

ANALYSIS OF SOIL MICROBIAL COMMUNITY STRUCTURE AND ENZYME ACTIVITIES ASSOCIATED WITH NEGATIVE EFFECTS OF *PSEUDOSTELLARIA HETEROPHYLLA* CONSECUTIVE MONOCULTURE ON YIELD

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Abstract

Pseudostellaria heterophylla is an important medicinal plant in China. However, cultivation of *P. heterophylla* using consecutive monoculture results in significant reductions in yield and quality. In this study, terminal-restriction fragment length polymorphism (T-RFLP) analysis and measurement of soil enzyme activities were used to investigate the regulation of soil micro-ecology to identify ways to overcome the negative effects of *P. heterophylla* consecutive monoculture. T-RFLP analysis showed that rice/*P. heterophylla* (RP) and bean/*P. heterophylla* (BP) crop rotation systems increased the number and diversity of microbial groups in *P. heterophylla* rhizosphere soil. In particular, the RP and BP crop rotations increased the number and abundance of beneficial bacterial species compared with two-year consecutive monoculture of *P. heterophylla*. The presence of these beneficial bacteria was positively correlated with soil enzyme activities which increased in rhizosphere soils of the RP and BP crop rotation systems. The results indicated that crop rotation systems could increase activities of key soil enzymes and beneficial microbial groups and improve soil health. This study could provide a theoretical basis to resolve the problems associated with *P. heterophylla* consecutive monoculture.

Key words: *Pseudostellaria heterophylla*; Crop rotation; Rhizosphere microorganisms; Soil enzyme activity; Consecutive monoculture

Introduction

The root of *Pseudostellaria heterophylla*, a member of the *Caryophyllaceae* family, is a common and widely used traditional Chinese medicine and is recorded in the Chinese Pharmacopoeia. Before the 1970s, this herb was collected mainly from wild sources. Today, *P. heterophylla* is cultivated in suitable areas within Fujian, Guizhou, Anhui, Jiangsu, and other provinces (Zeng *et al.*, 2012). The total area of cultivation is increasing in response to rapidly growing demand. High-quality *P. heterophylla* is produced predominantly in Ninde, Fujian Province, which has the best climatic and soil conditions for cultivation. However, as with other medicinal plants, consecutive monoculture of *P. heterophylla* results in decreased productivity (Zhang *et al.*, 2011). To compensate for the decreased yields and lower product quality resulting from consecutive monoculture, farmers often expand their *P. heterophylla* plantings into less desirable farmland.

Studies during the past few decades have shown that consecutive monoculture leads to soil nutrient imbalance (Grattan & Grieve, 1998; Minh, 2005; Ndabamenye *et al.*, 2013), the generation of autotoxins (Friedman & Waller, 1985; Bertin *et al.*, 2003; Yoneyama & Natsume, 2010), and/or changes in soil microbial community structure (Yang *et al.*, 2000; Mithufer, 2002; Lin Wenxiong *et al.*, 2007; Berendsen *et al.*, 2012). Plants release substances into the soil that alter the physical and chemical properties of the soil and contribute to the development of diverse microbial groups in the rhizosphere (Wardle *et al.*, 1999; Bardgett *et al.*, 1998; Dobermann *et al.*, 2000; Hawes *et al.*, 2003). As a consequence, the altered microbial community affects nutrient circulation and energy flow thereby impacting plant growth and yield (Hinsinger *et al.*, 2006; Wu *et al.*, 2011, 2013; Berendsen *et al.*, 2012). Bacteria

represent the largest group of soil microbes that regulate soil ecosystem function. The dynamics of bacterial population composition and diversity affect geochemical cycles, organic matter formation and degradation, soil structure, and biological interactions (Wang *et al.*, 2011). Continuous monoculture of *P. heterophylla* and other medicinal herbs leads to increased levels of soil pathogens and imbalance in the bacterial community structure (Zhang *et al.*, 2009).

