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Structure and function of rhizosphere and non-rhizosphere soil microbial community respond differently to elevated ozone in field-planted wheat

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ABSTRACT

To assess the responses of the soil microbial community to chronic ozone (O₃), wheat seedlings (*Triticum aestivum* Linn.) were planted in the field and exposed to elevated O₃ (eO₃) concentration. Three treatments were employed: (1) Control treatment (CK), AOT40 = 0; (2) O₃-1, AOT40 = 1.59 ppm•h; (3) O₃-2, AOT40 = 9.17 ppm•h. Soil samples were collected for the assessment of microbial biomass C, community-level physiological profiles (CLPPs), and phospholipid fatty acids (PLFAs). eO₃ concentration significantly reduced soil microbial carbon and changed microbial CLPPs in rhizosphere soil, but not in non-rhizosphere soil. The results of the PLFAs showed that eO₃ concentrations had significant effects on soil community structure in both rhizosphere and non-rhizosphere soils. The relative abundances of fungal and actinomycetous indicator PLFAs decreased in both rhizosphere and non-rhizosphere soils, while those of bacterial PLFAs increased. Thus the results proved that eO₃ concentration significantly changed the soil microbial community function and composition, which would influence the soil nutrient supply and carbon dynamics under O₃ exposure.

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Introduction

In the troposphere, particularly at the surface of the earth, O₃ is a widespread phytotoxic air pollutant. O₃ has been shown to be harmful to human health, vegetation and certain materials (Bytnerowicz et al., 2007; Serengil et al., 2011). Background concentrations of O₃ in the troposphere have continuously increased since the preindustrial age, although control measures on the emission of its precursors have reduced O₃ peaks (Vingarzan, 2004; Derwent et al., 2007). China has undergone rapid industrialization and urbanization in recent

decades. Information on ground level O₃ concentrations in China is limited, but the available data suggest that these concentrations may attain potentially damaging levels, exceeding the Chinese National Air Quality Standard (based on U.S. standards) of 80 nL/L for many hours during the summer (Zheng et al., 1998; Chen et al., 2013).

Current ambient O₃ levels may cause severe effects on plants, including visible foliar injury or growth reduction, impairment of physiological traits and impacts on resource allocation and reproduction (Paoletti, 2007; Feng et al., 2008; Zhang et al., 2011; Niu et al., 2011; Chen et al., 2008). In recent

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years, considerable (or increasing) attention has been paid to the below-ground effects of eO_3 (Chen et al., 2009). This is because plants live in close association and interaction with soil microorganisms, particularly in their rhizospheres and during the decay of plant material (Kogel-Knabner, 2002). eO_3 not only causes losses to crop yield but can also significantly reduce the allocation of assimilates into the root. This reduction in root biomass would influence the entry of organic matter into the soil (Andersen, 2003). eO_3 reduces litter quality and rates of decay. The types and amounts of organic root exudates can be changed when the shoots are exposed to high levels of O_3 , and these changes may affect rhizosphere microbial activity, potentially altering the ecological and nutritional dynamics in the rhizosphere (McCrary and Andersen, 2000).

