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Time-dependent responses of soil CO_2 efflux components to elevated atmospheric [CO_2] and temperature in experimental forest mesocosms

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Abstract

We previously used dual stable isotope techniques to partition soil CO_2 efflux into three source components (rhizosphere respiration, litter decomposition, and soil organic matter (SOM) oxidation) using experimental chambers planted with Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings. The components responded differently to elevated CO_2 (ambient + 200 μmol mol^{-1}) and elevated temperature (ambient + 4 °C) treatments during the first year. Rhizosphere respiration increased most under elevated CO_2, and SOM oxidation increased most under elevated temperature. However, many studies show that plants and soil processes can respond to altered climates in a transient way. Herein, we extend our analysis to 2 years to evaluate the stability of the responses of the source components. Total soil CO_2 efflux increased significantly under elevated CO_2 and elevated temperature in both years (1994 and 1995), but the enhancement was much less in 1995. Rhizosphere respiration increased less under elevated temperature in 1995 compared with 1994. Litter decomposition also tended to increase comparatively less in 1995 under elevated CO_2, but was unresponsive to elevated temperature between years. In contrast, SOM oxidation was similar under elevated CO_2 in the 2 years. Less SOM oxidation occurred under elevated temperature in 1995 compared with 1994. Our results indicate that temporal variations can occur in CO_2 production by the sources. The variations likely involve responses to antecedent physical disruption of the soil and physiological processes.

Abbreviations: ANOVA – Analysis of variance; C – Carbon; CO_2 – Carbon dioxide; FDA – fluorescein diacetate; HCl – Hydrochloric acid; O – Oxygen; ‰ – Parts per thousand; PDB – Pee Dee Belemnite; PVC – polyvinyl chloride; SE – Standard error; SOC – Soil organic carbon; SOM – Soil organic matter; V-SMOW – Vienna Standard Mean Ocean Water

Introduction

The gradual increase in atmospheric CO_2 concentration and potential climatic changes are likely to affect plant, soil and ecosystem processes, including carbon (C) flux from plants to soil and from soil to atmosphere (Curtis et al., 1994; Pajari, 1995; Peterjohn et al., 1993; Vose et al., 1995). In a typical forest ecosystem, the components of soil CO_2 efflux include (1) respiration due to litter decomposition, (2) root respiration, (3) rhizo-microbial respiration (i.e. microbial respiration utilizing C directly derived from living roots), and (4) microbial respiration utilizing native soil organic matter (SOM) (Cheng, 1999). Because the C require-

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ments, trophic position and requisite soil physical and chemical properties of the organisms responsible for producing soil CO_2 differ significantly, we expect that the soil CO_2 efflux components will respond differently to changes in atmospheric CO_2 concentration and climatic conditions. Although several studies have examined the effects of elevated atmospheric CO_2 concentration or temperature on soil CO_2 efflux in a number of forest ecosystems (e.g. Peterjohn et al., 1993; Pajari, 1995; Van Veen et al., 1991; Vose et al., 1995; 1997), there is very limited information available concerning which components of the efflux are most sensitive to changes in these climatic drivers (see reviews by Cheng, 1999; Paterson et al., 1997).

Lin et al. (1999) showed that three components of soil CO_2 efflux (root/rhizo-microbial respiration, respiration due to litter decomposition, and respiration from oxidation of SOM) in our Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco *var. menziesii*) chambers could be partitioned by using a dual stable isotope approach that combined analyzing stable isotope ratios of the C of plants, litter and SOM, and the oxygen (O) in soil-respired CO_2 and soil water at different depths. We tested and demonstrated the feasibility of this approach by examining the effects of elevated atmospheric CO_2 concentration and temperature treatments on the different components of soil CO_2 efflux during the first year of exposure to the treatments. First-year results indicated that rhizosphere respiration increased the most under elevated CO_2, while SOM oxidation was largely affected by elevated temperature. However, it is not clear whether the responses we observed in the first year would be maintained over a longer period of exposure.

Under conditions of elevated atmospheric CO_2 concentrations and associated climate change variables, numerous processes and potential interactions can occur that will affect the overall acquisition and allocation of C in ecosystems. Included in these processes, and interactions among them, are (1) the biphasic response (i.e. downregulation as described by Rogers et al., 1994) for physiological processes, (2) the potential for higher root growth, root respiration and root exudation (Curtis et al., 1994; Johnson et al., 1994; Norby et al., 1992; Rogers et al., 1994), and (3) increased allocation of labile C to soil as substrate for microbial processes relative to the total and available nitrogen in soil (Zak et al., 1993; review by Cheng, 1999; Zak and Pregitzer, 1998). Determining the fate of C in ecosystems is further complicated by the number of pools and fluxes of C in the soil foodweb, the time-dependent movement of C from the primary producers through all the consumer pools, and the great variability in processing rates and life cycles of foodweb organisms. Our chamber experiment using Douglas-fir seedlings and a reconstructed soil provided the opportunity to evaluate several of these processes and interactions that affect the temporal fate of C through a low nitrogen system. In this study, we focused our attention to some of the processes and interactions indicated above, at a system level, by evaluating longer-term responses (2 years). We expected that total soil CO_2 efflux and its three components could respond differently to the climate treatments in the 2 years. Some of the results in Lin et al. (1999) are presented herein to make the longer-term analysis more straightforward.

Materials and methods

Description of Douglas-fir mesocosms

The study was conducted using 12 sun-lit controlled-environment chambers at the US Environmental Protection Agency's Western Ecology Division Laboratory in Corvallis, Oregon, USA. Detailed descriptions of the facility, its monitoring equipment and performance can be found in Tingey et al. (1996), Apple et al. (1998) and Lin et al. (1999). Each chamber has a canopy volume of 3.18 m^3 and a soil lysimeter volume of 2.0 m^3 (2 m wide $\times$ 1 m front-to-back $\times$ 1 m deep). Fourteen Douglas-fir seedlings (1+1) were grown in reconstructed native forest soil in each mesocosm. Randomly-chosen chambers were assigned to one of the following climatic treatments with three replicates (mesocosms) each: (1) ambient CO_2 and ambient temperature, (2) elevated CO_2 (ambient plus 200 μmol mol^{-1}) and ambient temperature, (3) ambient CO_2 and elevated temperature (ambient plus 4 °C), and (4) elevated CO_2 and elevated temperature. Soil temperature was not controlled but followed the changes in air temperature in each mesocosm.

