INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–0713/2019/22–5–1173–1180
DOI: 10.17957/IJAB/15.1184
http://www.fspublishers.org

Full Length Article

Effects of *Funneliformis mosseae* on Mycorrhizal Colonization, Plant Growth and the Composition of Bacterial Community in the Rhizosphere of Continuous Cropping Soybean at Seedling Stage

Weiguang Jie[1,2†], Jixiang Lin[1†], Na Guo[1,2], Baiyan Cai[3] and Xiufeng Yan[1*]
[1]*Key Laboratory of Saline-alkali Vegetation Ecology Restoration, Ministry of Education, Alkali Soil Natural Environmental Science Center, Northeast Forestry University, Harbin, 150040, China*
[2]*Department of Food and Environment Engineering, Heilongjiang East University, Harbin, 150066, China*
[3]*College of Life Sciences, Heilongjiang University, Harbin, 150080, China*
[*]For correspondence: xfyan@nefu.edu.cn; jieweiguang2007@126.com
[†]Weiguang Jie and Jixiang Lin contributed equally to this work

Abstract

This study aimed at exploring the effects of *Funneliformis mosseae* on soybean growth and the composition of bacterial community in the rhizosphere of continuous cropping soybean. Here, the colonization rate of arbuscular mycorrhizal (AM) fungi and soybean growth were analyzed and high throughput sequencing was applied to study whether *F. mosseae* could change the composition of bacterial community in the roots and rhizosphere soil of continuous cropping soybean at seedling stage. The results showed that colonization rate increased after the inoculation of *F. mosseae*. Increasing continuous cropping regimes was beneficial to mycorrhizal colonization. Inoculation of *F. mosseae* significantly improved soybean growth. However, the relative abundance of bacterial community in the rhizosphere soil was not influenced by inoculation. The dominant phylum was Proteobacteria in all the samples. At the genus level, both inoculation and continuous cropping regimes had significant effects on the dominant genus and their relative abundances in the roots. The major genera among rhizosphere soils were same and their relative abundances were only slightly different. The results provide new insights into the interactive effects of AM fungi and rhizobacteria and contribute to further study the effects of *F. mosseae* on alleviating replant diseases. © 2019 Friends Science Publishers

Keywords: Arbuscular mycorrhizal fungi; Bacterial community; Continuous cropping; High throughput sequencing; Soybean

Introduction

Soybean is a major grain crop and currently the primary oilseed crop throughout the word. It is cultivated in more than 35 countries and mostly concentrated in America, Brazil, Argentina and China (Gawade *et al*., 2017). Soybean produces more oil and protein than other leguminous crops, and oil and protein can make up approximately 60% of the dried soybean weight (Cui *et al*., 2019). In addition, soybean also contains vitamin E, isoflavone, saponin, oligosaccharides and minerals, which are all beneficial for human health (Dass and Bhattacharyya, 2017; Yao *et al*., 2018). Due to the above positive reasons, soybean consumption has been increasing these years.

Continuous cropping of soybean has been widely adopted in Northeastern China because of the relatively low cost. However, it can also lead to substantial reductions by 70–80% or even no harvest, and result in large damages to soybean production (Liu *et al*., 2012). It can change the soil pH from neutral to acidic (Liu *et al*., 2017). Thus, it inhibits the growth and reproduction of bacteria and actinomycetes, which eventually leads to fungi becoming the dominant community. In addition, it also inhibits the growth of some beneficial microorganisms and favors the growth of some harmful microorganisms (Sun *et al*., 2012).

Rational utilization of beneficial microorganisms is another feasible alternative to improve soil nutrients and crop yields (Juliana *et al*., 2017). Arbuscular mycorrhizal (AM) fungi constitute a major component of rhizosphere microorganisms, and accounting for approximately 30% of the soil microbial biomass (Gai *et al*., 2018). In addition, it can colonize more than 90% of terrestrial plants, including major crop species, such as rice, wheat, potato and soybean (Zeng *et al*., 2019). AM fungi can generally improve plant performance, nutrient absorption and tolerance to both biotic and abiotic stresses (Zhang *et al*., 2019). In order to cope with the negative effects of chemical fertilizers and pesticides on crop production, environmentally-friendly measures have been explored in recent years (Zhu *et al*., 2017). The effects of AM fungi on the growth of various

To cite this paper: Jie, W., J. Lin, N. Guo, B. Cai and X. Yan, 2019. Effects of *Funneliformis mosseae* on mycorrhizal colonization, plant growth and the composition of bacterial community in the rhizosphere of continuous cropping soybean at seedling stage. *Intl. J. Agric. Biol*., 22: 1173–1180

crops have been verified (Ma *et al*., 2019). AM fungi can also significantly suppress the incidence of soybean root rot (Jie *et al*., 2015). Nevertheless, the effects of *F. mosseae* on the composition of bacterial community in the roots and rhizosphere soils of soybean under different continuous cropping regimes are never reported.

Therefore, the purposes of this study are to explore (1) whether inoculation of *F. mosseae* could exert a positive effect on the growth of soybean under different continuous cropping regimes, (2) whether *F. mosseae* could affect the composition, the diversity and richness of bacterial community in the roots and rhizosphere soils of soybean under different continuous cropping regimes?

