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Short communication

Shifts in soil microbial community, soil enzymes and crop yield under peanut/maize intercropping with reduced nitrogen levels

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ABSTRACT

A field experiment with five nitrogen (N) application rates (120, 140, 160, 180, 200 kg hm^{-2}) under peanut/maize intercropping regime was conducted to evaluate cropping patterns and N application rates on yield, soil enzyme activities, and rhizosphere microbial community structure. The field experiment showed that the land equivalent ratio (LER) in maize/peanut intercropping system was not decreased as compared with the monoculture pattern, though the total N supply was approximately reduced by 20%. Terminal restriction fragment length polymorphism (T-RFLP) results showed that the treatments of intercropping (IMP100%, IMP80% and IMP60%) increased the abundance of *Rhizobium hainanense*, *Rhizobium leguminosarum* and *Frankia* which are associated with nitrogen fixation as compared to monoculture pattern (M100% and P100%). The qPCR results revealed that the copy number of *nifH* was significantly higher in the treatments of intercropping than in monoculture pattern. The result was also consistent with the T-RFLP analysis. In conclusion, peanut and maize under the condition of intercropping can promote the population of microorganisms associated with nitrogen-fixing in the rhizospheric soil. Our results provide a theoretical basis and technical support to soil N utilization, development of low carbon agriculture and protection of farmland ecological environment.

1. Introduction

Interspecific interactions in intercropping systems can facilitate the promotion of nitrogen and phosphorus uptake for increased yield compared to sole cropping systems (Li et al., 2003; Xia et al., 2013). During intercropping systems small land holdings farmers used a large amount of nitrogenous fertilizers to get a higher yield (Zhang et al., 2011, 2004). More than 50% of nitrogen-containing fertilizers are not taken by the agricultural crops when applied to the fields (Sylvester-Bradley and Kindred, 2009), causing nitrates sequestration which finally held responsible for N deficiency in plants and causes soil and water pollution (Davies and Sylvester-Bradley, 1995; Misselbrook et al., 2000). Therefore, the introduction of better cropping is very critical for efficient use of soil nutrients and to optimize the level of chemical fertilizers in long-term sole fields.

A plant root interacts with physical, chemical and biological properties of soil, and is affected by activity and presence of roots and microbial community (Richardson et al., 2009). Intercropping can act the significant role in underground interactions and their roots have a selective effect on microorganisms in the rhizosphere (Hinsinger et al., 2011; Zhang and Li, 2003). Interspecific root interaction affects nutrient mobilization in the rhizosphere soil and attribute efficiently to nutrient uptake by legume- intercropping (Inal et al., 2007; Wasaki et al., 2003). In most intercropping systems, phosphorous mobilization is enhanced by the activation of soil enzymes and by carboxylates present in root exudates when legume crops such as peanut, pea, and beans etc. are intercropped with long-term sole plants (Ehrmann and Ritz, 2014; Hinsinger et al., 2011; Neumann and Römheld, 1999).

Soil microorganism analysis in various cropping systems and cultural practices is an important aspect because soil microbial communities quickly respond to environmental dynamic and is an important bio-indicator for soil health (Avidano et al., 2005; Costantini et al., 2015). Soil microbial activities act important roles in the nutrients mobilization and mineralization which are essential for plant growth. Plants secrete different kinds and amounts of root exudates that exert specific effects on soil microbial communities (Arafat et al., 2017;

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Baudoin et al., 2003). However, fewer studies have been reported about the micro-biological properties in the rhizosphere of intercropping systems with low nitrogen treatment.

Various molecular techniques are commonly used for nucleic acid extraction from soil microbes and can be resulted independently for high taxonomic groups such as phylum, class, order, families and genera in the rhizosphere and even for species (Nannipieri et al., 2003). The goal of this study was to understand the consequences of different nitrogen levels on microbial community structure and their related activities during intercropping of maize with peanut. We used T-RFLP and qPCR techniques to provide quantitative data on changes in relative abundance of different bacterial taxa and functional groups for bio-monitoring of highly diverse bacterial community.

2. Material and methods

2.1. Experiment design

The experiments were conducted at the experimental field station located at Fujian Agricultural and Forestry University (26°05′9.60″ N, 119°14′3.60″ E), China. The average temperature was 25 °C–32 °C. An agricultural soil was classified as sandy loam soil with pH 6.2, total nitrogen (TN) 1.20 g kg^{-1}, available nitrogen (AN) 40.6 mg kg^{-1}, total phosphorus (TP) 0.25 g kg^{-1}, available phosphorus (AP) 12.6 mg kg^{-1}, total potassium (TK) 4.55 g kg^{-1} and available potassium (AK) 31.6 mg kg^{-1}.

Two crops maize (*Zea mays* L.cv. Zhengda 12) and peanut (*Arachis hypogaea* L.cv. Minhua 8) were intercropped with five different nitrogen levels in this study. i.e., (Fig. S1, Fig. S2) intercropping maize/peanut with normal (200 kg hm^{-2}) dose of nitrogen fertilizer (IMP100%), 90% nitrogen (IMP90%), 80% nitrogen application (IMP80%), 70% nitrogen level (IMP70%), 60% nitrogen application (IMP60%) respectively. Sole maize (M100%) and sole peanut with normal nitrogen level (P100%) were used as a control with 100% of normal nitrogen fertilizer. Same fertilization (including 52 kg hm^{-2} $Ca(H_2PO_4)_2$ and 75 kg hm^{-2} KCl) and field management were applied to all treatments during the whole experimental period. All treatments with different nitrogen levels were planted in the pot (four replicates) for collecting soil samples at the same time (Fig. S1, Fig. S2).

