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Impact of elevated tropospheric ozone on soil C, N and microbial dynamics of winter wheat

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ABSTRACT

Tropospheric O_3 can impact soil systems mediated by changing root biomass and exudates. This study investigated how elevated O_3 alters soil CO_2 and N_2O emissions from a winter wheat field. Winter wheat (*Triticum aestivum* L.) were fumigated using elevated O_3 concentration (EO_3) and using ambient air within open-top chambers during 2015 and 2016. Soil CO_2 and N_2O emissions were measured using a static closed-chamber technique. Rhizosphere soils were collected to determine dissolved organic carbon (DOC) content, ammonium-nitrogen (NH_4^+-N) and nitrate-nitrogen (NO_3^--N) concentrations, microbial biomass carbon (MBC) and nitrogen (MBN), and activities of nitrate reductase (NR), nitrite reductase (NiR) and hydroxylamine reductase (HR). Moreover, volumetric soil water content (θ_v) was monitored and plant dry biomass was quantified. The EO_3 treatment exhibited decreased CO_2 and increased N_2O emissions from winter wheat soil. Soil NH_4^+-N, NO_3^--N, NR and NiR increased under EO_3, whereas DOC, MBC, MBN and HR decreased. Soil θ_v was higher under EO_3 before irrigation, due to reduced transpiration. Elevated O_3 decreased soil CO_2 flux potentially due to reduced root biomass and associated carbon input. It increased N_2O fluxes apparently through enhanced denitrification due to greater substrate availability and soil θ_v, despite the inhibition of nitrification-related routes.

1. Introduction

Tropospheric ozone (O_3) concentrations in China have been rising by 1%–3% per year since 2000 due to rapid urbanization and industrialization (Verstraeten et al., 2015). Such increases will be more rapid if anthropogenic activities such as massive fossil fuel consumption continue unabated in the future (Stocker et al., 2013). As an extremely phytotoxic air pollutant, O_3 has been shown to suppress plant growth, and to significantly reduce yield and quality in several plant species. Ozone damage to plants is estimated to have caused economic losses on the order of several billion dollars (Ashmore, 2005; Fuhrer, 2009; Fuhrer and Booker, 2003; Fuhrer et al., 1992; Wilkinson et al., 2012; Yi et al., 2016). Influence of elevated O_3 on agro-ecosystem has been studied widely from an above-ground perspective, and in recent years, increasing attention has been paid to the effects of elevated O_3 on below-ground functional processes, such as root growth, carbon allocation and soil nitrogen status (Andersen, 2000; McCrady and Andersen, 2000; Schrader et al., 2009). However, the responses are not always predictable because of the complexity of the belowground ecosystem (Andersen, 2000).

Ozone is a highly reactive and oxidative pollutant and is destroyed rapidly when interacting with various surfaces. Plants and soil remove most O_3 from the atmosphere, producing a vertical gradient of decreasing O_3 concentrations towards the ground and preventing any appreciable penetration of O_3 into the soil (Blum and Tingey, 1977; Turner et al., 1974). Therefore, direct effects of O_3 on roots or soil microbes are unlikely (Blum and Tingey, 1977). The effects of elevated tropospheric O_3 on the below-ground system are likely to be mediated indirectly through altering the quantity and quality of plant C inputs and resource allocation (Andersen, 2003; Kanerva et al., 2006; Kasurinen et al., 2005). The general consensus is that elevated O_3 decreases the allocation of assimilates into the root, reduces plant litter

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inputs and rates of decay, and alters the quantity and quality of root exudates. As a result of these changes, elevated O_3 could potentially lead to significant alterations in microbial community structure and function and thus modify microbial governed nutrient transformations (for example, C and N fixation, N mineralization, nitrification, denitrification, and greenhouse gas emission). This is because soil microbial processes depend on the forms and availability of soil C and N substrates (Chen et al., 2015b; Feng et al., 2015; Formanek et al., 2014; Jones et al., 2009; Kanerva et al., 2008; Kasurinen et al., 2005; Li et al., 2013). Particularly, ozone has a much greater effect on rhizosphere microbial processes than on those of non-rhizosphere soil due to the decreased C exudates from the roots of the O_3-exposed crop (Chen et al., 2015b).

However, the effects of elevated O_3 on soil CO_2 and N_2O emissions have received sparse attention, hampering the understanding of factors driving soil C and N cycling (Andersen, 2003; Wu et al., 2016). Emissions of CO_2 and N_2O are major sources of atmospheric greenhouse gases generated from upland agro-ecosystems, which may play important roles in regional or global climate change (Watson et al., 1995). Nitrous oxide is of particular interest in conjunction with O_3-impact research because it is a potent greenhouse gas with a relative global warming potential 298 times that of CO_2 at a 100-year time horizon, and it also causes stratospheric O_3 depletion. However, knowledge of the effect of tropospheric ozone levels on CO_2 and N_2O emissions from soil are lacking and there is controversy regarding the direction and extent of O_3 impacts on these gas fluxes (Decock et al., 2012; Edwards, 1991; Kanerva et al., 2007; Kou et al., 2015; Tang et al., 2014).

Winter wheat is a major staple food crop in China with annual production of over 120 million tons employing a planted land area of more than 22.6 million hectares (National Bureau of Statistics of the People's Republic of China, 2015). Winter wheat fields are considered to be one of the major sources of N_2O in the North China Plain (Xing, 1998), and winter wheat is among the most sensitive crops to O_3 damage (Pleijel and Uddling, 2012). Advancing our understanding of the impact of increased tropospheric O_3 on soil CO_2 and N_2O emissions from winter wheat fields is of considerable importance for predicting feedback effects on sustainable agro-ecosystems under global climate change.