Materials and Methods

Plant Materials

Suinong 26 (SN26) that has been widely cultivated in Heilongjiang Province was used in this study. It has an average protein and fat contents of 38.80% and 21.59%, respectively.

Mycorrhiza Inocula

Funneliformis mosseae was obtained from the rhizosphere soil of soybean in Heilongjiang Province of China by our group, and preserved at Wuhan institute of microbiology, China (CGMCC No.3013). Mycorrhiza inocula consisted of infected root segments, spores, hyphae and substrates, containing 20–30 spores per gram.

Experimental Design and Growing Conditions

The soil used in this experiment was collected from the Experimental Station of the Research Institute of Sugar Industry (126°36′E, 45°39′), Heilongjiang Province, China. This area is characterized by a middle temperate zone, continental monsoonal climate. Average annual precipitation is 569 mm and the annual average temperature is 4.5°C. Soils were labeled as experimental groups and control groups with 0, 1 and 3 years of continuous cropping for soybean, respectively. The physicochemical parameters of the soils are given in Table 1. In this study, potted plants were used, each pot containing 4 kg of air-dried and sieved through a 4 mm mesh.

Soybean seeds were superficially disinfected according to Valetti *et al.* (2016). The disinfected soybean seeds were placed in sterile wet cotton and kept in darkness at 28°C until the soybean radicle reached about 2 cm.

A pot experiment was used for soybean planting in this study. Each pot was filled with air-dried soil which was sampled from the soybean rhizosphere at Year 0 of continuous cropping, Year 1 of continuous cropping and Year 3 of continuous cropping. The *F. mosseae* inoculant (45 g) was added to each pot, and thoroughly mixed into the soil. The control group was identical but without *F. mosseae* inoculation. Each treatment was repeated nine times. After planting, pots of the same size, also sown with soybeans, were placed to surround the experimental pots as a protection barrier to prevent marginal effects.

Samples Collection and Processing

The roots and rhizosphere soil samples of soybean were randomly collected with a puncher after 30 days of planting (seedling stage). Three pots were randomly selected from each experimental treatment. The soils were collected at 0–20 depth to form samples. Some of the roots and soil samples were stored at -80°C, while other samples were used for other analyses.

Mycorrhizal Colonization and Plant Growth Measurements

The degree of mycorrhizal colonization was assessed as described previously (Phillips and Hayman, 1970).

The response of soybean plants to *F. mosseae* was estimated by determination of plant height, fresh and dry weight of shoot and root.

The collected seedlings were washed with distilled water at least three times. The shoot and roots of each plant were separated and the fresh weight of each plant was measured. The shoots and roots were dried at 70°C to a constant weight. Dry weights were finally measured, respectively.

Amplification of 16S rDNA Sequences

Roots DNA was extracted according to Long *et al.* (2005), while rhizosphere soil DNA was extracted with PowerSoil® DNA Isolation Kit (MOBIO, U.S.A.). The 16S rDNA V3 and V4 regions were amplified using the primers 338F (5′ -ACTCCTACGGGAGGCAGCA-3′) and 806R (5′ -GGACTACHVGGGTWTCTAAT-3′) (Dong *et al*., 2016), which were provided by Biomarker Technologies (Beijing). The 50 μL PCR reaction system contained 5 × FastPfu buffer 10 μL , 2.5 m*M* dNTPs 5 μL, 5 μM primer 338F 2.0 μL, 5 μM primer 806R 2.0 μL, 1.0 U Taq polymerase 0.2 μL, BSA 1.0 μL, template DNA 2.0 μL and ddH_2O 27.8 μL. PCR conditions were as follows: 95°C for 5 min, 25 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 40 s, followed by 72°C for 7 min. The fragment size of amplification products was about 450 bp. The amplification products were purified using a GeneJET Gel Extraction Kit (Thermo Scientific, U.S.A.).

Analysis of the Composition of the Bacterial Community

Illumina HiSeq 2500 (BioMarker Technologies Co., Ltd., Beijing, China) was used to analyze the responses of the

Table 1: Soil physicochemical parameters

Continuous cropping regimes	Organic matter g/kg	Total nitrogen g/kg	Total phosphorus g/kg	Total potassium g/kg	Ammonium nitrogen mg/kg	Nitrate nitrogen mg/kg	pH
0Y	25.69 ± 0.27^{b}	2.65 ± 0.18^{a}	5.61 ± 0.18^{a}	31.81 ± 0.29^{a}	1.16 ± 0.05^{a}	0.91 ± 0.05^{a}	7.49±0.01^{c}
1Y	28.34 ± 0.30^{a}	2.55 ± 0.18^{a}	5.37 ± 0.11^{a}	33.53 ± 0.33^{b}	1.08 ± 0.04^{ab}	0.85 ± 0.03^{a}	7.22±0.02^{b}
3Y	26.14 ± 0.23^{b}	2.27 ± 0.11^{a}	5.15 ± 0.10^{a}	34.84 ± 0.21^{c}	0.95 ± 0.06^{b}	0.73 ± 0.06^{a}	6.91±0.01^{a}

Note: 0 Y, 1 Y and 3 Y represent zero year, one year and three years of continuous cropping, respectively
Different letters indicate significant differences at different continuous cropping regimes ($P < 0.05$)

composition of bacterial community to *F. mosseae* and different continuous cropping regimes.

