Effects of elevated CO_2 and N fertilization on soil respiration from ponderosa pine (*Pinus ponderosa*) in open-top chambers

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Abstract: We measured growing season soil CO_2 evolution under elevated atmospheric CO_2 and soil nitrogen (N) additions. Our objectives were to determine treatment effects, quantify seasonal variation, and determine regulating mechanisms. Elevated CO_2 treatments were applied in open-top chambers containing 3-year-old ponderosa pine *(Pinus ponderosa* Dougl. ex Laws.) seedlings. Nitrogen applications were made annually in early spring. The experimental design was a replicated factorial combination of CO_2 (ambient, +175, and +350 $\mu L \cdot L^{-1}$ CO_2) and N (0, 10, and 20 $g \cdot m^{-2}$ N as ammonium sulfate). Soils were irrigated to maintain soil moisture at >25%. Soil CO_2 evolution was measured over diurnal periods (20-22 h) in April, June, and October 1993 using a flow-through, infrared gas analyzer measurement system. To examine regulating mechanisms, we linked our results with other studies measuring root biomass with destructive sampling and root studies using minirhizotron techniques. Significantly higher soil CO_2 evolution was observed in the elevated CO_2 treatments in April and October; N effects were not significant. In October, integrated daily values for CO_2 evolution ranged from 3.73 to 15.68 g $CO_2 \cdot m^{-2} \cdot day^{-1}$ for the ambient CO_2 + 0 N and 525 $\mu L \cdot L^{-1}$ CO_2 + 20 $g \cdot m^{-2}$ N, respectively. Soil CO_2 flux among treatments was correlated with coarse root biomass (r^2 = 0.40; $p > F$ = 0.0380), indicating that at least some of the variation observed among treatments was related to variation in root respiration. Across all sample periods and treatments, there was a significant correlation (r^2 = 0.63; $p > F$ = 0.0001) between soil CO_2 evolution and percent fungal hyphae observed in minirhizotron tubes. Hence, some of the seasonal and treatment variation was also related to differences in heterotrophic activity.

Resume : Nous avons mesure l'évolution du CO_2 edaphique pendant une saison de croissance en conditions de CO_2 atmosphérique eleve et d'ajouts d'azote (N) au sol. Nos objectifs etaient de determiner les effets des traitements sur cette variable, d'en quantifier la variabilité saisonnière et d'en élucider les mecanismes de controle. Le traitement de CO_2 eleve a ete applique dans des chambres d'exclusion a ciels ouverts contenant des semis de pins ponderosa *(Pinus ponderosa* L.) ages de 3 ans. Les applications de N etaient faites annuellement, tot au printemps. Le dispositif experimental était un factoriel complet de concentrations en CO_2 (ambiant, +175 et +350 $\mu L \cdot L^{-1}$ CO_2) et de taux d'application de N (0, 10 et 20 $g \cdot m^{-3}$ sous forme de sulfate d'ammonium). Les sols etaient irrigués de façon a maintenir leur taux d'humidité plus élevée que 25%. L'évolution du CO_2 edaphique a ete mesuree sur des périodes de 20 a 22 h en avril, juin et octobre 1993 au moyen d'un système de mesure a debit continu muni d'un analyseur infrarouge. Nous avons lie nos résultats a d'autres etudes portant sur des mesures destructives de biomasse racinaire et des observations racinaires au moyen de minirhizotrons afin d'examiner les mecanismes de controle.

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Le traitement de CO_2 élevé a entraïné une augmentation significative du CO_2 edaphique en avril et en octobre; l'effet du N n'était pas significatif. En octobre, l'évolution journalière du CO_2 etait de 3,73 g $CO_2 \cdot m^{-2} \cdot d^{-1}$ avec le CO_2 ambiant et 0 N, et de 15,68 g $CO_2 \cdot m^{-2} \cdot d^{-1}$ avec 525 $\mu L \cdot L^{-1}$ de CO_2 et 20 $g \cdot m^{-2}$ de N. Le flux de CO_2 edaphique etait corrélé avec la biomasse des racines grossières (r^2 = 0,40; $P > F$ = 0,0380), indiquant qu'au moins une partie de la variation observée entre les traitements etait due a une variation de la respiration racinaire. Toutes périodes et tous traitements regroupes, nous avons obtenu une correlation significative (r^2 = 0,63; $P > F$ = 0,0001) entre 1'evolution du CO_2 du sol et le pourcentage d'hyphes de champignons mesure avec les minirhizotrons. Une partie de la variation au cours de la saison et entre les traitements etait donc aussi causee par des differences dans l'activité hétérotrophique.
[Traduit par la Redaction]

Introduction

The evolution of carbon dioxide (CO_2) from soils is due to the combined metabolic activity of roots and free-living and symbiotic heterotrophs (i.e., fungi, mycorrhizae, and soil micro- and macro-organisms). In terrestrial systems, estimates of carbon (C) recycled to the atmosphere from belowground sources range from 70 (Raich and Schlesinger 1992) to >100 $Pg \cdot year^{-1}$ globally (Musselman and Fox 1991). This represents a major component of C flux in the global C cycle. Belowground C cycling processes and subsequent soil CO_2 fluxes are both important at ecosystem scales. We have limited knowledge of the magnitude of fluxes within an across ecosystems. Increased knowledge of the magnitude of C fluxes, as well as the factors that regulate these fluxes, is critical for understanding ecosystem C cycling and potential responses to factors such as climatic change.

