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Soil urease and catalase responses to ozone pollution are affected by the ozone sensitivity of wheat cultivars

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Abstract Understanding the effects of elevated O₃ (EO₃) on belowground process such as soil enzyme activities is essential to evaluate plant physiological reaction and soil processes (e.g. carbon and nitrogen turnover) under predicted increases in atmospheric O₃. In this study, O₃-induced changes in soil urease (UA) and catalase activities (CTA) under two contrasting wheat cultivars (O₃-sensitive vs. O₃-tolerant) were investigated using a free-air O₃ enrichment (O₃FACE) facility in China. EO₃ (60 ppb compared to 40 ppb in ambient O₃) generally increased UA under the O₃-tolerant cultivar but reduced it under the O₃-sensitive cultivar for different soil depths and growth stages. In contrast, the effects of EO₃ on CTA were not consistent, and varied with soil depths and growth stages. These results suggest that the O₃-sensitivity of wheat cultivars plays an important role in determining the effects of EO₃ on soil enzyme activities. The contrasting responses of soil UA and CTA to EO₃ may alter the effect of projected increase in tropospheric O₃ on soil carbon and nitrogen turnover.

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Keywords soil enzyme; ozone pollution; wheat; ozone-sensitivity; agroecosystem

Abbreviation

O₃: ozone

EO₃: elevated O₃

AO₃: ambient O₃

O₃FACE: free-air O₃ enrichment

UA: Soil urease

CTA: Soil catalase

Introduction

Tropospheric O₃ is predicted to increase by 40-70% by the year 2100 from the current level of 25-40 ppb in the Northern Hemisphere (Biswas et al. 2008, Zeng et al. 2008). The current tropospheric O₃ concentration may endanger terrestrial ecosystems through reducing photosynthetic rates, growth and biomass production in many crop, grass and forest species (Wang et al. 2004, Cao et al. 2009, Ashmore et al. 2006, Kou et al. 2009) and ecosystem C storage (Loya et al. 2003, Sitch et al. 2007). It has been widely reported that O₃ exposure alters the ratio of root to shoot biomass (Grantz et al. 2006, Chen et al. 2015) and belowground C and N allocation (McCrary and Andersen 2000, Kanerva et al. 2006, Kou et al. 2012). This could affect soil properties (Kou et al., 2014) and structural and functional aspects of soil biodiversity (Dinesh et al. 1998, Andersen, 2003) through changing nutrient dynamics in soil profiles (Kou et al. 2014, Wang et al. 2017) and soil microbial community (Sami et al. 2008, Li et al. 2012, Bao et al. 2015). As soil processes are driven by soil organisms (e.g. enzyme, microbial) and their interactions with plants and soil abiotic conditions, O₃ pollution has the potential to influence belowground organisms mediating these processes.

Soil enzyme is a sensitive indicator of soil quality and may provide early warning signals in response to climatic and environmental changes (Burns and Dick 2001) via soil biochemical process (e.g. C and N cycling) regulated by enzymatic reaction (Li et al. 2008). UA and CTA are important constituents of soil hydrolytic and oxidoreductase enzymes, respectively. UA is one of the key enzymes that affect N transformation in soil (Burns and Dick 2001). CTA reflects soil health status in response to environmental stress through decomposing hydrogen peroxide in soil and is involved in humus formation (Burns and Dick 2001). However, the influences of O₃ on soil enzymes are not fully

understood. To our knowledge, studies that reported on soil enzymes response to EO₃ from wheat (Li et al. 2008, Zheng et al. 2009, Huang et al. 2013, Chen et al. 2015), green vegetable (Shi et al., 2016) and ponderosa pine (Scagel and Andersen 1997) systems were few and showed inconsistent findings. Only four studies reported that the response of UA to EO₃ was either positive (Zheng et al. 2009), negative (Scagel and Andersen 1997, Shi et al. 2016) or neutral (Huang et al. 2013). The effects of EO₃ on CTA were generally positive at the wheat late growth stage (Li et al. 2008, Zheng et al. 2009). The limited knowledge on EO₃ effects on soil enzyme (UA and CTA) in agroecosystems restricts better understanding on soil belowground processes and C and N cycles in response to predicted future higher tropospheric O₃ environments.

