Elevated O$_3$ and wheat cultivars influence the relative contribution of plant and microbe-derived carbohydrates to soil organic matter

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**A B S T R A C T**

Soil carbohydrates are sensitive to changes in soil C inputs because of their fast turnover rates. However, the effects of elevated O$_3$ on the content and composition of soil carbohydrates are rarely reported in agroecosystem. The objectives of this study were to investigate the effects of elevated O$_3$ on the content and composition of soil neutral sugars in the two wheat cultivars with different O$_3$-tolerance. Our results showed that elevated O$_3$ decreased the total soil neutral sugars. At the wheat ripening stage, elevated O$_3$ increased the contents of galactose (Gal), arabinose (Ara) and mannose (Man) in the O$_3$-tolerant wheat and decreased the contents of xylose (Xyl), Gal and Ara in the O$_3$-sensitive wheat. Significant interactive effects between elevated O$_3$ and wheat cultivar were found in the ratios of (Man + Gal)/(Ara + Xyl) and Man/(Ara + Xyl). These two ratios increased with elevated O$_3$ at the wheat ripening stage in both wheat cultivars, with higher ratios observed in the O$_3$-sensitive wheat relative to the O$_3$-tolerant wheat. Our results indicated that elevated O$_3$ decreased the total neutral sugars and altered the relative contribution of plant- and microbe-derived carbohydrates to soil organic matter. Microbe-derived carbohydrates were dominant contribution to the total carbohydrates in the O$_3$-sensitive wheat. These changes in the accumulation and origin of soil carbohydrates will influence the accumulation and decomposition of soil organic matter and ecosystem functioning in agroecosystem.


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**1. Introduction**

Tropospheric ozone concentration (O$_3$) has been rising at the rate of 0.5–2% per year due to human activity (Feng et al., 2010; Singh et al., 2013) and is predicted to increase further (Vingarzan, 2004). As the most damaging and widespread phytotoxic air pollution, tropospheric O$_3$ poses a great threat to crop yields (Feng et al., 2008) and ecosystem carbon storage (Sitch et al., 2007), and affects the sustainable development of agroecosystem (Chen et al., 2009; Schrader et al., 2009). Although some studies have evaluated the effects of elevated O$_3$ on the aboveground subsystem, relatively little attention has been paid to the direct and indirect effects on soil-crop systems, especially for the accumulation and decomposition of labile soil organic matter (SOM) (Jones et al., 2009; Chen et al., 2009, 2010).

The influences of elevated O$_3$ on belowground are indirectly mediated by alterations in plant processes and C allocation (Andersen, 2003). Elevated O$_3$ has been reported to decrease the carbon allocation to roots and reduced carbohydrates levels and storage pools in O$_3$-exposed plants (Andersen et al., 1997). Changes in the quantity or quality of carbon flux into the soil will influence the interactions among soil organisms (Li et al., 2012; Li et al., 2013) and then alter carbon retention and mineralization in soil ecosystem (Andersen, 2003). As the labile fraction of SOM, soil carbohydrates account for about 5–25% of total SOM (Stevenson, 1994; Zhang et al., 2007). Soil carbohydrates are highly responsive to changes in C inputs to the soil because of their fast turnover rates (Schmitt and Glaser, 2011). However, the effects of elevated O$_3$ on the content and composition of soil carbohydrates are rarely reported in agroecosystem.

As a kind of non-cellulosic carbohydrates, soil neutral sugars initially originate from plant materials (including large proportions of pentose sugars-arabinose and xylose), however, soil microorganisms can re-synthesize a large amounts of hexoses (galactose and mannose) and deoxy sugars (rhamnose and fucose) and release...
them into the soil (Cheshire, 1979; Bock et al., 2007). Therefore, both the contents and compositions of soil neutral sugars can be used to evaluate the plant-microbe relationship on SOM dynamics (Amelung et al., 1999; Medeiros et al., 2006). Despite the significant indication of soil carbohydrates on accumulation and decomposition of SOM, the knowledge about the effects of elevated O₃ on the accumulation and origins of soil carbohydrates in SOM is still lacking (Andersen, 2003).

