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Soil microbial food web responses to free-air ozone enrichment can depend on the ozone-tolerance of wheat cultivars

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ABSTRACT

Soil processes are driven by soil organisms and their interactions with plants and soil abiotic conditions. Climate changes may directly or indirectly alter soil processes and the organisms mediating these processes. Although aboveground influences of ozone have been studied widely on agroecosystems, the effects on belowground processes are poorly understood. This study aimed to investigate the effects of elevated ozone concentration [O_3] on the components of soil microbial food webs and compare their responses between ozone-sensitive and ozone-tolerant wheat cultivars. The responses of soil biota to elevated [O_3] varied between the two wheat cultivars. Fungal PLFA and the fungi to bacteria ratio decreased following elevated [O_3], especially in the rhizospheric soil of ozone-tolerant wheat. Nematode functional guilds were sensitive to elevated [O_3] and cultivar effects. At wheat jointing stage, bacterivores belonging to *K*-strategies decreased following elevated [O_3], while fungivores exhibited a reverse trend. The abundance of flagellates decreased in ozone-tolerant wheat, but increased in ozone-sensitive wheat following elevated [O_3]. However, an opposite trend was found in the bacterivores belonging to *r*-strategies. In conclusion, wheat cultivars play an important role in determining the effects of elevated [O_3] on soil food web. The responses of soil biota to elevated [O_3] were greater in ozone-tolerant wheat than in ozone-sensitive wheat, which may in turn have influenced soil organic matter decomposition and nutrient turnover.

1. Introduction

Tropospheric ozone concentration [O_3] in the Northern Hemisphere has increased from about 10 ppb to the present background concentrations of 25–40 ppb as a consequence of rapid economic development (Vingarzan, 2004; Biswas et al., 2008), and is predicted to increase by 40–70% by the year 2100 (Zeng et al., 2008). Tropospheric ozone is recognized as the most damaging and widespread pollutant affecting agricultural and forest ecosystems (Häikiö et al., 2009; Schrader et al., 2009; Chen et al., 2009; Nikolova et al., 2010), which poses a great threat to crop yields (Feng et al., 2008; Feng and Kobayashi, 2009), forest productivity (Karnosky et al., 2007; Wittig et al., 2007) and ecosystem carbon storage (Loya et al., 2003; Sitch et al., 2007). Although aboveground influences of elevated [O_3] have been studied widely on agroecosystems (Feng and Kobayashi, 2009), direct and indirect effects on belowground processes are poorly understood (Andersen, 2003; Schrader et al., 2009; Chen et al., 2009). Since soil biota depend on carbon inputs from plants, altered C flux to soil due to ozone stress could also change the structure and function of soil biological communities (Andersen, 2003). Despite the importance of soil invertebrates in decomposition and nutrient cycling, relatively few researches have paid attention to the influence of elevated [O_3] on soil food webs. Several studies focused on the response of microbial community (Chen et al., 2009; Manninen et al., 2010) or soil fauna (Schrader et al., 2009), but rarely studied the response of soil food web to elevated [O_3]. Khan and Khan (1998) investigated interactions between root-knot nematodes and tomato in an open-top chamber experiment, and found the damage of *Meloidogyne incognita* to tomatoes was increased following elevated [O_3]. However, Qiu et al. (2009) found that soil ozone fumigation could reduce the abundance of *Meloidogyne javanica* and free-living nematodes on a sandy loam soil. Tingey et al. (2006) in a chamber experiment found no effects of ozone on soil respiration, fine root biomass and soil fauna including nematodes, collembolans and mites. Using an open-top chamber experiment, Schrader et al. (2009) found that the density of enchytraeids, collembolans

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and soil mites decreased significantly in the rhizosphere of winter wheat after ozone exposure for two years and concluded that elevated [O_3] has the potential to affect belowground biodiversity in agroecosystems. These examples indicate that our understanding on the possible effects of elevated [O_3] on the structure and function of soil food web is limited.

Wheat (*Triticum aestivum* L.) is sensitive to ozone. Elevated [O_3] generally results in severe detrimental effects such as visible foliar injury, accelerated senescence, growth reduction and yield loss (Biswas et al., 2008; Feng et al., 2008). However, the extent of the effects varies by species or even cultivars (Morgan et al., 2006; Biswas et al., 2008; Feng et al., 2008; Zhu et al., 2011). Biswas et al. (2008) using open-top chambers compared the sensitivity of 20 winter wheat cultivars and found that ozone-sensitive cultivars had higher stomatal conductance, larger reduction in antioxidative capacity and lower levels of dark respiration. Using the FAOE (free-air ozone enrichment) platform, Cao et al. (2009) also found that the ozone-sensitive cultivar Yannong 19 showed larger reductions in photosynthetic rate, stomatal conductance and transpiration rate than the ozone-tolerant Yangmai 16. Similar findings were also reported by Zhu et al. (2011) in the same experimental field, who found that the decrease in grain yield and individual grain mass of Yannong 19 was higher than those of Yangmai 16 due to elevated [O_3]. Since plant species are an important determinant of ecosystem properties (Wardle et al., 1997; Zhao et al., 2011), different plant species/crop cultivars may differ in the quantity and quality of resources that they return to soil which may have important effects on the components of soil biota and the processes that they regulate (McCrady and Andersen, 2000; Wardle et al., 2004). Based on the differences in the growth responses to elevated [O_3] between tolerant and sensitive wheat cultivars, we hypothesized that the effects of ozone enrichment on soil microbial food webs will depend on the tolerance of wheat cultivars, greater effects will be observed in the ozone-sensitive cultivar due to relatively lower crop residue or plant C input to the soil; and the responses of bacterial and fungal based food webs will be different owing to associated differences in their food resources.