Wheat (*Triticum aestivum* L.) is sensitive to O₃ (Feng et al. 2008). EO₃ was found to reduce photosynthetic rate of wheat and accelerate its senescence, impairing growth and yield (Biswas et al. 2008, Feng et al. 2008, Kou et al. 2012). However, compared to O₃-tolerant counterparts, O₃-sensitive winter wheat cultivars showed larger reductions in antioxidative capacity, dark respiration (Biswas et al. 2008), photosynthetic rate, stomatal conductance, transpiration rate (Cao et al. 2009), grain yield and individual grain mass (Zhu et al. 2011). This intraspecific variation in growth response to EO₃ was also observed in the quantity and quality of C assimilation allocated to belowground components (McCrary and Andersen 2000, Kou et al. 2012, 2017). Li et al. (2012) found that the O₃-tolerance of cultivars affected the component of bacterial and fungal based soil microbial food webs. However, Chen et al. (2015) reported that the O₃-sensitivity of cultivars affected wheat biomass allocation, above- and belowground N uptake and soil N content but not the nitrifying and denitrifying enzymes at the ripening stage. It is however unclear whether the differential above- and belowground responses to EO₃ between O₃-sensitive and O₃-tolerant cultivars may further influence soil enzyme (e.g. UA, CTA) in different soil depths and growth stages. We therefore investigated the effects of EO₃ on UA and CTA in soil grown with O₃-sensitive and O₃-tolerant wheat cultivars under an O₃FACE fumigation system.

Materials and methods

Experimental site and ozone fumigation

The experimental site was located in Jiangdu City, Jiangsu province of China (32°35'5" N, 119°42'0" E). The region's mean annual precipitation and annual temperature are 980 mm and 14.9 °C,

respectively. The soil is classified as a Shajiang Aquic Cambiosol (Cooperative Research Group on Chinese Soil Taxonomy, 2001) with a sandy-loamy texture, with 13.6% clay (<0.002 mm), bulk density 1.16 g cm^{-3} , SOM 18.4 g kg^{-1} , total N 1.45 g kg^{-1} , and pH 7.2 (Kou et al. 2014). An O_3 FACE experiment was established in 2007 (Tang et al. 2011) with continuous O_3 exposure from March to November each year during the crop growth period in a rice (*Oryza sativa* L.) –wheat rotation that has been used in this location for over 100 years. Winter wheat was sown in November and harvested in May of the next year, whereas summer rice was transplanted in June and harvested in October. No additional organic matter was incorporated the field except for rice /wheat residuals. Three octagonal rings (14 m in diameter) were maintained at a mean O_3 concentration of 60 ppb from 9:00 am to 18:00 pm each day except for rainy days (hereinafter referred to as EO_3) and three control rings at 40 ppb (hereinafter referred to as AO_3) were used. All of the rings were located > 70 m apart to avoid O_3 cross contamination. Two winter wheat cultivars viz. Yangmai 15 (Y15) and Yannong 19 (YN19), known to be resistant and sensitive to O_3 (Zhu et al. 2011), respectively, were grown in two subplots (82 m^2) of each experimental ring since 2007. This study was conducted during the wheat growing season in 2013. Nitrogen (urea), phosphorus (superphosphate) and potassium (potassium chloride) were applied as 478 kg ha^{-1} , 625 kg ha^{-1} and 125 kg ha^{-1} , respectively. Nitrogen fertilizer was applied prior to sowing, at the jointing stage, and at the booting stage of wheat in a ratio 5:1:4. All phosphorus and potassium fertilizers were applied prior to sowing. Field management closely followed the local agronomic practices.

Soil sampling and analyses

Soil samples (0-5, 5-10 and 10-20 cm) were collected at the jointing stage (April 16), the heading stage (May 1) and the milky stage (May 23). Each soil sample was homogenized from eight soil cores of 2.5 cm diameter sampled at random locations for each plot. Fresh soil samples were passed through an 8 mm sieve and stored at 4°C until further analyses. UA and CTA were analyzed by the indophenol blue colorimetric method and the potassium permanganate titration method, respectively, according to Burns and Dick (2001).

Statistical analysis

Statistically significant differences were identified by analysis of variance (ANOVA) using SPSS 11.5

software (Windows version 11.5; SPSS inc, Chicago, IL) and Tukey's HSD test at $P = 0.05$.

