MICROBIAL ACTIVITY AND COMMUNITY DIVERSITY IN TOBACCO RHIZOSPHERIC SOIL AFFECTED BY DIFFERENT PRE-CROPS

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Abstract. In this study, we used both culture-dependent physiological profiling and culture-independent DNA-based approaches to characterize the bacterial communities of tobacco rhizospheric soils affected by different pre-crops (soybean, maize and tobacco). Using Biology-Eco plates, we found that the bacterial metabolic activity in soybean-tobacco and maize-tobacco rotation soils were higher than in tobacco monocropping soil. Across all soil samples, bacterial communities were dominated by *Proteobacteria*, *Acidobacteria* and *Actinobacteria* at the phylum level. However, the diversity and composition of the bacterial communities varied significantly between tobacco rotations and monocropping soil. The estimated bacterial diversity (Shannon diversity index) was higher in the maize-tobacco and soybean-tobacco soils than in tobacco monocropping soil. The populations of *Proteobacteria*, *Acidobacteria*, *Actinobacteria* and *Bacteroidetes* were found in variable proportions, depending on the different pre-crops. The highest percentages of *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* were found in soybean-tobacco soil, whereas *Acidobacteria* occurred at higher percentages in tobacco monocropping soil. Collectively, crop rotation influenced soil biodiversity by change of composition and abundance of individual species, and soils under cereal-tobacco rations had higher bacterial activity and diversity than soils under tobacco monocropping.
Keywords: *soybean, maize, monocropping, rotation, bacterial community structure, bacterial community function*

Introduction

Microorganisms are ubiquitous in the environment and play an essential role in the biogeochemical cycles that sustain all life on Earth (Zarraonaindia et al., 2013; Su et al., 2012). Soil microbial communities are responsible for soil structure maintenance,

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organic matter decomposition, nitrogen fixation, the breakdown of toxic compounds, and inorganic compound transformation (Dick, 1992; Nannipieri et al., 2003). Previous studies have shown that the community structure, abundance and activity of soil microorganisms can be affected by various physical disturbances in the soil (Dick, 1992; Lienhard et al., 2013). Thus, land-use management practices, including crop rotation and tillage (Quadros et al., 2012), fertilizer regime (Qiu et al., 2012), irrigation (Qiu et al., 2012), and continuous cropping (Li et al., 2010) are logical case studies for soil microbial diversity studies.

Tobacco (*Nicotiana tabacum* L.) is among the most important economic crops in the world, and one million acres of tobacco are planted each year in China (Hecht et al., 1984). Tobacco quality is determined by genetic factors, environmental factors and cultivation measures. Cereal-tobacco rotation is the dominant practice in the northeast of China. Soybean (*Glycine max* L.) and maize (*Zea mays* L.) are the major cereal crops grown most often in rotation with tobacco. However, research on the relative effects of the preceding crops on the soil quality of subsequent tobacco in cereal-tobacco rotation systems is rare. It is well known that rotation improves soil quality, including soil biota structure, organic matter content and moisture retention capacity (Ponge et al., 2013). Soils under crop rotation with a high input and diversity of organic materials contain high microbial biomass content and enzyme activity compared with monoculture soils (Trasar-Cepeda et al., 2008). Many studies have found that crop sequence plays a major role in soil C retention (Morari et al., 2006; Varvel, 2008; Wright and Hons, 2005). In particular, the effects of preceding crops on subsequent crops can relate to residual nutrients and water as well as to disease control (Kirkegaard et al., 2008). The decomposition rate of plant debris was found to be mainly governed by the C/N ratio. There is growing evidence that the variety of pre-crops can become factors in the microbial population and enzymes in the soils for subsequent crops.

In this study, two different techniques (Biolog-Eco and 454 pyrosequencing) for estimating the soil bacterial community were adopted. To build a sustainable crop-tobacco rotation sequence, the objective was to investigate the bacterial community structure and function of subsequent tobacco in soils in response to three preceding crops (soybean, maize and tobacco) and to observe differences among bacterial communities.

Materials and Methods

Field plots and sample collection

Soil sampling was performed in September 2011, at Tobacco Research Institute of Mudanjiang, Ningan city of Heilongjiang province (latitude, 44°85′N; longitude, 129°60′E, Northeast China). The average annual temperature in the region is 4°C. The mean annual precipitation is 427.50 mm, and the evaporation is 1635 mm per year. The soil is classified as a river silt soil. Selected soil properties were as follows: pH 6.85; organic matter 27.70 $g \cdot kg^{-1}$; total nitrogen 1.90 $g \cdot kg^{-1}$; total phosphorus 1.62 $g \cdot kg^{-1}$; total potassium 1.61 $g \cdot kg^{-1}$; available nitrogen 86.50 $mg \cdot kg^{-1}$; available phosphorus 36.40 $mg \cdot kg^{-1}$; available potassium 300.00 $mg \cdot kg^{-1}$.

The experimental design consisted of three blocks. Each block was divided into three plots representing the three plantation systems. Each plot comprised 8 rows that were 6.00 m long and 8.80 m wide; thus, each was 52.80 m^2 in size. Treatments levels included (1) tobacco monocropping, (2) soybean-tobacco rotation and (3) maize-tobacco rotation. The cultivars of tobacco, soybean and maize were Longjiang 911, Heinong 34 and Kenfeng 1, respectively. Soil samples were collected from all

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plots, three individual rhizosphere soils randomly collected for each treatment in a plot. Tobacco rhizosphere soils, which adhered to the roots (Nazih et al., 2001), were collected by shaking the soil off the roots. After removal of the vegetation, roots, and stone (>2 mm), samples were placed into sterile centrifuge tubes under ice and transported to the laboratory within 24 hours. Finally, samples were stored at 4°C prior to microbial functional (Biolog™ ECO technic) and structural (454 pyrosequencing) analyses.

