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Research highlights

► The transformation velocity of amino sugars was evaluated for the first time by isotope technique. ► The 15N enrichment in amino sugars can clue on the renewal of bacterial and fungal cells in soils. ► Amino sugars can be decomposed as C sources, but being compound-specific and substrate-dependent.
Temporal responses of soil microorganisms to substrate addition as indicated by amino sugar differentiation

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Abstract

Amino sugars are one of the important microbial residue biomarkers which are associated with soil organic matter cycling. However, little is known about their transformation kinetics in response to available substrates because living biomass only contributes a negligible portion to the total mass of amino sugars. By using 15N tracing technique, the newly synthesized (labeled) amino sugars can be differentiated from the native portions in soil matrix, making it possible to evaluate, in quantitative manner, the transformation pattern of amino sugars and to interpret the past and ongoing changes of microbial communities during the assimilation of extraneous 15N. In this study, laboratory incubations of soil samples were conducted by using 15NH4 as nitrogen source with or without glucose addition. Both the 15N enrichment (expressed as atom percentage excess, APE) and the contents of amino sugars were determined by an isotope-based gas chromatography—mass spectrometry. The significant 15N incorporation into amino sugars was only observed in glucose plus 15NH4 amendment with the APE arranged as: muramic acid (MurN) > glucosamine (GlcN) > galactosamine (GalN). The dynamics of 15N enrichment in bacterial-derived MurN and fungal-derived GlcN were fitted to the hyperbolic equations and indicative for the temporal responses of different soil microorganisms. The APE plateau of MurN and fungal-derived GlcN represented the maximal extent of bacterial and fungal populations, respectively, becoming active in response to the available substrates. The different dynamics of the 15N enrichment between MurN and GlcN indicated that bacteria reacted faster than fungi to assimilate the labile substrates initially, but fungus growth was dominant afterward, leading to integrated microbial community structure over time. Furthermore, the dynamics of labeled and unlabeled portions of amino sugars were compound-specific and substrate-dependent, suggesting their different stability in soil. GlcN tended to accumulate in soil while MurN was more likely degraded as a carbon source when nitrogen supply was excessive.

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1. Introduction

The transformation of nitrogen (N) between the organic and inorganic phases in soil is primarily a biological process, but whether inorganic N is immobilized to organic forms depends on microorganism’s requirement (Dilly, 2004; Paul and Clark, 1996). Available substances such as glucose can shift soil microorganisms from dormancy to activity, increasing nutrient demand and leading to the significant utilization of extraneous N (e.g., NH4) (Blagodatskaya et al., 2007; Brant et al., 2006; De Nobili et al., 2001; Mondini et al., 2006). Consequently, the microbial cell walls are rapidly formed during biological metabolism and accumulated in soil as an important part of microbial residues (Engelking et al., 2007; Nicolardot et al., 1994; Paul and Clark, 1996).

