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Elevated ozone effects on soil nitrogen cycling differ among wheat cultivars

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ABSTRACT

Elevated O_3 (eO_3) has strong effects on natural and managed ecosystems, including decreased plant growth, plant tissue quality, species richness, plant litter inputs, decomposition, and root turnover. However, the effects of eO_3 on soil nitrogen (N) cycling are poorly understood. Here, a free -air O_3 enrichment experiment was conducted from 2007 to 2012, in which two O_3-tolerant wheat cultivars and two O_3-sensitive cultivars were grown under ambient O_3 (aO_3, 40 ppb) and eO_3 (60 ppb). We used a ^{15}N pool dilution technique to investigate N transformation rates and N availability in the soils in 2012. Both gross and net N transformation rates were significantly decreased ($P < 0.05$) by eO_3 in soils growing sensitive wheat cultivars, but were unchanged in soils growing tolerant cultivars. Compared with aO_3, NH_4^+ and NO_3^- concentrations were significantly increased ($P < 0.05$) by eO_3 in soils growing sensitive cultivars but not in soils growing tolerant cultivars. Ammonia monooxygenase activity was significantly increased by eO_3 while nitrate reductase activity was significantly decreased. Additionally, redundancy analysis (RDA) suggested that microbial community structure was largely shaped by soil and plant characteristics such as DOC, root C/N ratio, MBC/MBN, shoot/root ratio, root N, soil N and pH. In conclusion, wheat cultivars play an important role in determining the effects of elevated O_3 on N transformations. This study provides new insights into our understanding of how changes in microbial diversity and metabolism as mediated by plants will alter N cycling and ecosystem N availability in response to eO_3 and suggests that these effects will differ among different plants.

1. Introduction

The concentrations of ozone (O_3) in the troposphere have been rising from about 10 ppb during pre-industrial times to 25–40 ppb today, due to rapid industrial development and anthropogenic activities, and are predicted to increase by 40–70% by the year 2100 (Dentener et al., 2006). Ozone is an extremely phytotoxic pollutant that could decrease plant growth, yield, quality and cause loss of some species (Ainsworth, 2008; Feng et al., 2008) by suppressing photosynthesis, inducing foliar damage and accelerating leaf senescence (Dermody et al., 2006). Elevated O_3 may significantly change plant litter inputs, decomposition, root turnover and carbon exudation and alter belowground microbial ecological processes, leading to altered ecosystem C and N cycling rates (Wittig et al., 2009; Betzelberger et al., 2010; Simpson et al., 2014). However, little is known about the effects of elevated O_3 on soil N dynamics, and most previous studies have focused on forest species (Holmes et al., 2003, 2006).

Global economic losses induced by elevated O_3 on four major crops (wheat, soybean, rice and maize) were estimated to range from 14 to 26 billion US dollars on the basis of world market prices for the year 2000, and about 40% of this damage occurred in China and India (Van Dingenen et al., 2009). By 2030, total global agricultural losses (soybean, maize, and wheat) are estimated to reach 17–35 billion US dollars (Avnery et al., 2011). Wheat is one of the staple food crops in the world supporting nearly two-thirds of the world population, with an annual production of more than 650 million metric tons and a harvested area of over 200 million

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hectares worldwide (FAO, 2008). Elevated O_3 significantly decreased wheat growth and yield (Pleijel et al., 2006; Sarkar and Agrawal, 2010; Feng et al., 2011; Zhu et al., 2011). Wheat grain yield loss induced by elevated O_3 lies in the range of 18–29% according to a meta-analysis of 53 published studies between 1980 and 2007 (Feng et al., 2008). Elevated O_3 has been shown to decrease photosynthesis, stomatal conductance, transpiration, and dry matter production (Andersen, 2003; Biswas et al., 2008; Zhu et al., 2011) in O_3-sensitive species and cultivars, causing reduced plant litter input and C allocation to roots (Bortier et al., 2000).

