



Higher rates of manure application lead to greater accumulation of both fungal and bacterial residues in macroaggregates of a clay soil



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ABSTRACT

Microbial residues represent a significant soil organic matter pool and participate in soil aggregation. The addition of organic manure is known to modify soil aggregation and strongly influence soil microbial residues. How manure application influences the spatial distribution of microbial residues in soil aggregates is largely unknown. This study attempts to determine the effect of manure application at various rates on the content and distribution of microbial residues among aggregates of different sizes. We used a long-term manure application experiment in a Mollisol in northeastern China, where manure has been applied since 2001 at rates of 0, 7.5, 15, and 22.5 Mg ha⁻¹ yr⁻¹ (dry weight). The abundance of microbial residues was indicated by amino sugar analysis. Glucosamine and muramic acid were used as biomarkers for fungal and bacterial residues, respectively. Amino sugars were examined within four aggregate fractions: large macroaggregate (>2000 μm), small macroaggregate (250–2000 μm), large microaggregate (53–250 μm) and small microaggregate (<53 μm). Application of manure at 15 and 22.5 Mg ha⁻¹ yr⁻¹ provided significantly higher proportions of macroaggregates and mean weight diameter (MWD) than non-manure treatment and manure applied at 7.5 Mg ha⁻¹ yr⁻¹. Manure application, especially at higher rates, significantly stimulated the accumulation of total amino sugars in both macroaggregates and large microaggregates and more amino sugars were found in >250 μm macroaggregates compared with microaggregates. However, effects of manure application rates on amino sugar accumulation in larger aggregates were limited when manure rate was increased from 15 to 22.5 Mg ha⁻¹ yr⁻¹. The response of fungal- and bacterial-derived amino sugars to manure application rates differed among aggregate fractions, i.e., glucosamine associated with macroaggregates increased more than that of microaggregates, whereas the enhancement of muramic acid was prominent in both macroaggregates and large microaggregates. The mass proportions of macroaggregates and MWD showed significant positive correlations with amino sugar contents, indicating that these microbial residues are involved in the formation and stabilization of aggregates. Manure applications greatly increased the contribution of microbial residues to soil organic C (SOC) in small macroaggregates and large microaggregates ($P \leq 0.05$). We conclude that higher manure input may promote soil aggregation and higher SOC storage, which is closely related to a greater microbial residues-mediated improvement of soil aggregate stability. Our results also suggest that measurement of amino sugar content is a useful approach to assess fungal and bacterial contributions to soil aggregation.

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1. Introduction

Sequestration of carbon (C) in soil is critical for agriculture and the environment, in particularly sustainability of agroecosystem and food security (Lal, 2004). Soil C dynamics are closely related to catabolic and anabolic activities of microorganisms (Liang et al.,

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2011; Schimel and Schaeffer, 2012). The microbial contribution to soil C pool is directly related to microbial community dynamics and the balance between production and degradation of microbial products (Six et al., 2006). The great relevance of microbial residues (i.e., necromass) within soil organic matter (SOM) has been increasingly recognized within the last decade (Miltner et al., 2012). Reports indicate that microbial residues represent a significant source of stable C pool and may play a greater role in long-term C sequestration in soils than traditionally believed (Simpson et al., 2007; Liang et al., 2011). Increasing the potential for agricultural soils to sequester C, therefore, requires a better understanding of contribution of microbial necromass to soil organic C (SOC).

Manure application effects on the amount and size of water-stable aggregates and organic matter associated with aggregates have been reported by Haynes and Naidu (1998) and other researchers (Aoyama et al., 1999; Mikha and Rice, 2004; Mellek et al., 2010). Soil aggregates governs the spatial heterogeneity of soil physical and chemical properties, and consequently, the heterogeneous distribution of microorganisms and their activity among aggregates of different sizes (Gupta and Germida, 1988; Schutter and Dick, 2002). Studies regarding the effects of organic matter input (manure or crop residues) on soil microbes mainly focus on changes in the biomass and composition of microbial communities (Kong et al., 2011; Le Guillou et al., 2012; Hurisso et al., 2013). The effects of manure application on microbial residues, particularly their distribution in aggregates, have received very limited attention (Simpson et al., 2004; Ding and Han, 2014). Nevertheless, it was suggested that soil microbial residues may help form or stabilize soil aggregates and their effects on soil aggregates may be more persistent than living biomass (Tisdall and Oades, 1982; Chantigny et al., 1997). In turn, the degree to which microbial residues accumulate in soil depends largely on the extent of physical protection of the soil aggregate structure (Six et al., 2006). Therefore, it is important to isolate the microbial residues from soil and determine their distribution within soil aggregate structure. This information will improve our mechanistic understanding of the impacts of agricultural management practices on SOC storage and turnover (Simpson et al., 2004; Six et al., 2006), and further predict the sustainability of particular crop management systems (Liang et al., 2013).