2.2. Soil sample

Soil samples were taken from five replicates at the flowering stage of maize (60 days after sowing) in 2013. Plants were selected randomly and uprooted with a forked spade, the loosely adhering soil was shaken off carefully, and the tightly adhering soil was brushed up and collected as rhizosphere soil from each plant. After this, each rhizosphere soil was sieved through 1 mm mesh to remove plant roots and leaf residues. Each soil sample was divided into two parts: one stored at 4 °C for soil enzymes and nutrients, and other one stored at −20 °C for DNA extraction.

2.3. Analysis of soil nutrition (NPK) and soil enzymatic activity

A glass electrode pH meter was used to determined soil pH (1: 2.5 soils to water suspensions) (Thomas, 1996). The available and total amounts of main soil nutrient such as nitrogen, phosphorus, and potassium were measured using the methods described by (Jackson, 1958; Watanabe and Olsen, 1965). Soil urease [EC 3.5.1.5] activity was determined by incubating 5 g soil with 30 mL of extracting solution at 37 °C for 24 h. The formation of ammonium was measured spectrophotometrically at 578 nm (Wang et al., 2009). Soil invertase [EC 3.2.1.26] activity was determined by incubating 5 g soil with 15 mL of 8% sucrose solution at 37 °C for 24 h. The suspension reacted with 3, 5-dinitrosalicylic acid and absorbance was measured at 508 nm (Wang et al., 2009). Phosphomonoesterase [EC: 3.1.3.2] activity was determined based on a modified method adopted by Sardans et al. (2006).

2.4. Extraction of DNA and 16S rRNA gene amplification

Five soil samples (IMP100%, IMP80%, IMP60%, M100% and P100%) were selected for analysis of bacterial communities in this study. Soil (1 g) from five treatments was used to extract DNA by using the BioFast Soil Genomic DNA Extraction Kit (Hangzhou, China). Extracted DNA was subsequently stored at −20 °C until further use. The fluorescent carboxyfluorescein (6-FAM)-labeled forward primer 27f (5′-AGAGTTTGATCMTGGCTCAG-3′) and unlabeled reverse primer 1492r (5′- GGTTACCTTGTTACGACTT −3′) were used for 16S rRNA amplification. PCR reactions were carried out in the 50 μL volume containing 25 μL of 2 × EasyTaq® PCR SuperMix (Transgen, China), 1 μL of each primer (10 pmol mL^{-1}), 22 μL sterile water and 1 μL of the template (approximately 100 ng). The PCR program consisted of 5 min initial denaturation step at 95 °C, followed by 35 cycles of 95 °C for 1 min, 60 °C for 1 min and 72 °C for 90 s, a final 10 min extension step at 72 °C. All PCR products were visualized by 1.2% agarose gel electrophoresis and were purified using a Gel Extraction Kit (OMEGA Bio-Tek, USA) according to the manufacturer's instructions.

2.5. Determination of bacterial communities

The bacterial communities in the soil were analyzed by the terminal restriction fragment length polymorphism (T-RFLP) method. In this method, the purified PCR products were first digested separately with four enzymes (*Hae*III, *Msp*I, *Alu*I and *Afa*I). The digestion mixture contained 8 μL of PCR products, 1.5 μL of enzyme buffer, and 1.5 μL of restriction endonuclease and 4 μL sterile water. After this, the digested products were sent to Shanghai Sheng Gong company for sequencing (ABI automated sequencer analyzer; Model 3130 Applied Bio systems).

Furthermore, T-RFLP profiles were analyzed by Gene Marker software (Version 1.2). The terminal fragments between 30 and 600 bp were chosen for further analysis. The relative abundance of terminal restriction fragments (TRFs) in a profile was measured as a proportion of the total peak area of all the TRFs in a profile.

2.6. Quantification of functional communities involved in nitrogen cycling by qPCR

Real-time PCR quantifications of genes (*amoA* (AOA and AOB), *narG*, *nirK*, *nirS* and *nifH*) encoding the key enzymes of ammonia oxidation, nitrate reduction, denitrification and nitrogen fixation respectively were used to estimate the richness of functional communities involved in nitrogen cycling by using specific primers and conditions described in Supplementary Table S1.

2.7. Statistical analysis

Microsoft excel 2003 were used to processed all experimental data. Statistical analysis was conducted by using SPSS software. Analysis of variance (ANOVA) was used to determine significance difference at $P < 0.05$ for multiple comparisons by using DPS software version 7.05.

3. Results

3.1. Plant yields

Plant yields of maize and peanut were measured in 2012 and 2013 (Table 1). In 2012 the yield in treatments IMP100% and IMP75% of maize were significantly higher than sole maize (M100%) by 37.21% and 25.51% while, the yield of sole-peanut significantly higher than other treatments. In 2012, Land Equivalent Ratio's (LER) results