2000

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ELSEVIER

Environmental Pollution 107 (2000) 465–472

ENVIRONMENTAL POLLUTION

www.elsevier.com/locate/envpol

The effect of ozone on below-ground carbon allocation in wheat☆

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Received 28 October 1998; accepted 13 May 1999

Abstract

Short-term $^{14}CO_2$ pulse and chase experiments were conducted in order to investigate the effect of ozone on below-ground carbon allocation in spring wheat seedlings (*Triticum aestivum* L. 'ANZA'). Wheat seedlings were grown in a sand-hydroponic system and exposed to either high ozone (38–40 ppm-h) or low ozone (23–31 ppm-h) for 21 days in a series of replicated experiments. Following the ozone exposures, the plants were pulsed with $^{14}CO_2$ and allocation of ^{14}C-labeled photosynthate was measured in the plant and growth media. Soluble root exudates were measured, without disturbing the plant roots, 24 h after the $^{14}CO_2$ pulse. Shoot biomass was reduced by 17% for the high ozone and 9% for the low ozone exposures, relative to control treatments. Root biomass was reduced by 9% for the high ozone exposures, but was not significantly different than the controls for the low ozone. The amount of ^{14}C activity in the shoot and root tissue 24 h after the $^{14}CO_2$ pulse, normalized to tissue weight, total $^{14}CO_2$ uptake, or the total ^{14}C retention in each plant, was not affected by either high or low ozone exposures. The amount of ^{14}C activity measured in the growth media solution surrounding the roots increased 9% for the high ozone exposures, and after normalizing to root size or root ^{14}C activity, the growth media solution ^{14}C activity increased 29 and 40%, respectively. Total respiration of $^{14}CO_2$ from the ozone-treated plants decreased, but the decrease was not statistically significant. Our results suggest that soluble root exudation of ^{14}C activity to the surrounding rhizosphere increases in response to ozone. Increased root exudation to the rhizosphere in response to ozone is contrary to reports of decreased carbon allocation below ground and suggests that rhizosphere microbial activity may be initially stimulated by plant exposure to ozone. Published by Elsevier Science Ltd.

Keywords: Ozone; Carbon allocation; Root exudation; Rhizosphere microbial activity

1. Introduction

Tropospheric ozone stress reduces carbon acquisition by plants and subsequent allocation of carbon to roots. Since ozone does not penetrate the soil (Turner et al., 1973; Blum and Tingey, 1977), effects on the below-ground system are the indirect result of altered plant processes (Andersen and Rygiewicz, 1991). Previous work with ozone stress has shown that photosynthate is preferentially retained in the shoot at the expense of other plant sinks, such as roots (Cooley and Manning, 1987; Gorissen and Van Veen, 1988; Spence et al., 1990). McCool and Menge (1983) found reductions in carbon allocation to tomato (*Lycopersicon esculentum* Mill.) roots. Gorissen and Van Veen (1988) found that ozone delayed the allocation of ^{14}C-labeled photosynthate to roots of Douglas-fir seedlings and its subsequent release through respiration. Andersen and Rygiewicz (1995) found that ozone decreased carbon allocation to mycorrhizal hyphae of ponderosa pine seedlings. Reduced root growth and root carbohydrate concentrations have been reported in several species exposed to ozone (Manning et al., 1971; Tingey et al., 1976; Hogsett et al., 1985a; Andersen and Rygiewicz, 1991).

Decreased allocation of carbon to roots would be expected to decrease carbon inputs to soil through exudation, sloughing and turnover. However, the mechanistic linkage between root dysfunction and altered rhizosphere processes in ozone-stressed systems is poorly understood. Despite results showing the negative impact of ozone on plant root systems, recent results from our laboratory with ponderosa pine suggest that ozone exposure may increase carbon inputs to soil (Andersen and Scagel, 1997; Scagel and Andersen,

☆ The information in this article has been funded by the US Environmental Protection Agency (EPA). It has been subjected to the Agency's peer and administrative review, and it has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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0269-7491/00/$ - see front matter Published by Elsevier Science Ltd.
PII: S0269-7491(99)00122-0

1997). Soil CO_2 release and O_2 uptake both increased in ponderosa pine seedlings exposed to ozone for two growing seasons. The extent to which the increased CO_2 flux was the result of increased root respiration, microbial respiration, root turnover or some other mechanism has not been determined.