Previous studies showed that O_3-induced changes in aboveground and belowground productivity, plant litter inputs and decomposition, root turnover and carbon exudation impacted belowground microbial ecological processes and N cycling rates significantly (Wittig et al., 2009). Therefore, eO_3 should alter N cycling. However, there are limited studies on the effects of eO_3 on N cycling. It was reported that eO_3 had significant effects on soil microbial community structure (Kanerva et al., 2008; Li et al., 2013), and function (Zak et al., 2007). For example, eO_3 has been found to diminish cellobiohydrolase activity and N acetylglucosaminidase activity in soils (Phillips et al., 2002) because of less fine root input. Therefore, soil microbial community structure and function may be altered by the influence of eO_3 on plant litter production and root exudates, which provide substrates for microbial decomposition (Andersen, 2003; Li et al., 2012, 2013). Elevated O_3-induced decreases in substrate availability may also affect soil N cycling, but there are only a few studies about eO_3 effects on microbial mediated N cycling in soil (Holmes et al., 2003; Holmes et al., 2006; Kanerva et al., 2006; Pereira et al., 2011; Decock and Six, 2012). Those studies indicated that eO_3 had minor or negative effects on soil N cycling with different plants/ecosystems. However, most of those studies focused on net N transformation, and only one study (Holmes et al., 2003) examined gross N transformation rates in a forest ecosystem. Gross N turnover rates are frequently an order of magnitude greater than net N turnover rates and relationships between net and gross N turnover are often lacking (Verchot et al., 2001; Wu et al., 2012b). In order to understand how eO_3 impacts N cycling through changes in plant substrate availability, it is important to examine gross N transformation rates mediated by soil microbial community composition and structure.

Elevated O_3 effects on soil N dynamics may vary by species and cultivars because of differences in their susceptibility to O_3 damage. The effects of elevated O_3 on belowground processes are likely to be regulated indirectly through altered plant responses, substrate input and resource allocation (Andersen, 2003; Paterson et al., 1997). Previous studies showed that the response of plant characteristics to O_3 varied by species or cultivars (Biswas et al., 2008; Feng et al., 2008; Cao et al., 2009; Zhu et al., 2011). Therefore, plant species or cultivars may have important effects on soil microbial activities and the ecological processes that they drive (McCrady and Andersen, 2000; Wardle et al., 2004). In previous studies we showed that elevated O_3 decreased wheat grain yield, individual grain mass and litter input (Zhu et al., 2011), and altered soil microbial food webs and microbial community structure using Free Air Concentration Enrichment (FACE) technology (Li et al., 2012). However, the magnitude of these responses varied between O_3-sensitive and O_3-tolerant cultivars. All of these changes in microbial community structure, functions and activities might strongly impact soil organic matter decomposition and nutrient cycling.

In this study, our main objective was to investigate how eO_3 alters soil N transformations. We predicted that eO_3 would decrease soil N transformation rates in soils grown with O_3-sensitive cultivars, but show no significant effects in soils grown with O_3-tolerant cultivars. To test this prediction, we examined the effect of eO_3 on soil gross N transformation, net N transformation, inorganic N pools and microbial community structure in soils grown with O_3-sensitive and O_3-tolerant cultivars.

2. Materials and methods

2.1. Study site and experiment description

Our study was conducted in the O_3-FACE experiment, located near the town of Jiangdu, Jiangsu province, China (119°42′E, 32°35′N). The soil is classified as a sandy-loamy, Shajiang Aquic Cambosols (Chinese Soil Taxonomy) with 15 g kg^{-1} total C, 1.59 g kg^{-1} total N, pH 6.8 and a bulk density of 1.2 g cm^{-3} at 0–15 cm depth (Zhu et al., 2011). The site is in a subtropical marine climatic zone with total annual sunshine hours of >2000 h, and a frost-free period of >230 days. Mean annual precipitation is 1100–1200 mm and mean air temperature is 16 °C. More information can be found in Zhu et al. (2011) and Li et al. (2012).

The FACE ecosystem was set up in 2007 and has been maintained since then, with winter wheat sown in early November and harvested in late May or early June of the next year. The experimental design was based on completely randomized plots allocated to either aO_3 (40 ppb) or eO_3 (60 ppb) with 3 replicates, and split into subplots of wheat cultivars (treatment plots). In brief, three replicate rings (14.5 m in diameter) with elevated ozone (60 ppb from 9:00 am to 18:00 pm), were installed randomly within a uniform area of 4 ha. Three other rings of the same size were supplied with ambient air (about 40 ppb). The rings were located at 70 m distance from each other to prevent ozone spilling from one ring to another. There are four wheat cultivars, two of them are sensitive to O_3 (Yangfumai 2: Y2; Yannong 19: Y19) and two of them are tolerant to O_3 (Yangmai 15: Y15; Yangmai 16: Y16) (Cao et al., 2009; Bao et al., 2015). All cultivars were planted every year between 2007 and 2012. Seeds were hand-sown with a seeding density of ~2.25 million seeds ha^{-1} and a row space of 25 cm. Nitrogen was applied as urea (N = 46%) and di-ammonium phosphate at a total rate of 210 kg N ha^{-1}. Phosphorus (P) and potassium (K) were applied as di-ammonium phosphate and potassium chloride, respectively, at a rate of 90 kg P_2O_5 ha^{-1} and 90 kg K_2O ha^{-1}, respectively (Zhu et al., 2011). No pesticide was applied. On the basis of the wind direction and wind speed, O_3 was released achieving the elevated concentration of 60 ppb within 15% of the set point for 90% of the time, and within 20% of the set point for 95% of the time. See more information in Zhu et al. (2011).

