Hall, Corvallis, OR 97331. H. Tian, School of Forestry and Wildlife Sciences, Auburn Univ., AL 36849. J.W. Tang, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun Town, Mengla County, Yunnan Province, 666303, P.R. China.

Abbreviations: 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.

et al., 2001, 2005; Finzi et al., 2001; Finzi and Schlesinger, 2002; Weatherly et al., 2003; Johnson et al., 2004). Norby et al. (2001) also suggested that the altered litter quality produced under elevated CO_2 has a limited effect on C dynamics.

The meta-analyses conducted by Chen et al. (2001) and Norby et al. (2001) similarly focused on aboveground plant materials. Few studies have examined changes in substrate quality of tree roots under elevated CO_2 concentration and effects on subsequent decomposition (Cotrufo and Ineson 1995; Robinson et al., 1997; Dilustro et al., 2001; Matamala and Schlesinger, 2000). The decomposition response of roots produced under elevated CO_2 concentrations may differ from that of leaves produced under a similar environment as observed for perennial grass species (Gorissen and Cotrufo, 2000). More studies on substrate quality of tree roots that develop under elevated atmospheric CO_2 concentrations and their subsequent decomposition are needed.

The role of altered global climate to affect litter quality and the carbon and nitrogen cycles is particularly complex because it involves simultaneous increases in atmospheric CO_2 and temperature (IPCC, 2001), coupled with variable responses in precipitation patterns (Weltzin and Tissue, 2003). Many previous studies focused on the effects of a single factor such as elevated CO_2 concentrations (Couteaux et al., 1999; Norby et al., 2001) or warming of terrestrial ecosystems (Rustad et al., 2001; Melillo et al., 2002). Fewer experimental studies have been done examining the interactive effects of multiple factors (e.g., elevated CO_2 + warming) on substrate quality of plant materials (Olszyk et al., 2003; Parsons et al., 2004; Norby and Luo 2004; Henry et al., 2005). It remains unclear how elevated atmospheric CO_2 and warming together influence substrate quality of plant tissues, especially roots.

Results collected in short-term decomposition studies may differ from those obtained using longer-term incubation times. Many decomposition studies of litters produced under altered climate environments were conducted during short periods, ranging from 20 to 300 d for laboratory microcosm incubations and up to 1 yr for field studies (Chen et al., 2001). The longest decomposition studies of litter produced under elevated CO_2 environments lasted for 2 yr (Finzi and Schlesinger, 2002; Booker et al., 2005). Short-term studies are problematic to interpret because litter decomposition curves are exponential and time to achieve most (more than half) of the weight loss of lignified materials generally is much longer than these periods. For example, Chen et al. (2002) found the decomposition rates of fine roots after two years of field incubation were 50% lower than the first-year rates. Thus, longer-term decomposition experiments are needed to determine the potential effects of climate change on substrate quality of plant tissues and their subsequent decomposition.

Even though Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is the most important timber species in the Pacific Northwest of the United States and southwestern Canada (Little, 1971), its growth responses under elevated atmospheric CO_2 concentrations and global warming have been studied only minimally (Olszyk et al., 2003). To understand more comprehensively the interactive effects of elevated atmospheric CO_2 and temperature on this species, a 4-yr exposure experiment was conducted using seedlings at the USEPA Western Ecology Division in Corvallis, OR, USA (Tingey et al., 1996). As a component of the larger project, this study considered how the substrate quality of roots of Douglas-fir produced in the controlled-environment chambers was affected by the treatments and how the fine and small root fractions decomposed in the field during a 3-yr incubation that followed the exposure study in the chambers.

Materials and Methods

Elevated Atmospheric Carbon Dioxide and Warming Experiment

A study of ecosystem responses to elevated atmospheric CO_2 and temperature was initiated in July 1993 using 12 outdoor, sunlit controlled-environment chambers at the EPA lab in Corvallis, OR which control and monitor climatic and edaphic conditions while maintaining natural diurnal and seasonal variability (see Tingey et al. [1996, 2003] for details of the facility and its operation). Each chamber consisted of a water-tight soil lysimeter with a 1 × 2 m footprint and a 1-m depth which was covered with an aluminum-framed canopy (1.1 to 1.3 m height) that was covered with a clear stretched Teflon skin to form the aboveground chamber. The mineral soil and litter layers were obtained from the perimeter of an old-growth Douglas-fir stand located at 1200 m on the western slope of the Oregon Cascade Mountains. The soil was a coarse-loamy, mixed, frigid, Typic Hapludand. It was removed from the collection site by horizon, well mixed by horizon at the EPA chamber facility, sieved to 2.5 cm, and then the soil was placed by horizon into the lysimeters and tamped to achieve a bulk density similar to that measured at the soil collection site. Six cm of air-dried litter layer was added to the top of the mineral soil. The litter layer consisted of sieved (2.5 cm) forest floor that was also collected at the soil collection site. Fourteen 2-yr-old Douglas-fir seedlings, grown from seeds collected from open-pollinated trees in five low-elevation (< 500 m) seed zones in the mountains surrounding Corvallis, were planted in each chamber in June 1993 and grown as described in Olszyk et al. (2003). Initial soil N concentration was low with a value of 0.18%. No fertilizer was added during the exposure period. Instead, seedlings relied on the native nutrient capital of the soil and the nutrients from mineralization. The annual N uptake of the site where soils were collected for the experiment is about 3.5 g $N/m^2/yr$. This was estimated based on the N requirement of new tissue, i.e., net primary production (NPP) per foliage (litterfall), wood (tree rings), and fine roots (based on turnover estimates) (R. McKane, personal communication, 2007).

A 2 × 2 factorial design with three replicates of each treatment was used, with the chambers serving as the experimental unit. The four treatments were: ambient CO_2 and ambient temperature (ACAT), ambient CO_2 and elevated temperature (ambient + 3.8°C) (ACET), elevated CO_2 (ambient + 180 ppm) and ambient temperature (ECAT), and elevated CO_2 and elevated temperature (ECET). Ambient conditions were determined at the on-site meteorological tower located adjacent to the chambers. Details about the precision and accuracy of

the facility to provide the treatment target values during the first year of exposure are presented in Tingey et al. (1996).

Preparation of Roots

After 4 yr of treatment the Douglas-fir seedlings were harvested in the summer 1997 for intensive measurements on the soil and fresh plant material (see Olszyk et al. [2003] for details). Briefly, the entire root system of three of the 14 seedlings in each chamber was carefully removed from the soil by horizon and separated from the soil by sieving. They were rinsed, sorted by diameter (fine roots with diameters ≤ 2 mm; and small roots, diameter 2–5 mm), and then air dried at room temperature to a constant weight. Moisture contents of air-dried fine roots averaged 6.0% with a standard deviation of 0.5% (n = 5); comparable values for small roots were 9.2 and 0.7% (n = 5). All twelve chambers were sampled over a 7 wk final harvest period to allow time for intensive measurements to be made on the seedlings and soil from each chamber. As the trees in the chambers not yet harvested were still growing during the 7 wk harvest, presenting the possibility that additional seedling growth could affect the results among treatments, chambers were sampled according to the block design established for the exposure study such that one replicate per treatment was harvested in each of the beginning, middle, and final phases of the harvest.