Crop rotation is a common and useful agricultural production method for overcoming problems associated with continuous monoculture (Guo *et al.*, 1990; Leroux *et al.*, 1996; Kelley *et al.*, 2003; Zhao *et al.*, 2005; McDonald and Peck *et al.*, 2009; Verhulst *et al.*, 2011). How crop rotation alters bacterial community diversity and function in the rhizospheres of different crops remains unclear. Crop rotation may result in changes in the rhizosphere bacterial community by introducing root exudates detrimental to pathogenic bacteria with a concomitant increase in beneficial bacteria. In this study, we carried out rice/*P. heterophylla* (RP) and bean/*P. heterophylla* (BP) crop rotations in a “good agriculture practices” (GAP) experimental field and analyzed changes in soil microbial communities using terminal-restriction fragment length polymorphism (T-RFLP) analysis and measurement of soil enzyme activities.

T-RFLP analysis is a technically simple, relatively rapid, and culture-independent technique that is used widely to study the structure and functions of soil microbial communities (Marsh *et al.*, 2000; Lord *et al.*, 2002; Marsh, 2005; Widmer *et al.*, 2006; Hodgetts *et al.*, 2007; Joo *et al.*, 2010). We used T-RFLP analysis to explore the effects of different crop rotation systems on the relationship between changes in the abundance of bacterial groups and soil enzyme activities and the productivity of *P.*

heterophylla. Our results may aid in the development of specific microbial fertilizers or farming methods to improve the soil microbial community structure and ecosystem to promote the growth and yield of medicinal plants and other crops.

Materials and Methods

Experimental design, field planting, and soil sample collection:

The experiment was conducted using GAP in an experimental field in Zherong County, Ninde Municipality, Fujian Province, P.R. China, during 2009–2011. The *P. heterophylla* cultivar ‘Zheseng-2’ used for this study was planted in December and harvested each year in July. The field trial was composed of three replicates of five treatments including one-year monoculture (OM), two-year consecutive monoculture (TM), rice/*P. heterophylla* rotation (RP), bean/*P. heterophylla* rotation (BP), and fallow treatment (CK) as a control. The experimental plots were 5 × 5 m (25 m²) for each treatment. Individual *P. heterophylla* roots were planted with a spacing of 5*10 cm (5 cm between each plant in a row and 10 cm between rows) between plants. The one-year monoculture *P. heterophylla* plots were planted on December 23, 2010. *Pseudostellaria heterophylla* was planted in the two-year consecutive monoculture plots on December 23, 2009, and in the RP and BP rotation plots on December 23, 2010. Rice and beans were planted in July 2010 and harvested in November 2010. The *P. heterophylla* harvests from all the cropping systems took place in July 2011. The yields of the different cropping systems are presented in Table 1.

Table 1. The yield of *P. hereophyll* under different cropping patterns.

Cropping pattern	Yield (kg/667m ²)
One-year monoculture	473.57±16.41a
Two-year consecutive monoculture	272.136±12.9b
Rice- <i>P. heterophylla</i> rotation	437.06±18.08ab
Bean- <i>P. heterophylla</i> rotation	306.805±13.2b

The lowercase letters behind data represented significant differences ($p < 0.05$)

Pseudostellaria heterophylla germinated completely in March 2011. Soil samples were collected from five random locations in each plot in May 2011 during the peak growth peak period for *P. heterophylla*. Fresh plants were carefully uprooted from the soil with a forked spade. The roots were shaken to remove loosely attached soil.

Rhizosphere soil samples (adhering to the roots and rhizomes after shaking) were sieved to remove impurities and only the portions that passed through 80 mesh were retained and stored at -80°C for analysis. The physicochemical properties of soil collected from the different cropping systems are presented in Table 2.

Detection of soil enzyme activity: Soil urease, invertase, polyphenol oxidase, phosphomonoesterase, and catalase activities were measured by sodium phenoxide, nitrosalicylic acid, gallic acid, 4-nitrophenyl phosphate disodium salt hexahydrate colorimetry, and permanganate titration, respectively, using previously reported methods (Guan, 1986).

Nucleic acid amplification and restriction digestion:

Total microbial DNA was extracted from soil samples using the high salt/SDS method (Zhou *et al.*, 1996). Bacterial 16S rRNA genes were amplified by PCR using the general bacterial primers 8-27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 926-907R (5'-CCG TCA ATT CMT TTR AGT TT-3'). PCR reactions and purification of PCR products followed the method of Wang *et al.* (2004).

