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Nutrient availability alters belowground respiration of ozone-exposed ponderosa pine

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Summary Exposure to ozone (O_3) and changes in soil fertility influence both the metabolism of plant roots and their interaction with rhizosphere organisms. Because one indication of altered root metabolism is a change in belowground respiratory activity, we used specially designed measurement chambers to assess the effects of O_3 and nutrient availability on belowground respiratory activity of potted three-year-old ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.). Seedlings were exposed to a factorial combination of three O_3 treatments and three fertilization treatments in open-top O_3 exposure chambers.

Ozone exposure decreased and high nutrient supply increased total plant dry weight, but root/shoot ratios were not affected. In general, exposure to O_3 increased rates of belowground O_2 uptake and CO_2 release and the respiratory quotient (RQ, CO_2/O_2), although seasonal differences were detected. In October, following the second season of O_3 exposure, rates of belowground O_2 uptake and CO_2 release and RQ were increased in trees in the high-O_3 exposure treatment by 22, 73 and 32%, respectively, over values in control trees in charcoal-filtered air. Increasing nutrient supply resulted in decreasing rates of belowground O_2 uptake and CO_2 release but it had little effect on RQ. In the high-nutrient supply treatment, rates of belowground O_2 uptake and CO_2 release were decreased by 38 and 39%, respectively, compared with rates in the low-nutrient supply treatment. At the end of the second growing season, the high-nutrient supply treatment had decreased lateral root total nonstructural carbohydrates by 22% compared with the low-nutrient supply treatment.

Nutrient availability altered the belowground respiratory response to O_3, such that the response to O_3 was greatest in the low-nutrient supply treatment. Significant O_3 effects on belowground respiratory activity were apparent before any reduction in total plant growth was found, suggesting that roots and rhizosphere organisms may be early indicators of physiological dysfunction in stressed seedlings.

Keywords: carbon dioxide, microbial respiration, nutrient availability, respiration quotient, root respiration, soil fertility.

Introduction

Tropospheric ozone (O_3) adversely affects the growth of trees (Hogsett et al. 1985*a*, Miller 1987, Taylor et al. 1994). In most studies, emphasis has been placed on understanding the relationship between tree photosynthesis and growth in response to O_3 (Coyne and Bingham 1981, Clark et al. 1995). However, because 20 to 50% of the carbon fixed in photosynthesis may be translocated belowground (Cheng et al. 1993, Hendrick and Pregitzer 1993) and as much as 25% lost through belowground respiration (Snellgrove et al. 1982, Schumacher and Smucker 1985, Cheng et al. 1993, Rygiewicz and Andersen 1994), it is important to understand how O_3 affects belowground respiration.

Because O_3 pollution and low nutrient supply often co-occur in the field, various combinations of O_3 stress and low nutrient availability have been studied in several plant species; however, no consistent pattern of response has been observed (Greitner and Winner 1989). Tjoelker and Luxmoore (1991) found that current-year needle biomass was reduced by O_3 in loblolly pine (*Pinus taeda* L.) grown at high N concentrations, but not at low N concentrations, whereas Pääkkönen and Holopainen (1995) found the opposite response in birch (*Betula pendula* Roth.). Similarly, Heagle (1979) found that soybean plants were more sensitive to O_3 at low fertilization rates than at high fertilization rates. In aspen (*Populus tremuloides* Michx.), Greitner et al. (1994) found no significant interaction between N and O_3 on photosynthesis, whereas Pell et al. (1995) found that N limitation decreased biomass losses due to O_3.

In many plant species, O_3 reduces root growth more than shoot growth (Horsman et al. 1980, Reinert and Gray 1980, Hogsett et al. 1985*a*, Cooley and Manning 1987, Nouchi et al. 1991). Although decreased photosynthetic rates resulting from O_3 exposure reduce the amount of carbon available to both aboveground and belowground plant processes, this does not necessarily require a concomitant decrease in the proportion of total carbon lost as CO_2 through belowground respiratory processes. A few studies have observed O_3-induced decreases in root respiration (Hofstra et al. 1981, Ito et al. 1985, Edwards

1991), and C-allocation to roots (Gorissen et al. 1991, Andersen and Rygiewicz 1995); however, most of these studies were of short duration and none included simultaneous measurements of rates of O_2 uptake and CO_2 release.

Estimates compiled by Wiant (1967) suggest that root respiration makes up at least 33% of total soil CO_2 evolution in forests, and represents a significant proportion of carbon fixed annually. Despite the importance of nutrient supply and O_3 on belowground fluxes of carbon, there is little information on the interaction of these two stresses. If O_3 decreases photosynthesis, but also changes belowground respiratory patterns, the effect of O_3 on forest productivity could be even greater than indicated solely by reductions in photosynthesis. We report a three-year study examining the combined influences of O_3 exposure and soil fertility on belowground respiration of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.). We tested the hypothesis that nutrient availability alters the respiratory response to ozone of the belowground system of ponderosa pine.

Materials and methods

Seedling culture

Ponderosa pine (*Pinus ponderosa*) seedlings, grown from seed originating in Butte County, CA (Zone 521, USDA Forest Service) at an elevation of 769–923 m, were obtained from the California Department of Forestry as one-year-old container-grown stock in December 1990. Seedlings were transplanted to 7 × 25 cm pots filled with perlite supplemented with 3 g of slow-release fertilizer (17,6,10 N,P,K plus micronutrients) and grown in a nursery for one year.

In April 1992, seedlings were transplanted to PVC pipe pots (10-cm diameter × 30-cm depth) containing a 1/1 (v/v) mixture of a commercial growing medium and perlite amended with either 12 g (full rate), 8 g (2/3 rate) or 4 g (1/3 rate) of slow-release fertilizer (17,6,10 N,P,K plus micronutrients). Calculated nutrient concentrations for these fertilization rates are given in Table 1. Plants were grown for two months in an open nursery before being placed in O_3-exposure chambers for four months (June to October 1992). The plants were then moved to an open nursery to overwinter.