2.2. Soil and plant analyses

Five 10-cm-depth soil cores were collected from each treatment plot at the jointing stage (April 8 in 2012) using a 2.5-cm-diameter auger and then mixed. Soil samples were sieved (2 mm mesh width) to remove organic debris and rocks and to homogenize the samples. The total carbon (TC) and nitrogen (TN) of plants and soils were determined by a TruSpec CN Elemental Analyzer (Leco Corporation, St. Joseph, MI, USA). Soil moisture was determined by drying the soil sample at 105 °C for 24 h. Soil pH was determined with a glass electrode in a 1:2.5 (soil:water) solution (w/v). Microbial biomass C and N was estimated using the chloroform fumigation-extraction (FE) method as described in detail by Wu et al. (2011). For the application of the FE method, soil samples from single treatment plots were divided into paired subsamples of 10 g. One subsample was immediately extracted with 30 ml 0.5 M K_2SO_4 for 60 min on a rotary shaker at 150 rpm. The second sample was fumigated under chloroform vapor for 24 h in a desiccator and then extracted as described above. Extracts were frozen under −20 °C and total organic carbon and total chemically bound nitrogen were analyzed using a Multi N/C 3100 analyzer

(Jena Corporation, Germany). Dissolved organic carbon (DOC) was also determined using the above method. Fifteen winter wheat plants from each treatment plot were harvested and separated into shoots and roots. The shoots and roots were dried at 65 °C until a constant weight was obtained, and then weighed. We only measured plant and soil variables of Y2 and Y15 to represent sensitive and tolerant wheat cultivars respectively.

2.3. Gross ammonification and nitrification

Gross ammonification and nitrification were determined using the ^{15}N pool dilution technique described previously by Wu et al. (2012a). Briefly, 100 g of each soil sample was homogeneously labeled immediately after sieving with 3 ml $(^{15}NH_4)_2SO_4$ or $K^{15}NO_3$ solution at 30 atom% ^{15}N enrichment (1 mg N kg^{-1} soil). Then, two 30 g subsamples of fresh soil were separately placed into parafilm-sealed 250 ml plastic bottles and buried in-situ close to the sampling location after labeling. Eighteen hours (time 1) after labeling, 60 ml 1 M KCl solution was added into half of the bottles to extract the NH_4^+ and NO_3^-, with the remaining set of bottles extracted after 42 h (time 2). The remaining 40 g soil was dried at 105 °C to determine the soil water content after labeling. Total NH_4^+ and NO_3^- concentrations in the extracts were determined using a flow injection autoanalyzer (FIAstar 5000 Analyzer, Foss Tecator, Denmark). Diffusion protocols for trapping on acid filter traps and subsequent GC-IRMS analyses for ^{15}N-enrichment were performed as described in Dannenmann et al. (2009). Calculations of gross ammonification and nitrification followed the equations given by Kirkham and Bartholomew (1954).

2.4. Net ammonification, nitrification and mineral nitrogen

Net ammonification and nitrification were determined after soil incubation at 25 ± 0.5 °C for two weeks. Thirty g soil was extracted immediately with 1 M KCl (soil to solution ratio 1:2) for mineral N concentrations. After two weeks, the incubated soil was harvested for the second extraction as described above. The NH_4^+-N and NO_3^--N concentrations from the first harvesting date are referred to in-situ soil ammonium and nitrate concentrations. Net ammonification and nitrification were calculated as the difference in ammonium and nitrate (including nitrite) concentrations between the two sampling dates divided by the incubation time (Wu et al., 2012b).

2.5. Ammonia oxidation and nitrate reductase activity

Ammonia oxidation activity was determined by spectrophotometric measurement of soil NO_2^- after soil samples were treated with $(NH_4)_2SO_4$ at 25 °C for 5 h. Two M KCl was used as the extracting solution and the colorimetric assay (2,4-Dinitrophenol) was conducted at 520 nm (Hart et al., 1994). Nitrate reductase activity was determined by the spectrophotometric measurement of NO_2^- after the soil samples were treated with KNO_3 as substrate at 25 °C for 24 h. Four M KCl was used as the extracting solution and the colorimetric assay was conducted at 520 nm (Abdelmagid and Tabatabai, 1987).