The raw paired-end sequences were merged and quality filtered using FLASH v1.2.7 and QIIME v1.8.0, respectively (Magoč and Salzberg, 2011). All the effective sequences were clustered into different operational taxonomic units (OTUs) based on 0.97 sequence similarity level with UCLUST v1.2.22 (Edgar *et al*., 2011). These sequences were classified and identified by NCBI database.

The richness index ACE and Chao l were measured using software mothur v1.30.1 (ACE http://www.mothur.org/wiki/Ace, Chao1 http://www.mothur.org/wiki/Chao). Simpson and Shannon diversity indexes were evaluated using alpha diversity analysis (Simpson http://www.mothur.org/wiki/Simpson, Shannon http://www.mothur.org/wiki/Shannon).

The composition of bacterial community was analyzed at the phylum and genus levels, respectively. A heatmap was calculated to show the relative differences in OTU abundances among the twelve samples (Bai *et al*., 2015). The raw sequences have been submitted to the NCBI Sequence Read Archive (SAR) database (Accession number SRP137794).

Statistical Analysis

All data obtained were assessed by analysis of variance (ANOVA) using S.P.S.S. 19.0 statistical software (S.P.S.S. Inc., Chicago, I.L., U.S.A.). The treatment effects were assessed by two-way ANOVA. Significant differences were evaluated with Tukey's test (Honestly significant difference, HSD) ($P < 0.05$).

Results

The Effect of Inoculation on Mycorrhizal Colonization

Natural AM was observed in all soybean roots (Table 2). The colonization rate was significantly affected by the inoculation of *F. mosseae*, continuous cropping regimes and their interactions (Table 3). The colonization rate was significantly higher in all the *F. mosseae* inoculated treatments than those in control. Also, significant differences were found in mycorrhizal colonization under different continuous cropping regimes. From Table 2, we found that the colonization rate was the highest in three years of continuous cropping during seedling stage, followed by one year of continuous cropping and finally zero year of continuous cropping.

Table 2: Colonization rate of soybean under different treatments

Index	Continuous cropping years	Non-inoculated with *F. mosseae*.	Inoculated with *F. mosseae*.
Colonization rate (%)	0	3.10 ± 0.47^{e}	14.95 ± 1.33^{c}
	1	6.03 ± 0.44^{de}	23.82 ± 1.66^{b}
	3	8.32 ± 0.60^{d}	30.90 ± 1.26^{a}

Note: Different letters indicate significant differences from different treatments ($P < 0.05$)

Table 3: Two-way ANOVA for inoculation with *F. mosseae* (I), continuous cropping regimes (Y) and their interactions on colonization rate of AM fungi and soybean growth

Index	Item	inoculation with *F. mosseae* (I)	continuous cropping regimes (Y)	I×Y
Colonization rate (%)	df	2	1	2
	MS	0.02	0.14	0.01
	F-ratio	57.48**	480.08**	15.71**
Plant height (cm)	df	2	1	2
	MS	8.77	18.87	0.03
	F-ratio	133.58**	287.34**	0.45^{NS}
Shoot fresh weight (g)	df	2	1	2
	MS	3.49	5.10	0.01
	F-ratio	105.52**	154.30**	0.21^{NS}
Shoot dry weight (g)	df	2	1	2
	MS	0.26	0.48	0.01
	F-ratio	97.72**	180.55**	2.02^{NS}
Root fresh weight (g)	df	2	1	2
	MS	1.19	2.23	0.09
	F-ratio	154.23**	289.18**	11.72**
Root dry weight (g)	df	2	1	2
	MS	0.09	0.17	0.07
	F-ratio	92.76**	165.35**	5.64*

Note: ^{NS} Factors are not significant.
* Factors are significant at $P < 0.05$ level
** Factors are significant at $P < 0.01$ level

The Effect of Inoculation on Soybean Growth

Two-way ANOVA results showed that soybean growth (plant height, shoot and root weights) was significantly affected by the inoculation of *F. mosseae* and the continuous cropping regimes (Table 3). The plant height of soybean increased significantly for all the plants inoculated with *F. mosseae* compared with control. The average plant height was 1.2-fold for all the three treatments inoculated with *F. mosseae* in comparison with the average values of control (Table 4). Similar trends were also found in the shoot and root biomass. We observed that soybean plant that inoculated with *F. mosseae* maintained higher shoot and root weights under different continuous cropping

