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Fungal communities and functions response to long-term fertilization in paddy soils

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ABSTRACT

Soil fungi play an important role in agricultural ecosystems, yet the understanding of the responses of fungi community and function to different fertilization is limited. Here we report the responses of fungal communities and functions to 34 years of application of inorganic fertilizer (NPK), rice straw combined with inorganic fertilizer (NPKS), and no fertilizer (CK). The fungal communities were identified by Illumina high-throughput sequencing and the functional groups were inferred with FUNGuild. Three predominant Phyla of Ascomycota, Basidiomycota, Zygomycota were identified in three treatments. A significantly lower proportion of Ascomycota (47%) but higher relative abundance of Basidiomycota (28%) were observed in the NPKS treatment compared with the CK (71% and 14%, respectively). A low proportion of Basidiomycota (10%), and no significant changes in Ascomycota (74%) were found in the NPK treatment in comparison to the CK (14% and 71%, respectively). The relative abundance of Zygomycota in the NPKS (8%) and NPK (9%) treatments were significantly higher than in the CK (5%). Diversity analysis showed that indexes including Shannon, Simpson, Chao 1 and ACE all significantly declined in NPK but increased in NPKS compared with CK. FUNGuild revealed that symbiotrophic fungi in the NPKS treatment (8%) were significantly higher than in the NPK (3%) and CK (3%) treatments, and dominated by the genera *Redeckera* and *Tricholoma*, the species *Trichoderma atroviride* and the order *Scleroderma*. However, a higher proportion of pathotrophic fungi in the NPK treatment (19%) were detected than in the NPKS (11%) and CK (10%) treatments, and dominated by the species *Schizangiella serpentis*. Redundancy analysis (RDA) showed that fungal community structure and function were more strongly related to moisture, EC and porosity in the soil. We suggest that the sole application of inorganic fertilizer results in great changes in fungal community composition and the hazard of excess growth of pathogenic fungi, whereas combined organic fertilizer and rice straw is beneficial to maintain a healthy ecological environment and the diversity of fungal communities in paddy soil.

1. Introduction

Traditionally, fungi are considered as obligate aerobes (Grahl et al., 2012; Taniwaki et al., 2009). Then researchers found that fungi can survive in permanently waterlogged and poorly aerated soil, such as submerged paddy soils (Hao et al., 1981), muds and marshes (Apinis and Chesters, 1964; El-Wahid et al., 1982). Soil fungi are widely distributed in agricultural ecosystems (Tedersoo et al., 2014). They show diverse ecologies and display a crucial part as a source and regulator of energy flow and nutrient transformation in soil (Harris, 2009; Igiehon and Babalola, 2017; Smith and Barker, 2002; Steenwerth et al., 2002). It has been widely suggested that fungi are more important than bacteria in decomposing soil organic matter (SOM), especially in acidic ecosystems (Shortle and Smith, 2012; Thormann, 2006).

Higher microbial diversity is a symptom of good soil quality, indicating better substrate use efficiency and higher nutrient availability (Bending et al., 2004; Liu et al., 2009; Stenberg, 1999). Poor management practices and environmental changes may affect microbial biodiversity and potential threats to soil health and productivity (Peixoto et al., 2006; Shen et al., 2010). Thus some microbial indicators such as

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microbial biodiversity are supposed to be more dynamic than those physic-chemical characters, and may potentially serve as primary signals of soil degradation or amendment (Bell et al., 2005; Cardinale et al., 2006). Moreover, understanding the function of microbe could develop more precise management practices with which processes may have positive impacts on sustainability and productivity in agricultural ecosystems. So far it is not easy to illustrate the interactions among fungi and associated them with ecological processes and functions (Deng et al., 2016). Recently, FUNGuild, a methodology to analyze trophic modes of fungi, has been applied in determining the functional roles of fungi (Nguyen et al., 2016). FUNGuild relies on OTU taxonomic assignments, which datasets were obtained from high throughput sequencing rather than genetic loci, and provides a new way to comprehensively investigate the fungal function from an ecological perspective. These may successfully reveal fungal functions in agricultural soils. To the best of our knowledge, the effects of different fertilization regimes on fungal functions in paddy soils have not yet been fully explored.

Rice is one of the most globally important food crops for more than 50% of the world's population, and paddy soil cover 115 million hectares (ha) of the Earth's surface (Zhu et al., 2011). China is the largest rice producer (http://beta.irri.org/statistics) and fertilizer consumer (FAO 2015, available at www.fao.org/publications/sofa) in the world. In agricultural production, various fertilizers have been applied in paddy fields to increase rice yields over the last decades. Among these, mineral fertilizers i.e., urea, super phosphate, and potassium chloride, are commonly used in flooded paddy soil (Yuan et al., 2013). In addition, rice straw is a common organic material incorporated into paddy fields (Nakamura et al., 2003). However, the effects of these inorganic fertilizer and/or organic material on fungi communities and functions remain largely unknown.

Hence, in this study, both the fungal communities and functions were examined with high-throughput sequencing and FUNGuild to investigate the effects of inorganic and combined organic fertilizer and rice straw on diversities, compositions and functions of fungi in paddy soils from Southern China.