In the few cases where soil microbial community responses to O_3 stress have been examined, these responses have been variable. The effects of eO_3 on microbial functional diversity of both the rhizosphere and bulk soil in potted wheat were analyzed by using CLPPs, which showed that eO_3 significantly affected the carbon degradative microbes in rhizosphere soil, while not in bulk soil (Chen et al., 2009). CLPP and PLFA analyses showed that carbon degradative microbes and the microbial structure of surface soil under paddy rice were altered in response to eO_3 , and PLFAs showed much more change than CLPPs (Chen et al., 2010). Seven meadow species were exposed to eO_3 for three growing seasons, and the PLFA results of bulk soil showed that bacterial, actinobacterial, and fungal PLFAs and the fungal:bacterial PLFA ratio were decreased (Kanerva et al., 2008). In the same study, the decrease in the total microbial PLFA biomass in the bulk soil of *Lathyrus pratensis* under eO_3 and eCO_2 (elevated CO_2) (both $p = 0.050$) arose from reductions in the PLFA biomass of indicator subgroups for actinobacteria (29% and 31%), bacteria (26% and 33%) and mycorrhiza (i.e., AM fungi) (31% and 35%) in particular. Active fungal biomass in bulk soil of *Agrostis capillaris* was also significantly reduced under eO_3 and CO_2 combined (21%), while this significant effect of O_3 and CO_2 was not found in the rhizosphere soil (Manninen et al., 2010). This insignificant effect of O_3 on the overall functional structure of rhizosphere soil was also found in winter wheat, using an O_3 FACE fumigation system (Li et al., 2013). In the study, functional genes in the wheat rhizosphere microbial community were analyzed using GeoChip 3.0, which showed that the overall functional diversity and structure of the rhizosphere microbial community appeared not to be significantly altered by eO_3 (60 nL/L), while the results showed that the abundance of specific functional genes involved in C fixation and degradation, nitrogen (N) fixation, and sulfite reduction did significantly ($p < 0.05$) alter in response to eO_3 (Li et al., 2013). These results showing no significant effect of O_3 on the rhizosphere soil microbial community were in contradiction with common knowledge, which holds that eO_3 affects the rhizosphere soil microbial community much more than bulk soil.

Wheat (*T. aestivum* Linn.) is one of the most O_3 -sensitive crops and is among the most important crops in China. Ground-level O_3 pollution has already decreased global wheat yields by 8.5% to 14% (Wilkinson et al., 2012). A previous meta-analysis indicated that eO_3 decreases wheat grain yield, grain weight, grain number per ear, ear number per plant and harvest index by 26%, 18%, 11%, 5% and 11%, respectively,

relative to ambient air (Feng et al., 2009). Our previous results have shown that eO_3 levels may alter microbial community function in the rhizosphere soil, but not in the bulk soil, under potted wheat (Chen et al., 2009). However, it remains unclear how the soil microbial community under field-planted wheat responds to eO_3 and if this response is the same as that observed under potted wheat.

In this study, we hypothesize: (1) that soil carbon degradative microbes and soil microbial community structure would alter after wheat is exposed to eO_3 ; (2) that the effects of eO_3 on the rhizosphere soil community are more significant than for non-rhizosphere soil; and (3) that PLFA analysis could discriminate more differences between rhizosphere and non-rhizosphere soil community response to eO_3 than CLPPs. To test these hypotheses, both rhizosphere and non-rhizosphere soils of field-planted wheat were analyzed for CLPPs and PLFAs.

1. Materials and methods

1.1. Experimental site

The experimental site was located at the Shuangqiao Farm (31°53'N, 121°18'E) in Jiaxing City, Zhejiang Province, approximately 100 km from Shanghai, China's largest city. This site is in the center of the Yangtze Delta and represents one of the most important crop production areas in China. The area is influenced by the Asian monsoon climate system, with cold, dry winters and warm, wet summers. Mean temperature and annual precipitation are 15.5°C and 1199 mm, respectively. The prevailing cultivation rotations are wheat-rice and rape-rice.

1.2. Crop management

On 15 November, 2006, wheat (*T. aestivum* L. Jia 002) seeds were sown in the field. Open-top chambers were placed on blocks on 20 March, 2007. These plots consisted of three rows, with four plots in each row. Protective belts of approximately 3 m in width were established between the plots. Before transplanting, 60 kg/ha pure nitrogen (N), 60 kg/ha P_2O_5 and 60 kg/ha K_2O were applied, and 69.3 kg/ha pure N was reapplied during the tillering stage and the jointing stage. The crops were harvested on 28 May.

1.3. O_3 exposure and experimental design

The experiments were conducted in closed octagonal open-top chambers (OTCs), 2 m in diameter and 2.2 m in height. The boxes were connected to filter boxes, either without filters or with activated charcoal and a fan, and run at two air changes per minute.