Root exudates include a variety of soluble organic materials, including sugars, amino acids, organic acids, hormones and vitamins, that provide substrate for large populations of microorganisms (Whipps, 1990). Many factors influence the types and amounts of organic root exudates, including microbial development on the root surface, soil chemistry, plant species and physiological development, and various environmental conditions such as light and temperature (Rovira and McDougall, 1967; Curl and Truelove, 1986; Whipps and Lynch, 1986). Diffusible water-soluble root exudates are the primary carbon source for most of the microbial activity in the rhizosphere soil (Kraffczyk et al., 1984). According to Rovira and McDougall (1967), insoluble root cell debris also support microorganisms, but in experiments with pea and oat plants grown in quartz sand, Rovira (1956) reported greater amounts of soluble root exudates than insoluble cell debris. Changes in soluble root exudation in response to ozone stress may affect rhizosphere microbial activity, potentially altering the ecological and nutrient dynamics in the rhizosphere.

Here we test the hypothesis that ozone exposure does not alter the amount of below-ground carbon released to the soil solution surrounding the roots. In order to test the hypothesis, we selected a model system of wheat, and developed an approach that allowed the measurement of ^{14}C photosynthate released into the soil solution without manipulation of or damage to the roots. For quantifying soluble root exudates, and identifying various chemical species in the root exudate, we selected a sand-nutrient solution system and $^{14}CO_2$ feeding experiments, since they have the advantage of eliminating the chemical analysis of complex soil matrices and allow relatively easy quantitation of labeled exudates. Nutrient solution-based systems have been used to study the effects of anoxia, water stress, cultivar, nutrient sources, and various environmental conditions on root exudation (Hale and Moore, 1979; Wiedenroth and Poskuta, 1981; Lee and Gaskins, 1982; Shone et al., 1983; Kraffczyk et al., 1984; Kipe-Nolt et al., 1985; Smucker and Erickson, 1987). The system allowed us to measure ^{14}C-labeled photosynthate released into the rhizosphere without disturbing the root tissue.

2. Experimental methods

2.1. Plant culture

Spring wheat seedlings (*Triticum aestivum* L. 'ANZA') were grown individually in plastic cells using a growth medium consisting of 20-mesh Monterey beach sand and nutrient solution. The 2.2-cm diameter cells were filled with sand, previously washed with nutrient solution, to within 2 cm from the top of each cell. Each plastic cell contained ca. 78 g of dry sand. The plants were germinated in a greenhouse mist chamber. When the seedlings were approximately 1 cm high, they were transferred to the ozone exposure chambers. The bottom of each plant cell was immersed in nutrient solution which was changed three times per week. The plants were also watered from the top three times per week in order to flush excess salt build-up from the growth media.

2.2. Ozone exposures

Five separate and independent experiments were conducted at different times over the course of approximately 1 year. Each experiment exposed wheat seedlings to a simulated ozone profile for 21 days in ozone chambers which were placed inside two replicate Conviron growth chambers (Andersen and Rygiewicz, 1991). Each Conviron growth chamber contained two ozone chambers, one control and one ozone treatment.

The exposure chambers used in these experiments were a modification of the design of Heck et al. (1968). Each chamber was $90\times62\times62$ cm high and covered with clear Teflon film. Ozone was generated using compressed air and ultraviolet light in a laboratory reaction tank. Computerized ozone monitors and mass flow controllers continuously delivered ozone to each chamber in a predetermined pattern of ozone exposure. Temperature, humidity, and CO_2 levels were recorded hourly.

In the first two experiments, a low ozone exposure profile was used which had a pattern of episodic occurrence that varied in concentration of the daily peaks and was constructed using air quality monitoring data from the midwest and western USA (Hogsett et al., 1985b). This profile was constructed by proportionally scaling the hourly ozone concentrations for a composite profile representative of ambient air quality in the midwest using a sigmoid weighting function. These episodic ozone exposures consisted of a steadily increasing ozone concentration throughout the day resulting in a 1 h daily peak in the early afternoon. The episodic daily peaks varied in concentration from 0.03 to 0.22 ppm. The total cumulative ozone values for the two low ozone experiments, calculated by adding the measured hourly means over each 21-day exposure period, were 23 and 31 ppm-h.