Decomposition Field Sites

The 3-yr decomposition study was conducted at two Douglas-fir forested field sites on the western slope of the Oregon Cascade Mountains. These two sites are at different elevations with one site at 537 m (low site, 44°23'37" N, 122°22'18" W) and the other at 1200 m (high site, 44° 25'29" N, 122°01'54" W). There were two replicate plots for the decomposition study at each site. The mineral soil and litter used in the chambers was obtained from the high elevation site. More detailed edaphic characteristics of the sites are presented in Tingey et al. (2005). Briefly, the high-site soil was derived from similar parent material as found at the low site, but appears to be much younger. The soil at the high site is classified as a Medial-skeletal, Typic Fulvicryands, formed from a parent material of volcanic ash over volcanic ash mixed with glacial till, and has a total soil N of 0.15%. The low-site soil is classified as a fine-loamy, mesic, Andic Dystrudepts, formed from a parent material of volcanic ash over weathered volcanic ash over weathered tuff and breccia bedrock. The soil has 0.25% total soil N. The region experiences a maritime climate with wet relatively mild winters, and dry cool summers (Fig. 1). The average annual precipitation of the high and low elevation sites between 1999 and 2002 was similar with values of 1162 mm and 1203 mm, respectively (Fig. 1A), with a greater percentage of the precipitation falling as snow at the high site. The average monthly air temperature for a 12 consecutive month period for the high and low elevation sites was 6.5 and 8.9°C, respectively (Fig. 1B). Similarly, comparable values for average monthly soil temperature at 15-cm depth were 5.9 and 8.8°C, respectively. Soil temperature (Campbell 107B soil thermistor) was measured continuously at 15 cm at one location

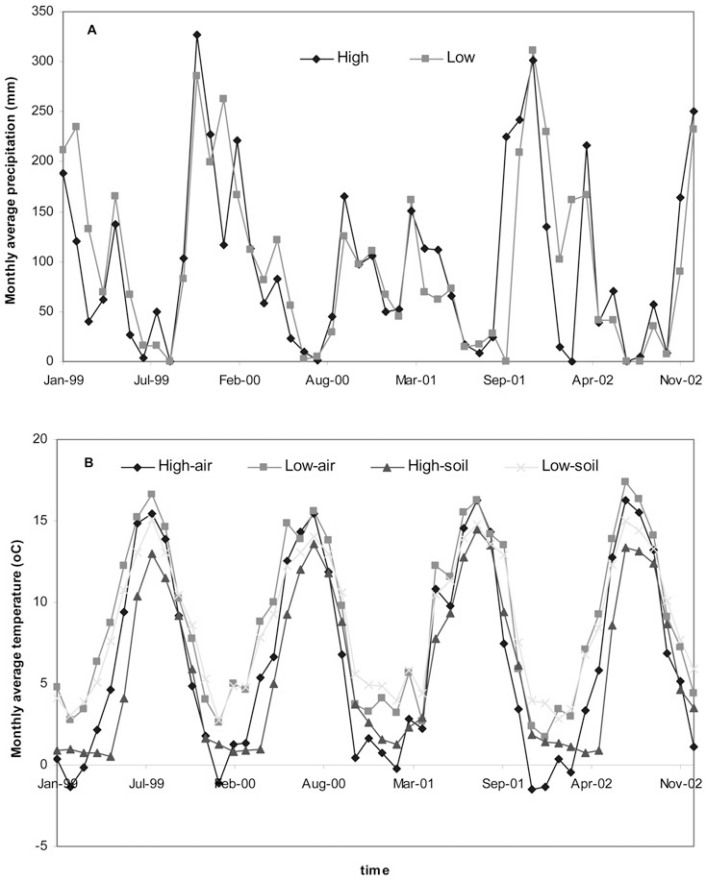

Fig. 1. Environmental conditions at the two root litterbag incubation field sites. (A) Monthly average precipitation during 1999–2002; and (B) Monthly average air and soil temperature (15 cm) during 1999–2002.

at each forest site and data were stored as hourly means on CR10 data loggers (Campbell Scientific, Logan, Utah).

Long-Term Root Decomposition Experiment

The 3-yr root decomposition study used the litterbag approach. Before deploying the litterbags in the field, approximately either 4.5 or 8 g of air-dried fine and small roots, respectively, were put into 20 × 20 cm dacron cloth litterbags with an effective mesh size of 50 μm. Each litterbag was tagged with a uniquely numbered aluminum tag, and then the labeled bags were tethered with nylon line for each site according to the date of harvest. The tethered labeled bags were placed in angled slots cut in the soil to about 20 cm with a shovel at each of two replicate plots at each of the two elevation sites in September 1999. Following placement of a litter bag the shovel was removed and the soil was tamped to close the slot and to ensure good soil-litterbag contact. A total of 128 fine root litterbags and 64 small root litterbags were buried at the four plots (two low elevation plots and two high elevation plots) for the 3-yr study. Harvests of fine root litterbags occurred eight times: 3, 6, 9, 12, 18, 24, 30, and 36 mo after being buried. Harvests of small root litterbags occurred 6, 12, 24, and 36 mo after burial. After being removed from the soil at harvest each litterbag was placed into a sealable plastic bag on site to reduce moisture loss from the roots and then placed in a

cooler. Upon returning to the lab, roots were carefully brushed free of soil and other debris, and new roots that had grown into the litterbags were removed. Samples were dried to a constant mass at 65°C and then weighed. Dried root samples were ground in a Wiley mill and passed through a fine screen (1 mm). Samples were stored in 20-mL vials to reduce moisture changes before analyzing them for ash content and N concentration. Mass loss of roots was estimated from changes in ash-free dry mass of roots based on sample harvests at different time intervals.

Analyses of ash and N concentrations of roots including the initial roots and all the decomposed roots harvested from the two sites were performed at the Soils Laboratory of the Department of Forest Science, Oregon State University. Ash concentration was determined by heating samples in a muffle furnace at 500°C for 4 h. Results for ash content were used to calculate the ash-free weights reported herein. N concentration of roots was measured by Carlo-Erba C-N analyzer. In addition, the initial organic constituents of roots were analyzed by proximate carbon analysis (n = 3; Chen et al., 2002). The constituents analyzed included nonpolar extractives (NPE: fats, oils, and waxes) using dichloromethane as the extratant (Effland 1977), hot water–soluble extractives (Chen et al., 2002), the acid-hydrolyzable fraction (cellulose, hemicellulose, and starch; hydrolysis followed by the phenol-sulfuric acid assay; Dubois et al., 1956), and the acid-resistant fraction (Effland 1977). Although the acid-resistant fraction includes recalcitrant carbon compounds besides lignin (e.g., suberin), we simply refer to this fraction as "lignin."

Statistical Analysis

A two-way analysis of variance (ANOVA) was used to compare the effects of the elevated CO_2 and warming treatments on the chemical quality of roots of Douglas-fir produced after 4 yr of exposure. The chemical quality indices examined included total carbon concentration (%C), total nitrogen concentration (%N), C/N ratio, nonpolar extractable (%NPE), water-soluble extractives (%WSE), acid-soluble cellulose and hemicellulose (%cellulose), acid-insoluble carbohydrate (%lignin), and lignin/N ratio. We used a three-way ANOVA to determine how the treatment conditions under which the roots were produced, root size, and the field incubation sites influenced root mass loss after various lengths of incubation during 3 yr. The two-way and three-way statistical analyses were conducted using SAS (SAS Institute Inc., 2002). Differences between means were detected using Fisher's protected least significant difference (LSD).