The soil used had a medium granular structure and was classified as a coarse-loamy, mixed, frigid Typic Hapludand. A detailed description of how the native forest soil was obtained, processed and placed into the chambers can be found in Rygiewicz et al. (2000). Briefly, in 1992 the B and C horizons of the soil were removed from an old-growth Douglas-fir forest situated in the Cascade Mountains in Oregon, transported to Corvallis, sieved to 2.5 cm, and then placed in

the soil lysimeters maintaining the master horizons. We allowed these horizons to rest and recover for 14 months. During 1992, the chambers were planted with the nitrogen-fixing cover crop *Vicia villosa* Roth. to provide inputs of carbon, sequester mineralized nitrogen and develop vertical channels for improved hydraulic properties. The cover crop and the top 2 cm of the B horizon were removed in May 1993. In June 1993, the A horizon of the soil was removed from the same general location in the Cascade Mountains from which the deeper horizons were removed, and subsequently it was processed in the same manner as were the deeper horizons. Within 72 h of removing the A horizon from the old growth site, it was placed into the soil lysimeters and the seedlings were planted. Forest floor (the litter layer consisting of Oi, Oe and Oa horizons) was collected from an old-growth Douglas-fir forest, adjacent to the soil collection sites, and sieved to 2.5 cm. Shortly after the trees were planted, a similar mass of homogenized, sieved forest floor was added to each chamber to achieve a litter layer thickness of 6 cm (Rygiewicz et al., 2000). The initially uniform litter layer material became colonized by fungi, bacteria and mesofauna of the foodweb of the reconstructed mineral soil and forest floor such that Oi and Oa horizons developed during the exposure period.

Seedlings, litter and soil were exposed to the climatic treatments beginning in August 1993. At the beginning of the exposure, soil C content averaged 2.8% for the A horizon and 2.4% for the B horizon. Throughout the exposure, the seedlings were irrigated with the same source of local purified water (reverse osmosis) to maintain typical seasonal patterns in soil moisture, i.e. soil moisture was highest in the winter and gradually decreased to a minimum during late summer (Tingey et al., 1996).

Because the chambers were maintained as closed systems most of the time (opened only for maintenance and taking samples or physiological measurements), commercial tank CO_2 and dump valves were used to maintain the desired CO_2 concentrations in all chambers (Lin et al., 1999; Tingey et al., 1996). Efforts were made to use tank CO_2 with relatively low and constant ^{13}C content throughout the exposure period. Consequently, photosynthate produced in the chambers and allocated to leaves, roots, microbes, fauna and soils had different ^{13}C contents than the amounts in the materials formed in the Cascade Mountains and used to initiate the experiment. Repeated measurements of the tank CO_2 and outside atmosphere confirmed that isotope compositions of tank and outside atmospheric CO_2 remained constant throughout the study period. The mean $\delta^{13}C$ values (see below for δ definition) of tank CO_2 and outside atmosphere were -35.77 ± 0.09 ‰ (n=8) and -8.55 ± 0.13 ‰ (n=8), respectively.

The air temperature in each chamber was maintained at treatment levels by using electrical strip heaters and chilled-water heat exchangers. Increases in soil temperature in the elevated temperature treatments occurred indirectly and were caused by heating the air in the headspace of the chambers (Tingey et al., 1996). Chamber soil temperatures followed day-to-day and seasonal variations in air temperature and solar radiation, and were similar to soil temperatures measured near the terracosms, indicating that chamber soil temperatures were typical for the area (Tingey et al., 1996). Also, chamber soil temperatures were similar to those we measured in plantations of Douglas-fir seedlings of similar age and located in the central Oregon Cascade Mountains (unpublished data).

Soil CO_2 efflux measurements

During eight sampling periods in 1994 and 1995, soil CO_2 efflux in each chamber was measured at two locations using a LI-6000-09 soil respiration chamber (LI-COR Inc.) and PVC collars (10 cm diameter $\times$ 7 cm height). PVC collars were installed in late March 1994 through the forest floor/litter layer to a depth of 5 cm in the mineral soil and left undisturbed throughout the 2-year study. At each sampling period, the change in CO_2 concentration in the PVC collars over 1–2 min was measured using the CO_2 analyzer of a LI-6200 portable photosynthesis system to calculate soil CO_2 efflux. In addition, soil temperature at 5-cm depth was measured with a thermocouple sensor attached to the LI-6000-09 soil respiration chamber.

Isotope sampling and analysis

After soil CO_2 efflux was measured, CO_2 released from the soil was collected. A water trap in a 2-L air flask (which had been previously filled with dry nitrogen gas) was inserted in series in the closed-loop of the LI-6200 system and LI-6000-09 soil respiration chamber (Lin et al., 1999). Chamber atmospheric CO_2 captured in the PVC (polyvinyl chloride) collars was removed using the soda lime scrubber in the by-pass system of the measurement equipment. Depending on the soil CO_2 efflux, 20–45 min were needed to collect CO_2 to a concentration of 350 μmol mol^{-1} in

the air flasks. After filling with CO_2 released from the soil, the air flasks were moved to a vacuum extraction line and the collected CO_2 was extracted cryogenically within 10 min. The CO_2 samples were analyzed for both $\delta^{13}C$ and $\delta^{18}O$ on an isotope ratio mass spectrometer (either a Delta S, Finnigan MAT, Breman; or a PRISM II, VG Isotope Inc., Manchester – both operated in dual inlet mode). Results are expressed in delta notation ($\delta^{13}C$ or $\delta^{18}O$) in ‰ (parts per thousand) relative to the international standard (Pee Dee Belemnite (PDB) for C, and Vienna Standard Mean Ocean Water (V-SMOW) for oxygen):

$$\delta\ (‰) = (R_{sam}/R_{std} - 1) * 1000 \quad (1)$$

where R_{sam} and R_{std} are $^{13}C/^{12}C$ or $^{18}O/^{16}O$ for the sample and standard, respectively. The external precision was ±0.11 ‰ based on repeated measurements of several standards. The two mass spectrometers were cross-calibrated using a common CO_2 standard and the maximum deviation was < ±0.1 ‰.

