PROMOTED AMINO SUGAR ACCUMULATION IN THE RHIZOSPHERE AND BULK SOIL OF A PADDY FIELD UNDER STRAW APPLICATION

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Abstract. Effects of straw application on amino sugars (a significant soil organic matter pool) in upland fields have received much attention, however, those in paddy fields remain unknown. We conducted a three-year experiment including three treatments: one without straw application (CK) and straw application at rates of 2500 (SA1) and 5000 (SA2) kg ha⁻¹ yr⁻¹. SA2 stimulated a higher increase in glucosamine (GluN), muramic acid (MurA), galactosamine (GalN), fungal-derived GluN (GluNF), and total amino sugars than SA1 in the rhizosphere and bulk soil in the first year. Generally, the positive effects of SA1 and SA2 on amino sugars did not differ in the last two years. Rhizosphere soil maintained a significantly higher content of MurA at 10.95%–16.35% than bulk soil. In total, rhizosphere soil accumulated insignificantly more GluN, GluNF, and total amino sugars than the bulk soil, indicating a limited positive effect of the rhizosphere on microbial-derived carbon. SA1 and SA2 notably increased the ratio of GluNF/MurA in bulk soil, implying that straw application increased the relative contribution of fungal-derived residues to soil organic carbon. This work may contribute to understanding the effect of straw applications on microbial residues and their contribution to soil organic carbon in paddy fields.

Keywords: amino sugars, straw application, microbial residue, soil organic carbon, paddy soil

Introduction

Agricultural soil contains large amounts of organic carbon (C), variations of which is critical concerning climate change and the sustainable development of agriculture (Manlay et al., 2007). Soil organic C (SOC) is very susceptible to the activity of soil microorganisms, which play a decisive role in regulating SOC (Chaparro et al., 2012). Microbial biomass and microbial residues are effective indicators for assessing the contribution of microbial processes to SOC accumulation (Liang et al., 2017). Although microbial biomass is sensitive to environmental changes (Koponen and Baath, 2016) and drives SOC turnover, microbial residues reside much longer in the soil than living microbial biomass and are believed to be a significant source of the stable C pool (Simpson et al., 2007). In the context of agriculture, soil requires SOC sequestration to maintain fertility, and better knowledge on the contribution of microbial residues to SOC is imperative.

Straw application not only proves to be highly efficient for SOC accumulation (Liu et al., 2014; Zhao et al., 2018), but could also lead to simultaneous variations in soil microbial communities and biomass (Chen et al., 2017a). However, the majority of previous studies on soil microorganisms have concentrated on the effects of straw
application on microbial biomass and community composition (Hurisso et al., 2013; Fan et al., 2016; Zhao et al., 2016; Chen et al., 2017a; Wang et al., 2018). Information on the effects of straw application on microbial residues remains scarce (Simpson et al., 2004; Liu et al., 2019). The dynamics of microbial residues and their contribution to SOC can be examined and quantified by soil amino sugars (Zhang et al., 1999) due to their high abundance in microbial cells (>90%) compared to dead microbial cells (Amelung et al., 2001) and their stability against fluctuations (Glaser et al., 2004). Thus, the analysis of amino sugars could provide insight into the fate and sequestration of C and N in microbial residues and also into long-term shifts in soil organic matter quality (Joergensen et al., 2010). Among various amino sugars, galactosamine (GalN) accounts for a significant fraction of the total amino sugar pool (Glaser et al., 2004), but its origin in soil is still debated (Engelking et al., 2007). Glucosamine (GluN) originates mainly from the chitins of fungal cell walls, though a small amount originates from bacterial peptidoglycan and exoskeletons of soil invertebrates (Amelung et al., 2001; Joergensen, 2018). Muramic acid (MurA) is a unique bacterial residue biomarker because it originates exclusively from the peptidoglycans of bacterial cell walls (Engelking et al., 2007). As a result of the different origins of GluN and MurA, their ratios (GluN/MurA) have been widely used to evaluate the relative contribution of fungal- and bacterial-derived residues to SOC (van Groenigen et al., 2010).

