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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 ^{15}N 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 ^{15}N. In this study, laboratory incubations of soil samples were conducted by using $^{15}NH_4^+$ as nitrogen source with or without glucose addition. Both the ^{15}N 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 ^{15}N incorporation into amino sugars was only observed in glucose plus $^{15}NH_4^+$ amendment with the APE arranged as: muramic acid (MurN) > glucosamine (GlcN) > galactosamine (GalN). The dynamics of ^{15}N 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 ^{15}N 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.

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., NH_4^+) (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, 1982). 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

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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 it 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 $^{15}N{-}NH_4^+$ 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) Mollisol sample (Typic Hapludoll) (Soil Survey Staff, 2003) was collected from Gongzhuling, Jilin Province, China (124°48′E, 43°30′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 ($NH_4^+ + NO_3^-$) 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 ^{15}N-containing substrate (He et al., 2006). The amendments included $(^{15}NH_4)_2SO_4$ (^{15}N 98% atom, Cambridge Isotope Laboratories, Inc. USA) addition alone (T1) and glucose plus $(^{15}NH_4)_2SO_4$ 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). KH_2PO_4 (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 ^{15}N 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 MurA under the two ionization modes. Therefore, the ^{15}N enrichment in GlcN and GalN was evaluated on EI mode according to the intensity ratio of m/z 98 and 99; whereas the ^{15}N 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 ^{15}N enrichment in soil amino sugars and the content of isotope-containing fraction

When ^{15}N-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 ^{15}N enrichment in each amino sugar is expressed as atom percentage excess (APE) and calculated as follows:

$$\mathrm{APE} = (\mathrm{Re} - \mathrm{Rc})[1 + (\mathrm{Re} - \mathrm{Rc})] \times 100 \quad (1)$$

where Re is the isotope ratio of incubated samples and $\mathrm{Re} = [A_{(F+1)}/A_{(F)}]$ (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 ^{15}N-labeled amino sugars can be obtained based on both APE and the concentration of individual compounds, which was expressed as:

$$C_L = C_T \times \mathrm{APE}/100 \quad (2)$$

where C_T is the total concentration of each amino sugar determined by GC–EI/MS and C_L represents the content of the ^{15}N-labeled portion in the compound.