Separating the contributing sources (i.e., roots vs. heterotrophs) of soil CO_2 evolution has proven difficult. In forests, estimates of the relative contribution of roots versus other soil components vary between 33 and 62% of the total CO_2 evolved (Edwards and Harris 1977; Ewel et al. 1987; Bowden et al. 1993). Factors known to influence the rate of CO_2 evolution include soil temperature and moisture (Wiant 1967; Garrett and Cox 1973; Edwards 1975; Schlentner and Van Cleve 1985; Naganawa et al. 1989; Hanson et al. 1993; Peterjohn et al. 1993, 1994), soil N content (Söderstrõm et al. 1983), and root biomass (Behera et al. 1990). Hence, changes in root biomass and (or) activity related to elevated CO_2 should directly influence the total CO_2 evolution from forest soils. Other factors related to carbon source and amount (e.g., fine root turnover and exudates) could also influence soil CO_2 evolution by altering microbial activity. Increased soil N availability could alter soil CO_2 flux by changing root (Ryan 1991) and microbial activity and (or) biomass (Soderstrom et al. 1983). Because many of these controlling factors vary temporally (diurnally and seasonally), considerable variation in soil CO_2 evolution results (Garrett and Cox 1973; Edwards and Sollins 1973; Vose et al. 1994; Schlentner and Van Cleve 1985; Hanson et al. 1993).

Several techniques are available for measuring soil CO_2 evolution, including static chambers (e.g., Cropper et al. 1985; Raich et al. 1990), soil CO_2 concentration profiles (i.e., pCO_2) (de Jong and Schappert 1972), and open and closed dynamic chamber methods (e.g., Hanson et al. 1993; Garrett and Cox 1973; Edwards and Sollins 1973; Vose et al. 1994). Studies comparing measurement techniques have found wide disparity among these methods (Edwards and Sollins 1973; Cropper et al. 1985; Raich et al. 1990; Rochette et al. 1992; Norman et al. 1992). In general, static chamber techniques provide lower (i.e., 10-30%) estimates of CO_2 evolution than dynamic chamber techniques (Ewel et al. 1987; Rochette et al. 1992), while pCO_2 techniques provide higher CO_2 evolution estimates than dynamic chamber techniques (de Jong et al. 1979). Although more difficult and expensive to conduct, dynamic, IRGA (infrared gas analyzer) based techniques are considered more accurate (Ewel et al. 1987) and can be configured to quantify diurnal patterns (Edwards and Sollins 1973; Vose et al. 1994).

This study is part of long-term, multi-investigator project assessing the impacts of elevated CO_2 and N on a variety of above- and below-ground processes (Ball and Johnson 1993). The specific objectives of the present study were (*i*) to examine the impacts of elevated atmospheric CO_2 and nitrogen fertilization on soil CO_2 evolution, *(if)* to quantify seasonal patterns in soil CO_2 evolution, and (*iii*) to relate treatment and temporal variation in soil CO_2 evolution to indices of fungi and mycorrizhae population size, root density, and root biomass.

Methods

Site description

The study was conducted at the USDA Forest Service Institute of Forest Genetics in Placerville, Calif. (39°N, 121°W). The site elevation is 843 m, receives an average of 1000 mm of annual precipitation, and has a mean annual temperature of 18°C. The soil is Aiken clay loam (Xeric Haplohumult) derived from andesite. Extensive sampling prior to study establishment indicated uniform soil chemical and textural characteristics across the study area. Bulk density of the soil averaged 1.14 g/cm^3, porosity was 54%, reaction was moderately acidic (pH in CaCl = 5.1 in upper 18 cm), and base saturation (1 M NH_4Cl extraction) was 50-60%. Soil N content in unfertilized soil was 900 $\mu g \cdot g^{-1}$.

Experimental design and treatments

The experiment used open-top chambers (8.4 m^2; hexagonal shape) to elevate atmospheric CO_2 concentration (Ball and Johnson 1993). Air was delivered to the chambers using a 45 cm diameter plastic plenum at three air changes per minute. The experimental design consisted of three levels of N (0, 10, and 20 $g \cdot m^{-2} \cdot year^{-1}$ of N as ammonium

sulfate, applied to the soil surface in early spring), and four continuous CO_2 treatments (ambient, no chamber; ambient, chamber; +175 $\mu L \cdot L^{-1}$; and +350 $\mu L \cdot L^{-1}$). Each of the chambered treatments was replicated three times, and the unchambered treatment was replicated twice. Because of cost limitations, the 10 $g \cdot m^{-2} \cdot year^{-1}$ N with + 175 CO_2 treatment was omitted. Hence, there were a total of 11 treatments. Each chamber contained 21 ponderosa pine (*Pinus ponderosa* Dougl. ex Laws) seedlings (grown from seed in the chambers) equally spaced in the ground at about 0.3 m in all directions. At the time of sampling, seedlings had been grown in the chambers under treated conditions for 3 years. Soils were irrigated weekly with sufficient water to maintain soil water potential at greater than -0.07 MPa. This corresponds to a soil moisture of >25%. To assess spatial variation (i.e., among chambers) in soil moisture, we measured soil moisture (averaged over 15 cm depth) in each chamber in June 1993 using a TRASE time domain reflectometry measurement system (Soil Moisture Instrument, Santa Barbara, Calif.). Results from those measurements indicated no significant differences in soil moisture across chambers (mean = 28.2%; standard error = 0.85).