In agroecosystems, microorganisms (bacteria and fungi), microbivores (protozoa and nematode) and predators (nematode) are constituents of soil microbial food webs (Wardle, 1995; Niwa et al., 2011), which are regarded as key members of detrital food webs. They are directly involved in nutrient mineralization and organic matter decomposition (Ingham et al., 1985; Wardle, 2002). Soil food webs are typically dominated by either bacteria or fungi, which are considered as separate channels in relation to energy flows (De Ruiter et al., 1993; Moore and Hunt, 1998; Drigo et al., 2008). Given the different roles played by bacteria and fungi in organic matter degradation and nutrient cycling, shifts in bacterial or fungal based food web might have a strong impact on soil function (Drigo et al., 2008). Despite their significance in biogeochemical cycles, our knowledge about the impact of elevated [O_3] on soil food web is scarce (Schrader et al., 2009). The objectives of this research were to evaluate the influences of elevated [O_3] on the structure and function of soil microbial food webs and to compare how tolerance of the wheat cultivars may influence soil food web components.

2. Materials and methods

2.1. Experimental site and ozone fumigation

The experimental site is located in a suburb of Jiangdu city in Jiangsu province of China (32°35′ N, 119°42′ E). The soil at the study site is a Shajiang Aquic Cambosols (Chinese Soil Taxonomy; Li et al., 2009) with a sandy-loamy texture, with 15 g kg^{-1} total C, 1.5 g kg^{-1} total N, pH 6.8, and 25.1% clay (<0.001 mm) and bulk density 1.2 g cm^{-3} at 0–15 cm depth (Zhu et al., 2011). The climate conditions are temperate with average annual temperature and precipitation at 14.9 °C and 980 mm, respectively (Li et al., 2009). An experimental platform of free-air ozone enrichment (FAOE) was established in 2007 over a rice-wheat rotation system, with rice transplanted in mid-June and harvested in middle-to-late October and winter wheat was sown in early November and harvested in late May or early June of the next year. Rice/wheat straw from the previous season was incorporated in which the rice/wheat was growing. No additional organic matter was incorporated during the wheat growing seasons. Different treatments were established in 2007 and have been maintained since then. Three replicate FAOE rings, each with 14.5 m in diameter, were set randomly within a uniform area of 4 ha to continuously provide an elevated [O_3] of 60 ppb over the ambient conditions (about 40 ppb) from 9:00 am to 18:00 pm (this setup is hereinafter referred to as E-O_3), while three replicate rings, each with the same size, were set randomly within the same area for the ambient [O_3] treatment (hereinafter referred to as A-O_3). All of the rings were far enough apart to prevent ozone from spilling over from one ring to another. Each plot under ambient and elevated [O_3] conditions was split into two subplots planting with two winter wheat cultivars [ozone-sensitive cultivar, Yannong 19 (Y19), and the ozone-tolerant cultivar, Yangmai 16 (Y16)]. Nitrogen was applied as urea ($N = 46\%$) and diammonium phosphate at a total rate of 210 kg N ha^{-1}, which was split into basal application at planting (60%), side-dressings at early tillering (10%) and elongation stages (30%). P and K were applied as diammonium phosphate and potassium chloride, respectively, at a rate of 90 kg P_2O_5 ha^{-1} and 90 kg K_2O ha^{-1} which were split-applied with 60% at planting and 40% at elongation stage, respectively (Zhu et al., 2011). This experiment was conducted during the wheat growing season of 2010, after exposure to elevated [O_3] for 3 years (from March 5 to May 27, about 83 days of each year). Soil samples were collected from 0 to 15 cm depth in the soil of Y19 and Y16 at the jointing stage (March 30 in 2010) and ripening stage (June 10 in 2010). Each soil sample was pooled from five soil cores of 2.5 cm diameter; the soil corer was placed near a plant within a plant row to be sure of sampling the rhizosphere. Soil samples were stored at 4 °C until further analyses.

2.2. Soil and plant analyses

The total carbon (C) and nitrogen (N) of plant or soil were determined by a TruSpec CN Elemental Analyzer (Leco Corporation, USA). Dissolved organic carbon (DOC) was determined by Multi N/C 3100 analyzer (Jena Corporation, Germany). Soil pH was determined with a glass electrode in 1:2.5 soil:water solution (w/v). 15 winter wheat plants from each subplot were harvested on 10 June 2010, and partitioned into grain and litter. Litter and grain samples were dried at 65 °C until a constant weight was obtained, and then weighted for 1000 kernel weight (grain weight) and individual plant (plant weight).

2.3. PLFA and protozoa analysis

The soil microbial community was characterized using phospholipid fatty acids (PLFAs) analysis as described by Bligh and Dyer (1959) with slight modifications. Eight gram freeze-dried soil samples were extracted with a single-phase mixture of chloroform:methanol:citrate buffer (30.4 mL at a 1:2:0.8 volume basis) on a horizontal shaker (250 rpm) for two and half hours at room temperature. After centrifugation for 10 min at 4000 rpm, the supernatant was transferred to another glassware tube and the soil

vortexed and re-extracted for another 2 h with an additional volume of extractant (7.6 mL). The combined supernatant was split into two phases by adding citrate buffer (16 mL) and chloroform (16 mL) and left overnight to separate. The $CHCl_3$ layer was then transferred to a new tube and dried under N_2 at 30 °C. Phospholipids were separated from neutral lipids and glycolipids on a 5 mL standard SPE tube (Supelco Inc., Bellefonte, PA). The tube was first conditioned with $CHCl_3$ (5 mL). The lipids were then transferred into the tube with $CHCl_3$ (3 × 250 μL). Neutral lipids and glycolipids were eluted with chloroform (8 mL) and acetone (16 mL) separately. Phospholipids were obtained from methanol elution (8 mL) and dried under N_2.