In December 1992, plants were transplanted to PVC pipe pots (15-cm diameter × 40-cm depth) containing the same growing medium supplemented with either 37, 25 or 12 g of slow-release fertilizer. Nutrient application rates and maximum calculated concentrations are given in Table 1. Plants were grown for five months in an open nursery before being subjected to a second four-month exposure to O_3 (May to October 1993). In November 1993, plants were transplanted to large PVC pipe pots (20-cm diameter × 40-cm depth) and supplemented with either 66.7, 44.5, or 22.2 g of slow-release fertilizer (Table 1). Plants were subsequently moved to the open nursery for the winter. Subsets of plants were harvested in September 1992 after one O_3 exposure season, October 1993 after the second O_3 exposure season and September 1994 after one season of growth without O_3. Throughout the study, seedlings were watered as needed, generally at 3–5 day intervals, based on the gravimetric measurement of a subset of plants.

Table 1. Nutrient concentration of the high-nutrient supply treatment based on pot volume for each year from 1992 to 1994.[1]

Nutrient	High-nutrient supply (g equivalent vol^{-1})[2]		
	1992	1993	1994
NH_4	0.495	0.509	0.482
NO_3	0.429	0.442	0.418
P_2O_5	0.326	0.336	0.318
K_2O	0.543	0.560	0.529
Ca	0.082	0.084	0.079
Mg	0.054	0.056	0.053
S	0.217	0.224	0.212
B	0.001	0.001	0.001
Cu	0.003	0.003	0.003
Fe	0.022	0.022	0.021
Mn	0.005	0.006	0.005
Mo	0.00005	0.00006	0.00005
Zn	0.003	0.003	0.003
Pot size (liter)	2.3	6.7	12.6
Fertilizer (N,P,K 17,6,10) (g)	12.5	37.5	66.7

[1] Assuming release for 8 to 9 months at a soil temperature of 20 °C.
[2] Values are for the high-nutrient supply treatment only. Other treatment rates can be approximated by multiplying nutrient supply by 2/3 or 1/3.

Pesticides were applied as needed to control aphids and spider mites.

Ozone exposures

Seedlings were exposed to O_3 for a total of 122 days during 1992 (June 1 to September 30, 1992) in six modified open-top fumigation chambers in Corvallis, OR. During 1993, plants were exposed to O_3 for a total of 142 days (May 15 to September 30, 1993) in nine open-top chambers. Plants not harvested after the second year of exposure (1993) were not exposed to O_3 in 1994. Fumigations were monitored and controlled by an automated gaseous pollutant exposure system (Hogsett et al. 1985*b*). Ozone treatment profiles were developed to reflect ambient regional air quality for the Midwest United States (Lefohn et al. 1986, 1987) and consisted of episodic patterns (EP) of varying daily peak concentrations on 28-day cycles as described by Clark et al. (1995).

Three treatments were replicated two (1992) or three (1993) times: CF (control, charcoal-filtered air), EP-23 (23 μmol mol^{-1} O_3 per cycle) and EP-31 (31 μmol mol^{-1} O_3 per cycle). Total O_3 exposure values (SUM00) were calculated for each chamber by summing the hourly mean concentrations for 122 days in 1992 and 142 days for 1993 (Table 2).

Morphological measurements

Total plant height (H) and root collar diameter (D) were measured at the same time as belowground measurements were taken. Total aboveground plant size, calculated as D^2H, was used to estimate root biomass to adjust gas exchange measures when plants were not destructively harvested. Subsets of plants

Table 2. Total O_3 exposure (SUM00) and duration of fumigation periods for each year of the ponderosa pine study. Ozone treatments were: CF = charcoal-filtered air; EP-23 = 23 μmol mol^{-1} O_3 per cycle; and EP-31 = 31 μmol mol^{-1} O_3 per cycle.

Ozone treatment	SUM00 ozone exposure (μmol mol^{-1}·h)		
	1992 Total[1]	1993[2] August	1993 Total
CF (Control)	27.833[3]	47.638	57.230
EP-23	99.623	177.425	217.665
EP-31	133.442	238.770	293.051
Duration (days)	122	93	144

[1] Period in exposure chamber per exposure season. Total = all season; August = until Exposure Day 93 in August.
[2] The 1993 values are cumulative over both exposure years.
[3] Values are averaged over chamber replicates (2 for 1992 and 3 for 1993).

were harvested in September 1992 and October 1993. At harvest, plants were partitioned into needle, stem, taproot and lateral root components and oven-dried weights obtained.

Root respiration instrumentation

Specially designed PVC pot enclosures similar to those described by Edwards (1991) were used to measure CO_2 and O_2 fluxes from the soil surface with a Micro-Oxymax 4.2 System with CO_2 and O_2 sensors (Columbus Instruments, Columbus, OH). The Micro-Oxymax System is a closed system designed to detect low amounts of O_2 uptake and CO_2 release and to calculate the respiratory quotient (RQ) of sequential samples. Air in the enclosed headspace above the soil surface was pumped through the gas sensors and returned to the pot enclosure where gas fractions were measured periodically and changes in the amounts were used to compute O_2 uptake and CO_2 release (normalized to 0 °C and 760 mm Hg). A reference chamber was used to recalibrate the sensors before each measurement. The oxygen sensor operated as an oxygen battery (fuel cell) measuring O_2 percentage directly, and the CO_2 sensor was a single-beam nondispersive infrared device. The system was fully automated and computerized for the sequential measurement of up to 20 plants at one time.

To make measurements, PVC enclosures were attached to pots containing trees by encircling the stem of the tree with enough closed-cell foam to create a tight seal between the stem and the hole in the center of the top of the PVC enclosure. Closed-cell foam seams on the top of the PVC enclosures were pressed together and attached to the base of the PVC enclosure with spring-loaded clamps that encircled the top of the pot. Two small hose connectors on the top of the PVC enclosure were used to connect the enclosure to the Micro-Oxymax System. Sampling volume of each pot was measured automatically.