Prior to planting the seedlings, four replicates of mature needles and of roots of two size classes (0–1 mm, 1–2 mm) were collected. Also, litter samples and soil samples at four depths (A, B1, B2 and C horizons) were collected from four randomly-selected mesocosms. These T_0 samples were dried and stored until they were analyzed for C isotope ratios.

On the same days that soil CO_2 efflux samples were collected, newly-formed needles from six seedlings were collected in each mesocosm, pooled and then immediately frozen. Litter (0–6 cm below the surface of the litter) from four positions surrounding each soil collar was collected, pooled and then sealed in screw-cap vials with parafilm. Similarly, mineral soil from the A horizon (6–14 cm below the litter surface) was collected from four positions around the soil collars, pooled and then sealed in screw-cap vials. In addition, twice each year, soil samples of the A, B1, B2 and C horizons and root samples from two size classes (0–1 mm, 1–2 mm) were collected from each chamber using a soil coring device (see Rygiewicz et al., 2000 for details).

The needle and root samples were dried at 70 °C for 48 h and then ground to pass through a 60-mesh sieve. For the litter and soil samples, water was cryogenically extracted in a vacuum line and then stored at 4 °C for O isotope analysis. Following this, each dried litter and soil sample was ground to pass through a 60-mesh sieve and stored for C isotope analyses. Prior to grinding, soil samples were cleaned of obvious plant materials and then treated with 1 N HCl to remove any carbonates.

The C isotope ratio of organic materials (needles, roots, liter, soil) was determined using the Delta S isotope ratio mass spectrometer (during 1994) or the Optima isotope ratio mass spectrometer (during 1995). The external precision for C isotope analyses of these samples was ±0.16 ‰. The O isotope ratios ($\delta^{18}O$) of litter and soil water were determined with the CO_2-H_2O equilibrium method modified by Socki et al. (1992). The external precision for the O isotope analysis was ±0.23 ‰.

Partitioning of soil CO_2 efflux

Partitioning the soil CO_2 efflux into three component sources was done using a 3-end-member triangular model for $\delta^{13}C$ values and a 2-end-member linear model for $\delta^{18}O$ values (Lin et al., 1999). In order to partition soil CO_2 efflux, calculations were made for the expected C and O isotope ratios of the different C sources in the soil profile. We assumed that (1) CO_2 flux from litter decomposition had the same $\delta^{13}C$ as litter, (2) CO_2 from the root/rhizosphere respiration had similar $\delta^{13}C$ to the new needles, (3) SOM CO_2 flux had the same $\delta^{13}C$ as bulk soil organic carbon (SOC) in the surface layer, (4) $\delta^{18}O$ of litter CO_2 flux was in equilibrium with litter water, and (5) $\delta^{18}O$ of CO_2 from root/rhizosphere respiration and SOM oxidation was in equilibrium with soil water (see details in Lin et al., 1999). The isotope compositions of CO_2 released from the soil were used to constrain the relative contributions of the different source components, and the absolute rates of these components were estimated by multiplying the percentage of their contribution with the total soil CO_2 efflux.

During both years, we did not observe a large percentage of the total root mass in the litter layer in the chambers (see '*Responses of rhizosphere respiration*', 'Discussion' section). We did not have a reliable method to separate root respiration from rhizomicrobial respiration because the substrate C used in the two processes had similar stable isotope ratios. Although root turnover may be a significant component of soil CO_2 efflux, our method could not account quantitatively for this process because the isotope signals of CO_2 from root turnover could be similar to those of either SOM oxidation or root/rhizosphere respiration. Thus, we partitioned soil CO_2 efflux into three major components: (1) litter decomposition, (2) rhizosphere respiration (root respiration plus micro-

bial respiration of C directly derived from the roots), and (3) SOM oxidation.

Statistical analyses

Temporal changes in soil CO_2 efflux and its isotope compositions, and the isotope compositions of plant tissues and soils were evaluated using one-way analysis of variance (ANOVA). Effects of elevated CO_2 and temperature on soil CO_2 efflux, and the partitioning of released CO_2 into its component source over the eight sampling dates were evaluated using 3-way ANOVA for each year. An additional 4-way ANOVA was done for all the data combined. Differences in isotope compositions of the CO_2 released from soils, the newly-formed needles, litter, SOM and water in the litter and soil among the four climatic treatments were evaluated using paired *t*-tests. All statistical analyses were performed using the SYSTAT 7.0 software package (SPSS Inc., Chicago).

