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Effect of nitrogen fertilization on the fate of rice residue-C in paddy soil depending on depth: ^{13}C amino sugar analysis

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Abstract

A 100-day incubation experiment was conducted to (i) trace the fate of rice residue-derived ^{13}C in the amino sugar (AS) pool in 0–1-cm (oxic) and 1–5-cm (anoxic) layers of paddy soil and (ii) evaluate the effects of inorganic N ($(NH_4)_2SO_4$) fertilization on the formation of AS at early and late incubation times (5 and 100 days, respectively). The accumulation of rice residue-derived AS occurred at 5 and 100 days in both soil layers as a result of AS stabilization. Inorganic N addition increased the contents of rice residue-derived muramic acid, glucosamine, and galactosamine in the 0–1-cm soil layer for both incubation times by average on 14.7–20.8%, 23.7–31.8%, and 11.6–23.3%, respectively. In contrast, no effects of N fertilization on AS content in the 1–5-cm soil layer were found. The amount of rice residue-derived AS was higher in the 1–5-cm than in the 0–1-cm soil layer at early incubation time, probably due to the higher contents of ammonium here compared to the upmost oxic layer where nitrate was the dominated N form. Thus, the preferential uptake of ammonium but not nitrate by microorganisms led to the higher formation of rice residue-derived AS in the anoxic soil layer. The ratio of fungal to bacterial residues (fungal glucosamine/muramic acid) ranged between 1.0 and 1.7 for rice residue-derived AS and was 12.5–14.6 for total AS, indicating that fungi and bacteria have similar contributions to the decomposition of fresh rice residue whereas native soil organic matter (SOM) is a fungi-predominated process. This study emphasized that coupling of C and N cycles in paddy soils is different in oxic and anoxic layers, resulting in variation of plant residue decomposition and formation of SOM.

Keywords Microbial biomarkers · Amino sugar · ^{13}C compound-specific isotope labeling · Inorganic N fertilization · Soil depth · Paddy soil

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Introduction

Paddy fields cover an area of approximately 165 million hectares worldwide (Lal 2004; Pan et al. 2004), accounting for 30% of total world production (http://beta.irri.org/statistics), with rice grown under flooded lowland conditions. Flooded paddy soil is not completely anoxic and has a special surface layer—"oxic"—due to the diffusion of dissolved O_2 from the overlying water to surface soil via soil pores (Frenzel et al. 1992; Kögel-Knabner et al. 2010; Liesack et al. 2000). The thickness of the oxic zone may range from several millimeters after flooding, or to several centimeters when the rice plants are fully grown (Frenzel et al. 1992). This surface layer has higher redox potential, water flux, and pH (Janssen and Lennartz 2007; Liesack et al. 2000), which makes it different from the underlying bulk soil. Such conditions stimulate (i) development of specific microorganisms (Liesack et al. 2000; Noll et al. 2005; Wu et al. 2014) and (ii) unique oxidative

processes of carbon (C) and nitrogen (N) in this zone compared to the anoxic belowground layers (Hoque et al. 2002; Inubushi et al. 2003).

It is commonly accepted that paddy soils have great potential to sequester organic C (Atere et al. 2017; Kögel-Knabner et al. 2010; Pan et al. 2004). Organic amendment, such as incorporation of rice residues, is an efficient practice to promote the soil organic matter (SOM) pool in paddy ecosystems (Li et al. 2010; Zhang et al. 2012). The transformation and fate of organic residues are largely determined by both catabolic and anabolic activities of microorganisms (Ding et al. 2010; Engelking et al. 2007; Rousk et al. 2016). Organic residues are decomposed by exoenzymes to monomers, which are later taken up by the living microorganisms with partial mineralization to CO_2 and assimilation into cell polymers, with final contribution to SOM in the form of microbial residues (Ding et al. 2010). One of the main microbial derived substances, which are stabilized in SOM, is amino sugars (AS) which can be used as indicators of microbial residues in soil (Amelung et al. 1999; Glaser et al. 2004); muramic acid (MurN) is used as an indicator of bacterial residue (Chantigny et al. 1997); glucosamine (GlcN) occurs in mainly fungal and bacterial cell walls (neglecting archaea and invertebrates), and a certain part might occur in extracellular polysaccharides (Chantigny et al. 1997; Glaser et al. 2004); the origin of galactosamine (GalN) as an indicator is not clear (Amelung et al. 2001; Engelking et al. 2007). Even if the microbial community composition of paddy soils was intensively studied before (Bai et al. 2015; Balasooriya et al. 2016; Ge et al. 2017; Li et al. 2017), the contribution of plant residue-derived C to SOM via microbial residues is not known.