Purified bacterial 16S rRNA fragments were digested with the restriction endonucleases *MspI* and *HaeIII* for 5 h at 37°C. The *MspI* restriction digest reaction contained 10 µl purified 16S rRNA fragments, 2 µl T buffer, 2 µl of 10 × BSA (Takara Bio, Otsu, Japan), and 1 µl *MspI* (5U) in a final volume of 20 µl. The *HaeIII* restriction digest reaction contained of 10 µl purified 16S rRNA fragments, 2 µl H buffer, and 1µl *HaeIII* (5U) in a final volume of 30 µl.

Terminal-restriction fragment (T-RF) isolation:

A 2 µl aliquot of each restriction digest reaction was mixed with 12 µl of formamide and 0.5 µl of standard (GeneScan-1000ROX, Applied Biosystems, Foster City, CA, U.S.A.). Samples were denatured at 96°C for 4 min, chilled on ice, and then run on an automated ABI DNA sequencer (model 3130, Applied Biosystems) to determine fragment sizes.

Data analysis: T-RFs were identified and quantified using GeneMarker Version 1.2 software. T-RFs that differed within ± 1 bp were considered to be identical. T-RFs ranged in size from 50 to 600 bp. The relative abundance of each T-RF was calculated as the individual T-RF peak area divided by the total area of all T-RF peaks. The fragments were classified using online T-RFLP analysis at the Ribosomal Database Project II website (Wang *et al.*, 2011).

Table 2. Soil physicochemical properties of *P. heterophylla*.

Cropping pattern	Organic matter (g/kg)	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	Available N (mg/100g)	Available K (mg/kg)	Available P (mg/kg)	pH
Fallow	38.31	5.01	0.47	19.77	19.78	21.07	185.89	5.7
One-year monoculture	38.88	2.02	0.47	20	18.2	30.09	174.52	5.18
Rice - <i>P. heterophylla</i> rotation	26.74	1.90	0.54	19.55	23.8	38.42	242.79	5.24
Bean - <i>P. heterophylla</i> rotation	33.38	1.68	0.76	23.85	15.4	54.94	211.18	5.59
Two-year consecutive monoculture	30.53	4.48	0.60	24.31	20.3	61.23	197.27	5.49

Table 3. Soil enzymes activity in the rhizosphere soil of *P. heterophylla*.

Cropping system	SUC	UR	ACP	PPO	CAT
Fallow	8.09 ± 0.57a	1.08 ± 0.09b	3.96 ± 0.22a	0.24 ± 0.02a	1.45 ± 0.13a
One-year monoculture	5.04 ± 0.73b	1.27 ± 0.13a	1.59 ± 0.23c	0.18 ± 0.01b	0.85 ± 0.09c
Two-year consecutive monoculture	2.30 ± 0.32e	0.28 ± 0.03e	2.05 ± 0.12b	0.11 ± 0.01d	1.02 ± 0.10b
Rice- <i>P. heterophylla</i> rotation	4.23 ± 0.21c	0.41 ± 0.05d	2.07 ± 0.09b	0.13 ± 0.02c	1.00 ± 0.07b
Bean- <i>P. heterophylla</i> rotation	3.12 ± 0.11d	0.54 ± 0.03c	1.96 ± 0.15b	0.11 ± 0.01d	1.00 ± 0.05b

SUC: invertase, UR: urease, ACP: Acid Phosphomonoesterase, PPO: polyphenol oxidase, CAT: catalase.

The units of SUC, UR, ACP, PPO and CAT are respectively mg glucose per g of soil, mg NH₃-N per g of soil, mg Phenol per g of soil, mg gallnut per g soil and mg Gallic vegetarian per g of soil. The lowercase letters behind data represented significant differences (p<0.05)

Statistical analysis of T-RFLP profiles was performed on the basis of complete sample profiles. The T-RFLP profile matrix analysis program from the RDP II website was used to determine the similarity among T-RFLP profiles (Maidak *et al.*, 1999). The proportion of shared terminal fragments from T-RFLP analysis was used to calculate similarity coefficients (Nunan *et al.*, 2005). Subsequently, three commonly used measures (Shannon's diversity index, Margalef index, and evenness index) were calculated using the software package Bio-Dap following standard procedures (Magurran, 2004). SSPS software was used to calculate the correlation between bacterial groups and soil enzyme activities and the productivity of *P. heterophylla*.