A single-exponential model was fitted to the mass of roots remaining during 3 yr of decomposition using the least squares method of the GLM procedure of SAS (SAS Institute Inc., 2002):

$$Y_t = Y_o * e^{-k*t}$$

where Y_o is the percentage of initial mass of roots, Y_t is the percentage of initial mass of roots remaining at time t, and k is the decomposition rate constant of the roots. The k value was calculated from the linear regressions of the natural logarithm (ln) of the percentage of initial mass of roots remaining vs. time. For roots from each of the four exposure treatments, the mean percentage of initial root mass remaining, at the various times, for the two plots at each site, rather than values for remaining root mass of individual litterbags was used in these regressions. The half-life (time for half of the initial mass to be lost) and the 95% time (time for 95% of the initial mass to be lost) of the roots produced in the various exposure treatments were calculated from the single-exponential models. The effects of initial root substrate quality on k were evaluated by using the p value of a simple regression using the GLM procedure of SAS (SAS Institute Inc., 2002). A linear regression was developed with k as the dependent variable and each initial root quality index (as indicated above) as the independent variable.

Results

Initial Litter Chemistry of Roots

After nearly 4 yr of continuous exposure, elevated CO_2 significantly increased %WSE of roots of both size fractions (Table 1). Similarly, elevated CO_2 significantly affected initial %NPE of roots, although the effect varied with root size. For fine roots (≤ 2 mm diameter) under elevated CO_2, %NPE decreased while %NPE of small roots (2–5 mm diameter) increased. However, elevated CO_2 did not affect %N, %C, C/N ratio, %lignin, and lignin/N ratio of the roots, regardless of root size.

Elevated temperature significantly affected %WSE and %lignin of fine roots (Table 1). Average %WSE of fine roots was higher in the warming treatment compared with the fine roots from the ambient temperature control. In contrast, the average %lignin of fine roots produced under elevated temperature was lower than comparable values for the control. The %C of small roots decreased slightly under elevated temperature. For most initial substrate quality indices of roots, elevated temperature had no significant effect. No significant interactions were found between the CO_2 and temperature treatments on the initial substrate quality of roots except for %WSE of fine roots.

Mass Loss during Shorter-Term Decomposition

During the first 9 mo of field incubation, decomposition of roots was significantly affected by the exposure treatments. The roots from the double ambient control (ACAT) had the slowest decomposition rates on the two sites, losing 8.6, 9.2, and 12.2% of their initial mass on average after 3, 6, and 9 mo of decomposition, respectively (Table 2, Fig. 2). The decomposition of roots from the other three treatments (ACET, ECAT, and ECET) did not differ from each other and all were higher than the control. For example, fine roots produced in the ACET treatment lost 11.9, 12.9, and 16.7% of initial mass after 3, 6, and 9 mo of decomposition, respectively.

Generally, during the first 9 mo of decomposition, the incubation site did not significantly affect root decomposition rates (Table 3). The only exception to this was the root mass loss data after 6 mo of decomposition. There were no interactive effects of exposure treatment, root size, and incubation site on weight loss during the first 9 mo.

Mass Loss during Longer-Term Decomposition

Roots from the various exposure treatments showed no statistically significant difference in decomposition between 9 and 36 mo of field incubation (Tables 2, 3 and Fig. 2). The % fine root mass remaining for the ACAT, ACET, ECAT, and ECET treatments was 65.6, 57.8, 66.9, and 64.8, respectively, after 36 mo of decomposition (Fig. 2A). The % of small root mass remaining for the ACAT, ACET, ECAT, and ECET treatments was 68.8, 65.5, 69.8, and 70.4%, respectively, for the same period. It is interesting to note that the size of the roots did not significantly affect weight loss except during the first 6 mo of incubation (Tables 2 and 3).

The effect of the incubation site on weight loss started to appear after the roots were buried for 1 yr (Table 3). Roots at the low elevation site decomposed significantly faster than the ones at the high elevation site regardless of exposure treatment or root size (Table 2). The effect of incubation site on weight loss lasted much longer than did exposure treatment effects (Table 3). After 3 yr of decomposition at the low elevation site, the % fine root mass remaining for the ACAT, ACET, ECAT, and ECET treatments was 64.9, 54.9, 61.6, and 62.1%, respectively. However, the % fine root mass remaining for the ACAT, ACET, ECAT, and ECET treatments at the high elevation site were 66.2, 60.6, 72.1, and 67.6, respectively, for the same period. There was no significant interaction between exposure treatment and incubation site on decomposition rates during the 36-mo incubation except at one harvest date (Table 3). No other significant interactions were observed.

Table 1. Chemical characteristics of roots of Douglas-fir grown for 4 yr under elevated atmospheric CO_2 and (or) elevated temperature.

Substrate quality index†	Litter type‡	Treatment§				*P* value¶		
		ACAT	ACET	ECAT	ECET	CO_2	Temp	CO_2 x Temp
%C	FS	51.8 ± 0.5	51.3 ± 0.3	51.3 ± 0.4	51.6 ± 0.6	ns	ns	ns
	SS	50.9 ± 0.4	49.9 ± 0.5	50.7 ± 0.2	50.4 ± 0.2	ns	*	ns
%N	FS	0.47 ± 0.05	0.46 ± 0.02	0.45 ± 0.02	0.41 ± 0.03	ns	ns	ns
	SS	0.25 ± 0.02	0.24 ± 0.02	0.25 ± 0.02	0.25 ± 0.02	ns	ns	ns
C/N ratio	FS	110 ± 14	111 ± 5	114 ± 5	126 ± 9	ns	ns	ns
	SS	201 ± 14	210 ± 15	207 ± 15	200 ± 18	ns	ns	ns
%NPE	FS	5.8 ± 0.4	5.6 ± 0.3	4.7 ± 0.8	4.2 ± 0.6	**	ns	ns
	SS	2.4 ± 0.5	1.8 ± 0.4	3.3 ± 0.3	3.1 ± 0.3	**	ns	ns
%WSE	FS	15.2 ± 4.9	23.8 ± 0.9	24.1 ± 0.5	26.1 ± 3.5	*	*	*
	SS	19.2 ± 3.3	16.3 ± 3.6	21.4 ± 3.7	25.3 ± 1.7	*	ns	ns
%Cellulose	FS	41.9 ± 3.3	38.6 ± 2.7	35.0 ± 1.5	39.2 ± 4.0	ns	ns	ns
	SS	47.1 ± 3.5	50.8 ± 1.3	43.8 ± 6.2	42.5 ± 1.5	*	ns	ns
%Lignin	FS	37.0 ± 1.3	32.0 ± 2.6	36.2 ± 1.8	32.5 ± 0.8	ns	**	ns
	SS	31.3 ± 0.3	31.1 ± 3.1	31.5 ± 2.8	29.2 ± 2.7	ns	ns	ns
Lignin/N ratio	FS	79 ± 12	69 ± 3	80 ± 7	79 ± 5	ns	ns	ns
	SS	124 ± 9	131 ± 20	128 ± 6	115 ± 5	ns	ns	ns

† NPE, nonpolar extractable(fats, oils, and waxes); WSE, water-soluble extractives; cellulose, acid-soluble cellulose and hemicelluloses; and lignin, acid-insoluble carbohydrate. The total of these four chemical components is equal to 100%.

‡ FS, fine roots (< 2 mm in diameter); SS, small roots (2–5 mm in diameter).

§ 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. Values are means ± SD of three replicates.

¶ $*P < 0.05$; $**P < 0.01$; Temp refers to temperature.