In the final three experiments a higher ozone exposure profile was used. This profile had a simulated diurnal pattern with a daily ozone peak of 0.12 ppm which was repeated over the 21-day exposure. The ozone concentration in the diurnal pattern increased from 06:00 to a peak ozone concentration at 10:00 (0.12 ppm). The peak ozone concentration was maintained for 5 h and

slowly decreased to a night-time low of 0.04 ppm. The total cumulative ozone values for the three high ozone experiments were 38, 40 and 39 ppm-h, respectively. The air used for the control chambers was unfiltered air containing negligible concentrations of SO_2 and NO_x. The measured cumulative ozone values for the control treatments ranged from 2 to 8 ppm-h.

2.3. Experimental design

Two replicated ozone exposure chambers and two identical replicated control chambers were used for each experiment. Each experiment consisted of 15 randomly selected plants placed inside each of the four exposure chambers. One ozone chamber and one control chamber were located inside each of two replicate Conviron growth chambers.

The Conviron growth chambers were operated on a 12-h photoperiod, 22:15°C day:night temperatures, and 65% relative humidity (RH). The measured temperature inside each ozone chamber was 27:15°C day:night, and the RH was ca. 75:34% day:night. The photosynthetic photon flux density measured at the top of the ozone chambers was 600 μmol m^{-2} sec^{-1}.

Identical ozone profiles, either low ozone or high ozone, were applied to two of the chambers while the remaining two chambers were maintained under ambient control conditions. Two of the exposure chambers, one ambient control and one ozone treatment, were located inside the first of two Conviron growth chambers. The remaining two exposure chambers, the replicate ambient control and the replicate ozone treatment, were located inside a second Conviron growth chamber. In subsequent experiments the ozone and control treatments were allocated to different chambers so that the ozone exposures were not always conducted in the same exposure chamber. Five individual experiments were conducted at different times over the course of approximately 1 year.

2.4. $^{14}CO_2$ pulse

Immediately following the ozone exposure all of the plants were removed from the ozone and control chambers and transferred to a 0.1-m^3 plexiglass pulse box (54×38×46 cm) located inside a chemical fume hood equipped with growth lights. The photosynthetic photon flux density measured 10 cm above the top of the plants was 300–400 umol m^{-2} sec^{-1}. A pump was used to circulate air out of the pulse box, through a bottle containing 20 ml of 85% lactic acid, then back into the pulse box. The circulating air flow through the pulse box was 15 l min^{-1} Two fans were placed near the bottom of the pulse box to mix the air inside the box. The plants were not exposed to ozone during the $^{14}CO_2$ pulse or during the 24-h chase period.

For each experiment, 15 plants from each of the four exposure chambers were combined and randomly placed inside the pulse box. The $^{14}CO_2$ pulse was started by injecting 7.4×10^3 kBq of ^{14}C-labeled sodium bicarbonate (specific activity 2.0×10^6 kBq $mmol^{-1}$) into the bottle of lactic acid. The evolved $^{14}CO_2$ was allowed to circulate through the pulse box for 25 min. After the pulse the plants were removed from the pulse box and kept inside the chemical fume hood under growth lights.

Twenty plants, five plants from each of the four exposure chambers, were harvested immediately after the pulse (0 h) in order to measure initial $^{14}CO_2$ uptake and plant biomass. Root exudates were collected from the remaining 40 plants after 24 h, followed immediately by the final plant harvest.

2.5. Root exudates

Soluble root exudates were collected, without disturbing the roots, by slowly adding 50 ml of nutrient solution to the top of each plant cell, and collecting the eluate as it drained out the bottom of the cell. The pre-tared collection bottles contained 0.5 ml of 10 N NaOH in order to trap and preserve $^{14}CO_2$ in the eluate. Some of the plants were eluted with a second 50 ml of nutrient solution resulting in approximately 90% elution efficiency of soluble ^{14}C activity with the first eluate. Greater than 90% of the total ^{14}C activity in the sand media was removed using this procedure as demonstrated by measuring the total ^{14}C activity in the sand after removing the plant roots. Some of the eluates were collected without adding NaOH and analyzed over time to determine the rate of $^{14}CO_2$ lost from the soil solution. In the last experiment, approximately 25% of the 68 root eluates collected were acidified and purged with air to remove dissolved $^{14}CO_2$ before the measurement of ^{14}C activity. After acidification and purging with air, the ^{14}C activity remaining in the eluates was assumed to be a non-volatile fixed root exudate.