Inorganic N fertilization, which is recommended to satisfy the microbial demand of mineral N, affects plant residue decomposition in paddy soils (Chen et al. 2010; Pan et al. 2009). The application of inorganic N could affect the composition of microbial communities in arable, grassland, and forest soils with different responses of bacteria and fungi, as reflected by AS and microbial biomass contents (Ding et al. 2010, 2013; Ge et al. 2017; Treseder 2008). However, little is known about the microbial communities participating in rice residue decomposition in response to addition of inorganic N in paddy ecosystems, which could be reflected by the alteration of individual AS.

The objective of this study was to evaluate the effects of (i) soil depth and (ii) inorganic N ($(NH_4)_2SO_4$) fertilization on the formation of AS from ^{13}C-labeled rice residues. The hypotheses were the following: (1) due to higher oxygen content in the top thin layer (0–1 cm), transformation of C and N will be higher here than in the lower layer of paddy soil; (2) due to the fact that the addition of inorganic N promotes microbial biomass development (Ge et al. 2017; Hoque et al. 2002; Zhong and Cai 2007), the total as well as rice residue-derived individual AS accumulations are expected to be higher in fertilized treatment with N addition compared to non-fertilized soil.

Methods and materials

Soil collection

Soil was collected in the fallow season from the 0–15-cm surface layer in Taoyuan County, Hunan Province of China (29° 15′ 22.0″ N and 111° 31′ 38.1 ″E), from a paddy field under single-rice management for at least 30 years. The soil was a clay loam (6.3% sand, 63.1% silt, and 30.6% clay) (Ultisol, USDA soil taxonomy) developed from a quaternary red earth; it had a pH in water of 5.36 (soil to water ratio of 1:2.5) and contained 17.0 g C kg^{-1} soil, 1.6 g N kg^{-1} soil, and 138.2 mg alkali-hydrolysale N kg^{-1} soil. The content of AS was 1187 mg kg^{-1} soil, with 49 mg kg^{-1} MurN, 725 mg kg^{-1} GlcN, and 414 mg kg^{-1} GalN (Table S1). The sampled paddy soil was thoroughly mixed, and visible rice residues were removed. The soil was later sieved through a 2-mm mesh screen. Visible discrete pieces of plant residues were removed, and then were stored at 4 °C prior to incubation.

Production of ^{13}C-labeled substrates

[13]C-labeled rice (*Oryza sativa* L.) was produced in a growth chamber (110 cm length, 250 cm width, 180 cm height) with temperature set at 22 °C at night and at 28 °C during the day. After germination, rice seedlings were transplanted into pots (20 cm inner diameter and 25 cm height) containing paddy soils derived from quaternary red clay and amended with basal fertilizers, consisting of $(NH_4)_2SO_4$, $Ca(H_2PO_4)_2$, and KCl at the rates of 40 mg N, 20 mg P, and 80 mg K kg^{-1} soil. After 25 days, the pots were put into the growth chamber, containing a beaker of 1 L H_2SO_4 (0.5 M). Then, the chamber was sealed, and 200 mL 1.0 M $NaH^{13}CO_3$ (50 atom % [13]C; Cambridge Isotope Laboratories, Tewksbury, MA, USA) was initially injected into the beaker with H_2SO_4. To maintain a CO_2 concentration in the growth chamber sufficient for the growth of rice, 40 mL of the $NaH^{13}CO_3$ was added daily into the H_2SO_4 solution. Every 7 days, the chamber was opened, and the solution in the beaker was replaced. Distilled water was occasionally added to the trays to maintain a submerged soil surface. After 1.5 months, the tops of the rice were harvested, dried at 70 °C, and ground to a size less than 0.25 mm. The rice material contained 44.69% C, 2.83% N, and 2.39% atom ^{13}C excess.

Experimental design

Sieved paddy soil was placed into plastic containers. Distilled water was added into the soil, and then the soil was thoroughly mixed as a slurry. Flooded paddy soil was pre-incubated at 25 °C for 1 week with a ~ 2-cm water layer maintained on the soil surface. Then, the upper water layer was removed. Two treatments were prepared, with each having eight replicates