Soil CO_2 evolution sampling

We measured diurnal patterns of soil CO_2 flux using an automated, flow-through, IRGA measurement system. The system measured flux sequentially from 10 soil chambers. Soil chambers were constructed of PVC pipe (10 cm diameter, 10 cm height, 785 cm^3 volume), sharpened on the open end and driven approximately 2 cm into the soil surface with a rubber mallet. All tubing was 5-mm (i.d.) flexible PVC. Air was passed through the chambers via inlet and outlet fittings attached to the upper sides of the chamber. Airflow through the chambers was regulated with a dual-sided air pump (Spec-Trex Corp., Redwood, Calif.) which balanced flow into and out of the chambers. Actual flow rate ($mL \cdot min^{-1}$) was controlled by varying voltage (0–12 V DC) supplied to the pump and was measured and logged electronically with a flow meter and data logger (Campbell 21×, Campbell Scientific, Logan, Utah). An air flow rate of 1000-1500 $cm^3 \cdot min^{-1}$ provided stable readings within 7-8 min. Chamber sampling was controlled with a multiplexer, data logger, and solenoids that opened sequentially (chambers 1-10) at 10-min intervals. Carbon dioxide concentrations of air entering and exiting the chambers was measured and logged electronically with an IRGA (ADC LCA3, Hoddeson, U.K.) operating in differential mode and a data logger (Campbell 21×), respectively. Soil CO_2 evolution rate (g $CO_2 \cdot m^{-2} \cdot min^{-1}$) was calculated based on the difference in CO_2 entering and exiting the chamber, the ground area sampled beneath the chamber, and the flow rate. To allow for equilibration between chambers, only data from the last minute of sampling were used in flux calculations.

Sampling was conducted over three 6-day periods in April, June, and October 1993. On each day, two soil respiration chambers were randomly placed in each of five treatment-replication combinations, with the restriction that soil respiration chambers could be no closer than 2.5 cm from a seedling. This restriction was imposed to ensure that seedlings were not damaged in the course of installing the chambers. Soil CO_2 evolution was measured for 22-24 h (i.e., a diurnal cycle) on each day. Sampling was initiated in early morning (≈09:00) and concluded between =08:00 and 09:00 the following day. On each successive day, the chambers were moved to a new set of treatment-replication combinations and the diurnal measurements repeated until all treatments and replications were sampled. On each day, treatment-replication combinations selected for sampling were chosen to span the factorial (e.g., all high CO_2 treatments were not sampled on the same day) combinations of CO_2 and N treatments. Using this sampling approach, we assumed that there would be minimal day to day variation in soil CO_2 evolution and (or) if day to day variation did occur, the selection of representative treatment replication combinations would minimize any potential bias. Climatic conditions were generally constant (i.e., no rain, cloudless skies, and comparable soil temperatures) throughout the 6-day measurement period. For example, in April and October there were no significant trends (i.e., nonsignificant linear regression) in soil temperature across the 6-day measurement period. In June, there was a slight but statistically significant ($p < 0.05$; linear regression) increase (1-2°C) in soil temperature across the 6-day measurement period, with most of the increase occurring between the third and fourth measurement day.

Soil temperature

Soil temperature at 10 cm depth was measured for one complete diurnal cycle at each soil respiration chamber location. Measurements were made with Type-T thermocouples connected to a data logger (Campbell 21×) and multiplexer.

Root mass

In October 1993, one seedling of average size was harvested with a shovel from each chamber (including the unchambered treatments). Removal of the seedling root system from the soil was facilitated with water sprayed under moderate pressure from a garden hose. Seedlings were transported to the laboratory where the root systems were separated from the shoots, sorted into fine (<2 mm) and coarse (≥2 mm) root fractions, oven-dried to a constant weight, and weighed to the nearest 0.1 g.

Minirhizotrons

During the week of 17 August 1992, minirhizotron tubes (clear plastic tubes, 5 cm inside diameter) were installed at an angle 45° from vertical with the bottom of the tubes extending into the Bt (argillic) horizon. Three, 1m long tubes, each fitted with a watertight PVC plug on the soil end, were placed in each of the open-top chambers. Tubes were installed parallel to three of the four ordinal directions and halfway between a target tree and its nearest neighbor tree. The aboveground portion of each tube was painted to exclude light and covered with a closed-cell foam rubber cap to exclude moisture and minimize heat exchange between the tube and the air. Application of minirhizotron technology has been described in detail elsewhere (Brown and Upchurch 1987).

Table 1. Average integrated soil CO_2 evolution ($g \cdot m^{-2} \cdot day^{-1}$) per treatment in April, June, and October 1993.