Results

Soil urease activity

UA generally increased with soil depth, regardless of cultivar or O_3 concentration; it also increased with growth stage, except for the wheat cultivar YN19 grown under AO_3 (Fig. 1). EO_3 increased UA in the plots grown with Y15, but decreased it in the plots grown with YN19 across all soil layers and key growth stages, respectively, except for non-significant negative effect at the 10-20 cm soil depth at the milky stage (Fig. 1, Tables 1 and 2). Specifically, EO_3 significantly increased UA by 13-45% in the 0-10 cm layer in Y15 but significantly decreased it by 6-26% in the 0-20 cm layer in YN19 at the jointing stage. At the heading stage, EO_3 significantly increased UA by 23-48% in the 0-20 cm layer in Y15 but significantly decreased it by 8-25% in the 0-10 cm layer in YN19. At the milky stage, EO_3 significantly increased UA by 45-50% in the 0-10 cm layer in Y15. For YN19, EO_3 significantly decreased UA by 7-19% in the 0-10 cm layer but significantly increased that by 19% in the 10-20 cm layer.

Soil catalase activity

CTA responses to EO_3 were controlled by cultivar, growth stage and soil depth (Fig. 2, Tables 1 and 2). At the jointing stage, EO_3 significantly increased CTA by 13% and 6% in the 10-20 cm layer in Y15 and YN19, respectively, but only significantly decreased it by 22% in the 5-10 cm layers in Y15. At the heading stage, EO_3 significantly increased CTA by 6-49% in the 0-20 cm layer in Y15. EO_3 significantly increased it by 33% and 40% in the 0-5 cm and 10-20 cm layers, respectively, and significantly decreased it by 15% in the 5-10 cm layer in YN19. At the milky stage, EO_3 significantly increased CTA by 34% in the 0-5 cm layer and by 36% in the 10-20 cm layer and significantly decreased it by 28% in the 5-10 cm layer in Y15. EO_3 significantly decreased CTA in the 0-10 cm layer and significantly increased it by 42% in the 10-20 cm in YN19.

Discussion

We found that the responses of UA, an enzyme involved in N mineralization, to EO_3 were affected by

the O₃-sensitivity of wheat cultivar. This indicates that wheat cultivars would play an important role in determining the effects of EO₃ on soil UA and its regulating processes. While soil enzymes are mainly associated with microbial and plant C sources (Burns and Dick 2001), the O₃-sensitivity of the wheat cultivar affected the quantity and quality of C input to soil (Kou et al. 2012, 2014) and subsequently the soil biota (Li et al. 2012, Bao et al. 2015) in response to EO₃. Since the C and N assimilation allocation between above- and belowground was affected by EO₃ (Kou et al. 2012, 2017, McCrady and Andersen 2000, Chen et al. 2015), the intraspecific variation in O₃ tolerance (Zhu et al. 2011, Huang et al. 2013) altered the substrate availability for enzymes via plant and microbial C resources. In particular, the contrasting responses of UA to EO₃ (Table 1) were consistent with the findings that EO₃ increased the ratios of root to shoot biomass and microbial biomass in Y15 but decreased them in YN19 (Kou et al. 2012, Zhang et al. 2014, Bao et al. 2015). The activities of UA could reflect soil nitrogen-supplying capacity (SNSC) (Burns and Dick 2001). Our findings suggest that SNSC via organic matter decomposition under elevated O₃ environments may be enhanced in an O₃-tolerant wheat cropping system but reduced in an O₃-sensitive one.

Unlike UA, CTA responses to EO₃ in each cultivar were not consistent among soil depths or growth stages. This implies that CTA was affected not only by enzyme substrate availability but also by other factors. As an oxidoreductase CTA is also affected by soil environment (abiotic factors) such as the redox potential (Burns and Dick 2001). Since the sources of CTA via assimilation of resources (McCrady and Andersen 2000, Kou et al. 2012) and soil organisms (Li et al. 2012) were changed under O₃ stress, the differences in CTA at different soil depth and growth stages under EO₃ might be attributed to the differential responding mechanisms of the two wheat cultivars to EO₃ (Kou et al. 2012) and soil reducing condition (Chen et al. 2015). The positive responses (10-20 cm layer) of CTA to EO₃ in both cultivars would increase the capacity of hydrogen peroxide decomposition, decreasing the toxic effects on soil biology (Wang and Feng 2006). The more negative (0-10 cm layer) responses of CTA in the O₃-sensitive than O₃-tolerant wheat systems indicates that growing an O₃-sensitive cultivar may decrease the ability of hydrogen peroxide decomposition, which may increase the reducing condition in the upper soil layer and O₃-stress to the plants. While CTA is highly associated with soil organic matter content, as is UA with N mineralization (Burns and Dick 2001), the contrasting responses of UA and CTA to EO₃ between the O₃-tolerant and O₃-sensitive cultivars would likely affect soil C and N cycling.