(assuming two destructive sampling points, each having four replications). These consisted of soil receiving ^{13}C rice residue alone (S) and soil receiving ^{13}C rice residue and inorganic N fertilizer (S + N). The replicate soil portions (each containing the equivalent of 400 g oven-dried soil) were weighed into 1-L glass bottles. Rice residues were added to soils at a rate of 4 g C kg^{-1} dry soil (equivalent to 8.95 g rice residue kg^{-1} soil). Inorganic N was added as ammonium sulfate $(NH_4)_2$ SO_4 at a rate of 150 mg N kg^{-1} soil. After addition of the rice residue and N, the soil was thoroughly mixed in each bottle. Then, distilled water was gently injected from the top of inner wall to scour soils adhering on the inner wall. This yielded a depth of soil mixture of approximately 5 cm. On the top of soil, a 2-cm-depth water layer was maintained during incubation by supplying distilled water every 2 days. The open bottles containing soil mixtures were incubated at 25 °C for 100 days. During the incubation, the redox potential (Eh) of the 0–1-cm and 1–5-cm soil layers were measured periodically (at days 0, 5, 20, 50, and 100) using a platinum electrode against a standard Ag/AgCl electrode (Mettler Toledo, Switzerland). The platinum electrode was inserted into the middle depth (~ 0.5 cm) of the upper soil layer. After reading, the platinum electrode was pushed into the middle depth (~ 3 cm) of the lower soil layer. At day 5 (designated as early incubation time) and day 100 (designated as late incubation time), four replicated bottles from each treatment were used to collect stratified samples as follows: the water above the soil was gently syringed off; then, the 0–1-cm soil layer (equivalent to 150 g moist soil) was collected using a scoop. The remaining soil in the bottom of bottles was collected as the 1–5-cm soil layer. Each of the stratified soil samples was separated into two parts. The first part (~ 50 g) was spread on a sheet, air-dried, and ground for analysis of AS and physicochemical soil properties. The second part (~ 50 g) was stored at 4 °C for NO_3^- and NH_4^+ analyses, which were carried out within 24 h.

Analysis methods

Amino sugars were extracted from freeze-dried soils according to Zhang and Amelung (1996). Briefly, the air-dried soils were ground to pass a 100-mesh stainless steel sieve using a porcelain ball grinder. Grounded soil samples were hydrolyzed with 6 M HCl for 8 h at 105 °C and the solution was filtered, adjusted to pH 6.6~6.8 with a 0.4 M KOH solution, and then centrifuged (2000×g for 10 min). The supernatant solution was freeze-dried, and then AS was washed out from the residues with methanol. The recovered AS was transformed into aldononitrile derivatives and extracted with

dichloromethane from the aqueous solution. Excess of anhydride was eliminated with 1 M HCl and water. The AS derivatives were redissolved in hexane and ethyl acetate mixture ($v:v = 1:1$) for final analysis after removing dichloromethane using N_2 stream. AS was quantified relative to the internal standards, myo-inositol (first internal standard), and methyl-glucamine (second internal standard), and determined by a GC-FID (Agilent 6890A, USA) equipped with a HP-5 fused silica column (30 m × 0.25 mm, an inner dimeter of 0.25 μm).

The ^{13}C composition of individual AS (GlcN, GalN, and MurN) was determined by a GC-combustion-isotope ratio MS (GC-C-IRMS; Delta plus, Thermo Finnigan MAT 253, Germany) equipped with a DB-5MS capillary column. The detailed procedures and conditions to determine and calculate ^{13}C labelled individual AS were described in the reference of Jia et al. (2017).

Calculations and statistical analysis

Since the area ratio of ^{12}C to ^{13}C in mass spectrum was already known, the amounts of newly formed AS from the rice residue-C were calculated by the following equation:

$$AS_{new} = \lfloor AS_{total} \times (AE_{sample} - AE_{control}) \rfloor / (AE_{residue} - AE_{control})$$

where AS_{new} is the amounts of newly formed individual AS derived from rice residue-C; AS_{total} is the total content of individual AS; AE_{sample} is the ^{13}C atom % excess in soil samples where ^{13}C rice residues were added; $AE_{control}$ is the ^{13}C atom % excess in the soil control; $AE_{residue}$ is the ^{13}C atom % excess in the added rice residues.

The calculation was based on the assumption that the proportion of labeled ^{13}C and unlabeled C synthesized by microorganisms was the same as the respective proportion ^{13}C and unlabeled C in the rice residues. The total AS derived from rice residue was calculated by the sum of the individual AS of the soil.

Fungal-derived GlcN, an index for fungal residues, was calculated by subtracting bacterial GlcN from total GlcN, assuming that MurN and GlcN occur at a 1:2 ratio in bacterial (Engelking et al. 2007). The calculation is as follows:

$$\text{Fungal GlcN } (mg\ kg^{-1})$$
$$= \left[\text{GlcN } (mg\ kg^{-1})/179 - 2 \times \text{MurN } (mg\ kg^{-1})/251 \right]$$
$$\times 179$$

where Fungal GlcN represents fungal-derived glucosamine, and 179 and 251 are the molecular weights of GlcN and MurN, respectively.

Ratio of ^{13}C-labeled fungal GlcN/MurN is used to represent ratio of fungal/bacterial residues in newly formed AS, whereas total fungal GlcN/MurN ratio is used to represent ratio of fungal/bacterial residue in total AS (Amelung et al. 2001).