As important constituents in microbial cell walls, amino sugars are considered as a storage pool for both the immobilized N and stable soil organic carbon (C) although they account for small proportion in soil organic matter (Amelung, 2001; Stevenson, 2002). Hence, their dynamics intensively depended on the C and N status in soil (Amelung, 2003; Liang et al., 2007; Paul and Clark, 1996). Additionally, amino sugars are reliable microbial residue biomarkers due to their different origins (Amelung, 2001; Parsons, 1981; Stevenson, 1982). Among the identified amino sugars, muramic acid (MurN) originates exclusively from bacteria, being...
a component of the peptidoglycan in bacterial cell wall (Amelung, 2001; Glaser et al., 2004; Parsons, 1981). The origin of soil galactosamine (GalN) is currently tricky. It was generally considered to be derived mainly from bacteria (Amelung, 2001; Glaser et al., 2004), but some evidence suggests fungi contributing much larger percentages of GalN than bacteria (Engelking et al., 2007). Glucosamine (GlcN) in soil is mainly in the form of chitin in fungal cell walls, while bacterial cell walls and the exoskeletons of soil invertebrates also make some contribution (Parsons, 1981). Amino sugars in soil are mainly contained in dead microbial residue which was derived from the living biomass, thereby they can reflect theoretically the changes in historical and current community structure of microorganisms (Glaser et al., 2004; Liang et al., 2008). Unfortunately, the mass ratios of GlcN/MurN and GlcN/GalN, which are commonly used to indicate the relative contribution of bacterial and fungal residues to soil organic matter turnover, were often in discordance due most likely to the difference in the dynamics of GalN and MurN (Glaser and Gross, 2005; Liang et al., 2007; Zhang et al., 1999). A better understanding of the production, stabilization, and turnover of amino sugars is thus required when relating amino sugar abundance to microbial community structure (Brant et al., 2006; Liang et al., 2007). Such knowledge can only be gained by differentiating the newly synthesized microbial residues from the soil native portions by isotope tracing techniques (Decock et al., 2009; Glaser and Gross, 2005). Recently, an isotope labeling based gas chromatograph/mass spectrometry (GC/MS) was developed and can be used to evaluate the isotope enrichment in amino sugars and to differentiate the labeled and the unlabeled portions in soil samples (He et al., 2006; Liang and Balser, 2010). The method offers an opportunity to investigate the dynamics of soil amino sugars as influenced by extraneous substrate additions.

Therefore, laboratory incubations of soil samples were conducted by using 15N–NH4 as N source with or without glucose addition. The pattern of isotope incorporation into each amino sugar was determined, and thus the newly synthesized and soil native amino sugars were differentiated. The objectives of our investigation were to evaluate the transformation rates and dynamics of individual amino sugars affected by available substrates, specific response of soil bacteria and fungi to available substrates, and the indication of the isotope dynamics of amino sugars to microbial succession over time.

2. Materials and methods

2.1. Soil sample and laboratory incubations

Surface layer (0–20 cm) Molisol sample (Typic Hapludoll) (Soil Survey Staff, 2003) was collected from Gongzhuling, Jilin Province, China (124°48.4′, 43°30.7′ N). The contents of soil organic C and total N were 17.4 g kg−1 and 1.65 g kg−1, respectively. The original soil had a pH in water of 6.3 (soil:water = 1:2.5) and the content of inorganic N (NH4 + NO3) was 40.3 mg N kg−1. The sieved (<2 mm) air-dried soil samples (ca. 8 g) were pre-incubated at 25 °C with 20% of water for 2 weeks, and then were used for the incubation with 15N-containing substrate (He et al., 2006). The amendments included [(15)NH4]2SO4 (98% atom, Cambridge Isotope Laboratories, Inc. USA) addition alone (T1) and glucose plus [(15)NH4]2SO4 addition (T2). Five hundred microliter of substrate solution was added into soil microcosms once a week till the end of the incubation. The addition amount each time was 0.1 mg N and 1.0 mg C per gram soil (C:N = 10). KH2PO4 (0.9 mg g−1 soil) was added at the beginning of the incubation to ensure adequate supplies of P and K. The containers were covered with perforated plastic lids, and distilled water was supplied after each substrate addition to maintain soil moisture at 20% (oven-dry soil basis). The soils were sampled and air-dried after 1, 2, 3, 4, 6, 9, 12, 15, 18 and 21 weeks, respectively. Both of the treatments were in triplicates for each sampling time and the air-dried original soil sample was used as a control.