Belowground respiration measurements

Measurements of CO_2 release and O_2 uptake, and respiratory quotient (RQ, CO_2/O_2; Minchin and Witty 1990) from the soil surface of pots were obtained for a subset of plants in September 1992 (after the first exposure season), April 1993 (before the second exposure season), August 1993 (after full expansion of four-year-old needles), October 1993 (after the second exposure season) and April 1994. Soil temperature and soil water content were monitored at the time of measurement. Also, at each harvest date (September 1992 and October 1993), rates of CO_2 release and O_2 uptake and RQ were measured on a subset of plants, the plants were detopped and pots containing roots and soil were kept at 5 °C. After seven days at 5 °C, rates of CO_2 release and O_2 uptake and RQ were remeasured to provide an estimate of basal respiration rates (Marshall and Perry 1987).

Root carbohydrate analyses

Fine roots were sampled in the fall of 1993 after the second O_3 exposure season and in 1994 after shoot elongation was completed. All samples removed for carbohydrate analyses were frozen at −70 °C and lyophilized within 72 h of harvest. Lyophilized tissue samples were weighed, ground in a Wiley mill (40 mesh), bottled, and redried before removing a subsample for carbohydrate analysis. Samples were extracted and concentrations of tissue starch, glucose, sucrose, fructose and monosaccharides were determined as described previously (Wilson et al. 1995).

Statistical analyses

We used a split-plot design with whole-plot treatments of three O_3 concentrations randomized within two (1992) or three (1993) chambers per O_3 treatment. Subplot treatments included three nutrient supply rates with five (1992) or four (1993) plants per nutrient supply treatment per chamber, for a total of 90 (1992) or 108 (1993) plants. Data were log transformed (i.e., $y = \log_e(y + 1)$) to reduce the heterogeneity of variances between treatment groups that was detected by Bartlett's Test (Bartlett 1937).

Analysis of variance (ANOVA) was performed on rates of belowground CO_2 release and O_2 uptake and RQ, morphological, harvest and root carbohydrate data separately for each of the measurement dates. Orthogonal polynomial contrasts based on the SUM00 O_3 were used to test for trends in the effects of O_3 on plant response. Polynomial contrasts based on nutrient supply rate (full, 2/3 and 1/3 rates) were used to test for trends in fertilized plants. Differences in mean response between the control treatment (CF at full-rate fertilization) and other treatment combinations were tested by Bonferroni's mean separation test (Miller 1981).

Repeated measurements of rates of belowground O_2 uptake and CO_2 release and RQ taken at pre-harvest and post-harvest dates were analyzed by multivariate ANOVA (MANOVA) to test for O_3 and nutrient supply effects and their interaction. Post-MANOVA tests included orthogonal polynomial contrasts for O_3 and fertilization main effects to detect linear and quadratic trends in plant response.

Results

Morphological responses to O_3 and soil fertility

By the end of the first season of growth in September 1992, low-nutrient supply had caused a significant linear reduction in total plant biomass (Table 3). After two seasons of growth with the low-nutrient supply, there was a significant reduction in the biomass of all seedling components (Table 4). Ozone exposure did not significantly reduce biomass during the first season of exposure (Table 3); however, during the second season, O_3 reduced the biomass of all seedling components (Table 4). Both O_3 and nutrient main effects were significant after the second exposure season, but there were no interactions between the treatments.

Premature needle senescence of the oldest foliage age classes was observed in the EP-23 and EP-31 O_3 treatments by the end of the study in 1994. Control plants retained approximately 76% of their 3-year-old needles, whereas seedlings in the EP-23 and EP-31 O_3 treatments retained 43 and 0%, re-

Table 3. Summary of ANOVA for ponderosa pine biomass responses to O_3 exposure and nutrient supply in September 1992 after one season of exposure to O_3. Ozone treatments were: CF = charcoal-filtered air; EP-23 = 23 μmol mol^{-1} O_3 per cycle; and EP-31 = 31 μmol mol^{-1} O_3 per cycle.

Factor	Ozone	Nutrient supply	Needle[1]	Stem	Above-ground	Total Root	Total Plant	Root: Shoot
LS Means of biomass (g_{dw})	CF (Control)	1/3	20.40	20.58	40.71	24.30	65.51	0.59
		2/3	18.77	18.47	37.25	19.38	57.51	0.51
		Full	20.18	17.91	37.69	23.12	61.38	0.59
	EP-23	1/3	13.71*	19.67	32.32	24.93	58.73	0.76*
		2/3	16.10	20.41	35.25	36.06	62.86	0.71*
		Full	21.61	22.48*	43.46	25.96	70.38	0.58
	EP-31	1/3	12.59*	17.99	30.61	21.16	52.15	0.57
		2/3	16.52	22.91*	38.79	26.76	66.79	0.65
		Full	22.33	21.96*	41.87	24.55	69.78	0.56
P > *F*	O_3		0.504	0.311	0.759	0.346	0.849	0.181
	SUM00-Linear[2]		0.300	0.171	0.518	0.298	0.698	0.164
	SUM00-Quadratic		0.705	0.608	0.808	0.306	0.712	0.180
	Fertility		0.003	0.483	0.042	0.855	0.154	0.007
	Fertility-Linear		0.001	0.269	0.012	0.578	0.055	0.002
	Ferility-Quadratic		0.372	0.629	0.858	0.980	0.871	0.767
	O_3 × Fertility		0.184	0.069	0.105	0.293	0.190	0.031

[1] An asterisk indicates that LS means were significantly ($P < 0.05$) different from CF controls in the high-nutrient supply treatment.
[2] Where SUM00 refer to contrasts based on exposure indices given in Table 2.

Table 4. Summary of ANOVA for ponderosa pine biomass responses to O_3 exposure and nutrient supply in October 1993 after two seasons of exposure to O_3. Ozone treatments were: CF = charcoal-filtered air; EP-23 = 23 μmol mol^{-1} O_3 per cycle; and EP-31 = 31 μmol mol^{-1} O_3 per cycle.

