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Organic amendments shift the phosphorus-correlated microbial co-occurrence pattern in the peanut rhizosphere network during long-term fertilization regimes

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ABSTRACT

Root-associated microbial communities play important roles in driving the microbial transformation of soil carbon and nitrogen cycling, but evidence of how these communities shape microbial co-occurrence and change phosphorus (P) correlated microbial interactions is still lacking. In this study, a random matrix theory-based network analysis of 16S rRNA genes was used to identify bacterial networks associated with the peanut rhizosphere and bulk in four long-term fertilization schemes. High-throughput sequencing data revealed that with the decrease of rhizosphere bacterial diversity, its positive covariation in the network increased, indicating stronger commensal and mutual assemblage with less functional redundancy in the rhizosphere. P availability is the major variable modulating the peanut rhizosphere microbial community in acidic soil. Two strongly positive P-correlated rhizosphere OTUs (closely related to *Chitinophaga pinensis* and *Nitrospira moscoviensis*), which have the potential to promote carbon-phosphorus and nitrogen-phosphorus synergistic conversion, respectively, were used to investigate the advantages of manure amendment in promoting rhizosphere P cycling in peanut planting agrosystems.

1. Introduction

Soil available phosphorus (P) is crucial for plant growth, but its deficiency is a problem in tropical acid soil, where P is easily converted into poorly soluble Fe and Al phosphates and is thus unavailable to plants (Ziadi et al. 2013). Until recently, traditional nutrient management for preserving high crop productivity has been mainly based on external fertilizer inputs (Pii et al. 2015). East Asia is the area that has contributed the largest cumulative global P surplus due to the low P use efficiency and the largest P fertilizer application (MacDonald et al. 2011). Excessive use of P input poses threats for ecosystem function, as the enrichment can contribute to water eutrophication and biodiversity loss (Aubriot et al. 2011). Therefore, there is substantial interest in improving phosphorus supply utilization in the agro-ecosystem.

Rhizosphere processes associated with microorganisms have an intrinsic biological potential to improve crop nutrient uptake capacity (Pii et al. 2015). Many reports have found that such variable success of carbon and nitrogen uptake are typically attributed to the complexity of microbial communities and their interactions in the zone immediately surrounding the roots (Ai et al. 2015, 2017; Francioli et al. 2016). In the case of phosphorus, another essential element for crop growth, the relationship of its availability and rhizosphere microbial interaction is still unclear. Castrillo et al (2017) demonstrated that the root synthetic bacterial community drives direct integration of phosphate stress in *Arabidopsis thaliana*, implying that plant roots have the potential to assemble specific microbial groups to benefit P cycling. However, there is still little information available regarding rhizosphere effects on P-correlated microbial interactions in agricultural soils.

Advances in culture-independent techniques have provided tools for the study of phosphorus transforming microbial communities in the environment. Since the first probe (ALPS primers) targeting alkaline phosphatase were developed, researchers not only improved new sets of specific primers to target a larger diversity of alkaline phosphatase genes (such as *pho*D), but had various insights into alkaline phosphatase diversity affected by crop management and fertilizers application (Sakurai et al. 2008; Wang et al. 2012; Tan et al. 2013; Fraser et al. 2015). However, some reports noted that ALPS primers likely have an amplification bias, resulting in an overrepresentation of

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Alphaproteobacteria (Tan et al. 2013). These studies focused on alkaline phosphatase but ignored the organic P mineralization from acid phosphatase (EC.3.1.3.2) and inorganic P dissolution. A new approach is required to provide a more comprehensive evaluation of microbial activation of phosphorus.

An ecological network, which is represented by complicated positive (e.g., commensalism and mutualisms) and negative (e.g., predation and competition) interactions (Chow et al. 2014), provides a standardized framework for understanding the interactions among microbial populations in complex systems (Poisot et al. 2012; Toju et al. 2017). Documenting inter-taxa and intra-taxa associations modulated by specific environmental factors across complex microbial communities may help predict the functional roles, habitat affinities and shared physiologies that can guide more focused studies or experimental settings (Cardinale et al. 2015). Jiang et al. (2017b) reported nematode predacious promotion of bacterial diversity and functional (alkaline phosphomonoesterase producing) bacterial abundance through network interaction, indicating this developing approach may forecast the interactions between specific functional microbes (such as P-transforming microorganisms) and the entire microbial community.

Peanuts are the fifth most important oil seed crop and are widely cultivated in tropical and subtropical regions of Asia, Africa and North and South America, areas facing excessive P input but relative low P use efficiency (FAOSTAT 2014). It is important to evaluate the coordinated effects of long-term fertilization schemes and rhizosphere effects on peanut rhizosphere P-correlated specific microbial assemblages. In acidic soil regions, the main fertilization scheme of peanut planting includes (1) traditional chemical fertilization and (2) inorganic (chemical) and organic (straw or manure) combined application. To further develop ecological and circular agriculture, the replacement of chemical fertilizer with manure or straw is the current popular modern eco-agricultural management. We hypothesized that fertilization schemes and rhizosphere effects cooperatively shaped the variation in the rhizosphere microbial community structure and construct the specific network interactions in the millimeter-sized habitat to activate rhizosphere nutrients, such as P. Furthermore, we expected to discover P-correlated microorganisms that are specifically regulated by rhizosphere effects and explore their niche position in the microbial ecological networks.