Substrate utilization patterns and data analysis

Substrate utilization patterns were measured using Biolog™ ECO plates (BIOLOG, Inc.). A 10^{-1} microbial suspension was prepared by suspending 10 g of fresh soil in 100 mL 0.85% NaCl solution. The slurry was processed with a Vortex mixer for 1 min at maximum speed and centrifuged for 10 min at 500×g (Rutgers et al. 2006). Tenfold serial dilutions were performed, and 150 µL of the 10^{-3} dilutions were pipetted into microplates using an 8-channel micropipette. Microplates were incubated at 28°C for 216 h. The color development at OD_{578} nm was read for each well at 24-h intervals. Negative values were set to zero. The average well color development (AWCD) value of the Biolog data was calculated for each sample at each time point by dividing the sum of the optical density data by 31 (number of substrates), as described by Garland (1996).

The 96-h data were used to measure the functional and species diversity of the soil microbial community, and the following parameters were calculated using the equations below:

Shannon-Wiener index $$H = -\sum Pi \times \ln(Pi) \quad \text{(Eq.1)}$$

Simpson index $$D = 1 - \sum (Pi)^2 \quad \text{(Eq.2)}$$

McIntosh index $$U = \sqrt{\left(\sum Ni^2\right)} \quad \text{(Eq.3)}$$

where *Ni* is the relative OD in each carbon source well, and *Pi* is calculated by subtracting the control from the absorbance of each substrate and then by dividing this value by the total color change recorded for all 31 substrates.

DNA extraction, amplification of 16S rRNA genes, and pyrosequencing

Total microbial community DNA was isolated from 0.25 g of soil per sample using soil DNA extraction kit (Omega Bio-Tek, Atlanta, USA). The extracted DNA was examined following electrophoresis in 1% agarose gel, and the DNA was normalized to the same concentration prior to amplification. A ~455 bp region of the 16S rRNA gene covering the V1-V3 region was selected to construct the community library through tag pyrosequencing. The V1-V3 region was amplified using the universal primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 533R (5'-TTACCGCGGCTGCTGGCAC-3') containing the A and B sequencing adaptors (454 Life Sciences). The PCR mixture (final volume, 50 µL) contained 5 µM of each primer, ~5 ng of template DNA, 5×FastPfu PCR buffer, and 2.5 U of FastPfu DNA Polymerase (MBI, Fermentas, USA). The amplification conditions consisted of an

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initial denaturation at 95°C for 2 min and 25 cycles of denaturation at 95°C at 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, followed by a final extension period at 72°C for 5 min. During amplification, negative control reactions lacking template DNA were also performed to check for experimental contamination. The amplicons were then purified once by gel electrophoresis/isolation and twice more using the Wizard SV Geland PCR Clean-Up System (Promega, Madison, Wisconsin, USA). Next, 454 pyrosequencing was conducted on a Roche massively parallel 454 GS-FlX sequencer according to standard protocols.

454 Pyrosequencing and data analysis

Pyrosequencing flowgrams were converted to sequence reads using the MOTHUR software (http://www.mothur.org) and analyzed. The acquired sequences were filtered by evaluating data quality and removing primers and barcodes. Sequences were filtered by the following methods: (1) selecting sequences that contained the barcode and forward primer and eliminating sequences with even a single base pair; (2) removing sequences shorter than 150 bp, with ambiguous base pairs, or with more than two wrong matches in the primer; and (3) eliminating barcodes and forward primers. After filtering, the effective sequences were clustered into operational taxonomic units (OTUs) based on phylum, class, order, family, genus and species levels using MOTHUR.

Data analysis

To detect OTUs, optimized sequences were reduced to 300 bp in length and compared using silva108. Rarefaction curves based on an identified OTU, the species richness estimator Chao 1, were generated for each sample using MOTHUR. The taxonomic assignment of sequences was performed using the Ribosomal Database Project (RDP) classifier (minimum confidence of 80%). After phylogenetic allocation of the sequences to the phylum and genus levels, the relative abundance of a given phylogenetic group was set to the number of sequences per sample. Hierarchical cluster (Heatmap) analyses were generated in MOTHUR using the gplots package of R. We used SPSS for windows (version 19) to test for significance ($P<0.05$) between treatments of relative abundances, Alpha diversity and richness of bacterial communities were analyzed using Duncan post-hoc test at 95% confidence level. The Duncan test method was conducted for multiple comparisons to assess the significance level of substrate utilization among treatments.

Results

Community level physiological profile

As expected, the average well color development (AWCD) increased with incubation period (*Fig. 1*). The soybean-tobacco rotation consistently exhibited the highest AWCD at all periods, followed by maize-tobacco rotation. There was no significant difference in soil bacterial functional diversity between the soybean-tobacco and maize-tobacco treatments. The tobacco monocropping treatment constantly had the lowest AWCD throughout all period. The soil bacterial functional diversity index (*H*, *D* and *S*) was significantly affected by the pre-crop treatment (*Table 1*). Among rotation treatments, there was no significant difference between the soybean-tobacco and maize-tobacco treatments.