There are six C atoms in a GlcN molecule, and thus GlcN C is calculated as:

$$\text{GlcN C} \ (\text{mg kg}^{-1}) = \text{GlcN} \ (\text{mg kg}^{-1}) \times 72/179$$

GalN is the epimer of GlcN, and thus the calculation was expressed as:

$$\text{GalN C} \ (\text{mg kg}^{-1}) = \text{GalN} \ (\text{mg kg}^{-1}) \times 72/179$$

There are nine C atoms in a MurN molecule, and thus muramic acid-derived C (MurN C) is calculated as:

$$\text{MurN C} \ (\text{mg kg}^{-1}) = \text{MurN} \ (\text{mg kg}^{-1}) \times 108/251$$

Total amino sugar-derived C (mg kg^{-1}) is calculated as the sum of GlcN C, GalN C, and MurN C.

Statistical analysis of the effect of inorganic N addition on individual AS was performed using standard analysis of variance (ANOVA). Statistical testing of the differences of individual AS between the two soil layers was performed using one sample T test. The contents of individual AS in the 0–1-cm soil layer were subtracted from those in the 1–5-cm soil layer, and these values were tested for their difference from "0" Homogeneity of variances was tested by Levene's test; normal distribution of residuals was tested by the Shapiro test. Statistical testing of the effect of incubation time on individual AS was performed using repeated measures ANOVA. The data for outliers and distribution were checked before the analysis was performed, and Mauchly's test was performed to check the sphericity. All statistical tests were performed by the software package SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard errors. Figures were plotted using Origin 8.0.

Results

Content of individual AS ranked as follows: MurN < GalN < GlcN (Fig. 1). Inorganic N addition increased the amounts of GlcN, GalN, and total AS ($P < 0.01$) (Fig. 1). Incubation time and soil layer did not affect the contents of total and individual AS ($P > 0.05$) (Fig. S1 and S2).

The amount of newly formed AS with their C derived from rice residue ranked as follows: MurN < GalN < GlcN (Fig. 2). The amounts of these AS were higher at the later than at the early incubation time ($P < 0.01$). The soil layer affected the amounts of GlcN, MurN, and total AS: at the early incubation time, the contents of total AS, GlcN, and MurN were higher in the 1–5-cm than in the 0–1-cm soil layer ($P < 0.01$); N
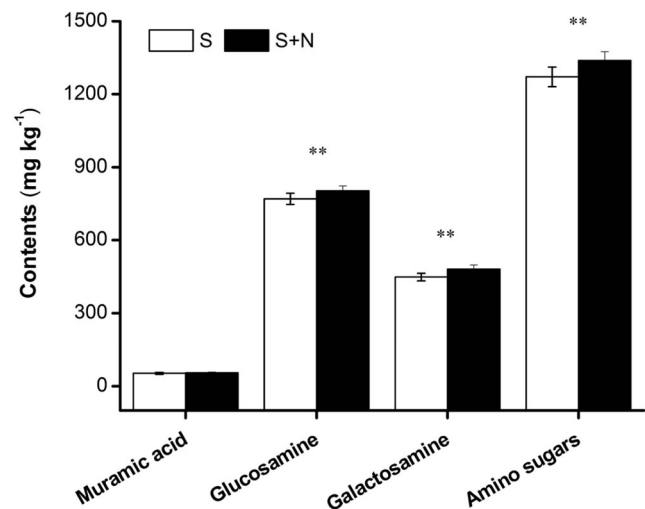

Fig. 1 Effect of inorganic N on total amino sugar content in the paddy soil. Data are the mean of replicates from 0–1-cm and 1–5-cm soil layers and from early and late incubation times ($n = 16$) since the amounts of amino sugars were not significantly different between the two soil layers and between the two incubation times (Fig. S1 and Fig. S2). Double asterisks (**) indicate significant effect from inorganic N at $P < 0.01$

addition stimulated the synthesis of AS in the 0–1-cm soil layer and the amounts of MurN increased by 14.7 and 20.8%, GlcN by 31.8 and 23.7%, GalN by 23.3 and 11.6%, and total AS by 26.3 and 20.0% at the early and late incubation times, respectively (Fig. 2).

The ratio of fungal GlcN/MurN ranged from 12.5 to 14.6 for total AS, whereas it was between 1.0 and 1.7 for newly formed AS (Fig. 3). This ratio for both newly formed and total AS under N addition treatment was significantly higher in the 0–1-cm than in the 1–5-cm soil layer ($P < 0.05$) (Fig. 3). Moreover, this ratio for newly formed AS was higher at the later than at the early incubation time ($P < 0.01$) (Fig. 3).

