Soil Biology & Biochemistry 109 (2017) 50–58

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

The fate of soybean residue-carbon links to changes of bacterial community composition in Mollisols differing in soil organic carbon

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ARTICLE INFO

Article history:
Received 21 July 2016
Received in revised form
31 December 2016
Accepted 31 January 2017

Keywords:
SOC fractions
^{13}C-labelled residue
Pyrosequencing
Bacterial community
Priming effect

ABSTRACT

Quantifying residue carbon (C) incorporation into soil organic C (SOC) fractions, and underpinning microbial community in the decomposition process of crop residues are essential for improving SOC management in agricultural systems. However, the fate of residue-C and associated responses of microbial communities remain unclear in Mollisols in north-eastern China, where SOC varies geographically. A 150-day incubation experiment was conducted with ^{13}C-labelled soybean residue (4%) amended into two Mollisols differing in SOC (SOC-poor and SOC-rich soils). The ^{13}C abundances in SOC fractions and the CO_2–C efflux from soil were determined, and bacterial community composition was analyzed with MiSeq sequencing. The amounts of residue-C incorporated into the coarse particulate organic C (POC), fine POC and mineral-associated C (MOC) fractions were 4.5-, 4.3- and 2.4-fold higher in the SOC-rich soil than in the SOC-poor soil, respectively. Residue amendment led to negative SOC priming before Day 50 but positive priming thereafter. The primed CO_2 per unit of native SOC was greater in the SOC-poor soil than in the SOC-rich soil. This indicates that the contributions of residue-C to the POC and MOC fractions were greater in the SOC-rich soil while residue amendment had stronger priming effect in the SOC-poor soil, stimulating the C exchange rate between fresh and native SOC. A principal coordinates analysis (PCoA) showed that the shift of bacterial community structure in response to residue amendment varied between the two soils. Genera *Verrucosispora*, *Xanthomonadales* and *Steroidobacter* were mainly enriched in the residue-amended SOC-poor soil while *Anaerolineaceae_uncultured* was dominant in the SOC-rich soil. The canonical correspondence analysis (CCA) revealed that the relative abundance of the bacterial operational taxonomic unit (OTU) among residue treatments was significantly associated with soil characteristics, especially C content in coarse POC and MOC fractions ($p < 0.01$), implying that the shift of bacterial community composition in response to residue amendment contributes to the sequestration of residue-C in SOC fractions.

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1. Introduction

As the largest pool of carbon (C) on Earth, soils contain approximately 2344 Gt of C (Stockmann et al., 2013), and it is possible to substantially increase this amount (Macias and Arbestain, 2010). However, it is not fully understood how farming practise and soil properties affect the accumulation and mineralization of soil organic C (SOC) in agricultural fields (Comeau et al., 2013). The return of crop residues to soil is an effective way to sustain the SOC content (Rui et al., 2009). For example, incorporation of crop residues for 43 years increased the SOC content by 21–29% in a wide range of soils (Pituello et al., 2016).

Physical fractionation of the SOC is a commonly-used approach to understanding the impact of residue C on the SOC dynamics because various soil fractions play different roles in SOC stabilization (Shinjo et al., 2000; Six et al., 2004). The particulate organic C (POC) corresponds to relatively young transformed C, while the mineral-associated organic C (MOC) is more stable over time due to chemically binding with mineral constituents of the soil (Mazzilli

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http://dx.doi.org/10.1016/j.soilbio.2017.01.026
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et al., 2015). Using ^{13}C-labelled materials allows the incorporation of the residue-C into various C fractions to be quantified (Buyanovsky et al., 1994; Hassink, 1997; Aita et al., 1997). For example, Aita et al. (1997) investigated the decomposition of ^{13}C-labelled wheat plant residues in the field, and found that more residue-C was incorporated into the fine soil fraction in the topsoil than in the subsoil. The dynamics of crop residues has been studied in a number of soils including Argiudol and Cambisol (Balesdent and Balabane, 1996; Comeau et al., 2013; Mazzilli et al., 2015), but the information is limited for Mollisols, of which soil properties fundamentally differ from other soils.

Mollisols are important cropping soils in Northeast China and have a wide range of SOC contents, ranging from 9.5 to 58.1 mg g^{-1} (Jin et al., 2013). The SOC contents of Mollisols have continuously decreased over the past decades (Fan et al., 2011; Liu et al., 2012), with an annual decline of about 0.9% in the 0- to 90-cm soil profile due to erosion, intensive tillage and burning of crop residues (Liu et al., 2006). In order to minimize or prevent this decline, the return of crop residues to the field is an important practice, especially the residue of soybean that is a major crop in rotation with cereal crops in Mollisols (Qin et al., 2010). A limited number of studies have shown that the return of crop residue could maintain or increase SOC in Mollisols (Liu and Herbert, 2002; Liu et al., 2012), but the effect of crop residues on SOC content and stability may vary between different Mollisols due to the biochemical variation in soils, which mediates the decomposition process (Lützow et al., 2006).

