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Conservation tillage positively influences the microflora and microfauna in the black soil of Northeast China

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ABSTRACT

Soil food webs are important in maintaining agricultural productivity and ecosystem health. However, our understanding is still limited with respect to the influences of tillage transitions on soil food webs. The present study aimed to quantify the response of microflora and microfauna, and their linkage to different tillage treatments: no tillage (NT), ridge tillage (RT) and conventional tillage (CT). Soil samples were collected from 0 to 20 cm depth in April of 2011 after 10 years of conservation tillage. The abundance and richness of bacteria and arbuscular mycorrhizal fungi were greater in NT and RT than in CT. In case of microfauna also, similar patterns were observed with greater protozoa, bacterivores and omnivores–carnivores in NT and RT compared to CT. The connectance of the bacterial and predator–prey pathways was greater in NT and RT than in CT and that of fungal pathway was greatest in RT. The trophic relationship of the bacterial and predator–prey pathways was strengthened due to the higher water content of soil and the lower NO_3^--N after the conversion of CT to NT and RT. Our study suggested that 10 years of conservation tillage can effectively enhance the structure and function of soil food webs through bottom–up effects in the black soil region of Northeast China.

1. Introduction

Soil food webs deliver important ecosystem services, which are necessary to maintain agricultural productivity and ecosystem health (Minoshima et al., 2007; Sánchez-Moreno and Ferris, 2007). The abundance and trophic relationship of food web components, including microorganisms (bacteria and fungi), microbivores (protozoa and nematodes) and predators (nematodes) (Li et al., 2012; Scharroba et al., 2012), highly depend on soil management (Coleman, 2008). Changes in soil food webs can affect mineralization of nutrients and decomposition of organic matter (Wardle, 2002; Li et al., 2012). Therefore, understanding the changes in microflora and microfauna, and their linking across contrasting soil managements could lead to a precise regulation of soil organisms for sustainable agroecosystems (Treonis et al., 2010; Wall et al., 2012).

Tillage affects soil organisms by changing the soil physical environment and the food supply (Kladivko, 2001; Kladivko and Clapperton, 2011; Sánchez-Moreno et al., 2011). In conventional tillage (CT), soil communities typically consist of bacteria and herbivorous nematodes (Govaerts et al., 2006; Kuntz et al., 2013); whereas, the communities in conservation tillage support a high proportion of fungi and predatory nematodes (Minoshima et al., 2007; van Capelle et al., 2012; Zhang et al., 2012). Until now, little research has investigated the response of soil microflora and microfauna simultaneously to different tillage treatments.

Conventional tillage in the black soil (Typic Hapludoll, USDA Soil Taxonomy) of Northeast China has been widely used in this region for decades. Continuous moldboard plowing and the removal of postharvest residues from the CT have seriously degraded the soil (Liu et al., 2010). Conservation tillage, including no tillage (NT) and ridge tillage (RT), has been proposed to farmers to replace CT in part, but large-scale application of conservation tillage can only be achieved by demonstrating their benefits for soil and plants.

To reveal the relationships between structure and functioning, three food web pathways were compartmented accounting for the main flux of C through the web: (1) the bacterial pathway, in which

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C flows from bacteria to their grazers (protozoa and bacterivorous nematodes); (2) the fungal pathway, in which C flows from fungi to fungivorous nematodes; and (3) the predator–prey pathway, in which C flows from nematode prey (protozoa, microbivorous and herbivorous nematodes) to nematode predators (Holtkamp et al., 2008; Sánchez-Moreno et al., 2011; de Vries et al., 2012). We hypothesize that (1) NT and RT, compared with CT, positively affect the components of the food webs, with the fungal-based decomposition pathway dominant; and (2) NT and RT increase the stability and strengthen the trophic relationship of the food webs.

2. Materials and methods

2.1. Experimental site

The tillage experiment was initiated in fall 2001 at the experimental station (44°12′N, 125°33′E) of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences. The station is located in Dehui County, Jilin Province in the northeastern part of China. The mean annual temperature is 4.4 °C, the mean annual precipitation is 520.3 mm with more than 70% of the precipitation between June and August. The soil is classified as black soil (Typic Hapludoll, USDA Soil Taxonomy) with a clay loam texture (the average soil texture was 36.0 g kg^{-1} clay, 24.5 g kg^{-1} silt and 39.5 g kg^{-1} sand). Before the tillage trail initiated, the field has been used for continuous maize (*Zea mays* L.) with conventional tillage for more than 20 years (Liang et al., 2007).

2.2. Experimental design

The tillage experiment consisting of no tillage (NT), ridge tillage (RT) and conventional tillage (CT) was arranged in a completely randomized design with a maize–soybean (*Glycine max* Merr.) rotation system (Liang et al., 2007). Each treatment area having four replications was 5.2 m × 30 m with 5 m buffer rows between treatments. The crops were sown in May and harvested in October followed by fallow for 6 months. Treatments in the CT consisted of moldboard plowing (20 cm) in the fall followed by secondary seedbed preparation by disked (7.5–10 cm) and harrowed in spring. For the NT, the soil was undisturbed, except when the crop was planted using a KINZE-3000 NT planter (Williamsburg, Iowa). In RT, ridges were maintained year-to-year with a cultivator (John Deere Company, US) in each June, and a modified lister and scrubber was used to form and press the ridge (16 cm in height and 75 cm in width). Maize and soybean were planted with a no-till planter. After harvest, the maize straw in RT and NT was cut into pieces of approximately 30 cm leaving a 30–35 cm stubble stand, and the straw pieces were then returned to the soil surface; soybean residues in RT and NT were directly returned to the soil surface.

