Effects of elevated CO_2 and O_3 on soil amino sugar from wheat straw decomposition in a meadow brown soil of Northeast China

Caiyan Lu[A], Yi Shi[A] and Mingfen Niu[B]

[A]Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, PR China, Email microyan76@126.com, shiyi@iae.ac.cn
[B]Shenyang Jianzhu University, Shenyang 110168 , China, Email hj_nmf@sjzu.edu.cn

Abstract
Amino sugars, being predominantly of microbial origin, can help elucidate the role of microbes in decomposition of crop residues in soil. A 12-month CO_2 and O_3 enrichment field experiment was conducted in open-top chambers in Shenyang suburb of Northeast China to study the dynamic changes of soil amino sugar during the decomposition of spring wheat straw. Compared with an ambient treatment, a significantly higher amount of soil glucosamine (GluN), galactosamine (GalN), and muramic acid (MurA) was observed in treatment elevated O_3 across the 12 months and in treatment elevated CO_2 in the first 4 months, which illustrated the stimulation effects of elevated O_3 and CO_2 on the proliferation of soil microbes. The GluN/MurA ratio under elevated O_3 increased and decreased under elevated CO_2, suggesting that elevated O_3 favoured the dominance of fungi decomposition of spring wheat, while elevated CO_2 stimulated the bacterial population and its decomposition.

Key Words
Elevated CO_2 and O_3; amino sugar; wheat residue decomposition; meadow brown soil.

Introduction

Amino sugar is a major constituent of microbial cell wall (Turrión *et al.* 2002; Roberts *et al.* 2007). Its amount in soil can be used to estimate soil microbial mass (Amelung 2001; He *et al.* 2006). Soil glucosamine (GluN) is the dominant component of soil amino sugar, and exists in fungal cell wall. Muramic acid (MurA) uniquely originates from terrestrial bacteria (Amelung 2001), and galactosamine (GalN) has dubious microbial origins. The summation of these three amino sugars represents soil microbial mass, and the relative abundance of MurA and GluN has been successfully used to assess the relative contribution of soil bacteria and fungi to the turnover of organic matter in many soils (Zhang *et al.* 1998; Amelung *et al.* 2001; Dai *et al.* 2002; Glaser *et al.* 2004; 2006; Liang et al, *et al.* 2007, 2008). Elevated CO_2 and O_3 have definite effects on soil microbial community structure (Hu *et al.* 2006; Shi *et al.* 2006; Yue *et al.* 2007; Van Groenigen *et al.* 2007; Kanerva *et al.* 2008). Glaser *et al.* (2006) investigated the effects of elevated pCO_2 on the bacterial and fungal-derived C in Lolium perenne pasture soil by using compound-specific isotope analysis ($\delta^{13}C$) of soil amino sugar. Van Groenigen *et al.* (2007) studied the effects of elevated CO_2 on the fungal decomposition pathway by quantifying the contents of soil GluN, MurA, and Gal N in three terrestrial ecosystems, but few studies were made on the effects of elevated O_3 on soil microbial community by soil amino sugar analysis. Returning wheat straw to farmland is an important agricultural practice in China. To understand the effects of elevated CO_2 and O_3 on the relative contribution of soil bacteria and fungi to the turnover of amended wheat straw, a 12-month CO_2 and O_3 enrichment experiment with meadow brown soil, an important agricultural soil in Northeast China, was conducted, using the summation of GluN, MurA and GalN to represent soil microbial mass, and the GluN/MurA ratio to characterize the relative contribution of soil fungi and bacteria to the decomposition of amended wheat straw.

Methods

Study site and treatments

The enrichment experiment was conducted in open-top chambers (OTCs, 3 m in diameter and 2.8 m in height) at the National Field Observation and Research Station of Agro-ecosystems in Shenyang suburb of Northeast China (41°31′N, 123°24′E). The annual mean air temperature is 7-8 °C, annual mean precipitation is 700 mm, and frost-free period is 147-164 d. Meadow brown soil is the main soil type for agricultural production. Three treatments were installed, i.e., control (ambient, ~342 μmol CO_2/mol and ~40 nmol O_3/mol), elevated CO_2 (550 μmol CO_2 /mol), and elevated O_3 (80 nmol O_3 /mol). They were tri-replicated, and arranged in a randomized complete block design. CO_2 was provided 24 h/d, and O_3 was provided 8 h at day time (08:00-12:00 and 14:00-18:00) from 27 April to 27 June. The CO_2 and O_3 concentrations in OTCs

were measured by ES-D infrared CO_2 sensor and S-900 O_3 analyser, with the variance being ± 4 and ± 9%, respectively (Ruan *et al.* 2006). Chinese spring wheat (Triticum aestivum L. cv. Liaochun 17) was sown in pots (23 cm in diameter and 20 cm in height) on 2 April, and harvested on 30 June 2006. 20 plants at three-leaf stage were established in each pot. The pots were watered periodically to prevent water deficit. Ammonium-based N fertilizer and P fertilizer were applied as basal at 150 kg N hm^{-2} and 10 kg P hm^{-2}, respectively. Each OTC contained 25 pots.