An improved, innovative O_3 distribution system was employed in this study. A rotatable transparent pipe with many small holes (diameter of 10 mm at intervals of 10 cm) released either charcoal-filtered air (CF air) or O_3 above the crop canopy. The CF air or the O_3 + CF air was driven from a centrifugal blower. O_3 was generated from pure oxygen by high-voltage electric discharge (Yuyao Shenglete Company, Zhejiang, China). A series of solenoid valves, linked online with a programmable log controller (PLC K80S, LG, LSIS,

Gyeonggi-Do, Korea), were used to control the gas meters and provide oxygen based on the established relationship between the O₃ concentrations within the OTCs and oxygen volume. The O₃ concentrations within the OTCs were monitored by an O₃ analyzer (Model 9810B, Teledyne Monitor Labs, Englewood, USA). A data logger (21× (L), Campbell Scientific, Inc. Logan, Utah, USA) stored O₃ concentration data and the temperature within the OTCs, which was measured with a constantan–copper thermocouple.

According to the monitoring of ambient O₃ concentrations during March and May 2006 in the experimental site, the maximum instantaneous O₃ concentration was 108 nL/L, maximum hourly average O₃ concentration was 94 nL/L, maximum 8-hour average O₃ concentration was 68 nL/L, and 8-hour average O₃ concentration was 45 nL/L. Three different O₃ exposure scenarios were employed, consisting of charcoal-filtered air alone (CK) and filtered air with the addition of two levels of daily changing O₃ (O₃-1 and O₃-2). In the O₃-1 treatment, the expected O₃ changes were as follows: 9:00–10:00, 50 nL/L; 10:00–12:00, 100 nL/L; 12:00–14:00, 150 nL/L; 14:00–16:00, 100 nL/L; and 16:00–17:00, 50 nL/L. In O₃-2, the same O₃ change trend was used with higher concentrations of 100, 150, and 200 nL/L. O₃ was generated from 9:00 to 17:00 local time. Nine chambers were used, providing three replicates for each of the three O₃ treatments. The O₃ treatments began on 26 March 2007, and were terminated on 19 May 2007, covering the period from the three-leaf stage until maturity. Because ambient O₃ concentrations are generally low during extended rainy periods, fumigations were not carried out, and 30 full days of fumigation occurred. The AOT40 (accumulated exposure over a threshold of 40 ppb or nL/L) values were 0, 1585, and 9172 ppb•h for the treatments CK, O₃-1 and O₃-2, respectively.

1.4. Soil sampling

On 13 May, samples of non-rhizosphere soil from the surface layer (0–10 cm) of the Chinese red soils were collected at random using 2 cm cores, with ten pooled soil cores at each OTC. The soil that remained adhered to the root hairs after gentle shaking was sampled as the rhizosphere soil, as classified by the operational definition (Lynch, 1990). Field moist soils were sieved to <2 mm, and large pieces of plant material and soil animals were removed before further analysis.

1.5. Soil microorganism assessment

1.5.1. Microbial biomass

The fumigation–extraction method (Vance et al., 1987) was used to determine the soil microbial biomass C of the soil samples. The content of K₂SO₄-extracted C from the CHCl₃-treated and untreated soils was determined by an automated TOC analyzer.

1.5.2. Community-level physiological profiles (CLPPs) obtained by sole C source utilization tests

ECO plates, which are based on 31 different C sources and contain built-in triplicates for better replication, were employed in this study. Triplicate 10 g soil samples were combined with 90 mL 0.85% sterile NaCl solution and vibrated for 30 min, and

the 10^{−3} dilutions were then used to inoculate the Biolog plates. A 150 μL dilution aliquot was inoculated into each well of a plate (Biolog Inc., Hayward, California, USA) and incubated at a constant 25°C. The plates were scanned twice daily at 590 nm on a special reader for 10 days. The raw absorbance data for each reading was converted to net absorbance data by subtracting the control well reading. The average well color development (AWCD) for each plate reading was calculated by averaging all responses as a measure of total activity as follows.

$$AWCD = \sum (C-R)/n$$

where, C is the value of absorbance in every well with a carbon source; R is the value of the absorbance in the blank well; and n is 31 carbon sources. For different C sources, n has different values: according to category, the values are 10, 7, 6, 2, 4, and 2 for carbohydrates, carboxylic acids, amino acids, amides/amines, polymers and miscellaneous compounds, respectively (Choi and Dobbs, 1999). In this study, only three main types of compounds, carbohydrates, carboxylic acids and amino acids, were used to indicate microbial activity.