The polar lipids were trans-esterified to the fatty acid methyl esters by a mild alkaline methanolysis (Bossio et al., 1998) with a methanol:toluene (1 mL) and potassiumhydroxide methanol solution (0.2 M, 1 mL) and heated at 35 °C for 15 min. After methylation, H_2O (2 mL) and acetic acid (0.3 mL) were added. Fatty acid methyl esters were extracted in hexane (2 × 2 mL) and dried under N_2. Samples were then dissolved in hexane (60 μL) and analysed in an Agilent 6850 series Gas Chromatograph with MIDI peak identification software (Version 4.5; MIDI Inc., Newark, DE). The column was an Agilent 19091B-102 (25.0 m × 200 μm × 0.33 μm) capillary column. Hydrogen was the carrier gas. The GC temperature progression was set by the MIDI software. The fatty acid 19:0 was added as an internal standard before methylation and fatty acid methyl esters were identified automatically by the MIDI peak identification software.

The sum of the following PLFAs was used as the measure of bacterial biomass: i15:0, a15:0, 15:0, i16:0, 16:1ω7c, i17:0, a17:0, 17:0, cy17:0, and cy19:0. The fatty acid 18:2ω6 and 18:1ω9c was used as an indicator of fungal biomass (Bååth and Anderson, 2003). Other PLFAs such as 16: 1ω5c for AM fungi, 20:4ω6c for the protozoa, the ratio of 18:2ω6 and 18:1ω9c to total bacterial PLFAs (F: B), the cy17:0 to 16:1ω7c ratio (cy:precursor) and the iso to anteiso branching ratio (i:a) (Bardgett et al., 1996; Frostegård and Bååth, 1996) were used to analyze the composition of microbial community in the PCA analysis. Taken together, all of the PLFAs indicated above were considered to be representative of the total PLFAs of soil microbial community.

The most-probable-number method was used to determine flagellate populations by the serial dilution method (Singh, 1975; Rodriguez-Zaragoza et al., 2005). Soil extract at a dilution of 1:5 (working solution) was used for the most-probable number procedure. The soil extract was prepared by homogenizing 200 g of soil in 1000 mL of tap water under continuous heating at 60 °C for 2 h; then, the extract was filtered and autoclaved for 15 min (Singh, 1975). Twenty-four-well tissue culture plates were inoculated as follows. A soil–water mixture was prepared by homogenizing 1 g of soil in 10 mL of soil extract. Five 15-s pulses of vigorous shaking in a vortex achieved homogenization. The homogenate was left undisturbed for 15 min. Then, 10-fold serial dilutions were made, beginning with 10^{-2} and ending with 10^{-7}. These dilutions were prepared by placing 100 μL of the soil mixture in the first row of a 24-well tissue culture plate, which was first filled with 900 μL of soil extract. Six dilutions were prepared, with four replicates for each dilution. The plates were incubated at 28 °C for 7–10 days and reviewed with an inverted microscope for the presence of flagellates.

2.4. Nematode community analysis

Nematodes were extracted from 100 g of soil (fresh weight) by a modified cotton-wool filter method (Oostenbrink, 1960; Townshend, 1963). Nematode populations are expressed as number of nematodes per 100 g dry soil and at least 150 nematodes from each sample were identified to genus level using an inverted compound microscope. The nematodes were assigned to the following trophic groups characterized by feeding habits (1) bacterivores (Ba); (2) fungivores (Fu); (3) omnivore-carnivores (Om-Ca) and (4) plant parasites (H) following Yeates et al. (1993).

The following nematode community indices were calculated:

$$\text{Shannon–Wiener diversity index}: H' = -\sum p_i \ln(p_i) \quad (1)$$

$$\text{Dominance index}: \lambda = \sum p_i^2 \quad (2)$$

$$\text{Evenness index}: J = H'/\ln(S) \quad (3)$$

where p_i is the proportion of individuals in the ith taxon and S is the number of taxa (Yeates and Bongers, 1999);

$$\text{Channel index CI} = 100 \times (0.8\text{Fu}_2)/(3.2\text{Ba}_1 + 0.8\text{Fu}_2) \quad (4)$$

$$\text{Structure index SI} = 100 \times \left(\sum k_s n_s / \left(\sum k_s n_s + \sum k_b n_b\right)\right) \quad (5)$$

$$\text{Enrichment index EI} = 100 \times \left(\sum k_e n_e / \left(\sum k_e n_e + \sum k_b n_b\right)\right) \quad (6)$$

where k_b is the weight assigned to guilds Ba_2 and Fu_2 and n_b is the abundance of nematodes in guilds Ba_2 and Fu_2, which indicate basal

Table 1
Soil and plant physiochemical variables in ozone-sensitive (Y19) and ozone-tolerant (Y16) wheat cultivars under ambient (A-O_3) and elevated ozone (E-O_3) conditions during wheat growing season (mean ± SD).

	Growing season	Y19		Y16		Effect		
		A-O_3	E-O_3	A-O_3	E-O_3	Treatment (T)	Cultivar (C)	T × C
SM (%)	Jointing	38.59 ± 2.13	36.35 ± 2.51	37.49 ± 2.56	38.11 ± 3.56	ns	ns	ns
	Ripening	31.16 ± 1.55	30.69 ± 1.94	30.19 ± 4.53	33.25 ± 1.48	ns	ns	ns
pH	Jointing	6.32 ± 0.27	6.69 ± 0.37	6.53 ± 0.39	6.78 ± 0.67	ns	ns	ns
	Ripening	6.16 ± 0.09	6.40 ± 0.30	6.15 ± 0.10	6.44 ± 0.12	ns	ns	ns
DOC (μg g^{-1})	Jointing	421.44 ± 56.66	459.40 ± 50.72	431.34 ± 19.98	461.14 ± 65.88	ns	ns	ns
	Ripening	499.50 ± 96.19	355.55 ± 16.20	389.97 ± 6.29	313.73 ± 2.08	0.024	0.033	ns
SOC (g kg^{-1})	Jointing	15.90 ± 1.11	14.67 ± 1.42	16.43 ± 0.96	13.93 ± 1.12	ns	ns	ns
	Ripening	15.17 ± 1.70	15.30 ± 1.32	17.10 ± 1.30	15.30 ± 1.25	ns	ns	ns
TN (g kg^{-1})	Jointing	1.70 ± 0.09	1.61 ± 0.13	2.07 ± 0.47	1.48 ± 0.20	ns	ns	ns
	Ripening	1.59 ± 0.19	1.60 ± 0.26	1.78 ± 0.07	1.65 ± 0.18	ns	ns	ns
Plant C (%)	Ripening	40.63 ± 0.46	41.00 ± 0.10	40.40 ± 0.10	40.73 ± 0.06	ns	ns	ns
Plant N (%)	Ripening	2.09 ± 0.24	2.43 ± 0.09	2.16 ± 0.13	2.18 ± 0.16	ns	ns	ns
Grain weight (g)	Ripening	39.38 ± 3.69	32.50 ± 4.68	44.87 ± 2.33	37.97 ± 2.44	0.001	ns	ns
Plant weight (g)	Ripening	3.52 ± 0.51	3.22 ± 0.47	3.81 ± 0.47	3.43 ± 0.35	ns	ns	ns