The percentage of total AS-C in soil organic C (SOC) was maintained at 3.0–3.2% during the incubation (Fig. 4). The percentage of newly synthesized AS in total AS ($\text{AS}_{\text{new}}/\text{AS}_{\text{total}} \times 100$) ranged from 0.9 to 1.7% (Fig. 5). Among the three AS, the newly synthesized MurN had the highest percentage (5.3–7.7%), compared to GlcN (0.9–1.6%) and GalN (0.5–1.1%) (Fig. 5). The addition of N only increased the percentage of newly synthesized AS (except for GalN) in the 0–1-cm soil layer ($P < 0.01$); additionally, these percentages were higher at later time of incubation compared to early time ($P < 0.01$) (Fig. 5).

Discussion

Effect of soil depth and N addition on total and newly synthesized amino sugars

The present study showed that inorganic N addition significantly promoted the synthesis of both rice residue-derived

Fig. 2 Effect of inorganic N addition on the newly formed amino sugars from the added ^{13}C-labeled rice residues at 5 and 100 days in the paddy soil
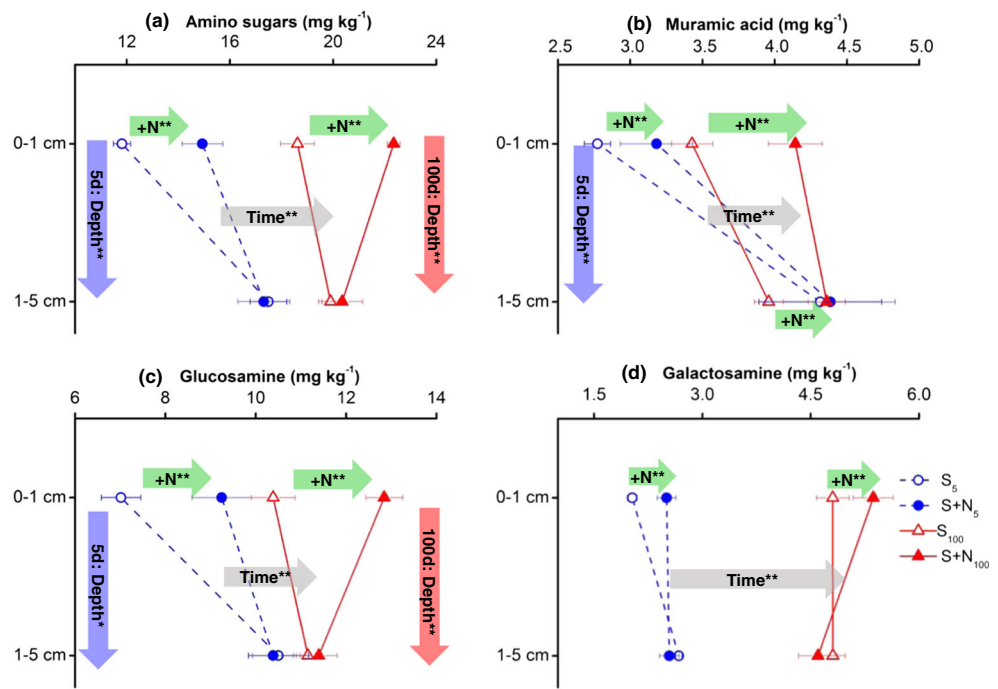

and total AS in the paddy soil (Figs. 1 and 2). Due to stimulation of microbial growth by N, microbial residues were accumulated (Ding et al. 2010; Hoque et al. 2002). The N addition stimulated the formation of newly AS only in the 0–1-cm soil layer (Fig. 2), suggesting a sensitive response of microbial utilization of rice residue-C to N only here. Despite the fact that Eh did not strongly change with depth (Fig. 6a), which may result from the large amount of rice residues added to soil, higher contents of NO_3^- in the 0–1-cm layer (Fig. 7b) indicated that aerobic conditions occurred here as compared with the 1–5-cm layer. Probably, anaerobic, aerobic, and facultative anaerobic/aerobic microorganisms can live in the oxic zone of the 0–1-cm layer (Liesack et al. 2000; Lüdemann et al. 2000) and differ for their N uptake (Schmidt et al. 2002) which can explain the differences in AS formation in responding to N addition between the two soil layers. Additionally, the lower soil pH in the 1–5-cm layer, especially at the late incubation time (Fig. 6b), may inhibit the NH_4^+ uptake by microorganisms

to some extent (Cheng et al. 2013), thus limiting microbial growth and AS formation.

At early incubation time, the amount of newly synthesized AS was higher in the 1–5-cm than in the 0–1-cm soil layer, but this difference was narrowed at the late incubation time (Fig. 2). Such phenomenon can also be explained by conversion of the part added NH_4^+–N to NO_3^-–N in the uppermost layer (Fig. 7) (Wang et al. 2015). The preferential uptake of NH_4^+–N but not NO_3^-–N by soil microorganisms (Burger and Jackson 2003; Recous et al. 1990), especially by bacteria (as suggested from MurN, Fig. 5) (Myrold and Posavatz 2007), could explain the higher microbial AS content in the 1–5-cm compared to the 0–1-cm soil layer at the early incubation time.