Table 4: Effects of inoculation with *F. mosseae* on soybean growth

Index	Continuous cropping years	Non-inoculated with *F. mosseae.*	Inoculated with *F. mosseae.*
Plant height (cm)	0	11.15 ± 0.11^{c}	13.28 ± 0.19^{a}
	1	9.94 ± 0.12^{d}	12.07 ± 0.15^{b}
	3	8.85 ± 0.13^{e}	10.74 ± 0.17^{c}
Shoot fresh weight (g)	0	5.10 ± 0.10^{b}	6.11 ± 0.13^{a}
	1	4.19 ± 0.08^{d}	5.24 ± 0.14^{b}
	3	3.52 ± 0.06^{e}	4.66 ± 0.10^{c}
Shoot dry weight (g)	0	1.27 ± 0.03^{c}	1.67 ± 0.03^{a}
	1	1.07 ± 0.03^{d}	1.37 ± 0.03^{b}
	3	0.91 ± 0.02^{e}	1.20 ± 0.02^{c}
Root fresh weight (g)	0	2.05 ± 0.05^{c}	3.04 ± 0.07^{a}
	1	1.66 ± 0.04^{d}	2.27 ± 0.06^{b}
	3	1.41 ± 0.03^{e}	1.93 ± 0.04^{c}
Root dry weight (g)	0	0.62 ± 0.02^{c}	0.88 ± 0.03^{a}
	1	0.51 ± 0.01^{d}	0.68 ± 0.02^{b}
	3	0.43 ± 0.01^{e}	0.57 ± 0.02^{c}

Note: Different letters indicate significant differences from different treatments ($P < 0.05$)

regimes. Moreover, fresh and dry weights of soybean root were affected by AM colonization compared with non-inoculation seedlings during the same continuous cropping regime. The highest fresh and dry weights were obtained from the soybean inoculated with *F. mosseae* in zero year of continuous cropping (Table 4).

We found that the fresh and dry weights of the shoot were affected by the increasing of continuous cropping regimes. The lowest shoot weight was observed in the non-inoculated seedlings for three years of continuous cropping. The production of the shoot and root weights inoculated with *F. mosseae* were at least 1.2 times higher than those of controls (Table 4). Overall, the results indicated that *F. mosseae* significantly improved the soybean plant growth under different continuous cropping regimes.

Characteristics of Sample Sequences

A total number of 571, 436 sequences were obtained after the quality control of raw sequences obtained by Illumina HiSeq 2500 sequencing. 47, 620 sequences were randomly selected from each sample for data analyses. As shown in Table 5, the Simpson index of the root samples was higher than those of the soil samples in the same experimental treatment, while the OTU richness, Ace, Chao1 and Shannon indexes were opposite. Furthermore, the OTU richness, Ace, Chao1 and Shannon indexes were all lowest in in1YRB, but the Simpson index was the highest in in1YRB.

Taxonomic Complexity and Comparisons of Bacterial Community

Taxonomic analysis showed the population of predominant bacteria in the twelve samples. At the phylum level, ten identified phyla were discovered and Proteobacteria was the predominant phylum (Fig. 1a). Moreover, the relative abundance of Proteobacteria occupied more than 60.51% of the total amount in all the roots. Acidobacteria was the second predominant phylum in all the soil samples, but

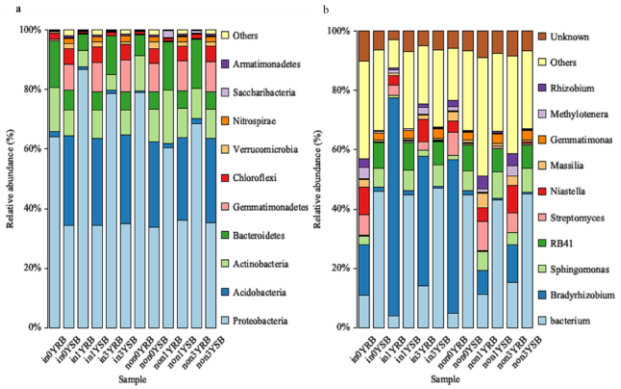

Fig. 1: Composition of the different bacterial communities at the phylum (**a**) and genus (**b**) levels in the twelve samples
Note: non represents non-inoculated with *F. mosseae*
in represents inoculated with *F. mosseae*
0 Y, 1 Y and 3 Y represent zero year, one year and three years of continuous cropping, respectively
RB represents bacteria in root samples
SB represents bacteria in soil samples

Bacteroidetes or Actinobacteria was the second predominant phylum in the roots. Interestingly, the relative abundance of Actinobacteria in the roots was significantly lower than that in the soil samples, whereas the relative abundance of Proteobacteria in the soil samples was significantly lower than that in the roots. In addition, three important phyla (Gemmatimonadetes, Verrucomicrobia and Nitrospirae) were observed in all the soil samples. However, there was no significant difference in the relative abundances of the ten identified phyla among the soil samples.