2.6. Microbial community structure

The soil microbial community was characterized using phospholipid fatty acids (PLFAs) analysis as described by Bligh and Dyer (1959) with slight modifications. Lipids were extracted from 8 g of lyophilised soil in a chloroform–methanol–phosphate buffer mixture (1:2:0.8), and the phospholipids were separated from neutral lipids and glycolipids on a SPE tube (Supelco Inc., Bellefonte, PA). The phospholipids were subjected to mild-alkaline methanolysis and the samples were extracted in hexane and dried under N_2. The resulting fatty acid methyl esters were dissolved in hexane and separated with an Agilent 6850 gas chromatograph using the MIDI eukaryotic method of Sherlock software (Version 4.5; MIDI Inc., Newark, DE, USA). 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 a measure of bacterial biomass. The fatty acids 18:2ω6 and 18:1ω9c were used as an indicator of fungal biomass (Bååth and Anderson, 2003). Other PLFAs such as 16: 1ω5c representing AM fungi, the ratio of 18:2ω6 and 18:1ω9c to total bacterial PLFAs representing the fungal:bacterial ratio (F:B), the cy17:0 to 16:1ω7c ratio representing the ratio of cyclopropyl fatty acids and monoenoic precursors (cyc:prec) 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 RDA analysis. Taken together, all of the PLFAs indicated above were considered to be representative of the total PLFAs of soil microbial community. Each PLFA and the sum of all PLFAs were expressed as nmol PLFA g^{-1} dry soil.

2.7. Statistics and calculations

The data were analyzed with a general linear model in a split-plot design to determine the effects of O_3 concentration (ambient vs. elevated), different wheat cultivars (Y2, Y19, Y15, Y16) and their interactions with ozone and cultivars as fixed factors and replicate as random factor. Means between treatments were compared using Tukey's HSD test. The response ratio of PLFA was calculated using the following equation: $(eO_3 - aO_3)^*100/aO_3$, where aO_3 and eO_3 were the average value of PLFA detected at aO_3 and eO_3. Their difference was tested by ANOVA. All the above statistical analyses were performed by SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Redundancy analysis (RDA) was performed to determine how microbial community structure based on PLFA data was related to microbial function variables such as N flux and enzyme activities using Canoco v4.5 (Biometris – Plant Research International, Wageningen, The Netherlands).

3. Results

Ozone significantly decreased gross ammonification ($P = 0.038$ and 0.007 for Y2 and Y19 respectively; Fig. 1A), gross nitrification ($P = 0.013$ and 0.044; Fig. 1B), net nitrification ($P = 0.026$ and 0.028; Fig. 1D) and increased NH_4^+ concentration ($P = 0.047$ and 0.030; Fig. 1E), NO_3^- concentration ($P = 0.041$ and 0.045; Fig. 1F) in O_3 sensitive cultivars (Y2, Y19), while no significant changes were observed in O_3 tolerant cultivars (Y15, Y16). Ozone also increased (less negative) net ammonification ($P = 0.031$; Fig. 1C) in Y2 but not in other cultivars. Ammonia monooxygenase activity significantly increased ($P = 0.010$; Fig. 1G) in Y19 and Y16, while nitrate reductase activity was generally lower in all wheat cultivars and effects were most pronounced in Y2 ($P = 0.001$), Y19 ($P < 0.001$), and Y15 ($P = 0.035$; Fig. 1H) under eO_3.

Ozone showed significant effects on soil microbial community structure ($P < 0.05$). However, we found a general difference between sensitive cultivars and tolerant cultivars, i.e., response ratios of microbial community to eO_3 were generally negative for sensitive cultivars while positive for tolerant cultivars (Table 1). Ozone decreased all microbial community factors estimated for sensitive cultivars with the exception of fungi for Y2. However, ozone increased most microbial community factors for tolerant cultivars with two exceptions for G^+ bacteria and branched chain

