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The interactive effects of elevated ozone and wheat cultivars on soil microbial community composition and metabolic diversity

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ABSTRACT

Human-induced global changes have important impacts on terrestrial ecosystems. Although aboveground influences of elevated ozone have been widely studied, relatively little attention has been paid to the belowground subsystem, nevertheless it is critical to study belowground effects in determining the long-term consequences of ozone exposure to ecosystems. Here, we investigated the effects of elevated ozone on soil microbial community structure and functional diversity using the free-air ozone enrichment platform (FAOE). We detected that soil fungal phospholipid fatty acid and the fungal/bacterial ratio were significantly ower under elevated ozone than under ambient ozone at the wheat ripening stage. Through determining soil microbial metabolic diversity as evaluated by variations in the microbial utilization rates of different carbon sources among different wheat cultivars, we found that soil microbial communities inhabiting the rhizosphere of ozone-tolerant cultivars preferred to consume easily degradable carbon sources, while more complex carbon sources were preferably utilized by those associated with ozone-sensitive cultivars. These changes may in turn promote (ozone-tolerant wheat cultivars)/inhibit (ozone-sensitive wheat cultivars) plant growth through alterations in nutrient availability and resource distribution.

1. Introduction

Tropospheric ozone [O_3] concentration has risen from about 10 ppb to current 40 ppb in the Northern Hemisphere due to anthropogenic emissions (Biswas et al., 2008), which is recognized as one of the most important phytotoxic air pollutants and poses a great threat to crop plants (Avnery et al., 2013; Booker et al., 2009; Feng and Kobayashi, 2009; Mills et al., 2011). Although responses of the aboveground subsystem to elevated [O_3] have been widely studied, relatively little attention has been paid to the direct and indirect effects of elevated [O_3] on the belowground subsystem. Microbes play a vital role in biogeochemical cycling (Malik and Grohmann, 2011), and the effects of elevated [O_3] on the belowground may in fact be more critical than aboveground effects in determining the long-term consequences of [O_3] exposure to ecosystems.

Studies have reported that elevated [O_3] can influence soil biotic and abiotic conditions via the changes in timing and quantity of carbon input to the soil (Andersen, 2003). McCrady and Andersen (2000) observed that [O_3] exposure increased root exudation in spring wheat seedlings, leading to an increase in substrates available to soil microbes. Several studies found negative effects of elevated [O_3] on soil microbial biomass and community structure (Kanerva et al., 2008; Li et al., 2012; Manninen et al., 2010). However, there are few available reports on the responses of soil microbial functional diversity or microbial metabolic activity. Since microbial community structure and functioning in cropping system shed insights into the changes in crop physiology and plant nutrient dynamics in a changing climate, it is important to examine the elevated [O_3] effects on the microbial community structure and metabolic potentials of rhizosphere microbial communities.

Previous findings from free-air ozone enrichment (FAOE) and open-top chamber (OTC) experiments suggest that there is genotypic variability in [O_3] sensitivity of wheat plants (Biswas et al., 2008; Feng et al., 2008, 2012; Sarkar and Agrawal, 2010; Zhu et al., 2011). Cao et al. (2009) reported that the ozone-sensitive wheat showed larger reductions in photosynthetic rate, stomatal conductance and transpiration rate than the ozone-tolerant wheat.

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Zhu et al. (2011), in the same experiment, reported that the decrease in individual grain mass was significantly larger in the ozone-sensitive wheat (22–25%) than those in the ozone-tolerant wheat (14–16%). In the belowground subsystem, Li et al. (2012) found that soil biota in ozone-tolerant wheat were more sensitive to elevated [O_3] than those in ozone-sensitive wheat. All studies suggest that effects of elevated [O_3] on crop physiological characters, yield components and soil biota might depend on the ozone-tolerance of wheat cultivars, but hitherto, there have been no quantitative studies about the effects of elevated [O_3] on the overall microbial community structure and microbial catabolic activity traits of wheat cultivars with different ozone-tolerance, which is critical in determining the long-term influence of [O_3] exposure on belowground ecosystem.

The objectives of this study were to explore the effects of elevated [O_3] on soil microbial communities and to determine if the changes in microbial community structure result in variations in metabolic diversity. We hypothesized that: (1) elevated [O_3] would alter the microbial community structure and consequently be reflected in the physiological responses of soil microbial communities to carbon substrate supply; (2) although overall, ozone will negatively affect plant growth, the level of the above-mentioned responses in soil microbial communities will exhibit cultivar dependence.