To verify our speculation, peanut rhizosphere and bulk soil were collected from four long-term fertilization schemes (a no-fertilization control; inorganic nitrogen, phosphorus and potassium, NPK; NPK plus organic manure, NPKM; NPK plus straw, NPKS). The 16S rRNA gene amplicon sequencing coupled with co-occurrence networks analysis were used to present the systematic framework for rhizosphere and bulk microbial diversification and ecological networks. We filtered the P-strongly correlated OTUs through a Pearson-correlation analysis, and we further explored their functional ecological interactions through matching OTUs to the co-occurrence networks and compared their relative abundance in different fertilization schemes.

2. Materials and methods

2.1. Experimental sites and sample collections

A long-term fertilization experiment was initiated in 1988 at the Red Soil Ecological Experimental Station of the Chinese Academy of Sciences located in Yingtan, Jiangxi Province, China (28°15′N, 116°55′E). This region has a subtropical, humid monsoon climate with an annual average temperature and precipitation of 17.6 °C and 1795 mm, respectively. The soil is an acid loamy clay that is derived from quaternary red clay (Udic Ferralsols in Chinese Soil Taxonomy and Ferric Acrisols in the FAO classification system). Four fertilization treatments (triplicate soil blocks for each) were established with a peanut-rape rotation from 1989 to 1995 and peanut cropping since 1996. Treatments consisted of soil without fertilizer (control, CK), fertilizer N, P and K (NPK), fertilizer NPK plus organic manure (NPKM), and fertilizer NPK plus peanut straw (NPKS) in a randomized plot design (5.3 m in width × 6.5 m in length × 1.0 m in depth). For the NPK treatment, fertilizers N, P and K were applied in the form of urea (120 kg N ha^{-1}), superphosphate (75 kg P_2O_5 ha^{-1}) and potassium chloride (75 kg K_2O ha^{-1}), respectively. For NPKM or NPKS treatment, the chemical fertilizer application was 70% of the regular amount of NPK fertilizer (84 kg N ha^{-1} y^{-1}, 52.5 kg P_2O_5 ha^{-1} y^{-1}, and 52.5 kg K_2O ha^{-1} y^{-1}) plus 30% of the same content present in manure or peanut straw (contain 36 kg N ha^{-1} y^{-1}, 22.5 kg P_2O_5 ha^{-1} y^{-1}, and 22.5 kg K_2O ha^{-1} y^{-1}).

The rhizosphere and bulk soil samples were collected during the peanut plant flowering stage in June 2015, which is when the rhizosphere effects tend to be pronounced (Shi et al. 2016). Rhizosphere soil was collected by gently shaking the entire plant root system to remove loosely attached soil (Ai et al. 2015). Six soil samples (three from the rhizosphere and the other three from the bulk) from each treatment (in total, 24 samples) were placed in polythene wrappers, chilled on ice following their collection in the field, and immediately transported to the laboratory, where they were sieved to 4 mm to remove visible roots and residue. Each soil sample was then divided into two subsamples and stored at either 4 °C for measurements of their physical and chemical properties or −80 °C for the microbial community analysis.

2.2. Soil physical and chemical properties measurement

The soil pH was determined with a glass electrode in a water-to-soil ratio of 2.5:1 (v/w). Soil total nitrogen and nitrate (NO_3^- − N) and ammonium nitrogen (NH_4^+ − N) were analyzed with a flow injection auto-analyzer (Auto Analyzer 3; Seal Analytical, Norderstedt, Germany). The soil total phosphorus (TP) and available phosphorus (AP) were extracted by sodium carbonate and sodium bicarbonate, respectively; both were quantified using the molybdenum blue method (Olsen et al. 1954). Total potassium (TK) and available potassium (AK) concentrations were determined using flame photometry after their extraction with sodium hydroxide and ammonium acetate, respectively (Kanehiro and Sherman, 1965). Soil organic carbon (SOC) content was measured by wet digestion using the potassium dichromate oxidation method. The soil moisture was determined by drying the soil for 48 h at 105 °C. The cation exchange capacity (CEC) was measured in an ammonium acetate solution at pH 7 (Chapman, 1965).

2.3. 16S rRNA gene amplicons and high-throughput sequencing

The microbial genomic DNA was extracted from 1.0 g of soil for each sample using a Power Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA) according to the manufacturer's instructions. The bacterial 16S rRNA genes were amplified using primers 515F (5′-GTG CCAGCMGCCGCGG-3′) and 806R (5′-GGACTACHVGGGTWTCT AAT-3′), which target the V4 hypervariable regions. The PCR was performed in 50 μl reaction volumes containing 25 μl of Premix Taq DNA polymerase, 0.5 μl of forward primer (20 μM), 0.5 μl of reverse primer (20 μM), 23 μl of doubly distilled water (ddH_2O), and 1 μl of DNA template (20 ng of total soil DNA). The thermal cycling conditions were as follows: an initial denaturation at 94 °C for 5 min, followed by 30 cycles at 94 °C for 45 s, 56 °C for 30 s and 72 °C for 45 s, with a final extension at 72 °C for 7 min. Illumina libraries were constructed using the MiSeq Reagent Kit V3 according to manufacturer's instructions. High-throughput, paired-end sequencing was performed on the Illumina MiSeq PE250 platform. Sequencing data were deposited in the European Nucleotide Archive under the accession number PRJEB22659.

2.4. Analysis of sequencing data

Based on the MiSeq Reagent Kit Preparation Guide (Illumina, San