2.2. Analysis of amino sugars and determination of isotope incorporation by GC/MS

The air-dried soil samples were ground to <0.25 mm for amino sugar analysis. The hydrolysis, purification and derivatization of soil amino sugars were processed according to Zhang and Amelung (1996) and myo-inositol, as an internal standard, was added before the filtration of the hydrolyte. The amino sugar derivatives were separated on a DB-5MS column (30 m × 0.25 mm × 0.25 μm) and the 15N incorporation into individual amino sugars was identified by GC/MS (Finnigan trace, Thermo Electron Co., Ltd., USA). The temperature of the electron impact (EI) and chemical ionization (CI) sources were set at 200 °C, and 180 °C, respectively. The electron energy was 70 eV and the interface temperature was 250 °C. Helium was used as carrier gas with the flow rate set at 0.8 mL min−1 (He et al., 2006). The GC temperature program under the EI mode was set according to Zhang and Amelung (1996) and the split ratio was 30:1. Under the chemical ionization negative mode (CI−), from 230 to 250 °C the column temperature was increased at a rate of 5 °C min−1 and the split ratio was raised to 40:1 for baseline separation between GalN and MurN. Methane was used as the reaction gas and the flow rate was 1.5 mL min−1 (He et al., 2006). In the full scan mode of mass 50–500, the intensities of the N-containing fragments (F) and also the corresponding F plus 1 (F + 1) were measured considering only 1 N atom in an amino sugar molecule.

The concentrations of individual amino sugars in both original and incubated soils were quantified by total ion current chromatogram on EI mode (GC-EI/MS) and calculated by the internal standard method. According to He et al. (2006), there was no method-specific effect for the isotope evaluation of GlcN, GalN and MurN under the two ionization modes. Therefore, the 15N enrichment in GlcN and GalN was evaluated on EI mode according to the intensity ratio of m/z 98 and 99; whereas the 15N incorporation into MurN was estimated on CI− mode by monitoring the intensity changes of m/z 264 and 265 (He et al., 2006).

2.3. Calculations of 15N enrichment in soil amino sugars and the content of isotope-containing fraction

When 15N-labeled N was immobilized by microorganisms, the newly synthesized amino sugars contain the heavy isotope and thus can be differentiated from the inherent portions. Accordingly, the 15N enrichment in each amino sugar is expressed as atom percentage excess (APE) and can be calculated as follows:

\[
\text{APE} = (\text{Re} - \text{Re}_c) / (1 + (\text{Re} - \text{Re}_c)) \times 100
\]

where Re is the isotope ratio of incubated samples and Re_c = [A_F + 1] / A_F (A is the area of the selected ion). Rc represents the corresponding ratio obtained from original soil analyzed on the same GC/MS assay (He et al., 2006).

Because the calculated APE represents the percentage of isotope-containing fraction relative to the total amount of the target compound, the content of 15N-labeled amino sugars can be obtained based on both APE and the concentration of individual compounds, which was expressed as:

\[
C_1 = C_f \times \text{APE} / 100
\]

where C_f is the total concentration of each amino sugar determined by GC−EI/MS and C_f is the content of the 15N-labeled portion in the compound.
2.4. Statistical analysis

The effect of substrate amendment on the concentrations and 15N enrichment of individual amino sugars during incubations was analyzed using a one-way analysis of variance (ANOVA) and the LSD method at a 95% confidence level. The curve fitting of the dynamic change in APE was performed by Origin 8.0 to describe the transformation kinetics of amino sugars. The linear regression and various non-linear curve fitting models including Gauss, Boltzmann, Lorentz and hyperbolic functions were tested. The coefficient of determination ($R^2$) and the Pearssons chi-square test ($\chi^2$) were applied to evaluate the goodness of fit.

3. Results

3.1. The isotope incorporation pattern in soil amino sugars

As shown in Table 1, the 15N enrichment of individual amino sugars (represented as APE) was quite low in the soil samples with weekly addition of (15NH4)2SO4. The APE of both GlcN and GalN in T1 was less than 1.31 and that of MurN was no more than 2.70 during 21-week incubation. The 15N enrichment in MurN was detectable shortly after the substrate addition, but it was not found in GlcN and GalN until the 3rd and 6th weeks, respectively. The slight increase in APE for GlcN and MurN mainly occurred before the first six weeks and then remained almost unchanged during the incubation ($P > 0.05$).