At the genus level, the relative abundance of *Bradyrhizobium* (73.44%) in in1YRB was much higher than the other five root samples (Fig. 1b). *Niastella* (3.43%), *Streptomyces* (3.37%), *Methylotenera* (0.92%), *Rhizobium* (0.55%), *Sphingomonas* (0.80%), RB41 (0.08%), *Massilia* (0.80%) and *Gemmatimonas* (0.06%) were also detected in in1YRB. However, the relative abundance of *Bradyrhizobium* decreased significantly, remaining at around 8.07% in non1YRB. The predominant identified

Table 5: Estimator parameters for alpha diversity in different samples

Index	Continuous cropping years	Samples	OTU	ACE	Chao1	Simpson	Shannon
Inoculated with *F. mosseae.*	0	RB	922	1,213.9601	1,223.6642	0.0413	4.5462
		SB	1,779	1,810.2898	1,836.0978	0.0057	6.4137
	1	RB	674	1,011.3424	1,024.2642	0.5374	1.8988
		SB	1,766	1,794.5323	1,815.0323	0.0064	6.3606
	3	RB	877	1,178.8744	1,192.1562	0.1956	3.4558
		SB	1,778	1,804.4604	1,814.1132	0.0057	6.4102
Non-inoculated with *F. mosseae.*	0	RB	711	1,259.4616	1,119.4639	0.2718	2.9817
		SB	1,767	1,791.0399	1,802.9381	0.0059	6.4036
	1	RB	1,098	1,436.0514	1,433.175	0.0224	4.8778
		SB	1,782	1,812.3259	1,822.2797	0.0077	6.3389
	3	RB	1,095	1,465.8245	1,475.3846	0.0294	4.7283
		SB	1,776	1,798.7309	1,812.2021	0.0066	6.3942

RB represents bacteria in root samples
SB represents bacteria in soil samples

bacteria were *Streptomyces* (9.85%), *Bradyrhizobium* (8.07%) and *Sphingomonas* (6.26%) in non1YRB. The genus level analysis demonstrated that the treatment with *F. mosseae* could favor *Bradyrhizobium* over *Streptomyces* and *Sphingomonas* in in1YRB. Moreover, all root samples except non1YRB (*Streptomyces* was predominant) were characterized by high relative abundance of *Bradyrhizobium*. As shown in Fig. 1b, in0YRB was different from other root samples inoculated with *F. mosseae*. The relative abundance of *Bradyrhizobium* maintained a low level about 16.91% in in0YRB. In addition, non0YRB was also different from non1YRB and non3YRB. The predominant genera were *Bradyrhizobium* (51.77%) and *Streptomyces* (7.66%) in non0YRB. However, the predominant genera were *Bradyrhizobium* and *Niastella* in in3YRB and non3YRB. In light of this, both inoculation and continuous cropping regimes had significant effects on the dominant genus and their relative abundances in the root samples. Compared with root samples, lower amount of *Bradyrhizobium* were observed in the soil samples. Furthermore, the relative abundances of *Niastella* and *Streptomyces* decreased significantly, remaining at around 0.36% and 0.77% in the soil samples, respectively. Comparison of the genus-level proportional abundances showed that the dominant genera in the soil samples were same, and the relative abundances were only slightly different. The predominant identified bacteria were RB41, *Sphingomonas* and *Gemmatimonas* in the soil samples. It indicated that *F. mosseae* might have no significant effect on the composition of bacterial community in the rhizosphere soil in this study.

According to the heatmap diagram of the bacterial community, the soil samples were classified into the following three clusters: non1YSB did not cluster with other soil samples; non0YSB, in0YSB and in1YSB clustered together; non3YSB and in3YSB clustered together (Fig. 2). As shown in Fig. 2, all root samples were also divided into three clusters, one for non1YRB, another for non0YRB and in1YRB, and the remaining samples clustered together. It was consistent with the composition of the bacterial community at the phylum level. Those

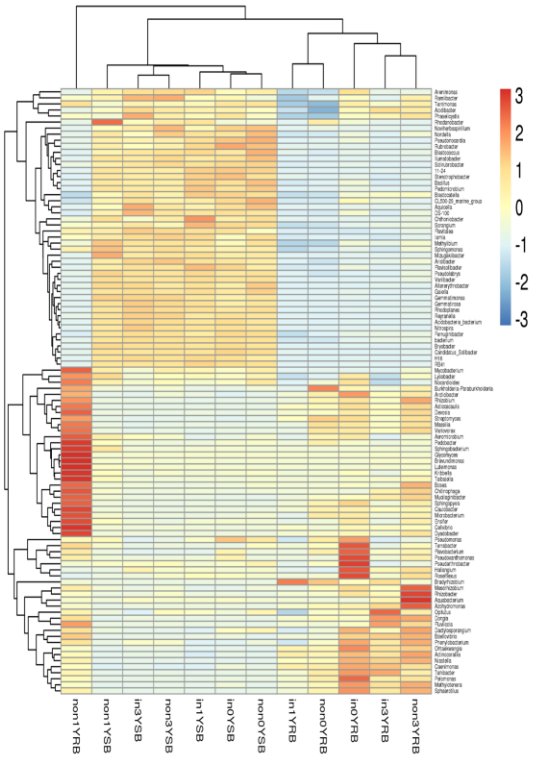

Fig. 2: Heatmap analysis of the 100 most abundant bacterial genera among the twelve samples

above showed that both the inoculation of *F. mosseae* and continuous cropping regimes could significantly affect the dominant genera and their relative abundances in the root samples.

Discussion

Microbial communities indirectly influence agroecosystem productivity, major nutrient cycles and soil carbon dynamics (Bardgett *et al*., 2008). Plant-microbe relationship is decisive for plant growth and survival (Heijden *et al*., 2008).