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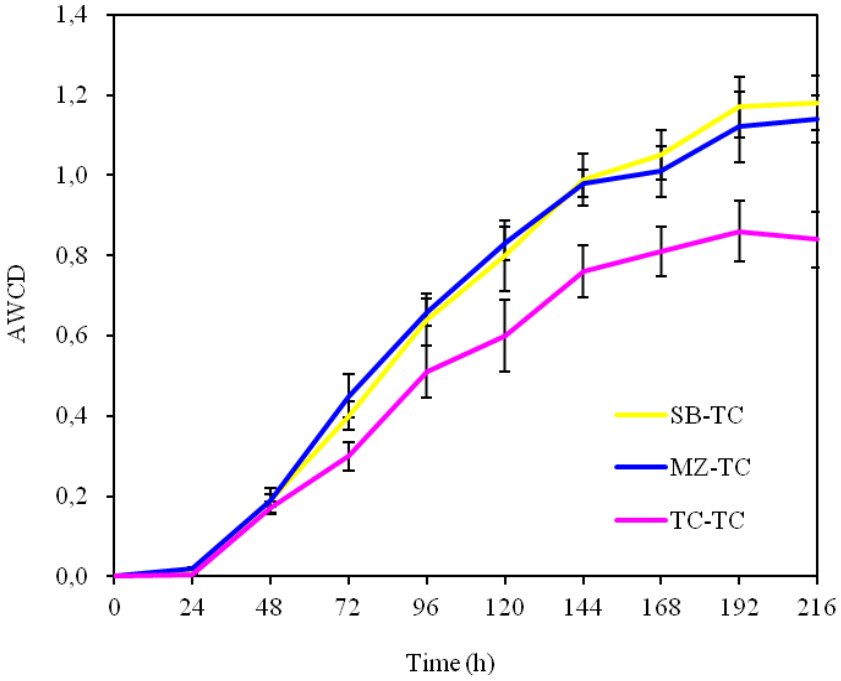

Figure 1. *Average well color development (AWCD) obtained by Biolog-Eco Plate™ incubation of all treatments. SB-TC, soybean-tobacco; MZ-TC, maize-tobacco; and TC-TC, tobacco monocropping*

Table 1. *Bacterial diversity indices of soils based on community level physiological profile. Treatment: SB-TC, soybean-tobacco; MZ-TC, maize-tobacco; and TC-TC, tobacco monocropping. Different letters indicate significant difference at $P<0.05$*

Treatment	Shannon-Wiener index(*H*)	Simpson-diversity index(*D*)	Substrate richness (*S*)	McIntosh index(*U*)
SB-TC	1.31±0.01a	0.94±0.00 a	23.33±0.58 a	4.71±0.57 a
MZ-TC	1.32±0.05 a	0.94±0.01 a	24.33±1.53 a	4.64±1.18 a
TC-TC	1.24±0.03 b	0.92±0.01 b	21.33±0.58 b	4.97±0.62 a

Principal component analysis of bacterial community

Microbial consumption of polymers, amino acids, amines/amides and miscellaneous compounds were significantly higher in soybean-tobacco rotation soils compared to that found in tobacco monocropping soil (*Fig. 2*). On the contrary, the consumption of carbohydrates was lower in soybean-tobacco soils than in tobacco monocropping soil. However, similarly to soybean-tobacco rotation treatment, soil microbial communities of maize-tobacco rotation treatment showed large polymers utilization.

The principal component analysis (PCA) of bacterial substrate utilization patterns (*Fig. 3*) explained 79.42% of the total variance, with the first principle component having the greatest power of separation (70.21%). The PCA score plot revealed that the soybean-tobacco and maize-tobacco rotation treatments were clustered together and separated from the tobacco monocropping treatment.

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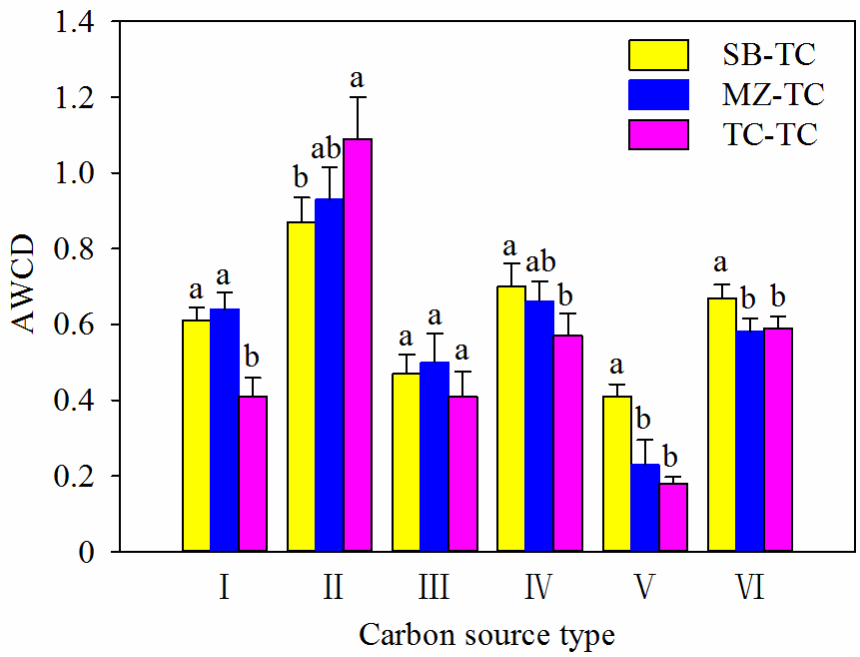

Figure 2. *Relative use efficiency of soil microbial community on different carbon sources. I :Polymers; II: Carbohydrate; III: Carboxylic acids; IV: Amino acids; V: Amines/amides; VI: Miscellaneous. SB-TC, soybean-tobacco; MZ-TC, maize-tobacco; and TC-TC, tobacco monocropping. Different letters indicate significant difference at P<0.05*