The addition of glucose plus 15NH4+ pronouncedly enhanced the 15N incorporation into amino sugars. The 15N enrichment of the three amino sugars in T2 increased significantly with the time of incubation ($P < 0.05$); however, the dynamics were compound-specific (Fig. 1). The APE of MurN increased rapidly within the first six weeks and then reached the maximum of 42.3 after nine weeks of incubation. The APE in GlcN increased continuously and did not approach to the plateau of 35.7 until the 18th week. The APE discrepancy between MurN and GlcN in T2 was the largest during the 2nd to 4th week and decreased with incubation time. GalN had the lowest 15N enrichment, with values less than 10.2. Yet its enrichment increased linearly ($R^2 = 0.9776$, $P < 0.0001$) during the incubation.

3.2. Total content and 15N-labeled portion of individual amino sugars

3.2.1. Muramic acid

Despite the small degree of 15N incorporation, the total content of MurN in T1 increased within the first two weeks by up to 19% of the initial value (45.2 mg kg$^{-1}$, Fig. 2). Then, the content declined gradually during the incubation, and only 23.6 mg kg$^{-1}$ of the original MurN remained in the soil matrix after the 21-week incubation.

The content of MurN in T2 increased significantly at the early stage of the incubation ($P < 0.05$) and reached the maximal value of 62.5 mg kg$^{-1}$ at the 6th week, showing an increase by 29% (Fig. 2). The amount of MurN remained at the same level during the 6–12th weeks and then decreased gradually. By the end of the incubation, the content declined to 47.8 mg kg$^{-1}$, which showed no significant difference from the original level ($P > 0.05$). The amount of 15N-labeled MurN in T2 increased rapidly and then remained largely unchanged after approaching to the maximum (about 23 mg kg$^{-1}$) at the 9th week (Fig. 2, Table 2). However, except for the initial increase, the concentration of unlabeled MurN in T2 decreased gradually. At the end of the incubation, the content of the unlabeled portion dropped to 63% of the original level.

3.2.2. Glucosamine

The content of GlcN in T1 increased slightly within the first two weeks but showed no significant difference from the level in original soil ($P > 0.05$). Thereafter, the concentration of GlcN declined gradually with a significant loss of 16% (115 mg kg$^{-1}$) after

---

**Table 1**

<table>
<thead>
<tr>
<th>Incubation time (week)</th>
<th>APE (%)</th>
<th>GlcN</th>
<th>GalN</th>
<th>MurN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>1.56 ± 0.19 a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>2.01 ± 0.22 b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>0.56 ± 0.06 a</td>
<td>2.35 ± 0.25 bc</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>0.64 ± 0.07 a</td>
<td>2.69 ± 0.22 c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>1.25 ± 0.11 b</td>
<td>2.67 ± 0.27 c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>1.23 ± 0.12 b</td>
<td>2.56 ± 0.28 bc</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>1.30 ± 0.10 b</td>
<td>2.70 ± 0.30 c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>1.29 ± 0.12 b</td>
<td>2.63 ± 0.16 c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>1.26 ± 0.08 b</td>
<td>2.67 ± 0.29 c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>1.31 ± 0.15 b</td>
<td>2.58 ± 0.18 c</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Values reported as means ± standard error ($n = 3$). Different letters within each column indicate significant differences between sampling intervals ($P < 0.05$).

---

Fig. 1. 15N enrichment in amino sugars during the incubation amended with glucose and (15NH4)2SO4 MurN: muramic acid; GlcN: glucosamine; GalN: galactosamine.
21 weeks (Fig. 3). In the glucose plus \((^{15}\text{NH}_4\text{})_2\text{SO}_4\) amendment, the concentration of GlcN increased throughout the incubation, reaching an increase of 615 mg kg\(^{-1}\) (equivalent to 86% of the original value) at the final sampling time (Fig. 3). The contribution of labeled and unlabeled portions to GlcN accumulation was time-dependent. After one week incubation, the content of GlcN was 270 mg kg\(^{-1}\) greater than that in the original soil, though the \(^{15}\text{N}\)-containing portion accounted for less than 15% of the augment (Fig. 3). The content of the labeled portion increased with the incubation and played dominant role (more than 85%) in the final increase of soil GlcN compared to the initial sampling. Concomitantly, the concentration of unlabeled GlcN declined gradually to 833 mg kg\(^{-1}\) at the 6th week despite its initial increase to 940 mg kg\(^{-1}\). During the whole incubation course, the content of unlabeled GlcN in T2 remained stable and significantly higher than that in original soil \((P < 0.05)\).

