Specific response of soil fungi and bacteria to carbon availability indicated by the transformation dynamics of soil amino sugars

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Abstract
Microorganisms in terrestrial ecosystems are usually in dormancy due to carbon starvation. Labile carbon sources enhance microbial assimilation of extraneous N but carbon availability regulates the specific response of fungus and bacterium populations as well as the community structure of the microbiota. The dynamics of ^{15}N incorporation into fungus-derived glucosamine (GluN) and bacterium-originated muramic acid (MurN) can reflect the ever-active response of these two populations during the immobilization of extraneous ^{15}N-NH_4^+. For glucose amendment, the more rapid ^{15}N transformation into MurN than GluN indicated that bacteria were more competitive initially than fungi, but the successional growth of fungi was dominant over time, resulting in the continuous increase of ^{15}N enrichment in GluN. However, the addition of maize stalk was of more benefit to the reproduction of fungi and retarded the rapid growth of bacteria in soil, thus the ^{15}N enrichment in GluN and MurN was increased initially and then became stable. After being incubated with maize stalks for 18 weeks, the assimilation capacity of reproduced fungi to simple substrates was enhanced while that of bacteria was weakened in soil microcosms. The findings indicate that soil fungi and bacteria respond to different carbon sources specifically.

Key Words
Amino sugar, ^{15}N transformation, carbon availability, bacterium and fungus, response

Introduction
Native microorganisms in terrestrial ecosystems are usually in dormancy and restricted in reproduction mostly due to carbon deficiency (Mondini *et al*., 2006). Therefore, the assimilation of nutrients, especially N by soil microorganisms is highly depended on carbon availability (Paul and Clark, 1996). If large amounts of inorganic N remained in soil, there would be an environmental risk due to N losses. Labile carbon sources enhance microbial assimilation of extraneous N but the carbon availability regulates the specific, response of fungus and bacterium populations as well as the community structure of the microbiota (Brant *et al*., 2006; Schneckenberger *et al*., 2008; Rasul *et al*., 2009). Because amino sugars are recognized as microbial residue biomarkers with heterogeneity, i.e., muramic acid (MurN) is uniquely derived from bacteria and glucosamine (MurN) is mainly of fungus origin (Parsons, 1981; Zhang *et al*., 1999), the dynamics of inorganic N incorporation into these two compounds can reflect the ever-active response of different communities to the added nitrogen. However, this can only be done when newly incorporated N (labeled) can be differentiated from soil inherent N (unlabeled) by the isotope tracing technique (He et al., 2006). Therefore, laboratory incubations with ^{15}N-labeled ammonium were conducted with addition of either glucose or maize stalk as carbon sources. The ^{15}N enrichment in GluN and MurN was traced periodically and the specific response of bacteria and fungi to different carbon availability was evaluated.

Methods
Soil and incubations
A fresh surface (0-20 cm) Mollisol sample was collected from Gongzhuling, Jilin Province, China (124º48´E, 43º30´N) and sieved to <2 mm. Portions of soil were weighed into plastic containers and $^{15}NH_4^+$ solution was added once a week at 0.1 mg N/g soil each time. For the organic material amendment, maize stalk (<2 mm, 452 mg C/g) was mixed initially with soil samples at the weight percentage of 4%, while for glucose amendment, glucose was added at 1 mg C/g soil together with $^{15}NH_4^+$ once a week till the 18th week to ensure equal carbon input between the two treatments. The incubated soils were sampled after 1, 2, 4, 6, 9, 12, 15 and 18 weeks, respectively.
In the other incubation, the soil sample was first incubated with NH_4^+ addition once a week with maize stalk as the carbon source. After 18 weeks, glucose plus $^{15}NH_4^+$ was added into the microcosm once a week and the incubated soils were sampled after 1, 2, 4, 6 and 9 weeks, respectively.

Analysis of soil amino sugars and the determination of ^{15}N enrichment

The air-dried soil samples were ground to <0.25 mm and the pretreatments of amino sugars including hydrolyses, purification and derivatization were conducted using the method of Zhang & Amelung (1996). The amino sugar derivatives were separated on a DB-5MS column and the ^{15}N incorporation into individual amino sugars was identified by gas chromatography/mass spectrometry (GC/MS) (He et al, 2006).

Calculations and statistic analysis

The ^{15}N enrichment in each amino sugar was expressed by the term of atom percentage excess (APE) and calculated as follows:

$$APE = (Re - Rc)/[1 + (Re - Rc)] \times 100$$

Where Re is the isotope ratio of incubated samples and Re = $[A_{(F+1)}/A_{(F)}]$ (A is the area of the selected ion). Rc represents the corresponding ratio obtained from original samples analyzed on the same GC/MS assay (He *et al*., 2006).