The absence of incubation time and soil depth effects on total AS (Figs. S1 and S2 and Table S1) is due to the fact that most of AS are stored in the pool of microbial residues, with turnover time ranging from hundreds of days to several years (Glaser et al. 2006), although the precise turnover times of

Fig. 3 Ratio of fungal GlcN to MurN for the newly formed (**a**) and total (**b**) AS at 5 and 100 days in the paddy soil. AS, amino sugars
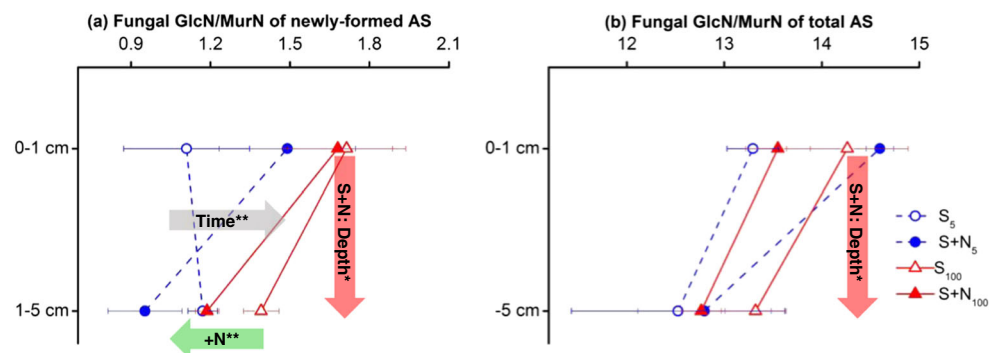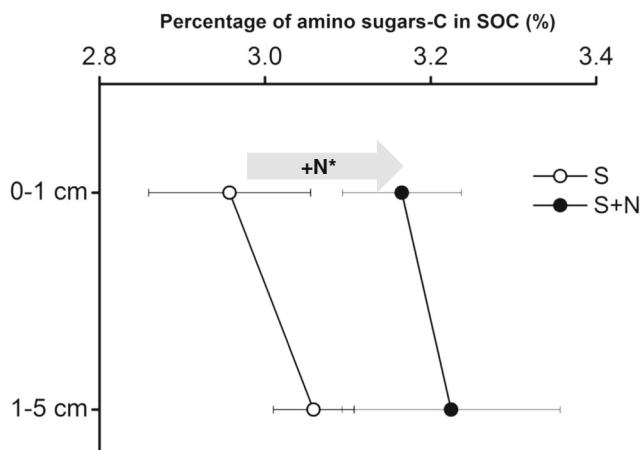
Fig. 4 Percentage of total amino sugar C in the SOC pool at the end of incubation

individual substances are not known (Gunina et al. 2017). In contrast, all newly formed individual AS in both soil layers (obtained based on the ^{13}C analysis) accumulated significantly from the early to the late incubation time (excluding GalN), confirming their continuous formation with time.

Discrepancy of bacteria- and fungal-derived amino sugars in responding to inorganic N addition

The inconsistent responses of three individual AS to N addition suggest that bacterial and fungal communities behaved differently to substrate availability. It is generally believed that N addition causes a shift from fungal-dominated to bacteria-dominated communities (Strickland and Rousk 2010). At the early incubation time, with the addition of rice residue, the total fungal residues (as inferred from fungal GlcN content) and bacterial residues (as inferred from MurN content) increased from their initial levels by 6.6 and 10.2%, respectively (Table S1). This indicates that bacteria may respond faster in the fresh rice residue decomposition as compared to fungi (Nakamura et al. 2003). Inversely, N addition promoted the amount of total fungal residues by 4% but did not significantly change bacterial residues (Fig. 1 and Table S1), suggesting a stronger response of fungal community to the increase of N availability in comparison to bacteria.