GlcN is mainly present as the monomer of the chitin in fungal cell walls, but a small portion is in the bacterial peptidoglycan linked to MurN derivatives (Amelung, 2001; Paul and Clark, 1996). Fungal-derived \(^{15}\text{N}\)-GlcN was calculated by subtracting the bacterial portion of \(^{15}\text{N}\)-GlcN from the total labeled amount, assuming that GlcN and MurN occur at a molar ratio of 7:1 (equivalent to mass ratio 5:1) in the steady state of growth in the selective bacterial cultivation (Glaser et al., 2004). And then, the \(^{15}\text{N}\) enrichment of GlcN derived respectively from bacteria and fungi was calculated. As shown in Table 2, the concentration of \(^{15}\text{N}\)-labeled GlcN linked to \(^{15}\text{N}\)-MurN increased initially and remained constant after 6 weeks \((P < 0.05)\). The \(^{15}\text{N}\) enrichment of bacteria-derived GlcN exhibited the same trend. The concentrations and \(^{15}\text{N}\) enrichment of fungal-derived GlcN increased significantly \((P < 0.05)\) and reached a plateau after 15 weeks.

### 3.2.3. Galactosamine

In both T1 and T2 treatments, the content of GalN tended to increase at early stage of incubations and then declined gradually (Fig. 4). However, except for the sampling in the 3rd week, the ranges of augment or decline of GalN did not exceed 20%, exhibiting no significant difference from the original value \((P > 0.05)\). Although large amounts of glucose and labeled NH\(_4\)\(^+\) were added into the microcosm, the content of labeled GalN in T2 increased slowly and the unlabeled portion contributed significantly to the increment of GalN, particularly within the first two weeks (Fig. 4). Later, the unlabeled GalN tended to decompose simultaneously with the synthesis of newly labeled GalN, but there was no significant difference during the incubation \((P > 0.05)\).

### 4. Discussion

#### 4.1. Transformation rates of individual amino sugars evaluated by \(^{15}\text{N}\) incorporation

As heterogeneous microbial residue biomarkers, the turnover of different amino sugars has attracted much attention as a tool for probing microbial contributions to soil organic matter cycling.

---

**Table 2**

<table>
<thead>
<tr>
<th>Incubation time (week)</th>
<th>(^{15}\text{N})-labeled amount (mg kg(^{-1}))</th>
<th>APE of GlcN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MurN</td>
<td>Fungus-derived GlcN</td>
</tr>
<tr>
<td>1</td>
<td>4.0 ± 0.2 a</td>
<td>8.2 ± 1.1 a</td>
</tr>
<tr>
<td>2</td>
<td>10.7 ± 1.2 b</td>
<td>13.3 ± 1.2 b</td>
</tr>
<tr>
<td>3</td>
<td>13.3 ± 1.2 c</td>
<td>15.0 ± 1.5 b</td>
</tr>
<tr>
<td>4</td>
<td>16.2 ± 0.9 d</td>
<td>6.8 ± 0.9 c</td>
</tr>
<tr>
<td>5</td>
<td>20.7 ± 1.4 e</td>
<td>11.2 ± 1.3 d</td>
</tr>
<tr>
<td>6</td>
<td>22.9 ± 2.3 e</td>
<td>236 ± 22 e</td>
</tr>
<tr>
<td>7</td>
<td>24.1 ± 2.0 e</td>
<td>353 ± 23 f</td>
</tr>
<tr>
<td>8</td>
<td>23.5 ± 1.3 e</td>
<td>370 ± 28 f</td>
</tr>
<tr>
<td>9</td>
<td>22.6 ± 1.6 e</td>
<td>377 ± 30 f</td>
</tr>
</tbody>
</table>