Conclusions

Our findings indicate that EO_3 can affect soil UA and CTA in wheat cropping systems, but the effect is strongly dependent on the O_3 sensitivity of the wheat cultivar. The responses of UA and CTA to EO_3 were mainly positive for an O_3 -tolerant cultivar but negative for an O_3 -sensitive cultivar. This study highlights the need for further investigation on the impacts of O_3 on soil enzyme activities to better explain the belowground processes associated with C and N cycling under projected rising tropospheric O_3 .

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1 Table (1-2):

2 **Table 1** Significances of elevated O₃ effects on urease and catalase activities in soils under winter wheat
3 cultivars Y15 and YN19

Soil depth (cm)	Growth stage	Urease activity		Catalase activity	
		Y15	YN19	Y15	YN19
0-5	Jointing stage	(+) *	(-) *	(-)	(-)
	Heading stage	(+) **	(-) *	(+) *	(+) **
	Milky stage	(+) **	(-) *	(+) **	(-) *
5-10	Jointing stage	(+) **	(-) **	(-) **	(-)
	Heading stage	(+) **	(-) **	(+) *	(-) *
	Milky stage	(+) **	(-) **	(-) **	(-) *
10-20	Jointing stage	(+)	(-) *	(+) *	(+)
	Heading stage	(+) *	(-)	(+) **	(+) **
	Milky stage	(-)	(+) *	(+) **	(+) **

4 (+) and (-) refer to the positive and inhibitory effects of elevated O₃ on enzymes activities. *, **
5 indicate significant difference at P < 0.05 and P < 0.01, respectively

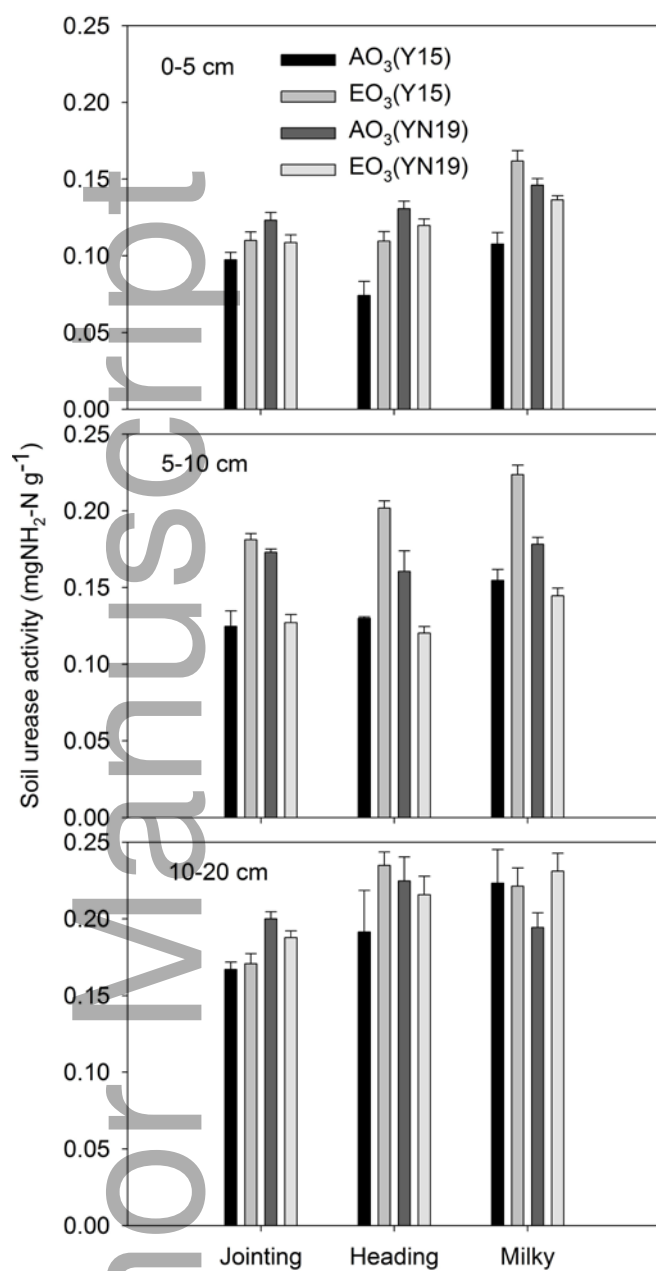
6 **Table 2** Analysis of variance for urease and catalase activities in response to elevated O₃

