



## Long-term manure amendments enhance neutral sugar accumulation in bulk soil and particulate organic matter in a Mollisol



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### ABSTRACT

Manure application generally increases soil organic matter (SOM) and particulate organic matter (POM) content in soil. Free and occluded POM (fPOM and oPOM) can be quantified by combining density and ultrasonic dispersion approaches, but it remains unclear which fraction of POM is more responsive to manure application, and whether manure treated soils have a more pronounced effect on POM content than unmanured soils (no or chemical fertilizer treated soils). Because neutral sugars in POM can be attributed to either plant- or microbial-derived compounds, we analyzed the pattern and ratio of different neutral sugars to clarify effects of different fertilizations on quality of POM in a study over two decades. Soil samples (0–20 cm) were collected from six fertilization treatments in a 25-year long fertilization experiment including no fertilizer (CK), low manure (M1), high manure (M2), chemical nitrogen, phosphorus and potassium fertilizers (NPK), and combined manure and chemical fertilizers (M1NPK, M2NPK). Our results showed that manure application generally led to higher organic carbon concentrations in bulk soil (M2NPK > M2 > M1NPK > M1 > CK > NPK), fPOM (M2NPK > M2 > M1 > M1NPK > NPK > CK) and oPOM (M1 > M2 > M1NPK > M2NPK > NPK > CK), respectively. As compared with unmanured treatments, manure amendments induced 48, 21 and 107% greater increases on average in neutral sugar concentrations in bulk soil, fPOM and oPOM, respectively. More plant-derived organic compounds were enriched in fPOM than oPOM and bulk soil, and the enrichment was more pronounced in manure treated soils than the unmanured soils. This study suggests that long-term use of manure enhanced microbial routing of specific monosaccharides into different POM fractions. Clearly, manure amendments improved labile SOM content and SOM quality in the Mollisol thus maintaining soil productivity over decades.

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### 1. Introduction

Black soils, typical Mollisols with a high organic matter content, are mainly distributed in the northeast of China (Chen et al., 2010). The soils have been primarily used for maize and soybean cultivation for more than 100 years (Zhao et al., 2006). Mollisols play an important role in Chinese grain production due to their fertile and productive nature (Chen et al., 2010). However, the productivity of the soils is declining due to improper management practice under

long-term conventional tillage. In the Chinese conventional tillage system, all aboveground plant biomass are typically removed for energy use or livestock feed. The removal of crop residue resulted in declines of soil organic matter (SOM), deterioration of soil structure, and serious soil erosion. The similar adverse effect can be induced via over-use of chemical fertilizers (Chen et al., 2010; Yan et al., 2012). Increasing organic inputs (e.g. manure application) increases SOM content (Haynes and Naidu, 1998) and thus is one of most crucial soil amendment practices for minimizing the deterioration of soil quality.

Relative to total SOM, labile SOM fractions have been found to be more sensitive to soil management, including fertilization, rotation and tillage management (Besnard et al., 1996; Aoyama et al., 1999).

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Particulate organic matter (POM) is an important part of labile SOM and is composed of relatively undecomposed or partly degraded plant residues (Mirsky et al., 2008; Kölbl et al., 2005). POM is usually categorized to the free and occluded fractions (fPOM and oPOM) based on physical fractionation method, providing important information about the fate of plant-derived C in the course of SOM decomposition (Golchin et al., 1994, 1995; Kölbl et al., 2005). Furthermore, POM is closely linked to aggregation (Aoyama et al., 1999) and its decomposition can enhance soil microbial activity and consequently the production of transient binding agents (Golchin et al., 1994; Puget et al., 1995). Accordingly, POM is an effective indicator of soil quality (Cambardella and Elliott, 1992).

Carbohydrates are labile fractions of SOM and influence soil biological, physical and chemical processes (Cheshire, 1979). Carbohydrates are usually represented by neutral sugar monomers with 5–25% of the total soil organic carbon (SOC) (Cheshire, 1979). The neutral sugars originate from both plants and soil microorganisms (Jolivet et al., 2006) including eight monosaccharides (Xylose, Arabinose, Galactose, Mannose, Rhamnose, Fucose, Ribose and Glucose). Plant-derived pentoses (i.e. xylose and arabinose) and microbially-derived hexoses (i.e. galactose and mannose) are used to differentiate the relative contributions of plants and microbes in regulating SOM decomposition and formation (Oades, 1984; Jolivet et al., 2006; Bock et al., 2007). The carbohydrate origin could be roughly estimated by the monosaccharide ratios. Oades (1984) suggested that the ratio, defined as (Gal + Man)/(Xyl + Ara) in soil, is >2 for microbe-derived and <0.5 for plant-derived. Other ratio expressions e.g. Man/Xyl (Hu et al., 1995) (Rha + Fuc)/(Ara + Xyl) (Glaser et al., 2000) and (Gal + Man + Rha + Fuc)/(Ara + Xyl) (Jolivet et al., 2006) were also used to interpret the carbohydrate origin. The neutral sugars have well been used as sensitive indicators of soil C turnover (Guggenberger et al., 1994; Nacro et al., 2005; Trouve et al., 1996; Navarrete and Tsutsuki, 2008).

