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Ozone Stress and Changes Below-Ground: Linking Root and Soil Processes

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S u m m a r y

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Physiological changes in roots in response to tropospheric ozone can lead to altered below-ground processes, and responses are not always predictable due to the complexity of the belowground ecosystem. Previous experiments have shown that ozone reduces carbon allocation to roots and mycorrhizal hyphae, and reduces root starch in ponderosa pine. Ozone was found to increase CO_2 flux from soils containing ponderosa pine seedlings in controlled exposures, a response seemingly inconsistent with results showing decreased allocation to roots. We hypothesize this apparent paradox is due to a combination of factors including altered root metabolism and increased soil microbial respiration. Increased microbial respiration is hypothesized to result from increased root exudation, which was observed in trials with wheat, and possibly increased root mortality and turnover. Increased microbial respiration was supported by findings of increased bacterial and fungal populations in soil of plants exposed to ozone. Responses are expected to be short term, since chronic ozone exposure would lead to lower standing root biomass over time and decreased CO_2 flux from soil.

Changes in carbon allocation to roots and mycorrhizae, reduced root growth, and altered carbon release to soil are all important factors that affect carbon fluxes into and out of forested ecosystems. The dependence of the soil organisms on carbon substrates from plants illustrates the potentially important role that ozone may play in altering ecosystem carbon fluxes. Ozone may alter nutrient availability, soil moisture holding capacity, and ultimately plant productivity through indirect effects on soil chemical and physical characteristics. Studies are currently underway in naturally regenerated stands of ponderosa pine to better understand how natural and anthropogenic stresses such as ozone affect roots and soil processes, and how changes occurring below-ground affect ecosystem productivity and sustainability.

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Introduction

There is still considerable uncertainty about the short- and long-term effects of tropospheric ozone on forested ecosystems (Air Quality Criteria for Ozone and Related Photochemical Compounds, U.S. EPA, 1996). Much of the uncertainty is due to our relative lack of understanding of the changes occurring in roots of plants exposed to ozone and how those changes affect carbon fluxes into and out of the belowground ecosystem. LAW & al. 1999 estimated that 76% of ecosystem respiration from an old growth ponderosa pine ecosystem (*Pinus ponderosa* Laws.) originated from root and microbial respiration of soils. In order to understand how ozone affects forested ecosystems, a better understanding of mechanistic processes occurring in plant roots and subsequent changes in the below-ground ecosystem is necessary.

The combination of stresses present in natural ecosystems makes it difficult to determine the role ozone may play in controlling ecosystem productivity. The goal of the research presented here is to understand physiological processes in roots, mycorrhizae and soil foodweb organisms of ponderosa pine in order to understand possible mechanisms of ozone response in natural ecosystems. Several controlled experiments have been conducted using ozone to follow carbon transport to roots and mycorrhizal fungi, and to soils to better understand how ozone may effect carbon flux from soil. Collectively, the results show that ozone decreases allocation of carbon to roots and mycorrhizal fungi, and decreases root biomass (ANDERSEN & al. 1991, 1997, ANDERSEN & RYGIEWICZ 1991, 1995). Carbon dioxide flux from soils of ozone-exposed pine seedlings increases, despite decreased allocation below-ground (ANDERSEN & SCAGEL 1997, SCAGEL & ANDERSEN 1997). These results suggest that one rapid response to ozone may be increased C lost from soil even though less carbon is being allocated below-ground. It is hypothesized that a transient, short-term response to ozone is increased carbon flux to microbial foodwebs and subsequent loss as CO_2 from ponderosa pine ecosystems. Carbon dioxide flux from soils will decrease over longer time intervals in systems exposed to chronic ozone exposure due to the cumulative negative effects of ozone on root system size. The purpose of this paper is to discuss the possible short- and long-term effects of ozone on soil CO_2 flux, and to illustrate how ozone may disrupt free-living soil organisms and ecosystem fluxes of carbon.

Materials and Methods

Three experiments were conducted in open-top chambers in Corvallis, OR, USA (HOGSETT & al. 1985a). Ponderosa pine used in the experiments were obtained from the California State Nursery (Magalia, CA) as 2-0 bare-root stock and transplanted into PVC pots (15 cm diameter x 38 cm deep). Two root substrates were used in the experiments for comparison. The first consisted of a potting mixture of Sunshine mix (SunGro Horticulture Inc., Bellevue, WA) and perlite (Supreme Perlite Co., Portland, OR) amended with slow release fertilizer (17-6-10 N, P, K Plus minor nutrients). The second substrate was a sandy loam soil collected from a ponderosa pine forest in Bend, OR, and was not amended with fertilizer. The details of maintenance and culture of these plants have been published elsewhere (ANDERSEN & al. 1991, ANDERSEN & SCAGEL 1997, SCAGEL & ANDERSEN 1997).