Although some studies have investigated the effects of straw application on amino sugars in agricultural soil (Ding et al., 2013, 2015; Liu et al., 2019), they did not differentiate the effects of straw application between rhizosphere and bulk soil. By root exudates, plant roots play a vital role in regulating rhizosphere microecosystems, such as shaping rhizosphere microbiota (Wang et al., 2012; Hu et al., 2018; Zhlnina et al., 2018) and stimulating nitrogen (N) transformation (Yin et al., 2013). The effect could be appear as impacting rhizosphere bacterial communities associated with shifts in carbon metabolism (Staley et al., 2017), and facilitating or inhibiting SOC decomposition (Cheng et al., 2014). At the end, the accumulation of amino sugars in the rhizosphere and their contribution to SOC could be further influenced. An adequate description of the accumulation of amino sugars in the rhizosphere contributes to accurately evaluating the contribution of microbial residues to SOC. However, comparatively few studies have focused on amino sugar accumulation in the rhizosphere of agricultural systems as compared to bulk soil. In addition, straw application affects not only microbial biomass and community structure (Li et al., 2012; Wang et al., 2018; Jin et al., 2019; Yang et al., 2019), but also root biomass (Ma et al., 2019) and growth (Gao et al., 2018). These factors may all further alter the accumulation of amino sugars in the rhizosphere soil directly or indirectly.

Moreover, previous studies on the effects of straw application on amino sugars have focused on upland fields (i.e., maize fields, mung bean fields) (Praveen et al., 2002; Liang et al., 2007; Ding et al., 2015; Liu et al., 2019), while the effects on paddy fields remain mostly unexplored. Paddy fields in Northeast China provide the second largest grain yield in this region with large amounts of rice straw available after harvesting. In recent years, it was recommended to return rice straw to the field for maintaining fertilization and contributing to sustainable agriculture instead of conventional burning to avoid environmental pollution. The objective of this study is to reveal the effect of straw application on the amino sugar accumulation in the rhizosphere and bulk soil of paddy fields.
Materials and Methods

A three-year experiment from October 2016 to August 2019 was conducted in Daan City (123°08′45″-124°21′56″E, 44°57′00″-45°45′51″N), Western Jilin province, Northeast China. The weather of study site is typical of a semi-arid and semi-humid continental monsoon climate. The average annual temperature is 4.3 °C, and the average annual precipitation is about 414 mm. The basic properties of the soil before the trial are shown in Table 1.

Table 1. Soil properties at 0–15 cm depth of the experimental site (October 2016)

<table>
<thead>
<tr>
<th>SOC (g kg⁻¹)</th>
<th>Bulk Density (g cm⁻³)</th>
<th>Total N (g kg⁻¹)</th>
<th>Total P (g kg⁻¹)</th>
<th>Total K (g kg⁻¹)</th>
<th>Hydrolysable-N (mg kg⁻¹)</th>
<th>pH</th>
<th>Particle size distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.31</td>
<td>1.23</td>
<td>1.56</td>
<td>0.35</td>
<td>18.90</td>
<td>105.63</td>
<td>7.68</td>
<td>sand</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sand</td>
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</tbody>
</table>

Crop management

The growth period of rice lasts from about mid-May to the end of September. For the rest of the year, the fields lie fallow. Before 2016, conventional tillage without straw application was practiced for over 25 years.

The rice seeds were sown in the greenhouse for nursery raising and seedlings were transplanted manually to the field at 30 × 18 cm plant spacing. The same fertilization rates and timing for all treatments were applied during 2017, 2018, and 2019. 150 kg N ha⁻¹ (urea) was applied, 20% of which was for base fertilization, 50% of which was for the tillering period, and 30% of which was for the heading period. 100 kg P₂O₅ ha⁻¹ (superphosphate) and 60 kg K₂O ha⁻¹ (potassium chloride) were applied as base fertilizers. 30 kg K₂O ha⁻¹ were applied during the heading period.