Notes: SM, soil moisture; DOC, dissolved organic carbon; SOC, soil organic carbon; TN, total nitrogen.

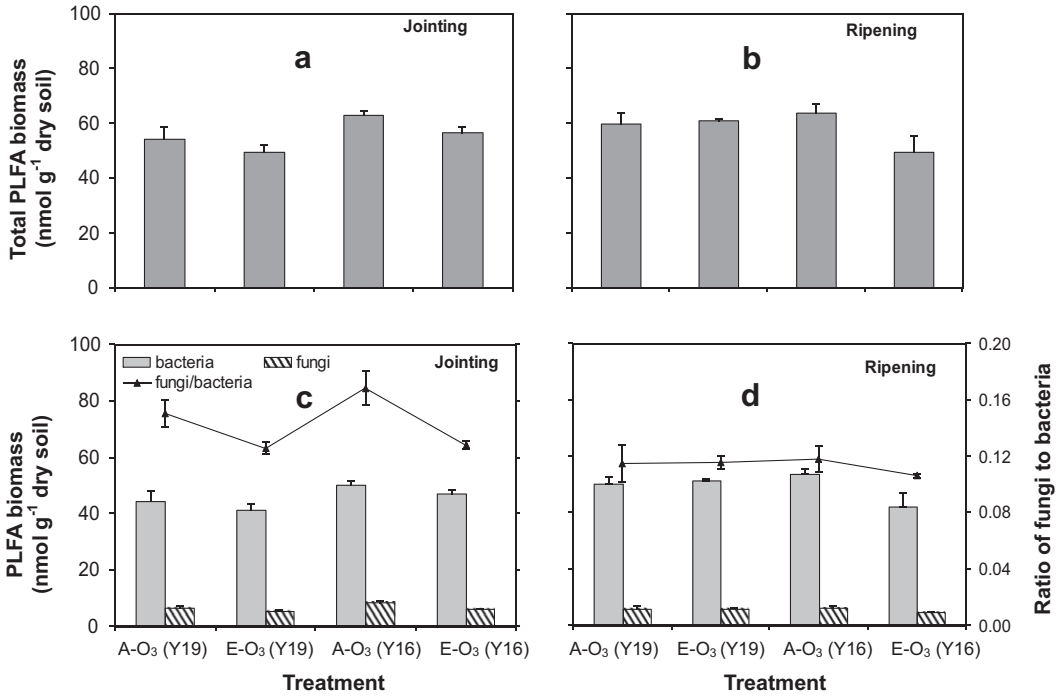

Fig. 1. Total phospholipid fatty acid (PLFA) biomass (a, b) and the PLFA biomasses for indicator subgroups of bacteria, fungi and the fungi:bacteria ratio (c, d) in ozone-sensitive (Y19) and ozone-tolerant (Y16) wheat cultivars under ambient (A-O_3) and elevated ozone (E-O_3) conditions.

characteristics of the food web; k_s the weight assigned to guilds Ba_3–Ba_5, Fu_3–Fu_5, Om_4–Om_5 and Ca_2–Ca_5, n_s is the abundance of nematodes in these guilds, which represent the structure condition of the food web; k_e the weight assigned to guilds Ba_1 and Fu_2, and n_e is the abundance of nematodes in these guilds, which represent an enriched condition of the food web (Ferris et al., 2001). Ba_x, Fu_x, Ca_x, Om_x, (where $x = 1$–5) represent the functional guilds of nematodes that are bacterivores, fungivores, carnivores and omnivores where the guilds have the characters indicated by x on the colonizer-persister (cp) scale (1–5) following Bongers and Bongers (1998).

2.5. Statistical analysis

Nematode and protozoa abundances were $\ln(x + 1)$ transformed prior to statistical analysis. To test the main effects and interactions of treatment and cultivar effects on soil properties and biota, general linear model analysis of variance designed for split plot were performed, with ozone and cultivars as fixed factors and replicate as random factor. All statistical analyses were performed by SPSS statistical software (SPSS Inc., Chicago, IL). Difference at $P < 0.05$ level was considered to be statistically significant. Principal component analysis (PCA) and Canonical correspondence analysis (CCA) were performed to study soil microbial community structure based on PLFA data and the relationships between soil detrital food web and environmental parameters using CANOCO software (ter Braak, 1988).

3. Results

3.1. Soil physiochemical parameters

After 3 yr exposure of elevated [O_3], most soil physicochemical parameters did not fluctuate between two wheat cultivars. The content of DOC was the only character that showed response to both treatment and cultivar effects. At wheat ripening stage, the content of DOC decreased by 28.8% in ozone-sensitive wheat and 19.6% in ozone-tolerant wheat, respectively, following elevated [O_3]. Higher contents of DOC were observed in ozone-sensitive wheat than in ozone-tolerant wheat under both ambient and elevated [O_3] conditions.