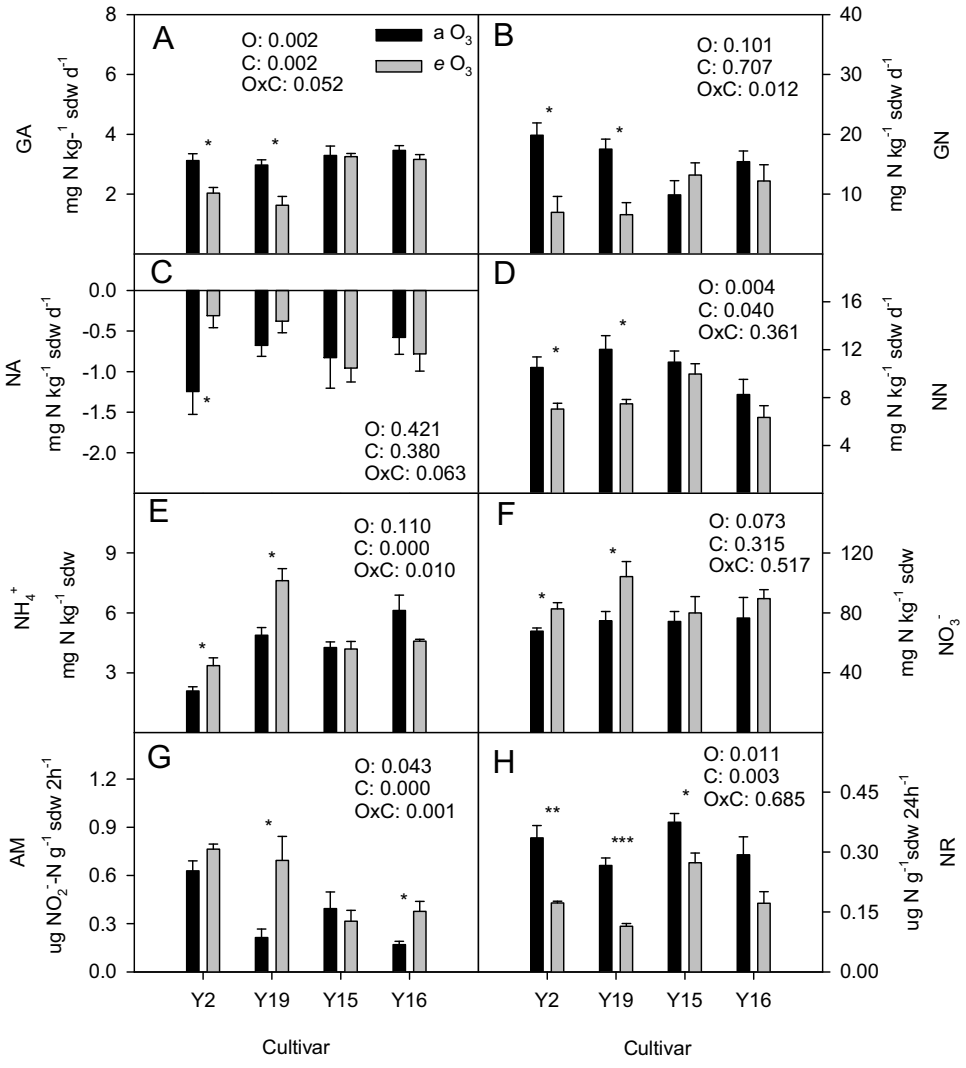

Fig. 1. Nitrogen turnover, inorganic N content, ammonia monooxygenase and nitrate reductase affected by elevated O_3 and wheat cultivars. (A) GA: gross ammonification; (B) GN: gross nitrification; (C) NA: net ammonification; (D) NN: net nitrification; (E) NH4+: ammonia concentration; (F) NO_3^-: nitrate concentration; (G) AM: ammonia monooxygenase and (H) NR: nitrate reductase. Y2 and Y19 are two O_3-sensitive cultivars, while Y15 and Y16 are two O_3-tolerant cultivars. P-values shown in the figure are based on split-plot ANOVA (O: O_3; C: cultivar; O × C: O_3 × cultivar). Error bars show standard error of the mean (n = 3). Asterisks denote significant differences between treatments, * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

Table 1
Effects of elevated O_3 and wheat cultivars on soil microbial community.

	RR_{SC}	RR_{TC}	*F*	*P* values
Total PLFA	−5.08 ± 1.93	2.42 ± 1.56	9.139	0.013
Bacteria	−5.22 ± 1.78	2.11 ± 1.64	9.181	0.013
Fungi	2.2 ± 3.72	10.34 ± 2.73	3.114	0.108
AMF	−9.48 ± 2.65	2.64 ± 2.82	9.805	0.011
G+	−4.4 ± 1.04	−1.13 ± 1.59	2.977	0.115
G-	−5.19 ± 2.81	4.76 ± 1.14	10.80	0.008
SATFA	−5.32 ± 1.63	1.22 ± 1.66	7.953	0.018
MUFA	−5.42 ± 2.55	3.14 ± 1.28	9.04	0.013
Branched	−5.19 ± 1.07	−1.02 ± 1.27	6.304	0.031

RR_{SC} is response ratio (RR) of sensitive cultivars, and RR_{TC} is RR of tolerant cultivars. RR was calculated using the following equation: $(eO_3 - aO_3)*100/aO_3$, where aO_3 and eO_3 were the average value of PLFA detected at aO_3 and eO_3. Response ratios are shown as mean ± standard error. *P* values are based on ANOVA. Y2 and Y19 were combined as sensitive cultivars, and Y15 and Y16 were combined as tolerant cultivars. Arbuscular mycorrhizal fungi, AMF; gram positive, G+; gram negative, G−; saturated fatty acid, SATFA; monounsaturated fatty acid, MUFA; branched chain fatty acid, Branched.

fatty acids (although they decreased less than those for sensitive cultivars).

