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Research Paper

Alteration of soil bacterial interaction networks driven by different long-term fertilization management practices in the red soil of South China

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ABSTRACT

Interactions among soil bacteria occur widely and play important roles in the maintenance of soil functions. Long-term fertilization management practices have distinct effects on soil fertility and the soil microbial activity and community, which are closely associated with soil microbial interactions. Red soil is typically low-productivity soil in South China. Chemical nitrogen fertilization caused serious soil acidification and low-productivity (defined as the acidified soil group, Ac), whereas the application of lime to the acidified soil increased the soil pH (defined as the quicklime improvement soil group, Qlime). Long-term manure or fallow treatment maintained the soil pH and increased the soil fertility (defined as the high-productivity potential soil group, HPP). A molecular ecological network analysis method was used to analyze 454 pyrosequencing data of bacterial communities from the HPP, Ac and Qlime soils. Several major differences were observed among the three constructed networks. First, the HPP network contained the largest ratio of positive to negative correlations, whereas the Ac network contained the smallest. Second, the HPP and Ac networks shared only 8.67% of their operational taxonomic units (OTUs), whereas Ac and Qlime shared 27.04%. Third, the HPP network contained the most “module hubs” (A set of OTUs that have strong interactions or common functions are grouped as a “module” in network analysis. These OTUs are called “nodes”. And the nodes with high connectivity to many other nodes within the same module are “module hubs”.), whereas Ac contained the fewest. These results demonstrated that the bacterial community of HPP was a better organized or a better operated community than Ac and that quicklime application helped to order the bacterial community. By comparing the topological roles of nodes in different networks, we proposed that there should be more module hubs in the networks of higher-productivity soils and hypothesized that these OTUs could be indicators of high-productivity.

1. Introduction

Soil biodiversity is usually associated with ecosystem functions (Hooper et al., 2005; Naeem and Wright, 2003; Petchey and Gaston, 2006). Fuhrman (2009) attempted to link marine microbial community structures to their functional implications. However, the interactions among different microorganisms were ignored. Soil is a complex ecological environment in which microorganisms do not exist in isolation (Faust and Raes, 2012). Tens of thousands of bacterial species (Torsvik et al., 1990) in soils may form complex ecological interaction networks through various interaction types (Lidicker, 1979) (e.g., mutualism, commensalism, parasitism, predation, and competition).

The ecological interaction pattern of a network can reflect the general situation of a whole community structure (Freilich et al., 2010) or even the ecosystem functions (Zhou et al., 2010). Biological networks were previously constructed at the intracellular levels for protein-protein, protein-DNA, and protein-metabolite interactions (Barabási and Oltvai, 2004; Han et al., 2004; Maslov, 2002; Zhang and Horvath, 2005) or in complete systems like food-web structures (Cattin et al., 2004; Dunne et al., 2002). Global networks can be constructed from different data sets independently (e.g., 16S rRNA datasets) (Chaffron et al., 2010).

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Network analysis (Zhou et al., 2010) is performed to represent the ecological interactions of different species in a complex bacterial community. Moreover, random matrix theory (Wigner, 1967) is a powerful approach for studying the interactions of complex systems. Therefore, the combination of these two techniques will be a very good analytical tool for microbial ecology. Briefly, after estimating the pairwise correlations between any two OTUs according to their relative abundances, their similarity is measured using the absolute value of the pairwise correlation coefficient. Then, based on an RMT approach, a similarity threshold is applied to transform the similarity matrix into adjacency matrix, which provides the strengths of the connections between nodes. Module analysis and network characterization are then performed according to the adjacency matrix. The topological network parameter, for example, connectivity, is estimated to represent how strongly that node is connected to all of the other nodes in the network. Additionally, the topological roles of nodes are evaluated to understand their importance in driving community functions. Previous works (Deng et al., 2012; Zhou et al., 2011) studied the responses of soil microbial communities to elevated CO2 levels and provided good examples of phylogenetic molecular ecological networks based on the random matrix theory conceptual framework. Recently, microbial association networks have been used to study various complex microbial ecological systems inferred for a range of communities from the ocean (Aylward et al., 2015; Fuhrman et al., 2015; Steele et al., 2011) to natural soils (Barberán et al., 2012; Eldridge et al., 2015) to the human body (Kelder et al., 2014; Lagkouvardos et al., 2015). Microbial association networks have also been widely applied in agricultural soils. Nielsen et al. (2014) compared microbial communities from soils with enhanced biochars and traditional fertilizers and found that enhanced biochar application provided similar sweet corn yields although the community composition and networks were quite different compared with standard fertilizers. Menezes et al. (2014) demonstrated that fungi and bacteria were co-correlated and formed distinct associations in soils.

