



Tillage and crop succession effects on soil microbial metabolic activity and carbon utilization in a clay loam soil

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ABSTRACT

Soil microorganisms play important roles in nutrient cycling and ecosystem functioning. Knowledge on the responses of soil microbial metabolic characteristics to agricultural practices is critical to better understand soil microbial processes in agroecosystems. The study aimed to explore the responses of soil microbial metabolic activities and their carbon utilization under different tillage and crop succession systems in a 12-year field experiment in a clay loam soil of Jilin Province, China. This experiment was set up in a split-plot design with four replicates including no-tillage (NT) and conventional tillage (CT) as main plots and continuous corn (CC) and corn-soybean succession (CS) treatments as subplots. Soil samples were collected from 0 to 5 cm and 5–15 cm depths. The results showed that compared with CT, NT increased soil organic carbon, soil microbial biomass carbon, basal respiration and microbial coefficient at 0–5 cm depth. Greater basal respiration was observed under CC than under CS at both depths. Compared with NTCC, NTCS decreased microbial functional diversity at 0–5 cm depth and increased microbial metabolic quotient at 5–15 cm depth. Redundancy analysis indicated that crop succession management induced the changes in soil microbial carbon utilization. Compared with other soil environmental factors, NO_3^- -N and soil moisture significantly affected soil microbial metabolic activities. No significant correlation was found between microbial carbohydrate utilization and soil organic carbon in this study. Structural equation modeling analysis suggested that corn-soybean succession practice could stimulate the consumption of aromatic acids, which indirectly affected the accumulation of soil organic carbon.

1. Introduction

Soil fertility is generally mediated by soil microorganisms because of their important roles in soil nutrient cycling especially in affecting soil organic matter (SOM) turnover [1,2]. During SOM turnover, soil microbial activities can be responsible for the accumulation and decomposition of soil organic carbon (SOC) [3]. Microbial processes are affected by agricultural practices due to the changes in the quantity as well as quality of crop residues returned to soil, and variations in the soil physicochemical conditions [4]. As a result, understanding the relative contribution of the important regulatory factors of soil microbial activity is necessary for further predictions of soil carbon cycling in response to agricultural managements.

In agroecosystems, agricultural management such as tillage practice generates significant impact on the sustainability of crop production due to their effects on soil environment [5]. Moreover, previous researches have documented that soil biological activities are mainly related to the changes of soil physicochemical characteristics induced by tillage practices [6,7]. It has been broadly reported that intensive tillage managements like conventional tillage (CT) can decrease the stability of soil aggregates and the content of soil organic carbon, which lead to the decline of soil microbial biomass [8]. However, conservation agriculture practices, i.e. no-tillage (NT) with crop residue retention has been regarded as an effective management to maintain the productivity of farmland, improve soil structure and increase soil organic matter content as well as crop yields [7,9]. Aziz et al. [10] also concluded that

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large storage of diverse plant biomass on the undisturbed surface existed in NT system, which might provide sufficient carbon source for the microbial community utilization and resulted in efficient microbial activity.

Crop succession practice, as another main driving factor also strongly affected soil microbial communities and their metabolic activities [11,12]. On one hand, crop succession can increase the input of organic carbon and nitrogen into soils, and consequently soil fertility [13]. On the other hand, crop succession with diverse crop residues being returned to the soil can also affect the habitat, biomass, activity and diversity of soil microorganisms [11,12,14]. In addition, the responses of soil microbial communities to the crop residues largely depend on the substrate complexity [15]. For example, under high N supply, in comparison with bacteria, fungi are the main consumers of polymeric carbon sources [16]. Therefore, it is necessary to understand the responses of soil microbial characteristics to different crop succession practices and their carbon utilization, which are important for SOC dynamics and the sustainability of agroecosystems.

Until now, most researches have paid much attention on either the influences of different tillage managements or the effects of crops on soil microbial community and microbial activity. It is known that tillage practice combined with crop succession is regarded as a complete system applied in the agricultural production [14]. However, few studies have focused on the interaction between tillage and crop succession on soil microbial metabolic characteristics. Moreover, the knowledge on the responses of soil microbial metabolic activities and their carbon utilization to agricultural practices is important to better understand soil microbial processes in agroecosystems. Consequently, we used the MicroResp™ system [17,18] to analyze the responses of soil microbial metabolic activity, diversity and their carbon utilization to the interaction between different tillage and crop succession systems. The objective of this study was to elucidate the impacts of conservation tillage and conventional tillage with different crop succession practices on soil microbial metabolic characteristics in relation to soil organic carbon accumulation.

2. Materials and methods

2.1. Experimental site

The experiment was conducted at the Experimental Station (44°120' N, 125°33' E) of the Northeast institute of Geography and Agroecology, Chinese Academy of Sciences, in Dehui County, Jilin Province, China. The station is located in a continental temperate monsoon zone with the mean annual temperature of 4.4 °C. The mean annual precipitation is 520.3 mm with about 70% falling from June to August. The soil is classified as black soil (Typic Hapludoll according to the USDA Soil Taxonomy) with a clay loam texture consisting of 36.0% clay, 24.5% silt and 39.5% sand. Before the experiment was initiated, the cultivation of corn with a conventional tillage management was practiced in the field for more than 20 years [19].