Variable source	Urease activity		Catalase activity	
	F value	P value	F value	P value
O ₃	13.376	<0.001	369.139	<0.001
Cultivar (C)	0.417	ns	4.104	0.046
Soil depth (D)	258.775	<0.001	949.539	<0.001
Stage (S)	30.145	<0.001	38.889	<0.001
O ₃ * C	74.566	<0.001	4.899	0.030
O ₃ * D	0.073	ns	325.61	<0.001
O ₃ * S	3.479	0.036	153.942	<0.001
C * D	12.001	<0.001	18.599	<0.001
C * S	4.387	0.016	2.017	ns
O ₃ * C * D	20.375	<0.001	40.13	<0.001

O ₃ * C * S	1.838	ns	14.435	<0.001
C * D * S	3.502	0.002	37.791	<0.001
O ₃ * C * D * S	1.387	ns	26.943	<0.001

7 ns indicates no significant difference at P< 0.05.

1 Figure (1-2):



2

3 **Fig. 1** Soil urease activities in topsoil (0-20 cm) at three key growth stages of two wheat cultivars under

4 elevated (EO₃) and ambient O₃ (AO₃) concentrations across soil depths. Values are means±1SE (n=3)

5

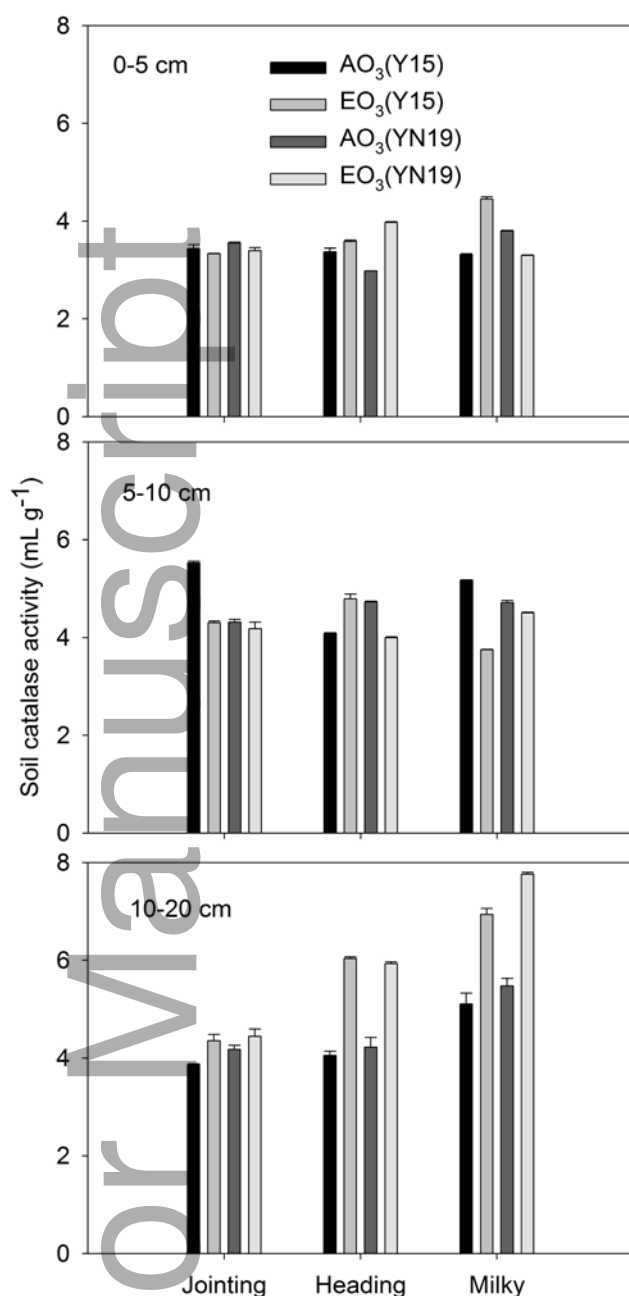


Fig. 2 Soil catalase activities in topsoil (0-20 cm) at three key growth stages of two wheat cultivars under elevated (EO₃) and ambient O₃ (AO₃) concentrations across soil depths. Values are means±1SE (n=3)