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Effects of elevated O_3 and CO_2 on the relative contribution of carbohydrates to soil organic matter in an agricultural soil

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ABSTRACT

As labile fraction of soil organic matter, soil carbohydrates are sensitive to environmental changes due to their high turnover rates. The effects of elevated O_3 and CO_2 on the concentration and composition of soil carbohydrates in agroecosystems have been rarely studied. The present experiment was conducted to investigate the dynamics of the concentration and composition of neutral sugars in soil under the influence of elevated O_3 and CO_2 with different N application rates (150 kg N ha^{-1} and 225 kg N ha^{-1}). Our results showed that elevated O_3 decreased soil carbohydrate accumulation, with the effect being more pronounced with high than with low N application rate. Elevated O_3 significantly increased the ratio of (mannose + galactose)/(arabinose + xylose), indicating that elevated O_3 promoted the relative contribution of microbe-derived carbohydrates to soil organic matter (SOM). The high N application rate further increased the contribution of microbe-derived carbohydrates to SOM under elevated O_3 conditions. However, elevated CO_2 had no effect on soil carbohydrate accumulation and little effects on carbohydrate composition in the study soil, regardless of N application rates. The elevated O_3-induced effects were not altered by elevated CO_2 at the low N application rate, and the change in composition mediated by elevated CO_2 was observed only at the high N application rate. Our results suggest that elevated O_3 affected soil carbohydrate accumulation and their composition and that the effects on composition were mediated by elevated CO_2 and N application rates.

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1. Introduction

As one component of global climate changes, increases in atmospheric carbon dioxide (CO_2) and tropospheric ozone (O_3) concentrations are of particular interest to researchers (Yang et al., 2007; Pleijel and Uddling, 2012; Kumari et al., 2013; Sun et al., 2014). Since the beginning of the industrial revolution, the concentration of atmospheric CO_2 has risen from approximately 280 ppm to the current level of 395 ppm (NOAA, 2013). Along with the increase in the CO_2 concentration, the concentration of tropospheric O_3 has also been rising at a rate of 0.5–2% per year from about 10 ppb to the present concentration of 25–40 ppb (Feng et al., 2010; Li et al., 2012; Singh et al., 2013) and is predicted to increase in future (Vingarzan, 2004). Elevated O_3 and CO_2 have substantial impacts on photosynthesis, carbon allocation to soil-crop systems, and on the sustainable development of agro-ecosytems (Sitch et al., 2007; Chen et al., 2009; Schrader et al., 2009). Many studies have been conducted to evaluate the effects of elevated O_3 and CO_2 on the aboveground subsystem. However, the effects on soil processes, especially the accumulation and decomposition of labile soil organic matter (SOM), still remain poorly investigated (Jones et al., 2009; Chen et al., 2010).

The effects of elevated O_3 and CO_2 on belowground subsystems are mediated indirectly by altering plant processes and C allocation (Grantz and Yang, 2000; Andersen, 2003). Elevated CO_2 could stimulate plant photosynthetic process (Amthor, 2000; Morgan et al., 2004), and thus increase aboveground net primary production and crop yield (Amthor, 2001; Morgan et al., 2005; Li et al., 2008; Qiao et al., 2014), which in turn increases plant residue returns and root biomass, and consequently SOM stocks (Kimball et al., 2002; Rodriguez et al., 2004; Peralta and Wander, 2007). As a detrimental air pollutant, elevated O_3 may have opposite effects, impairs stomatal function, accelerates leaf

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senescence, and reduces plant photosynthesis (Feng et al., 2008; Singh et al., 2013), which ultimately decreases the crop yield and C allocation to soil by restricting photosynthetic carbon fixation, plant residue, and root returns (Fiscus et al., 2005; Feng et al., 2008; Ainsworth et al., 2012). Additionally, the effects of elevated CO_2 on crop yield and SOM dynamics have shown to depend on different N supply levels (Daepp et al., 2000; Kimball et al., 2002; Ainsworth and Long, 2005; Ziska and Bunce, 2007). Until now, it remains unknown if the effects of elevated O_3 are related to N supply.

Soil carbohydrates are the labile fraction of SOM, accounting for approximately 5–25% of total SOM (Stevenson, 1994; Zhang et al., 2007; Schmitt and Glaser, 2011). Due to their fast turnover rates, soil carbohydrates are highly sensitive to changes in C inputs to the soil and thus act as a measurable indicator before the detection of changes in other components in total SOM (Cochran et al., 2007; Schmitt and Glaser, 2011). As non-cellulosic carbohydrates, soil neutral sugars consist of plant-derived (including large proportions of glucose, arabinose and xylose) and microbial-derived (including large proportions of glucose, mannose, galactose, fucose and rhamnose) origins (Bock et al., 2007). Consequently, the amount and composition of soil neutral sugars are used to evaluate the relationship between plant and microbe on SOM dynamics (Amelung et al., 1999; Medeiros et al., 2006). It is commonly believed that O_3-induced damages could be counteracted by the positive effects of elevated CO_2, which are supported by some studies which have evaluated the effects of elevated O_3 and CO_2 on the foliar organic carbon content, plant growth and yield of different tested plants (Liu et al., 2005; Kumari et al., 2013). However, relatively little is known about the effects of elevated O_3 and CO_2 on the accumulation and composition of neutral sugars in agricultural soils under different levels of N fertilization.

The objectives of present work were to (1) investigate if elevated O_3 and CO_2 affect the concentration and composition of soil neutral sugars, and (2) determine if their effects are influenced by N fertilization dose. We hypothesized that elevated O_3 would negatively affect the concentration of soil neutral sugars and such negative effects of elevated O_3 would be mediated/offset by elevated CO_2 or N fertilization.