Results

Soil enzyme activity: Soil enzyme activity was closely related to soil properties and types and was used as an evaluation criterion for soil quality and productivity. Soil enzyme activities measured in different soil samples are shown in Table 3. The enzymatic activities of invertase, polyphenol oxidase, and urease were higher by 220%, 170%, and 450%, respectively, in OM soil than in TM soil. Phosphatase and catalase activities were higher in TM soil than in OM soil. These results demonstrated that continuous *P. heterophylla* monoculture caused significant changes in soil enzyme activity.

The enzymatic activities of invertase, polyphenol oxidase, and urease were higher by 180%, 120%, and 150%, respectively, in the RP rotation soil than in the TM soil and were higher by 140%, 105%, and 190%, respectively, in the BP rotation soil than in the TM soil. There was no significant difference in phosphatase or catalase activity between the soils from the RP rotation, the BP rotation, and the TM.

Bacterial community composition in different soil samples: According to the T-RFLP analysis, two groups of T-RFs were generated by digestion with *MspI* and *HaeIII* (Fig. 1). Digestion with *MspI* and *HaeIII* produced 113 and 105 bp T-RFs, respectively, from CK soil, 94 and 99 bp T-RFs from OM soil, 81 and 77 bp T-RFs from TM soil, 115 and 110 bp T-RFs from RP rotation soil, and 108 and 111 bp T-RFs from BP rotation soil.

Sequence comparison with the Ribosomal Database Project II identified 155, 182, 102, 196, and 245 types of bacteria in the CK, OM, TM, RP rotation, and BP

rotation soil samples, respectively. Most bacteria in the five soil sample types belonged to 15 phyla, 89 classes, and 445 species. Other bacteria belonged to unknown phyla. The fifteen phyla included proteobacteria, actinobacteria, firmicutes, bacteroidetes, spirochaetes, tenericutes, fusobacteria, chloroflexi, clostridium, nitrospira, planctomycetes, chlamydiae, cyanophyta, aquificae, and deferribacteres.

Diversity index analysis of bacterial communities:

Good microbial structure and diversity could improve the stability and harmony of the soil micro-ecological system and increase the capacity of the soil to buffer against ecological deterioration. The diversity indices of OM soils were significantly higher than those of TM soils (Table 3).

The diversity indices were significantly higher for soil samples collected from the crop rotation plots than from the TM plots. The diversity indices for the BP rotation were similar to those of OM and higher than for the RP rotation. The bacterial community diversity indices of the different soil samples indicated that crop rotation could enrich the soil microbial composition.

Correlation analysis of bacterial community diversity and growth of *P. heterophylla*:

Correlation analysis of the percentages of T-RFs produced by *Hae* digestion (which represent the total T-RFs digested by *Hae*) and growth indices of *P. heterophylla* showed that nine T-RFs had significantly positive correlations with *P. heterophylla* growth (Table 4) with higher amounts of T-RFs detected in OM, RP rotation, and BP rotation soils than in TM soil. Six T-RFs of 203, 231, 238, 292, 309, and 313 bp were not detected in TM soil. Nine T-RFs showed significantly negative correlations with *P. heterophylla* growth (Table 5) all of which were detected in TM soil. The 204, 212, 243, and 297 bp T-RFs were not detected in the RP rotation soil and the 243 and 295 bp T-RFs were not detected in the BP rotation soil.

T-RFs that correlated with *P. heterophylla* growth were identified using the Ribosomal Database Project II. Bacterial species that showed a positive correlation with *P. heterophylla* growth fell into five categories according to functions in the nitrogen cycle (*Nostoc*, *Bacillus*, and *Nitrospira*), in the carbon cycle (*Methylorhabdus*, *Caryophanon*, *Clostridium*, and *Paenibacillus*), in the sulfur cycle (*Desulfovibrio* and *Achromatium*), as probiotics (*Bacillus* and *Paenibacillus*), and as a pathogen (*Helicobacter*).

