



Dynamics of physically- separated soil organic carbon pools assessed from $\delta^{13}\text{C}$ changes under 25 years of cropping systems



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ARTICLE INFO

Keywords:

Continuous maize cropping
Maize–soybean rotation
 $\delta^{13}\text{C}$
Soil aggregates
Density fractionation
Soil C turnover

ABSTRACT

Quantification of the dynamics of soil organic carbon (SOC) pools under the influence of different cropping systems is essential for predicting carbon (C) sequestration. We combined soil fractionation with C isotope analyses to investigate the SOC dynamics of the various soil fractions in a black soil of Northeast China after 25 years of tillage. Soil samples from the initial condition (CK) and 2 cropping treatments including continuous maize cropping (MM), maize–soybean rotation (MS) were separated into 4 aggregate sizes (< 53 μm , 250–53 μm , 2000–250 μm , and > 2000 μm) and 3 density fractions: free light fraction (LF), intra-aggregate particulate organic matter (iPOM), and mineral-associated organic matter (mSOM). The 25 years of cropping with manure application significantly increased the SOC storage, mainly by enhancing the soil C of the macroaggregates (2000–250 μm), with most of the C stored in the iPOM (62.01–90.32%). The MS system was more beneficial for the SOC accumulation in macroaggregates (> 250 μm) than the MM system because of enhanced SOC in heavier fractions (iPOM and mSOM); this was probably induced by the differentiation of the below-ground humification rate between soybean and maize roots, while the MM system may be a more effective measure for future soil C sequestration because most of the stable C is stored in the small size fraction (< 53 μm). The $\delta^{13}\text{C}$ values indicated that, among aggregate sizes, the fastest soil C turnover occurred in microaggregates (250–53 μm). Moreover, C in the MS soils had a faster turnover rate than in the MM soils.

1. Introduction

Soil organic carbon (SOC) in agricultural ecosystems plays an important role in soil fertility, soil tilth, nutrient cycling, soil sustainability and crop production by affecting soil physical, chemical and biological properties (Mazzilli et al., 2014, 2015; Tian et al., 2015). In soil-crop ecosystems, SOC storage reflects the net balance between ongoing carbon (C) accumulation resulting from inputs of crop biomass (aboveground, belowground) and/or exogenous organic matter (e.g., manure, straw) and soil C decomposition processes due to microbial oxidation (Tian et al., 2015; Dou et al., 2016a; Meng et al., 2016). When C inputs to the soil exceed the C outputs from the soil, a positive imbalance occurs, and consequently results in soil C sequestration (Jastrow et al., 2007; Mazzilli et al., 2014; Manns et al., 2016). Soil and crop management practices (e.g., crop type and variety, rotation, chemical fertilizer and manure application) could strongly affect the SOC of cultivated land, by altering the quantity and quality of crop residues in soil, the microbial dynamics and the supply of nutrients (McDaniel

et al., 2014; Dou et al., 2016a; Tian et al., 2015). Thus, studies assessing the effects of management practices on SOC in agricultural soils have often contrasted continuous and rotation cropping systems, which usually show differences in soil C inputs and decomposition rates of soil C resulting from crop residues.

Negative and positive effects of cropping practices on SOC storage and stability have been observed under different cropping systems (Kou et al., 2012; Mi et al., 2014; Kabiri et al., 2015). Mostly, inappropriate cropping practices can result in dramatic SOC losses and soil quality deterioration, while rational cropping practices, such as manure application and conservation tillage, can enhance SOC storage and soil quality (Lehuger et al., 2010; Meng et al., 2016). As a commonly recommended and widely used tillage practice, crop rotation [e.g., maize (*Zea mays* L.)–soybean (*Glycine max* L.) rotation] has been considered as an effective approach to improve the soil quality and C sequestration potential of cropland ecosystems (Kou et al., 2012; Gaudin et al., 2015). However, some observations showed adverse conclusions pertaining to C sequestration in soils under a maize–soybean rotation system in the

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U.S. Midwest and Northeast China (Grant et al., 2007; Kou et al., 2012). These incompatible results imply that the entire cropping system might need to be considered to calculate the C balance, which should focus on the effects of different cropping systems on SOC dynamics (Lehuger et al., 2010; Kou et al., 2012).