Factor	Ozone	Nutrient supply	Needle[1]	Stem	Above-ground	Root	Plant	Root: Shoot
LS Means of biomass (g_{dw})	CF (Control)	1/3	67.79*	76.13*	144.69*	61.85	206.78*	0.42
		2/3	85.70	93.19	180.57	66.34	247.17	0.36
		Full	105.00	103.35	209.09	84.09	293.78	0.40
	EP-23	1/3	38.69*	56.69*	96.62*	39.19*	136.24*	0.41
		2/3	56.56*	68.10*	124.77*	45.78*	173.99*	0.39
		Full	75.55	95.96	172.25	62.75	236.07	0.37
	EP-31	1/3	23.94*	40.89*	66.75*	25.93*	93.08*	0.39
		2/3	51.19	69.58*	121.79*	46.86*	169.70	0.39
		Full	44.14	84.20	133.71*	57.54	191.39*	0.43
P > *F*	O_3		0.043	0.058	0.042	0.019	0.032	0.914
	SUM00-Linear[2]		0.019	0.026	0.189	0.008	0.014	0.900
	SUM00-Quadratic		0.520	0.772	0.702	0.883	0.735	0.711
	Fertility		0.000	0.000	0.000	0.000	0.000	0.475
	Fertility-Linear		0.000	0.000	0.000	0.000	0.000	0.652
	Ferility-Quadratic		0.182	0.548	0.318	0.929	0.409	0.257
	O_3 × Fertility		0.566	0.249	0.419	0.278	0.362	0.475

[1] An asterisk indicates that LS means were significantly ($P < 0.05$) different from CF controls in the high-nutrient supply treatment.
[2] Where SUM00 refer to contrasts based on exposure indices given in Table 2.

spectively, of their 3-year-old needles by the end of the study in August 1994 (data not shown). The loss of foliar biomass due to early senescence was reflected in the October 1993 harvest (Table 4).

Root carbohydrate responses to O_3 and soil fertility

High nutrient supply significantly increased sucrose concentrations and decreased starch concentrations of lateral roots after the second season (October 1993) (Table 5). Although lateral root glucose, fructose and monosaccharide concentrations were not significantly influenced by nutrient supply, total nonstructural carbohydrates (TNC) decreased with increasing nutrient availability.

To follow the carry-over effect of O_3 exposure, a harvest was conducted in August 1994, the year following the second exposure season (Table 5). Lateral root glucose, fructose and monosaccharide concentrations all decreased significantly with increasing nutrient availability (Table 5). Lateral root TNC, glucose, fructose, sucrose, monosaccharides and starch concentrations were all significantly higher in O_3-exposed plants than in control plants.

Respiration responses to O_3 and soil fertility

On all sample dates, O_3 significantly increased the rate of belowground O_2 uptake expressed per m^2 soil surface area (Table 6). In April, August and October 1993, the rate of belowground O_2 uptake generally showed a linear increase with increasing exposure to O_3. The rate of belowground CO_2 release, expressed per m^2 soil surface, was significantly affected by O_3 in September 1992, and April and August 1993. In general, increased exposure to O_3 resulted in increased RQ; however, the effect was only significant on the October 1993 sampling date (Figure 1). The nutrient supply treatments significantly affected the rates of belowground O_2 uptake and CO_2 release in April and August 1993, but did not alter RQ ratios at any date. Belowground respiratory rates were lower in the high-nutrient supply treatment than in the low-nutrient supply treatment in April 1993 and 1994, whereas in August 1993, belowground respiratory rates were higher in the high-nutrient supply treatment than in the low-nutrient supply treatment. There were significant interactions between O_3 and nutrient availability on three sample dates for rate of belowground O_2 uptake, and two sample dates for rate of belowground CO_2 release. There was no apparent interaction between O_3 and nutrient availability on RQ values (Figure 1).

The O_3 and low-nutrient supply treatments significantly increased belowground O_2 uptake rates on four of five sample dates when the data were expressed on a root dry weight basis (Table 7). The rate of belowground CO_2 release was also significantly greater in O_3-exposed plants than in control plants on three of the five sample dates, and the low-nutrient supply treatment significantly increased the rate of belowground CO_2 release on three of the five sample dates (Figure 2, Table 7). Significant interactions between O_3 and nutrient availability on the rate of belowground CO_2 release were observed in April 1993 and 1994. In general, there were linear increases in the rates of belowground O_2 uptake and CO_2 release with increasing exposure to O_3 (Figures 2 and 3, Table 7). On all measurement dates, belowground respiratory rates were higher in the low-nutrient supply treatment than in the high-nutrient supply treatment (Figures 2 and 3).

Comparison of pre- and post-harvest respiration

Immediately following the respiration measurements in September 1992 and October 1993, plants were detopped and soil gas exchange was remeasured after 5 days at 5 °C to estimate basal metabolic rates. Ozone had significant effects on changes in the rates of pre- versus post-harvest belowground O_2 uptake

Table 5. Summary of ANOVA of effects of O_3 exposure and nutrient supply on ponderosa pine lateral root carbohydrate concentrations in October 1993 and August 1994. Ozone treatments were: CF = charcoal-filtered air; EP-23 = 23 μmol mol^{-1} O_3 per cycle; and EP-31 = 31 μmol mol^{-1} O_3 per cycle.