We only measured plant and soil variables of Y2 and Y15 to represent sensitive and tolerant wheat cultivars, respectively. After 5 years of treatment, we observed that eO_3 significantly decreased DOC (Fig. 2A), MBN (Fig. 2C) and increased MBC/MBN ($P < 0.05$; Fig. 2D) in soils growing Y2 (sensitive cultivar), while eO_3 showed no significant effect on Y15 (tolerant cultivar).

RDA was used to further explore what environmental factors largely shaped the functional structure of microbial communities (Fig. 3). Overall, the first two axes of the RDA analysis explained most of the total variance (81.8% by the first axis and another 2.9% by the second axis, $P < 0.05$) by a Monte Carlo permutation test in Canoco. The results showed that DOC was the most important factor shaping microbial community structure, followed by root C/N ratio, MBC/MBN ratio, shoot/root ratio, root N, soil N and pH (Fig. 3).

The relationship between N transformation variables and microbial community structure as affected by eO_3 and cultivars, was also analyzed by RDA (Fig. 4). Overall, the first two axes of the

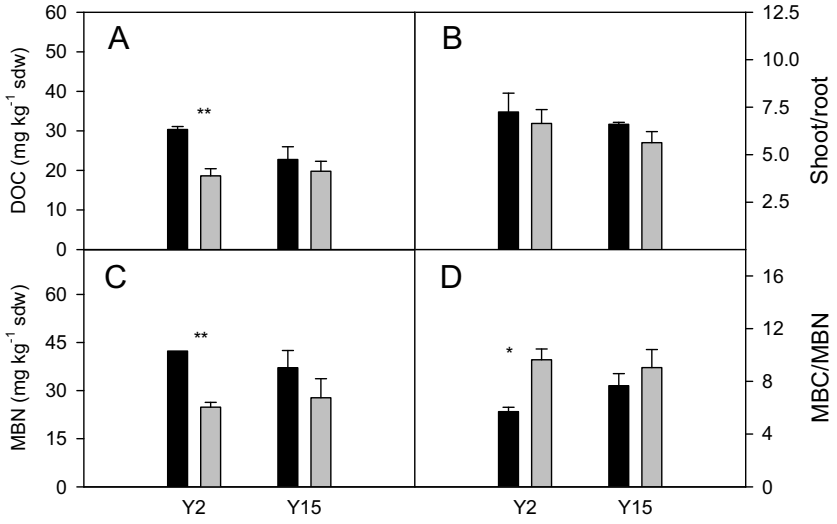

Fig. 2. Soil and plant properties under ambient (black bars) and elevated O_3 (gray bars) with Y2 (O_3-sensitive) and Y15 (O_3-tolerant) wheat cultivars. Error bars show standard error of the mean (n = 3). Asterisks denote significant differences between treatments, * ($P < 0.05$), ** ($P < 0.01$).

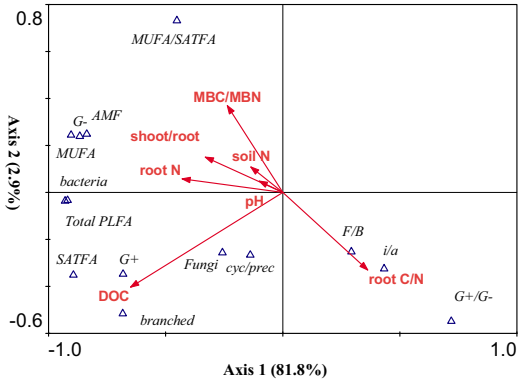

Fig. 3. Redundancy analysis (RDA) of soil microbial community structure and environmental variables, including DOC, root C/N ratio, MBC/MBN, shoot/root ratio, root N, soil N and pH for Y2 and Y15 under ambient and elevated O_3. Fungi/bacteria, F/B; iso to anteiso branching ratio, i/a; cy17:0–16:1ω7c ratio, cyc/prec. Arrow length indicates the importance of environmental variables on microbial communities.