In the agricultural soil of Southern China, different fertilizations practices have significantly altered crop yields and soil characteristics (Xun et al., 2016), resulting in the formation of the major low-productivity and high-productivity soils. Soil bacteria are indispensable maintainers of soil productivity (Smith and Paul, 1990). Thus, elucidating the ecological network of the bacterial communities from soils with different productivities is meaningful. However, differences between the networks of the microbial communities in the major low-productivity and high-productivity arable soils are poorly understood.

To investigate the alteration of soil bacterial interaction networks driven by different long-term fertilization management practices, we collected samples, from soils that could be identified as high-productive potential soils (HPP), low-productivity soils (Ac) and artificially remediated soils (Qlime), and analyzed the 16S rRNA genes using 454 high-throughput pyrosequencing. We hypothesized that several specific OTUs might play mainstay roles in a holonomic biological network and could serve as indicators of soil productivity.

2. Materials and methods

2.1. Experimental site description

This study was established in the Red Soil Experiential Station (RSES) of the Chinese Academy of Agricultural Sciences, Qiyang (111°53′E, 26°45′N), Hunan Province, southern China. Red soil, which developed from Quaternary red clay, is one kind of Ferralsols according to the World Reference Base for Soil Resources (WRB) (IUSS Working Group, 2014).

After three years of homogenization in one field by performing annual rotations of winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.), the experimental field was divided into successions of plots (20 m × 10 m × 0.4 m). All fertilization treatments were organized in a randomized design with two replications and began in 1990 with the same rotation system. The experiment began with ten original treatments: (i) control (CK), no fertilizer application; (ii) chemical nitrogen (N); (iii) combined chemical nitrogen-potassium fertilizer (NK); (iv) combined chemical nitrogen-phosphorus fertilizer (NP); (v) combined chemical nitrogen-phosphorous-potassium fertilizer (NPK); (vi) combined chemical phosphorus-potassium fertilizer (PK); (vii) combined of swine manure and chemical nitrogen-phosphorus-potassium fertilizer (NPKM); (viii) swine manure (M); (ix) combined of straw and chemical nitrogen-phosphorus-potassium fertilizer (NPKS); and (x) fallow (Fallow). Five treatments (N, NK, NP, NPK and NPKS) resulted in strong acidification after 20 years (2010) of fertilization. These plots were split into two parts. Half-plot was fertilized as usual, and quicklime (CaO) was added into the other half-plot. More detailed description of the treatments and fertilizations can be found in my previous publication (Xun et al., 2016).

2.2. Soil sample characteristics

Soil samples were collected in May, 2012. Each plot was divided into two parts, and fresh samples were obtained from the upper 20 cm. Each replicate was a mix of 12 soil cores that were 5 cm in diameter. Four soil samples of each treatment were obtained; three of the samples were randomly selected for analysis. All samples were sieved through a 2 mm sieve. The subsamples used to measure physico-chemical properties were air-dried, and the subsamples used for the molecular analyses were stored at −80 °C prior to DNA extraction.

Several physico-chemical and biological properties were measured to assess soil fertility: (i) The soil pH was determined using a PHS 3C mv/pH detector (Shanghai, China) at a soil-to-water ratio of 1:5; (ii) Available N (AN) was measured using the NaOH pervasion method (Bao, 2000); (iii) Available K (AK) in the soil was extracted with ammonium acetate and determined by flame photometry, and available P (AP) in the soil was extracted with sodium bicarbonate and then determined using the molybdenum blue method (Olsen et al., 1954); (iv) Soil organic matter (SOM) (Schollenberger, 1931) was determined by the potassium dichromate volumetric method; and (v) The microbial biomass C (MBC) (Vance et al., 1987) concentration was measured using the chloroform fumigation-extraction method and these values were then transformed to microbial biomass using a kEc factor of 2.64 (Zhong and Cai, 2007).

2.3. 454 pyrosequencing analysis

Soil DNA was extracted from 0.25 g subsamples using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA), and three successive DNA extractions of each sample were pooled before PCR. The DNA quality was assessed with a NanoDrop ND-2000 Spectrophotometer (NanoDrop ND2000, Thermo Scientific, Wilmington, DE, USA).

454 pyrosequencing analysis of the V1-V3 hypervariable regions of the bacterial 16S rRNA gene (Zhao et al., 2014) was performed on a 454 GS-FLX Titanium System (Roche, Switzerland) by Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China). The pyrosequencing data were processed using Mothur (version 1.27.1) (Schloss et al., 2009) following the Schloss standard operating procedure (SOP) (http://www.mothur.org/wiki/454_SOP). More detailed description of the raw sequences analysis can be found in my previous publication (Xun et al., 2016).

2.4. Soil grouping and network analysis

The crop yields, soil properties (Table S1) and bacterial communities were different among these fifteen fertilization managements (Xun et al., 2016). With pH values higher than 5.0 and greater yields, the CK, Fallow, NPKM, M and PK treatments were defined as the high-productivity potential group (HPP); with low pH values and low-