Microtiter wells with optical densities of 0.1 or greater were considered positive for determining Biolog richness (total number of positive wells). The Shannon–Wiener diversity index (H') was calculated as follows:

$$H' = -\sum (P_i \times \log P_i)$$

where, P_i = (C − R)/Σ(C − R). The functional richness index and diversity index were determined from the data gathered after 96 hr of incubation.

1.5.3. Ester-linked phospholipid fatty acid (PLFA) analysis

PLFA analyses were performed using the modified Bligh and Dyer method (Frostegård et al., 1993), as described in Chen et al. (2010).

Known amounts of methyl nonadecanoate (19:0) were added before analysis by GC–MS. The total PLFA was calculated using 19:0 as the internal standard. The PLFA 18:2ω6,9 was taken to indicate predominantly fungi. The PLFAs 18:0(10Me) and 19:0(10Me) were taken to indicate actinomycetes. The amount of bacterial PLFAs was calculated as the sum of the PLFAs 14:0, 15:0, a15:0, 16:0, i16:0, 16:1ω7, a17:0, 17:0, cy17:0, 18:0, 18:1ω7, 18:1ω9 and cy19:0, as according to Frostegård et al. (1996).

1.6. Statistical analyses

The O₃ effects on microbial biomass C, AWCD, Biolog richness, and Shannon–Wiener diversity were tested by Analysis of Variance (ANOVA) using SPSS 11.5 (SPSS Inc., Chicago, Illinois, USA). The LSD for the 95% confidence interval (LSD_{0.05}) was used for multiple comparisons. Student's t-test was used to compare the treatment means of microbial biomass C, functional diversity indices, PLFA% and the relative PLFA indicators. Differences were considered significant at p ≤ 0.05. The values were normalized by dividing by the AWCD for the plate reading at 96 h of incubation, and these values were analyzed by principal component analysis (PCA). The PLFAs, expressed as percentages, were analyzed by PCA, and the effects of treatments on the different indicator PLFAs (%) were

tested by ANOVA using SPSS 11.5 (SPSS Inc., Chicago, Illinois, USA). In order to find if PCs could discriminate between the treatments, the scores of PCs were analyzed by ANOVA using SPSS 11.5 (SPSS Inc., Chicago, Illinois, USA).

2. Results

2.1. Microbial biomass carbon

In the CK and low O₃ treatments, the microbial biomass of rhizosphere soil is higher than for non-rhizosphere soil. In the high O₃ treatment, however, the microbial biomass of rhizosphere soil decreased rapidly, and that of the non-rhizosphere soil did not decrease much. No significant effects of O₃ were observed on the microbial biomass C of non-rhizosphere soil under field-planted wheat, while both O₃ treatments significantly reduced the soil microbial C of rhizosphere soil (by 16% for O₃-1 and 50.7% for O₃-2) compared to the control treatment (Table 1).

2.2. Community level physiological profiles

2.2.1. Principal component analysis

Principal component analyses were conducted to determine the microbial community structures of wheat soils under the different O₃ treatments. PC1 and PC2 explained 38.4% and 17.7% of the variance, respectively, and PC1 clearly discriminated between the treatments for rhizosphere soil ($F = 20$, $p \leq 0.002$ for PC1); however, both PCs did not discriminate between the treatments for the non-rhizosphere soil (Fig. 1).

2.2.2. Functional diversity indices

In the CK and low O₃ treatment, the functional diversity indices were similar between rhizosphere and non-rhizosphere soils, although the functional diversity indices were lower in rhizosphere than those in non-rhizosphere soil. High O₃ treatment (O₃-2) significantly reduced the Shannon–Wiener diversity of both non-rhizosphere and rhizosphere soils, but O₃ only had an evident effect on richness in the rhizosphere soil (Table 2).

2.2.3. Utilization of main carbon sources

EO₃ had no effects on the three main C sources of non-rhizosphere soil under field-planted wheat. However, the high O₃ treatment (O₃-2) had an obvious negative effect on the utilization of the main compounds compared to both the control and the low O₃ treatments (Fig. 2).