2. Materials and methods

2.1. Experimental site and design

This experiment was conducted at a National Field Observation and Research Station of Agro-ecosystems in Shenyang (41°31′ N, 123°24′ E), a member station of the Chinese Ecosystem Research Network (CERN) established in 1987. The station is located in the Lower Liao River Plain, with a humid and semi-humid continental monsoon climate in warm-temperate zone. Mean annual temperature is 7.8 °C with minimum and maximum monthly mean temperatures of −12.6 °C in January and 24.4 °C in July, respectively. The duration of the frost-free season is 147–168 days. Mean annual precipitation is 692 mm. The tested soil is classified as Hapic-Udic Alfisols in US soil taxonomy with a silt-loam texture, with 11.28 g kg^{-1} total C, 1.20 g kg^{-1} total N. It had a pH of 6.7, 177 g sand kg^{-1} (2–0.05 mm), 594 g silt kg^{-1} (0.05–0.002 mm), 229 g clay kg^{-1} (<0.002 mm), and a bulk density of 1.18 Mg m^{-3} in the 0–20 cm layer.

A total of 12 open-top chambers were used with four different treatments: ambient air as control (CK, 40 nmol mol^{-1} and 342 μmol mol^{-1} for O_3 and CO_2, respectively), elevated O_3 (O_3, 60 nmol O_3 mol^{-1}, 7 h day^{-1}, 9:00–12:00 am, 14:00–18:00 pm), elevated CO_2 (CO_2, 550 μmol CO_2 mol^{-1}, 24 h day^{-1}), and elevated O_3 and CO_2 (O_3 + CO_2, 550 μmol CO_2 mol^{-1} plus 60 nmol O_3 mol^{-1}), with three replicates for each treatment. Each open-top chamber with an internal diameter of 3 m and a height of 2.8 m was divided into two subplots for two N application levels (Li et al., 2008). Nitrogen as urea (*N* = 46%) and diammonium phosphate were applied via basal application at 150 kg N ha^{-1} (40 kg P ha^{-1}, low N application rate, LN) or 225 kg N ha^{-1} (40 kg P ha^{-1}, high N application rate, HN), respectively. Potassium (K) as potassium chloride was also applied as basal fertilizer at 60 kg K ha^{-1}.

2.2. Soil and plant sampling

At 0–15 cm depth, soil samples were collected during the wheat ripening stage of 2011 (July 11 in 2011), after exposing the plots to elevated O_3 and CO_2 for 2 years. Each composite soil sample consisted of five 2.5 cm diameter soil cores. Fresh soil samples were mixed homogeneously and stored at 4 °C until further analyses. About 150 g of fresh subsamples were used to determine the concentration of soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), dissolved organic carbon (DOC), NH_4^+-N, NO_3^--N and soil moisture (SM). About 20 g subsamples were air-dried first, and then ground and passed through a 100 mesh (<0.15 mm) sieve before measuring soil organic carbon (SOC) (Nelson and Sommers, 1996), total nitrogen (TN) and neutral sugars.

At the same time, we set three 30 cm × 30 cm quadrants in each subplot to collect wheat plant materials. We separated above-ground materials into straw (including leaf) and grain, and collected all roots from 0 to 15 cm soil layer in each quadrant. All plant materials from the same subplot were pooled and then oven dried at 65 °C to a constant weight (>48 h). All plant parts (approximately 85 g straw, 60 g grain and 20 g root) were ground and sieved (<0.25 mm) for the analysis of total C and N concentrations.

2.3. Chemical analysis

Total organic C and N of soil and plant samples were determined with a TruSpec CN Elemental Analyzer after air-drying and sieving (Leco Corporation, USA). MBC and MBN were measured using a chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). DOC was extracted with water for 0.5 h and determined with a Multi N/C 3100 Analyzer (Jena Corporation, Germany). NH_4^+-N and NO_3^--N were measured by extracting fresh soil samples with 2 M KCl and the MgO-Devarda alloy distillation method (Keeney and Nelson, 1982). SM was determined by weight loss after drying at 105 °C for 48 h.

Soil neutral sugars were determined following the method of Zhang et al. (2007). Briefly, air-dried soil samples were hydrolyzed with 4 M trifluoroacetic acid (TFA) and the hydrolyte was filtered. The filtrate was vacuum-dried, and the residue was re-dissolved into deionized water. The solution was adjusted to pH 6.6–6.8 and centrifuged. The supernatant solution was dried again, and the neutral sugars were subsequently re-dissolved in distilled water, transferred to a Reacti-VialTM of 5 ml and then freeze-dried completely under vacuum for derivation. Derivation reagent was added to the Reacti-VialTM. The capped vial was shaken and heated for 30 min at 75–80 °C, and then cooled to room temperature, and acetic anhydride was added. The vial was closed, shaken again, and heated for 20 min at 75–80 °C. After cooling, the derivatives were extracted with dichloromethane, and excessive derivation reagents were removed with 1 M HCl and distilled water. The final neutral sugar derivatives were re-dissolved in the mixture of hexane and ethyl acetate solvent (v:v = 1:1), and detected with a gas chromatograph (GC-14B, Shimadzu, Japan) equipped with a DM-1 (30 m × 0.25 mm × 0.25 μm) fused silica capillary column and flame ionization detector (FID).

Eight types of neutral monosaccharide (ribose (Rib), rhamnose (Rha), arabinose (Ara), xylose (Xyl), fucose (Fuc), mannose (Man),

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