Treatment	April	June	October
Ambient CO_2 + 0 N	1.602 (1.231)	1.704 (0.364)	3.733 (1.049)
Ambient CO_2 + 10 N	5.641 (1.421)	2.126 (0.297)	8.584 (1.049)
Ambient CO_2 + 20 N	1.485(1.421)	1.071 (0.297)	5.636 (1.211)
Ambient + 175 CO_2 + 0 N	4.595 (1.231)	1.628 (0.297)	12.194 (1.285)
Ambient + 175 CO_2 + 20 N	3.501 (1.421)	2.976 (0.297)	15.675 (1.211)
Ambient + 350 CO_2 + 0 N	3.692 (1.421)	2.383 (0.364)	6.120 (1.049)
Ambient + 350 CO_2 + 10 N	2.275 (1.231)	4.723 (0.364)	9.424 (1.049)
Ambient + 350 CO_2 + 20 N	9.558 (1.231)	1.584 (0.364)	10.557 (1.049)
Open + 0 N	1.675 (2.132)	2.058 (0.514)	1.863 (1.285)
Open + 10 N	1.936 (1.507)	1.784 (0.364)	6.536 (1.817)
Open + 20 N	2.051 (1.846)	1.798 (0.514)	3.235 (1.817)

Note: Average values are least-square means with standard errors given in parentheses. $n = 2$ for chamberless (Open) treatments and $n = 3$ for all others. Open, chamberless.

Minirhizotron images were recorded seven times (between October 1992 and October 1993), on S-VHS tape using a minirhizotron camera (Bartz Technology Company, Santa Barbara, Calif.). In this study, only measurements from April, June, and October 1993 (taken within ±1 week of the soil CO_2 evolution measurements) were used. The camera was remote focusing, with a white light source and equipped with an indexing handle that locked into position in an index hole in each minirhizotron tube. The indexing handle had a ratchet advancing mechanism and regularly spaced detents to reliably advance the camera from one field of view (frame) to the next. The indexing handle system insured that the camera was returned to the same position in each tube and traveled along the same viewing line each time images were collected. Root images were recorded on the uppermost surface of the minirhizotron tubes beginning at the bottom of the tubes. In this application, the camera had a field of view of about 1.8 cm^2. Forty-five frames were recorded in each tube, for a total of 135 frames per open-top chamber per sampling event.

Root data were extracted from the video tapes using ROOTS, an interactive PC-based software program developed at Michigan State University (Hendrick and Pregitzer 1992). The software allows the user to review all images and trace various root features (length and diameter) and annotate mycorrhizae and fungal hyphae occurrence. In this study, we summarized the data based on the occurrence of roots (percent roots), fungal hyphae (percent fungi), and mycorrhizae (percent mycorrhizae) as measured by the percentage of the total minirhizotron frames in which they occurred. Data from the three minirhizotron tubes in each open-top chamber were averaged to provide a chamber-level estimate.

Statistical analysis

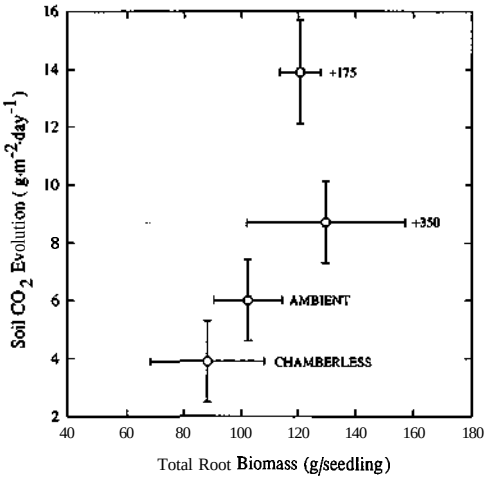
We used integrated values of diurnal measurements to estimate daily soil CO_2 flux ($g\ CO_2 \cdot m^{-2} \cdot day^{-1}$) for each open-top chamber treatment combination. Integrated values were calculated by determining the area under each segment of two consecutive sample points and then summing the segments for a total daily flux rate. When the sampling interval was <24 h, the values were extrapolated to 24 h by connecting the last sample point to the first sample point of the sampling period. These values were used in analysis of variance (ANOVA) to test for treatment effects (SAS Institute Inc. 1987). A reduced error term (chamber(treatment)), which accounted for the subsample of two soil CO_2 evolution chambers per open-top chamber, was used in the ANOVA to test for treatment effects. Because we had an unbalanced experimental design, contrast statements (Snedecor and Cochran 1980) were constructed to determine the effects of CO_2, N, and CO_2 X N interaction on soil CO_2 evolution. A repeated measures ANOVA was used to test for seasonal (time) effects on soil CO_2 evolution (SAS Institute Inc. 1987). We performed a separate analysis for each CO_2 treatment averaging across N additions. If an overall significant F-value was found, tests for significant differences among time (April, June, and October) were made using the appropriate orthogonal contrasts. Scatterplots were used to examine the relationships between soil CO_2 evolution and soil temperature, root biomass, percent roots, percent fungi, and percent mycorrhizae. For obvious linear relationships, linear correlation (PROC CORR, SAS Institute Inc. 1987) was used for analyses. For nonlinear relationships, natural logarithm transformations on both the dependent and independent variables were used to linearize the relationship.