Values reported as means ± standard error \((n = 3)\). Different letters within each column indicate significant differences between sampling intervals \((P < 0.05)\).
However, the dynamics of amino sugar have not been well understood due to the lack of effective techniques to assess their transformation rate. When combining \(^{15}\text{N}\) tracing technique with GC/MS identification, amino sugars newly formed from extraneous N can be differentiated from native soil ones (He et al., 2006).

In the glucose plus \((^{15}\text{NH}_4)^2\text{SO}_4\) amendment, the APE dynamics of amino sugars were compound-specific, demonstrating that the retention characteristics were different among different soil amino sugars (Amelung, 2001; Guggenberger et al., 1999; Liang et al., 2007; Zhang et al., 1998, 1999). The much higher \(^{15}\text{N}\) enrichment in MurN than GlcN especially at the early stage of the incubation confirmed that the turnover of MurN was more rapid than GlcN in the experiment. This result was consistent with previous studies (Amelung, 2001; Glaser and Gross, 2005; Liang et al., 2007; Zhang et al., 1999). The APE plateaus in both GlcN and MurN represented the maximal extent conversion from the extraneous N to these two biological compounds (Glaser et al., 2004; Liang et al., 2007). As shown in Fig. 1, the contribution of the extraneous N to GlcN- and MurN-nitrogen in soil was no more than 35% and 42%, respectively, regardless of frequent additions of glucose and \((^{15}\text{NH}_4)^2\text{SO}_4\).

In contrast to the widespread understanding to the turnover of GlcN and MurN, much controversy remains on the dynamics of GaN. In most previous publications, GaN in soils was thought to be less stable and decompose rapidly, similar to MurN (Amelung, 2001; Poulton et al., 2004; Zhang et al., 1999). However, Zhang et al. (1999) pointed out that the long-term (decades) stability of MurN was significantly lower than GaN. Based on the compound-specific \(^{13}\text{C}\) of amino sugars, Glaser and Gross (2005) reported a significantly faster turnover of MurN than GaN in the short-term (weeks). The low \(^{15}\text{N}\) enrichment of GaN in our isotopic incubation indicated that the cycling of GaN in soil was quite slow even in the presence of amended substrates.

4.2. Indication of the isotope dynamics of amino sugars

Soil microbial biomass survives as a largely dormant population in soil due to the limited availability of substrates especially in terms of C and energy (Demoling et al., 2007; Mondini et al., 2006). Therefore, the immobilization of extraneous N was severely restricted in T1 (Blagodatskaya et al., 2009; McFarland et al., 2002) and only a small quantity of microbial residues such as amino sugars were formed and accumulated in the microcosm.

Glucose addition strongly promoted the activity of microorganisms, leading to the increase in the immobilization of mineral nutrients in microbial biomass (Dilly, 2004; Joergensen and Schu, 1999; Mondini et al., 2006; Vinten et al., 2002). Amino sugars originally in soil have been known to be mainly included in dead microbial residues (Brant et al., 2006; Glaser et al., 2004). Along with the addition of \(^{15}\text{NH}_4\), the recovered \(^{15}\text{N}\) in soil amino sugars was derived from two ways, the direct incorporation of \(^{15}\text{NH}_4\) and the indirect conversion from other \(^{15}\text{N}\)-containing compounds previously transformed from \(^{15}\text{NH}_4\). Such transformation paths cannot be differentiated even by N isotope tracing techniques. Nevertheless, the formation of amino sugars was dominantly driven by soil microorganisms (Amelung, 2001; Wander et al., 2007), thus the \(^{15}\text{N}\)-labeled amino sugars essentially represented the cumulative microbial response to the extraneous substrates regardless of the transformation ways.