Sample preparation
Soil samples were taken from the pots after spring wheat harvested, and air-dried and sieved to < 2 mm, with their properties listed in Table 1. The harvested aboveground part of spring wheat was crushed into ~1 mm pieces, and mixed with 150 g soil at a rate of 3% of dry soil. The soil-wheat mixture was adjusted to 60% water-holding capacity, and placed into 300-mesh sieve nylon bags. A total of 144 bags were buried at 10 cm depth in 9 OTCs on 7 July 2006. In 2007, the bags were exposed to elevated CO_2 and O_3 during spring wheat growth period, and two bags per OTC were sampled at the 0, 1st, 2nd, 3rd, 4th, 9th, 10th, 11th and 12th month (i.e. 7 July, 11 August, 11 September, 8 October and 8 November 2006, and 9 April, 5 May, 8 June and 9 July 2007, the 5th- 8th month was frozen period) for amino sugar analysis, respectively.

2.3 Amino sugar extraction and derivatization. Soil amino sugar was extracted and purified according to Zhang and Amelung (1996). Briefly, 100 μg myoinositol was added as an internal standard to the samples containing about 0.3 mg N. The samples were hydrolyzed with 10 mL 6 M HCl at 105°C for 8 h, and the released amino sugar was separated from impurities by neutralization (pH 6.6-6.8) with 0.4 M KOH. Before derivatization, 100 μg N-methyl-glucamine was added as a recovery standard.

The aldononitrile derivatives of amino sugar were prepared according to Guerrant and Moss (1984). Samples were dissolved in 0.3 mL derivatization reagent, and heated at 75-80°C for 30 min. After acetylation with 1 mL acetic anhydride at 75-80°C for 20 min, dichloromethane was added, and excess derivatization reagent was removed by washing with 1 mL 1 M HCl for four times, air-dried at ambient temperature, and finally dissolved in 0.3 mL ethyl acetate-hexane (1:1 v/v). The aldononitrile derivatives were analysed on an Agilent 6890 gas chromatograph equipped with an Agilent DB-5MS (30 m × 0.25 m × 0.25 μm) column and a flame ionization detector. The optimized carrier gas flow and oven temperature programs for successful separation of aldononitrile derivatives on the DB-5MS chromatograph column were as follows: 0.8 mL/min constant gas flow, 1.0 μL injection volume at 1:10 split ratio, 250 and 120°C injector and oven temperatures, respectively, at injection time. For further details on temperature program, see Zhang and Amelung (1996).

Data analysis
All data were analysed by repeated measures ANOVA. The metadata were analysed by using SPSS 13.0 and Excel 2003 software.

Results
During 12 months decomposition of spring wheat straw, the average summation of soil GluN, GalN and MurA across all treatments was 813-1234 mg/kg soil. A significantly higher summation of soil GluN, GalN, and MurA was observed in treatment elevated O_3 from the 2nd month to the end of the experiment, and in treatment elevated CO_2 from the 2nd to the 4th month, compared with that in treatment ambient ($p<0.05$, Figure 1). GluN was the dominant amino sugar and generally mirrored the total pattern of soil amino sugar (Figure 1). For example, GluN, GalN, and MurA contributed 68.0%, 29.7%, and 2.3% in treatment elevated O_3, 67.0%, 28.9%, and 4.2% in treatment elevated CO_2, and 62.8%, 34.0% and 3.2% in treatment ambient, respectively, which were similar to the findings of other studies (Glaser *et al.* 2006; Van Groenigen *et al.* 2007).

The average amount of GluN in treatments elevated CO_2 and O_3 was significantly higher than that in treatment ambient ($p<0.05$). Elevated O_3 significantly increased the average amount of GalN and but decreased that of MurN, compared with elevated CO_2 ($p<0.05$). The ratio of GluN to MurA has been successfully used to track the relative contributions of fungi and bacteria to the decomposition of spring wheat straw. In present study, the GluN/MurA ratio increased by 47.9% under elevated O_3, but decreased by 20.7% under elevated CO_2, compared with that under ambient (Figure 2), suggesting that elevated O_3 favoured the dominance of fungi decomposition of spring wheat, while elevated CO_2 stimulated bacterial population and its decomposition.