At wheat ripening stage, grain weight decreased 17.5% in ozone-sensitive wheat, and 15.4% in ozone-tolerant wheat, respectively, following elevated [O_3]. However, grain weights were higher in

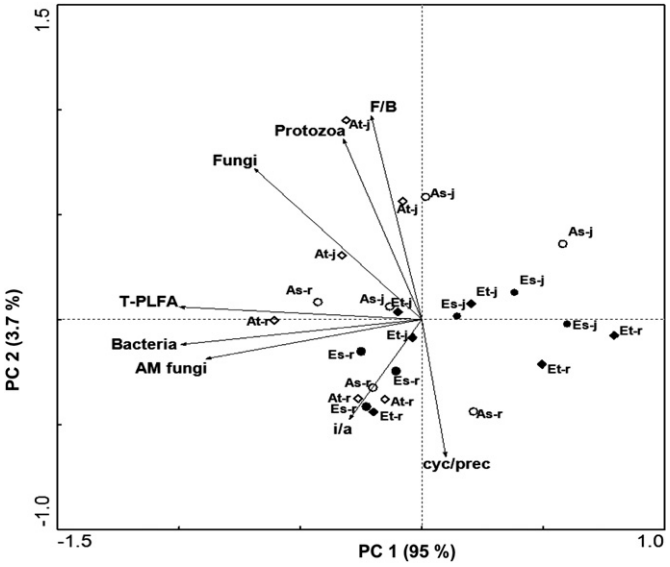

Fig. 2. PCA analysis of microbial PLFA indicators of ozone-sensitive (Y19) and ozone-tolerant (Y16) wheat cultivars under ambient and elevated ozone conditions (◇, ○, represent ozone-tolerant and ozone-sensitive wheat cultivars under ambient (open) and elevated ozone (filled) conditions, respectively; A, A-O_3; E, E-O_3; t, ozone-tolerant wheat (Y16); s, ozone-sensitive wheat (Y19); j, jointing stage; r, ripening sage; T-PLFA, total PLFA).

ozone-tolerant wheat than in ozone-sensitive wheat under both ambient and elevated [O_3] conditions, but no significant differences were detected (Table 1).

3.2. Soil microbial community

The cultivar effects on soil microbial functional groups were more obvious than the treatment effects. At wheat jointing stage, total PLFA and bacterial PLFA were higher in ozone-tolerant wheat than in ozone-sensitive wheat ($P < 0.05$) (Fig. 1a and c). Similar trends were also observed in the fungal PLFA and the fungi:bacteria ratio (F/B ratio) ($P < 0.05$). Relative to the ozone-sensitive wheat, greater treatment effects were observed in the rhizospheric soil of ozone-tolerant wheat. Following elevated [O_3], fungal PLFA in the ozone-tolerant wheat were decreased by 28.5% and 29.9%, respectively, at wheat jointing and ripening stages ($P < 0.05$) (Fig. 1c and d), similar pattern was also observed in the F/B ratio at wheat jointing stage ($P < 0.05$) (Fig. 1c).

The principal components analysis (PCA) of PLFAs showed differences in the composition of total PLFAs between wheat growing seasons. Principal component PC1 and PC2 accounted for 98.7% of the variation among soil samples (Fig. 2). Soil microbial community in ozone-tolerant wheat occupied different portions of ordination space under ambient and elevated [O_3] conditions, indicating that soil microbial community composition in the ambient [O_3] differed substantially from the treatment of elevated [O_3], especially at wheat jointing stage (Fig. 2).

The abundance of flagellates was sensitive to cultivar effect at wheat jointing and ripening stages, with higher abundance found in ozone-tolerant wheat ($P < 0.05$) (Fig. 3a). The interactive effects between elevated [O_3] and wheat cultivars were observed at wheat jointing stage ($P < 0.05$). The abundance of flagellates decreased in ozone-tolerant wheat, but increased in ozone-sensitive wheat following elevated [O_3].

3.3. Soil nematode community

During the study period, no obvious treatment effects were observed in the abundance of total nematodes and different trophic groups (Fig. 3b–f). However, nematode functional guilds showed response to the treatments and wheat cultivar effects. At wheat jointing stage, bacterivores belonging to cp-4 guilds (Ba_4) decreased following elevated [O_3] ($P < 0.05$), while an opposite trend was observed in the fungivores of cp-4 guilds (Fu_4), which increased following elevated [O_3] ($P < 0.05$). At wheat ripening stage, the abundance of plant parasites belonging to cp-2 guilds (H_2) were sensitive to cultivar effect, with higher abundance found in ozone-tolerant wheat ($P < 0.01$) (Table 2). Significant interactive effects between treatment and wheat cultivar were only observed in bacterivores belonging to cp-1 guilds, which increased in ozone-