Wheat (Triticum aestivum L.) is the second largest food crop with an annual production of about 650 million metric tons which is sensitive to the elevated O₃ (Zhu et al., 2011). In the Yangtze River Delta region, elevated O₃ reduced the yield of wheat by 10% in 1999 as predicted by Feng et al. (2003). Recently, some O₃-tolerant wheat cultivars have been reported in China, which may avoid yield reduction in a high O₃ environment (Cao et al., 2009; Zhu et al., 2011). Different physiological characters and yield components responses to elevated O₃ have been reported in O₃-sensitive and O₃-tolerant wheat cultivars (Cao et al., 2009; Zhu et al., 2011). These distinct responses of different wheat cultivars to elevated O₃ would lead to differences in the quality and quantity of plant litter and/or roots which may in turn influence the C inputs to SOM. The objectives of this research were to investigate the effects of elevated O₃ on the contents and compositions of soil neutral sugars in the two wheat cultivars with different O₃-tolerance. We hypothesized that (1) the effects of elevated O₃ will negatively affect the contents of soil carbohydrates and would subsequently be reflected in the relative contribution of plant and microbial derived carbohydrates to SOM; (2) the level of the above-mentioned changes in soil neutral sugars will exhibit cultivar dependence.

2. Materials and methods

2.1. Experimental site and O₃-FACE treatments

The experiment was conducted 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, Zhu et al., 2011) with a sandy-loamy texture, 15.0 g kg⁻¹ total C, 1.59 g kg⁻¹ total N, pH 6.8, 9.2% sand (1–0.05 mm), 65.7% silt (0.05–0.001 mm), 25.1% clay (<0.001 mm), and bulk density 1.2 g cm⁻³ at 0–15 cm depth (Zhu et al., 2011). The climate conditions are temperate with annual temperature and precipitation averages at 16°C and 1100–1200 mm, respectively, and a frost-free period of >230 days (Zhu et al., 2011).

The experimental design was a split plot with the main plots being ambient O₃ or elevated O₃, and sub plots being wheat cultivars (Tang et al., 2011; Zhu et al., 2011). Three replicate elevated O₃ rings, each with 14.5 m in diameter, were set randomly within a uniform area of 4 ha to continuously provide an elevated O₃ of 60 ppb over the ambient conditions (about 40 ppb), while three replicate rings, each with the same size, were set randomly within the same area for the ambient O₃ treatment. All of the rings were far enough apart to prevent O₃ from spilling from one ring to another. Each plot under ambient and elevated O₃ conditions was split into two subplots planting with two winter wheat cultivars (Triticum aestivum L.) [O₃-sensitive cultivar, Yannong 19 (Y19), and the O₃-tolerant cultivar, Yangmai 16 (Y16)]. The experimental

![Fig. 1. The ratio of microbial biomass C to soil organic C in soil planted with O₃-sensitive (Y19) and O₃-tolerant (Y16) wheat under ambient (A-O₃) and elevated O₃ (E-O₃) conditions. Within each stage, capital and lowercase letters represent the significant differences between ambient and elevated O₃ for O₃-sensitive and O₃-tolerant wheat cultivars, respectively, bars with the same letters suggested nonsignificant difference in t-test (P < 0.10).](image-url)

### Table 1

<table>
<thead>
<tr>
<th>Soil and plant physicochemical variables in the soil planted with O₃-sensitive (Y19) and O₃-tolerant (Y16) wheat under ambient (A-O₃) and elevated O₃ (E-O₃) conditions during wheat growth season (mean ± SD).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage</strong></td>
</tr>
<tr>
<td><strong>MBC</strong> (mg kg⁻¹)</td>
</tr>
<tr>
<td><strong>SOC</strong> (mg kg⁻¹)</td>
</tr>
<tr>
<td><strong>TN</strong> (%)</td>
</tr>
<tr>
<td><strong>Soil C/N</strong></td>
</tr>
<tr>
<td><strong>TSN/SOC (%)</strong></td>
</tr>
<tr>
<td><strong>Grain yield (kg ha⁻¹)</strong></td>
</tr>
</tbody>
</table>

- **A-O₃** indicates ambient O₃ conditions.
- **E-O₃** indicates elevated O₃ conditions.
- **MBC** indicates microbial biomass carbon.
- **SOC** indicates soil organic carbon.
- **TN** indicates total nitrogen.
- **TSN/SOC** indicates the ratio of total soil neutral sugars to soil organic carbon.
platform of free-air O3 enrichment was established in 2007 over a rice-wheat rotation system, with rice transplanted in mid-June and harvested in mid- 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 in which the wheat/rice was growing. No additional organic matter was incorporated during the wheat growth season. Nitrogen as urea (N=46%) and diammonium phosphate at a total rate of 210 kg N ha\(^{-1}\) was split into basal application at planting (60% of the total N), side-dressings at early tillering (only urea, 10% of the total N) and elongation stages (30%). P and K as diammonium phosphate at 90 kg P\(_2\)O\(_5\) ha\(^{-1}\) and potassium chloride at 90 kg K\(_2\)O ha\(^{-1}\) were split-applied with 60% at planting and 40% at elongation stage, respectively (Zhu et al., 2011).