Decomposition Rate Constant (*k*)

The decomposition rate constant values varied with the exposure treatment from which the roots originated and decreased over the length of incubation, especially during the first 9 mo (Table 4). After 3 mo of decomposition, the values of *k* of fine roots in increasing order were 0.36, 0.40, 0.48, and 0.51/year for ACAT, ECAT, ECET, and ACET, respectively. Similar relative patterns for *k* of fine roots occurred after 6 and 9 mo of incubation. The effect of exposure treatment on *k* value decreased as the length of incubation increased until it disappeared after 9 mo. The average *k* values of fine roots, derived from the regression of a single-exponential model using data from all the sampling times of the 36 mo incubation period were 0.14, 0.13, 0.15, and 0.16/year for ACAT, ECAT, ECET, and ACET, respectively. The *k* values of fine roots were 0.15, 0.15, 0.15, and 0.17/year for ACAT, ECAT, ECET, and ACET at the low elevation site, in comparison with 0.13, 0.12, 0.12, and 0.15/year for ACAT, ECAT, ECET, and ACET at the high elevation site, respectively.

Table 2. Percent of mass remaining of roots during 3 yr of decomposition.

Treatment†	Size	Site	Decay period (months)							
			3	6	9	12	18	24	30	36
ACAT	Fine	Low	91.1	90.5	88.8	83.2	82.5	71.9	61.5	64.9
		High	91.6	91.0	86.9	88.4	84.8	73.6	68.7	66.2
	Small	Low	NA	93.1	NA	84.7	NA	62.3	NA	59.8
		High	NA	92.6	NA	89.2	NA	82.4	NA	77.7
ACET	Fine	Low	87.7	86.4	83.4	84.9	75.3	67.2	64.8	54.9
		High	88.5	87.8	83.3	85.2	76.3	74.6	66.2	60.6
	Small	Low	NA	88.6	NA	82.6	NA	66.0	NA	64.6
		High	NA	89.9	NA	84.4	NA	83.9	NA	66.5
ECAT	Fine	Low	88.7	87.7	86.4	81.0	79.2	67.6	69.6	61.6
		High	92.3	88.7	85.7	87.8	79.3	70.5	68.4	72.1
	Small	Low	NA	90.5	NA	83.7	NA	76.1	NA	59.6
		High	NA	93.0	NA	86.8	NA	84.2	NA	80.0
ECET	Fine	Low	88.5	87.9	86.7	85.2	75.6	71.3	64.3	62.1
		High	89.0	87.9	86.9	85.0	77.0	75.6	71.5	67.6
	Small	Low	NA	89.9	NA	84.5	NA	76.5	NA	62.4
		High	NA	90.2	NA	84.2	NA	78.6	NA	78.5

† 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.

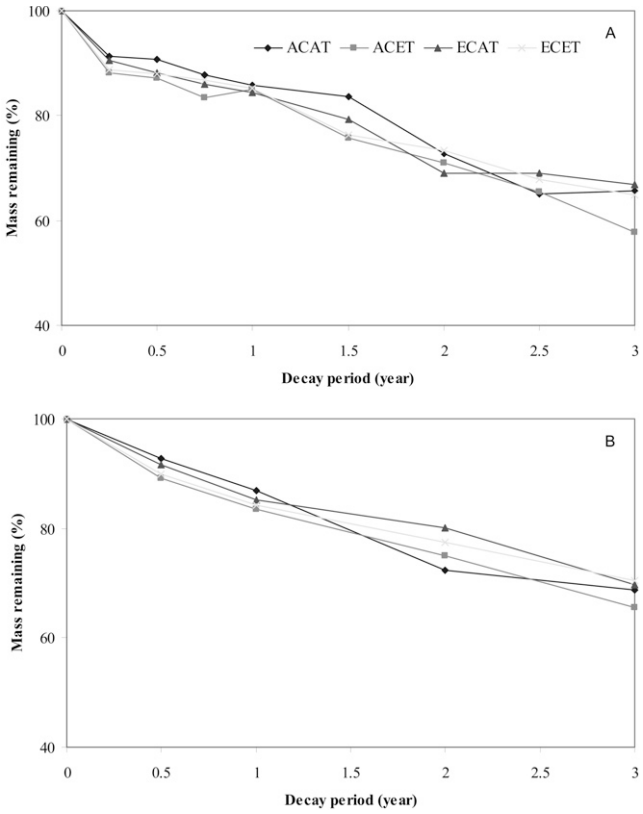

Fig. 2. Root decomposition as indicated by percent of mass remaining of initial roots. (A) Fine roots (≤ 2 mm diameter) and (B) Small roots (2– 5 mm diameter).

After 6 mo of decomposition, the values of *k* of small roots in increasing order were 0.14, 0.16, 0.17, and 0.18/year for ACAT, ECAT, ECET, and ACET, respectively. Only the *k* value of small roots from ACAT was significantly different from the values for roots originating from the other exposure treatments. After 3 yr of decomposition, there was no significant difference in the *k* value for small roots with values of 0.13, 0.11, 0.11, and 0.13/year for ACAT, ECAT, ECET, and ACET treatments, respectively (Table 4). The *k* values of Douglas-fir small roots were greater at the low elevation site compared with the comparable values at the high elevation site (Table 5).

Effects of Initial Substrate Quality on *k* Value

Decomposition rate constants of roots were significantly correlated with several of the initial root substrate quality indices that were measured on the roots soon after they were harvested from the chambers (Table 6). After the first 3 mo of decomposition, none of the eight initial substrate quality indices of the roots was significantly correlated with the *k* value of fine roots. However, after 6 mo of incubation, initial %WSE and %C were significantly and positively correlated with *k* values of fine roots and small roots, respectively. The *k* value of fine roots removed after 9 mo of decomposition were significantly correlated with three out of the eight initial substrate quality indices, including %WSE, %C, and lignin/N ratio. After 3 yr of decomposition, initial lignin/N ratio was the only index significantly correlated (negatively) with *k* value of fine roots. For small roots after 3 yr of incubation, none of the initial substrate quality indices were correlated with *k* the value.

Table 3. Significance levels of variation in loss of litter mass at the eight collection dates determined by multiple ANOVA.†

	Decay period (months)							
Source of variation	**3**	**6**	**9**	**12**	**18**	**24**	**30**	**36**
Root source (RS)	*	**	*	ns	ns	ns	ns	ns
Site	ns	*	ns	***	ns	*	*	**
Size	NA	*	NA	ns	NA	ns	NA	ns
RS x site	ns	ns	ns	*	ns	ns	ns	ns
RS x size	NA	ns	NA	ns	NA	ns	NA	ns
Site x size	NA	ns	NA	ns	NA	ns	NA	ns
RS x site x size	NA	ns	NA	ns	NA	ns	NA	ns

† *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$; ns, not significant ($P > 0.05$); NA, not applicable because only fine roots were collected after 3, 9, 18, and 30 mo of decomposition.

Table 4. Regression coefficients of the single-exponential model used to estimate the decomposition rate constant (*k*) of roots that decomposed at the two field incubation sites in Oregon, USA.†

Root diameter class	Root source‡	*k*§ first 0.25 yr	*k* first 0.5 yr	*k* first 0.75 yr	Regression coefficients			
			yr^{-1}		*k*	SD	R^2	N¶
Fine	ACAT	0.36	0.19	0.17	0.14	± 0.01	0.95***	9
	ACET	0.51	0.28	0.24	0.16	± 0.01	0.97***	9
	ECAT	0.40	0.25	0.20	0.13	± 0.01	0.95***	9
	ECET	0.48	0.26	0.19	0.15	± 0.01	0.97***	9
Small	ACAT	NA	0.14	NA	0.13	± 0.01	0.96**	5
	ACET	NA	0.18	NA	0.13	± 0.01	0.98**	5
	ECAT	NA	0.16	NA	0.11	± 0.01	0.98**	5
	ECET	NA	0.17	NA	0.11	± 0.01	0.96**	5

† *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$;

‡ 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.