The effect of carbon availability on the ^{15}N enrichment of individual amino sugars at different sampling time was analyzed using a one-way analysis of variance (ANOVA) and LSD method at a 95% confidence level.

Results

Specific response of fungus- and bacterium-derived amino sugar to different C sources

When glucose plus ^{15}N-labeled NH_4^+ was added into the soil samples in a week, the ^{15}N enrichment in MurN increased rapidly and then tended to reach the maximal level of 42 after 9 week incubation. The extraneous ^{15}N incorporation into bacterial MurN was more rapid at the first week in maize stalk amendment and showed no significant difference compared with that in glucose amendment (P>0.05). However, the plateau of ^{15}N enrichment in MurN was found shortly after the incubation with the value less than 13 for maize stalk treatment. The ^{15}N enrichment for fungal GluN increased also for glucose amendment but the velocity and magnitude were much lower than those for MurN. The transformation from $^{15}NH_4^+$ into GluN for the first two weeks was more rapid for the maize stalk amendment than for the glucose treatment and it became stable after 9-week incubation with the maximal magnitude of 15%, which was much lower than that for the glucose amendment (Figure 1). Interestingly, there were different responses of fungal and bacterial amino sugars to different C source addition.

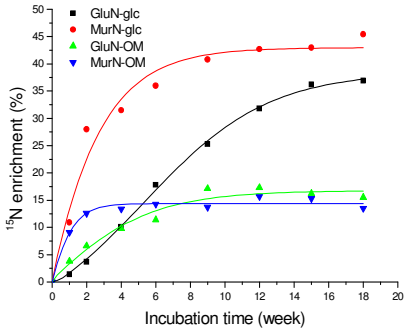

Figure 1. ^{15}N enrichment of muramic acid (MurN) and glucosamine (GluN) during incubations amended with maize stalk (OM) or glucose (glc).

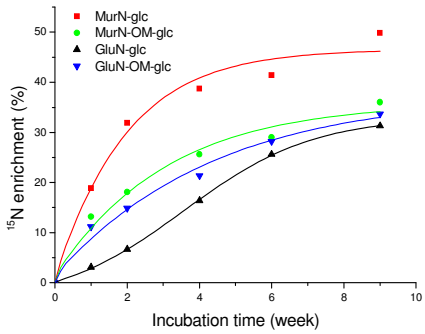

Figure 2. ^{15}N enrichment of muramic acid (MurN) and glucosamine (GluN) after glucose-$^{15}NH_4^+$ addition into the soil microcosm amended with maize stalk (OM-glc).

Specific response of fungus- and bacterium-derived amino sugars to the change of C source

When glucose plus $^{15}NH_4^+$ was added into the soil samples having been incubated with maize stalk and NH_4^+, the ^{15}N enrichment in both MurN and GluN was increased rapidly the first 4 weeks and then tended to become stable. In this case, the magnitude of ^{15}N enrichment between the two compounds showed no significant difference during the whole incubation (P>0.05). Compared with the incubation with glucose addition initially, the ^{15}N incorporation into bacterial MurN was decreased significantly (P<0.05); whereas, the ^{15}N enrichment for fungal GluN was significantly higher, especially before the first 6 weeks (P<0.05).

Conclusion

The availability of C sources determined the microbial immobilization of extraneous N and the specific response of bacteria and fungi as well as the changes in the community structure was indicated by the ^{15}N enrichment dynamics of heterogeneous amino sugars. Active carbon, for instance, glucose and the labile components including soluble saccharide, organic acids and amino acids released from maize stalk, enhanced

nitrogen assimilation and the transformation of the structural compounds; whereas, the recalcitrant carbon in organic residue can only enable survival of the microorganisms in soil matrices and thus the nutrient transformation was restricted. The significant higher 15N enrichment of MurN than GluN for glucose amendment reflected explicitly that bacteria were more competitive initially than fungi to assimilate the substrate of high availability, but the successional growth of fungi was dominant over time. However, in the maize stalk amendment, the nutrient competition between bacterium and fungus populations was diminished because fungi have higher ability to span microsites and decompose more recalcitrant substrates to compensate intensive carbon requirement, leading to the relatively retarded growth of bacteria. Therefore, the less available substrate of crop residue was of more benefit to the biodiversity of soil microorganisms, especially the reproduction of fungi in the soil microcosms. As a result, the assimilation capacity of the stimulated fungi to simple substrates was enhanced while that of bacteria was weakened. The findings indicate that soil fungi and bacteria respond to different C sources specifically. In order to reduce the environmental risk of extraneous N losses from both agricultural and natural ecosystems, enough C sources with different availability should remain.

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