Our results showed that application of microbial inoculants to rhizosphere soil was an appropriate strategy for soybean growth. In the present study, soybean height, shoot and root weights were significantly affected by the inoculation of *F. mosseae*, indicating that it had positive effects on soybean growth (Table 4). In addition, increasing the continuous cropping regimes was beneficial to mycorrhizal colonization in soybean at seedling stage. According to Qian *et al.* (2015), mycorrhizal colonization can help soybean resist pathogens to some extent. It is worthwhile to mention that AM fungi were not able to avoid phytopathogens infection, but they were able to relief damage generated by the infection, boosting a defensive response mechanism in soybean. The inoculation effects of AM fungi in maize (Dhawi *et al*., 2015), wheat (Zhu *et al*., 2017) and soybean (Spagnoletti *et al*., 2017) have been proved. The interactive effects of *Azospirillum lipoferum* and *Piriformospora indica* on growth attributes and secondary metabolites production of sesame (*Sesamum indicum* L.) under saline condition was studied. The data indicated that the applied microorganisms raised the ability of sesame in absorption of essential nutrients (Khademian *et al*., 2019).

AM fungi not only increased soybean growth, but also changed the composition of bacterial community in roots and rhizosphere soil of continuous cropping soybean. The beneficial effects of AM fungi may due to their interactions with rhizosphere bacteria (Artursson *et al*., 2006). Allison and Martiny (2008) showed that microbial communities were susceptible to external perturbation. In addition, alteration of the rhizosphere bacterial community has also been considered as a mechanism responsible for plant growth promotion by AM fungi. Meanwhile, the interactions of plant-fungus complex may affect the bacterial community in rhizosphere soil. Although Vestergård *et al.* (2008) proved that AM fungi changed the bacterial community in the rhizosphere soil by DGGE approach, the technique has a serious resolution limitation, resulting in just a general overview of changes in microbial communities. Thus, the sequencing-based methods, such as high throughput sequencing provides more precise tools for the further study of soil microbial communities. However, the effects of *F. mosseae* on the composition of bacterial community in the roots and rhizosphere soils of soybean under different continuous cropping regimes during the seedling period were never reported using such molecular tools.

In this study, we found that *F. mosseae* could affect the composition of bacterial community. However, the inoculation of *F. mosseae* did not cause significant changes in the species richness estimators and alpha-diversity indices in soil samples. The Simpson index was the lowest in in0YSB and in3YSB. As a result, there may be more beneficial bacteria that could promote soybean growth in these soil samples. We found that the bacterial community between the rhizosphere soil samples inoculated and non-inoculated with *F. mosseae* changed slightly under the same continuous cropping regime, but those changes did not contribute to a progressive convergence or divergence between the two treatments. Variation in the composition of bacterial community due to the inoculation of *F. mosseae* may be related to the different continuous cropping regimes (Fig. 1). The variations are most likely to be related to the changes of soil physical and chemical properties, which need further research.

Several studies have reported that microbial communities improved rhizosphere soil nutrients and plant tolerance (Li *et al*., 2014; Dhawi *et al*., 2016). Most of these functions might be directly or indirectly influenced by the interactions of rhizosphere microbes. *F. mosseae* could repress or promote the recruitment of some bacteria in the rhizosphere of continuous cropping soybean. In this study, it also showed that inoculation of *F. mosseae* only had little effect on the relative abundance of the bacterial community in the root and soil samples. Its importance in the bacterial community of the inoculated soybean was reflected at the phylum level, as Proteobacteria, was an indicator of these rhizospheres microbes. Nitrospirae was not the most abundant indicator of the rhizosphere microbes in the root samples. Contrary to our results, Rodríguez-Caballero *et al.* (2017) found that Nitrospirae was the most abundant indicator of the rhizosphere microbes.

At the genus level, *Bradyrhizobium* was an indicator of rhizosphere microbes in leguminous plants. Compared with root samples, lower amount of *Bradyrhizobium* were observed in the soil samples, which may be related to the living habits of *Bradyrhizobium*. The introduction of *F. mosseae* may have affected negatively the relative abundance of *Sphingomonas*, an indicator genus of the non-inoculated root samples. The relative abundance of *Sphingomonas* in the three inoculated root samples was lower than those in the three non-inoculated root samples. In this study, the relative abundances of *Gemmatimonas* and RB41 were less than 0.1% in in1YRB and non0YRB. *Gemmatimonas* have been reported as polyphosphate accumulating bacteria and associated with enhancement of wheat yield (Yang *et al*., 2013). As is known, AM fungi are also polyphosphate accumulating organisms. It indicated that AM fungi may outcompete and replace *Gemmatimonas* when functional redundancy occurs. Moreover, AM fungi can increase plant resistance to biotic and abiotic stress and also increase the stability of soil aggregates (Islas *et al*., 2016). Many studies have indicated that some microbial communities coexisting with AM fungi in rhizosphere soil could be potentially efficient to improve plant growth (Imaz *et al*., 2014). Islas *et al.* (2016) reported that the capacity of mycorrhizal nutrition is more sensitive to soil properties than the influence of agricultural land use. Furthermore, microbial interactions involving AM fungi, plant growth-promoting rhizosphere bacteria, and rhizosphere indigenous bacterial community can promote the growth of continuous cropping soybean.