Experiment design

The experiment consisted of three treatments: 1) no straw application (CK); 2) straw application at a rate of 2500 kg ha⁻¹ year⁻¹ (SA1); 3) straw application at a rate of 5000 kg ha⁻¹ year⁻¹ (SA2). All the aboveground plant residues in all the plots were removed after the annual harvest. The rice straw was chopped into 5-cm-long pieces and evenly strewed onto the soil surface in SA1 and SA2 treatments by hand, then integrated into the 0–20-cm layer using a shovel and followed by raking after harvest in 2016, 2017, and 2018. The 0–20-cm soil layer in the CK treatment was mixed using a shovel and raking in the same way and at the same time as in the SA1 and SA2 treatments. The treatments were arranged in a randomized complete block design with three replicates. Each plot measured 2.56 m² (1.6 m × 1.6 m), and the space between adjacent plots was at least 1 m. Chambers (1.6 m × 1.6 m × 0.55 m) without top and bottom made of polymethyl methacrylate for the plots were pressed into the soil to a depth of 40 cm to minimize the effects from outside the plots.

Sampling and measurements

Soil samples were collected during the heading period in 2017, 2018, and 2019. Three rice plants were randomly selected in each plot, and rice plants in one row on each side of all plots were not collected to avoid border effects. Soil in between 0–15 cm depth
5–10 cm distance from the rice shoot in the horizontal direction was collected as the bulk soil. Rice plants and the soil within 5 cm from their shoot in the horizontal direction were dug out to a depth of 15 cm. Rhizosphere soil was collected from soil adhering to the roots after they were thoroughly shaken. After visual plant residues and rocks were removed, soil samples were air dried, ground, and passed through a 0.25-mm sieve for SOC and amino sugar analyses.

Amino sugars were determined according to the method described by Zhang and Amelung (Zhang and Amelung, 1996). Briefly, soil samples (containing about 0.3 mg N) with 10 ml of 6 M HCl were heated for 8 h at 105 °C. After cooling, the hydrolysate with the addition of 100 μl inositol solution (1 μg ml⁻¹) was filtered. The filtrate was dried, and the residue was dissolved with distilled water and adjusted to a pH of 6.6–6.8. The solution was centrifuged and dried again. The residue was dissolved with methanol and centrifuged. The supernatant was transferred to a derivative bottle and dried using N₂ gas at 45 °C. Amino sugars were determined by gas chromatography with a flame ionization detector (Agilent 6890, Agilent Technologies, Inc., USA) after their conversion to aldononitrile acetates. The concentrations of total amino sugars were calculated as the sum of GluN, GalN, and MurN.

Previous studies showed that the ratio of MurN and GluN from bacteria is 1:2, therefore, the fungal-derived GluN (GluNF, mg kg⁻¹) can be calculated according to Eq. 1 (Engelking et al., 2007).

\[
\text{GluNF} = \left( \text{GluN} \frac{179}{251} - 2 \times \text{MurN} \frac{251}{251} \right) \times 179
\]  

(Eq. 1)

where GluN, MurN, and GluNF are the concentrations of glucosamine, muramic acid, and fungal glucosamine, respectively. The molecular weight of GluN and MurN is 179 g mol⁻¹ and 251 g mol⁻¹, respectively.

**Statistical analysis**

The differences between treatments and different years were tested with repeated measures analysis of variance using SPSS 22.0 (International Business Machines Corporation, New York, USA). All figures were drawn using Sigmaplot 12.5 (Systat Software Inc. San Jose, CA, USA).

**Results**

In CK and SA2, the contents of MurA, GalN, GluN, GluNF, total amino sugars, and GluNF/MurA did not differ during the trial in both rhizosphere and bulk soil (Fig. 1, Fig. 2, and Fig. 3). Although, the contents MurA, GalN, GluN, GluNF, and total amino sugars in SA1 treatment were increased from 2017 to 2018, no significant difference was observed between 2018 and 2019 at the rhizosphere or bulk soil. The rhizosphere soil of SA1 and SA2 in 2018 and 2019 did not differ with respect to the contents of MurA, GluN, GluNF, and total amino sugars, respectively, and the same was true for bulk soil.