Results and discussion

Treatment effects

Soil CO_2 evolution varied considerably among treatments (Table 1). The greatest difference was observed in October, where integrated daily values ranged from 1.86 (ambient CO_2 + 20 N) to 15.68 $g \cdot m^{-2} \cdot day^{-1}$ (+175 CO_2 + 20 N). In April and October, soil CO_2 evolution was significantly ($p < 0.05$) greater in the chambers receiving elevated atmospheric CO_2 (Table 2). Soil CO_2 evolution rates were generally greater in the elevated atmospheric CO_2 treatments in June (Table 1) as well; however, the differences were too small to detect statistically significant differences (Table 2). Nitrogen fertilization effects were not

Fig. 1. Total root biomass versus soil CO_2 evolution in October 1993. Data are means (averaged across N) and standard errors.



significant in any sampling period. In addition, there were no differences between the chamberless and ambient treatments (Table 2) indicating that the open-top chamber did not influence soil CO_2 evolution.

The rates observed in our study are in the range of those observed elsewhere; however, comparisons must be made with caution due to the differences in measurement techniques discussed previously (e.g., static vs. dynamic chambers differences of 10-30%). In our study, control (ambient and unchambered) values ranged from 1.7 to 3.7 g $CO_2 \cdot m^{-2} \cdot day^{-1}$ and treatment values (CO_2 and (or) N) ranged from 1.1 to 15.7 g $CO_2 \cdot m^{-2} \cdot day^{-1}$. In conifers, values range from 3.5 to 14.4 g $CO_2 \cdot m^{-2} \cdot day^{-1}$ (Schlentner and Van Cleve 1985; Weber 1985; Ewel et al. 1987). In hardwoods, values range from 1 to 26 g $CO_2 \cdot m^{-2} \cdot day^{-1}$ (Garrett and Cox 1973; Edwards and Sollins 1973; Weber 1990; Bowden et al. 1993; Hanson et al. 1993; Peterjohn et al. 1993).

The greater soil CO_2 evolution in the elevated atmospheric CO_2 treatment may be related at least in part to greater root biomass of the pine seedlings growing in the elevated CO_2 chambers. Nakayama et al. (1994) also found increased soil CO_2 evolution in response to elevated atmospheric CO_2 in field grown cotton (*Gossypium hirsutum* L.), which they attributed to greater root density and microbial populations under elevated CO_2. In our study, root biomass (fine and coarse root combined) in October 1993 was 30% greater in the elevated CO_2 treatments (averaged across N levels) relative to ambient CO_2 (126.0 g/seedling for elevated CO_2 versus 95.3 g/seedling for ambient CO_2) (Table 3) and there was a trend of increased soil CO_2 evolution with increased root biomass (Fig. 1). In addition to these general patterns, there was a weak, but statistically significant (r^2 = 0.40; F = 5.903; $p > F$ = 0.0380; n = 11) correlation between chamber-specific estimates of coarse root biomass and soil CO_2 evolution; however, there was no correlation between fine root mass and soil CO_2 evolution. In a related study, Johnson et al. (1994) also found significant correlations between soil pCO_2 respiration estimates and root biomass in 1991 and 1992.

Table 2. Analysis of variance tables for test of treatments with contrasts for CO_2 and N comparisons for April, June, and October 1993.

Source	df	SS	MS	F	$p > F$
		April			
Treatment	10	313.796	31.380	1.93	0.104
Chamber	1	5.415	5.415	0.33	0.572
Ambient vs. elevated CO_2	2	137.624	68.812	4.20	0.031
N	2	22.854	11.427	0.70	0.511
N control vs. N high	1	12.394	12.394	0.76	0.396
Error	19	311.575	16.399		
		June			
Treatment	10	42.298	4.230	1.47	0.246
Chamber	1	0.298	0.298	0.10	0.752
Ambient vs. elevated CO_2	2	2.415	1.208	0.42	0.664
N	2	11.423	5.711	1.99	0.174
N control vs. N high	1	0.052	0.052	0.02	0.894
Error	19	40.172	2.869		
		October			
Treatment	10	744.164	74.416	3.03	0.024
Chamber	1	22.116	22.116	0.90	0.357
Ambient vs. elevated CO_2	2	568.117	284.058	11.56	0.001
N	2	120.954	60.477	2.46	0.117
N control vs. N high	1	64.414	64.414	2.62	0.125
Error	19	393.059	24.566		

Note: Chamber tests for a difference between 350 CO_2 and chamberless treatments across levels of N. Because there is no significant difference between ambient CO_2 and chamberless treatments, then ambient vs. elevated CO_2 tests for a difference for the average of ambient CO_2 and chamberless treatments vs. the average of the ambient + 175 CO_2 and ambient + 350 CO_2 treatments across nitrogen level; the + 10 N nitrogen level is ignored. N tests for a difference for + 0 N vs. the average of the + 10 N and + 20 N treatments across CO_2 level; ambient + 175 CO_2 level is ignored. N control vs. N high tests for a difference between + 0 N and + 20 N treatments across CO_2; the + 10 N is ignored because it does not occur in every possible level of CO_2, i.e., no ambient + 175 CO_2 + 10 N treatment. The error term is chamber(treatment).