Date	Ozone	Nutrient supply	Glucose[1]	Fructose	Sucrose	Mono-saccharide	Starch	TNC[2]
October 1993	CF (Control)	1/3	47.46	52.99	72.08*	100.29*	281.21*	554.03
		2/3	40.09	44.40	85.75	84.58	180.47*	435.29
		Full	36.91	38.82	86.61	77.17	192.87	432.38
P > F		Fertility	0.297	0.294	0.018	0.030	0.016	
August 1994	CF (Control)	1/3	64.19	66.22	52.62*	130.42	397.07*	710.52
		2/3	65.84	66.83	69.63	132.67	437.19*	772.16
		Full	52.35	55.41	68.89	107.76	251.67	536.08
	EP-31	1/3	79.38*	86.71*	54.61*	166.09*	425.46*	812.25
		2/3	84.15*	84.09*	67.95	168.25*	321.55*	725.99
		Full	62.74	64.86	87.19*	127.61	317.29	659.69
P > F		O_3	0.041	0.079	0.082	0.070	0.827	
		Fertility	0.004	0.009	0.000	0.003	0.000	
		$O_3 \times$ Fertility	0.764	0.616	0.009	0.685	0.007	

[1] An asterisk indicates LS means significantly ($P < 0.05$) different from CF controls in high-nutrient supply treatment.
[2] Where TNC = Total nonstructural carbohydrates.

and CO_2 release in September 1992 (Figure 4). In September 1992, plants that received the EP-23 O_3 treatment showed large declines in rates of belowground O_2 uptake and CO_2 release following detopping, whereas plants in the EP-31 O_3 treatment showed opposite responses (Figure 4). Control plants showed no change in rates of belowground O_2 uptake and CO_2 release in response to detopping. After detopping, RQ increased in plants exposed to the EP-23 O_3 treatment, whereas RQ decreased in plants exposed to the EP-31 O_3 treatment.

By October 1993, changes in the rates of belowground O_2 uptake and CO_2 release and RQ in response to detopping were significantly affected by both nutrient availability and O_3 (Figure 5). Rates of belowground O_2 uptake decreased in all O_3 treatments following detopping in October 1993 (Figure 5). Although the rate of belowground CO_2 release tended to decrease following detopping, the rates were not significantly different from those of intact plants (Figure 5). Changes in the rates of belowground CO_2 release and O_2 uptake resulted in RQ values of control plants increasing dramatically following detopping, whereas plants exposed to the EP-31 O_3 treatment showed relatively small increases in RQ following detopping (Figure 5).

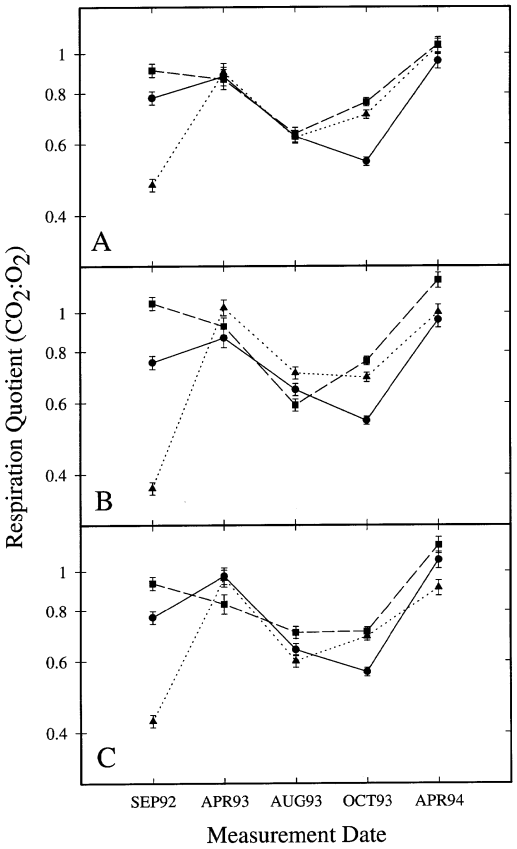

Figure 1. Respiratory quotient of soil surrounding roots of ponderosa pine grown at (A) 1/3, (B) 2/3 or (C) full fertilization and exposed to either EP-31 (31 μmol mol^{-1} O_3 per cycle) (■), EP-23 (23 μmol mol^{-1} O_3 per cycle) (▲) or charcoal-filtered air (●). Geometric LS means and standard errors plotted on a $\log_{10} y$-axis.

Discussion

There was a significant reduction in the biomass of all plant components after two seasons of exposure to O_3 (Table 4). In the EP-31 O_3 treatment, total plant biomass was reduced by 55% in response to low-nutrient supply compared with 35% in response to high nutrient supply, although no significant interactions were detected (Table 4). Although there have been other studies of tree responses to a combination of O_3 stress

Table 6. Summary of ANOVA for ponderosa pine respiratory responses (μM m^{-2} h^{-1}) to O_3 exposure and nutrient supply. RQ = respiratory quotient.

Response Variable	Date	Ozone effects Main	SUM00[1]		Nutrient supply effects Main	Level		Interaction $O_3 \times$ Fertility
			Linear[2]	Quadratic		Linear	Quadratic	
O_2 uptake rate	Sept 92	0.003	0.720	0.001	0.341	0.476	0.200	0.047
	Apr 93	0.085	0.045	0.299	0.000	0.000	0.033	0.000
	Aug 93	0.035	0.013	0.852	0.001	0.000	0.346	0.410
	Oct 93	0.013	0.006	0.126	0.735	0.443	0.883	0.921
	Apr 94	0.012	0.999	0.005	0.037	0.011	0.983	0.000
CO_2 release rate	Sept 92	0.001	0.643	0.000	0.288	0.306	0.230	0.235
	Apr 93	0.066	0.029	0.945	0.000	0.000	0.127	0.000
	Aug 93	0.003	0.001	0.657	0.006	0.005	0.122	0.379
	Oct 93	0.183	0.432	0.101	0.137	0.214	0.115	0.553
	Apr 94	0.177	0.472	0.094	0.644	0.378	0.725	0.004
RQ	Sept 92	0.795	0.949	0.534	0.308	0.141	0.668	0.132
	Apr 93	0.195	0.722	0.095	0.327	0.312	0.267	0.169
	Aug 93	0.993	0.916	0.977	0.890	0.680	0.805	0.424
	Oct 93	0.022	0.009	0.471	0.963	0.860	0.835	0.916
	Apr 94	0.516	0.427	0.427	0.858	0.686	0.715	0.495

[1] Where SUM00 refer to contrasts based on exposure indices given in Table 2.
[2] Where Linear and Quadratic denote linear and quadratic contrasts, respectively, for the O_3 and nutrient supply treatments.