The content of MurA in the rhizosphere soil of CK, SA1, and SA2 ranged from 42.65–44.39, 48.45–56.84, and 52.98–56.46 mg kg⁻¹ during the trial, which was significantly higher by 10.95%–13.21% (4.21–5.18 mg kg⁻¹), 13.52%–16.14% (5.77–7.90 mg kg⁻¹), and 12.99%–16.35% (6.44–7.83 mg kg⁻¹) than that in bulk soil, respectively (Fig. 1). The content of MurA in the rhizosphere and bulk soil of CK in 2017,
2018, and 2019 was significantly lower than that of SA1 and SA2, respectively. Compared to the content of MurA in the rhizosphere soil of CK (42.65 mg kg\(^{-1}\)) and SA1 (48.45 mg kg\(^{-1}\)) in 2017, that of SA2 increased significantly by 24.22\% and 9.35\%, respectively. The rhizosphere and bulk soil of SA1 in 2019 maintained a significantly higher content of MurA than that in 2017, respectively.

![Figure 1](image.png)

**Figure 1.** Variations of muramic acid (MurA) and galactosamine (GalN) in different treatments from 2017 to 2019. Different uppercase letters indicate significant differences between the same treatments in the different year, whereas different lowercase letters indicate significant differences among different treatments in the same year (P<0.05, multiple comparison test by least significant difference). **RS:** rhizosphere soil; **BS:** bulk soil; **CK:** no straw application; **SA1:** straw application at a rate of 2500 kg ha\(^{-1}\) year\(^{-1}\); **SA2:** straw application at a rate of 5000 kg ha\(^{-1}\) year\(^{-1}\). The same below.

The content of GalN in rhizosphere soil in the CK treatment ranged from 238.74–253.65 mg kg\(^{-1}\) during the trial, which was in general lower by 9.33\%–10.31\% (26.1–28.42 mg kg\(^{-1}\)) than that in bulk soil (**Fig. 1**). The values of GalN in rhizosphere and bulk soil in SA1 and SA2 peaked in 2019. Compared to the content of GalN in the rhizosphere and bulk soil of CK in 2019, that of SA1 increased by 149.35 mg kg\(^{-1}\) and 89.94 mg kg\(^{-1}\), and that of SA2 increased by 141.46 mg kg\(^{-1}\) and 87.74 mg kg\(^{-1}\). In SA1 and SA2, rhizosphere soil contained notably higher contents of GalN than bulk soil in 2018, and 2019, respectively.

Rhizosphere and bulk soil in the same treatment and year did not differ overall in GluN and GluN\(_F\). The rhizosphere soil in all treatments contained 38.69–60.15 mg kg\(^{-1}\) more GluN and 27.52–50.86 mg kg\(^{-1}\) more GluN\(_F\) than the bulk soil (**Fig. 2**). At the end of the experiment, the soil content found of GluN and GluN\(_F\) in the rhizosphere soil were higher by 192.36 mg kg\(^{-1}\) and 174.59 mg kg\(^{-1}\) at SA1 treatment, as well as by 208.78 mg kg\(^{-1}\) and 191.58 mg kg\(^{-1}\) at SA2 treatment than at CK treatment. Bulk soil in SA1 and SA2 in 2019 contained 201.05 mg kg\(^{-1}\) and 217.72 mg kg\(^{-1}\) more GluN, and 16 mg kg\(^{-1}\) and 202.39 mg kg\(^{-1}\) more GluN\(_F\) than the bulk soil of CK.
Total amino sugars among CK, SA1, and SA2 in 2017 varied significantly in both rhizosphere and bulk soil. It followed the order: SA2 (1224.95 mg kg\(^{-1}\), RS; 1140.49 mg kg\(^{-1}\), BS) > SA1 (1082.09 mg kg\(^{-1}\), RS; 1025.36 mg kg\(^{-1}\), BS) > CK (932.91 mg kg\(^{-1}\), RS; 901.36 mg kg\(^{-1}\), BS) (Fig. 3). SA1 and SA2 in 2019 maintained significantly higher contents of total amino sugars by 346.28 mg kg\(^{-1}\) and 370.19 mg kg\(^{-1}\) in the rhizosphere soil and by 298.53 mg kg\(^{-1}\) and 318.41 mg kg\(^{-1}\) in the bulk soil than in the CK treatment, respectively. Just at the SA1 treatment, the content of total amino sugars in either the rhizosphere or bulk soil was notably higher in 2019 than in 2017.
The values of GluNF/MurA in rhizosphere soil among the three treatments in the same year did not differ (Fig. 3). There were no significant differences in the values of GluNF/MurA among 2017, 2018, and 2019 in the rhizosphere soil in each of the three treatments. The values of GluNF/MurA in the bulk soil of SA1 and SA2 in 2017 were not significantly different from and, in 2019, markedly higher than those in the CK.