Table 1 – Microbial biomass carbon in rhizosphere and non-rhizosphere soil under different ozone treatments.

Treatments	Microbial biomass (mg C/kg dry soil)	
	Non-rhizosphere soil	Rhizosphere soil
CK	468.60 ± 1.6a	594.20 ± 14a
O ₃ -1	468.10 ± 12a	499.50 ± 29b
O ₃ -2	459.80 ± 7a	292.80 ± 6.3c

Data are presented as means ± SE ($n = 3$).

Different letters within the column indicate significant differences among treatments ($p \leq 0.05$).

2.3. Ester-linked phospholipid fatty acid analysis

2.3.1. PLFA profile expression

Sixteen total PLFAs, ranging from 14 to 19 Cs, were detected, including saturated, monounsaturated, polyunsaturated, branched and cyclopropyl fatty acids in all three treatments and both the rhizosphere and non-rhizosphere soils. The most abundant PLFAs were 15:0, 16:0, a17:0, 16:1 ω 7c and 18:1 ω 9c, which were characteristic of bacteria and comprised 60% of the total (Table 3).

2.3.2. Principal component analysis

In the field experiment, 57.7% of the total variance in the dataset was accounted for by PC 1 and 24.8% by PC 2, but only PC1 clearly discriminated among the PLFA profiles of non-rhizosphere soil from the different O₃ treatments ($F = 165$, $p \leq 0.001$). For rhizosphere soil, the two principal components captured 55% and 28.5% of the total variance, respectively, and PC1 significantly discriminated among the PLFA profiles of the different O₃ treatments ($F = 12$, $p \leq 0.01$) (Fig. 3).

2.3.3. Distribution of microorganism groups

The relative abundances of the microbial groups in the microbial community structure can be understood by examining the relative proportions of their characteristic fatty acids. The relative PLFA indicators of fungi, actinomycetes and bacteria can be calculated as percentages of the total. In non-rhizosphere soil, bacteria significantly increased and fungi increased after O₃ exposure, while actinomycetes had no change. In rhizosphere soil, only high O₃ treatment significantly increased bacteria and decreased fungi and actinomycetes (Table 4).

3. Discussion

O₃ itself is a bactericide widely used for disinfection (Khurana, 2003). However, soil and vegetation remove O₃ from the atmosphere, producing a vertical gradient of decreasing O₃ concentrations towards the ground (Turner et al., 1974) and preventing any appreciable penetration of O₃ into the soil (Blum and Tingey, 1977). Therefore, any direct effects of O₃ on soil microorganisms can be of only minor importance, and the indirect effects of O₃ on microbes arising from its impacts on plants are much more relevant. Among previous examinations of soil microbial communities, very few have considered rhizosphere soil microbial responses to O₃. The rhizosphere is the microbial habitat in the soil that is most strongly influenced by plants (Dohrmann and Tebbe, 2005). In several studies, the composition of root exudates, which mainly serve as C and energy sources for soil microorganisms (especially rhizosphere microbes), has been shown to strongly influence the structural and functional diversity of the microbial community (Kowalchuk et al., 2002; Miethling et al., 2000; Schmalenberger and Tebbe, 2003). O₃ has also been shown to change the quantity and quality of root exudates (McCready and Andersen, 2000), which primarily influence the microbial structure and function of the rhizosphere. In this study, the microbial community structures of both non-rhizosphere and rhizosphere soils were examined to determine if O₃ had a greater effect in the rhizosphere.