Table 7. Summary of ANOVA for ponderosa pine respiratory responses to O_3 and nutrient supply on a root dry weight basis ($\mu M\ h^{-1}\ g^{-1}$ root dry weight). Means for CO_2 and O_2 are shown in Figures 2 and 3, respectively.

Response Variable	Date	Ozone effects Main	SUM00[1]		Nutrient supply effects Main	Level		Interaction $O_3 \times$ Fertility
			Linear[2]	Quadratic		Linear	Quadratic	
O_2 Flux	Sept 92	0.005	0.425	0.002	0.405	0.514	0.243	0.117
	Apr 93	0.089	0.043	0.042	0.000	0.000	0.025	0.000
	Aug 93	0.948	0.783	0.884	0.039	0.011	0.989	0.339
	Oct 93	0.051	0.025	0.329	0.000	0.000	0.616	0.010
	Apr 94	0.025	0.054	0.017	0.000	0.000	0.076	0.000
CO_2 Flux	Sept 92	0.005	0.188	0.002	0.317	0.262	0.308	0.241
	Apr 93	0.112	0.051	0.932	0.000	0.000	0.084	0.000
	Aug 93	0.927	0.716	0.935	0.109	0.036	0.847	0.281
	Oct 93	0.025	0.011	0.602	0.000	0.000	0.974	0.225
	Apr 94	0.028	0.048	0.022	0.000	0.000	0.023	0.001

[1] Where SUM00 refer to contrasts based on exposure indices given in Table 2.
[2] Where Linear and Quadratic denote linear and quadratic constants, respectively, for the O_3 and nutrient supply treatments.

and nutrient availability, no consistent pattern has been observed across species or treatments (Greitner and Winner 1989). Tjoelker and Luxmoore (1991) found that O_3 reduced the biomass of current-year needles of loblolly pine grown at high N concentrations but not at low N concentrations, and suggested that loblolly was more sensitive to O_3 at high N

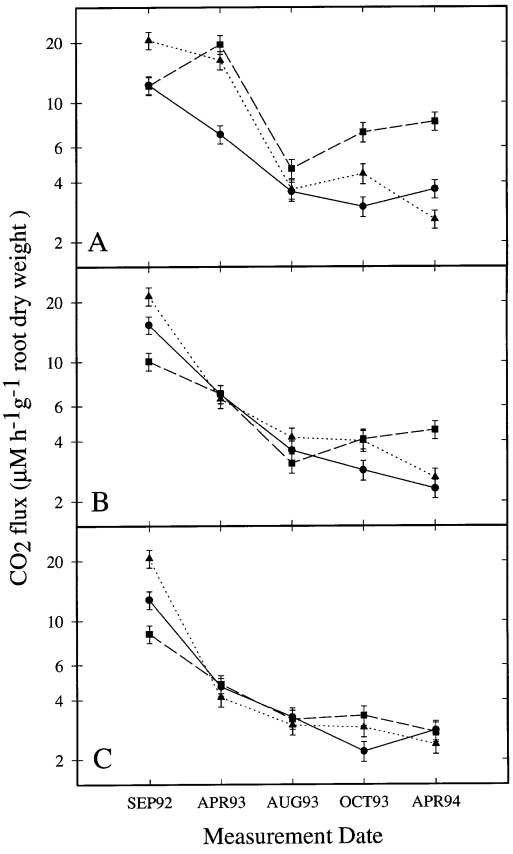

Figure 2. CO_2 production of soil surrounding roots of ponderosa pine grown at (A) 1/3, (B) 2/3 or (C) full fertilization and exposed to either EP-31 (31 μmol mol^{-1} O_3 per cycle) (■), EP-23 (23 μmol mol^{-1} O_3 per cycle) (▲) or charcoal-filtered air (●). Geometric LS means and standard errors plotted on a $\log_{10}$ *y*-axis.

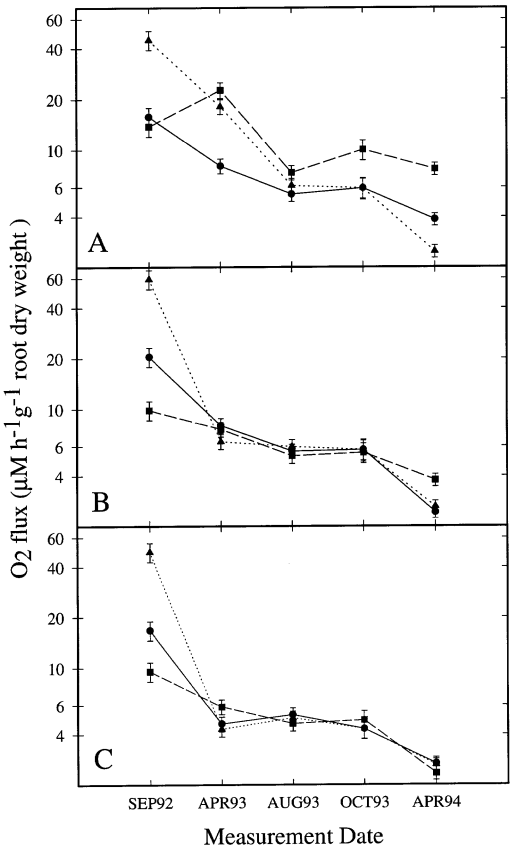

Figure 3. O_2 consumption of soil surrounding roots of ponderosa pine grown at (A) 1/3, (B) 2/3 or (C) full fertilization and exposed to either EP-31 (31 μmol mol^{-1} O_3 per cycle) (■), EP-23 (23 μmol mol^{-1} O_3 per cycle) (▲) or charcoal-filtered air (●). Geometric LS means and standard errors plotted on a $\log_{10}$ *y*-axis.