Results

Carbon isotope ratios of needles, litter, roots and soils

The initial mean $\delta^{13}C$ value of Douglas-fir needles was -27.5 ± 0.2 ‰ (n=4). During exposure to the ^{13}C-depleted CO_2 of the source (tank CO_2), the seedlings attained progressively lower $\delta^{13}C$ values in the newly-formed needles (Figure 1A). By the end of 1995, mean $\delta^{13}C$ values for needles were -32.5 ± 0.3 ‰ in the control chambers, -38.5 ± 0.2 ‰ under elevated CO_2, -33.8 ± 0.1 ‰ under elevated temperature, and -38.3 ± 0.3 ‰ under elevated CO_2 and elevated temperature. Differences in $\delta^{13}C$ values between the two CO_2 treatments were highly significant ($p<0.001$), but there were no significant differences in $\delta^{13}C$ values between the two temperature treatments under either of the two CO_2 treatments ($p>0.05$). The $\delta^{13}C$ values of the litter were not significantly different among the treatments nor among the sampling dates (Figure 1B). In fine roots (<1 mm), $\delta^{13}C$ values decreased significantly during exposure to the treatments, from -27.0 ± 0.2 ‰ at the beginning, to -30.1 ± 0.1 ‰ (control), -35.3 ± 0.1 ‰ (elevated CO_2), -31.1 ± 0.2 ‰ (elevated temperature), and -34.7 ± 0.5 ‰ (elevated CO_2 and elevated temperature) at the end of the study (Figure 1C). The $\delta^{13}C$ values of roots in the elevated CO_2 treatments decreased more compared with the change in values for the ambient CO_2 treatments. Differences in $\delta^{13}C$ values of fine roots between CO_2 treatments were highly significant ($p<0.001$), but there were no significant differences in $\delta^{13}C$ values of roots between temperature treatments ($p>0.05$). The $\delta^{13}C$ values of 1-2 mm roots also decreased during the exposure period. Differences were significant between the two CO_2 treatments but not between the two temperature treatments (Figure 1D).

The $\delta^{13}C$ values of SOC in all horizons were not significantly different between either CO_2 treatments or temperature treatments ($p>0.05$, Figure 2). The A and B1 horizons decreased slightly in $\delta^{13}C$ values (1–1.5 ‰) during the exposure period (Figure 2A, B). The $\delta^{13}C$ values of soil in the B2 and C horizons did not significantly change during the same period, except for the last sampling date when $\delta^{13}C$ apparently started to decrease (Figure 2C, D).

Oxygen isotope ratios of litter and soil water

On individual sampling dates, there were no significant differences in $\delta^{18}O$ values for litter water among treatments (Figure 3A). In 1994, there was a strong seasonal trend in $\delta^{18}O$ values of litter water in all treatments ($p<0.001$), increasing from April to August and then decreasing from August to October. In 1995, the $\delta^{18}O$ values of litter water again decreased significantly (-5 ‰ to -7 ‰) during the growing season ($p<0.01$). The $\delta^{18}O$ values for soil water in the upper part of the A horizon were not significantly different among the four treatments, nor among the four sampling dates within each year (Figure 3B). The $\delta^{18}O$ values for soil water in the A horizon in 1995 (-10.2 to -9.3 ‰) were slightly higher than in 1994 (-12.1 to -11.2 ‰). The $\delta^{18}O$ values of soil water were significantly lower than those of litter water in all treatments across all sampling dates ($p<0.001$).

Soil temperature and soil CO_2 efflux rates

Soil temperature at 5 cm depth in the A horizon varied seasonally, increasing from April to either June or August, and then decreasing thereafter in both years (Figure 4A). Within each temperature treatment, soil temperatures were not affected by the CO_2 treatment. However, there were consistent differences in soil temperature between the ambient and elevated temperature treatments ($p<0.001$). Differences in soil temperatures between the two temperature treatments (2.1 – 2.9 °C) were slightly lower than differences in air temperatures between treatments (3.7 – 3.9 °C). This was due to the indirect heating of the soil since only the air temperatures of the headspace in the

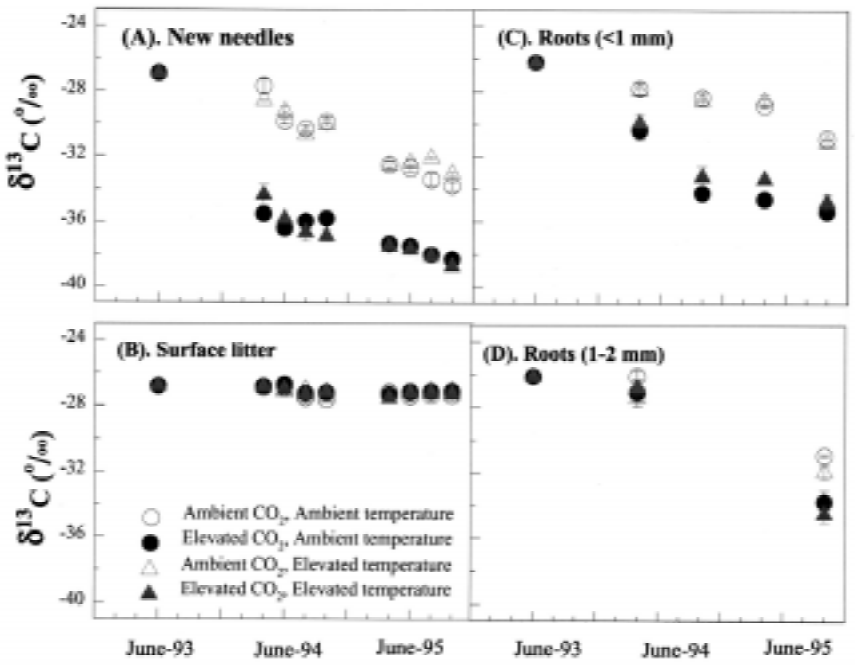

Figure 1. Changes in C isotope ratios ($\delta^{13}C$) of newly-formed needles (A), surface litter (B), and roots of two size classes (C, D) in the chambers planted with Douglas-fir seedlings under different CO_2 and temperature treatments. Values are the mean ± SE (n=3).