RDA analysis explained most of the total variance (57.2% by the first axis and another 6.4% by the second axis, $P < 0.05$) and separated the microbial community structures of Y2, Y19 and Y15, Y16 samples in both aO_3 and eO_3 treatments. Microbial communities in eO_3 were negatively correlated with gross and net N transformations but positively correlated with inorganic N concentration and ammonia monooxygenase activity, while microbial communities in aO_3 displayed the opposite pattern, indicating that microbial communities from aO_3 plots had higher activity. Microbial communities under aO_3 and eO_3 of ozone tolerant cultivars (Y15, Y16) were closely clustered together indicating that eO_3 had little effect. On the other hand, microbial communities of O_3 sensitive cultivars (Y2, Y19) differed between aO_3 and eO_3 treatments indicating that N transformations of O_3 sensitive cultivars were largely reduced by eO_3.

4. Discussion

Understanding the effects of eO_3 on soil N cycling is important to predict soil N availability for sustainable agroecosystems under climate change. In this study, we investigated N cycling, plant and soil properties, and soil microbial community structure for four wheat cultivars under aO_3 and eO_3. We predicted that eO_3 would decrease soil microbial activity including N cycling due to lower crop productivity and belowground inputs (Zhu et al., 2011; Simpson et al., 2014). However, we predicted that the effects would depend on the sensitivity of wheat cultivars, with eO_3 only decreasing N cycling in sensitive wheat cultivars, but not in tolerant wheat cultivars.

Our results were consistent with our predictions, and showed that eO_3 significantly decreased N cycling in sensitive wheat cultivars (Y2, Y19) but had no effects on tolerant wheat cultivars (Y15, Y16). Specifically, our results showed that gross ammonification, gross nitrification and net nitrification were significantly decreased by eO_3 in Y2 and Y19 but not in Y15 and Y16. Net ammonification became less negative under eO_3 in Y2 and Y19, but not in Y15 and Y16, most likely because nitrification and ammonium immobilization decreased more than gross ammonification. Our results are consistent with the decreases in gross ammonification observed in forest (Holmes et al., 2006), meadow (Kanerva et al., 2007) and cropland ecosystems (Pereira et al., 2011). However, the inhibition of nitrification in the soils with sensitive cultivars may improve N retention as NH_4^+ is less mobile compared to NO_3^- that can be leached or removed by surface runoff. As far as we know, our study is the only report of eO_3 effects on gross nitrification, which reduced only in the sensitive cultivars. The only previous study that explored responses of N cycling to eO_3 for different plant species found no effects of species (Holmes et al., 2003). Further studies should be conducted to explore eO_3 effects on N cycling with more plant species with different sensitivity to O_3.

There are several mechanisms that could explain the contrasting effects of O_3 on soil N cycling between sensitive and tolerant cultivars. In general, fungal biomass increased under eO_3 for the tolerant cultivars, and bacterial biomass declined under eO_3 for the sensitive cultivars. The abundance of fungi was an important predictor of N mineralization (Fraterrigo et al., 2006) and fungi generally decomposed substrates more efficiently than bacteria (Allison et al., 2005). Increased fungal biomass by eO_3 with lower biosynthetic requirement for N may therefore dampen negative effects of eO_3 on N mineralization for tolerant cultivars. Also, the results generally indicate that the microbial communities from sensitive cultivars may be inhibited by eO_3 while microbial

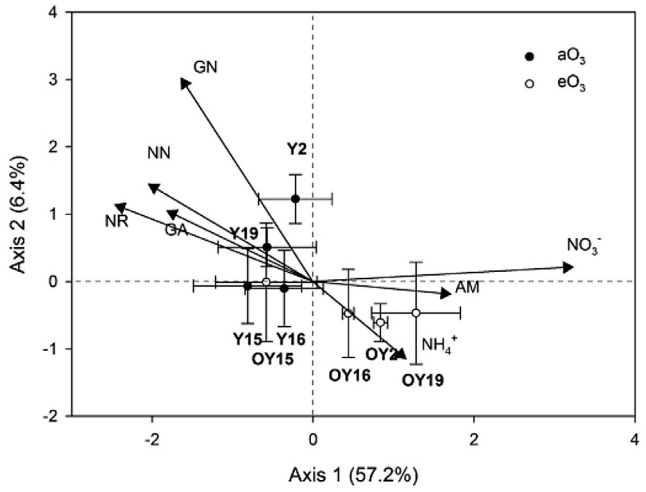

Fig. 4. Redundancy analysis (RDA) of microbial community structure and N transformation variables. Arrow length indicates the importance of each N transformation variable and their relationships with microbial communities. Cultivar with O represents cultivar with elevated O_3. For abbreviations of N transformation variables see Fig. 1. Open and closed circles show the mean ± standard error (n = 3).

communities from tolerant wheat cultivars may be more tolerant under eO_3 conditions.

