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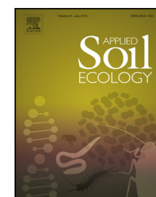

Impacts of inorganic and organic fertilization treatments on bacterial and fungal communities in a paddy soil

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ABSTRACT

Soil microbes play an integral role in agricultural production, and there is limited understating of the complex responses of microbial diversity to organic and conventional farming. Here we report the response of soil microbiome, of a paddy field, to ten years application of inorganic fertilizer (CF) and organic fertilizer (OF), also including a no fertilizer control (CK). The bacterial and fungal communities were examined using high-throughput sequencing. Our results revealed that compared with CK and OF, the CF treatment had lower richness of bacteria, but higher richness of fungi. To utilize the fertilizers efficiently and adaptions to soil conditions, bacterial and fungal compositions changed under different fertilizations. The application of CF increased the abundance of some oligotrophic bacteria such as *Bacteroidetes* and *Acidobacteria*, and increased the relative abundance of *Zygomycota* phylum of fungi. The application of OF increased the abundance of the copiotrophic bacteria such as *Proteobacteria* phylum, and the relative abundance of *Agaricomycetes* and *Orbiliomycetes* classes of fungi. The OF also had the sparsest and most discrete network indicated that soil microbes tended to be less interacted with each other after organic fertilizers inputs. This study suggested that the soil microorganisms respond differently to the inputs of inorganic and organic fertilizers in paddy soil, which offers novel insights into the potential of managing soil microbiomes for sustainable agricultural productivity.

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1. Introduction

For concern of the increasing population, agricultural systems have evoloved to maintain less biodiversity and have been more intensively managed for half a century (Dick, 1992). Farmers manage their fields with high fertilizer application to maintain the soil productivity. Mineral fertilizer and organic fertilizer are the main forms of nutrients for the arable soils. Many studies have reported the effects of fertilizations on soil properties and the crop yields. For example, Pernes-Debuyser and Tessier (2004) reported that the application of manure could maintain the soil organic matter, while the ammoniacal fertilizers strongly decreased the soil pH and cation exchange capacity. Belay et al. (2002) revealed that the total organic carbon (C) was decreased by the long-term inorganic fertilization soil, while the maize grain yield was increased significantly.

Soil microbes play important roles in energy flow and nutrient cycling, such as the decomposition of organic matter and the biogeochemical cycling of C, nitrogen (N), phosphorus (P) and sulphur (S) (Morris and Blackwood, 2015). They also supply the essential nutrients for crop growth. Soil microbial biomass and diversity are potential indicators of the soil quality (Bending et al., 2004), and are sensitive to the changes of soil nutrients, pH and organic matter content (Mele and Crowley, 2008; Zhong et al., 2010). In addition, the growth and activities of bacteria and fungi are flexible with the crop yield and the soil chemical, physical and biological properties. Many researches have reported the shifts of soil microbiota after fertilizer application. For example, Zhong and Cai (2007) reported that mineral fertilizations increased microbial biomass and diversity. Marschner et al. (2003) revealed that the bacterial community was affected by different fertilization treatments.

With the increasing accumulation of microbial communities sequence data, it is not enough to focus only on the alpha- or beta-diversity patterns. These represent the bulk of sequence-based microbial communities and are unable to document the

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interactions and functional roles of microbial populations that are coexisting in soils (Barberán et al., 2012). The interactions of soil microorganisms could be mutualism, predation, competition, parasitism and commensalism (Faust and Raes, 2012), however, these interactions are difficult to explore in natural conditions. Documenting these relationships across complex and diverse communities may help to understand the functional roles or environmental niches occupied by uncultured microorganisms (Barberán et al., 2012). It is not easy to describe the interactions among microbes and link them to ecosystem processes and functions (Deng et al., 2015). Recently, ecological network analysis as a methodology to holistically analyze environmental interactions has been used in exploring the ecological roles of microorganisms (Fuhrman, 2009; Raes and Bork, 2008; Zhou et al., 2010). These may successfully reveal the interactions within complex ecosystems. In a network, the heterogeneous relationships are simplified into a single integrated net system based on their co-occurrence or mutual-exclusion patterns (Deng et al., 2015). To the best of our knowledge, the effects of organic and inorganic fertilizations on ecological network patterns have not been fully explored. In the present study, both the bacterial and fungal communities were examined using high-throughput sequencing under the long-term fertilization experiment field to reveal the effects of inorganic and organic fertilizations on the α-diversities, compositions and interactions (ecological network) of bacteria and fungi.

2. Materials and methods

2.1. Site description

The long-term field experiment of wheat-rice rotation system is situated in Changshu, Jiangsu province, China (31°18′N, 120°37′E, 6 m above sea level), and was established in 2005. The climate of the site is humid, subtropical monsoon with an average annual rainfall of ≈1063 mm. The annual temperatures were 2 °C and 31 °C in 2015, respectively (Chen et al., 2016; Wang et al., 2016).

Crops were harvested manually at the ground level by sickle, and the above-ground biomass was removed. After the harvesting of wheat and rice, the plots are ploughed to a depth of 20 cm and a basal fertilizer was applied before sowing wheat, and before transplanting for rice (Wang et al., 2016, 2012).