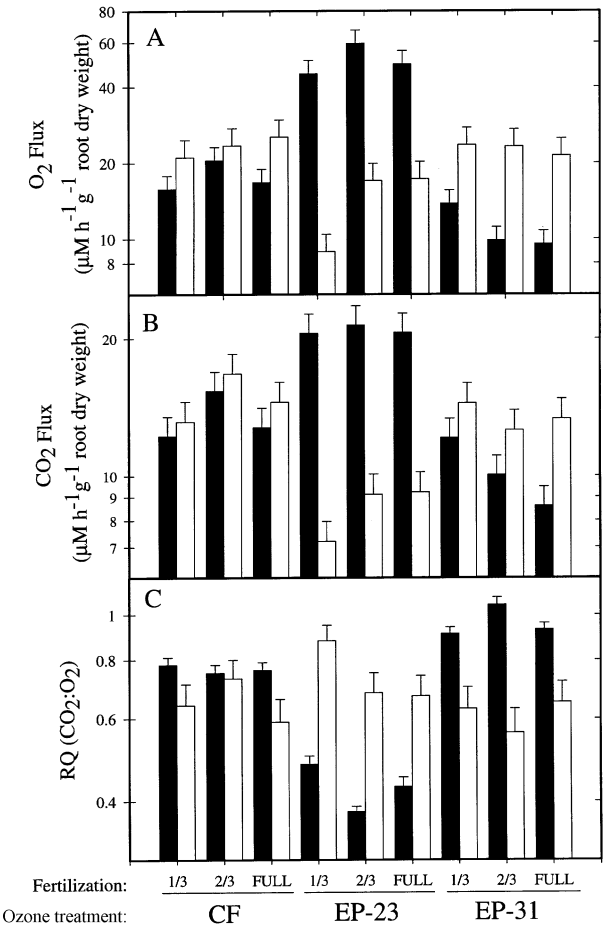

Figure 4. Comparison of September 1992 pre-harvest (filled) and post-harvest (open) O_2 consumption (A), CO_2 production (B), and respiratory quotient (RQ) (C) of soil surrounding roots of ponderosa pine grown at 1/3, 2/3 or full fertilization and exposed to either EP-31 (31 μmol mol^{-1} O_3 per cycle), EP-23 (23 μmol mol^{-1} O_3 per cycle) or charcoal-filtered air (CF). Geometric LS means and standard errors plotted on a $\log_{10}$ *y*-axis.

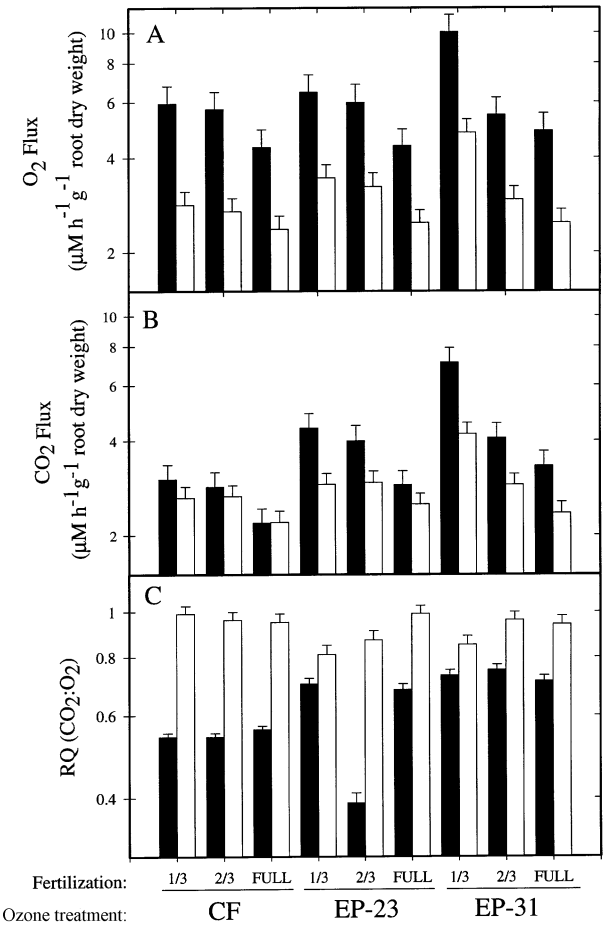

Figure 5. Comparison of October 1993 pre-harvest (filled) and post-harvest (open) O_2 consumption (A), CO_2 production (B), and RQ (C) of soil surrounding roots of ponderosa pine grown at 1/3, 2/3 or full fertilization and exposed to either EP-31 (31 μmol mol^{-1} O_3 per cycle), EP-23 (23 μmol mol^{-1} O_3 per cycle) or charcoal-filtered air (CF). Geometric LS means and standard errors plotted on a $\log_{10}$ *y*-axis.

concentrations. Pääkkönen and Holopainen (1995) found the opposite response in leaf biomass and root dry weight in birch (*Betula pendula* Roth.), with increased resistance to O_3 with increasing N concentration. Greitner et al. (1994) did not find a significant interaction between N and O_3 on leaf area or photosynthesis in aspen, but hypothesized that a lower N treatment might have been necessary to detect a significant O_3 by N interaction. Heagle (1979) found that soybean plants exposed to O_3 were more sensitive at low-nutrient supply rates than at high-nutrient supply rates. In hybrid poplar, fertilization with one-half the recommended rate of N,P,K fertilizer was associated with increased O_3-induced foliar injury compared with unfertilized plants or those receiving the full fertilization treatment (Harkov and Brennan 1980). We found that the effects of O_3 and nutrient availability on ponderosa pine biomass were additive, although there was a trend of greater O_3 response at low-nutrient supply.