This experiment was conducted during the wheat growth season of 2010, after exposure to the elevated O3 for 3 years (about 83 days per year, from March 5 to May 27). Soil samples were collected from 0 to 15 cm soil layer at jointing stage (March 30 in 2010) and ripening stage (June 10 in 2010). Each composite soil sample was made from five soil cores of 2.5 cm diameter, and the soil corer was placed near a plant within a plant row. Soil samples were stored at 4 °C until further analyses.

2.2. Analysis of soil and plant samples

Fresh soil samples were mixed homogeneously, and about 100 g fresh subsamples were used to determine soil microbial biomass carbon, dissolved organic carbon and moisture content. About 20 g subsamples were air-dried and sieved (<15 mm) for measuring soil organic carbon, total nitrogen and neutral sugars. At maturity (June 10 in 2010), grain yield was determined from a 2 m\(^2\) patch in the middle of each subplot to minimize the boundary effect, and the plant biomass excluding grain mass (the litter per plant, averaged for 15 plants) was determined after drying at 65 °C until a constant weight was obtained. All grain and litter samples were ground and sieved (<0.25 mm) for total C and N analysis.

Total organic C and N of soil or plant samples were determined by a TruSpec CN Elemental Analyzer (Leco Corporation, USA). Microbial biomass carbon (MBC) was measured using a chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). Dissolved organic carbon (DOC) was extracted with water for 0.5 h, and determined by Multi N/C 3100 analyzer (Jena Corporation, Germany). Soil moisture (SM) was determined by weight loss after drying at 105 °C for 48 h.

2.3. Analysis of soil neutral sugars

Soil neutral sugars were determined according to 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 derivatization. Derivation reagent was added to the Reacti-VialTM, and 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 derivatization 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 by gas chromatograph (GC-14B, Shimadzu, Japan).
Table 3
The ratios of (Man + Gal)/(Ara + Xyl), Man/(Ara + Xyl) and Man/Xyl in the soil planted with O₃-sensitive (Y19) and O₃-tolerant (Y16) wheat under ambient (A-O₃) and elevated O₃ (E-O₃) conditions during wheat growth season (mean ± SD).

<table>
<thead>
<tr>
<th>Growth Stage</th>
<th>A-O₃</th>
<th>E-O₃</th>
<th>Effect*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y19</td>
<td>Y16</td>
<td>C</td>
</tr>
<tr>
<td>(Man + Gal)/(Ara + Xyl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jointing</td>
<td>0.82 ± 0.07</td>
<td>0.90 ± 0.03</td>
<td>ns</td>
</tr>
<tr>
<td>Ripening</td>
<td>0.81 ± 0.06</td>
<td>0.79 ± 0.03</td>
<td>ns</td>
</tr>
<tr>
<td>Man/(Ara + Xyl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jointing</td>
<td>0.39 ± 0.01</td>
<td>0.41 ± 0.01</td>
<td>ns</td>
</tr>
<tr>
<td>Ripening</td>
<td>0.36 ± 0.03</td>
<td>0.34 ± 0.01</td>
<td>ns</td>
</tr>
<tr>
<td>Man/Xyl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jointing</td>
<td>0.61 ± 0.04</td>
<td>0.69 ± 0.02</td>
<td>ns</td>
</tr>
<tr>
<td>Ripening</td>
<td>0.60 ± 0.05</td>
<td>0.53 ± 0.01</td>
<td>ns</td>
</tr>
</tbody>
</table>

* The effects were significant at P < 0.10; ns represents not significant.