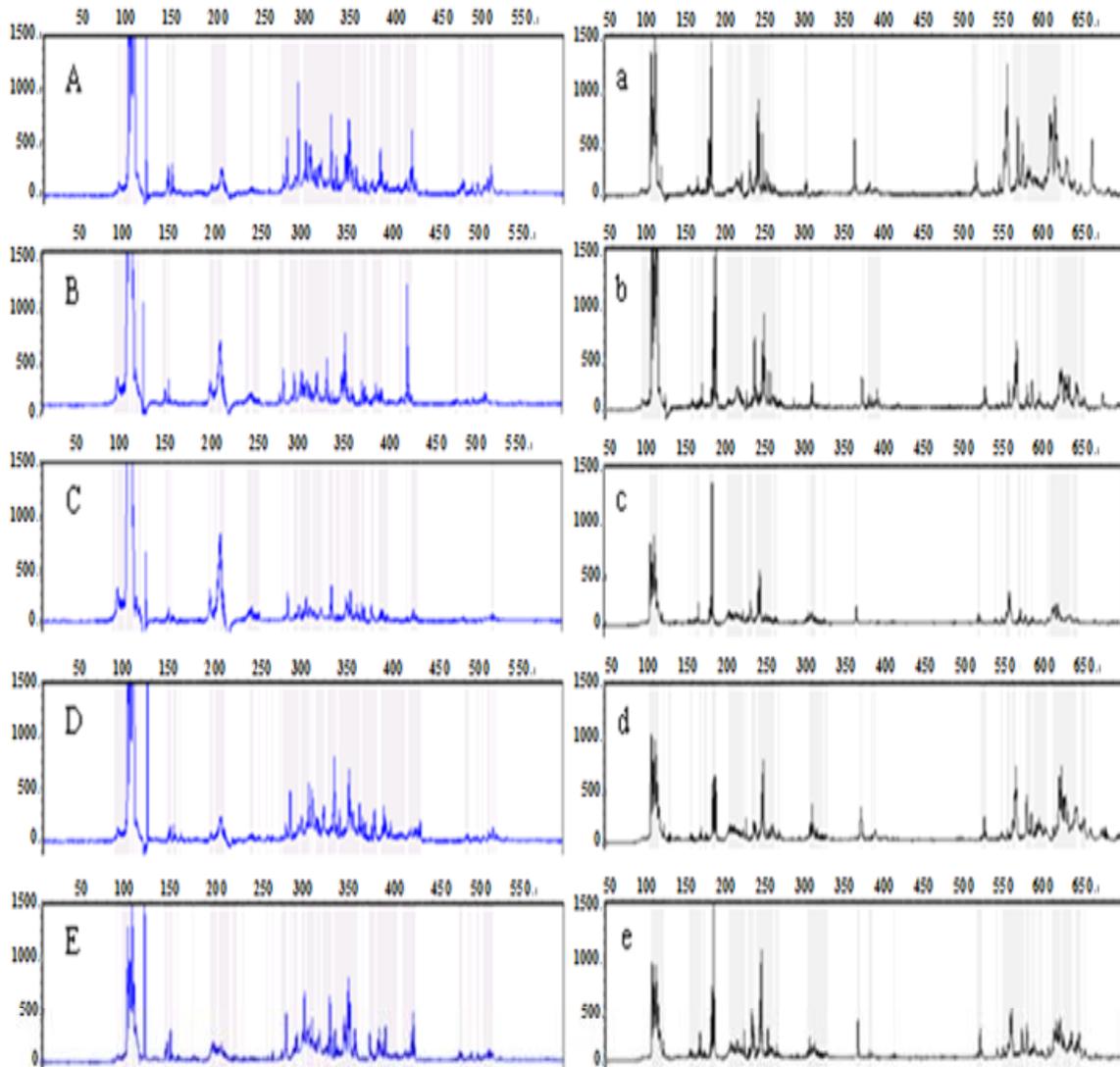


Fig. 1. T-RFLP profile of the bacterial community digestion by *Hae* III & *Msp* I in the rhizospheric soils of different cropping patterns. Note: the figure A, B, C, D and E were respectively T-RFLP profile of fallow, one-year monoculture, two-year consecutive monoculture, rice-*P. heterophylla* rotation and bean-*P. heterophylla* rotation digested by *Hae* III; the figure a, b, c, d and e were respectively T-RFLP profile of fallow, one-year monoculture, two-year consecutive monoculture, rice-*P. heterophylla* rotation and bean-*P. heterophylla* rotation digested by *Msp* I.