Fig. 1 – Biplot of soil microbial community structure generated by principal component analysis (PCA) of BIOLOG data under different ozone treatments. *The capital letter plus number indicates the corresponding substrate in ECO-plate with 8 lines (A–H) and 4 rows (1–4). A1, Water; A2, β -methyl-D-glucoside; A3, D-galactonic acid γ -lactone; A4, L-arginine; B1, pyruvic acid methyl ester; B2, D-xylose; B3, D-galacturonic acid; B4, L-asparagine; C1, Tween 40; C2, L-erythritol; C3, 2-hydroxy benzoic acid; C4, L-phenylalanine; D1, Tween 80; D2, D-mannitol; D3, 4-hydroxy benzoic acid; D4, L-serine; E1, α -cyclodextrin; E2, N-acetyl-D-glucosamine; E3, γ -hydroxybutyric acid; E4, L-threonine; F1, glycogen; F2, D-glucosaminic acid; F3, itaconic acid; F4, glycyl-L-glutamic acid; G1, D-cellobiose; G2, glucose-1-phosphate; G3, α -ketobutyric acid; G4, phenylethyl-amine; H1, α -D-lactose; H2, D,L-a-glycerol; H3, D-malic acid; H4, putrescine.

In our study, eO_3 significantly reduced microbial biomass C in rhizosphere soil, but no effects of O_3 on this variable were observed in non-rhizosphere soil. This result is coincident with the soil microbial biomass response to eO_3 under potted wheat of the same species (*T. aestivum* L. Jia 002) (Chen et al., 2009). Another of our previous experiments also showed that eO_3 reduced soil microbial C under rice (Chen et al., 2010). O_3 significantly decreased soil microbial biomass in the fall, after one season of exposure, in a wheat (*T. aestivum*) and soybean (*Glycine max*) system (Islam et al., 2000). However, in ponderosa pine (*Pinus ponderosa*), the total fungal and bacterial biomasses increased at low O_3 levels, and the total microbial biomass decreased at high O_3 levels, compared to the biomasses under the control treatment (Scagel and Andersen, 1997). O_3 has also been reported as having only slight effects on soil biomass in field-grown silver birch exposed to eO_3 in OTCs for 3 years (Kasurinen et al., 2005). O_3 may shift microbial structure without altering total microbial biomass, as has previously been found under eCO_2 (Phillips et al., 2002). These inconsistent results require further investigation.

CLPP by sole C source utilization tests is a good method for assessing microbial community metabolic diversity using Biolog microplates (Garland, 1997). In this study, eO_3 did not change the C utilization pattern of non-rhizosphere soil microorganisms, but

did significantly impact the utilization pattern of rhizosphere microbes. The effect of O_3 on the diversity index calculated from the Biolog plates was also significant in the rhizosphere soil, but not in the non-rhizosphere soil. O_3 also reduced the utilization of the three main substrates in rhizosphere soil but had no effect in non-rhizosphere soil. The CLPP results showed that the soil microbial community under field-planted wheat responds in the same manner to eO_3 as does that under potted wheat (Chen et al., 2009). All of these effects showed that O_3 has much greater effects on rhizosphere soil carbon degradative microbes than on those of non-rhizosphere soil. This difference may be due to the decreased C exudates from the roots of the O_3 -exposed crop. The changes in root exudation may affect rhizosphere microbial activity, potentially altering the ecological and nutrient dynamics in the rhizosphere (Bardgett et al., 1999). Edwards' study showed that the exudation of organic compounds from the roots of O_3 -exposed plants declined due to the reduced translocation of photosynthate to the roots, thereby decreasing the nutrient supply to soil microorganisms and resulting in lower microbial metabolism (Edwards, 1991). The difference in utilization of C sources suggests a change in substrate availability between the CK and eO_3 treatments, as Biolog plates are capable of detecting changes in microbial community metabolic function resulting from actual C source availability (Grayston et al., 1998). The

Table 2 – Effects of ozone on soil microbial community functional richness and diversity.

	Shannon–Weinner diversity		Richness	
	Non-rhizosphere soil	Rhizosphere soil	Non-rhizosphere soil	Rhizosphere soil
CK	1.34 $\pm$ 0.01a	1.32 $\pm$ 0.01a	24.70 $\pm$ 0.33a	24.00 $\pm$ 1.15a
O_3 -1	1.31 $\pm$ 0.02ab	1.32 $\pm$ 0.01a	23.00 $\pm$ 1.53a	24.70 $\pm$ 1.20a
O_3 -2	1.28 $\pm$ 0.01b	1.19 $\pm$ 0.03b	22.00 $\pm$ 0.00a	18.3.0 $\pm$ 0.67b

Data are presented as means $\pm$ SE ($n = 3$).