2.6. Plant harvest

Immediately after the pulse and at the end of the root exudation period (24 h), plants were harvested in order to determine plant tissue biomass and ^{14}C activity. The shoots were removed, transferred to a pre-tared vial, and weighed. The roots were removed from the wet sand and washed with nutrient solution to remove the residual sand. Residual water was removed by squeezing the root tissue between sorbent paper. The root tissue was transferred to a pre-tared vial and weighed. Approximately 1 cm of plant tissue (the crown), consisting of the junction between the green shoot tissue and the fibrous roots, was removed and weighed. Plant tissue was dried at 80°C for 72 h and re-weighed to determine dry weights. The dried tissue samples were

cut into 5-mm pieces and saved for analysis of ^{14}C activity.

2.7. ^{14}C analysis

A Packard Tri-Carb® liquid scintillation analyzer (LSA) was used to analyze duplicate 2-ml aliquots of the root eluates. Packard® quenched standards were used to calibrate the instrument for every set of samples. Direct oxidation of dried plant tissue was done with a Packard Tri-Carb® sample oxidizer. Duplicate subsamples (50–100 mg) of each dried tissue were oxidized and analyzed by LSA. The coefficient of variation for the measured specific activity of the duplicate tissue samples was less than 10% and the mean specific activities were used in the data analysis.

2.8. Statistical analysis

The data for plant biomass, $^{14}CO_2$ uptake, shoot and root tissue ^{14}C specific activity, ^{14}C retention, and root exudation from replicate experiments having similar ozone treatments (either high or low ozone) were combined into an overall analysis of variance (ANOVA) in which experiments were regarded as a blocking variable. The testing procedure was adapted from the procedure given by Cochran and Cox (1957) and Snedecor and Cochran (1967). The mean squares for the interactions involving experiment and/or growth chambers were pooled to form a single denominator for the *F*-test of ozone treatments. Prior to pooling, the presence of an interaction was tested and, when an interaction term was significant, it was not included in the pooled error term. Consequently, the pooled error term used in the *F*-test for ozone treatments had varying degrees of freedom. A one-sided *t*-test was used instead of the *F*-test whenever the direction of ozone treatment response was known a priori. The significance levels for the two sets of experiments were combined using procedures developed by Tippett (1931), Fisher (1932) and Wilkinson (1951) to test the null hypotheses of no ozone treatment effect overall.

3. Results

3.1. Plant Growth

The plant dry weights and ANOVA results for the combined three experiments using high ozone exposures and the combined two experiments using low ozone exposures are shown in Table 1. The shoots, roots, and total plant dry weights from the high ozone exposures were all significantly reduced by 17%, while the root/shoot ratios remained unchanged relative to the control treatments. The low ozone treatments reduced shoot dry weight 9%, but the root weights, relative to the controls, were not affected. The root/shoot ratio for the low ozone treatments increased 9% and was significant at the 10% level ($p = 0.076$). When the *p*-values for the two sets of experiments were combined, ozone effects on plant dry weight were highly significant and consistent across the two sets of experiments.

3.2. $^{14}CO_2$ uptake

The plant uptake of $^{14}CO_2$ from the pulse was calculated from the measured specific activities (kBq g^{-1} DWt tissue) of the shoot, root, and crown tissue from each plant harvested immediately after the pulse (0 h). The specific activity of each tissue was multiplied by the tissue dry weight, and the total shoot, root, and crown activities were added together to determine the total uptake per plant. The mean $^{14}CO_2$ uptake and specific activities of the shoot and root tissue for the combined experiments are shown in Tables 2 and 3.