§ k was calculated based on a single-exponential model using one time harvest of roots. $Y_t = Y_o e^{-kt}$ where Y_t is the mass remaining of roots after decomposition of *t* year, Y_o was the initial mass, and *k* is the decomposition rate of constant. NA: not applicable.

¶ Each data point is the mean of two samples at each site.

Discussion

Effects of Elevated Atmospheric Carbon Dioxide Concentrations and Warming on Substrate Quality of Roots

Several studies have reviewed the literature on the chemistry of litter produced under elevated atmospheric CO_2 concentrations (Couteaux et al., 1999; Chen et al., 2001; Norby et al., 2001). Chen et al. (2001) conducted a metadata analysis and found that the average %N of tree litters including senescent leaves and green leaves produced in elevated CO_2 environments were about 20% lower than the average for the control CO_2 level, ranging from 1.1 to 51.5% less than the control. However, naturally senesced leaves in field experiments showed that the N concentration in leaf litter was 7.1% lower in elevated CO_2 concentration than that under ambient CO_2 concentration (Norby et al., 2001). Our data indicate that 4 yr of exposure to elevated atmospheric CO_2 concentrations had no effect on %N of Douglas-fir roots (Table 1). A similar finding was reported by Tingey et al. (2003) for a different sample of roots produced in these chambers. However, the %N of Douglas-fir needles from the same

project decreased under elevated CO_2 treatment compared with the levels found for control needles (Lewis et al., 2001; Olszyk et al., 2003). Lewis et al. (2004) further reported that elevated CO_2 led to photosynthetic acclimation, which may account for a decreased requirement for N by needles under elevated CO_2. Tingey et al. (2003) suggested that under elevated CO_2 needle N was allocated to other tissues as an explanation of why the %N decreased in the elevated CO_2 treatment. Our study suggests that %N of roots is less responsive to elevated CO_2 exposure than %N of needles produced by seedlings growing on this N-poor soil. For this plant-soil system, needle %N of Douglas-fir was less than 1% while soil N content in the A horizon was as low as 0.11% (Johnson et al., 2006). As our data indicate that the %N of roots was not altered by elevated CO_2, the putative needle N translocated to other tissues suggested by Tingey et al. (2003) may have been allocated to the extramatrical hyphae of ectomycorrhizal fungi as shown by Hobbie et al. (2007).

Surprising to us was the increased %WSE of roots produced under elevated CO_2 (Table 1) which primarily includes water-soluble sugars and water-soluble phenols (Chen et al., 2002). Most of the compounds that constitute the water-soluble extractives are easily decomposed while the constituents of the water-soluble phenols are more recalcitrant (Chen et al., 2002). Although the constituents of these two extract groups were not characterized in our study, content of water-soluble sugar and water-soluble phenols generally is similar; about 25% of WSE based on carbon fraction data of fine roots of Douglas-fir was found in a separate study in the Pacific Northwest (Chen et al., 2002). Tingey et al. (2003) reported the principal carbon constituents of several size classes (< 1 mm to > 10 mm dia.) of a separate cohort of these roots, also sampled at the final harvest of the exposure study. The water-soluble extract was further characterized into total reducing sugars and phenols. No significant CO_2 or temperature effects were found in any root size fraction for total reducing sugars and phenols constituents.

It is not clear why elevated CO_2 differentially affected %NPE of fine roots and small roots. The %NPE of fine roots was significantly lower in the elevated CO_2 treatment while the %NPE of small roots was higher under the same treatment (Table 2). The %NPE fraction was determined using dichloromethane as the extratant, and includes fats, oils, and waxes (Effland, 1977) which usually are difficult for microbial organisms to decompose.

Out of the eight substrate quality indices examined in this study, the elevated temperature treatment significantly affected %lignin and %WSE of fine roots (Table 1). Elevated temperature significantly decreased %lignin of fine roots, a result consistent with those of Tingey et al. (2003). Elevated temperature also increased %WSE of fine roots as EC treatment (elevated CO_2) did on fine roots. There were no significant interactions between CO_2 and temperature on root tissue quality except for %WSE of fine roots, which is consistent with the results of Olszyk et al. (2003). Thus, elevated CO_2 and temperature treatments generally increased the substrate quality of Douglas-fir roots by increasing %WSE and decreasing %lignin, a finding quite different from previous literature reviews (Chen et al., 2001; Norby et al., 2001).

Table 5. Regression coefficients of the single-exponential model used to estimate the decomposition rate constant (*k*) of roots incubated in Oregon, USA.†

Root diameter class	Root source‡	Site	Regression coefficients				95% mass loss	
			k	SD	R^2	N§	Half-life	
			yr^{-1}				yr	
Fine	ACAT	Low	0.15	± 0.02	0.94***	9	4.6	20.0
	ACAT	High	0.13	± 0.01	0.96***	9	5.3	23.1
	ACET	Low	0.17	± 0.01	0.96***	9	4.1	17.6
	ACET	High	0.15	± 0.01	0.96***	9	4.6	20.0
	ECAT	Low	0.15	± 0.01	0.94***	9	4.6	20.0
	ECAT	High	0.12	± 0.02	0.89***	9	5.8	25.0
	ECET	Low	0.15	± 0.01	0.97***	9	4.6	20.0
	ECET	High	0.12	± 0.01	0.95***	9	5.8	25.0
Small	ACAT	Low	0.19	± 0.03	0.94**	5	3.6	15.8
	ACAT	High	0.08	± 0.01	0.97**	5	8.6	37.5
	ACET	Low	0.15	± 0.03	0.93**	5	4.6	20.0
	ACET	High	0.12	± 0.02	0.90**	5	5.8	25.0
	ECAT	Low	0.16	± 0.01	0.98**	5	4.3	18.8
	ECAT	High	0.07	± 0.01	0.89**	5	9.9	42.9
	ECET	Low	0.15	± 0.01	0.98**	5	4.6	20.0
	ECET	High	0.08	± 0.02	0.81*	5	8.6	37.5

† *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$.

‡ 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.

§ Each data point is the mean of two samples at each site.

Controls on Short-Term Root Decomposition

Litter decomposition is profoundly influenced by substrate quality, climate, and the decomposer community (Heal et al., 1997; Chen et al., 2001, 2002). However, the relative importance of these factors at different stages of decay is poorly understood. During short-term (≤ 9 mo) root decomposition in our study, the exposure treatments that the roots were grown under significantly affected decomposition rates; roots from the elevated CO_2 and temperature exposure decomposed faster than roots from the respective control (Table 2, 3, 4 and Fig. 2). Both the mass of decomposing roots remaining (Table 2) and the decomposition rate constant (*k*) (Table 4) confirm this pattern. The fast decomposition rate is likely due to the higher %WSE because

Table 6. Initial substrate quality index showing significant correlation with different decomposition rate constant (*k*) of roots incubated in Oregon, USA.

k of roots	Initial substrate index§	F	DF	*P*	R
Fine roots after 6 mo of decay†	WSE%	5.84	1, 7	0.050	0.71
Small roots after 6 mo of decay†	C%	10.85	1, 7	0.017	0.80
Fine roots after 9 mo of decay†	WSE%	5.84	1, 7	0.050	0.68
	C%	22.65	1, 7	0.003	0.89
	Lignin/N	10.18	1, 7	0.019	–0.79
Fine roots after 3 yr of decay‡	Lignin/N	6.47	1, 7	0.044	–0.72

† *k* was calculated based on a single-exponential model using one time harvest of roots. $Y_t = Y_o e^{-kt}$ where Y_t is the mass remaining of roots after decomposition of t year, Y_o was the initial mass, and *k* is the decomposition rate of constant.

