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Responses of fungal–bacterial community and network to organic inputs vary among different spatial habitats in soil

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ABSTRACT

Interactions among the species in microbial communities are important for organic matter turnover and nutrient cycling in the soil. Their responses to organic amendments have been studied recently but the co-occurrence patterns in different spatial soil habitats such as those with different sized aggregates are still unclear. Thus, we investigated networks comprising bacteria and fungi after the application of a cover crop for 9 years. The microbial community compositions and their co-occurrence networks were examined in the whole soil and different sized soil aggregates (> 0.25 mm, 0.053–0.25 mm, and < 0.053 mm). The microbial community compositions and their responses to the cover crop varied in the whole soil and aggregate fractions. Network analysis in the whole soil and different sized aggregates showed that the competition between fungi and bacteria in the whole soil increased due to the annual organic material input, but the fungi–bacteria relationships varied among different sized aggregates. In particular, the competition between fungi and bacteria increased in macroaggregates but decreased in silt + clay due to organic material inputs. Thus, the co-occurrence networks determined for the fungal and bacterial communities in various soil aggregates were very different from those in the whole soil, and their responses to organic inputs also varied in different spatial habitats in the soil.

1. Introduction

Soil fungi and bacteria are important for soil biochemical processes and functions. In soils, various species of fungi and bacteria live together to form a complex system of inter-species interactions rather than living in isolation (Freilich et al., 2010). Thus, it is important to understand the interactions among community members and the organization of fungal and bacterial communities as well as the abundance and diversity of each taxon in order to explore the functioning of the soil (Deng et al., 2012; Lu et al., 2013). Network analysis-based approaches have been used recently to study the co-occurrence of microorganisms in complex environments ranging from the human gut to oceans and soils (Ruan et al., 2006; Fuhrman and Steele, 2008; Faust and Raes, 2012; Chow et al., 2013). Using this technique, the characteristic co-occurrence patterns have been determined at various taxonomic levels and keystone microbial groups have been identified in different soils (Lupatini et al., 2014).

Several studies have shown that the soil microbial community structure and network can be influenced by the soil pH, organic matter content, and soil disturbance level (Eldridge et al., 2015; Creamer et al., 2016). In many cases, the levels of soil nutrients, such as the soil carbon and nitrogen contents, are the key factors related to shifts in the soil microbial community structure and network. Organic amendment can affect the microbial diversity as well as the relative abundances of copiotrophic and oligotrophic bacteria (Trivedi et al., 2015; Brennan and Acosta-Martinez, 2017; Zhang et al., 2017). Moreover, organic material inputs significantly alter the network of fungal and bacterial communities, where the identities of the interacting species are driven by resources rather than being species-specific (Banerjee et al., 2016b). However, previous studies have mainly focused on the microbial networks in the whole soil, and thus the responses of microbial networks to changes in the soil environment, especially organic material inputs, are still unclear in different soil aggregates.

Soils have a complex hierarchical structure where they contain different sized aggregates. These soil aggregates generally vary in terms of their nutrient availability and environmental conditions, and they can provide spatially heterogeneous habitats for microorganisms (Jiang et al., 2013, 2017). Previous studies have shown that each aggregate represents a different ecological niche for microbial colonization (Trivedi et al., 2015). In soil aggregates with different sizes, the

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macroaggregates generally contain more labile substrates derived mainly from plant residues (Bronick and Lal, 2005), whereas the microaggregates are characterized by higher recalcitrant carbon contents and they provide a protective microenvironment for the growth of microbes (Six et al., 2000b). Consequently, the different carbon sources and amounts in various soil aggregates probably lead to variations in the microbial community structure and different interactions between members of microbial communities, as well as changes in the keystone species in the co-occurrence pattern.

In this study, we investigated the responses of the soil microbial networks in the whole soil and different soil aggregate fractions to organic material inputs in a cover cropped apple orchard. Soil samples were collected in a 9-year orchard experiment with a split-plot design and they were separated into three aggregate size fractions (> 0.25 mm, 0.053–0.25 mm, and < 0.053 mm). The carbon and nitrogen contents, enzyme activities, and microbial (bacteria and fungi) community structures were determined in the whole soil and different soil aggregates. We investigated the following hypotheses. (1) The bacterial and fungal community structures vary in the whole soil and different sized soil aggregates because their different carbon and nitrogen contents as well as their responses to carbon inputs can also vary across the whole soil and different sized soil aggregates. (2) The co-occurrence networks also differ in the whole soil and different sized soil aggregates, and their responses to organic inputs can vary across the whole soil and different sized soil aggregates. We aimed to understand how fungal and bacterial species interact with each other in different spatial habitats, and the impacts of soil organic inputs on the co-occurrence of soil fungi and bacteria in a complex soil matrix.

2. Materials and methods

2.1. Site description and experimental design

The experiment was conducted at the Weibei Dryland Experimental Station of Northwest A&F University (109°56′E, 35°21′N; altitude of 838 m) in Baishui County, Shaanxi Province, China. The soil in the apple orchard was silt loam (8% sand, 67% silt, and 25% clay) and it was classified as Haplustalfs (USDA textural classification system). The rainfall distribution in the study area is dominated by a monsoon climate, where the summer is hot and moist, and the winter and early spring are always cold and dry. The average rainfall at the experimental site was 570 mm, 60% of which occurred in the summer (July–September).