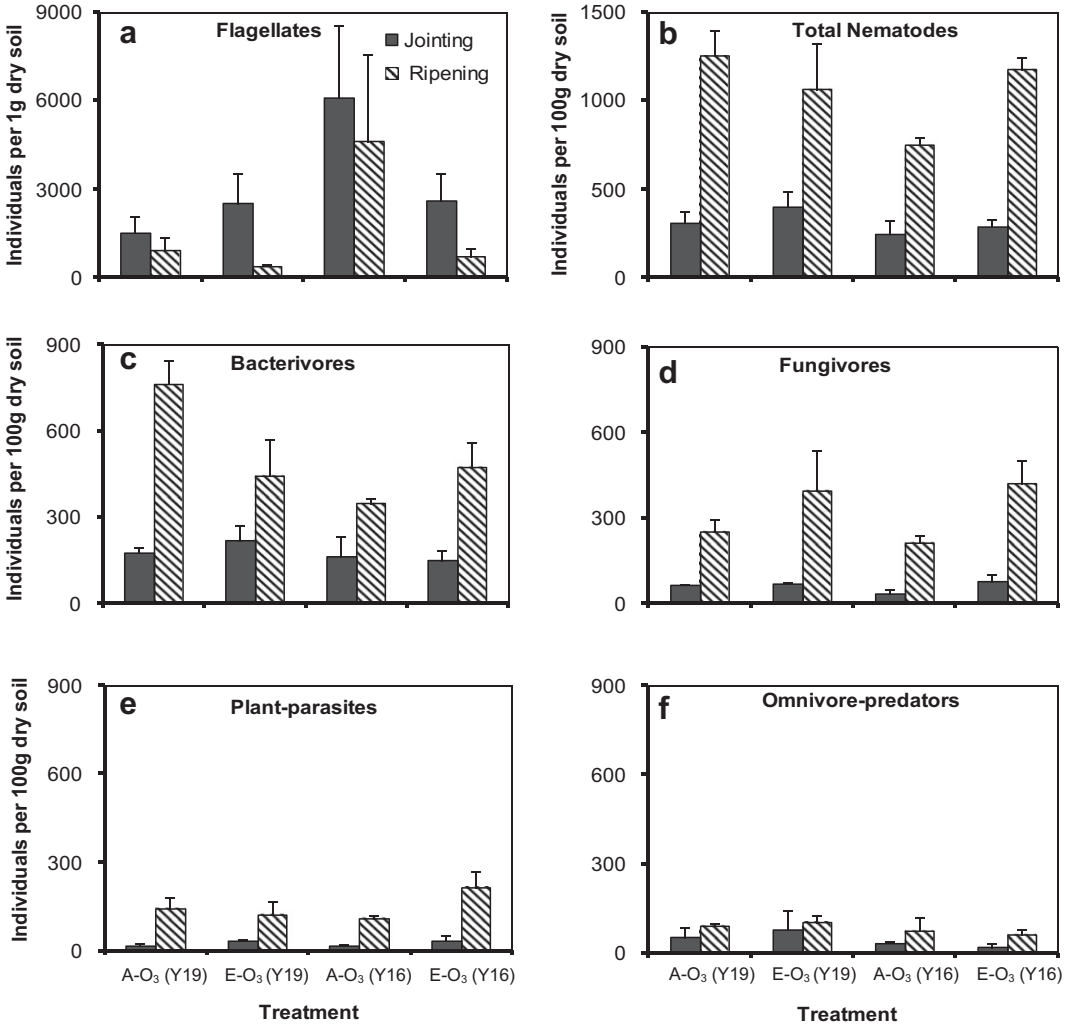

Fig. 3. The abundance of flagellates (a), total nematodes (b) and nematode trophic groups (c, d, e, f) in ozone-sensitive (Y19) and ozone-tolerant (Y16) wheat cultivars under ambient ($A\text{-}O_3$) and elevated ozone ($E\text{-}O_3$) conditions (bars represent the standard error).

Table 2
Abundance of nematode functional guilds in ozone-sensitive (Y19) and ozone-tolerant (Y16) wheat cultivars under ambient (A-O_3) and elevated ozone (E-O_3) conditions (Individuals per 100 g dry soil) (mean ± SE).

Guild	Growing season	Y19		Y16		Effect		
		A-O_3	E-O_3	A-O_3	E-O_3	Treatment (*T*)	Cultivar (*C*)	*T* × *C*
Ba_1	Jointing	64 ± 24	68 ± 30	64 ± 42	39 ± 17	ns	ns	ns
	Ripening	9 ± 5	0	11 ± 8	19 ± 3	ns	<0.001	0.001
Ba_2	Jointing	106 ± 14	153 ± 37	100 ± 27	111 ± 20	ns	ns	ns
	Ripening	736 ± 171	423 ± 105	337 ± 13	448 ± 93	ns	ns	ns
Ba_4	Jointing	5 ± 1	0	1 ± 1	0	0.011	ns	ns
	Ripening	17 ± 9	20 ± 20	2 ± 2	7 ± 4	ns	ns	ns
Fu_2	Jointing	62 ± 3	62 ± 8	34 ± 12	68 ± 18	ns	ns	ns
	Ripening	214 ± 31	385 ± 140	192 ± 30	415 ± 79	ns	ns	ns
Fu_4	Jointing	2 ± 2	8 ± 4	0	12 ± 2	0.042	ns	ns
	Ripening	41 ± 22	11 ± 6	22 ± 7	8 ± 4	ns	ns	ns
H_2	Jointing	7 ± 4	12 ± 3	4 ± 4	9 ± 3	ns	ns	ns
	Ripening	7 ± 7	5 ± 5	34 ± 11	39 ± 21	ns	0.008	ns
H_3	Jointing	7 ± 7	18 ± 3	8 ± 4	25 ± 14	ns	ns	ns
	Ripening	131 ± 47	115 ± 41	68 ± 13	169 ± 52	ns	ns	ns
H_4	Jointing	1 ± 1	2 ± 2	3 ± 3	0	ns	ns	ns
	Ripening	4 ± 4	2 ± 2	7 ± 0	7 ± 4	ns	ns	ns
Ca_3	Jointing	1 ± 1	0	3 ± 1	1 ± 1	ns	ns	ns
	Ripening	13 ± 1	7 ± 7	27 ± 23	0	ns	ns	ns
Ca_4	Jointing	30 ± 17	35 ± 27	17 ± 4	9 ± 4	ns	ns	ns
	Ripening	60 ± 15	47 ± 3	37 ± 19	43 ± 13	ns	ns	ns
Ca_5	Jointing	0	0	0	0	ns	ns	ns
	Ripening	0	4 ± 4	0	0	ns	ns	ns
Om_5	Jointing	23 ± 17	43 ± 38	14 ± 2	10 ± 10	ns	ns	ns
	Ripening	21 ± 11	45 ± 31	13 ± 7	22 ± 22	ns	ns	ns

Note: H, plant parasites; Ba, bacterivores; Fu, fungivores; Om, omnivore; Ca, carnivores; numbers following the functional guilds indicate the c-p values (Bongers and Bongers, 1998; Ferris et al., 2001).

tolerant wheat and decreased in ozone-sensitive wheat following elevated [O_3] (Table 2).