Bacterial species that showed a negative correlation with *P. heterophylla* growth fell into five categories according to functions in the nitrogen cycle (*Selenomonas ruminantium* and *Frankia*), in the sulfur cycle (*Sulfobacillus*), in carbohydrate synthesis (*Rhodopila globiformis*), as probiotics (*Desulfotobacterium dehalogenans* and *Sphingomonas*), and as pathogens (*Mycoplasma capricolum*, *Mycoplasma mycoides*, *Helcococcus kunzii*, *Flexibacter litoralis*, *Ureaplasma urealyticum*, and *Pirellula*).

The amounts of the bacterial species that were positively correlated with *P. heterophylla* growth were greater in the RP and BP rotation soils and lower in the TM soil. The amounts of the bacterial species that were negatively correlated with *P. heterophylla* growth were greater in the TM soil and lower in the RP and BP

rotation soils. The types and amounts of probiotics were higher in the crop rotation soils and contributed to nutrient cycling, improved soil texture, and inhibition of pathogen growth. These results indicated that crop rotation could reduce the amount of pathogens by increasing the types and amount of probiotics, improving the soil micro-ecological environment, and mediating the balance in soil micro-ecology. The increase in the types and amounts of probiotics in RP rotation soil was significantly greater than in BP rotation soil indicating that rotation with rice was more effective than rotation with bean. Beneficial bacteria in the two-crop rotation systems promoted soil enzyme activity in contrast to the pathogenic bacteria which inhibited soil enzyme activity (Table 6).

Table 6. The correlation analysis between microorganisms and soil enzymes.

Hae \square	Msp I	Function	SUC	UR	ACP	PPO	CAT
203	205	Sulfur cycle	0.95*	0.99**	0.95*	0.35	0.66
231	153	Probiotics	0.95*	0.98*	0.95*	1.00**	0.99**
238	235	Pathogen	0.56	0.55	0.65	-0.96*	-0.95*
292	150	Nitrogen cycle	-0.43	1.00**	0.67	0.98*	0.69
307	143	Probiotics	0.89	0.98*	0.96*	0.99**	0.98*
309	145	Probiotics	0.99**	0.83	0.86	0.75	0.95*
313	148	Improving soil texture	1.00**	0.65	0.95**	0.83	0.64
317	224	Sulfur cycle	0.58	0.21	0.95*	0.95*	0.98*
331	601	Nitrogen cycle	0.78	0.98*	0.96*	0.43	0.57
152	242	Nitrogen cycle	-0.43	0.95	0.55	0.21	0.42
204	146	Nitrogen cycle	-0.45	0.87	-0.76	0.21	0.75
212	152	Producing lactic acid	0.98*	-0.56	0.59	0.21	0.45
243	455	Carbohydrate synthesis	0.95*	0.47	-0.63	0.78	0.83
283	192	Pathogen	0.54	0.32	-0.43	-0.98*	-0.95*
295	152	Probiotics	0.55	0.95*	0.64	0.43	0.32
297	542	Pathogen	-0.43	-0.75	0.23	-0.86	-0.98*
318	542	Pathogen	-0.98*	-0.75	-0.95*	-0.85	-0.76
326	169	Pathogen	-0.47	0.19	0.56	-0.98*	-0.99**