The ratio of fungal GlcN/MurN for the newly formed and total AS can be used to indicate the microbial turnover process of the fresh and native organic residue in the paddy soil. The fungal GlcN/MurN ratio of newly formed AS ranged from 1.0 to 1.7 with higher value in later than in early incubation time. This suggests that fungi and bacteria have similar contributions to the decomposition of fresh rice residue and the contribution from fungi increased with incubation time in the paddy soil. Due to the turnover of bacterial residue that is faster than that of fungi (Gunina et al. 2017), the 12.5 to 14.6 times higher accumulation of fungi residue than that of bacteria (fungal GlcN/MurN ratio, Fig. 3) showed that the native organic matter decomposition should be a fungi-predominated process. The higher ratio of fungal

Fig. 5 Percentage of newly formed amino sugars in total amino sugars at 5 and 100 days in the paddy soil. AS, amino sugar; MurN, muramic acid; GlcN, glucosamine; GalN, galactosamine
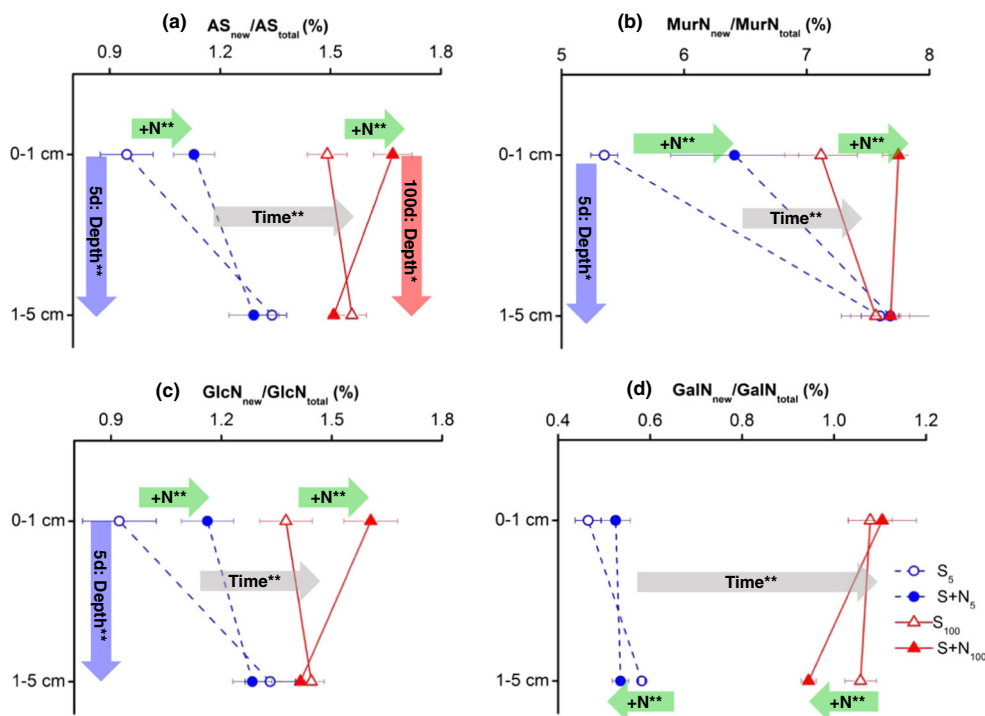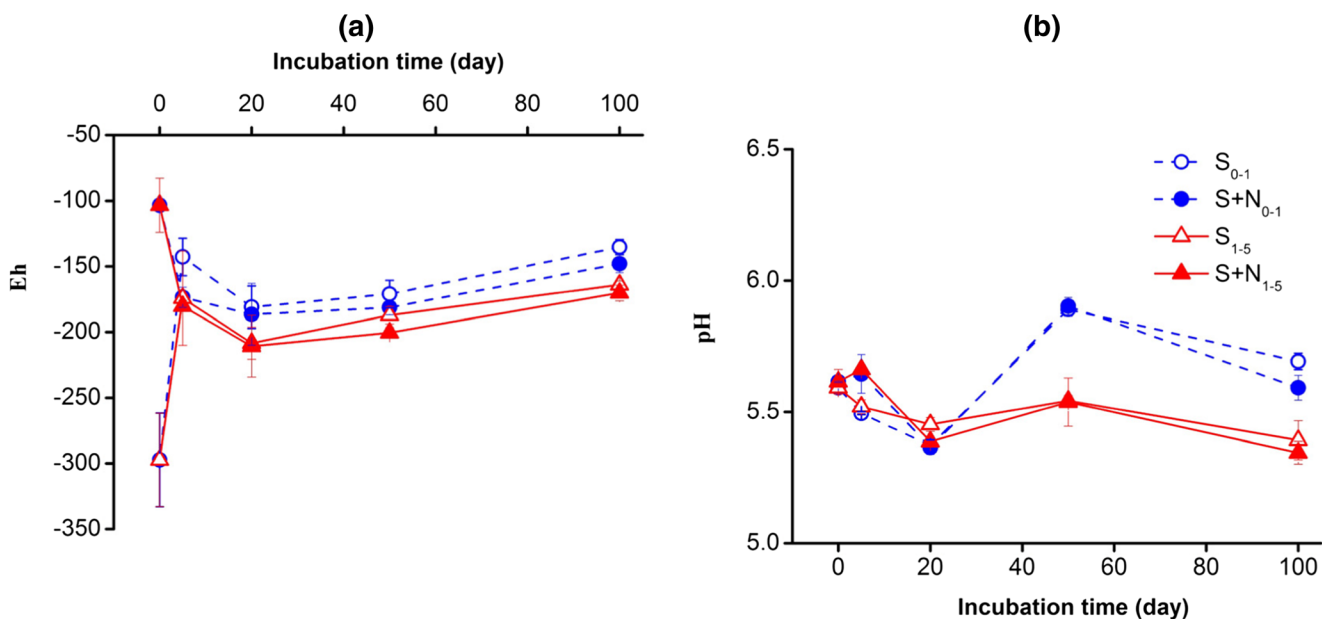
Fig. 6 Dynamic of Eh (**a**) and pH (**b**) in 0–1-cm and 1–5-cm soil layers during the incubation