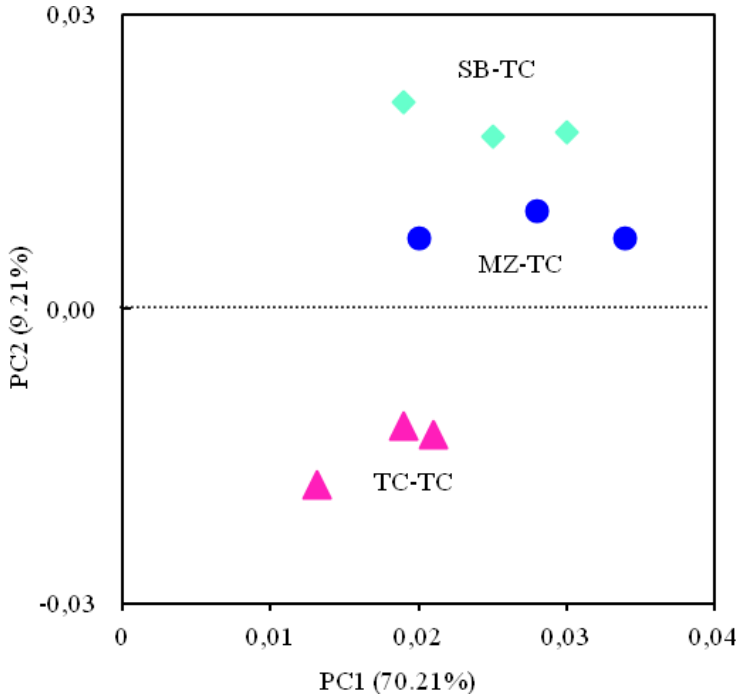

Figure 3. *Principal component analysis (PCA) of the use on different carbon sources for soil microbial community. SB-TC, soybean-tobacco; MZ-TC, maize-tobacco; and TC-TC, tobacco monocropping*

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Bacterial community analysis using pyrosequencing

In this study, we assessed and compared the composition of soil bacterial communities derived from the soybean-tobacco, maize-tobacco and tobacco monocropping libraries through the pyrosequencing-based analysis of 16S rRNA gene sequences. Pyrosequencing analysis of the V1-V3 region of the 16S rRNA genes resulted in 57,084 high-quality sequences reads with a read length of ≥300 bp across all samples. The average read length was 434 bp. The read numbers were uneven per sample, ranging from 18,343 to 19,911, with an average of 19,028 (*Table 2*). All samples were randomly reduced to the same size using MOTHUR based on the sample with the smallest number of reads.

Table 2. *Number of 16S rRNA gene sequences derived from three libraries. Treatment: SB-TC, soybean-tobacco; MZ-TC, maize-tobacco; and TC-TC, tobacco monocropping*

Treatment	No. obtained sequences ≥400 bp	Bases (bp)	Average length (bp)
SB-TC	18,343	5,934,166	426.2
MZ-TC	19,911	6,243,854	436.3
TC-TC	18,830	6,091,675	439.7

Bacterial alpha-diversity

To determine rarefaction curves and other measures of diversity, OTUs (operational taxonomic units) were identified at 3% genetic distance. The rarefaction curves indicated consistent differences among the three libraries (*Fig. 4*). At 3% genetic distance, the rarefaction curves suggested that the sequencing effort was not large enough to capture the complete diversity of these communities, as the curves did not reach the asymptote with increasing sample size. The same conclusion was observed in terms of coverage (*Table 3*). The coverage from the three libraries was below 95%, indicating that the sequencing reads were not sufficient for this analysis.

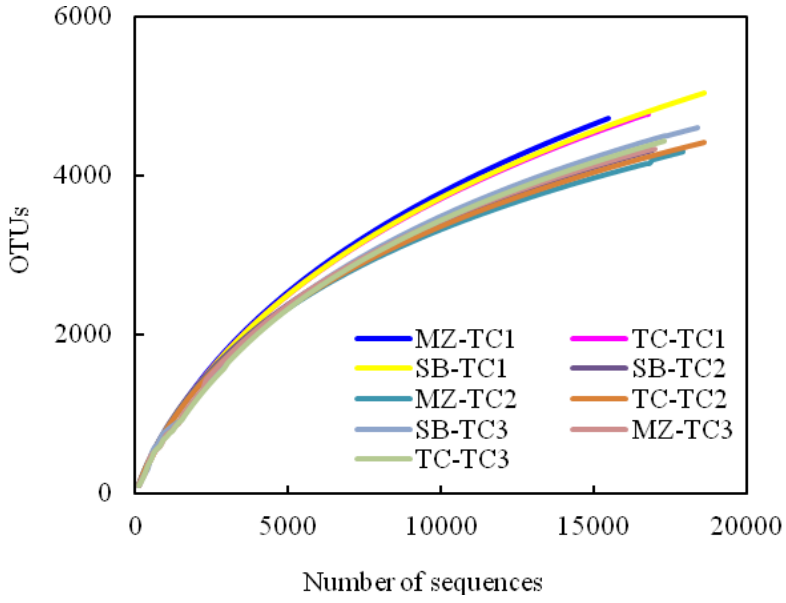

Figure 4. *Rarefaction curves indicating the observed number of OTUs at genetic distances of 3% in all libraries. SB-TC, soybean-tobacco; MZ-TC, maize-tobacco; and TC-TC, tobacco monocropping*

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Table 3. *Sequencing information and diversity estimated for three samples obtained by 454 pyrosequencing. Treatment: SB-TC, soybean-tobacco; MZ-TC, maize-tobacco; and TC-TC, tobacco monocropping*

Treatment	3% Genetic distance			
	Ace	Chao1	Shannon	Coverage (%)
SB-TC	8,806	7,221	7.78	86.6
MZ-TC	9,812	7,892	7.91	86.3
TC-TC	7,589	7,563	7.86	86.9

Comparing the mean Shannon diversity index of the three libraries revealed that the highest bacterial diversity at the analyzed genetic distances was found in the maize-tobacco soil, followed by the tobacco monocropping and soybean-tobacco soils. The Chao1 index varied among the three libraries, although not significantly. In addition, the Ace index increased significantly in tobacco rotations soils compared to the tobacco monocropping soil.