Discussion

The rhizosphere is a complex medium for plant roots, soil, and soil biota, which change to form temporarily stable systems. Bacteria account for 70% of the microorganisms in the rhizosphere and constitute the microbial fraction that is most active in promoting energy flow, information transmission, and nutrient cycling (Wang *et al.*, 2011). Changes in bacterial populations and activities could serve as an excellent indicator of changes in soil health (Kennedy and Papendick, 1995; Pankhurst *et al.*, 1997; Koga *et al.*, 2003; Jonassona *et al.*, 2006; Giller *et al.*, 2009; Pankhurst *et al.*, 2009; Berendsen *et al.*, 2012; Lorenzo *et al.*, 2013). In this study, we used T-RFLP analysis to identify bacterial populations and to assess bacterial diversity in the *P. heterophylla* rhizosphere soil from different cropping systems. T-RFLP is a fingerprinting technology for analyzing the composition of microbial communities and is sensitive enough to detect microbial groups with small populations (Wang *et al.*, 2004). T-RFLP analysis identified 155 bacterial species belonging to 51 genera in CK soil, 182 species belonging to 55 genera in OM soil, and 102 species belonging to 40 genera in TM soil. Previous studies of plant-soil microbe interactions showed that the microbial biomass and microbial community structure are affected by diverse substances derived from different organisms (Semenov *et al.*, 1999; McKinley *et al.*, 2005; Wu *et al.*, 2011; Lorenzo *et al.*, 2013; Ponge, 2013). Plants play an active role in the formation of their rhizosphere microbiome as indicated by the fact that different plant species grown on the same soil host specific and distinct microbial communities (Berendsen

et al., 2012). Consecutive monoculture has a negative influence on soil microbial diversity which in turn affects plant production (Chen *et al.*, 2008; Zhang *et al.*, 2009, 2011). The diversity index analysis in our study indicated that the microbial community diversity and evenness degree of OM soil were higher than for TM soil.

Correlation analysis of the presence and distribution of T-RFs with *P. heterophylla* growth was performed to clarify the relationship between key microbial groups and the detrimental effects of continuous cropping of *P. heterophylla*. The analysis revealed a correlation between the presence of multiple beneficial bacterial species in OM soil that participate in soil nutrient cycling, degradation of allelopathic autotoxicity substances, and improvement of soil texture with *P. heterophylla* growth. In rhizosphere soil, plant-microbe interactions can be broadly categorized as neutral, beneficial, or detrimental. Most microorganisms use organic compounds derived from plants or other organisms as substrates for energy production and play key roles in nutrient cycling and in modifying plant environments (Pankhurst *et al.*, 1997; Bloem *et al.*, 1997). Beneficial microbes could promote plant growth and/or suppress plant diseases in rhizosphere soil via a variety of mechanisms including improved nutrient acquisition, production of growth regulators, and biosynthesis of pathogen-inhibiting compounds (Lugtenberg *et al.*, 2009; Doornbos *et al.*, 2012; Berendsen *et al.*, 2012). Beneficial bacteria were the dominant microbial groups promoting *P. heterophylla* growth in the OM, RP rotation, and BP rotation cropping systems. Beneficial microbes were in a state of equilibrium with pathogens which was beneficial to *P. heterophylla* growth.

Consecutive monoculture disrupts the balance

between the rhizosphere microflora and plant pathogens that is crucial for plant health (Chen *et al.*, 2008; Zhang Zhongyi *et al.*, 2009, 2011; Berendsen *et al.*, 2012). Raaijmakers *et al.* (2009) described the rhizosphere as both a playground and battlefield for soil borne pathogens and beneficial microorganisms. The numbers of beneficial bacteria decreased and pathogenic bacteria such as *Mycoplasma* and *Pirellula* increased in TM soil. These changes were negatively correlated with *P. heterophylla* growth. Consecutive monoculture destroyed the original rhizosphere ecosystem and affected *P. heterophylla* growth similar to the reduction in beneficial bacteria, increase in pathogenic bacteria, and environmental deterioration in rhizosphere soil observed with consecutive monoculture of *Rehmannia* (Chen *et al.*, 2007). However, bacterial community diversity indices, evenness indices, and the abundance of bacteria all increased in the rhizosphere soil in the two-crop rotation systems compared with TM. The number and abundance of beneficial bacteria types increased in RP rotation and BP rotation soils. Pathogenic bacteria types and abundance decreased in RP rotation soil but increased in BP rotation soil. Generally, microorganisms respond more rapidly than higher organisms to environmental changes due to their high surface to volume ratio (Kennedy and Papendick, 1995; Elliott *et al.*, 1996). Changes in microbial populations or activities could precede changes in the physical and chemical properties of the soil and provide an early indication of soil improvement or an early warning of soil degradation (Pankhurst *et al.*, 1997). In our study, the bacterial community structure and diversity in the rhizospheres of the RP and BP crop rotation systems were similar to those in the rhizosphere of OM indicating that rice or bean cultivation could restore the soil microbial ecosystem destroyed by the consecutive monoculture of *P. heterophylla*.