The experiment was conducted in a Fuji apple (*Malus pumila* Mil.) orchard. Apple trees were planted in 2005 on M.26 (rootstock) at a density of 1200 plants per hectare. In 2008, the experiment was established with a split-plot design, which included two main plots and two subplots. The two main plots were the no cover crop treatment (C) and cover crop treatment (GC). For the C treatment, no cover crop was planted and weeds were controlled manually by farmers. For the GC treatment, crown vetch (*Coronilla varia* L.) was sown in each inter-row of the apple trees in the orchard at a depth of 1.5 cm and at a sowing rate of 6.0 kg per hectare. The crown vetch sprouted in late March each year and it was mowed in early July, August, and September, where the residues were left on the soil surface as mulch. The two subplots were no fertilizer treatment (CK) and fertilizer treatment (NPK). Urea (containing 46% N), calcium superphosphate (containing 12% P_2O_5), and potassium sulfate (containing 50% K_2O) were used as N, P, and K fertilizers, respectively, and 192 kg N ha^{-1}, 108 kg P_2O_5 ha^{-1}, and 168 kg K_2O ha^{-1} were applied each year for NPK. Each treatment was replicated three times. Each replicate comprised two rows with 12 apple trees in each row. The surface area of each replicate was approximately 200 m^2.

2.2. Soil sampling and aggregate fractionation

Soil sampling was conducted in late September (apple harvest time) during 2016 after nine years of cover cropping. Six soil cores were randomly collected from the inter-rows in each plot at a depth of 20 cm, and the six soil samples were then composited to obtain one soil sample and placed on ice, before they were immediately transported to the laboratory. Soil aggregate fractionation was performed using the wet-sieving technique according to Davinic et al. (2012). Three fractions were obtained for each sample: macroaggregates (> 0.25 mm; MA), microaggregates (0.053–0.25 mm; MI), and silt and clay (< 0.053 mm; SC).

2.3. Soil characteristics, enzyme activities, and microbial community analysis

Soil organic carbon (SOC), total nitrogen (TN), and enzyme activities were measured in the whole soil and different sized aggregates. The soil organic carbon content was determined using the K_2CrO_7-H_2SO_4 oxidation method. The soil total nitrogen was measured by acid digestion according to the Kjeldahl method. The soil enzyme activities (phosphatase, PHOS; β-N-acetylglucosaminidase, NAG; β-glucosidase, BG; cellobiohydrolase, CBH; and β-xylosidase, BXYL) were determined using microplate fluorimetry (Qi et al., 2016).

Before sequencing the 16S rRNA and internal transcribed spacer (ITS) gene sequences, DNA was extracted from the whole soil and aggregate fractions using MoBio PowerSoil™ DNA Isolation Kits (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The quantity and quality of the extracted DNA were assayed using a Nanodrop ND-2000 UV-VIS spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

For bacteria, the V4 region of the 16S rRNA gene was amplified using the primer pair comprising 515F (50-GTGCCAGCMGCCGCGGTAA-30) and 806R (50-GGACTACHVGGGTWTCTAAT-30) (Caporaso et al., 2011). For fungi, the primer pair comprising ITS2 (50-GCTGCGTTCTTCATCGATGC-30) and ITS5 (50-GGAAGTAAAAGTCGTAACAAGG-30) was used to amplify the ITS1 region (Bellemain et al., 2010; Lu et al., 2013). Amplification was performed using Thermo Scientific® Phusion High-Fidelity PCR Master Mix (New England Biolabs, UK). After amplification, the products obtained were purified using a Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing was performed using the Illumina HiSeq2500 platform at Novogene Bioinformatics Technology Co. Ltd, Beijing, China.

After removing the adaptor and primer sequences, the raw sequences were assembled for each sample according to a unique barcode. Paired end sequences for each sample were merged using FLASH V1.2.7 (Magoč and Salzberg, 2011). Quality filtering of the raw tags was performed under specific filtering conditions to obtain high-quality clean tags according to the QIIME (V1.7.0) quality control process (Bokulich et al., 2013; Caporaso et al., 2010). Row tags with more than three consecutive low quality base calls (Phred quality score ≤ 19) were truncated at the position where their quality began to drop, and only reads with > 75% consecutive high quality base calls, but without any ambiguous characters, were retained for further analyses. The tags were compared with the reference database (Gold database, http://drive5.com/uchime/uchime_download.html) using the UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) to detect chimeric sequences and the chimeric sequences were then removed. Finally, the effective tags were obtained. Sequence analysis was performed using UPARSE software (UPARSE v7.0.1001) (Edgar, 2013). Sequences with ≥97% similarity were assigned to the same operational taxonomic unit (OTU). For each representative sequence, the SILVA (bacteria) and UNITE (fungi) databases were used to annotate taxonomic information. OTU abundance information was normalized using a standard sequence number corresponding to the sample with the least sequences (47593 for bacteria and