GlcN/MurN under N addition treatment in the 0–1-cm than in the 1–5-cm soil layer (Fig. 3) indicated a relative richer fungal biomass existing at the 0–1-cm oxic horizon compared to subsoil.

Contributions of rice residue-derived amino sugars to total amino sugar pools as affected by inorganic N addition

The percentage of total AS-C in SOC maintained an unchangeable level from the early to the end of incubation (Fig. 4), suggesting an equilibrium between mineralization of AS from the microbial residues and consistent contribution of fresh AS from living microorganisms to SOC pool in the paddy soil. The addition of both rice residue and N promoted the accumulation of AS pool. However, the newly formed AS

contributed just 0.9–1.7% of the total AS, whereas the $MurN_{new}/MurN_{total}$ reached 5.3–7.7% at the end of incubation (Fig. 5). It demonstrates that bacteria can play important role in the fresh organic residue decomposition.

The percentages of newly formed AS in total individual AS were also promoted by the addition of inorganic N (Fig. 5), indicating that the microbial decomposition of fresh rice residue may require more mineral N than microbial turnover of native organic matter in paddy soils (Kuzyakov et al. 2000). This is supported by the facts that fungi have lower nutrient requirements than bacteria (Güsewell and Gessner 2009), and our finding that the contribution of fresh rice residue decomposition from bacteria is higher than that of native organic matter. This phenomenon occurred only in the 0–1-cm soil layer (Fig. 5), suggesting that the microbial immobilization of inorganic N is an oxygen-limited process.

Fig. 7 Content of NH_4^+–N (**a**) and NO_3^-–N (**b**) in 0–1-cm and 1–5-cm soil layers at 5 and 100 days
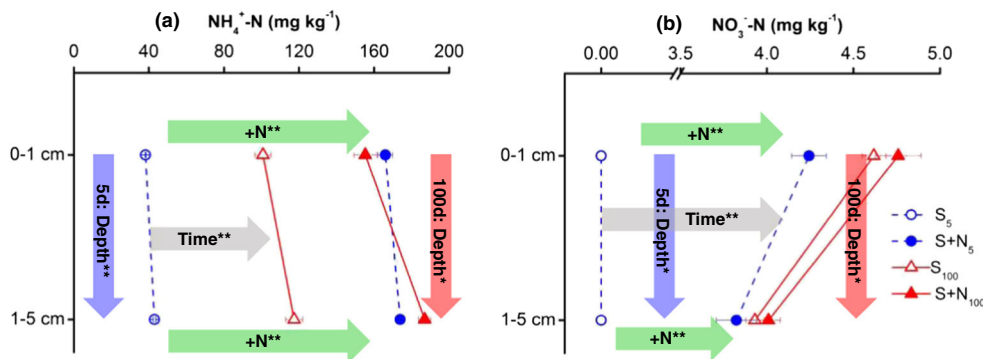
Conclusions

Decomposition of ^{13}C-labeled rice residue in paddy soil during 100 days showed that inorganic N addition increased the accumulation of rice residue-derived C within a pool of microbial residues based on AS analysis. Formation of AS was lower at the 0–1-cm oxic horizon compared to anoxic horizon (1–5 cm) at the early incubation time. Discrepancies between these two soil layers are due to the decrease of NH_4^+ and increase of NO_3^- contents in the oxic layer, which can result in slow growth of microorganisms and thus synthesis of AS. In contrast, absent differences between the two soil layers at the late incubation time are due to progressive plant residue decomposition in the oxic layer and suppression of microbial activity in anoxic. Therefore, although the thickness of the upper oxic or partly oxic layer in flooded paddy soil is only several millimeters, the discrepancies in C and N transformation here should be taken into account in future investigations.

Both fungi and bacteria participated in rice residue decomposition. The ratio of fungal GlcN/MurN for the ^{13}C-labeled and total AS clearly indicated that fungi and bacteria have similar contributions to the decomposition of fresh rice residue whereas native SOM is a fungi-predominated process.

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