Ozone exposures

Plants were exposed to ozone in open-top fumigation chambers in three separate experiments in Corvallis, Oregon, between 1989 and 1994 (ANDERSEN & al. 1991, 1997, ANDERSEN & SCAGEL 1997, SCAGEL & ANDERSEN 1997). In 1989 and 1990 (experiment 1), plants received 112 days of exposure (June 7 to September 26) and 120 days of exposure (June 5 to October 3), respectively. The second and third experiments were conducted in 1992, 1993, and 1994 where plants were exposed for a total of 122 days (June 01 to September 30, 1992), 142 days (May 15 to September 30, 1993) and 131 days (June 7 to October 13, 1994), respectively. Three treatments were employed during each exposure season: CF (control, charcoal-filtered air), Ep-23 (23 μmol mol^{-1} per cycle) and Ep-31 (31 μmol mol^{-1} per 28 day exposure cycle). Total O_3 exposure values (SUM00) were calculated for each chamber by summing the hourly mean concentrations each day for the entire exposure period. Three replicate chambers were used for each ozone treatment. Fumigations were controlled by an automated gaseous pollutant exposure system (HOGSETT & al. 1985a).

Plant measures

Plant growth and biomass were measured using standard procedures. For carbohydrate analysis, tissues were lyophilized and analyzed for glucose, fructose, sucrose, monosaccharides, and starch using HPLC coupled with a pulsed amperometric detector (PAD) (WILSON & al. 1995, ANDERSEN & al. 1997).

Specially designed PVC pot enclosures were used to measure CO_2 release and O_2 uptake from the soil surface of each pot using a Micro-Oxymax 4.2 System (Columbus Instruments, Columbus, OH, USA). 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. The Micro-Oxymax System is a closed system designed to simultaneously detect very low amounts of O_2 consumption and CO_2 production and to calculate RQ ($RQ= CO_2/O_2$). Measures of gas flux included both root and microbial respiration. 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 infra-red device. The system was fully automated utilizing an IBM compatible microcomputer and an expansion interface with 20 channels for sequential measurements for up to 20 plants at one time (ANDERSEN & SCAGEL 1997, SCAGEL & ANDERSEN 1997).

Results and Discussion

Ozone significantly reduced the biomass of all tissue components of ponderosa pine (Table 1). Ozone has been shown previously to decrease carbohydrate allocation to roots (MCCOOL & MENGE 1983, GORISSEN & VAN VEEN 1988, ANDERSEN & RYGIEWICZ 1995). Decreased allocation results in decreased root system size, and results have shown roots are more affected by ozone than shoots (HOGSETT & al. 1985b, MANNING & al. 1971). Reductions in biomass were associated with a reduction in starch concentration in fine and coarse roots. Over time, ozone would be expected to reduce the carbon released to soils due to a reduction in root system size. GRULKE & al. 1998 found decreased coarse, medium and fine root biomass with increased ozone exposure across an ozone gradient in S. California.

Table 1. Tissue component biomass (g) and starch (μmol glucose g^{-1}) of ponderosa pine seedlings exposed to three levels of ozone over two growing seasons. Asterisk denotes statistical significance from control treatment at $p < 0.01$. Data from ANDERSEN & al. 1997.

	Control	EP- 23	EP-31
Shoot Weight	37.2	19.5*	16.5*
Root Weight	18.9	8.5*	7.3*
Total Plant Weight	56.0	28.1*	23.7*
Needle Starch	53.2	46.1	41.2
Coarse Root Starch	456.4	249.2*	114.4*
Fine Root Starch	716.2	271.3*	199.6*

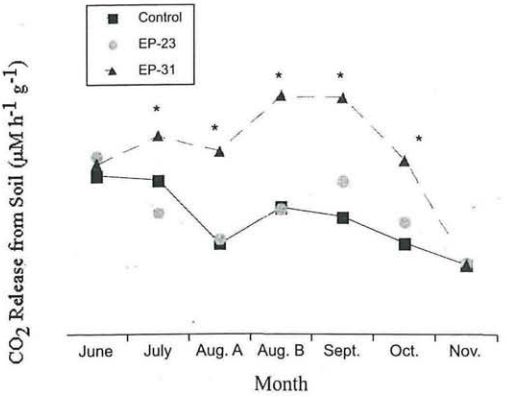

Fig. 1. CO_2 release from soil surface pots containing ponderosa pine seedlings exposed to three different levels of ozone. Asterisks denote statistical significance from control at $p<0.01$. Data from SCAGEL & ANDERSEN 1997.