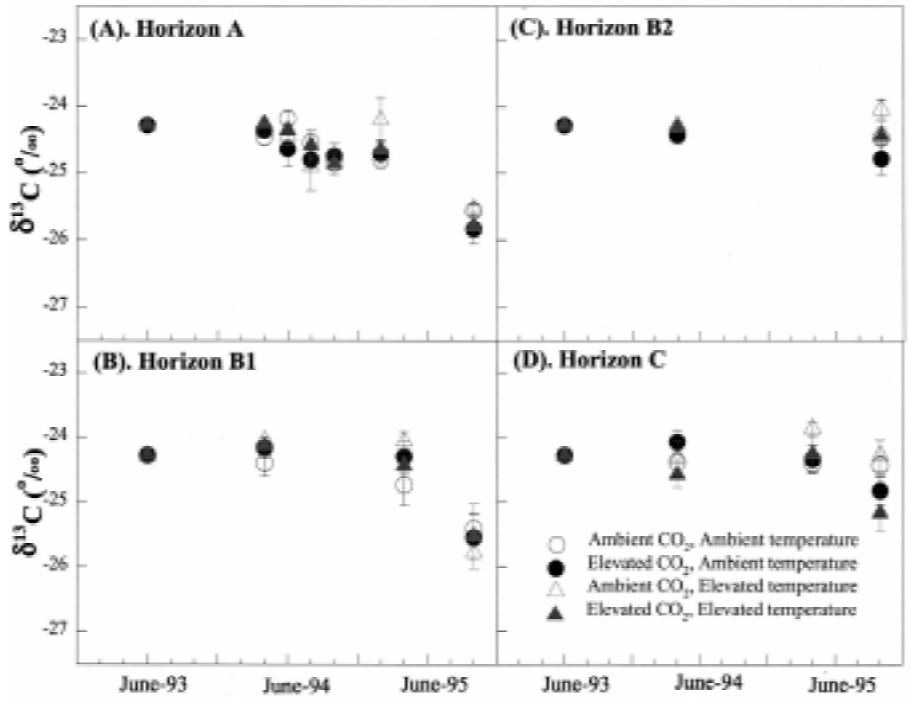

Figure 2. Changes in soil organic C in the soil horizons planted with Douglas-fir seedlings under different CO_2 and temperature treatments. Soil depths for each horizon are: A horizon, 10 cm; B1 horizon, 28 cm; B2 horizon, 30 cm; and C horizon, 20 cm. Notation is the same as in Figure 1.

Table 1. F values and significance levels of differences (ns: not significant at $p>0.05$; *: significant at $p<0.05$; **: significant at $p<0.01$; and ***: significant at $p<0.0001$) for the ANOVA analyses on effects of elevated CO_2, temperature, sampling month and year on total soil CO_2 efflux and its three component sources in the Douglas-fir seedling/soil system

Year	Factor	df	Rhizosphere Respiration	Litter Decomposition	SOM Oxidation	Total Soil CO_2 Efflux
1994	CO_2 (C)	1	37.38***	9.75**	25.27***	16.80***
	Temp (T)	1	11.12**	41.14***	30.38***	68.88***
	Month (M)	3	4.39*	23.42***	13.90***	16.19***
	C*T	1	0.01^{ns}	0.78^{ns}	6.45*	0.01^{ns}
	C*M	3	1.45^{ns}	0.06^{ns}	4.43*	0.29^{ns}
	T*M	3	7.17*	2.53^{ns}	12.50***	2.34^{ns}
	C*T*M	3	0.92^{ns}	0.68^{ns}	4.13*	1.73^{ns}
1995	CO_2 (C)	1	102.8***	3.41^{ns}	0.79^{ns}	29.21***
	Temp (T)	1	1.71^{ns}	20.48***	27.57***	19.26***
	Month (M)	3	43.48***	50.94***	17.56***	97.46***
	C*T	1	12.95**	0.52^{ns}	0.40^{ns}	2.09^{ns}
	C*M	3	5.12**	2.06^{ns}	2.13^{ns}	2.23^{ns}
	T*M	3	6.12**	2.09^{ns}	5.32*	1.47^{ns}
	C*T*M	3	10.58***	0.70^{ns}	7.75**	1.99^{ns}
All	CO_2 (C)	1	138.4***	18.57***	13.49***	45.96***
	Temp (T)	1	9.14**	54.82***	50.63***	70.27***
	Month (M)	3	33.94***	73.47***	8.94***	107.0***
	Year (Y)	1	35.06***	96.46***	46.16***	166.0***

chambers were controlled (see Tingey et al., 1996 for details).

During both years and under all treatments, there was a seasonal trend in soil CO_2 efflux (Table 1), with the highest rates occurring in June–August (Figure 4B). The efflux was higher in summer 1995 than in summer 1994. Mean rates were 20% greater under the elevated CO_2 treatments, 26% greater under the elevated temperature treatments and 54% greater under elevated CO_2 and elevated temperature treatments compared with the ambient treatment. There were no significant interactions between CO_2 and temperature treatments for total soil CO_2 efflux (Table 1).

Responses of rhizosphere respiration, litter decomposition and SOM oxidation

Litter decomposition was the dominant component of soil respiration, accounting for 60 – 65% of total soil CO_2efflux in both years (Figure 5). Carbon dioxide derived from root/rhizosphere respiration accounted for 23 – 32% in year 1 and 16 – 31% in year 2, while respiration from SOM oxidation accounted for 8 – 18% in year 1 and 7 – 16% in year 2.

Among the three components, rhizosphere respiration increased under both elevated CO_2 and elevated temperature treatments (Figure 5A, Table 1). Rhizosphere respiration also varied among the different sampling months in both years (Table 1). Litter decomposition, however, was affected more by elevated temperature than by elevated CO_2 (Figure 5B, Table 1). The effect of elevated CO_2 on litter decomposition was apparent only in 1994. Similar to litter decomposition, SOM oxidation increased mainly under elevated temperature (Figure 5C, Table 1).