The content and composition of neutral sugars are affected by management practices such as tillage (Puget et al., 1999), land use change (Martens et al., 2004; Jolivet et al., 2006; Navarrete and Tsutsuki, 2008) and long-term fertilization (Angers and Ndayegamiye, 1991). Jolivet et al. (2006) compared neutral sugars in whole soil and particle-size fractions and found that carbohydrates as indexed by neutral sugars decreased when soil use was converted from forest to maize cropping. Neutral sugar composition in two type of bulk soil and their density fractions revealed the role of polysaccharide-mineral interaction in the course of SOM stabilization (Rumpel et al., 2010). Dalal and Henry (1988) found that 27%–43% of carbohydrates were present in light fractions, but there was little explicit information about the allocation of carbohydrate (e.g. neutral sugars) in fPOM and oPOM fractions. This knowledge is very important to understand the rate of different organic components during SOM decay. Manure application usually increases SOM content including both particulate and mineral-associated SOM content (Aoyama et al., 1999). However, it remains unclear whether fertilizations differ in their influences on the concentrations of fPOM and oPOM and the neutral monosaccharides allocated within each POM. In addition, it remains unresolved whether quantification of neutral sugars in POM can be used to identify the origin of POM so that we can evaluate the extent of microbial recycling during SOM decay.

In this study, we quantified neutral sugar concentrations in fPOM, oPOM and bulk soil at a 25-year long manured and unmanured fertilization experiment in the northeastern China. We aimed to (1) assess the effects of long-term application of chemical fertilizers and manure separately or in combination on concentrations of fPOM, oPOM and SOM; (2) characterize the effects of long-term application of different fertilizers on the abundance and

composition of neutral sugars in the fPOM and oPOM; and (3) employ the neutral sugar ratio for assessing the relative contribution of plant- and microbial-derived organic matter in the POM. We hypothesized that manured soils harbor a greater amount of SOM, fPOM and oPOM than unmanured soils, and long-term manure application increased the proportion of plant-derived organic compounds than chemical fertilizer application. This study is expected to reveal effects of long-term fertilizations on SOM dynamics at the specific compound level thus it can shed new insights on long-term SOM transformation under intensive agricultural practice.

## 2. Materials and methods

### 2.1. Site characteristics and soil collection

A long-term field experiment was established in 1979 by Jilin Academy of Agricultural Sciences at Gongzhuling, Jilin Province, China (124°48'33"E, 43°30'23"N). The soil is a clay loam [*Typic Hapludoll* (Mollisol) in USDA Soil Taxonomy] with 39% sand, 30% silt and 31% clay at the beginning of the experiment. Each plot covers 400 m<sup>2</sup>, arranged in a randomized block design with three replicates. Prior to this experiment, the land had been intensively cultivated for at least 50 years (continuous corn for about 20 years), and then the experimental field was homogenized by growing maize (*Zea mays* L.) for 3 years without fertilizer addition. The primary soil chemical and physical properties were shown in Table 1. Since 1979, all plots have grown monoculture maize until present. Maize is sown in late April and harvested in late September. Above ground residues are removed at harvest. Six treatments were selected in this study including CK, NPK, M1, M1 + NPK, M2 and M2 + NPK. The CK is the control with cropping but no fertilizer applied; M1 and M2 are two pig manure treatments applied at the rate of 4.5 and 9.0 Mg ha<sup>-1</sup> in Spring once every year (dry weight basis), respectively; NPK are chemical N (urea), P (multiple superphosphate) and K (potassium sulfate) fertilizers added at the rate of 165 kg N, 82.5 kg P and 82.5 kg K ha<sup>-1</sup> per year, respectively. Three soil samples (0–20 cm) were collected from each treatment plot and composited in one sample prior to fertilization in April, 2005 after two and half decades' cultivation. The soil samples were air dried and passed through a 2-mm sieve before they were subject to physical and chemical analysis. The manure was collected before its application in April, 2005. Typical samples were air dried and grounded, and were quantified by analyzing the chemical composition of applied manure (i.e. C, N and neutral sugars concentrations, Table 2).

**Table 1**

Soil physiochemical properties (0–20 cm) at the long-term fertilization site in Gongzhuling, Jilin Province, China.

| Sampling time | Treatments | SOC  |     |     |                    | C:N  | pH  |
|---------------|------------|------|-----|-----|--------------------|------|-----|
|               |            | TN   | TP  | TK  | g kg <sup>-1</sup> |      |     |
| 1980          | Initial    | 16.3 | 1.9 | 1.4 | 19.7               | 8.6  | 7.6 |
| 2005          | CK         | 20.0 | 1.6 | 0.6 | 18.8               | 12.5 | 7.7 |
|               | NPK        | 19.4 | 1.6 | 0.7 | 17.8               | 12.1 | 7.6 |
|               | M1         | 27.0 | 2.5 | 1.1 | 21.1               | 10.8 | 7.5 |
|               | M1NPK      | 27.2 | 2.6 | 0.9 | 18.2               | 10.5 | 7.7 |
|               | M2         | 30.3 | 2.7 | 1.3 | 20.7               | 11.2 | 7.4 |
|               | M2NPK      | 35.5 | 2.4 | 2.2 | 18.8               | 10.4 | 7.3 |

SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium.

CK = Check; NPK = treatment receiving chemical N, P and K fertilizers; M1 = low Manure application rate; M2 = high Manure application rate; M1NPK, combination of M1 and NPK; M2NPK, combination of M2 and NPK. Other details are presented in the method section. Data in 1980 was adapted from Sun et al. (1991).

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