2.4. Statistical analysis

All data in the tables and figures are displayed as means ± standard deviations. To test the main effects and interactions of elevated O₃ and wheat cultivars on soil neutral sugars, general linear model for split-plot design was performed, with O₃ and cultivars as fixed factors and replicate as random factor. The t-test was conducted to compare the differences of soil neutral sugars between ambient and elevated O₃ treatments of different wheat cultivars at each growth stage. All statistical analyses were performed by SPSS 16.0 statistical software (SPSS Inc. Chicago, IL). Difference at P < 0.10 level was considered to be statistically significant. Redundancy analysis (RDA) was selected to study the relationships between soil neutral sugars and soil environmental parameters using CANOCO software with a Monte Carlo permutation tests, because the lengths of gradient was less than 3 in the Detrended correspondence analysis (DCA) (ter Braak 1988). We did RDA with soil neutral sugars as dependent variables and the soil properties as explanatory variables. The Monte Carlo tests were based on random permutations of the data, and the results were accepted as significant at P < 0.05.

3. Results

3.1. Soil and plant parameters

After 3-yr exposure of the elevated O₃, most soil parameters did not vary obviously between two wheat cultivars (Table 1, P > 0.10). The DOC was the only parameter that showed response to both treatment and cultivar effects. At the wheat ripening stage, elevated O₃ decreased the content of DOC by 28.8% in O₃-sensitive wheat (Y19) (Table 1, P < 0.05). Higher content of DOC was observed in the O₃-tolerant wheat under both ambient and elevated O₃ conditions (Table 1, P < 0.05). In comparison with the ambient O₃, elevated O₃ reduced the content of SOC by 15.2% at the wheat joining stage in the O₃-tolerant wheat (Table 1, P < 0.10). At the ripening stage, elevated O₃ increased the ratio of MBC/SOC in the two wheat cultivars (Fig. 1, P < 0.05).

No cultivar effects were observed in the crop yield under both ambient and elevated O₃ conditions (Table 1, P > 0.10). Elevated O₃ decreased the yield by 19.2% and 15.7% in the O₃-sensitive and O₃-tolerant wheat, respectively (Table 1, P < 0.001). No obvious treatment and cultivar effects were observed in the plant C and N (Table 1, P > 0.10).

3.2. Soil neutral sugars contents

Elevated O₃ significantly decreased the contents of total neutral sugars at the wheat jointing and ripening stages (Fig. 2, P < 0.10). However, no obvious cultivar effects were observed in the contents of total neutral sugars (Fig. 2, P > 0.10). Among the 8 individual neutral sugars, Glu was the dominant monosaccharide, followed in a decreasing order by Xyl-Gal-Ara-Man and Rha whereas Fuc and Rib were minor constituents of the soil neutral sugars determined (Fig. 3).

Individual neutral sugars showed different response to elevated O₃ or wheat cultivars (Fig. 3). At the wheat jointing stage, elevated O₃ decreased the content of Glu in the two wheat cultivars (Table 2, Fig. 3, P < 0.01). Significant interactive effects between elevated O₃ and wheat cultivar were observed in the contents of Fuc and Rib.
Elevated $O_3$ increased the content of Fuc in $O_3$-sensitive wheat, and decreased the contents of Fuc and Rib in $O_3$-tolerant wheat (Table 2, Fig. 3, P < 0.10).

At the wheat ripening stage, significant interactive effects were observed in individual neutral sugars, except that of Rha (Table 2, P < 0.10). Elevated $O_3$ decreased the contents of Glu-Fuc and Rib and increased the contents of Gal-Ara and Man in the $O_3$-tolerant wheat; and decreased the contents of Xyl-Gal and Ara in the $O_3$-sensitive wheat (Table 2, Fig. 3, P < 0.10).

3.3. Ratios of individual neutral sugars

No obvious treatment and cultivar effects were observed in the ratios of different neutral sugars at the wheat jointing stage Table 3. At the wheat ripening stage, significant interactive effects between elevated $O_3$ and wheat cultivars were found in the ratios of $(\text{Man} + \text{Gal})/(\text{Ara} + \text{Xyl})$ and $\text{Man}/(\text{Ara} + \text{Xyl})$, which increased with elevated $O_3$ in the two wheat cultivars. The ratios of $(\text{Man} + \text{Gal})/(\text{Ara} + \text{Xyl}), \text{Man}/(\text{Ara} + \text{Xyl})$ and $\text{Man}/\text{Xyl}$ in $O_3$-tolerant wheat were significantly lower than those in the $O_3$-sensitive wheat under the elevated $O_3$ condition ($t$-test, $P < 0.05$).