‡ The single-exponential model above fit the remaining mass of decomposing roots, which was collected 8 and 4 times for fine roots, small roots, respectively, during 3 yr of decomposition.

§ NPE, nonpolar extractable (fats, oils, and waxes); WSE, water-soluble extractives; cellulose, acid-soluble cellulose and hemicelluloses; and lignin, acid-insoluble carbohydrate. The total of these four chemical components is equal to 100%.

these compounds are easily decomposed or dissolved. In contrast, roots from the double ambient control (ACAT) always had the lowest %WSE (Table 1). This is confirmed by the positive correlation between the initial %WSE and the *k* value after the first 9 mo of decomposition (Table 6). During the first 9 mo, the incubation location did not generally affect root decomposition (Table 2 and 3). Further, results from multiple ANOVAs including only mass loss data of fine roots confirm this observation. Thus, the initial substrate quality of roots, especially that of the fine roots, not the incubation site, was the dominant driver controlling the short-term decomposition (≤ 9 mo). The differences in initial substrate quality, particularly %WSE, and caused by elevated CO_2 and temperature, were sufficiently large to show an effect on root decomposition after 9 mo of incubation.

Controls on Longer-Term Root Decomposition

Differences in root initial substrate quality among the exposure treatments were sustained through the first 9 mo of decomposition (Table 3). Over the longer-term incubation period (9–36 mo) the effects of initial substrate quality on root decomposition diminished. In contrast, we found that the incubation site significantly controlled root long-term decomposition (Table 3). The major difference between the two incubation sites was the annual mean air and soil temperatures (15-cm depth) (Fig. 1B) with the low elevation site being 2.4 and 2.9°C warmer than the high elevation site, respectively. The annual precipitation at the two sites is similar (Fig. 1A). The warmer conditions at the low elevation site enhanced decomposition regardless of the root sources or root sizes (Tables 2, 5) and was especially evident for small roots. Small roots at the low elevation site lost on average 38.4% of their initial mass versus 24.3% at the high elevation site after 3 yr of decomposition. In contrast, comparable values for fine roots were 39.1 versus 33.4% for the same time period. Only initial lignin/N ratio showed a significant and negative correlation with *k* value after 3 yr of decomposition (Table 6). As there was no significant difference in initial lignin/N ratio among the roots, this result further suggests that the difference in initial substrate quality exerted little influence on longer-term decomposition. Our findings are consistent with those of Norby et al. (2001); any differences in litter quality produced under elevated CO_2 had limited effect on C dynamics.

Evaluating the "Negative-Feedback" Hypothesis

The "negative-feedback" hypothesis predicts that elevated atmospheric CO_2 concentrations generally decrease substrate quality of living plant tissues mainly by decreasing %N and increasing the C/N ratio (Strain and Bazzaz, 1983; Mooney et al., 1991; Chen et al., 2001; Graaff et al., 2004); the resulting reductions in litter quality slows litter decomposition and litter-mediated nutrient recycling in the soil (e.g., Strain and Bazzaz, 1983). Our short-term (≤ 9 mo) decomposition results did not support this "negative-feedback" hypothesis. First, the elevated CO_2 and temperature exposures used to produce the roots actually increased the substrate quality of the Douglas-fir roots. Second, due to the increased substrate quality, roots produced by these altered climate treatments decomposed faster than roots originating from the ACAT control treatment. Although it is not clear exactly how litter-mediated nutrient cycling responded over time in our study, our results do not support the "negative- feedback" hypothesis. Instead, our data suggest that a "positive-feedback" mechanism may have occurred in this plant-soil system, at least in a short-term period. This is because roots produced in the elevated CO_2 and temperature exposures had increased substrate quality which apparently leads to faster rates of root decomposition and higher rates of release of carbon into the atmosphere from decomposition. Although our root decomposition study was not conducted in chambers in which the Douglas-fir seedlings were exposed to the climate treatments, Lin et al. (2001) reported that soil CO_2 efflux in these chambers increased about 20 and 17% under elevated CO_2 during Years 1 and 2 of the exposure, most likely due to increased decomposition of the various litters including dead fine roots. Although neither elevated atmospheric CO_2 nor elevated temperature affected fine-root production, mortality, or standing crop (Johnson et al., 2006), the increased soil CO_2 efflux in the Years 1 and 2 under elevated CO_2 may be in part due to the faster decomposition of dead fine roots, which had a higher %WSE under elevated CO_2 treatments.

Our longer-term (9–36 mo) decomposition results also did not support the "negative-feedback" hypothesis, given no significant differences in loss of root mass and *k* values were observed after 3 yr of decomposition. The initial differences in root substrate quality among the treatments diminished after 9 mo and then the incubation location became the dominate factor influencing root decomposition accompanied by a weak substrate-environment interaction.

Implications for Carbon Storage of Douglas-Fir Forests

How will elevated CO_2 and warming influence carbon storage of Douglas-fir forests in the Pacific Northwest? As we know that ecosystem carbon storage includes carbon in soil and plants, the amount of carbon stored in these two components is determined by many processes, among them photosynthesis and aboveground plant respiration, weathering, mineralization, and nutrient uptake, and soil respiration (e.g., root respiration, microbial respiration related to litter decomposition, and oxidation of soil organic matter) (Chapin et al., 2002). The chamber facility in Corvallis utilized a model reconstructed Douglas-fir seedling-native soil ecosystem and our study was part of this larger comprehensive project by considering the decomposition of roots grown under different CO_2 and warming conditions. Before addressing the question above, we review some of what was learned about this system. Lewis et al. (2001) reported that elevated CO_2 and temperature increased net photosynthesis. At the end of the 4-yr exposure period, Olszyk et al. (2003) found that neither elevated CO_2 nor temperature affected seedling biomass production or allocation (both in total and proportional amounts) to any plant organ, including leaves, fine and coarse roots, buds, branches, and stems. Johnson et al. (2006) further indicated that both elevated CO_2 and elevated temperature did not affect fine root production, mortality, or standing crop, although fine roots appeared to root deeper in the soil profile under elevated CO_2 and temperature treatments. These results suggest that any additional C fixed due to increasing photosynthesis was not allocated to plant biomass. In contrast to photosynthesis, soil CO_2 efflux increased about 20% with elevated CO_2 and 41% with

elevated temperature relative to controls during Year 1 of the study. However, these values decreased to 17 and 16%, respectively, during Year 2 of the study (Lin et al., 2001). By Years 3 and 4, neither elevated CO_2 nor elevated temperature stimulated soil CO_2 efflux (Tingey et al., 2006). In most cases, litter decomposition was the dominant component of soil CO_2 efflux in this system (Lin et al., 1999, 2001). Hobbie et al. (2004) used isotopic ^{13}C to estimate new carbon inputs into litter and soils and concluded that neither elevated CO_2 nor temperature affected soil carbon sequestration patterns in the A horizon under the nitrogen-limiting conditions. Our study adds information on the effects of elevated CO_2 and temperature on root substrate quality and short- and longer-term root decomposition. Taken collectively, these results indicate that elevated CO_2 had little effect on the carbon storage of the seedlings and suggest that Douglas-fir forests of the Pacific Northwest may respond similarly. The lack of treatment effects is probably due to N constraints on the plant-soil-litter system in this N-limited system as indicated by Hobbie et al. (2007). They concluded that the trends found of greater carbon allocation to mycorrhizal hyphae under elevated CO_2, or of increased carbon allocation to saprotrophic hyphae under elevated temperature would constrain overall system N availability. This would occur by favoring N uptake by the plants in the former situation, and N immobilization to support litter decomposition in the latter. Our findings are consistent with those found in the N-limited Duke Forest free-air CO_2 enrichment experiment, in that elevated CO_2 had little effect on carbon storage in woody tissues (Oren et al., 2001) and a very limited effect on increasing carbon storage in mineral soils in a loblolly pine forest without added nutrients (Schlesinger and Lichter, 2001).