2.2. Experimental design and soil sampling

Three fertilization treatments were established in a randomized block design: (1) no fertilizer control (CK), (2) NPK inorganic fertilizer (CF), in which 391 kg ha^{-1} urea (46% N), 750 kg ha^{-1} superphosphate and 183 kg ha^{-1} potassium chloride (mixed and pilled) were applied, and (3) organic fertilizer (OF), in which 4500 kg ha^{-1} organic fertilizer only (26.4% organic C, 2.5% total N, 1.6% P_2O_5 and 1.3% K_2O, made of composted pig manure and rice straw by Tianniang Ltd of Changshu, China) was applied. The randomized block plots were 6 × 7 m in size.

The bulk soil samples at a depth of 0–20 cm were collected on June 10th, 2015, immediately after the wheat was harvested. For each plot, 4 cores (5 cm in diameter) were collected. Soil samples from different treatments were mixed separately and sieved (2 mm) to remove the plant materials, roots and stones.

2.3. Soil properties analysis

Soil pH was determined with a soil-to-water ratio of 1: 2.5. Soil total carbon (TC) and total nitrogen (TN) were determined using Elemental Analyzer (Elementar Vario EL III, Germany). Soil nitrate (NO_3^-) and ammonium (NH_4^+) contents were extracted with 2 M KCl and were quantified by Bran + Luebbe GmbH − AutoAnalyzer 3 (Norderstedt, Germany). Soil available phosphorus (AP) was extracted using sodium bicarbonate and then measured by the molybdenum-blue method. Soil available potassium (AK) was measured using flame atomic absorption spectrophotometry. Soil moisture content was determined by drying the soil at 105 °C for 12 h.

2.4. DNA extraction

Soil DNA was extracted in triplicate from each soil site using MoBio PowerSoil™ DNA Isolation Kits (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions, totally 27 DNA samples (3 treatments × 3 biological replicates × 3 technical replicates) were obtained for the further analysis. The quantity and quality of DNA extracts were determined by a Nanodrop ND-2000 UV–VIS Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and the DNA was stored at −80 °C for future analyses.

2.5. Gene amplification and deep sequencing

The PCR reaction of bacterial 16S rRNA gene V3-V4 region was performed containing 5 μL of 5 × Q5 Reaction Buffer (New England BioLabs, Ipswich, Massachusetts), 5 μL of 5 × Q5 GC high Enhancer (New England BioLabs), 1 μL (10 μM) of 338F primer (5′- ACTCCTACGGGAGGCAGCA −3′)(Walter et al., 2000), 1 μL (10 μM) of 806R primer (5′- GGACTACHVGGGTWTCTAAT −3′) (Mcbain et al., 2003), 2 μL of dNTP (2.5 mM), 1 μL of DNA template (20 ng μl^{-1}), 0.25 μL of Q5 Polymerase (5 U μl^{-1}, New England BioLabs) (Voronova et al., 2012) and 9.75 μL of ddH_2O to a final volume of 25 μL. The PCR protocol was performed in triplicate using the following conditions: 5 min at 98 °C for initial denaturing, followed by 25 cycles of 98 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s with the final extension for 5 min at 72 °C. The PCR reaction of fungi ITS1 region was performed containing 5 μL of Q5 Reaction Buffer (5 ×), 5 μL of Q5 GC high Enhancer (5 ×), 1 μL (10 μM) of ITS1F primer (5′- CTTGGTCATTTAGAGGAAGTAA −3′)(Gardes and Bruns, 1993), 1 μL (10 μM) of ITS2 primer (5′- GCTGCGTTCTTCATCGATGC −3′)(Baldwin, 1992), 2 μL of dNTP (2.5 mM), 1 μL of DNA template (20 ng μl^{-1}), 0.25 μL of Q5 Polymerase (5U μl^{-1}) and 9.75 μL of ddH_2O to a final volume of 25 μL. The PCR protocol was performed in triplicate using the following conditions: 5 min at 98 °C for initial denaturing, followed by 27 cycles of 98 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s with the final extension for 5 min at 72 °C. The Illumina sequencing adapter ligated reverse primer contained a 6-bp barcode specific for sample identification (Caporaso et al., 2012). After amplification, the triplicate PCR products were pooled and purified using the PCR cleanup Kit (Axygen Biosciences, Union City, CA, USA). Bacterial and fungal PCR products were pooled separately to be sequenced in their runs, respectively. Sequencing was performed on a single lane of Illumina MiSeq platform at Personal Biotechnology Co., Ltd (Shanghai, China).

2.6. Bioinformatics and statistical analysis

Bacterial and fungal sequences were analyzed using the UPARSE pipeline (Edgar, 2013). Briefly, paired-end reads were merged into single sequences, the low-quality merged sequences (maximum expected error higher than 1 for bacterial and 0.5 for fungal, shorter than 370 bp for bacterial and 200 bp for fungal) were removed from downstream analysis. After removing the chimera, sequences with ≥97% similarity were clustered into operational taxonomic units (OTUs). The OTU representative sequences were assigned using the RDP classifier to identify bacterial taxonomies

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