Comparison of treatment effects between 1994 and 1995

Effects of elevated CO_2 and temperature were significantly less in 1995 than in 1994; the exception being that total soil CO_2 efflux values under elevated CO_2 were similar between the 2 years (Figure 6). On average, elevated CO_2 enhanced rhizosphere respiration by 81% and litter decomposition by 14%,

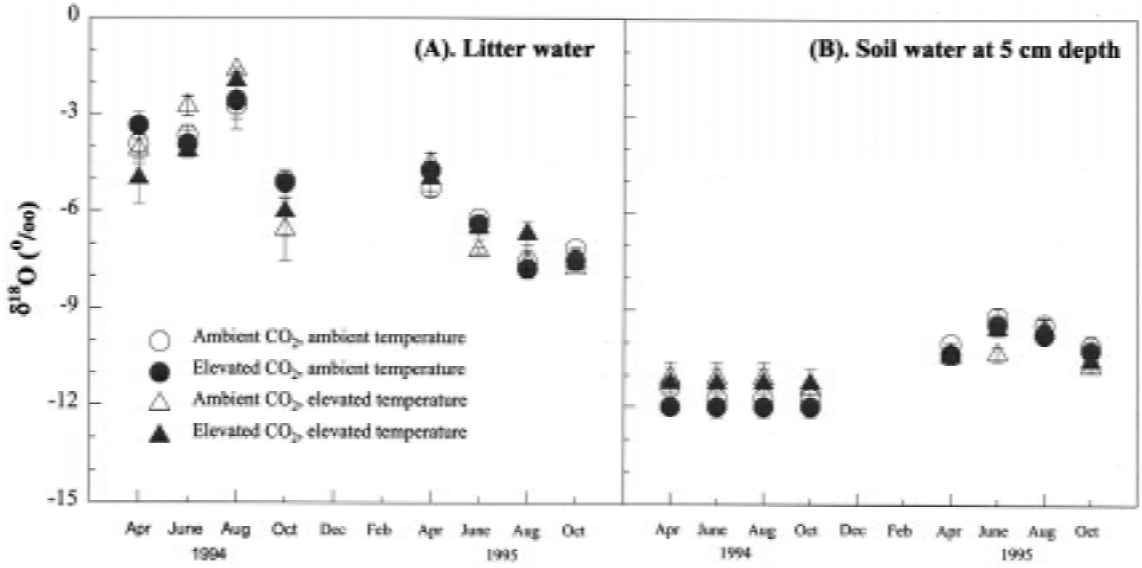

Figure 3. Oxygen isotope ratios ($\delta^{18}O$) of the litter water (A), and soil water at 5 cm depth (B) in the chambers planted with Douglas-fir seedlings under different CO_2 and temperature treatments. Notation is the same as in Figure 1.

but reduced SOM oxidation by 36% in 1994. One year later, the corresponding changes were 61%, 4% and −44%, respectively. For total soil CO_2 efflux, the stimulation by elevated temperature in 1995 was about one third of that calculated for 1994. Elevated temperature in 1994 increased rhizosphere respiration by 43%, litter decomposition by 34% and SOM oxidation by 116%, while in 1995, the corresponding enhancements were reduced substantially for rhizosphere respiration (to 28%) and for SOM oxidation (to 14%). Litter decomposition under elevated CO_2 in 1995 was not significantly different from that under the ambient treatment, while in 1994 the former was 16% higher. The combined effects of elevated CO_2 and elevated temperature were not significantly different between the two years for rhizosphere respiration (125% vs. 115%) and litter decomposition (59% vs. 51%), while SOM oxidation showed a 10% enhancement in 1994 but a 30% reduction in 1995.

Discussion

Responses of rhizosphere respiration

Release of newly-fixed C (via rhizosphere respiration) increased the most in response to elevated CO_2. It also increased, but to a lesser degree, under elevated temperature. There is increasing evidence in the literature for higher root, respiration and exudation under elevated CO_2 (Curtis et al., 1994; Johnson et al., 1994; Norby et al., 1992; Rogers et al., 1994). For the Douglas-fir seedlings, root biomass (unpublished data obtained from 5 cm i.d. soil cores, see below) increased under the elevated CO_2 treatments, but did not respond to the elevated temperature treatments. Root mass was similar among all treatments (96±5.3 g m^{-2}, mean of the four treatment means ± SE) in spring 1994. In fall 1994, root mass in the two ambient CO_2 treatments was unchanged (93±12 g m^{-2}, mean of the six chambers in the two treatments ± SE), while mass in the two elevated CO_2 treatments increased to 115±11 g m^{-2} (n=6). In spring 1995, mean mass was 134±27 g m^{-2} in the two ambient CO_2 treatments and 204±46 g m^{-2} in the two elevated CO_2 treatments. In fall 1995, mass in the ambient CO_2 treatments continued to increase (190±18 g m^{-2}), but decreased to 145±21 g m^{-2} under the elevated CO_2 treatments. These changes in root mass suggest that root production was higher in 1995 than in 1994, and greater under elevated CO_2 compared with ambient CO_2 during the 2-year period. Root turnover in 1995 may have been greater under elevated CO_2 compared with the ambient treatment. In addition, at the end of 1994, there was extensive proliferation of roots at the soil-litter interface with approximately 80% of the roots within the top 40 cm of soil (Tingey et al., 1996). Few roots were ever found in the litter layer. Even after

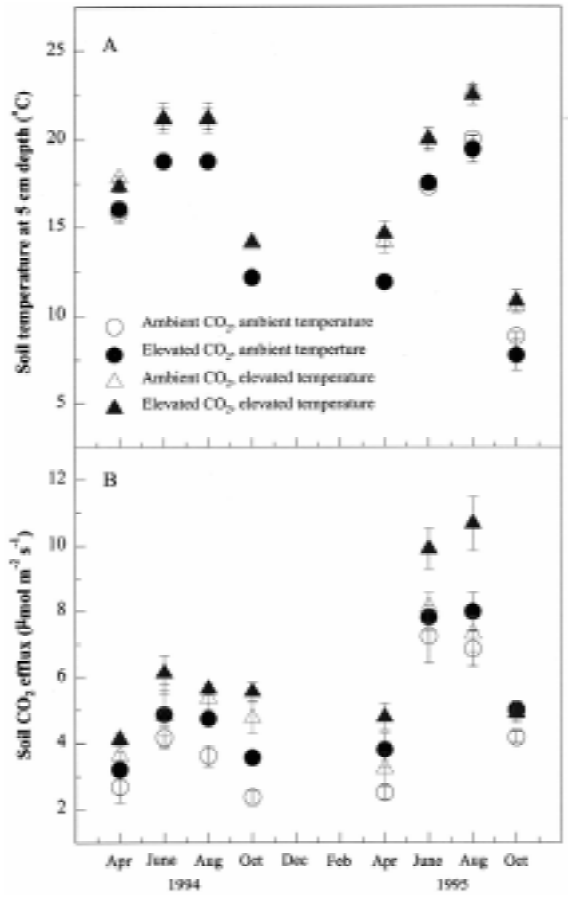

Figure 4. Mean soil temperature at 5 cm depth and CO_2 efflux in the chambers planted with Douglas-fir seedlings under different CO_2 and temperature treatments. Notation is the same as in Figure 1.