Discussion

Effects of straw application on amino sugar fractions

The present results demonstrate that straw application facilitated the accumulation of amino sugars (GalN, MurA, GluN, GluNF, and total amino sugars) in both the rhizosphere and bulk soil in rice fields, which was similar to the results reported for upland fields (Ding et al., 2013, 2015; Liu et al., 2019). The accumulation of amino sugars was ascribed to microbial biomass turnover as well as to the balance of microbial residue production and decomposition (Ding et al., 2013, 2015). Straw provided more available substrates and, therefore, maintained a higher microbial biomass (Chen et al., 2017b). Additionally, sugars, amino acids, and other nutrients risen from rice straw decomposition can favor a greater microbiology activity in the soil (Blagodatskaya and Kuzyakov, 2008). With continuous microbial reproduction and mortality, microbial cell wall components, such as amino sugars, are gradually released and subsequently accumulated more in the SA1 and SA2 treatments than in the CK treatment. On the other hand, association of amino sugars in aggregates can facilitate the maintenance of aggregate cohesion and stabilization (Chantigny et al., 1997), which, thus, protecting amino sugars from decomposition and reducing its decomposition, and thereby, increasing the potential of amino sugar accumulation. Xue et al. (2020) found that straw incorporation drives the formation of SOC-Fe (oxyhydr) oxides associations in aggregates, which contribute to SOC (including amino sugars) stabilization in paddy soils. The SA2 treatment could provide more available substrates, nutrients, and microbial cell wall components and better stimulate the amino sugar mediated formation of aggregates, thus, leading to an excessive accumulation of amino sugars in 2017. The lack of pronounced differences in the content of amino sugars between SA1 and SA2 in both 2018 and 2019 suggests a threshold effect of straw incorporation on microbial residue build-up in the present paddy soil. The threshold effect might result from the “saturation” of the soil aggregates with amino sugars, indicating that microbial amino sugars could be decomposed, and that the prerequisite for amino sugars contributing to a stable C pool is their combination with aggregates (Craig et al., 2018).

Although the straw application to paddy and upland fields have increased amino sugar accumulation, the degree of this accumulation is different. After a three-year straw application, compared to the treatment without straw application, our results show that total amino sugar contents increased by 32.27-38.98%. However, Liu et al. (2019) and Ding et al. (2013) reported that the content of total amino sugars in upland fields increased by 8.37% and 1.23%, respectively. These differences were perhaps due to a variation in soil aeration. The better soil aeration in upland fields greatly facilitate the decomposition of SOC (Chen et al., 2017a) (including amino sugars), thus, leading to a slower accumulation of amino sugars. Soil erosion, aggregate breakdown (Hao et al., 2019), and decreased SOC contents (Yao et al., 2019) caused by rainfall were also responsible for the increased loss of amino sugars in upland fields. Continuous flooding conditions in
paddy fields provide anaerobic and relatively stable conditions (Chen et al., 2017a) to protect the amino sugars and aggregates from decomposition and physical destruction, respectively.