Different letters within the column indicate significant differences among treatments ($P \leq 0.05$).

Fig. 2 – The effect of ozone on utilization of three main carbon sources of non-rhizosphere soil and rhizosphere soil under field wheat. Bars with different letters indicate differences ($p \leq 0.05$) between treatments within each main carbon source in non-rhizosphere or rhizosphere soil. The vertical thin bars are stand errors at 95% confidence intervals.

results from the C utilization experiments showed that rhizosphere soil microorganisms used fewer carbohydrates, carboxylic acids and amino acids under eO_3 , which may be attributed to the reduced exudation of these compounds from the roots when the

crops were exposed to eO_3 concentrations. Indeed, McCool and Menge (1983) have found that O_3 treatment decreases the amount of sugars and amino acids in root exudates.

CLPP is a simple and economical method for investigating carbon degradative microbes, but it has the disadvantages of being biased by cultural conditions and failing to consider the contributions of fungi and slow-growing bacteria (Smalla et al., 1998), which the PLFA method does not miss (Bååth et al., 1998; White and MacNaughton, 1997). Biolog plates present functional rather than structural information about the soil microbial community (Bossio and Scow, 1998), while PLFA results can express both structural and quantitative information (Yao et al., 2000). The relative abundances of the microbial groups in the microbial community structure can be understood by examining the relative proportions of their characteristic fatty acids (Bossio and Scow, 1998). In this study, the PLFA method was also used to investigate the effects of O_3 on microbial community structure.

The bacterial indicator PLFAs were the most abundant in the rhizosphere and non-rhizosphere soils. The patterns of the PLFA profiles were significantly different between the three O_3 treatments in both the rhizosphere and non-rhizosphere soils. After the wheat was exposed to O_3 , the relative abundances of fungi and actinomycetes decreased, and the relative abundance of bacteria increased. These observations are consistent with the results from an experimental platform of free-air O_3 enrichment in which fungal PLFA and the fungi to bacteria ratio decreased following exposure to eO_3 , especially in the rhizosphere soil of O_3 -tolerant wheat (Li et al., 2012). Active bulk soil fungal biomass and the ratio of active fungal to active bacterial biomass increased with increasing exposure to O_3 in ponderosa pine (*P. ponderosa*) (Scagel and Andersen, 1997). In another study, the relative proportions of gram-positive and gram-negative indicator PLFAs of bulk soil beneath aspen, birch, and maple trees (*Populus tremuloides* Michx., *Betula papyrifera* Marsh., and *Acer saccharum* Marsh.) were not affected after incubation at elevated levels of tropospheric O_3 over a period of

Table 3 – PLFA profiles (expressed as %) recovered from wheat soil samples after shoot exposed to different ozone dose.

PLFA ^a	Rhizosphere soil			Non-rhizosphere soil		
	CK	O ₃ -1	O ₃ -2	CK	O ₃ -1	O ₃ -2
14:0	3.73a	2.27b	2.75b	3.20a	0.00c	2.45b
15:0	10.83b	11.28a	11.43a	10.89b	8.88c	12.12a
16:0	14.07	15.98	15.97	13.21	11.29	15.14
17:0	3.12	2.67	3.21	3.14	3.27	2.61
18:0	2.76b	1.86c	3.61a	1.65b	2.59ab	3.07a
i14:0	0.00	0.00	0.00	0.00b	2.35a	0.00b
a15:0	6.65	7.01	7.09	6.20a	4.52b	7.05a
i16:0	6.40a	5.68ab	5.02b	5.40a	3.86b	5.67a
a17:0	10.95a	10.00b	10.08b	12.10b	26.07a	10.71b
16:1ω7c	13.98a	12.62b	11.21c	9.66	10.16	11.70
i17:1ω5t	0.00b	3.13a	2.86a	3.45a	0.00b	2.92a
18:1ω7t	1.28a	0.00b	0.00b	0.00	0.00	0.00
18:1ω9c	10.98c	12.61b	14.46a	12.59a	10.45b	13.73a
cy17:0	5.04a	4.52b	2.72c	4.22a	2.97b	2.68b
cy19:0	2.52	2.47	2.72	2.84	2.81	2.85
18:0(10Me)	2.91a	3.13a	1.54b	3.28ab	4.07a	2.03b
19:0(10Me)	2.39c	2.60b	2.81a	2.19b	1.82c	2.71a
18:2ω6,9	2.36	2.16	2.54	3.09	4.90	2.56

Different letters within the row indicate significant differences among treatments ($p \leq 0.05$).