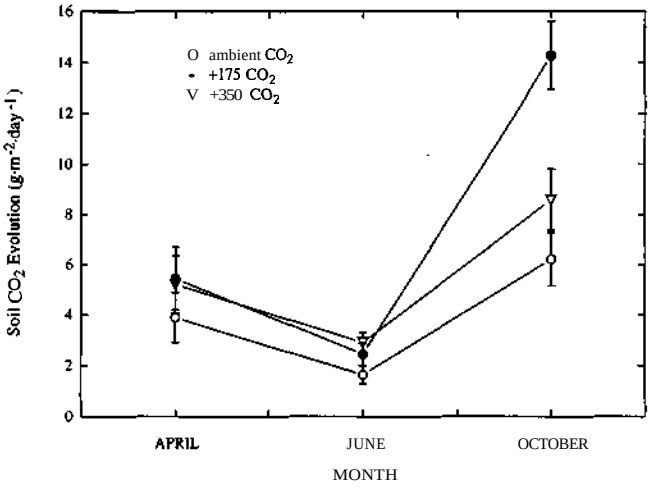
Other studies have also shown increased root biomass in response to elevated CO_2 (e.g., Rogers et al. 1992; Norby et al. 1987). Roots are an important contributor to soil CO_2 evolution because root respiration can contribute as much as 62% of the total (Ewel et al. 1987). In the elevated CO_2 treatments, the greater root biomass may directly translate into greater root respiration and indirectly translate into greater heterotrophic respiration in response to a larger rhizosphere (see discussion on seasonal patterns). Total root biomass (Table 3) was dominated by the coarse root fraction. While we would expect the respiration rate (mg·(g root weight)$^{-1}$) of coarse roots to be lower than

Table 3. Fine (<2 mm) and coarse (≥2 mm) root biomass (g/seedling) determined in October 1993 with destructive sampling.

Treatment	Coarse	Fine	Total
350 CO_2 + 0 N	72.5 (12.2)	6.4 (2.0)	78.9 (13.8)
350 CO_2 + 10 N	105.0 (15.2)	9.5 (1.9)	114.5 (16.4)
350 CO_2 + 20 N	108.6 (25.5)	5.2 (2.3)	113.8 (26.4)
525 CO_2 + 0 N	106.5 (25.4)	7.1 (1.6)	113.6 (27.0)
525 CO_2 + 20 N	117.2 (25.8)	10.6 (1.8)	127.8 (27.4)
700 CO_2 + 0 N	150.7 (27.1)	8.7 (1.6)	159.4 (28.5)
700 CO_2 + 10 N	68.9 (4.5)	6.8 (0.8)	75.7 (4.2)
700 CO_2 + 20 N	147.2 (32.0)	6.5 (2.2)	153.7 (30.1)
Open + 0 N	40.1 (8.0)	9.6 (3.3)	49.7 (11.3)
Open + 10 N	88.4 (20.2)	11.1 (0.6)	99.5 (20.9)
Open + 20 N	103.1 (48.9)	12.2 (0.1)	115.3 (48.8)

Note: Data are means (n = 2 for open; n = 3 for all others) with standard errors in parentheses. Open, chamberless.

Fig. 2. Seasonal variation in mean soil CO_2 evolution by CO_2 treatment. Data are means (averaged across N) and standard errors.



for fine roots (e.g., Behera et al. 1990), the 10-fold (Table 3) difference in coarse root versus fine root biomass may offset lower respiration rates. Additionally, the inherent difficulties in destructively sampling fine roots probably resulted in more sampling variability than with coarse roots and this may have reduced our ability to establish stronger relationships between soil CO_2 evolution and the fine root fraction.

The lack of N response may reflect the counteracting effects of N on autotrophic and heterotrophic processes. For example, plant tissue respiration rates have been shown to increase with increasing tissue N concentration (e.g., as a result of N fertilization) (Ryan 1991) and nitrate and ammonium uptake (Bloom et al. 1992), while heterotrophic rates may decrease with N additions (Söderström et al. 1983). Similar to our study, Castro et al. (1994) found no effect of N fertilization on soil CO_2 evolution from soils in a slash pine (*Pinus elliottii* Engelm.) plantation in Florida.

Other potential driving variables (e.g., soil temperature, litter depth, and quality) did not appear to contribute to the variation among treatments. For example, there was no litter layer in any of the chambers and minimal variation in soil temperature among treatments within a given sampling period (Table 5).

Seasonal patterns

Strong seasonal effects on soil CO_2 evolution were also apparent, where as much as a threefold difference was observed within a CO_2 treatment (Fig. 2). Time effects were statistically significant for all treatments (Table 4). Orthogonal contrasts indicated that October soil CO_2 evolution was greater than in June for all CO_2 treatments, and greater than in April in both the ambient and +175 CO_2 treatments (Table 4).