Ponderosa pine showed increased rates of belowground CO_2 release and O_2 uptake when exposed to O_3, and the effect of O_3 was greatest at low-nutrient supply (Figures 2 and 3). Increased belowground respiration in O_3-treated ponderosa pine may reflect altered root metabolism and fine root production (Temple et al. 1993), as well as accelerated rates of root turnover and associated decomposition by free-living soil organisms. Other studies have shown decreased or increased rates of belowground respiration, depending on the species examined, the duration of the ozone exposure, and the approach used to measure respiration. Using a technique similar to the approach employed here, Edwards (1991) found that O_3 exposure reduced root respiration of loblolly pine (*P. taeda* L.) seedlings. Gorissen et al. (1991) found that ^{14}C release from root plus soil respiration was significantly reduced in *Pseudotsuga menziesii* (Mirb.) Franco during the first one to two weeks after exposure to O_3, followed by a recovery period during which respiratory release rates returned to control values. An-

dersen and Rygiewicz (1995) found that, although total belowground respiration was not significantly altered by short-term exposure to O_3, the maximum rate of allocation to and respiratory release of ^{14}C by ponderosa pine roots was decreased. In rice (*Orzya sativa* L.), Nouchi et al. (1991) reported that a one-week exposure to 0.1 ppm O_3 reduced root respiration by 16%, whereas a 3- to 7-week period of exposure to O_3 resulted in elevated root respiration. The physiological mechanisms underlying increased or decreased respiration rates of roots and associated soil organisms of O_3-exposed plant systems have not been fully elucidated.

Although O_3 does not penetrate the soil beyond a few centimeters, several studies have shown indirect effects of O_3 on rhizosphere organisms. Reddy et al. (1991) observed an interaction between soil pH and O_3 on rhizosphere enzymes. Shafer (1988) found an interaction between O_3 and simulated acid rain on numbers of fungal propagules in the rhizosphere of sorghum. We obtained preliminary evidence that O_3 exposure of ponderosa pine shoots can increase the active fungal and bacterial populations in the rhizosphere (Andersen and Scagel 1995). Ozone also alters the symbiotic associations of mycorrhizal fungi (Reich et al. 1986, Stroo et al. 1988, Simmons and Kelly 1989, Meier et al. 1990). Although our belowground respiration data included fauna, microbial respiration and root respiration, we estimate that non-root respiration of CO_2 and O_2 accounted for less than 20% of the total flux (unpublished results). Furthermore, our data are comparable to values obtained by direct measurements (Ledig et al. 1976, Cropper and Gholz 1991). If O_3 increases root turnover and stimulates soil decomposition processes, then the contribution of free-living soil organisms to the total soil flux may be greater under conditions of elevated O_3.

Although we used a containerized system, our rates of CO_2 flux from the pot surface of 2 to 20 μmol m^{-2} s^{-1} were comparable to *in situ* gas-exchange measures taken from field soils (Table 6). Hanson et al. (1993) found CO_2 fluxes of 0.8 to 5.7 μmol m^{-2} s^{-1} from an oak forest soil. Early studies employing gas-exchange chamber techniques (Reiners 1968, Garrett and Cox 1973, Edwards 1975, Edwards and Harris 1977) yielded seasonal minimum and maximum values of 1.1 and 7.6 mol m^{-2} s^{-1}, and studies employing CO_2 absorption techniques (Froment 1972, Anderson 1973, Larkin and Kelly 1987) gave seasonal minimum and maximum values of 0.7 and 9.2. Hanson et al. (1993) found distinct seasonal trends in rates of soil CO_2 release that followed variations in soil temperature and were related to soil water content and coarse fraction. We have also observed seasonality in gas flux, with high rates of CO_2 release and O_2 uptake during periods of high root activity in the spring and fall (Scagel and Andersen 1997).

Plant detopping eliminates the source of current photosynthate for roots, inhibits protein synthesis, and has been used to estimate maintenance and basal respiration rates in roots (Marshall and Perry 1987). Changes in ponderosa pine root metabolism following seedling detopping may reflect patterns of root growth occurring at the time of detopping. In 1992, belowground RQ increased following detopping in the EP-23 O_3 treatment, whereas seedlings in the EP-31 O_3 treatment showed a decrease in belowground RQ following detopping (Figure 4). One possible explanation for the pattern observed is that, at the time of detopping in 1992, root growth was occurring in the EP-23 O_3 treatment but not in the EP-31 O_3 treatment. Detopping would disrupt belowground respiratory activity to a greater extent in seedlings undergoing active root growth than in seedlings with slow root growth. Nonautotrophic CO_2 fixation in roots is thought to provide carbon skeletons for amino acid synthesis in roots (Vuorinen et al. 1992), and in other tissues, as much as 20% of the carbon used for protein synthesis may be derived from nonautotrophic CO_2 fixation (Hunt and Fletcher 1976). Decreased CO_2 fixation associated with decreased root growth in response to detopping may partly explain the increase in belowground RQ in seedlings exposed to the EP-23 O_3 treatment.

In October 1993, patterns of respiration following detopping were similar among treatments, suggesting synchronized root phenology at the time of detopping (Figure 5). In most cases, rates of belowground CO_2 release and O_2 uptake decreased or did not change following detopping. Decreased respiration and increased RQ following detopping in 1993 are consistent with a reduction in root growth and associated protein synthesis. Respiratory quotient in all treatments approached 1 following detopping, consistent with the hypothesis that detopping reduced non-autotrophic CO_2 refixation by the root (Minchin and Witty 1990, Vuorinen et al. 1992).

In summary, belowground respiration was more sensitive to O_3 in the low-nutrient supply treatment than in the high-nutrient supply treatment. Ozone-induced increases in the rates of belowground CO_2 release and O_2 uptake may have resulted in part from increased root turnover and decomposition by soil organisms. The effects of O_3 and nutrient supply rate on root carbohydrate concentrations generally paralleled the effects of these treatments on belowground respiration rates. Values of RQ suggested that basal root metabolism was altered by exposure to ozone; however, additional studies will be necessary to determine the mechanisms involved. Because forest soils are often nutrient deficient, the significant interactions between soil fertility and O_3 must be considered when assessing the impact of O_3 on ponderosa pine.

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