Soil enzymes also play an important role in maintaining soil ecology, physical and chemical properties, fertility, and soil health (Nannipieri *et al.*, 1996). Soil enzyme activities serve as sensitive indicators of soil ecological quality because of their multiple functions in microbial activities, soil processes, and ecosystem responses to human behavior and global environmental change (Burns *et al.*, 2013). Soil enzymes are derived mainly from plant roots, soil microorganisms, and soil fauna which play important roles in determining soil enzyme activities (Martens *et al.*, 1992). Different plant species can shape unique microbial communities due to differences in the amounts and qualities of root exudates (Nguyen, 2003; Viketoft *et al.*, 2005; Yang *et al.*, 2007). The cultivation of multiple agricultural plant species may enhance soil microorganism complexity by increasing the heterogeneity of organic substrates during decomposition of litter and living roots (Broughton and Gross, 2000; Stephan *et al.*, 2000). Consecutive monoculture of a crop releases unique organic substrates into the soil resulting in low microbial community diversity and reduced soil enzyme activities (Zhang *et al.*, 2011). Our study showed that the soil enzymatic activities of invertase, polyphenol oxidase, and urease declined with continuous cropping of *P. heterophylla*. Phosphatase and catalase activities decreased in OM soil and increased slightly in TM soil. Invertase, polyphenol oxidase, and urease activities

increased in the soils of the two-crop rotation systems compared to TM soil. Correlation analysis between soil enzymes and key bacterial groups showed that the amount of beneficial bacteria was positively correlated with soil enzyme activity and that pathogen abundance was negatively correlated with soil enzyme activity. These results indicate that crop rotation could improve the soil micro-ecological system and promote the absorption of nutrients and the growth of *P. heterophylla*.

Consecutive monoculture of *P. heterophylla* had a detrimental effect on the microbial community structure in the rhizosphere soil causing a decrease in beneficial bacteria, an increase in pathogenic bacteria, deterioration of the soil ecological system, inhibition of nutrient transformation, and a decline in soil fertility leading to poor *P. heterophylla* growth. The use of crop rotation allowed the soil microbial community to adjust and recover a beneficial composition leading to improved *P. heterophylla* growth. The comparison of the two-crop rotation systems showed that the effect of the RP rotation was more significant than that of the BP rotation, which may be related with different farming practices used for rice and bean.

The planting of rice in paddies could dilute allelochemicals from root exudates and *P. heterophylla* degradation residue and slow the accumulation of allelochemicals. In addition, differences in nutrient demand and release of secondary metabolites were substantial between rice and *P. heterophylla*, which could have a large impact on the microbial community structure and promote the restoration of a benign soil ecosystem. Bean and *P. heterophylla* are both rain-fed crops grown using the same farming practices. Although there were certain differences in nutrient demand and release of secondary metabolites between bean and *P. heterophylla*, root exudates and *P. heterophylla* degradation residue could accumulate in the soil which could affect the microbial community structure to a lesser extent, degrade the soil micro-ecological environment, and affect *P. heterophylla* growth. The RP crop rotation was more effective than the BP rotation for promoting *P. heterophylla* growth.

The effects of consecutive monoculture on the yield and quality of *P. heterophylla* cannot be absolutely overcome by long-term crop rotation. It is important to use diverse methods to restore benign soil health for optimum *P. heterophylla* productivity. Building on this study, our future research will aim to develop rational rotation systems for *P. heterophylla* by selecting distantly related crops for rotation and isolating probiotics and bacteria antagonistic to detrimental microbes. In addition, specific microbial fertilizer and organic matter such as soybean meal, fish meal, and rapeseed meal could be used in the cultivation of *P. heterophylla* or other medicinal plants to restore soil microbial diversity, reduce the accumulation of autotoxic substances, improve the functional diversity of the microbial community, and control soil diseases.

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