2. Materials and methods

2.1. Field site and experimental design

The study site is located in a suburb of Jiangdu city in Jiangsu province of China (32°35′N, 119°42′E). The soil is a Shajiang Aquic Cambosols (Chinese Soil Taxonomy) with a sandy–loamy texture, with 15 g kg^{-1} total C, 1.5 g kg^{-1} total N, pH 6.8, 25.1% clay (<0.001 mm) and bulk density 1.2 g cm^{-3} at 0–15 cm depth (Zhu et al., 2011). This region has temperate climate with an average annual temperature of 14.9 °C and an annual precipitation at 980 mm (Li et al., 2009). Free-air ozone enrichment (FAOE) facility was established in 2007 over a rice–wheat rotation system. Rice was 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 into the soil. No additional organic matter was incorporated into the soil during the wheat growing season. The experiment was conducted during the wheat growing season of 2012, after exposure to elevated [O_3] for 4 years (from March 5 to May 27 exposure to elevated [O_3], about 83 days during each year). Three replicate FAOE rings, each with 14.5 m in diameter, were set within a uniform area of 4 ha to continuously provide an elevated [O_3] concentration of 60 ppb over the ambient conditions (about 40 ppb) from 9:00 am to 18:00 pm. While three replicate rings, each with the same size, were set within the same area for the ambient [O_3] treatment. Each ring was separated from others by about 70 m to prevent [O_3] from spilling over from one ring to another. The experimental design was based on completely randomized plots allocated to either ambient [O_3] or elevated [O_3], and split into subplots consisting of different wheat cultivars (Tang et al., 2011; Zhu et al., 2011).

We used four modern cultivars of winter wheat (*Triticum aestivum* L.): Yangfumai 2 (Y2), Yannong 19 (Y19), Yangmai 15 (Y15) and Yangmai 16 (Y16). The former two are ozone-sensitive wheat cultivars, and the latter two are ozone-tolerant wheat cultivars (Zhu et al., 2011). Seeds were hand sown with a basic seeding density of 2.25 million ha^{-1} and a row spacing of 25 cm. 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%), and side-dressings at early tillering (10%) and elongation stages (30%). Phosphorus (P) and potassium (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 the elongation stage, respectively (Zhu et al., 2011). Soil samples were collected from 0 to 15 cm depth at the jointing stage (April 9, 2012) and ripening stage (June 7, 2012). There were total of 24 soil samples (2 [O_3] level × 4 wheat cultivars × 3 replicates). Each soil sample was pooled from five soil cores (2.5 cm diameter). The soil core was placed near a plant within a plant row to be sure that the rhizosphere was sampled. Field moist soils were sieved <2 mm and visible pieces of plant material and soil macroorganisms were removed. Soil samples (about 30 g) were immediately frozen at −20 °C and dried prior to PLFA analysis; the remaining soil samples were stored at 4 °C for physiochemical charateristics and soil microbial metabolism analysis.

2.2. Soil and plant analyses

Soil moisture (SM) was determined as gravimetric method after drying soil at 105 °C for 24 h. Soil pH was determined with a glass electrode in 1:2.5 soil:water solution (w/v). Dissolved organic carbon (DOC) was extracted with water (soil:water = 1:2) for 0.5 h, and then determined by a Multi N/C 3100 analyzer (Jena Corporation, Germany). Soil microbial biomass was determined using the fumigation–extraction method (Vance et al., 1987). To destroy the cell membranes of soil microorganisms, 10 g of the moist soil were fumigated with alcohol-free chloroform at 25 °C for 48 h (the unfumigated soil was used as the control). The fumigated and non-fumigated samples were extracted with 30 ml 0.5 M K_2SO_4 and shaken for 1 h on a reciprocal shaker. The extracts were filtered, and soil microbial biomass C (MBC) and N (MBN) in the filtrate were determined using a TOC analyser (Multi C/N 3000, Analytik Jena, Germany).

Wheat plants were clipped from a 15 × 15 cm^2 patch in the middle of each subplot. Plant samples were partitioned into shoot and root biomass. Root biomass at 0–15 cm depth was measured by soil auger (8 cm in diameter). The soil was carefully removed from the root system and the roots were thoroughly rinsed. The dry masses of shoot and root samples were determined by over-drying at 65 °C until a constant weight.

2.3. Phospholipid fatty acid analysis (PLFA)

The soil microbial community was characterized using phospholipid fatty acids (PLFAs) analysis as described by Bligh and Dyer (1959). Specific modifications and GC conditions and nomenclature were as described by Certini et al. (2004). Briefly, lipids were extracted with 8 g of freeze-dried soil samples using a chloroform–methanol–citrate buffer mixture (1:2:0.8). The polar phospholipids were separated from neutral lipids and glycolipids on a solid phase extraction (SPE) columns (Supelco Inc., Bellefonte, PA). The phospholipids were trans-esterified to a mild alkaline methanolysis (Bossio et al., 1998) and the resulting fatty acid methyl esters were extracted in hexane and dried under N_2. Samples were then dissolved in hexane and analysed in an Agilent 6850 series Gas Chromatograph with MIDI peak identification software (version 4.5; MIDI Inc., Newark, DE). Calculations of quantitative concentrations (nmol PLFA g^{-1} dry weight soil) of single PLFAs were carried out as described in Palojärvi (2006).

The following biomarkers were used: gram-positive bacteria (i15:0, a15:0, i16:0, i17:1G, i17:0 and a17:0), gram-negative bacteria (16:1ω7c, cy17:0, 18:1ω8c/t, 19:1 (ω8) alcohol and cy19:0). The sum of gram-positive bacteria, gram-negative bacteria and non-specific bacteria (16:0, 18:0) was used as measure of bacterial biomass. The fatty acid 18:2ω6c was used as an indicator