Distribution of bacterial composition at the phylum level

The 57,084 classifiable sequences were affiliated with 15 bacterial phyla (*Fig. 5*).The dominant phyla across all samples were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes*, and *Bacteroidetes*, representing 28.10, 18.90, 17.50, 11.00, 7.59, and 5.47%, respectively, of all sequences that were classified below the domain level. These dominant bacterial phyla were found in all samples (*Fig. 6*). Other sequences belonged to *Nitrospirae*, *Armatimonaddetes*, *Firmicutes*, *Fibrobacteres*, *Chlorobi*, *Verrucomicrobia* and other bacteria, and they were always found in very low proportions (<5%).

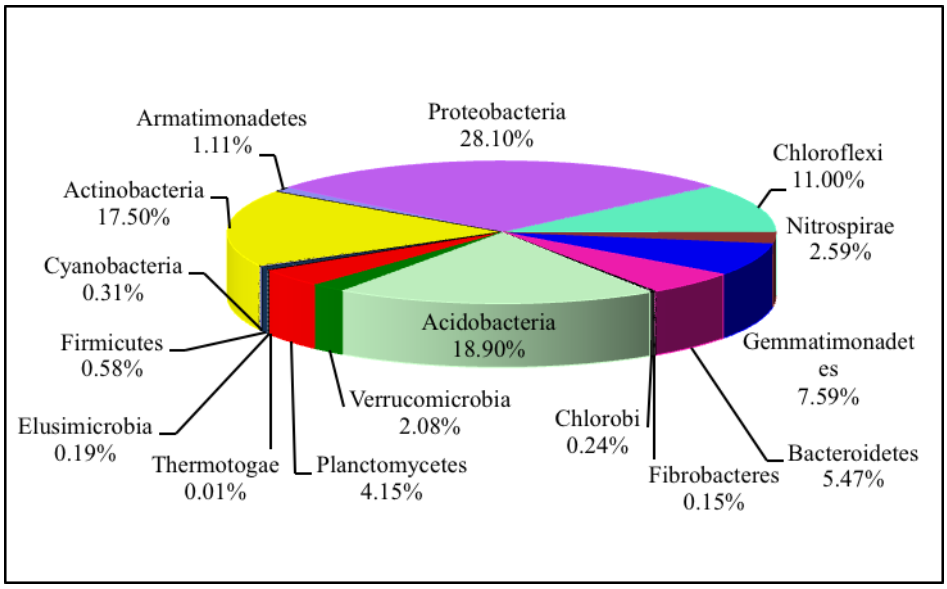

Figure 5. *Proportional distribution of different phyla.*

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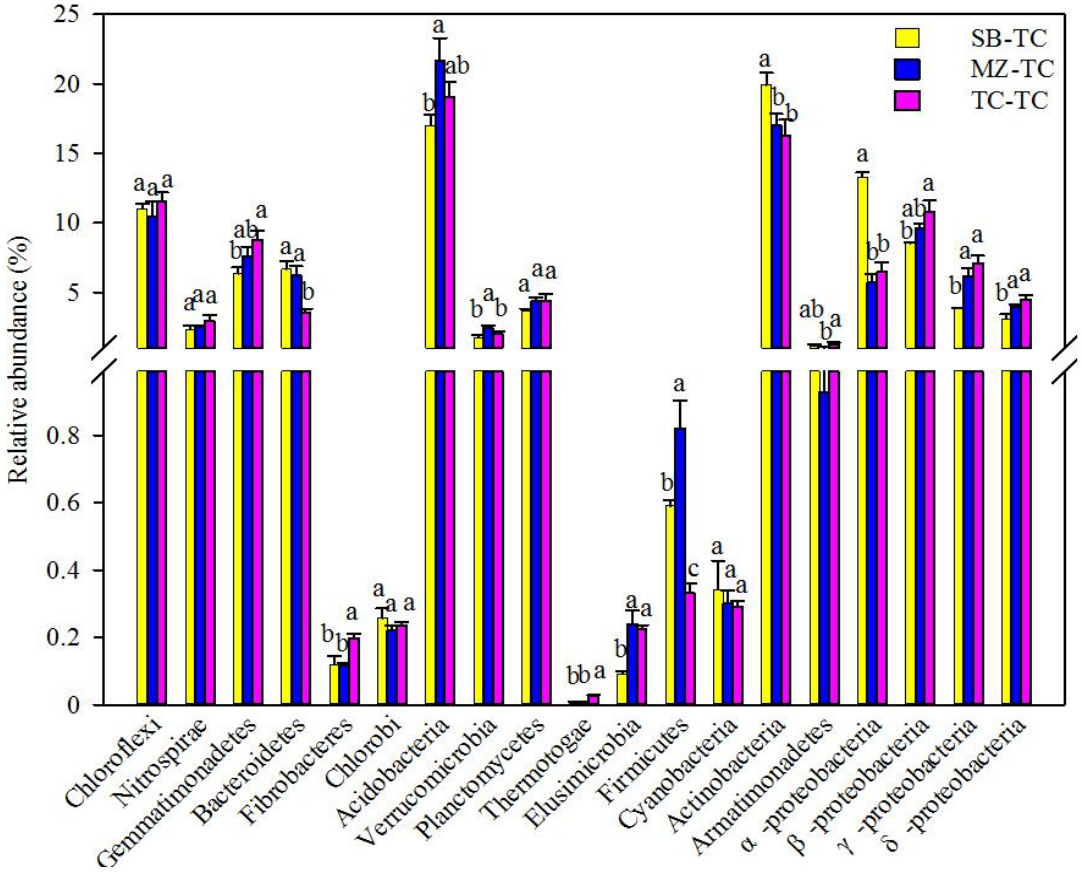