^a Fatty acids are designated in terms of the total number of C atoms: number of double bonds, followed by the position of the double bond from the methyl end of the molecule. Cis and trans configurations are indicated by c and t, respectively. The prefixes a and i indicate anteiso and iso branching, br indicates an unknown methyl branching position, 10Me indicates a methyl group on the 10th C atom from the carboxyl end of the molecule, and cy refers to cyclopropane fatty acids.

suppressed under eO₃, and those involved in C degradation and CH₄ generation remained unchanged under eO₃ (He et al., 2013). These variable responses of the microbial community to eO₃ may be attributed to numerous factors, including different species, different levels of O₃ exposure and different sampling procedures at different growth stages.

The results of the present study showed that eO_3 had similar effects on the soil microbial community under field and potted wheat (Chen et al., 2009), but some differences were observed between the field and potted experiments. The soil microbial biomass C in the field-planted wheat was much higher than that in the potted wheat, and the utilization of C sources in the field-planted wheat was also higher. Additionally, fungal indicator PLFAs were not detected in non-rhizosphere soil in the potted experiment. In the potted wheat study, the AOT40 values for the two eO_3 treatments were 21.4 ppm•h and 44.1 ppm•h, respectively, for O_3 -1 and O_3 -2, which were much higher than the levels for O_3 -1 (1.59 ppm•h) and O_3 -2 (9.17 ppm•h) employed in this field study. However, the effects of eO_3 on the soil microbial community in the field experiment were considerably more significant than in the pot experiment. The pot experiment may therefore have masked the real harm of eO_3 to vegetation. The effects of eO_3 on soil microbes were definitely especially prominent in rhizosphere soil, which means that O_3 affects the soil food web through the roots. In addition, the effect area is

	Non-rhizosphere soil			Rhizosphere soil		
	Bacteria	Actinomycete	Fungi	Bacteria	Actinomycete	Fungi
CK	88.54 ± 1.16b	5.47 ± 0.04	3.09 ± 0.02a	91.81 ± 0.50b	5.63 ± 0.23a	2.86 ± 0.07a
O ₃ -1	91.72 ± 1.01a	5.89 ± 0.80	2.40 ± 0.21b	91.61 ± 0.22b	5.73 ± 0.09a	2.66 ± 0.14ab
O ₃ -2	93.03 ± 0.17a	4.74 ± 0.01	2.22 ± 0.17b	93.10 ± 0.04a	4.36 ± 0.05b	2.54 ± 0.01b

Data are presented as means ± SE (n = 3).
Different letters within the column indicate significant differences among treatments (P ≤ 0.05).

limited, and further study is needed to determine whether the limited effect of O₃ on rhizosphere soil microbes would affect the nutrient utilization of vegetation.

4. Conclusions

This study showed that there is significant alteration in microbial biomass and community functions in rhizosphere soil, but not in rhizosphere soil, under eO₃ exposure. In addition, the microbial community structure of rhizosphere soil responds more significantly to eO₃ than non-rhizosphere soil. This strongly suggests that the effects detected in the rhizosphere microbial community came about due to the ozone effects on the plant, not as direct effects of ozone on the rhizosphere microbes. However, there are still some problems in this study. OTC can produce microclimates which might not reflect the real reaction of plant vegetation to O₃. Recent advances in molecular biological methods could provide more sophisticated tools to study the microbial community response to O₃ than the CLPP and PLFA methods used here. Many uncertainties remain in the soil microbial community response to O₃ stress. Long-term experiments should be carried on in order to determine if the O₃ impacts on the soil are accumulative.

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