More studies are needed to address the effects of elevated atmospheric CO_2 concentrations and associated climate change on carbon storage of Douglas-fir forests, specifically to understand the dynamic link between CO_2 fertilization and soil nutrient capital. In our 3-yr root decomposition study, roots decomposed significantly faster at the warmer (low elevation) site than at the colder (high elevation) site, suggesting global warming would enhance the decomposition process unless precipitation levels and/or soil moisture conditions were to become limiting. We need to understand better how the carbon and nitrogen cycles interact in such N-limited systems, which comprise about 30% of the Douglas-fir forests in the Oregon Cascade Mountains and which were represented by the reconstructed ecosystem studied herein.

Conclusions

After 4 yr of growth under conditions of elevated CO_2, roots of Douglas-fir seedlings had significantly increased water-soluble extractives concentration (%WSE) and nonpolar extractives concentration (%NPE). The elevated CO_2 conditions had little effect on the concentration of N, cellulose, and lignin of the root material. Elevated temperature showed no effect on root substrate quality except it increased %WSE and decreased %lignin of fine roots. Thus, exposure to elevated CO_2 and temperature slightly increased the substrate quality of Douglas-fir roots. No significant interactions were observed between CO_2 and temperature treatments on substrate quality of roots except for %WSE of fine roots. Short-term root decomposition (≤ 9 mo) in the field indicated that roots originating from the ambient CO_2–ambient temperature exposure decomposed the slowest compared with the roots from the other treatments. However, over a longer-term incubation period (9–36 mo) effects of initial substrate quality on root decomposition diminished. Instead, the incubation site significantly controlled root decomposition. This study suggests that increasing atmospheric CO_2 had little effect on the carbon storage of Douglas-fir old-growth forests of the Pacific Northwest.

Acknowledgments

We thank Jay Sexton for his technical assistance during the early phase of this study. We thank Mark Coleman for comments on an early draft of the manuscript. This study was supported by in part by National Science Foundation funding of the Andrews Forest Long-Term Ecological Research Program (DEB 0218088) and the Pacific Northwest Research Station. The senior author was also supported by a Collaborative Project Seed Funding program grant from the Univ. of Illinois at Springfield. The senior author thanks the U.S. Environmental Protection Agency in Corvallis for providing the roots used in this study. This study has been subjected to review by the National Health and Environmental Effects Research Lab.'s Western Ecology Div. and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

Angelis, P.D., K.S. Chigwerewe, and G.E.S. Mugnozza. 2000. Litter quality and decomposition in a CO_2–enriched Mediterranean forest ecosystem. Plant Soil 224:31–41.

Ball, A., and B.G. Drake. 1997. Short-term decomposition of litter produced by plants grown in ambient and elevated atmospheric CO_2 concentrations. Glob. Change Biol. 3:29–35.

Booker, F.L., S.A. Prior, H.A. Torbert, E.L. Fiscus, W.A. Pursley, and S. Hu. 2005. Decomposition of soybean grown under elevated concentrations of CO_2 and O_3. Glob. Change Biol. 11:685–698.

Chapin, F.S., P.A. Matson, and H.A. Mooney. 2002. Principles of terrestrial ecosystem ecology. Springer-Verlag, New York.

Chen, H., M.E. Harmon, J. Sexton, and B. Fasth. 2002. Fine-root decomposition and N dynamics in coniferous forests of the Pacific Northwest, USA. Can. J. For. Res. 32:320–331.

Chen, H., M.E. Harmon, and H.Q. Tian. 2001. Effects of global change on litter decomposition in terrestrial ecosystems. Acta Ecol. Sinica 21:22–37.

Cotrufo, M.F., P.D. Angelis, and A. Polle. 2005. Leaf litter production and decomposition in a poplar short-rotation coppice exposed to free air CO_2 enrichment (POPFACE). Glob. Change Biol. 11:971–981.

Cotrufo, M.F., and P. Ineson. 1995. Effects of enhanced atmospheric CO_2 and nutrient supply on the quality and subsequent decomposition of fine roots of *Betula pendula* Roth. and *P. icea sitchensis* (Bong.) Carr. Plant Soil 170:267–277.

Couteaux, M.M., C. Kurt, P. Bottner, and A. Raschi. 1999. Influence of increased atmospheric CO_2 concentration on quality of plant material and litter decomposition. Tree Physiol. 19:301–311.

Dilustro, J.J., F.P. Day, and B.G. Brake. 2001. Effects of elevated atmospheric CO_2 on root decomposition in a scrub oak ecosystem. Glob. Change Biol. 7:581–589.

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

Graaff, M.D., J. Six, D. Harris, H. Blum, and C.V. Kessel. 2004.

Decomposition of soil and plant carbon from pasture system after 9 years of exposure to elevated CO_2: Impact on C cycling and modeling. Glob. Change Biol. 10:1922–1935.

Effland, M.J. 1977. Modified procedure to determine acid insoluble lignin in wood and pulp. TAPPI 60:143–144.

Finzi, A.C., A.S. Allen, E.H. Delucia, D.S. Ellsworth, and W.H. Schlesinger. 2001. Forest litter production, chemistry, and decomposition following two years of free-air CO_2 enrichment. Ecology 82:470–484.

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.

Franck, V.M., B.A. Hungate, F.S. Chapin, III, and C.B. Field. 1997. Decomposition of litter produced under elevated CO_2: Dependence on plant species and nutrient supply. Biogeochemistry 36:223–237.

Gorissen, A., and M.F. Cotrufo. 2000. Decomposition of leaf and root tissue of three perennial grass species grown at two levels of atmospheric CO_2 and N supply. Plant Soil 224:75–84.

Heal, O.W., J.M. Anderson, and M.J. Swift. 1997. Plant litter quality and decomposition: An historical overview. p. 3–32. *In* G. Cadisch and K.E. Giller (ed.) Driven by nature: Plant litter quality and decomposition. CAB International.

Henry, H.A.L., E.E. Cleland, C.B. Field, and P.M. Vitousek. 2005. Interactive effects of elevated CO_2, N deposition, and climate change on plant litter quality in a California annual grassland. Oecologia 142:465–473.

Hirschel, G., C. Korner, and J.A. Arnone, III. 1997. Will rising atmospheric CO_2 affect leaf litter quality and in situ decomposition rates in native plant communities? Oecologia 110:387–392.

Hobbie, E.A., M.G. Johnson, P.T. Rygiewicz, D.T. Tingey, and D.M. Olszyk. 2004. Isotopic estimates of new carbon inputs into litter and soils in a four-year climate change experiment with Douglas-fir. Plant Soil 259:331–343.

Hobbie, E.A., P.T. Rygiewicz, M.G. Johnson, and A.R. Moldenke. 2007. ^{13}C and ^{15}N in microarthropods reveal little response of Douglas-fir ecosystems to climate change. Glob. Change Biol. 13:1386–1397.

Hu, S.J., M.K. Firestone, and F.S. Chapin, III. 1999. Soil microbial feedback to atmospheric CO_2 enrichment. Trends Ecol. Evol. 14:433–437.