Relative to the control plants, ozone reduced $^{14}CO_2$ uptake per plant by 22% (high ozone, $p = 0.003$) and 13% (low ozone, $p = 0.051$). The reduction in $^{14}CO_2$ uptake from ozone-treated plants was consistent with their significantly smaller size. When $^{14}CO_2$ uptake per plant was normalized by dividing by the shoot dry weight, low ozone reduced $^{14}CO_2$ uptake g^{-1} shoot by 5% ($p = 0.052$). High ozone reduced $^{14}CO_2$ uptake g^{-1} shoot by 6% but this was not statistically significant at the 0.05 level. Ozone did not affect the specific activities

Table 1
Plant biomass and test for ozone effects

Ozone level	Shoots (g dry wt) Mean (SE)	Roots (g dry wt) Mean (SE)	Plant (g dry wt) Mean (SE)	Root/shoot (ratio) Mean (SE)
Low ozone	0.150 (0.003)	0.113 (0.005)	0.287 (0.007)	0.756 (0.019)
Control	0.165 (0.003)	0.114 (0.005)	0.304 (0.007)	0.693 (0.019)
p-value[a]	0.027	0.901	0.178	0.076
High ozone	0.179 (0.005)	0.135 (0.006)	0.357 (0.015)	0.766 (0.021)
Control	0.215 (0.005)	0.162 (0.006)	0.430 (0.015)	0.759 (0.021)
p-value[b]	0.004	0.016	0.011	0.822

[a] *F*-test of ozone effects on plant tissue biomass based on two combined low ozone experiments (four low ozone chambers and four control chambers, five plants per chamber). Error term had four degrees of freedom.

[b] *F*-test of ozone effects on plant tissue biomass based on three combined high ozone experiments (six high ozone chambers and six control chambers, five plants per chamber). Error term had seven degrees of freedom.

Table 2
Plant uptake of $^{14}CO_2$ from the pulse and test for ozone effects

Ozone level	Uptake/plant (kBq) Mean (SE)	Uptake g^{-1} shoot (kBq g^{-1}) Mean (SE)
Low ozone	88.2 (3.3)	585 (6.7)
Control	101 (3.3)	615 (6.7)
p-value[a]	0.051	0.052
High ozone	132 (5.7)	747 (28)
Control	168 (5.7)	793 (28)
p-value[b]	0.003	0.293

[a] *F*-test of ozone effects on plant uptake of $^{14}CO_2$ based on two combined low ozone experiments (four low ozone chambers and four control chambers, five plants per chamber). Error term had four degrees of freedom.

[b] *F*-test of ozone effects on plant uptake of $^{14}CO_2$ based on three combined high ozone experiments (six high ozone chambers and six control chambers, five plants per chamber). Error term had seven degrees of freedom.

Table 3
Specific activity of plant tissue measured 24 h after the $^{14}CO_2$ pulse and test for ozone effects

Ozone level	Shoots (kBq g^{-1}) Mean (SE)	Roots (kBq g^{-1}) Mean (SE)
Low Ozone	227 (5)	177 (7)
Control	228 (5)	187 (7)
p-value[a]	0.868	0.379
High ozone	328 (12)	257 (12)
Control	353 (12)	265 (12)
p-value[b]	0.172	0.625

[a] *F*-test of ozone effects on specific activity of plant tissue based on two combined low ozone experiments (four low ozone chambers and four control chambers, 10 plants per chamber). Error term had four degrees of freedom.

[b] *F*-test of ozone effects on specific activity of plant tissue based on three combined high ozone experiments (six high ozone chambers and six control chambers, 10 plants per chamber). Error term had seven degrees of freedom.

(kBq g^{-1} DWt tissue), measured 24 h after the pulse, of the shoot, root, or crown (not shown) tissues.

3.3. ^{14}C retention

The ^{14}C activity retained in the shoots and roots was calculated by dividing tissue activity at 24 h by either the total activity recovered in each plant at 24 h, or the total $^{14}CO_2$ uptake by each plant at 0 h. It was not possible to directly measure the $^{14}CO_2$ uptake of the plants harvested at 24 h, which would have required destructively harvesting all of the plants at 0 h. Therefore, the $^{14}CO_2$ uptake for plants harvested at 24 h was estimated from the uptake values from the plants harvested at 0 h. The $^{14}CO_2$ uptake values for the ozone or control plants harvested at 0 h were first normalized for plant dry weight, and then the mean uptake g^{-1} was multiplied by the weight of the 24-h plants. Plant growth was assumed negligible during the 24-h chase period since the plant dry weights did not change significantly during this time.