**Amino sugars in rhizosphere and bulk soil**

Our results showed that living rice roots change the accumulation of amino sugars in the rhizosphere. Root exudates (i.e., organic acids, amino acids), which are major components contributing to the rhizosphere effect, appear to shape the rhizosphere microbial community (Zhalnina et al., 2018). Rice root exudates provided not only available C for microbes (Lu et al., 2004), but also affected microbial biomass. Kong et al. (2008) found that rice root exudates resulted in a higher number of bacteria, actinobacteria, and fungi in rhizosphere than in bulk soil. Furthermore, rhizosphere aggregate stability was higher than that of non-rhizosphere soil (Caravaca et al., 2002). These aspects might favor the higher contents of MurA, GluN, and total amino sugars in the rhizosphere soil of our study. The significantly higher contents of MurA in the rhizosphere probably resulted from the fact that minor variations would lead to significant impacts on MurA because of its low content. The lack of pronounced differences in the contents of GluN and total amino sugars between rhizosphere and bulk soil were probably due to the fact that microbes had enough substrates to feed on both in rhizosphere and bulk soil. The net increase of GluN and total amino sugars caused in the rhizosphere soil was diminished when expressed on their large background in the present soil. This indicated a limited rhizosphere effect on the content of GluN and total amino sugars.

The factors that caused variations in the content of GalN appeared to be more complex. The GalN content in rhizosphere soil was lower in CK, but higher in SA1 and SA2 than that in bulk soil, respectively. Compared with MurA and GluN, little information is known about the main origin of GalN in soil (Amelung et al., 1999) and its function within microbial cells or as metabolites (Engelking et al., 2007). Engelking et al. (2007) concluded that fungi contribute larger percentages of GalN to the amino sugar pool than bacteria, which contradicted the viewpoint stated in earlier publications (Kogel and Bochter, 1985). Joergensen (2018) reported that GalN occurred mainly in bacterial extracellular polymeric substances, fungal extracellular polymeric substances, and fungal cell walls, but he did not quantify it. Ding et al. (2013) found that GalN accumulation patterns within aggregates were different from those of GluN or MurA. Therefore, further investigation is necessary to elucidate the origin and function of GalN as well as its dynamics in soil.

**Variations in GluN<sub>F</sub>/MurA**

Previous studies showed that GluN<sub>F</sub> accumulated mainly in coarse particulate organic matter and macroaggregates, and 79% of the total MurA pool accumulated in the clay fraction (Pronk et al., 2015). Straw incorporation had a more positive impact on the macro-aggregate (> 2000 μm) and mid-aggregate (250-2000 μm) fraction than on the micro-aggregate fraction (<250 μm) in paddy fields (Huang et al., 2017). This could be responsible for the significantly increased GluN<sub>F</sub>/MurA ratio in the bulk soil of SA1 and SA2 in 2018 and 2019 in the current study, indicating that straw application increased the relative contribution of fungal- over bacterial-derived residues to SOC. The insignificant variations in GluN<sub>F</sub>/MurA in the rhizosphere soil of all treatments was probably due to the regulation of the bacterial and fungal communities and/or aggregates by rice root
exudates instead of the litter amendments. Therefore, the rhizosphere likely mediated the relative contribution of fungal- and bacterial-derived residues to SOC and kept it stable. However, the related mechanisms remain unclear.

Conclusions

Amino sugar analysis revealed that three-year straw application enhanced the accumulation of MurA, GluN, GalN, GluNF, and total amino sugars in rhizosphere and bulk soil. A high rate of straw application led to a high amino sugar accumulation in the first year only. Aggregates tended to be “saturated” with amino sugars in the last two years, indicating a limited positive effect of straw application on amino sugar accumulation. In the rhizosphere soil, the accumulation of MurA was promoted, and the contents of GluN, GalN, GluNF, and total amino sugars tended to be higher than those in the bulk soil, suggesting a limited effect of the rhizosphere on GluN, GalN, GluNF, and total amino sugars. Straw application increased the relative contribution of fungal-derived residues to SOC in bulk soil and did not affect the relative contribution of fungal- and bacterial-derived residues to SOC in rhizosphere soil. This work may contribute to understanding the effect of straw applications on microbial-derived C and their contribution to SOC in paddy fields. Future research should concentrate more strongly on the nature and mechanisms of microbial residue–C process during long-term agricultural practices (e.g. fertilization, water management, tillage).

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