Figure 6. *Relative abundance of bacterial phyla and classes of Proteobacteria for each soil library. SB-TC, soybean-tobacco; MZ-TC, maize-tobacco; and TC-TC, tobacco monocropping*

The bacterial composition at the phylum level differed between the rotations (soybean-tobacco and maize-tobacco) and the monocropping system. The major phyla were shared by all samples, but in different relative abundances (*Fig. 6*). In the maize-tobacco and tobacco monocropping soils, *Proteobacteria* and *Acidobacteria* were the most abundant phyla, representing 26.58%, 28.02% and 20.63%, 19.05%, respectively. However, the soybean-tobacco rotation systems were dominated by the phyla *Proteobacteria* and *Actinobacteria*, representing 29.20% and 19.91%, respectively.

Among the *Proteobacteria*, *α*-, *β*-, *γ*-and *δ-proteobacteria* were found in all soil libraries (*Fig. 6*). In the maize-tobacco and tobacco monocropping systems, *β-proteobacteria* showed the highest relative abundance (9.74% and 10.48%), followed by *γ-proteobacteria* (6.96% and 6.89%), *α-proteobacteria* (5.82% and 6.26%) and *δ-proteobacteria* (4.06% and 4.39%). The insignificant differences between the maize-tobacco and tobacco monocropping soils indicated that maize as a pre-crop did not significantly impact *Proteobacteria* in subsequent tobacco soil ($P>0.05$). Compared with the tobacco monocropping system, *Proteobacteria* showed significant differences in the soybean-tobacco rotation soil ($P<0.05$). The relative abundances of *Proteobacterial* sequences were 13.57% for *α-proteobacteria*, 8.67% for *β-proteobacteria*, 3.92% for *γ-proteobacteria* and 3.04% for *δ-proteobacteria*.

Considering the bacterial distribution in tobacco rotations and monocropping systems,

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variations in relative abundances were also observed for the phyla *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes* and *Bacteroidetes* (*Fig. 6*). The *Acidobacteria* showed relative abundances of 19.05% in tobacco monocropping soil, 16.98% in the soybean-tobacco rotation soil, and 20.63% in the maize-tobacco rotation soil. A statistical comparison across soil libraries revealed that the relative abundances of *Bacteroidetes* in soybean-tobacco and maize-tobacco rotation soils, amounting to 6.62% and 6.23%, respectively, were significantly higher than in tobacco monocropping soil (3.56%)(P<0.05). In contrast, the relative abundance of *Gemmatimonadetes* revealed a significant decrease in the soybean-tobacco (6.33%) rotation soils compared to the tobacco monocropping soil (8.80%)(P<0.05). Among the *Actinobacteria*, we observed no significant differences between the soybean-tobacco (19.91%) and maize-tobacco (17.02%) soils (P>0.05).

Distribution of bacterial composition at the genus level

Hierarchically clustered heatmap analysis based on 100 predominant bacterial communities at the genus level was used to identify the different compositions of these soil libraries (*Fig. 7*). Variations at the genus level were observed between soil from tobacco rotations and tobacco monocropping soils. Overall, among the *Proteobacteria*, the genera *Rhodoplanes* (0.41%), *Phenylobacterium* (0.53%) and *Sphingobium* (0.86%) revealed a significant increase in soybean-tobacco rotation soil compared to the corresponding genera in the tobacco monocropping soil (0.13%, 0.23% and 0.18%, respectively). In contrast, the relative abundances of *Nitrosomonadaceae* (1.90% and 2.83%), *Sorangium* (0.22% and 0.30%), *Lysobacter* (0.4% and 0.33%) and *Arenimonas* (0.12% and 0.25%) were lower in soybean-tobacco and maize-tobacco soils than in tobacco monocropping soil (3.29%, 0.36%, 0.60% and 0.32%, respectively). The phylum *Actinobacteria*, which contains the genera *Solirubrobacter*, *Aeromicrobium* and *Microbacterium*, showed significant abundance differences between tobacco rotations and tobacco monocropping soils. *Aeromicrobium* showed a higher relative abundance in the soybean-tobacco soil, and *Solirubrobacter* was present at lower relative abundances in the soybean-tobacco and maize-tobacco soils compared with the tobacco monocropping soil. The comparison of the relative abundances of *Solirubrobacter* revealed no significant difference between the soybean-tobacco and maize-tobacco soils. Within the *Bacteroidetes* and *Acidobacteria*, representatives of the genera *Adhaeribacter* and *Candidatus_Chloracidobacterium* were variational in the three libraries. Their relative abundances were significantly higher in the soybean-tobacco and maize-tobacco soils than in tobacco monocropping soil. Other genera, *Bacillaceae_Bacillus* (affiliated with *Firmicutes*), *Zavarzinella* (affiliated with *Planctomycetes*) and *Anaerolineaceae* (affiliated with *Chloroflexi*), showed higher relative abundances in the maize-tobacco soil compared to tobacco monocropping soil.