IPCC. 2001. Climate Change 2001: The scientific basis. *In* J.T. Houghton et al. (ed.) Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge Univ. Press, Cambridge, UK and New York.

Johnson, D.W., W. Cheng, and J.T. Ball. 2000. Effects of CO_2 and N fertilization on decomposition and N immobilization in ponderosa pine litter. Plant Soil 224:115–122.

Johnson, D.W., W. Cheng, J.D. Joslin, R.J. Norby, N.T. Edwards, and D.E. Todd. 2004. Effects of elevated CO_2 on nutrient cycling in a sweetgum plantation. Biogeochemistry 69:379–403.

Johnson, M.G., P.T. Rygiewicz, D.T. Tingey, and D.L. Phillips. 2006. Elevated CO_2 and elevated temperature have no effect on Douglas-fir fine-root dynamics in nitrogen-poor soil. New Phytol. 170:345–356.

King, J.S., K.S. Pregitzer, D.R. Zak, W.E. Holmes, and K. Schmidt. 2005. Fine root chemistry and decomposition in model communities of north-temperate tree species show little responses to elevated atmospheric CO_2 and varying soil resource availability. Oecologia 146:318–328.

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

Lewis, J.D., M. Lucas, D. Olszyk, and D.T. Tingey. 2001. Seasonal patterns of photosynthesis in Douglas-fir seedlings during the third and fourth year of exposure to elevated carbon dioxide and temperature. Plant Cell Environ. 24:539–548.

Lewis, J.D., M. Lucas, D. Olszyk, and D.T. Tingey. 2004. Relationships between needle nitrogen concentration and photosynthetic responses of Douglas-fir seedlings to elevated CO_2 and temperature. New Phytol. 162:355–364.

Lin, G., J.R. Ehleringer, P.T. Rygiewicz, M.E. Johnson, and D.T. Tingey. 1999. Elevated CO_2 and temperature impacts on different components of soil CO_2 efflux in Douglas-fir terracosms. Global Change Biol. 5:157–168.

Lin, G., P.T. Rygiewicz, J.R. Ehleringer, M.E. Johnson, and D.T. Tingey. 2001. Time-dependent response of soil CO_2 efflux components to elevated atmospheric CO_2 and temperature in experimental forest mesocosms. Plant Soil 229:259–270.

Little, E.L. 1971. Atlas of United States trees. Vol. 1. Conifers and important hardwoods. USDA Forest Service Publication 1146. USDA, Washington, DC.

Lutze, J.L., R.M. Gifford, and H.N. Adams. 2000. Litter quality and decomposition in *Danthonia richardsonii* swards in response to CO_2 and nitrogen supply over four years of growth. Glob. Change Biol. 6:13–24.

Matamala, R., and W.H. Schlesinger. 2000. Effects of elevated atmospheric CO_2 on fine root production and activity in an intact temperate forest ecosystem. Glob. Change Biol. 6:967–979.

Melillo, J.M., P.A. Steudler, J.D. Aber, K. Newkirk, H. Lux, F.P. Bowles, C. Catricala, A. Magill, T. Ahrens, and S. Morrisseau. 2002. Soil warming and carbon-cycle feedbacks to the climate systems. Science 298:2173–2176.

Mooney, H.A., B.G. Drake, R.J. Luxmoore, W.C. Oechel, and L.F. Pitelka. 1991. Predicting ecosystem responses to elevated CO_2 concentrations. Bioscience 41:96–104.

Norby, R.J., and M.F. Cotrufo. 1998. A question of litter quality. Nature 396:17–18.

Norby, R.J., M.F. Cotrufo, P. Ineson, E.G.O. Neill, and J.G. Canadell. 2001. Elevated CO_2, litter chemistry, and decomposition: A synthesis. Oecologia 127:153–165.

Norby, R.J., and Y.Q. Luo. 2004. Evaluating ecosystem responses to rising atmospheric CO_2 and global warming in a multi-factor world. New Phytol. 162:281–293.

Olszyk, D.M., M.G. Johnson, D.T. Tingey, P.T. Rygiewicz, C. Wise, E. VanEss, A. Benson, M.J. Storm, and R. King. 2003. Whole-seedling biomass allocation, leaf area, and tissue chemistry for Douglas-fir exposed to elevated CO_2 and temperature for four years. Can. J. For. Res. 33:269–278.

Oren, R., D.S. Ellsworth, K.H. Johnsen, N. Phillips, B.E. Ewers, C. Maier, K.V.R. Schafer, H. McCarthy, G. Hendrey, S.G. McNulty, and G.G. Katul. 2001. Soil fertility limits carbon sequestration by forest ecosystem in a CO_2–enriched atmosphere. Nature 411:469–472.

Parsons, W.J., B.J. Kopper, and R.L. Lindroth. 2003. Altered growth and fine root chemistry of *Betula papyrifera* and *Acer saccharum* under elevated CO_2. Can. J. For. Res. 33:842–846.

Parsons, W.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.

Robinson, C.H., A. Michelsen, J.A. Lee, S.J. Whitehead, T.V. Callaghan, M.C. Press, and S. Jonasson. 1997. Elevated atmospheric CO_2 affects decomposition of *Festuca vivipara* (L) Sm. litter and roots in experiments simulating environmental change in two arctic ecosystems. Glob. Change Biol. 3:37–49.

Rustad, L.E., J.L. Campbell, G.M. Marion, R. Norby, M. Mitchell, A. Hartley, J. Cornelissen, J. Gurevitch, and GCTE-NEWS. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126:543–562.

Schlesinger, W.H., and J. Lichter. 2001. Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO_2. Nature 411:466–469.

Strain, B.R., and F.A. Bazzaz. 1983. p. 177–222. *In* E.R. Lemon (ed.) CO_2 and plants: The responses of plants to rising levels of atmospheric carbon dioxide. Westview, Boulder, CO.

SAS Institute Inc. 2002. The SAS System for Windows, version 7 ed. SAS Institute Inc., Cary, NC.

Tingey, D.T., E.H. Lee, R. Waschmann, M.G. Johnson, and P.T. Rygiewicz. 2006. Does soil CO_2 efflux acclimatize to elevated temperature and CO_2 during long-term treatment of Douglas-fir seedlings? New Phytol. 170:108–117.

Tingey, D.T., R.B. McKane, D.M. Olszyk, M.G. Johnson, P.T. Rygiewicz, and E.H. Lee. 2003. Elevated CO_2 and temperature alter nitrogen allocation in Douglas-fir. Glob. Change Biol. 9:1038–1050.

Tingey, D.T., B.D. McVeety, R. Waschmann, M.G. Johnson, D.L. Phillips, P.T. Rygiewicz, and D.M. Olszyk. 1996. A versatile sun-lit controlled-environment facility for studying plant and soil processes. J. Environ. Qual. 25:614–625.

Tingey, D.T., D.L. Phillips, M.G. Johnson, P.T. Rygiewicz, P.A. Beedlow, and W.E. Hogsett. 2005. Estimates of Douglas-fir fine root production and mortality from minirhizotrons. For. Ecol. Manage. 204:359–370.

Weatherly, H.E., S.F. Zitzer, J.S. Coleman, and J.A. Arnone III. 2003. In situ litter decomposition and litter quality in a Mojave Desert ecosystem: Effects of elevated atmospheric CO_2 and interannual climate variability. Glob. Change Biol. 9:1223–1233.

Weltzin, J.F., and D.T. Tissue. 2003. Resources pulses in arid environments—Patterns of rain, patterns of life. New Phytol. 157:167–173.