Table 1. Chemical properties of test soil.

Treatments	TOC (g/kg)	Total N (g/kg)	C/N	GluN (mg/kg)	GalN (mg/kg)	MurA (mg/kg)	Total amino sugar (mg/kg)
Ambient	10.01	1.21	8.3	586.0	276.6	31.8	894.4
CO_2	10.18	1.18	8.6	586.1	272.6	35.3	894.0
O_3	9.94	1.27	7.8	629.0	271.8	24.2	925.0

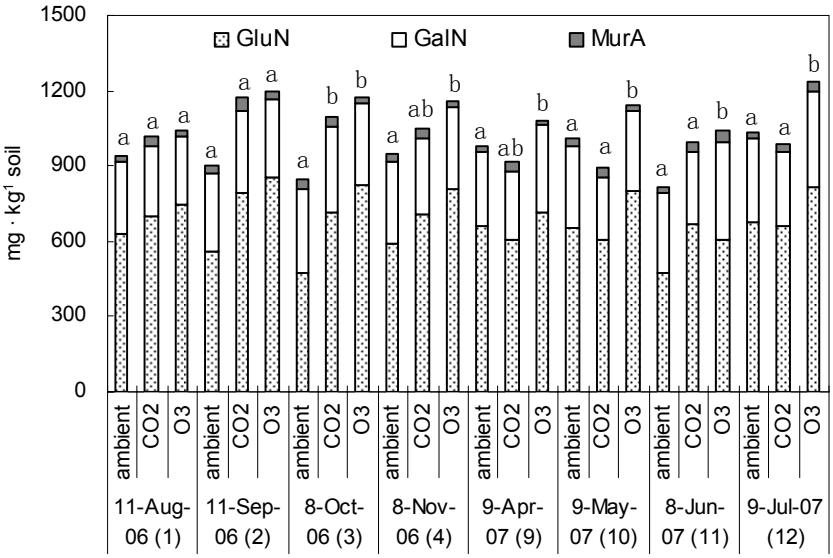

Figure 1. Amount changes of soil GluN, GalN, and MurA over time. Data are the means ± standard error of three replicates. Within a given date, the means with the same letter were not significantly different at P >0.05.

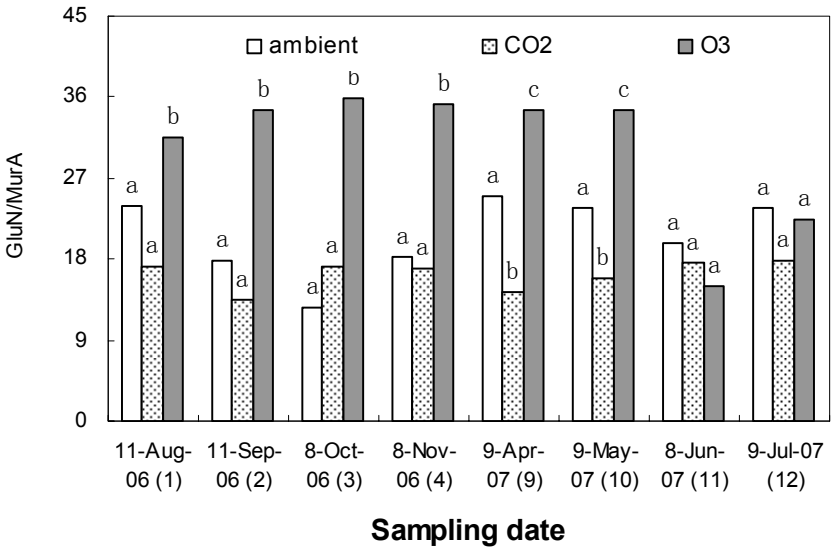

Figure 2. Ratio changes of GluN/MurA over time. Data are the means ± standard error of three replicates. Within a given date, the means with the same letter were not significantly different at P >0.05.

Conclusion

The increased summation of soil GluN, GalN and MurA in treatments elevated O_3 and CO_2 illustrated the stimulation effects of elevated O_3 and CO_2 on the proliferation of soil microbes. The larger GluN/MurA ratio under elevated O_3 than under elevated CO_2 suggested that elevated O_3 favoured the dominance of fungi over bacteria, while elevated CO_2 stimulated bacterial population and its decomposition.

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