Several abiotic and biotic factors can influence soil CO_2 evolution and contribute to this seasonal variation. For example, seasonal variation in soil moisture can be an important regulator (e.g., Reiners 1968; Schlentner and Van Cleve 1985; Gordon et al. 1987). However, as noted previously, the open-top chambers were irrigated to maintain soil moisture at >25% so it is unlikely that the seasonal variation was due to soil moisture differences. Soil CO_2 evolution rates are generally positively related to soil temperature (e.g., Reiners 1968; Edwards and Sollins 1973; Raich and Schlesinger 1992; Peterjohn et al. 1993; Hanson et al. 1993), so some of the seasonal variation may have been related to variation in soil temperature (Table 5). In our study, the lowest soil CO_2 evolution rates occurred when the soils were warmest (e.g., mean soil temperature in June = 21.2°C). This response is inconsistent with responses observed in undisturbed forests (e.g., Raich and Schlesinger 1992; Peterjohn et al. 1993; 1994; Hanson et al. 1993), but consistent with responses observed in some disturbed forests (e.g., Mattson and Swank 1989; Hendrickson et al. 1985). In a previous study (Vose et al. 1994), we established that soil CO_2 evolution responds to diurnal variation in soil temperature. However, two factors restrict our ability to quantify the effects of temperature on seasonal variation in the present study. First, our data did not include any winter measurements, so the responses to low soil temperatures (i.e., <10°C) were not quantified. Second, with only three growing season measurements and little spatial variation in soil temperature, the data were too restricted to develop functional relationships. It is noteworthy that laboratory incubations and soil pCO_2 analyses also showed consistently lower respiration activity in June than in other months (D.W. Johnson, personal observation).

In other forest soil studies, when soil temperature and moisture were modeled as dependent variables regulating soil CO_2 evolution, there was often a considerable amount of unexplained variation (e.g., r^2 = 0.50, Raich and Schlesinger 1992; r^2 = 0.32-0.72, Gorden et al. 1987; r^2 = 0.49-0.73, Hanson et al. 1993). Factors such as temporal (i.e., seasonal) and spatial variation in root growth and turnover, root exudate quality and quantity, fungi, mycorrihzae, and (or) variation in microbial populations and activity may be causal factors contributing to this variation. For example, Rygiewicz and Anderson (1994) determined that 19.4% of total soil CO_2 evolution in ponderosa pine

Table 4. Probability values for test of seasonal variation in soil CO_2 flux within a treatment.

		Contrasts		
Treatment	Time effects	April vs. June	April vs. October	June vs. October
Ambient CO_2	0.0009	0.0454	0.0003	0.0008
+175 CO_2	0.0001	0.0475	0.0012	0.0001
+350 CO_2	0.0116	0.1825	0.1049	0.0017

Note: Because N effects were not significant, data were averaged across N levels.

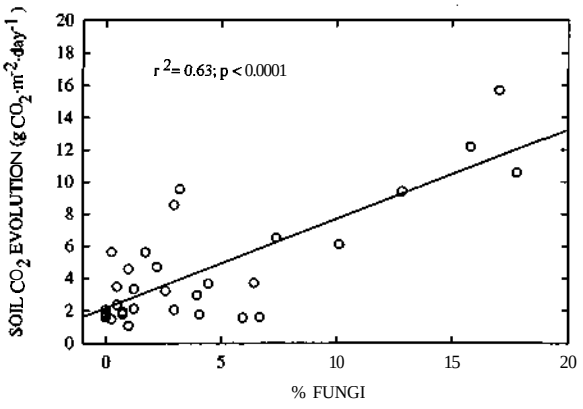
Table 5. Average soil temperature (°C) per treatment in April, June, and October 1993.

Treatment	April	June	October
Ambient CO_2 + 0 N	11.9 (0.86)	22.2 (1.67)	15.0 (0.28)
Ambient CO_2 + 10 N	12.0 (0.89)	22.2 (0.73)	15.0 (0.58)
Ambient CO_2 + 20 N	11.4 (0.71)	19.8 (1.27)	14.9 (0.68)
Ambient + 175 CO_2 + 0 N	11.4 (0.54)	20.0 (0.60)	14.1 (0.83)
Ambient + 175 CO_2 + 20 N	10.3 (0.27)	19.9 (0.62)	13.9 (0.61)
Ambient + 350 CO_2 + 0 N	11.9 (0.77)	22.0 (1.54)	15.9 (0.62)
Ambient + 350 CO_2 + 10 N	11.7 (0.25)	21.6 (1.21)	15.5 (0.34)
Ambient + 350 CO_2 + 20 N	10.9 (0.40)	20.2 (0.96)	15.4 (1.28)
Open + 0 N	13.6 (1.10)	22.6 (0.43)	13.7 (0.12)
Open + 10 N	12.8 (0.36)	21.9 (1.42)	13.3 (1.64)
Open + 20 N	12.0 (0.84)	21.4 (1.37)	12.5 (1.59)
Seasonal average	11.8 (0.27)	21.2 (0.32)	14.5 (0.32)

Note: Standard errors are given in parentheses. $n = 1$ for chamberless (Open) treatments and $n = 3$ for all others. Open, chamberless.

was directly attributable to fungal hyphae respiration. To examine the role of some of these factors in our study, we correlated soil CO_2 evolution values for all sample periods and treatments with percent fungi, percent mycorrizhae, and the percent roots observed in the minirhizotron tubes. There was a significant and moderately strong correlation ($r^2 = 0.63$; $F = 51.78$; $p > F = 0.0001$; $n = 33$) between soil CO_2 evolution and percent fungi (Fig. 3), while no other correlations were significant. Although cause and effect cannot be established, these results suggest that seasonal and treatment variation in decomposer populations or fungal hyphae are also important regulators of soil CO_2 evolution. In our study, percent fungi was greatest in October and in the elevated CO_2 treatments, while there was no consistent response to N (Fig. 4).