2. Materials and methods

2.1. Experimental background and soil sampling

Soil sampling was conducted at a long-term (34 years) fertilizer experiment station (119°04′10″ E, 26°13′31″ N) affiliated to Chinese Academy of Agricultural Sciences. The soil was a grey yellow paddy soil developed form hilly red earth slope deposits, and the main properties of the soil (0–20 cm) were as follows: pH (H_2O, 1:2.5), 4.90; total organic carbon (SOC), 12.5 g kg^{-1}; alkali-hydrolysable N, 141 mg kg^{-1}; available phosphorus, 12 mg kg^{-1}; and available potassium, 41 mg kg^{-1}. Three treatments: control (CK), inorganic fertilizer (NPK) (urea, super phosphate, and potassium chloride, respectively), and straw combined with inorganic fertilizer (NPKS) were evaluated for this study. For each treatment, there were three replicates. The experimental plots were 12 m^2 (3 × 4, m).

In the NPK treatment, a total of 103.5 kg N ha^{-2} yr^{-1}, 11.0 kg P ha^{-2} yr^{-1}, and 109.7 kg K ha^{-2} yr^{-1} were fertilized in the soil. Rice straw and equivalent inorganic fertilizer of NPK treatment were applied in NPKS treatment. All super phosphate was applied as a basal fertilizer, and 50% of the urea and potassium chloride were applied as base fertilizer but the remainder applied after 35 days of rice planting. The rice straw applied in NPKS treatment was approximately 4500 kg ha^{-2}, containing SOC 373.8 g kg^{-1}, N 11.0 g kg^{-1}, P 1.7 g kg^{-1} and K 16.6 g kg^{-1}. The rice straw was produced from the corresponding plots of NPKS treatment.

Five soil cores (0–20 cm) were sampled in June 2016 for each plot. Then the samples were mixed thoroughly and divided into three parts. One part was frozen in liquid N_2 then stored at −80 °C for molecular analysis, another part was used for moisture and ammonium analysis, and the other part was air-dried for the determination of pH, electrical conductivity (EC), SOC, and total N. Meanwhile, the bulk density circle was used for *in-situ* sampling for the analyzing of solid particles density and porosity of soil samples.

2.2. Physical and chemical analytical procedures

Moisture was analyzed by a moisture meter (MA100, Sartorius Company, Göttingen, Germany). Soil pH was determined by a pH-meter (PHS-3E, INESA Scientific Instrument Co., Ltd, Shanghai, China) in a 1:2.5 soil/H_2O suspension. Soil total N and SOC was detected with a total carbon analyzer (TOC-V CPH, SHIMADZU, Japan). Ammonium (NH_4^+) was extracted from soil with 2 mol L^{-1} KCl then analyzed by a flow injection analyzer (Lachat, CO, USA). Soil EC was explored with a Conductivity Meter (DDSJ-308F, INESA Scientific Instrument Co., Ltd, Shanghai, China) in a 1:5 soil/H_2O suspension. Soil solid particles density and porosity determination were determined as described by Lu (Rukun, 2000).

2.3. DNA extraction

Soil DNA was extracted from approximately 0.25 g wet soil with a PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA), in congruence with the manufacturer's instructions. The DNA concentration and quality were measured using a ND-2000 Spectrophotometer (Nanodrop Tech-nologies, Wilmington, DE, USA). DNA concentrations and purity were measured on 1% agarose gels. DNA was diluted with sterile water to a final concentration of 1 ng μL^{-1}.

2.4. Gene amplification and sequencing

The rDNA ITS1 (internal transcribed spacer 1 of rDNA) region of fungi were amplified using the high-coverage primers ITS11F and ITS2 (Toju et al., 2012). The PCR reaction mixture contained 15 μL Master Mix (NEB Inc., MA, USA), 0.2 μmol L^{-1} of each primer, 10 ng template DNA and dd H_2O to a final of 30 μL volumes. Thermal profile is started with the initial denaturation 1 min at 98 °C, followed by 30 cycles of 10 s at 98 °C, 30 s at 50 °C, 60 s at 72 °C, then finally 5 min at 72 °C. The PCR products and electrophoresis of 2% agarose gel were mixed with equal volumes of loading buffer (1X, containing SYB green) prior to detection. Samples with one bright main strip between 400 bp and 450 bp were selected for next step. Equivolume ratios of PCR products were mixed, then purified using GeneJET Gel Extraction Kit (Thermo Scientific, Schwerte, Germany). Sequencing libraries were performed with a DNA Library Prep Kit for Illumina (NEB Inc., MA, USA), in accordance with manufacturer's recommendations and index codes. The library validation and quantitation was evaluated using the Agilent Bioanalyzer 2100 system (Palo Alto, CA, USA) and Qubit 2.0 Fluorometer (Thermo Scientific, Schwerte, Germany). Finally, the sequencing library was performed on an Illumina MiSeq platform, generating 250 bp paired-end reads.

2.5. Bioinformatics

Fungal sequences analysis was assayed by UPARSE software package (Edgar, 2013). The α-diversity among samples were evaluated using in-house Perl scripts. Sequences assembly with more than 97% similarity were typically assigned to the same OTUs. For each OTU, a representative sequence annotated by RDP classifier were selected. The selected representative sequences were implemented in the UNITE database (http://unite.ut.ee) for molecular identification of fungi (Abarenkov et al., 2010). Four indexes including Shannon index, Simpson index, Chao 1 index and ACE index were calculated through OTU table to express the *α*-diversity of fungi community.