Several knowledge gaps remain regarding the clear mechanisms that determine changes in SOC dynamics as a consequence of changes in the quantity and composition of residue inputs (Mazzilli et al., 2014, 2015; McDaniel et al., 2014; Li et al., 2016). Accordingly, quantifying and tracking soil organic matter (SOM) pools under agricultural cropping systems can be difficult, as SOM is heterogeneous and consists of a complex mixture of soil fractions with different compositions and turnover rates (Cheng et al., 2011; Dou et al., 2013; Kabiri et al., 2015). Additionally, the long-standing theory usually suggested that SOM was composed of inherently stable and chemically unique compounds, while an emergent view showed that SOM was a continuum of progressively decomposing organic compounds (Lehmann and Kleber, 2015). Meanwhile, physical soil architectural traits were functionally linked to the organic carbon decomposition rate within aggregates (Rabbi et al., 2016). For instance, SOM associated with macro-aggregates is more sensitive and labile in response to tillage practices than that associated with microaggregates (Kabiri et al., 2015); Greater SOM concentrations and higher mineralization rates are often reported to be associated with macroaggregate fractions (Six et al., 1998; Liao et al., 2006). Meanwhile, although LF usually represents only a small proportion of total soil C, changes in C storage following shifts in crop species composition can be more pronounced in LF compared to bulk soil (Cheng et al., 2011; Dou et al., 2016b). In contrast, the iPOM and mSOM fractions are mainly relevant to the heavy and mineral-associated recalcitrant fractions that maintain physical and chemical stabilization (Six et al., 1998; Mazzilli et al., 2015). Originally, C₄ (e.g., maize, $\delta^{13}\text{C}$ ca. -12%) and C₃ (e.g., soybean, $\delta^{13}\text{C}$ ca. -28%) plants may produce detritus with different $^{13}\text{C}/^{12}\text{C}$ ratios because of their differences in utilizing C isotopes (Dalal et al., 2013; Zhang et al., 2015). The relative contribution of new SOC vs. old SOC can be estimated based on the mass balance of C isotope contents, and thus SOM turnover rate could then be estimated *in situ* (Zhang et al., 2015). A soil with an intermediate isotopic composition derived from mixed C₃ and C₄ vegetation ($\delta^{13}\text{C} = -18$ to -21%) allows researchers to simultaneously follow the depletion in soil $\delta^{13}\text{C}$ after the introduction of C₃ plants and its enrichment after the introduction of C₄ plants (Dalal et al., 2013; Mazzilli et al., 2014). Thus, SOM physical fractionation techniques, combined with the natural abundance of stable C isotopes, have been considered to be an effective approach for quantifying SOM dynamics under long-term cropping systems in agro-ecosystems (Dalal et al., 2013; Mazzilli et al., 2014; Wang et al., 2015).

Commercial grain production in Northeast China plays a vital role in Chinese food security (Kou et al., 2012). Black soils (Mollisols), with a rich organic matter content, are the most fertile and productive soils in Northeast China (Ling et al., 2014; Dou et al., 2016a). In recent years, the productivity of black soils has been declining as a result of unsustainable agricultural practices (Xie et al., 2014). In the agricultural tillage system of China, aboveground crop residue is usually removed for energy use or is used as livestock feed, which could result in a decline of SOM, a depletion of C stocks, soil erosion and deterioration. Thus, maize–soybean rotation plus manure application has been widely recommended in order to improve soil quality since 1990 (Kou et al., 2012; Ling et al., 2014). In this study, we hypothesized that 25 years of cropping practices would significantly change the organic C stored in soil fractions as well as the turnover rate of soil C (the proportion of soil new vs. old C). The objectives of this study were to examine the following issues: (1) how 25 years of cropping systems have potentially impacted the organic C pools in the SOM fractions and (2) how 25 years of cropping systems affect the new C inputs and decay rates of old C in the native SOM fractions.