In the present study, we investigated C and N dynamics in winter wheat rhizosphere soils under the influence of elevated O_3. Dynamics were evaluated in terms of soil dissolved organic carbon (DOC) content, soil ammonia nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) availability, microbial biomass C and N (MBC and MBN), as well as the activities of nitrate reductase (NR), nitrite reductase (NiR) and hydroxylamine reductase (HR). The main objective was to further our understanding of how elevated O_3 alters soil CO_2 and N_2O emissions.

2. Materials and methods

2.1. Experimental site and soil

The research was conducted at the Seed Management Station in Changping District, Beijing, located at 40°19'N and 116°13'E, at an altitude of 31.3 m above mean sea level. The station is situated north of Beijing under the influence of a warm temperate semi-humid continental monsoon climate. The average annual temperature and precipitation is 11.7 °C and 569.8 mm, respectively. The total annual sunshine duration and the frost-free period is 2640 h and 198 days, respectively. The soil texture at the experimental site is sandy loam with an average bulk density of 1.29 g cm^{-3}, pH of 8.3 (1:2.5 soil:water), organic C of 14.2 g kg^{-1}, total N of 0.81 g kg^{-1}, Olsen-P of 19.9 mg kg^{-1}, and effective K of 79.5 mg kg^{-1}.

2.2. Ozone treatments

An in-situ ozone fumigation experiment was carried out in six open-top chambers (OTC). The OTCs were framed with galvanized tubing (3.0 m diameter × 2.0 m tall), and covered with transparent PVC film. Air was delivered into the OTCs through a centrifugal blower (750 W, 1000 Pa, 14 m^3 min^{-1}, CZR, Fengda, China) through downward facing holes in a rotating pipe to maintain uniform air velocity. Ventilation rates were maintained at a rate that replaced the OTC volume every minute in order to minimize leaf boundary layer resistance and keep the OTC air temperature close to ambient. Each OTC was separated from others by at least 3 m to prevent mutual influence between adjacent chambers.

Two contrasting ozone treatments were set up in this study, namely, elevated ozone (hereinafter referred to as EO_3) and non-filtered ambient air (hereinafter named NF). Three replicates (i.e. OTCs) for each treatment were established. In the EO_3 treatment, ozone was generated from pure oxygen using a ceramic ozone generating tube (F-20-11A, Sankang Environmental Technology Co., Ltd., Jinan, China) and mixed with the ambient air ventilation to continuously provide a daytime ozone level of 40 nL L^{-1} above the NF treatment in the top of the canopy. The flow of pure oxygen was manually controlled by a mass flow controller (maximum flow rate of 300 mL min^{-1}, Shengye Co., Beijing, China) to obtain the target O_3 concentration. Ozone fumigation on winter wheat in 2015 began on April 15 at the early heading stage and ended on June 20 when wheat was ripe, then O_3 injection started again on March 20 at early jointing stage and ended on June 20 in 2016. The daily maximum fumigation period was 9 h (from 08:00 to 17:00) except when leaves were wet from rain or irrigation.

2.3. Crop management

The wheat cultivar used in the experiment was *Triticum aestivum* L. Zhongmai 12, obtained from Chinese Academy of Agricultural Sciences. This variety of wheat is widely planted in Northern China. The seeds was sown around September 25 each year. Prior to sowing, 750 kg ha^{-1} compound fertilizer (N:P_2O_5:K_2O = 18%:12%:15%, total fertility ≥ 45%) was applied to the field, where management (planting, harvest, weeds control, and pesticide application) followed local winter wheat agronomic practices during each growing season. Natural rainfall does not satisfy the water demand of wheat in this area, therefore each experimental field was intermittently irrigated following local irrigation practice using micro-spray facilities. Crops were harvested at maturity on June 21 each year.

2.4. Ozone and climate monitoring

The ozone concentration was monitored continuously by sampling air from the center of each OTC 10 cm above the plant canopy. Sampled air passed through an UV absorption ozone analyzer (Model 49i, Thermo Scientific, USA) via a Teflon solenoid valve switch system. The daylight 9-h average ozone concentrations for both treatments across fumigation days in the year 2015 and 2016 are shown in Fig. 1. Because the ozone release was discontinuous under certain conditions, the effective increase in 9-h mean ozone concentration was 34.91 ± 1.42 nL L^{-1} (mean ± SE) in the EO_3 compared with NF across both years. The accumulative O_3 exposure index AOT40 (accumulated hourly O_3 concentrations over a threshold of 40 nL L^{-1}) were 15.00 ± 0.40 μL L^{-1} h and 45.40 ± 2.55 μL L^{-1} h in EO_3 and NF respectively in 2015, and were 17.36 ± 0.16 μL L^{-1} h and 40.75 ± 2.07 μL L^{-1} h in EO_3 and NF respectively in 2016.

Air temperature (T), relative air humidity (RH), photosynthetic photon flux density ($PPFD$), as well as volumetric soil water content (θ_v) inside each OTC was recorded every 5 min using an automatic weather station (HOBO U30-NRC-SYS-PRO, Onset Computer Corporation, Bourne, MA, USA). As illustrated in Fig. 1, the daily average T inside OTC fluctuated from 9.0 °C to 28.4 °C, and from 5.6 °C to 28.4 °C during the fumigation period of 2015 and 2016, respectively. The daily average RH inside OTC fluctuated from 18.8% to 89.0%, and from