2.2. Experimental design

The long-term tillage and crop succession experiment was established in a split-plot design with four replicates and initiated in the fall of 2001. Tillage systems as the main plot were a no-tillage (NT) and a conventional tillage (CT) treatments, and crop succession as the subplot included continuous corn (CC) and corn-soybean succession (CS) (one year corn and one year soybean) [19]. The size of each subplot was 5.2 m × 20 m (width × length). Field crops were sown in May and harvested in October. A fallow period (about seven months) was followed after each harvest.

In NT, there were minimal human disturbances except for planting using a KINZE-3000 NT planter (Williamsburg, Iowa). After harvest, the corn straws were collected and cut into pieces of roughly 30 cm leaving

a 30–35 cm stubble stand, and then were returned to the soil surface. The residues of soybean were directly returned to the soil surface. The management in CT consisted of mouldboard plowing (20 cm depth) after harvest in fall, and disking (7.5–10 cm depth) and harrowing for the secondary seedbed preparation in spring of the following year. The aboveground crop residues in CT were all incorporated into the soil. For corn fields, 100 kg ha⁻¹ of N, 45.5 kg ha⁻¹ of P and 78 kg ha⁻¹ of K were applied as basic fertilizer during the sowing period and 50 kg ha⁻¹ of N as top dressing at the 6 leaves with collars (V-6 stage) each year. For soybean field, 40 kg ha⁻¹ of N, 60 kg ha⁻¹ of P and 80 kg ha⁻¹ of K were applied as starter fertilizer [19].

2.3. Soil sampling

Soil samples were collected from 0 to 5 cm and 5–15 cm depths in April 2012 (at the end of the fallow period after corn harvest in 2011). In each subplot, composite samples of five random sub-samples were collected with a soil auger (2.64 cm diameter). Totally, 32 soil samples were collected in this study. The fresh samples were collected into plastic bags and kept at 4 °C until lab analysis. The detail information of soil physical and chemical properties in this study were listed in the [Supplementary Tables S1 and S2](#).

2.4. Soil organic carbon, microbial biomass carbon, soil microbial metabolic characteristics

In this study, soil organic carbon (SOC) was determined by dichromate oxidation and titration with ferrous ammonium sulfate [20]. Microbial biomass carbon (MBC) was extracted using the chloroform fumigation and extraction method [21] and measured with a TOC analyzer (Multi C/N 3000, Analytik Jena, Germany). The MicroResp™ system was used to determine soil microbial metabolic diversity. The procedure was according to the methods of Campbell et al. [17] and Yu et al. [18]. Briefly, after the removal of visible plant residues and stones, soil samples were all gently sieved through a 2 mm screen. Ten g samples of different tillage and crop succession systems were separately weighed in a 20-mL vial, and distilled water was added to adjust soil moisture content to 40% of the water-holding capacity [18]. Before measurement, in order to recover kinetic response, tightly closed vials with soil samples were incubated in the dark at 25 °C for 72 h. The 25 µL of 15 different carbon resources (30 mg g⁻¹ of C) representing amino acids (L-arginine, γ-amino, butyric acid (GABA), L-alanine, L-cysteine-HCl, L-lysine-HCl and N-acetyl-glucosamine (NAGA)), aromatic acids (3,4-dihydroxybenzoic acid), carbohydrates (L-arabinose, D-fructose, D-galactose, D-glucose and trehalose), and carboxylic acids (L-malic acid, oxalic acid and citric acid) were added to the MicroResp deep well plates. Meanwhile, 25 µL distilled water was also separately added to the deep well plates to measure the basal respiration [22]. Subsequently, the preincubated soil samples (about 0.35 g fresh weight per well) were added to the deep well plates to generate a final substrate concentration of 8 mg g⁻¹ dry weight soil (mixed with 15 different carbon sources and distilled water, respectively) by using a special filling device [17,23]. The respired CO₂ was measured by dye plates - a colorimetric reaction with absorbent alkali [24] and tested by a spectrophotometer reading at 570 nm. The detection plates were read two times: before placement (t0) and after 3 h (t3). During that time, the plates were incubated in the dark at 25 °C. CO₂ emission rate was calculated according to the instructions of the manufacturer. The following formula converts the normalized t0/t3 h data (Ai) to %CO₂: %CO₂ = A + B/(1 + D × Ai). Where A = -0.23, B = -1.61, D = -6.77. The formula is for a linear-to-linear standard curve fit [23]. Microbial metabolic quotient (qCO₂) and microbial coefficient (MBC/SOC) of different tillage and crop succession systems were calculated in this study. In general, qCO₂ suggests the maintenance energy requirement of soil microbial communities and MBC/SOC represents the amount of living C composing the soil organic C [18,25]. In

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