5 years of conducting the experiment, only 8% of the fine roots were in the litter layer while 42% were found in the A horizon (unpublished data). While we do not know why the relative contribution of rhizosphere respiration is low (Figure 5) compared with respiration from litter decomposition, it may be related to the lower total root mass per unit soil surface area that is attained in seedling systems compared with mature forests.

The enhancement in rhizosphere respiration under elevated CO_2 was significantly higher in 1994 than 1995. This appears the consequence of an initial effect of elevated CO_2 on root growth (data presented above, this section). Higher growth responses can result in higher demand for water and nutrients, thereby stimulating root activity. Rogers et al. (1994) found that the effect of elevated CO_2 on plant growth is most intensive during the initial stage of imposing treatments and then it gradually decreases as plants downregulate under further exposure. Other results from this study corroborate the apparent effect of relatively greater root growth *versus* aboveground growth, in enhancing rhizosphere respiration. Over the course of our study, plant growth was initiated earlier and ceased earlier in the growing season in the elevated temperature treatments, while final plant height was reduced and there was no significant temperature effect on stem diameter (Olszyk et al., 1998). While under elevated CO_2, we found no significant effects on stem diameter or plant height (Olszyk et al., 1998).

Responses of litter decomposition

Litter decomposition responded mainly to elevated temperature, especially in 1995. An analysis of the foodweb (fungi, bacteria, mesofauna, etc.) was done twice yearly on soil and litter samples taken from either mesh bags containing soil or litter (mesofauna) or soil cores (bacteria, fungi, nematodes, etc.). Active fungal biomass (direct counts, expressed as dry matter of hyphae that reacted with the vital stain fluorescein diacetate (FDA), Lodge and Ingham (1991)) peaked in fall 1994 (unpublished data). Bacterial biomass (direct counts) was unaffected during the exposure period. Nematode populations (retrieved from water-saturated samples), including fungivorous nematodes, were higher in 1995 (unpublished data), indicating increased preying on the greater amounts of fungal hyphae previously produced. From these responses, we hypothesize that the higher litter decomposition in 1995 was likely due to increased C throughput in fungi.

The effect of elevated CO_2 on enhanced litter decomposition observed in 1994 was not evident in 1995. Lin et al. (1999) speculated that this effect of elevated CO_2 was indirect, caused by increased plant growth and activity (primarily root growth and exudation) setting up a positive feedback of increased nutrient release from litter to satisfy higher plant nutrient requirements caused under elevated CO_2. It is unclear why this effect on litter decomposition was not evident in 1995. It may be that, in 1995, the putative higher root turnover rate (standing crop of roots estimated from soil cores, see above), relative to root production, provided sufficient nutrients to support seedling growth.

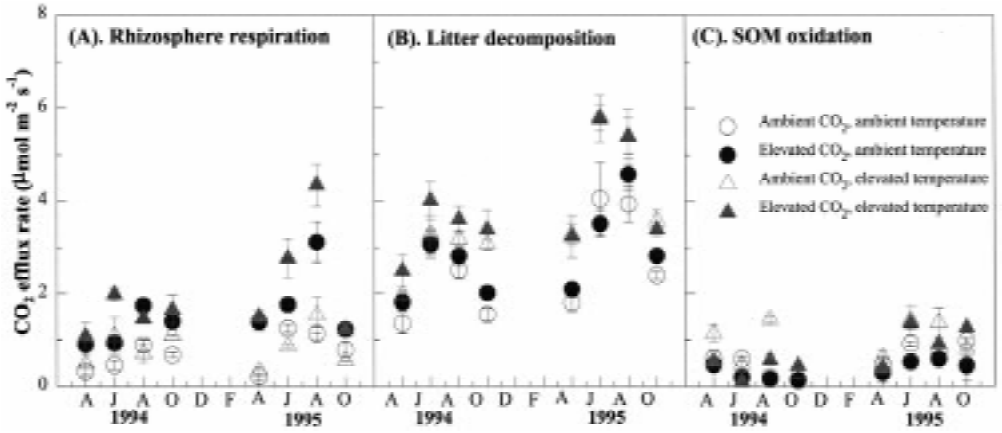

Figure 5. Estimated rates of rhizosphere respiration (A), litter decomposition (B), and soil organic matter (SOM) oxidation (C) in the chambers planted with Douglas-fir seedlings under different CO_2 and temperature treatments. Notation is the same as in Figure 1.