Nematode ecological indices were sensitive to wheat cultivar effect. At wheat jointing stage, the values of dominance were higher in ozone-tolerant wheat than in ozone-sensitive wheat under both ambient and elevated [O_3] conditions (Table 3). At wheat ripening stage, soil nematode diversity was higher and dominance was lower in ozone-tolerant wheat than in ozone-sensitive wheat under ambient condition. In addition, higher CI and lower EI were found in ozone-sensitive wheat than in ozone-tolerant wheat. Significant interactive effects between treatments and wheat cultivars were observed in the dominance, Shannon–Wiener and evenness indices at wheat ripening stage ($P < 0.05$). Following elevated [O_3], the values of dominance decreased and those of Shannon–Wiener and evenness indices increased in ozone-sensitive wheat, while opposite patterns were observed in ozone-tolerant wheat.

3.4. Correlations between soil biota and soil physicochemical parameters

The CCA analysis suggested that soil moisture was the most important parameter which contributed to the distribution of soil biota, and then was soil pH, elevated [O_3], SOC, total N and wheat cultivar (Fig. 4). The eigenvalues for the first and second axis were 0.013 and 0.002, respectively. The first axis explained 76.1% of the species–environment relations, and the two axes explained 90.5% of the species–environment relations.

4. Discussion

Soil processes, such as organic matter decomposition and nutrient cycling are driven by soil organisms and their interactions with plants and soil abiotic conditions (Wardle, 2002). Two primary ways that ozone alters carbon flux to soil are through altered

Table 3
Nematode ecological indices in ozone-sensitive (Y19) and ozone-tolerant (Y16) wheat cultivars under ambient (A-O_3) and elevated ozone (E-O_3) conditions during wheat growing season (mean ± SE).

Indices	Growing Season	Y19		Y16		Treatment (*T*)	Cultivars (*C*)	*T* × *C*
		A-O_3	E-O_3	A-O_3	E-O_3			
λ	Jointing	0.11 ± 0.02	0.11 ± 0.01	0.15 ± 0.02	0.12 ± 0.01	ns	0.033	ns
	Ripening	0.23 ± 0.03	0.15 ± 0.01	0.14 ± 0.00	0.15 ± 0.00	ns	0.029	0.029
H′	Jointing	2.46 ± 0.25	2.44 ± 0.18	2.21 ± 0.12	2.32 ± 0.11	ns	ns	ns
	Ripening	1.95 ± 0.15	2.23 ± 0.07	2.38 ± 0.05	2.23 ± 0.03	ns	0.038	0.038
J	Jointing	0.87 ± 0.01	0.87 ± 0.02	0.84 ± 0.05	0.91 ± 0.01	ns	ns	ns
	Ripening	0.73 ± 0.01	0.81 ± 0.02	0.80 ± 0.01	0.79 ± 0.01	ns	ns	0.019
CI	Jointing	25.71 ± 10.13	24.44 ± 8.10	41.96 ± 29.18	33.49 ± 6.59	ns	ns	ns
	Ripening	85.71 ± 8.25	100.00 ± 0.00	80.82 ± 12.72	84.18 ± 0.46	ns	0.014	ns
SI	Jointing	50.24 ± 18.23	41.41 ± 23.87	55.82 ± 11.82	39.49 ± 15.09	ns	ns	ns
	Ripening	39.59 ± 5.21	44.80 ± 13.39	40.16 ± 7.41	28.92 ± 5.57	ns	ns	ns
EI	Jointing	61.68 ± 10.29	57.32 ± 10.61	50.94 ± 19.28	53.42 ± 4.61	ns	ns	ns
	Ripening	21.36 ± 3.63	30.06 ± 4.25	31.14 ± 1.52	35.72 ± 4.98	ns	0.049	ns

Note: λ, dominance index; H′, Shannon–Wiener diversity index; J, Evenness index; CI, channel ratio; SI, structure index; EI, enrichment index.

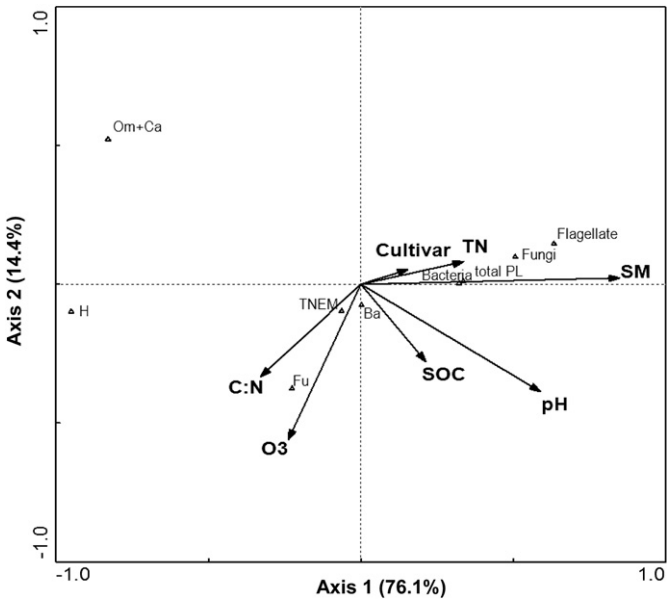

Fig. 4. Correspondence analysis between soil biota and environmental parameters (SM, soil moisture; SOC, soil organic carbon; TN, total nitrogen; C:N, the ratio of soil organic carbon to total nitrogen; TNEM, total nematodes; Fu, fungivores; Ba, bacterivores; H, plant parasites; Om-Ca, omnivore-carnivores; total PL, total PLFA; fungi, fungal PLFA; bacteria, bacterial PLFA; Flagella, Flagellates; O_3, treatment effect; cultivar, cultivar effect).