Conclusion

This work first reported the effects of *F. mosseae* on soybean growth and the composition of the bacterial community in the rhizosphere of continuous cropping soybean at seedling stage. It demonstrated that inoculation of *F. mosseae* significantly improved soybean growth and the composition of the bacterial community in the roots and rhizosphere soil of continuous cropping soybean. Therefore, inoculation of *F. mosseae* is a useful tool for stabilizing soybean growth and the composition of the bacterial community in the rhizosphere of soybean under different continuous cropping regimes. This study provides new insights into the interactive effects of AM fungi and rhizobacteria and also contributes to further study the effects of *F. mosseae* on alleviating continuous cropping obstacles.

Acknowledgements

This work was supported by a grant from Natural Science Foundation of China (31570487), Natural Science Foundation of Heilongjiang Province (YQ2019C016), Scientific Research Projects of Heilongjiang East University (HDFKY190104, HDFKY190120).

References

Allison, S.D. and J.B.H. Martiny, 2008. Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. U.S.A.*, 105: 11512–11519

Artursson, V., R.D. Finlay and J.K. Jansson, 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environ. Microbiol.*, 8: 1–10

Bai, L., J.Q. Cui, W.G. Jie and B.Y. Cai, 2015. Analysis of the community compositions of rhizosphere fungi in soybeans continuous cropping fields. *Microbiol. Res*., 180: 49–56

Bardgett, R.D., C. Freeman and N.J. Ostle, 2008. Microbial contributions to climate change through carbon cycle feedbacks. *ISME J*., 2: 805–814

Cui, G., S. Ai, K. Chen and S. Wang, 2019. Arbuscular mycorrhiza augments cadmium tolerance in soybean by altering accumulation and partitioning of nutrient elements, and related gene expression. *Ecotoxicol. Environ. Saf.*, 171: 231–239

Dass, A. and R. Bhattacharyya, 2017. Wheat residue mulch and anti-transpirants improve productivity and quality of rainfed soybean in semi-arid north-Indian plains. *Field Crop Res.*, 210: 9–19

Dhawi, F., R. Datta and W. Ramakrishna, 2016. Mycorrhiza and heavy metal resistant bacteria enhance growth, nutrient uptake and alter metabolic profile of sorghum grown in marginal soil. *Chemosphere*, 157: 33–41

Dhawi, F., R. Datta and W. Ramakrishna, 2015. Mycorrhiza and PGPB modulate maize biomass, nutrient uptake and metabolic pathways in maize grown in mining-impacted soil. *Plant Physiol. Biochem.,* 97: 390–399

Dong, W.W., F.L. Xuan, F.L. Zhong, J. Jiang, S.Q. Wu, D.H. Li and L.H. Quan, 2016. Comparative Analysis of the Rats' Gut Microbiota Composition in Animals with Different Ginsenosides Metabolizing Activity. *J. Agric. Food Chem.*, 65: 327–337

Edgar, R.C., B.J. Haas, J.C. Clemente, C. Quince and R. Knight, 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, 27: 2194–2200

Gai, J.P., J.Q. Fan and S.B. Zhang, 2018. Direct effects of soil cadmium on the growth and activity of arbuscular mycorrhizal fungi. *Rhizosphere*, 7: 43–48

Gawade, D.B., R.R. Perane, A.P. Suryawanshi and C.D. Deokar, 2017. Extracellular enzymes activity determining the virulence of *Rhizoctonia bataticola*, causing root rot in soybean. *Physiol. Mol. Plant Pathol.*, 100: 49–56

Heijden, M.G.A.V., R.D. Bardgett and N.M.V. Straalen, 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.*, 11: 296–310

Imaz, P.A., P.A. Barbieri, H. Echeverría, H.R.S. Rozas and F. Covacevich, 2014. Indigenous mycorrhizal fungi from Argentina increase Zn nutrition of maize modulated by Zn fertilization. *Soil Environ.*, 33: 23–32

Islas, A.J.T., K.H. Guijarro, M. Eyherabide, H.R.S. Rozas, H.E. Echeverría and F. Covacevich, 2016. Can soil properties and agricultural land use affect arbuscular mycorrhizal fungal communities indigenous from the Argentinean Pampas soils? *Appl. Soil Ecol.*, 101: 47–56

Jie, W., L. Bai, W. Yu and B. Cai, 2015. Analysis of interspecific relationships between *Funneliformis mosseae* and *Fusarium oxysporum* in the continuous cropping of soybean rhizosphere soil during the branching period. *Biocontr. Sci. Technol.*, 25: 1036–1051

Juliana, S.P., O. Fritz and D.P. Cicero, 2017. Diversity of arbuscular mycorrhizal fungi in the Brazilian's Cerrado and in soybean under conservation and conventional tillage. *Appl. Soil Ecol.*, 118: 178–189