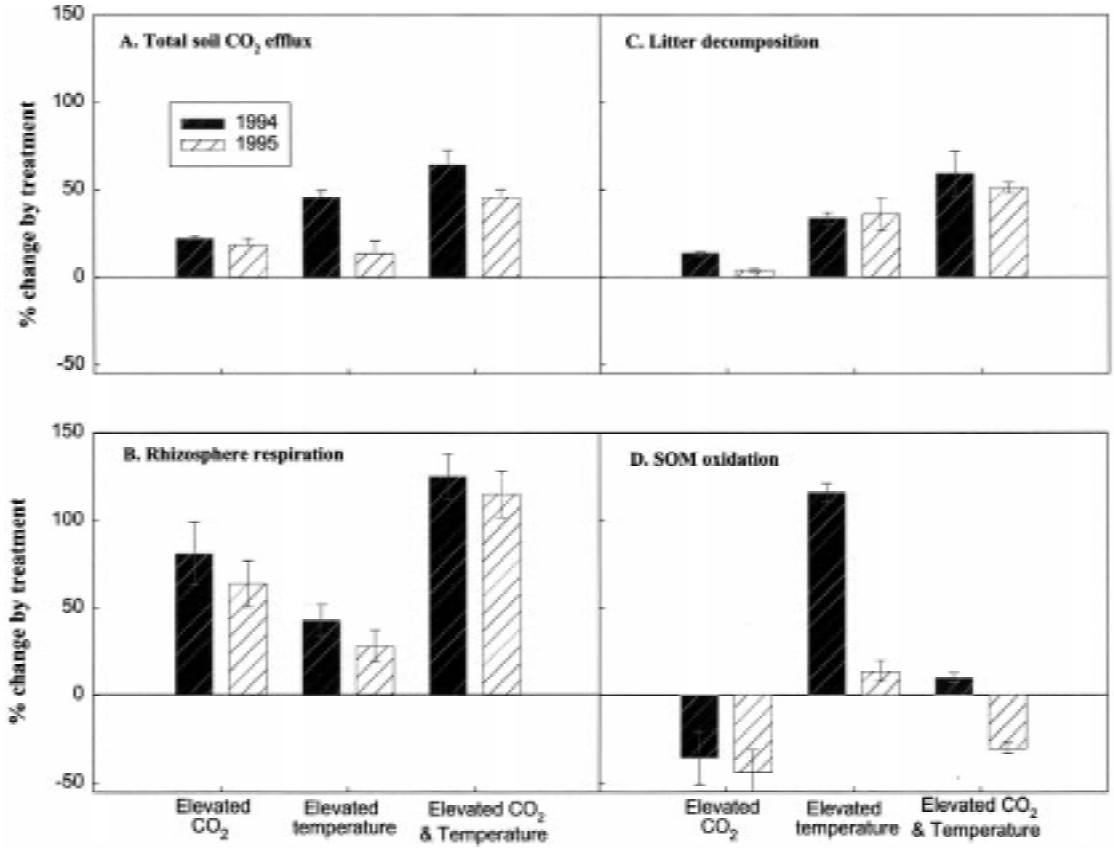

Figure 6. Comparison between 1994 and 1995 of effects of elevated CO_2 and elevated temperature treatments on total soil CO_2 efflux and its three component sources in the chambers planted with Douglas-fir seedlings. Percentages reflect changes due to the specified treatment relative to the corresponding rate in the control treatment.

Responses of SOM oxidation

Release of previously-formed SOM was reduced significantly by elevated CO_2 in both years, but increased under elevated temperature in most cases. The negative effect of elevated CO_2 on SOM oxidation can be expected since elevated CO_2 could result in more C allocation belowground and perhaps more C exudation from roots. The increased allocation of labile C to soil can serve as substrate for microbes, thus reducing their reliance on C from SOM and thereby leading to reduced SOM oxidation as we observed in this study and suggested by others (review by Cheng, 1999). It is worth noting the large change in the temperature effect on SOM oxidation between 1994 and 1995. In 1994, there was nearly a 120% increase in SOM oxidation under the elevated temperature treatment compared with the ambient treatment. By 1995, the increase in oxidation due to elevated temperature was greatly reduced (to about 20%). The B and C horizons were placed in the chambers in 1992 and allowed to rest and recover for 1 year prior to planting the seedlings. However, we needed to wait before placing the A horizon to increase the likelihood of establishing a complete and functioning foodweb. Since the A horizon, containing the greatest amount of C (2.8%, see 'Materials and methods'), was excavated, moved and placed into the chambers in June 1993, one might expect that the increased SOM oxidation under elevated temperature in 1994 resulted from decomposition of newly-available SOM caused by the physical disturbance. The relatively lower response in SOM oxidation to elevated temperature in 1995 may be due to less readily-available SOM resulting from the higher antecedent oxidation rates in 1994. Thus, the transient magnitude of the temperature effect likely was due to interactions involving the physical disturbance that was done to the soil. These results indicate that researchers may need to consider the potentially strong and latent effects that can occur when establishing an integrated system-level study in chambers, and that long-term scenarios for climate change studies, even with seedlings, may entail periods greater than 3–5 years.

The dual isotope technique was applied to controlled environment chambers where seedlings produced new photosynthate using CO_2 highly depleted in ^{13}C. Preliminary results of refining the technique in Corvallis indicate that the technique can be applied to field settings (J. Gregg, pers. com.). Since $\delta^{18}O$ is used to separate the CO_2 of litter decomposition from CO_2 of SOM oxidation, C isotope signatures of newly-formed roots are not required to be different from those of SOM. Furthermore, even if the difference in ^{13}C between roots and SOM minimally is 2 ‰, as suggested by Ehleringer et al. (2000), it is possible that the component rates can be determined as long as the standard deviation of the measurements of each source is less than 0.25 ‰ (Phillips and Gregg, 2001).

In summary, the three component sources (i.e. rhizosphere respiration, litter decomposition and SOM oxidation) that we could estimate and attribute to soil CO_2 efflux responded differentially to elevated CO_2 and temperature conditions likely to occur sometime in the 21st century in Douglas-fir forests in the northwestern US. Calculations made to predict ecosystem C balance in these forests will need to consider the rates of these processes and how they may change under future climates. In addition, there appears to be temporal variations in soil CO_2 efflux responding to elevated atmospheric CO_2 and temperature. These variations probably are caused by transient physiological responses (e.g. in the litter/soil foodweb) and physical disruptions to the soil. Temporal responses may be particularly important in newly planted forests where seedlings are growing in physically disturbed soil. The isotope technique appears to be sensitive, and allowed us to detect responses soon after treatments were imposed (within a year) and between consecutive years. However, some of the treatment effects observed in the earlier period of exposure to the climate treatments may have been artifacts caused by physically disturbing the soil. Although these results specifically apply to the experimental system used here, this technique may be applicable in field situations. Our results demonstrate the importance of undertaking long-term studies of plant and soil processes to understand how ecosystems respond to anthropogenic or natural perturbation.

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views and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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