



## Short communication

## Effects of contrasting agricultural management on microbial residues in a Mollisol in China

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## ABSTRACT

Microbial residue represents a significant amount of soil organic matter, and its component amino sugar can serve as time-integrated indicators that reflect chronic effects of agricultural management. We evaluated the influence of different land-use and fertilization treatments on the amounts and patterns of amino sugars (glucosamine and galactosamine) and muramic acid in a Mollisol (Udolls, USDA Soil Taxonomy System) in northeastern China. The treatments included: BL, bareland, without any vegetation; GL, restored grassland which allows plants to re-vegetate naturally; AL, arable land without any fertilizer; ALF, arable land with chemical fertilizer; and ALMF, arable land with chemical fertilizer and pig manure. The amino sugar concentrations differed significantly between various treatments after 26 years, with the order of ALMF > GL > ALF > AL > BL. This suggests that long-term contrasting management changed microbial residue accumulation in soil, which is strongly related to soil organic carbon content. The larger ratios of glucosamine to muramic acid in the GL plots than the AL and BL plots indicated a shift toward fungal-derived residues after 26 years of natural restoration. Our results suggested that different land-use and fertilization treatments clearly influenced amounts and patterns of microbial residues and their contribution to SOM accumulation, primarily due to differences in organic C inputs.

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## 1. Introduction

In terrestrial ecosystems, the dynamics of soil organic matter (SOM) are highly related to microorganisms due to their key roles in SOM turnover and nutrient cycling (Paul and Clark, 1989; Wardle et al., 1999; Glaser et al., 2006; Liang and Balser, 2008). Numerous studies have shown that the SOM content and associated fractions as well as microbial biomass and community composition are influenced by different land-use or fertilization treatments (Frey et al., 1999; Joergensen et al., 2010; Yang et al., 2012). However, one important but little-understood aspect is the accumulation of microbial residues in soils (Guggenberger et al., 1999; Glaser et al., 2006; Liang et al., 2012). Microbial residues have relatively long residence time in soils and are regarded as important part of the stable carbon (C) pool (Amelung, 2001; Kiem and Kögel-Knabner, 2003; Glaser et al., 2004; Simpson et al., 2007). The stable C pool is closely related to the role of soils as a terrestrial C sink (Falloo and Smith, 2000), thus the long-term sequestration of C in soil may be related to senesced microbial residues (Liang and Balser, 2008). Meanwhile, microbial residues serve as time-integrated indicators

that reflect chronic effect of agricultural management (van Groenigen et al., 2010; Ding et al., 2011).

The concentrations of amino sugars and muramic acid (MurA) have been routinely used to indicate the presence of microbial residues in soil and their contribution to SOM (Glaser et al., 2004; Amelung et al., 2008; van Groenigen et al., 2010). A significant proportion of amino sugars may accumulate in soil as a stable pool; but they also form part of the labile and mineralizable pool. The most important amino sugars in soil are glucosamine (GluN) and galactosamine (GalN), although other kinds of amino sugars also exist in soil (Stevenson, 1982; Amelung, 2001). Glucosamine is primarily originated from the chitin of fungal cell walls, although bacteria and soil invertebrates also make some contribution (Parsons, 1981; Chantigny et al., 1997). The origin of GalN is still uncertain, even though it is thought to generally derive from bacteria (Amelung, 2001). Muramic acid occurs exclusively in bacterial cell walls (Parsons, 1981). The ratio of GluN to MurA is generally used to differentiate the relative contribution of fungi and bacteria to SOM turnover and accumulation (Guggenberger et al., 1999; Amelung, 2001; Glaser et al., 2004).

Mollisols serve as a key base of grain production in China. It is mainly distributed in northeastern China with a total area of 5.9 ha × 10<sup>6</sup> ha, and about 4.4 ha × 10<sup>6</sup> ha of it was cultivated. Mollisols were once very fertile; however, the present SOC content

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has declined to 50% of its initial value due to intensive cultivation (Liu et al., 2003). The serious degradation scenarios have threatened sustainable crop production and even national food supply. Therefore, proper management of Mollisols to improve soil quality and its C storage has received much attention. Some research focused on considering organic manuring practice and conversion of some arable soils into secondary grassland with an aim to foster agricultural sustainability. Studies have shown that land-use change and fertilization practices could influence SOC level in the Mollisols (Li et al., 2007; Ding et al., 2012); little is known about the degree to which soil microbial residues persist and differ under different land-use and fertilization practices. Such information may help to improve our monitoring and assessment capacities of the sustainability and environmental impact of particular cropping system (Six et al., 2006; Liang et al., 2012).

Long-term field experiments could provide indispensable resources for the assessment of management-induced changes in microbial residues and their contribution to SOM (Rasmussen et al., 1998; Glaser et al., 2006). Here, we used amino sugars and MurA as biomarkers to investigate the dynamics of microbial residues. Our objective was to evaluate how different land-use and fertilization treatments affect the patterns of microbial residue accumulation in Mollisols and their contribution to SOM. We hypothesized that long-term contrasting management could lead to significant differences in the storage of microbial residues and hence their contribution to SOM due to differences in organic inputs and soil disturbance.