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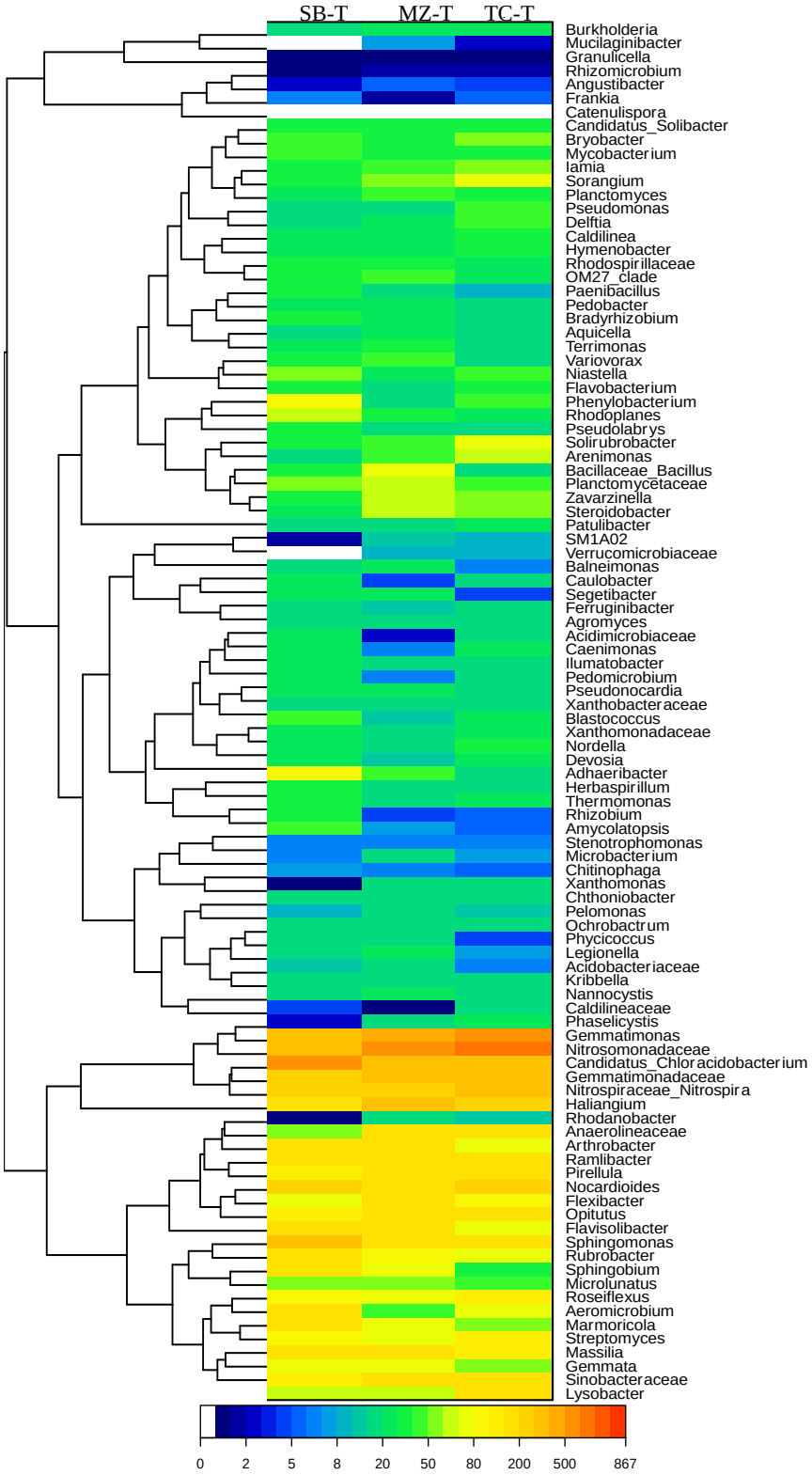

Figure 7. *Hierarchical cluster analysis of 100 predominant bacterial communities among the three samples. The Y-axis is the clustering of the most abundant OTUs (5% distance) in reads. The OTUs were ordered by genus. Sample communities were clustered based on complete linkage method. The color intensity of scale indicates relative abundance of each OTU read. Relative abundance was defined as the number of sequences affiliated with that OTU divided by the total number of sequences per sample*

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Discussion

The residue of the preceding crops has been found to strongly influence soil characteristics, including physic-chemical properties and enzyme activities, which are important for subsequent crops (Urbatzka et al., 2009; Preissel et al., 2015). Numerous bacteria have been known to flourish in the rhizosphere, which is mostly due to the supply of nutrients and the platform supplied by plant residues (Mendes et al., 2013; Bakker et al., 2012; Sugiyama and Yazaki, 2012). In this study, the rhizosphere bacterial communities for the tobacco crop (with pre-crops of soybean, maize and tobacco) were studied using both culture-dependent physiological profiling (Biolog-Eco) and culture-independent 16S rRNA metagenomics (454 pyrosequencing). Using the Biolog-Eco plates, we found that the bacterial activity and diversity in soil from tobacco rotations (soybean and maize) were higher than in the tobacco monocropping soil (*Fig. 1* and *Table 1*). This result was consistent with the findings of Yue (2013) that continuous monocropping in tobacco cultivation led to a decrease in soil bacterial abundance. Meanwhile, some studies have found that rotation systems stimulate soil microbial biomass, enzyme activities and functional diversity and abundance in the soil community structure (Orr et al., 2012; Insam et al., 2015; Plaza et al., 2004). Thus, we speculated that pre-crops of soybean and maize affected the rhizospheric bacterial communities of subsequent tobacco. Moreover, the results of principal component analysis suggested that the soybean-tobacco and maize-tobacco rotation treatments had similar rhizospheric bacterial communities, whereas the continuous cropping of tobacco showed different bacterial communities. The preceding crop effect of grain legumes providing nitrogen has been demonstrated (Hauggaard-Nielsen et al., 2009). As a consequence, this effect varies the bacterial communities for subsequent crops in the soil. In addition to legumes, many broad-leaved crops or summer cereals produce similar rotational benefits (Preissel et al., 2015). The bacterial activity and diversity data showed no significant differences between the soybean-tobacco and maize-tobacco rotation systems, although maize and soybean leave different residues in the field after the crops have been harvested. These results suggested that the plant species planted in the current year was more important in determining the bacterial community in soils than the pre-crop species.