3.4. Correlation between soil neutral sugars and soil physicochemical parameters

Among soil environment factors we tested, $O_3$ treatment ($P = 0.016$) was the most important parameter which contributed to the distribution of total neutral sugars, and then were TNSC/SOC, SOC, cultivar and MBC/SOC (Fig. 4, P < 0.05). The RDA analysis showed that total neutral sugars were negatively correlated with elevated $O_3$ and MBC/SOC, and positively correlated with DOC and TNSC/SOC. The eigen values for the first and second axis were 0.705 and 0.201, respectively. The first axis explained 75.0% of the species-environment relation ($P = 0.014$), and the two axes explained 96.4% of the species-environment relation ($P = 0.008$).

4. Discussion

4.1. The effects of elevated $O_3$ on total soil neutral sugars

The accumulation of neutral sugars in soil is a net increment between continuous production and decomposition processes. In present study, elevated $O_3$ reduced the content of total neutral sugars in the two wheat cultivars. The contents of plant derived neutral sugars Xyl and Ara were also decreased following the elevated $O_3$ in the $O_3$-sensitive cultivar at the wheat ripening stage. The decrease in soil neutral sugars might result from the decline of $C$ input. Our results also showed that soil neutral sugars were positively correlated with DOC. Following the $O_3$ exposure, decreased $C$ assimilation from aboveground led to the low availability of $C$ exporting to roots. At the same time, the repair processes of plant and the synthesis of antioxidants increased $C$ demand in leaves and then reduced the $C$ allocation belowground (Zheng et al., 2002; Zheng et al., 2002).

Another reason for the changes of soil neutral sugars might be due to a fast decomposition of soil organic C. Pascual et al. (1997) suggested that the ratio of MBC/SOC represents the mineralization potential of SOM. The lower the ratio of MBC/SOC, the lower was the tendency of SOM to be mineralized. Our results found that the contents of SOC decreased and the percentage of MBC/SOC increased following the elevated $O_3$. These findings might indicate the high decomposition of SOM under elevated $O_3$ conditions.

4.2. Effects of elevated $O_3$ on the plant and microbial derived neutral sugars

Soil neutral sugars included plant and microbial derived origins and could indicate the plant-microbial relationship in the SOM turnover. Oades (1984) reported that the ratio of plant-derived to microbe-derived carbohydrates is plant-derived dominant when the ratio is lower than 0.5 or microbe-derived dominant when the ratio is higher than 2. At the wheat ripening stage, the higher ratios of $(\text{Man} + \text{Gal})/(\text{Ara} + \text{Xyl})$ and $\text{Man}/(\text{Ara} + \text{Xyl})$ in our study suggested that elevated $O_3$ promoted the relative contribution of microbial process to the accumulation of soil carbohydrates.

On the other hand, the extent of the elevated $O_3$ effects on the origins of plant and microbial derived carbohydrates varied between different wheat cultivars. Relatively higher ratios of $(\text{Man} + \text{Gal})/(\text{Ara} + \text{Xyl}), \text{Man}/(\text{Ara} + \text{Xyl})$ and $\text{Man}/\text{Xyl}$ were observed in $O_3$-sensitive wheat. The greater $O_3$ damage to the $O_3$-sensitive wheat may result in fewer plant-derived carbohydrates allocation to the belowground. Cao et al. (2009) suggested that elevated $O_3$ decreased the net photosynthetic rate, stomatal conductance and transpiration rate, especially for the $O_3$-sensitive wheat. They also found that the extent of total soluble protein and rubisco changes were larger in $O_3$-sensitive wheat than those in $O_3$-tolerant wheat. These changes in aboveground plants could result in changes in the quantity and quality of plant litters and then influence the relative contributions of plant derived carbohydrates to SOM. In addition, our results also suggested that the microbe-derived carbohydrates were dominant contribution to the total carbohydrates and SOM under elevated $O_3$ conditions. Soil microorganisms are responsible for SOM turnover and nutrient cycling. Our previous study in the same experiment also confirmed that elevated $O_3$ promoted the relative contribution of bacteria to SOM turnover (Zhang et al., 2014). These changes in the accumulation and origins of soil carbohydrates will influence the accumulation and decomposition of SOM and soil ecosystem functioning in agroecosystem.

5. Conclusions

Elevated $O_3$ decreased soil total neutral sugars and altered the relative contribution of plant- and microbe-derived carbohydrates to SOM. Wheat cultivar is important in determining the relative contribution of plant-microbial relations in SOM, with microbial process dominant in the $O_3$-sensitive wheat. These changes in the accumulation and origins of soil carbohydrates may affect the cycling and transformation of $C$ and other nutrients in agroecosystem.

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