MurN is uniquely derived from bacteria, being one of the important components in cell wall peptidoglycan (Amelung, 2001; Parsons, 1981), thus the dynamics of the \(^{15}\text{N}\) enrichment in MurN can reflect the reproduction rate of bacteria. In the glucose plus \((^{15}\text{NH}_4)^2\text{SO}_4\) amendment, the relationship between the APE of MurN and the added \(^{15}\text{N}\) amount \((0.1 \text{ mg N kg}^{-1} \text{ once a week and in direct proportion to incubation time})\) was more fitted to a hyperbolic equation than other models \((R^2 = 0.9959, \text{Reduced } \chi^2 = 4.504, n = 12)\), in accordance with the microbial growth kinetics described as the Michaelis–Menten or Monod equation (Paul and Clark, 1996; Prescott, 2002). The plateau value of MurN APE represented the maximal extent (about 42%) of the bacterial population that became active in the microcosm. The low half-saturation constant \((K_m = 140 \text{ mg N g}^{-1} \text{ soil})\) indicated the high affinity of bacteria to the available substrates.

In bacterial biomass, the molar ratio of GlcN and MurN is theoretically 1:1 (Amelung, 2001). However, the actual ratio was significantly greater (about 2–8) mainly due to the faster degradation of GlcN than MurN (Amelung, 2003; Prescott, 2002). Therefore, the ratio of bacterial-derived \(^{15}\text{N}\)-GlcN and \(^{15}\text{N}\)-MurN changed during the cell proliferation of bacteria, but it was extremely difficult to estimate the ratio accurately over time. Because the \(^{15}\text{N}\) enrichment in MurN remained unchanged during the incubation of 6–21 weeks, the plateau value in the steady state of bacterial growth (i.e., mass ratio of 5:1) was useful to estimate the mean GlcN and MurN contributions in living and dead biomass (Glaser et al., 2004). Although the amount of GlcN initially originated from fungi was possibly underestimated, the transformation of GlcN within weeks was evidenced by high \(^{15}\text{N}\) enrichment and the continuous increase of GlcN APE after six weeks should be attributed to the fungal response to the extraneous N. The relationship between the APE of fungal-originated GlcN and the added \(^{15}\text{NH}_4\) amount could be fitted well to various non-linear models including Boltzmann, Gauss, Lorentz, and hyperbolic functions \((R^2 > 0.97)\). Considering the \(^{15}\text{N}\) enrichment in GlcN was associated with microbial processes, the hyperbolic fitting was applied here to express such a relationship \((R^2 = 0.9724, \text{Reduced } \chi^2 = 4.650, n = 12)\) because the biochemical meaning is clear. The APE plateau (about 28) represented the maximal extent of fungal activation in response to the glucose and \(^{15}\text{NH}_4\) addition and the initial lower APE suggested a low affinity of fungi to the substrates.

Much discussion regarding the origin of GaN was focused on its particular transformation dynamics. Based on our \(^{15}\text{N}\) tracing experiments, the APE dynamics of GaN during the incubation were found to be significantly different from MurN and GlcN, implying that the origin and the function of GaN within microbial cells or as metabolites are still ambiguous (Engelking et al., 2007). Hence, caution should be taken when using the ratio of GlcN to GaN to evaluate the relative contributions of microbial residues to the accumulation of soil organic matter. Therefore, only MurN and GlcN were considered when the effect of nutrient application on the temporal pattern of microbial community structure is probed.

4.3. Specific response of soil bacteria and fungi to available substrates

The significant higher \(^{15}\text{N}\) enrichment and the much lower half-saturation constant of MurN than fungal-derived GlcN suggested that bacteria responded faster than fungi to the available substrate (Engelking et al., 2007; Meidute et al., 2008; Paul and Clark, 1996). Oppositely, fungi had a less important role in the utilization of the added glucose in the short-term (days) soil amendment (Rinnan and Baath, 2009; Ziegler et al., 2005). However, the \(^{15}\text{N}\) enrichment of MurN changed slightly in T2 after six weeks, suggesting the rapid equilibrium between cell division and cell death of bacteria.