rhizodeposition and changes in litter quality or quantity (Andersen, 2003). Soil organisms are responsible for recycling nutrients and for maintenance of soil properties which may be affected by altered allocation patterns in plants exposed to ozone (Andersen, 2003). The PLFA analysis suggested that fungal PLFA and the fungi to bacteria ratio decreased following elevated [O_3], especially in the rhizospheric soil of ozone-tolerant wheat. Our results support the findings of Kasurinen et al. (2005) that ozone decreased the abundance of fungal PLFA. Kanerva et al. (2008) also found that ozone exposure reduced total, bacterial, and fungal PLFAs biomasses as well as the F/B ratio in the mesocosm bulk soil. The findings of Phillips et al. (2002) suggested that the fungi are possibly more responsive in the rhizosphere of ozone-stressed plants than bacteria. The decrease in fungal PLFA biomass and the F/B ratio following elevated [O_3] may be linked to a slowly changing substrate quality (Kandeler et al., 2008). Since fungi are regarded as the main decomposers of recalcitrant litter and fungal cell walls decompose more slowly than bacterial cell walls (Paul and Clark, 1996; Six et al., 2006), shifts in fungal communities might have a potential influence on soil functioning.

While changes in nematode functional guilds did not correspond with the microbial community, the abundance of Ba4 decreased and those of Fu4 increased following elevated [O_3]. Bacterivores and fungivores belonging to cp-4 guilds are persisters characterized by a long generation time and sensitive to disturbance (Bongers and Bongers, 1998); changes in their abundance indicated the variations in the structure of soil food webs. In general, lower trophic level organisms in soil food webs are controlled by top-down forces, whereas higher trophic level organisms are controlled by bottom-up forces (De Ruiter et al., 1995). Existing information of ozone effects on belowground processes mainly focused on soil microbiological aspects. Soil fauna mainly depend on the soil microbial composition for nutritional reasons and microfaunal grazing on primary decomposers may exert a major effect on the energy and nutrient flow through the soil system (Niwa et al., 2011). Changes in microbial community composition together with soil fauna lead to the suggestion that functional aspects of soil food webs might be indirectly changed through the effects of ozone on the plant (Schrader et al., 2009).

Different plant species could affect the bacterivores or fungivores indirectly through altered inputs of root exudates and crop residue, which firstly influence the soil microbial community and then the microbivorous nematodes (Wardle et al., 2004). These may help to explain the relatively higher microbial PLFA biomasses and the abundances of flagellates and nematode functional guilds (Ba_1 and H_2) in the ozone-tolerant wheat. Since root-feeding nematodes may be host specific (Van der Putten and van der Stoel, 1998), a change in plant species will also alter nematode composition in this feeding group (Yeates and Bongers, 1999).

Following elevated [O_3], different plant species may discharge different root exudates, resulting in plant-specific microbial communities (Johnson et al., 2003; Marschner et al., 2004). The PCA analysis of PLFA suggested that soil microbial community structure was more sensitive to elevated [O_3] in ozone-tolerant wheat than in ozone-sensitive wheat. Our results were in contrast to our hypothesis, that greater effects of elevated ozone on soil biota were observed in the ozone-sensitive wheat. Hofstra et al. (1981) concluded that belowground processes may be changed by elevated ozone before symptoms on plants become detectable. Since plant response to ozone is affected by nutrient availability (Whitfield et al., 1998; Utriainen and Holopainen, 2001), changes in nutrient cycling and plant availability may lead to feedback effects on plant sensitivity to ozone (Andersen, 2003).

Following elevated [O_3], the abundance of flagellates and nematode diversity and evenness indices decreased in ozone-tolerant wheat and increased in ozone-sensitive wheat. While an opposite trend was observed in the abundance of Ba_1 guilds and nematode dominance index. Understanding the effect of ozone on belowground processes is complicated due to the trophic complexity associated with soil food webs and our limited knowledge about the processes controlling resource acquisition and use in both individual plants and in ecosystems (Andersen, 2003). De Deyn et al. (2007) found that nematode community composition responded differently to specific plants, and the effects of plant species identity were most pronounced in trophic levels intimately interacting with the plants (plant feeders and microbivores) (De Deyn et al., 2004). Previous studies also suggest the important effects of plant species identity on multiple trophic levels in the soil food webs and ecosystem processes (Wardle et al., 2003). In a grazing experiment, Veen et al. (2010) found that plant community composition was the main determinant of nematode community composition relative to soil abiotic properties. In an open-top chamber experiment, Manninen et al. (2010) also found that the minor changes in the microbial biomass of rhizospheric soils were plant-mediated via quantity and quality of rhizodeposits (Marschner et al., 2001; Wiemken et al., 2001). Our findings also suggested that crop cultivars were important in determining the responses of soil biota to elevated ozone. Plant species effects on the response of detrital food webs can in turn feed back to the plant community (Bardgett and Chan, 1999).

Long-term ecosystem responses and subsequent feedbacks to climate change may depend on how the soil system responds to these perturbations. The CCA analysis indicated that the responses of soil food web to elevated [O_3] were also associated with certain abiotic factors, such as soil moisture, soil pH, carbon and nitrogen contents. Ozone may alter nutrient cycling in soils by altering litter quality and quantity and by altering rates of energy flow through soil food webs (Andersen, 2003).

In conclusion, elevated [O_3] affects various components in the soil microbial food web. The crop cultivars play an important role in determining the responses of soil food web exposure to ozone. The responses of soil biota were more sensitive to elevated [O_3] in

ozone-tolerant wheat than in ozone-sensitive wheat, which may in turn influence the decomposition processes and nutrient turnover in agroecosystems.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2011.12.012.

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