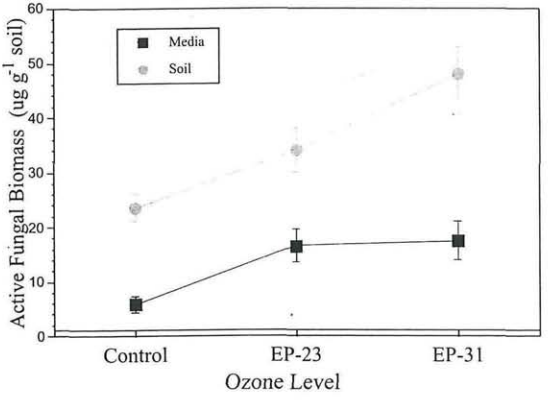

Fig. 2. Active fungal biomass in media and soil of ponderosa pine seedlings exposed to three levels of ozone. Bars represent one standard error of the mean. Data from SCAGEL & ANDERSEN 1997.

Although ozone reduced carbon allocation below-ground, ozone increased soil CO_2 flux (Fig. 1, ANDERSEN & SCAGEL 1997, SCAGEL & ANDERSEN 1997). Increased CO_2 release from soil was associated with an increase in O_2 uptake in ozone treated plants (not shown). Increased CO_2 flux appears to result both from physiological changes in the roots as well as changes in microbial respiration. A metabolic change in pine roots was suggested by a significant reduction in RQ (Respiratory Quotient = CO_2/O_2 ratio) after two seasons of ozone exposure (ANDERSEN & SCAGEL 1997). Decreased RQ may reflect a change in substrate utilization by roots of plants exposed to ozone. There also was evidence of a change in soil microflora in response to ozone. Ozone significantly increased total fungal and bacterial biomass in both potting mix and ponderosa pine soil (Fig. 2). Since ozone does not penetrate soil beyond a few centimeters, the change in microbial populations is likely the result of changes in root exudation and possibly root turnover (MCCRADY & ANDERSEN 2000).

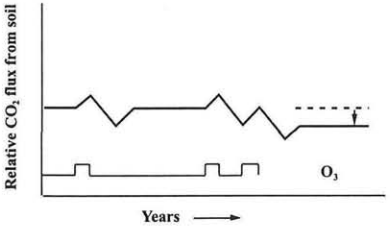

Fig. 3. Hypothesized flux of carbon from soils in response to ozone stress in ponderosa pine ecosystems. Dashed line represents long-term historical average CO_2 flux.

If ozone alters root exudation and root turnover in ponderosa pine ecosystems, then it may affect ecosystem carbon budgets. Fig. 3 shows hypothesized respiratory flux of CO_2 from soils of ponderosa pine exposed to ozone. Under typical conditions, CO_2 flux approaches an historical 'steady state', which is a function of temperature, moisture, phenology and age of the trees occupying a site, and characteristics of soil food web organisms. During years when ozone is high, CO_2 release from soil increases above 'steady state', due to altered root metabolism and increased exudation and turnover. Soil CO_2 flux increases as carbon is metabolized by soil microflora. Increased microbial activity can result in increased sequestration of N and other nutrients, potentially reducing plant available N (DIAZ & al. 1993). Chronic exposure to elevated levels of ozone is hypothesized to decrease respiratory release of CO_2 below the historical 'steady state' due to a reduction in belowground root biomass.

Although the effects of ozone on ponderosa pine individuals have been relatively well studied, the role that ozone plays in altering carbon flux into and out of ecosystems is poorly understood. Controlled seedling studies provide the mechanistic basis for identifying the role ozone may play in ecosystem carbon dynamics. Our results illustrate the importance of understanding both the short and long-term effects of ozone, and provide a basis to understand potential interactions that occur with other stresses in the field. The challenge will be to verify these effects in natural ecosystems exposed to a range of stresses over spatial and temporal scales that are not easily measured.

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