One of the reasons that the growth rate of bacteria did not rise further with increasing N addition is very likely due to the deficiency of available C. Additionally, too high concentration of accumulated NH\(_4\) in the microcosm (data not shown) resulted in the decline of soil pH from an initial value of 6.3 to 4.6 at the end of the...
incubation, thus exerting a negative effect on soil bacteria (Demoling et al., 2007; Yevdokimov et al., 2008).

Despite the initial lag phase, the continuous APE increase of fungal-derived GlcN indicated the dominant growth of fungi at the later stage of incubation because they have more ability to span microsites and decompose more recalcitrant constituents (Schimmel and Bennett, 2004). Therefore, even if a labile substrate does not stimulate a general response of the entire microbial community, specific groups of the community appear to be more involved in certain immobilization process than others (Brant et al., 2006; Meidute et al., 2008). In other words, the rapidly growing organisms were dominant initially, but replaced by more slowly growing organisms in succession (Bailey et al., 2002; Blagodatskaya et al., 2009; Schutter and Dick, 2001). The $^{15}$N enrichment dynamics of amino sugars could reflect both historical and current responses of the active microorganisms to extraneous N and hence integrated microbial community structure over time (Glaser et al., 2004; Liang et al., 2008).

4.4. Dynamics of amino sugar pool affected by available substrates

Because the native amino sugars were associated to varying degrees with soil organic matter cycling simultaneously with the formation of $^{15}$N-labeled portions (Liang et al., 2007; Paul and Clark, 1996), the dynamics of amino sugars were essentially the effect of the accumulation and decomposition, which was compound-specific and substrate-dependent.

Except for the initial priming effect, the input of large amount of NH$_4^+$ led to the exhaustion of the available C (McFarland et al., 2002). As a result, the significant loss of MurN and GlcN in T1 was mostly probably used as C and energy sources to maintain soil N turnover (Engelking et al., 2007, 2008). The application of glucose and $^{15}$NH$_4^+$ in T2 can meet the initial C and N requirement of a bacterial-dominated community (Paul and Clark, 1996), leading to an enhanced accumulation of amino sugars in soil. However, the rapid accumulation of inorganic N (data not shown) indicated the deficiency of available C after 4–6 week incubation. The unlabelled portion of GlcN accumulated in the micromoss while MurN declined along with the synthesis of the labeled portion, indicating the different stability of GlcN and MurN. MurN was more likely degraded to meet the microbial C demand. Inversely, GlcN tended to accumulate in soil organic matter (Paul and Clark; 1996; Six et al., 2006), and the partial decomposition occurred only in the case of intensive deficiency of available C (e.g., large amount inorganic N input).

Different from GlcN and MurN, the total content of GalN did not change significantly in both amendments ($P > 0.05$). By the differentiation between the labeled GalN and the unlabelled portion, the constant concentration of GalN was proved to be attributed to the identical rates of GalN formation and decomposition, except for the initial production (Engelking et al., 2008). In addition, considering the significant lower turnover rate than GlcN, GalN could be more stable and less involved in microbial metabolism in soil.

4.5. Conclusions

This study significantly improved the understanding to the turnover pattern of soil amino sugars and their indication on the microbial process in soil C and N cycling. The $^{15}$N enrichment in different amino sugars explicitly reflects the temporal response of different microbial populations to the substrates. The compound-specific pattern of isotope incorporation indicated that bacteria were more competitive initially than fungi to assimilate the simple substrate, but the successional growth of fungi became dominant over time. MurN was more likely degraded to compensate the microbial C demand while GlcN tended to accumulate in soil organic matter. Our investigation on the dynamics of amino sugars was conducted through laboratory incubation experiments with high amounts of available C and nutrient application, but it may be an important attempt to clarify the mechanism of microorganism-mediated soil organic matter turnover at the molecular scale.

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References