The dynamics of microbial residues and their contribution to SOM can be indicated by soil amino sugar analysis (Zhang et al., 1999; Amelung, 2001). Previous studies have shown that amino sugars are rather stable against fluctuations in living microbial biomass and primarily occur in dead microbial cells (Chantigny et al., 1997; Guggenberger et al., 1999a; Glaser et al., 2004). Amino sugars also serve as a time-integrated biomarker to indicate microbial community structure (Glaser et al., 2004). Muramic acid is uniquely synthesized by bacteria, whereas glucosamine predominantly originates from fungal cell walls (Parsons, 1981; Amelung, 2001; Appuhn and Joergensen, 2006). Galactosamine constitutes a significant fraction of the total amino sugar pool (Glaser et al., 2004), but its origin in soil is still debated (Amelung, 2001; Engelking et al., 2007). Glucosamine and muramic acid has been used to differentiate between fungal and bacterial contributions to soil aggregation (Chantigny et al., 1997). Simpson et al. (2004) observed a preferential accumulation of fungal-derived amino sugars in macroaggregates under no-tilled versus conventional-tilled soils. Manure application can enhance soil aggregation and provide a protective mechanism for organic matter even in annually tilled systems (Aoyama and Kumakura, 2001). Although some knowledge exists regarding the effects of manure application on fungal and bacterial residues in bulk soils (Scheller and Joergensen, 2008; Joergensen et al., 2010; Ding et al., 2013), the information on the long-term manure effects on content and distribution of amino sugars in aggregates and association of these

microbial residues with aggregate stability is very scarce. Recently, we found that long-term manure application ($15 \text{ Mg ha}^{-1} \text{ yr}^{-1}$) combined with chemical fertilizer significantly stimulated the accumulation of total amino sugars in all aggregate-size fractions as compared to chemical fertilization in Mollisols (Ding and Han, 2014). The unknown questions so far are: 1) whether higher level of manure inputs could further lead to a corresponding increase in microbial residues within all soil aggregate size classes? 2) how fungal and bacterial residues along aggregates of different size respond to higher levels of manure input?

The objective of this study was to investigate the influence of 11 years of continuous manure addition under a broad range of input rates (from 0 to $22.5 \text{ Mg ha}^{-1} \text{ yr}^{-1}$) on distribution of microbial cell wall residues within the aggregate structure of a clay soil in China. Our hypotheses were: 1) amino sugar concentrations in all aggregate-size fractions should be significantly higher in soils received higher manures versus those in non- or lower manure additions, with a greater magnitude of responses in macroaggregate-associated amino sugars, and 2) enhancement dynamics of fungal and bacterial residues in macro- and micro-aggregates with higher manure input would differ and the both ultimately approach to a saturation capacity.

2. Materials and methods

2.1. Study site

The study site is located at the National Observation Station of Hailun Agro-ecology System ($47^{\circ} 26' \text{ N}$, $126^{\circ} 38' \text{ E}$) in Heilongjiang province, China. The area experiences a typical temperate continental monsoon climate. The mean annual air temperature is 1.5° C . The mean annual precipitation is 550 mm, with approximately 65% occurring from June to August. The soils in this site are classified as Udolls according to the USDA Soil Taxonomy (Soil Survey Staff, 2010) and the respective WRB soil types are Phaeozems (World Reference Base for Soil Resources, 2006). The surface soils (0–20 cm) had an average texture of 258 g sand kg^{-1} , 332 g silt kg^{-1} , and 410 g clay kg^{-1} .

2.2. Experimental setup

The field experiment was initiated in the fall of 2001 using a randomized complete block design with three replicates and five treatments, which resulted in a total of 15 plots. Each plot was 12 m long and 5.6 m wide. The treatments selected for the present study were: (1) chemical fertilization with no manure application; (2) chemical fertilization with manure applied at $7.5 \text{ Mg ha}^{-1} \text{ yr}^{-1}$; (3) chemical fertilization with manure applied at $15 \text{ Mg ha}^{-1} \text{ yr}^{-1}$; and (4) chemical fertilization with manure applied at $22.5 \text{ Mg ha}^{-1} \text{ yr}^{-1}$. Urea and ammonium hydrogen phosphate (30 kg N ha^{-1} and 36 kg P ha^{-1} for soybean, 150 kg N ha^{-1} and 33 kg P ha^{-1} for maize, and 75 kg N ha^{-1} and 33 kg P ha^{-1} for wheat) were applied as basal fertilizers. Potassium sulfate was used as K fertilizer (30 kg K ha^{-1} for all crops). Chemical fertilizers were all applied in one time as basal fertilizer. The manure was collected from an open, commercial pig feedlot and stored for 1 year before application. Although all the manure came from the same feedlot, there were some variations in manure characteristics among years. The moisture content, pH, organic C, and nutrient composition of the manure applied during the last 3 years were reported in Table S1. On average, the total C input in the form of manure was equivalent to 2467, 4935 and $7402 \text{ kg C ha}^{-1} \text{ yr}^{-1}$ at rates of 7.5, 15, and $22.5 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, respectively, the amounts of added nitrogen (N) were 182.8, 365.5 and $548.3 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, respectively and the amounts of added phosphorus (P) were 19.6, 39.2, $58.8 \text{ kg P ha}^{-1} \text{ yr}^{-1}$, respectively. Each year manure was applied in one time by manually spreading

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