The specific activity (kBq g^{-1}) of the plant tissue, and the ^{14}C retention values and ANOVA results for the combined experiments are shown in Tables 3 and 4. Ozone did not affect either the specific activity of the plant tissue or the ^{14}C retention in the shoot and root tissue.

3.4. $^{14}CO_2$ respiration

The direct measurement of root- and shoot-respired $^{14}CO_2$ was not done in these experiments, but the total amount of respired $^{14}CO_2$ from each plant was estimated. The respired $^{14}CO_2$ lost from each plant was calculated by subtracting the ^{14}C activity recovered in each plant at 24 h from the amount of ^{14}C uptake at 0 h. Total plant respiration of $^{14}CO_2$ averaged 15–35% of $^{14}CO_2$ taken up by each plant. For the combined experiments, the average plant respiration was reduced 22% (high ozone) and 10% (low ozone), but neither estimate of reduced plant respiration was statistically significant at the 0.05 level.

3.5. Root exudation

ANOVA results for testing ozone effects on ^{14}C exudation are shown in Table 5. These results include both fixed ^{14}C activity and respired $^{14}CO_2$ recovered in the root eluate. The ^{14}C activity measured in the root eluate was normalized for plant weight and the total amount of ^{14}C activity recovered in the root plus the eluate.

The high ozone treatments significantly increased the amount of ^{14}C activity in the root eluates. When the ^{14}C activity in the root eluates was normalized to either the total ^{14}C activity recovered in the root plus the eluate, or to the root dry weight, the normalized eluate ^{14}C activities increased 29 and 40% and were highly significant ($p < 0.01$). The low ozone treatments did not significantly affect root eluate ^{14}C activity. When the significance levels were combined for all five experiments, the overall test of ozone treatment was statistically significant at the 5% level based on the Fisher and Tippett–Wilkinson tests.

Several experimental variations were conducted in order to elucidate the origin of the ^{14}C activity eluted from the root chambers. The eluted ^{14}C activity in our experiments could have come from several sources, including: (1) soluble non-volatile 'fixed' root exudates; (2) root respired $^{14}CO_2$; (3) microbially respired $^{14}CO_2$; (4) insoluble particulate matter originating from the root or microbial biomass; and (5) diffusion of $^{14}CO_2$ from the pulsed air into the sand/water growth medium.

Degradation experiments conducted with root eluates, in which NaOH was not added, indicated that the ^{14}C activity had a half-life of approximately 24 h, suggesting microbial degradation and/or loss of volatile

Table 4
^{14}C retention in shoots and roots 24 h after the ^{14}C pulse (normalized to either ^{14}C uptake or total ^{14}C recovered in the plant at 24 h), and test for ozone effects

Ozone level	Shoot kBq/kBq uptake Mean (SE)	Root kBq/kBq uptake Mean (SE)	Shoot kBq/kBq plant Mean (SE)	Root kBq/kBq plant Mean (SE)
Low ozone	0.394 (0.013)	0.230 (0.007)	0.604 (0.009)	0.352 (0.004)
Control	0.376 (0.013)	0.211 (0.007)	0.609 (0.009)	0.343 (0.004)
p-value[a]	0.380	0.127	0.712	0.153
High ozone	0.443 (0.018)	0.260 (0.011)	0.570 (0.008)	0.333 (0.007)
Control	0.448 (0.018)	0.249 (0.011)	0.580 (0.008)	0.325 (0.007)
p-value[b]	0.849	0.499	0.440	0.425

[a] *F*-test of ozone effects on ^{14}C retention in shoots and roots based on two combined low ozone experiments (four low ozone chambers and four control chambers, 10 plants per chamber). Error term had four degrees of freedom.
[b] *F*-test of ozone effects on ^{14}C retention in shoots and roots based on three combined high ozone experiments (six high ozone chambers and six control chambers, 10 plants per chamber). Error term had seven degrees of freedom.