Fig. 3. Relationship between percent fungi (percent occurrence in minirhizotron frames) and soil CO_2 evolution across all treatments and sampling periods. Analyses were performed on mean values ($n = 3$ for all treatments except chamberless, where $n = 2$).

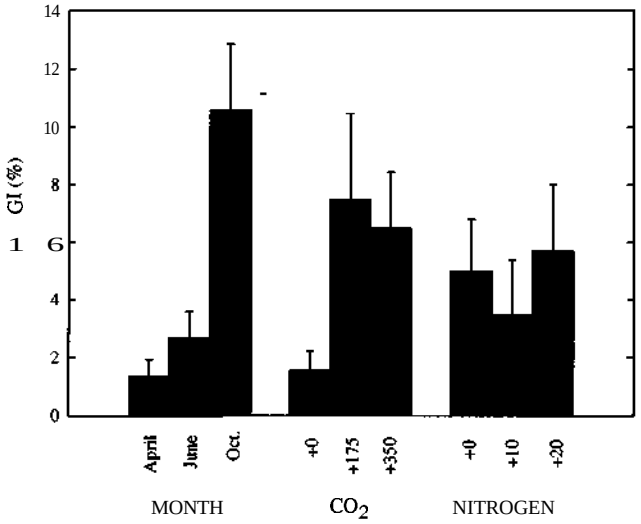


Growing season extrapolations

To obtain a growing season estimate for total C lost from the soil, we averaged daily soil CO_2 evolution values across growing season and N treatments and multiplied the values by 180 (i.e., a 6-month growing season) (Table 6). Values ranged from 188 g $C \cdot m^{-2}$ for ambient CO_2 treatments to 332 g $C \cdot m^{-2}$ for the +175 CO_2 treatment. Hence, there was as much as a twofold increase in C lost from the soil under elevated atmospheric CO_2. Additional soil C could be evolved in the winter months, where in cool climates, winter loss rates are roughly one-third of growing season loss rates (Raich and Schlesinger 1992).

Assuming that 50% (i.e., a midpoint value from the literature) of the soil CO_2 is derived from root respiration, approximately 90 to 170 g $C \cdot m^{-2}$ was derived from heterotrophic activity. The C source for this heterotrophic activity includes soil C pools, fine root turnover, and root exudates. To demonstrate the potential importance of fine-root turnover, we extrapolated seedling-level root data from Table 3 to obtain a maximum standing stock

Fig. 4. Variation in percent fungi (percent of occurrence in minirhizotron frames) by month (averaged across CO_2 and N), CO_2 treatment (averaged across months and N), and N (averaged across months and CO_2). Data are means and standard errors.



estimate of 30 $g \cdot m^{-2}$ for fine roots in October 1993 (e.g., (12.2 g/seedling X 21 seedlings/chamber) ÷ 8.4 m^2). In our systems, roots turnover about once per year (Tingey et al. 1995). Hence, fine roots could contribute one-third to one-sixth of the carbon evolved in heterotrophic activity.

Summary and conclusions

Exposure to elevated atmospheric CO_2 increased the rate of soil CO_2 evolution. Higher soil CO_2 evolution rates generally occurred in conjunction with greater coarse root biomass and increased occurrence of fungal hyphae. Hence, the higher rates appear to be a result of the combined effects of greater belowground root and heterotrophic respiration under elevated atmospheric CO_2. Seasonal variation was substantial but it was difficult to determine causal mechanisms. Across all treatments and sample periods, percent fungi was significantly correlated with flux rate, which suggests that seasonal and treatment variation in heterotrophic populations contribute to the variation observed. Extrapolating our rates to the entire growing season resulted in estimates of total belowground C losses ranging from 188 to 332 g $C \cdot m^{-2}$, and losses were twofold higher under elevated atmospheric CO_2. This does not necessarily imply an accelerated net loss of soil C pools under conditions of elevated CO_2, however, because higher C allocation below ground (with associated root mortality) and perhaps root C exudates may offset the greater soil C losses as CO_2. In fact, Johnson et al. (1994) found that, on average, increased C inputs to the soil under elevated CO_2 in 1991 and 1993 more than offset the concomitant increased C losses via belowground respiration.

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Table 6. Growing season estimates of soil C evolution.

Treatment	C flux (g $C \cdot m^{-2} \cdot 6$ months $^{-1}$)
Ambient CO_2	188
+ 175 CO_2	332
+ 350 CO_2	274
Chamberless	172

Note: Values were obtained by averaging daily flux rates across N levels and season (Table 1), multiplying by 180, and converting to C.

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