2. Materials and methods

2.1. Study area

A 25-year experiment to monitor SOC dynamics under different cropping systems has been conducted since 1989 in Gongzhuling, Jilin Province, China (124°48'33"E, 43°30'23"N) (Xie et al., 2014). This region has a northern temperate and semi-humid climate with an annual average temperature of 5.6 °C. The annual precipitation is approximately 562 mm, 80% of which falls between June and September (Song et al., 2015). The soil is a clay loam [Typic Hapludoll (Mollisol) in USDA Soil Taxonomy] developed from Quaternary loess-like sediments, with 39% sand, 30% silt and 31% clay at the beginning of the experiment (Xie et al., 2014). The 3 replicates were arranged in a randomized block design; each replicate plot covered an area of 130 m². The experiment included 3 treatments: (1) initial soil condition (CK), no plant materials and/or farmyard manure inputs during the 25 years; (2) continuous maize cropping (MM); and (3) maize–soybean rotation (MS) in a 3-year rotation sequence: 2-year maize and then 1-year soybean cultivation. The cropping regime was dominated by one crop (maize or soybean) per year (Ling et al., 2014). Primary tillage was followed by one pass of a stubble crushing machine to a depth of 0–20 cm approximately 3 days before planting in late April each year in both cropped plots. Maize and soybean were ridge-till planted and harvested in late September (Kou et al., 2012). In both cropping systems, aboveground plant residues were removed at harvest, and the land was kept weed-free manually (Song et al., 2015). Balanced inorganic fertilizers, i.e., 50 kg N ha⁻¹, 82.5 kg P₂O₅ ha⁻¹, and 82.5 kg K₂O ha⁻¹, plus farmyard manure at a rate of 2.3×10^4 kg ha⁻¹ (MNPK) were applied to the soils to a depth of 10 cm (Dou et al., 2016a). The sources of inorganic N, P, and K fertilizers were urea, triple superphosphate (TSP) and muriate of potash (MoP) (Song et al., 2015). The organic C content and N content of farmyard manure (mostly pig manure) were approximately 112 g kg⁻¹ and 5.0 g kg⁻¹, respectively; the $\delta^{13}\text{C}$ of farmyard manure had an average value of -21.59% (Xie et al., 2014; Dou et al., 2016a). The farmyard manure was applied in the MM and MS plots after crop harvesting in autumn each year. Prior to the long-term experiment, the field was homogenized by growing maize for 3 years without fertilizer application (Xie et al., 2014).

2.2. Field sample collection and soil fractionations

In August 2014, we randomly established 3 sub-plots (2 m × 2 m) around the maize and soybean root-spheres (i.e., the area within the canopy edge) within each treatment plot; the distances between the sub-plots were approximately 5 m. Soil samples (0–20 cm) from each treatment plot were collected using a 5-cm diameter stainless steel soil corer. Newly produced maize and soybean leaves were collected in each treatment plot. Root sampling blocks were excavated within a 30 × 30 cm quadrant at a soil depth of 0–20 cm and were washed clean carefully; leaves and roots were oven-dried to a constant weight at 65 °C in the laboratory in preparation for analysis. The soil samples were air-dried, after which large roots and stones were removed by hand.

The methods for aggregate separation and size density fractionations were adapted from Six et al. (1998). Four aggregate sizes were separated using wet-sieving through a series of sieves (2000, 250, and 53 μm). A 100 g air-dried sample was submerged for 5 min at room temperature in de-ionized water on top of the 2000-μm sieve. Aggregate separation was achieved by manually moving the sieve up and down 3 cm with 50 repetitions over a period of 2 min. After the 2-min cycle, the stable > 2000 μm aggregates were gently back-washed off the sieve into an aluminum pan. The floating organic material (> 2000 μm) was discarded, because this is by definition not considered soil organic matter (Six et al., 1998). The water and soil that passed through the sieve were poured into the next 2 sieves (one at a

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