The application rates of N, P and K were the same in all the three treatments. Each year, 100 kg N ha^{-1}, 45.5 kg P ha^{-1} and 78 kg K ha^{-1} were applied to maize as basal fertilizer. An additional 50 kg N ha^{-1} was applied as a top dressing at the V-6 stage (6 maize leaves with collars). For soybean, all fertilizers were applied as basal fertilizer, including 40 kg N ha^{-1}, 60 kg P ha^{-1} and 80 kg K ha^{-1}. An attachment to the no-till planter banded the basal fertilizers at planting.

2.3. Soil sampling

Soil samples from each treatment were collected from 0 to 20 cm depth in April of 2011. Each soil sample was pooled from six soil cores of 2.5 cm diameter. In the center of each treatment, bulk density was determined from the surface to 20 cm depth at 5 cm intervals using a 100-cm^3 cylinder (5 cm height × 5 cm diameter). Each sample was split into two subsamples. One was stored at 4 °C for <2 weeks for soil biological analysis, and the other was air-dried and sieved within one month for physical and chemical analysis.

2.4. Soil physical and chemical properties

Bulk density (BD) was determined using the core method (Grossman and Reinsch, 2002). Water content of soil (WCS) was measured gravimetrically. Soil pH was determined with a glass electrode in 1:2.5 soil:water solution (w/v). Soil inorganic N (NO_3^--N and NH_4^+-N) was first extracted with 2 M KCl, and then the filtrates were determined using a flow injection auto analyzer (FIAstar 5000 Analyzer, Foss Tecator, Hillerød, Denmark). The concentrations of soil inorganic N were calculated based on dry soil weight. Soil total carbon and total nitrogen (TN) contents were each determined using a FlashEA 1112 elemental analyzer (ThermoFinnigan, Italy). Because the soil was free of carbonate, the soil organic carbon (SOC) was assumed to be equal to the total carbon. The C/N ratio was calculated by dividing SOC by total N.

2.5. PLFA and protozoa analysis

The soil microbial community was characterized using phospholipid fatty acids (PLFAs) analysis as described by Bossio and Scow (1998) and Briar et al. (2011). Lipids were extracted from 8 g of freeze-dried soil using a chloroform–methanol–citrate buffer mixture (1:2:0.8). The polar lipids were separated from neutral lipids and glycolipids on a solid phase extraction columns (Supelco Inc., Bellefonte, PA). The phospholipids were trans-esterified to a mild-alkali methanolysis and the resulting fatty acid methyl esters were extracted in hexane and dried under N_2. Samples were then dissolved in hexane and analyzed in an Agilent 6850 series Gas Chromatograph with MIDI peak identification software (Version 4.5; MIDI Inc., Newark, DE).

The following biomarkers were used: Total PLFA (sum of all identified PLFAs; from C14 to C20); gram-negative bacteria (16:1ω7c, cy17:0, 16:1ω9c, 17:1ω8c, 18:1ω7c, cy19:0, 16:1 2OH); gram-positive bacteria (i14:0, i15:1, i15:0, a15:0, i16:0, i17:0, a17:0) (Aciego Pietri and Brookes, 2009; Bach et al., 2010); saprophytic fungi (18:1ω9c and 18:2ω6c) (Li et al., 2012; Dempsey et al., 2013); and arbuscular mycorrhizal fungi (AMF) (16:1ω5c) (McKinley et al., 2005; Bach et al., 2010). The sum of the gram-negative bacteria, gram-positive bacteria and non-specific bacteria (14:0, 15:0, 16:0, 18:0, 20:0) was expressed as the total bacteria. The PLFA richness was calculated as the number of different PLFAs detected per sample, and abundance was expressed in nmol g^{-1} dry soil.

The most-probable-number method was used to determine flagellate populations (Singh, 1975; Rodriguez-Zaragoza et al., 2005; Li et al., 2012). The assays were performed in 24-well cell culture plates and the growth medium in each well was 0.9 mL autoclaved and filtered soil extract (1:5, soil:water). The first well of each dilution series was inoculated with a 0.1 mL aliquot of 1:10 soil suspension shaken in a vortex for five 15-s pulses. Four replicates 10-fold dilutions to 10^{-7} were prepared for each soil sample. The plates were incubated at 28 °C for 7–10 days and reviewed with an inverted microscope for the presence of flagellates. Abundance and richness of flagellates were expressed as the number of individuals or taxa per gram of dry soil.

2.6. Nematode determination

Nematodes were extracted from a 50 g soil sample (fresh weight) by a modified cotton–wool filter method (Liang et al., 2009). After counting the total number of nematodes, 100 specimens per sample were randomly selected and identified to the