Microorganisms are the major driver of residue decomposition and turnover in soils, and play an important role in the incorporation of decomposed components into the SOC fractions (Rui et al., 2009; Marschner et al., 2011; Manjaiah et al., 2000). Residue chemistry and soil types can affect microbial populations and metabolisms (Kuzyakov et al., 2000; Semenov et al., 2012; Pascault et al., 2013). The effects of crop residue on microbial community have been studied in crop species such as wheat, maize and soybean (Bernard et al., 2007; Fan et al., 2014; An et al., 2015; Ramirez-Villanueva et al., 2015). For instance, the genus *Arthrobacter* belonging to Actinobacteria was found dominant as primary sequestrators in a Vertisol amended with soybean residue. However, such effects on C dynamics and associated microbial community in Mollisols remain largely unknown.

The objectives of this study were (1) to quantify the contribution of the soybean residues to various SOC fractions in two Mollisols differing in SOC content, and (2) to investigate the microbial community composition in response to residue amendment in the soils. A soybean residue labelled with ^{13}C was used to track residue-derived C in soils during a 150-d incubation period. The MiSeq pyrosequencing technique of the 16S rRNA genes was employed to examine the effect of residue addition on microbial community. We hypothesized that the fate of residue-C in various SOC fractions of different soils is likely attributed to microbial accessibility to crop residues and community in response to residue amendment. With residue amendment, the specific selection of microbial community from the community reservoirs of the soils might affect residue-C turnover and its contribution to SOC fractions.

2. Materials and methods

2.1. Soil and residues preparation

The Mollisols used in this study were taken from the tillage layer to about 0.1-m depth at two locations in northeast China. For each soil, 20 cores were collected from a farmer's paddock, bulked, air-dried and sieved through a 2-mm sieve. The SOC concentrations were 14 and 50 mg g^{-1} for the SOC-poor and SOC-rich soils, respectively. The chemical characteristics of the two soils are listed in Table 1.

The technology of $^{13}CO_2$ pulse-labelling was employed to produce the ^{13}C-labelled stalk residues of soybean. The plants were labelled for ten times across the growth stages and harvested at the fully matured stage. The details of ^{13}C labelling are referred to Lian et al. (2016). The $\delta^{13}C$ value of the stalk was 1903.9‰. The shoot residues (stalk) were dried at 70 °C and ground through 2-mm and 0.25-mm sieves. The residues left in between were for the following incubation. The residue had 429 mg g^{-1} of C, 13 mg g^{-1} of N, 33.4 mg g^{-1} of soluble sugar, 6.5 mg g^{-1} of starch, 305 mg g^{-1} of cellulose and 153 mg g^{-1} of lignin.

2.2. Experimental setup and soil incubation

An incubation experiment consisted of two soils (SOC-rich and SOC-poor Mollisols) and two residue treatments (with or without soybean residue). The soils were watered to 50% field capacity (FC) and pre-incubated at 25 °C for 15 days to recover microbial activity and function (Butterly et al., 2016). The ground shoot residue (0.8 g) was mixed with 20 g of soil which was placed into each PVC core (4.5-cm height, 2-cm diameter) with nylon mesh bottoms. The soil was then compacted to a bulk density of 1.1 g cm^{-3}. Each PVC core was placed into a 1-L mason jar together with a vial containing 10 mL of water to maintain the humidity inside the jar, and a 15-mL plastic vial containing 10 mL of 1 M NaOH as an alkali trap to capture evolved CO_2. There were 15 jars per treatment for 5 sampling dates. Three of them were sampled at each time point. Through weighing and watering, the soil water content was maintained at 80% FC. All jars were kept in a dark incubator at a constant temperature of 25 °C.

2.3. Respiration measurements and soil sampling

The NaOH traps were sampled and then renewed on days 4, 8, 12, 16, 20, 24, 28, 32, 39, 46, 53, 60, 70, 80, 90, 100, 110, 120, 130, 140 and 150 after incubation was initiated. Soil respiration was estimated by measuring the amount of CO_2 absorbed in the NaOH trap. Carbonates in NaOH were precipitated using an excess 1 M $SrCl_2$ solution and filtered (Shahzad et al., 2015). HCl (0.1 M) was then used to neutralize the excess NaOH using phenolphthalein as an indicator (Blagodatskaya et al., 2011). The neutralized solutions together with $SrCO_3$ precipitate were centrifuged for three times at 2000 *g* for 10 min. The precipitate was washed in between with deionized and degassed water. Then, $SrCO_3$ precipitate was dried at 105 °C (Blagodatskaya et al., 2011). The δ^{13} C value of carbonates (trapped CO_2) was determined using Mat 253 isotope ratio mass spectrometer (Thermo Fisher, Germany).

During incubation, three replicates in each treatment were destructively sampled on days 15, 30, 60, 90 and 150 after incubation. Each soil sample was separated into two or three parts: one part (approximately 2 g) was frozen immediately in liquid nitrogen, and then stored at −80 °C for DNA extraction and following pyrosequencing. This part was only collected on days 150. The second part (approximately 10 g) was maintained at 4 °C for measurements of microbial biomass and dissolved organic C. The remaining portion (approximately 8 g) was air-dried for the pH measurement and SOC fractionation. The pH was determined using a Wettler Toledo 320 pH meter in water (1:5 = w:v) after shaking the suspension for 30 min and centrifugation at 800*g* for 5 min.

2.4. Microbial biomass C and dissolved organic C

The chloroform-extraction method was employed to determine the microbial biomass C (MBC) in the soil (Vance et al., 1987). The

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