Table 5
^{14}C activity recovered in the root eluate (fixed root exudate plus $^{14}CO_2$), normalized to the total ^{14}C recovered in the root plus eluate and normalized to root dry weight

Ozone level	Eluate (kBq) Mean (SE)	Eluate/root + eluate (ratio) Mean (SE)	Eluate kBq/root dry weight (kBq g^{-1}) Mean (SE)
Low ozone	0.533 (0.030)	0.025 (0.001)	4.67 (0.30)
Control	0.543 (0.030)	0.025 (0.001)	4.88 (0.30)
p-value[a]	0.821	0.683	0.595
High ozone	0.227 (0.005)	0.007 (0.000)	1.72 (0.03)
Control	0.208 (0.005)	0.005 (0.000)	1.33 (0.03)
p-value[b]	0.039	0.007	0.0005

[a] *F*-test of ozone effects on ^{14}C recovered in the eluate based on two combined low ozone experiments (four low ozone chambers and four control chambers, 10 plants per chamber). Error term had four degrees of freedom.
[b] *F*-test of ozone effects on ^{14}C recovered in the eluate based on three combined high ozone experiments (six high ozone chambers and six control chambers, 10 plants per chamber). Error term had five degrees of freedom.

$^{14}CO_2$. In earlier experiments root chambers were eluted at 0, 4, 24, and 48 hours after the $^{14}CO_2$ pulse. The eluted ^{14}C activity at 0 h was initially high due to diffusion of $^{14}CO_2$ into the soil solution during the $^{14}CO_2$ pulse, but then dropped to background levels in 4 h. The eluate ^{14}C activity increased significantly at 24 h and then gradually increased during the last 24 h. The ^{14}C activity in the root tissue harvested at 0 h was negligible. These experiments indicated that the initial ^{14}C activity in the root eluate at 0 h was caused by diffusion of $^{14}CO_2$ from the pulsed air into the sand/water growth medium. Within 4 h, the dissolved $^{14}CO_2$ in the growth medium exchanged with ambient CO_2 in the air and returned to negligible levels. These conclusions were supported by purging $^{14}CO_2$ from acidified eluates collected at 0 h, resulting in a loss of over 90% of the ^{14}C activity.

In the last experiment, approximately 25% of the 68 root eluates collected were acidified and purged with air to remove dissolved $^{14}CO_2$ before the measurement of ^{14}C activity. Purging acidified eluates collected at 24 h resulted in a 30–50% loss of $^{14}CO_2$, suggesting that a significant amount (50–70%) of ^{14}C-activity was a non-volatile fixed root exudate. The high ozone exposure in the final experiment increased the average amount of fixed root exudate (normalized to root dry wt) by approximately 20%. However, the statistical analysis of this sub-set of plants from the final experiment had only one degree of freedom and the observed increase in fixed root exudation was not statistically significant at the 0.05 level.

4. Discussion

In our experiments, ozone did not affect the allocation of currently available ^{14}C-labeled photosynthate to the roots of wheat seedlings. This conclusion is supported by nearly identical root tissue specific activities and ^{14}C retention in the roots (Tables 3 and 4). However, our experiments suggest that exposure to ozone resulted in a statistically significant increase in ^{14}C- labeled photosynthate released into the soil solution surrounding the root tissue. The

^{14}C activity released into the soil solution included dissolved $^{14}CO_2$ from root respiration, microbially respired $^{14}CO_2$, and soluble fixed root exudates.

The overall pattern of root exudation in our experiments was similar to the research reported by McDougall and Rovira (1965). These authors characterized root exudation for wheat seedlings grown in sterile nutrient solution. They used a $^{14}CO_2$ pulse and chase technique to quantify soluble root exudates and both volatile and non-volatile ^{14}C activity was measured in the growth solution. Radioactivity first appeared in the growth solution in 4–5 h after the start of $^{14}CO_2$ exposure and 0.1% of the assimilated $^{14}CO_2$ was exuded by the roots in 24 h. The authors indicated that 80% of the exuded radioactivity was volatile, and suggested that the volatile component of soluble root exudate was $^{14}CO_2$ respired by the roots. Our results indicated higher amounts of non-volatile fixed root exudate (50–70% of the total ^{14}C activity measured in the root eluate).