In addition, significant decreases in MBN and increases in MBC/MBN in sensitive cultivars under eO_3 is also consistent with the observed shifts in most of the PLFA responses (Table 1) as well as fungal:bacterial ratios (Kanerva et al., 2008; Li et al., 2012, 2013; Zak et al., 2007). Soil pH is an important driver of soil microbial community diversity and structure (Fierer and Jackson, 2006; Pietri and Brookes, 2009; Cheng et al., 2013). However, no significant difference was found for soil pH in this study. Previous studies in the same site showed that eO_3 changed the soil food web structure with significantly lower fungal and bacterivore biomass (Li et al., 2012), which may decrease decomposition of plant litter and soil organic matter (Paul and Clark, 1996; Six et al., 2006; Kandeler et al., 2008). Furthermore, in another study in the same site, it was found that eO_3 altered the abundance of N cycling genes such as N fixation (nifH) in soil (Li et al., 2013).

The decreased microbial mediated gross and net N transformation in sensitive cultivars under eO_3 were likely driven by changed plant and soil properties since there is a strong connection between plant production, litter inputs and microbial activity (Wardle, 2002; Biswas et al., 2008; Feng et al., 2008; Zhu et al., 2011). The RDA analysis indicated that the diversity of the microbial community could be largely shaped by soil DOC, root C/N ratio, MBC/MBN ratio, shoot/root ratio, root N, soil N and pH. Our results showed that eO_3 significantly decreased soil DOC in sensitive cultivars, suggesting reduced availability of labile C, changing the activity and function of the microbial community (Bais et al., 2006; Dennis et al., 2010), hence, decreasing soil N transformations. Holmes et al. (2006) also observed decreased gross ammonification by eO_3 due to decreased organic substrate inputs to soil in a forest ecosystem.

We observed significantly higher NH_4^+ and NO_3^- concentrations under eO_3 in Y2, Y19, which was unexpected as decreased N cycling usually reduces soil inorganic pools (Kanerva et al., 2006; Pereira et al., 2011). This effect was likely driven by decreased plant N demand and microbial N immobilization as NH_4^+ availability was controlled by gross ammonification, microbial N immobilization, and plant NH_4^+ uptake (Schimel and Bennett, 2004). Similar to our observation, Swank and Vose (1990) found that eO_3 lead to greater NO_3^- –N levels in stream water because of less plant accumulation of cations in the forest ecosystem with white pine. Decock and Six (2012) also found that eO_3 significantly increased NH_4^+ and NO_3^- concentrations in a 2-day lab incubation experiment from a soybean-agroecosystem. Furthermore, we also observed reduced MBN under eO_3, suggesting reduced microbial immobilization. Increased NH_4^+ and NO_3^- content in soil with less plant and microbial N demand can result in greater mineral N loss from cropland ecosystems.

We observed that eO_3 significantly increased ammonia monooxygenase activity, but decreased nitrate reductase activity. The former response was likely affected by the higher ammonia concentration in the soil under eO_3 providing substrate for the nitrification reaction (Zhalnina et al., 2012). The decrease in DOC may have favored the nitrifying community as autotrophic nitrifying bacteria are more competitive than heterotrophic microbes in low C conditions (Verhagen et al., 1992). The nitrate reductase activity, associated with the abundance of denitrifying bacteria, is governed by soil moisture, the availability of C substrates, and NO_3^- concentrations in the soil (Barnard et al., 2005; Wallenstein et al., 2006). The decreased nitrate reductase activity under eO_3 may be due to the reduction in DOC, despite increased NO_3^- concentrations, suggesting that DOC is the key factor limiting the denitrifying bacteria in this system. In contrast to our results, Pereira et al. (2011) demonstrated that eO_3 in combination with elevated atmospheric CO_2 increased denitrification by the greater abundance of the denitrifier gene nosZ, which was driven by the higher soil organic C content with elevated CO_2 in the soybean FACE system. Increased ammonia monooxygenase activity, but decreased nitrate reductase activity will lead to high NO_3^- concentration in the soil and may cause NO_3^- leakage from the soil, resulting in surface and groundwater contamination and water eutrophication.

In summary, our results indicate that eO_3 is an important factor affecting microbial community structure and activity through altering plant and soil properties, which in turn will change N availability in agro-ecosystems and potentially increase N loss from soil. Wheat cultivars play an important role in determining the microbial function in response to eO_3. Microbial mediated N transformations significantly decreased under eO_3 in sensitive wheat cultivars, but were largely unchanged in tolerant wheat cultivars. Therefore, developing tolerant cultivars will diminish the threat of eO_3 to sustainable agro-ecosystems. This study provides new insight into our understanding of how change in microbial diversity and metabolism will alter N cycling and ecosystem N availability in response to eO_3 and wheat cultivars.

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