The 454 pyrosequencing analyses revealed 57,084 sequences obtained from the three soil libraries in this study, and *Proteobacteria*, *Acidobacteria* and *Actinobacteria* were the most abundant phyla, which generally agreed with several previous studies reporting that these phyla are capable of having various effects on plant health, including both beneficial and pathogenic interactions (Lee et al., 2008; Berendsen et al., 2012; Li et al., 2016). The rarefaction curves for these samples did not reach the asymptote. Therefore, the deeper sequencing may be required to avoid underestimation of microbial diversity in our samples. But the coverage values for all libraries were not too low (i.e. over *C*. 85%), and provide a basis for comparison with other studies. Indeed, our coverage values were largely over reported in oil-contaminated sediment (C=61%-83%). Our rarefaction curves based on 454 pyrosequencing did not saturate but we consider, on the basis of the above comparisons, that the majority of the bacterial diversity in our samples was correctly sampled.

Significant differences in bacterial community composition systems were observed between tobacco rotations and tobacco monocropping. The largest shifts in relative abundance were found for *Proteobacteria* and *Acidobacteria*. The increase in the relative abundance of *α-proteobacteria* in the soybean-tobacco rotation soil was driven by the increases in the genera *Rhodoplanes*, *Sphingobium* and *Phenylobacterium* (*Fig. 7*), suggesting that these genera are enriched by soybean as a pre-crop. The pH was the highest in the soybean-tobacco (7.02) soil compared to the tobacco monocropping (6.60)

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and maize-tobacco (6.87) soils. Previous research has demonstrated that *α-proteobacteria* and *γ-proteobacteria* showed positive relationships to soil pH (Rousk et al., 2010). The phylum *Chloroflexi* is ubiquitous in various soil samples, and its relative abundance was frequently higher than the abundance of *Bacteroidetes* (Roesch et al., 2007). This finding was further supported by the changes in our study. The relative abundance of *Acidobacteria* increased significantly in the maize-tobacco rotation soil compared to the soybean-tobacco soil. The abundance of the phylum *Acidobacteria* correlates with the soil pH (Hartman et al., 2008; Jones et al., 2009), and acidobacterial subgroups 1 and 2 decreased with increasing pH (Lauber et al., 2009), which is in consistent to our study. The biological functions of *Acidobacteria* are not well known because most microorganisms of this phylum have not been cultured (Yamada and Sekiguchi, 2009). However, as recent study found that *Acidobacteria* are capable of degrading plant litter in soils (Eichorst et al., 2011), the presence of maize residues in the maize-tobacco rotation soil may have contributed to the observed differences. Symbiotically fixed N may contribute to the nutrient availability, soil structure, and microbial activities of subsequent crops in rotation via the N mineralization of plant residues (Urbatzka et al., 2009). The relative abundance of *Actinobacteria* was strongly shaped by the pre-crop, and a significant increase was observed in the soybean-tobacco rotation soil compared to the tobacco monocropping soil. *Actinobacteria* members play an important role in the degradation of recalcitrant compounds and prevent infection with pathogenic microorganisms by secreting various antibiotics (Nour et al., 2003). In addition, previous studies have demonstrated that the *Bacillaceae_Bacillus* genus (affiliated with *Firmicutes*) was primarily involved in biological control against different soil-borne pathogens (Pal et al., 2004). Since the *Bacillaceae_Bacillus* genus showed higher relative abundances in soil from tobacco rotations with (soybean and maize) than in tobacco monocropping soil. The higher abundance of *Bacillaceae_Bacillus* in the tobacco rotation systems may contribute to disease suppression, and soybean as a pre-crop resulted in the highest bacterial community diversity and activity compared with maize and tobacco. Such results suggest that the soybean-tobacco rotation system, from a biological perspective, may represent a good strategy for enhancing microbial diversity and maintaining soil biological fertility.

Conclusion

Our research shows that the bacterial communities in the soybean-tobacco and maize-tobacco rotation soils are diverse, and various members can apparently be attributed to the pre-crops (compared to the corresponding bacterial communities in the tobacco monocropping system soil). The results obtained from the present field investigations, by using Biolog-Eco platess and next-generation sequencing from the three treatments, showed that (i) the bacterial activity and diversity in soil from tobacco rotations (soybean and maize) were higher than in tobacco monocropping soil; (ii) there were increased relative abundances of *Actinobacteria* and *Bacteroidetes*, particularly of the genera of *Rhodoplanes*, *Adhaeribacter*, and *Bacillaceae_Bacillus* in the soybean-tobacco and maize-tobacco rotation soils compared with the tobacco monocropping soil.

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