## 2. Materials and methods

### 2.1. Study site

The study site was located at the Hailun National Field Station, Chinese Academy of Sciences, in Heilongjiang province, China (47°26'N, 126°38'E). The climate of this region is humid continental according to the Köppen Climate Classification (Peel et al., 2007). The mean annual temperature (MAT) is about 1.5 °C. The mean annual precipitation (MAP) varies from 500 to 600 mm, with about 88% occurring through May to September. The soil is classified as Udolls based on the USDA Soil Taxonomy (Soil Survey Staff, 1998). The average texture in the 0–20 cm layer is 257 g sand kg<sup>-1</sup>, 324 g silt kg<sup>-1</sup>, and 408 g clay kg<sup>-1</sup>. The study site had been under crop cultivation for more than 100 years before 1985.

### 2.2. Experimental design

In 1985, three adjacent lands were included to set up the experiment. One land (180 m<sup>2</sup>) was treated as bareland (BL). The land was kept bare and wild vegetation was hoed manually once emerged. Another land (360 m<sup>2</sup>) was used for grassland restoration (GL). The land was vegetated naturally without any fertilizer inputs or tillage. *Leymus chinensis* has gradually become the dominant species. The last land (720 m<sup>2</sup>) was used as agricultural land, with a 3-year soybean (*Glycine max* L.)-maize (*Zea mays* L.)-wheat (*Triticum aestivum* L.) rotation cropping. The agricultural land included: no fertilizer control (AL), chemical fertilization (ALF), and application of pig manure plus chemical fertilizer (ALMF). The three fertilizer treatments were arrayed in a randomized block design with four replicates (60 m<sup>2</sup> for each treatment plot). Urea and ammonium hydrogen phosphate were added as the N sources to provide 30 kg N ha<sup>-1</sup> for soybean, 150 kg N ha<sup>-1</sup> for maize, and 75 kg N ha<sup>-1</sup> for wheat. Ammonium hydrogen phosphate was also used to supply 36 kg P ha<sup>-1</sup> for soybean, 33 kg P ha<sup>-1</sup> for maize, and 24 kg P ha<sup>-1</sup> for wheat. Pig manure, with an average composition of 265 g C kg<sup>-1</sup>, 21 g N kg<sup>-1</sup>, and 2.6 g P kg<sup>-1</sup> was added at a rate of 15,000 kg ha<sup>-1</sup> year<sup>-1</sup> (on a dry weight basis) for

all crops. The above rate of manure supplied 3975 kg C ha<sup>-1</sup>, 315 kg N ha<sup>-1</sup>, and 39 kg P ha<sup>-1</sup> annually. Further detailed information about the study site can be found in Li et al. (2007).

It should be mentioned that our experimental design was pseudoreplicated for BL and GL which were subdivided into four equal subplots to serve as four treatment replicates. Our specific interest was to identify the microbial residue accumulation and explore the potential for contrasting land management effects in site-specific soil management. Based on the random selection and low spatial variability in soil characteristics we presume that any significant differences between these plots could be attributed to long-term treatment effect.

### 2.3. Soil sampling

Six randomized soil cores (2.64 cm in diameter) per replicate were sampled from the 0–20 cm depth with a T sampler in April 2011. The soil cores from the same replicate plot were composited, placed in plastic bags in the field, and kept cool until processed in the laboratory. A total of 20 soil samples (5 treatments × 4 replicates) were collected. The soils were passed through a 4-mm sieve, homogenized, and stored at 4 °C. All visible roots and fresh litter materials were removed from samples prior to sieving. Field-moist samples were used for microbial biomass C analysis. Subsamples were air-dried for pH, available N, and available P analyses, and ground to pass a 0.25-mm sieve for total C and amino sugar analysis.

### 2.4. Analytical procedures

Total C and N contents in soil were determined by dry combustion with a VarioEL CHN elemental analyzer (Heraeus Elementar Vario EL, Hanau, Germany). Because these soils are free of carbonates, the total C content is equivalent to SOC content. Soil pH was measured in a 1:2.5 soil/water suspension with a combination reference glass electrode. Bulk density was calculated from the mass of oven-dried soil (105 °C) contained in a given volume of the soil sample. Soil available N was analyzed through quantification of alkali-hydrolysable N in a Conway diffusion unit with Devarda's alloy in the outer chamber and boric acid-indicator solution in the inner chamber (Shen et al., 2004). Available P was determined with the Olsen P method (Lu, 2000). Soil microbial biomass C was determined by chloroform fumigation-extraction technique (Vance et al., 1987).

Amino sugar analysis was carried out according to Zhang and Amelung (1996). Briefly, amino sugars were extracted and converted to aldononitrile acetates, and the derivatives were separated on an Agilent 6890A gas chromatograph (GC, Agilent Tech. Co. Ltd., USA) equipped with a HP-5 fused silica column and flame ionization detector. Amino sugars were identified manually by comparison with the peaks for standards and quantified based on the internal standard myo-inositol (100 µL at 1 mg mL<sup>-1</sup> per sample).

### 2.5. Data analysis

Because “replicates” of the BL and GL treatments are not true replicates but “pseudoreplicates”, the Kruskal–Wallis test is used to determine if there are significant differences among the treatment medians. The Kruskal–Wallis test is the nonparametric analog to One-way analysis of variance. To determine what significant differences are, the Kruskal–Wallis Multiple Comparisons with a MINITAB Macro Dunn's Test were performed. Although this approach may be somewhat lacking for statistical power, it should still be able to detect significant differences among treatments after 26 years.

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