In our experiments, the increase in total soluble ^{14}C activity measured in the root eluates in response to ozone could have been caused by increased root exudation of non-volatile fixed exudates, microbial respiration of fixed root exudates, or increased root respiration of ^{14}C-labeled photosynthate. Ozone decreased the total plant respiration by 10–20% in our experiments, although this trend was not statistically significant. Since root respiration is the major contributor to the $^{14}CO_2$ found in the root eluates, and total plant respiration decreased in response to ozone, then the increase in ^{14}C activity in the root eluates probably was not caused by increased root respiration.

Our results demonstrate a statistically significant increase in total ^{14}C activity in the root eluates of ozone-treated plants and that 50–70% of the measured ^{14}C activity is a fixed root exudate. We also observed trends, although not statistically significant, of decreased plant respiration for all of the ozone-treated plants and increased fixed root exudation in the final high ozone experiment. Therefore, we conclude that the increase in soluble ^{14}C activity in the root eluates was a result of increased fixed root exudation in response to ozone.

Since the specific activity of the root tissue and the ^{14}C retention in the roots were not affected by ozone, the increased fixed root exudation could be the result of ozone-induced changes in root physiology, such as different catabolic activities in the root tissue or changes in root membrane integrity.

It should be noted that the absolute amount of soluble ^{14}C activity recovered in the root eluates after the 24-h chase period was only 1–2% of the ^{14}C activity found in the root tissue, and only 0.1–0.6% of the total amount of assimilated $^{14}CO_2$. These relatively small amounts of root exudation would not be expected to affect the total carbon budget in the plants. However, if the absolute amount of fixed root exudation is significantly affected by ozone, and this root exudation is the primary carbon source for most of the microbial activity in the rhizosphere (Kraffczyk et al., 1984), then ozone exposure would be expected to alter the rhizosphere microbial activity.

Our results indicate that ozone exposure increases ^{14}C allocation to the rhizosphere in wheat and suggests that soluble fixed root exudation increases. These results differ from those of McCool and Menge (1983) who found that exposure of tomato (*L. esculentum* Mill.) to 300 ppb ozone resulted in small, although insignificant, decreases in reducing sugars in the root exudate of both mycorrhizal and non-mycorrhizal roots. These authors also reported a significant decrease in root exudation of amino acids for mycorrhizal roots and a small, but insignificant, decrease in root exudation of amino acids for non-mycorrhizal roots. Aside from using different plant species, we examined total allocation and release of recently fixed carbon, rather than exudation of total sugars and amino acids at a point in time. Clearly, additional experiments are necessary to understand rhizosphere processes in ozone-exposed plants.

Although few studies have measured root exudation directly, several studies have provided indirect evidence that ozone increases root exudation and carbon flux to the rhizosphere. Increased root exudation would be expected to promote mycorrhizal establishment (Graham et al., 1981) and Stroo et al. (1988) found increased mycorrhizal colonization in white pine (*Pinus strobus* L.) at low ozone exposures. Reich et al. (1986) found similar results in red oak (*Quercus rubra* L.) and concluded that ozone may stimulate mycorrhizal infection at low concentrations. Enzyme studies also provide indirect support for increased exudation in ozone-exposed plants. Shafer (1988) found that ozone tended to increase the number of fungal propagules in the rhizosphere, and that rhizosphere bacteria capable of phosphatase activity increased.

Under chronic ozone stress, the total carbon flux to soil would be expected to decrease due to less root biomass that results from less carbon being allocated below ground. Allocation studies have shown decreased allocation below ground, but most studies have not measured the amount of root exudation. The results from our experiments with wheat using relatively short-term ozone exposures suggest an increase in root exudation. Whether this response persists over time, and whether perennial species respond similarly needs to be examined. Increased root exudation and carbon flux to soils could stimulate microbial activity, and could increase populations of rhizosphere bacteria which may compete for nitrogen and other nutrients in nutrient limiting soils (Diaz et al., 1993). Although the response may be transient, it could shift the nutrient balance in favor of the soil biota, thus altering the rhizosphere ecological dynamics.

Acknowledgements

The authors would like to thank Dr. Henry Lee for assistance with the statistical analysis.

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