Khademian, R., B. Asghari, B. Sedaghati and Y. Yaghoubian, 2019. Plant beneficial rhizospheric microorganisms (PBRMs) mitigate deleterious effects of salinity in sesame (*Sesamum indicum* L.): Physio-biochemical properties, fatty acids composition and secondary metabolites content. *Indust. Crops Prod.*, 136: 129–139

Li, T., G. Lin, X. Zhang, Y.L. Chen, S.B. Zhang and B.D. Chen, 2014. Relative importance of an arbuscular mycorrhizal fungus (*Rhizophagus intraradices*) and root hairs in plant drought tolerance. *Mycorrhiza*, 24: 1–8

Liu, J.J., Z.H. Yu and Q. Yao, 2017. Distinct soil bacterial communities in response to the cropping system in a Mollisol of northeast China. *Appl. Soil Ecol.*, 119: 407–416

Liu, X., Y. Li, B. Han, Q. Zhang, K. Zhou and X. Zhang and M. Hashemi, 2012. Yield response of continuous soybean to one-season crop disturbance in a previous continuous soybean field in Northeast China. *Field Crops Res.*, 138: 52–56

Long, L.K., S.Z. Yang, Q. Yao and H.H. Zhu, 2005. DNA extraction from arbuscular mycorrhizal fungi and analysis by PCR-denaturing gradient gel electrophoresis. *Mycosystema*, 24: 564–569

Ma, X.N., W.Q. Luo and J. Li, 2019. Arbuscular mycorrhizal fungi increase both concentrations and bioavilability of Zn in wheat (*Triticum aestivum* L) grain on Zn-spiked soils. *Appl. Soil Ecol.*, 135: 91–97

Magoč, T. and S.L. Salzberg, 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27: 2957–2963

Phillips, J.M. and D.S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and cesicula-arbuscular mycorrhizal fungi for rapid, assessment of infection. *Trans. Brit. Mycol. Soc.*, 55: 158–161

Qian, L., W.J. Yu, J.Q. Cui, W.G. Jie and B.Y. Cai, 2015. *Funneliformis mosseae* affects the root rot pathogen *Fusarium oxysporum* in soybeans. *Acta Agric. Scand. B- Soil Plant Sci.*, 65: 321–328

Rodríguez-Caballero, G., F. Caravaca, A.J. Fernández-González, M.M. Alguacil, M. Fernández-López and A. Roldán, 2017. Arbuscular mycorrhizal fungi inoculation mediated changes in rhizosphere bacterial community structure while promoting revegetation in a semiarid ecosystem. *Sci. Total Environ.*, 584–585: 838–848

Spagnoletti, F., M. Carmona, N.E.T. Gómez, V. Chiocchio and R.S. Lavado, 2017. Arbuscular mycorrhiza reduces the negative effects of *M. phaseolina* on soybean plants in arsenic-contaminated soils. *Appl. Soil Ecol.*, 121: 41–47

Sun, X., X.Y. Chen, Z.P. Chai and J.P. Li, 2012. Features of the soil microorganism and its enzyme activities of orchard under different management measures in sourthern Xinjiang. *Pratacult. Sci.*, 29: 1023–1027

Valetti, L., L. Iriarte and A. Fabra, 2016. Effect of previous cropping of rapeseed (*Brassica napus* L.) on soybean (*Glycine max*) root mycorrhization, nodulation and plant growth, *Eur. J. Soil Biol.*, 76: 103–106

Vestergård, M., F. Henry, J.I. Rangel-Castro, A. Michelsen, J.I. Prosser and S. Christensen, 2008. Rhizosphere bacterial community composition responds to arbuscular mycorrhiza, but not to reductions in microbial activity induced by foliar cutting. *FEMS Microbiol. Ecol.*, 64: 78–89

Yang, C., C. Hamel, Y. Gan and V. Vujanovic, 2013. Pyrosequencing reveals how pulses influence rhizobacterial communities with feedback on wheat growth in the semiarid prairie. *Plant Soil*, 367: 493–505

Yao, Q.Z., W. Yan and J. Wei, 2018. Establishment of a quality test for arbuscular mycorrhizal inoculum. *Intl. J. Agric. Biol*., 20: 926−932

Zeng, H., W. Zhong, F. Tan, Y. Shu, Y. Feng and J. Wang, 2019. The influence of *Bt* maize cultivation on communities of arbuscular mycorrhizal fungi revealed by MiSeq sequencing. *Front. Microbiol.*, 9: 1–12

Zhang, F.G., M.H. Liu and Y. Li, 2019. Effects of arbuscular mycorrhizal fungi, biochar and cadmium on the yield and element uptake of *Medicago sativa*. *Sci. Total Environ*., 655: 1150–1158

Zhu, Y., G.C. Lv, Y.L. Chen, X.F. Gong, Y.N. Peng, Z.Y. Wang, A.T. Ren and Y.C. Xiong, 2017. Inoculation of arbuscular mycorrhizal fungi with plastic mulching in rainfed wheat: A promising farming strategy. *Field Crop Res.*, 204: 229–241

[Received 27 Apr 2019; Accepted 03 Jul 2019; Published (online) 10 Nov 2019]