



Full Length Article

Rhizosphere Fungal Diversity of Wild and Cultivated Soybeans in Field and Greenhouse Experiments

Jianfeng Zhang¹, Jiayu Zhang¹, Pengju Zhang¹, Peipei He¹, Haixia Song¹, Jiejing Zhang^{1*} and Chunjie Tian^{1,2**}

¹School of Life Sciences, Jilin Agricultural University, Jilin province innovation platform of straw comprehensive utilization technology, Changchun 130118, P. R. China

²Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology Chinese Academy of Sciences, Changchun, Jilin 130102, China

*For correspondence: zjjx124@163.com; tiancj@neigae.ac.cn

Abstract

In order to find out the microorganisms that can improve the environmental adaptability of soybeans, the rhizosphere microbial diversity of wild and cultivated soybeans was studied using the Illumina MiSeq sequencing method. There were no significant difference in the dominant fungal phyla and dominant fungal genera of wild soybean rhizosphere and cultivated soybean rhizosphere. However, there was a significant difference in arbuscular mycorrhizal fungi (AMF) between wild soybean rhizosphere and cultivated soybean rhizosphere. Under controlled conditions, the relative abundance ratio of *Paraglomus* in wild soybean rhizosphere was much larger than that in cultivated soybean rhizosphere. While, the relative abundance of *Funneliformis* and *Rhizophagus* in cultivated soybean rhizosphere were much higher than that in wild soybean rhizosphere. Interestingly, in fields, no AMF was found in cultivated soybeans rhizosphere, and only *Paraglomus* with drought resistance was found in wild soybeans rhizosphere. It was concluded that under different growth conditions, there were differences in the number and types of rhizosphere fungi between wild soybean and cultivated soybean, and the similarity of fungal community structure was also different. © 2019 Friends Science Publishers

Keywords: Wild soybean and cultivated soybean; Rhizosphere fungal diversity; Arbuscular mycorrhizal fungi; Field conditions

Introduction

Legumes are the second largest crop species, and their production accounts for about 27% of global crops (Smykal *et al.*, 2015). Soybeans are the most important legumes (Foyer *et al.*, 2016). However, the safety of genetically modified technology remains to be verified. According to genetic relationship, soybeans can be divided into cultivated soybeans and wild soybeans (Kim and Phillips, 2010). Cultivated soybean has higher nutritional value and larger yield but poorer environmental adaptability. Wild soybean has better environmental adaptability than cultivated soybean. Wild soybean is resistant to disease, drought, salt and alkali (Van and Krüger, 2002; Zhang *et al.*, 2009; Xue *et al.*, 2011). The excellent environmental adaptability of wild soybeans is due to its resistance genes such as drought resistance genes, salt and alkali resistance genes, disease resistance genes and so on (Hajjar and Hodgkin, 2007). However, the safety of genetically modified foods needs to be verified (Foyer *et al.*, 2016). Therefore, it is very necessary to seek other safe and reliable methods to improve the environmental adaptability of cultivated soybeans.

Some plant rhizosphere microorganisms have been proved to play an important role in improving the environmental adaptability of plants (Zhang *et al.*, 2018). Arbuscular mycorrhizal fungi (AMF) are one of the most important rhizosphere fungi. AMF can improve plants drought resistance, plants disease resistance and the absorption of plant nutrients (Tian *et al.*, 2013; Pellegrino and Bedini, 2014; Ortiz *et al.*, 2015). Some rhizosphere fungi can break down cellulose and chitin to provide sugar and nitrogen to plants (Deacon *et al.*, 2006). Some rhizosphere fungi can improve the environmental adaptability of legumes, such as *Hedysarum coronarium L* (Labidi *et al.*, 2015) and *Trifolium repens L* (Tuo *et al.*, 2017). Tuo *et al.* (2017) found that *Funneliformis mosseae* and *Paraglomus occultum* can improve the drought resistance and growth rate of *Trifolium repens L*. Shores *et al.* (2010) found that *Trichoderma* spp. and *Sebacinales* spp. have the ability to control numerous foliar, root, and fruit pathogens and even invertebrates such as nematodes. However, the differences in the rhizosphere microorganisms between wild soybean and cultivated soybean are not clear. Whether these differences will affect the adaptation of soybean to the environment is also unknown.

To solve the above problems, in this paper, using Illumina MiSeq sequencing method, we studied the composition and variation of rhizosphere fungal community structure of wild soybean and cultivated soybean under natural and controlled conditions respectively; explored the differences of rhizosphere fungi which could improve plant environmental adaptation between wild and cultivated soybeans under both natural and controlled conditions and the composition of fungal communities in wild and cultivated soybean rhizosphere. Then, based on these results, we studied the influence of environmental factors on microbial rhizosphere fungi of wild and cultivated soybean.

Materials and Methods

Soil Collection

Field soil samples were collected on 21 to 23 August 2017 in Fuyuan County, Heilongjiang Province, China. 3 sampling sites were chosen to collect the Field soil samples, and their geographic coordinates were A sampling sites (47°91'N, 134°47'E), B (47°97'N, 134°30'E), and C (47°99'N, 134°08'E), respectively. Meteorological data for each of the sampling sites (annual mean air temperature, mean annual rainfall, and annual accumulated temperature) were collected from local meteorological stations. Normally, in the 3 sampling sites, annual mean air temperature is 20.2°C and annual mean rainfall is 600 mm. At each sampling site, we selected three sampling regions which were wild soybean region (W), cultivated soybean region (Z), and non-vegetation coverage region (CK), respectively. Each sampling region's area is 100 m². Then, 4 plots (1 m²) were selected randomly in each sampling region for choosing plants. After that, we selected five plants (S' distribution) in each plot and scooped up these plants. And then, we collected the soil samples which had a 0-1 cm distance from the plant roots. The soil samples from wild soybean root in A, B and C sampling sites were marked as AW, BW and CW, respectively. Similarly, the soil samples from cultivated soybean root in A, B and C sampling sites were marked as AZ, BZ and CZ, respectively. In non-vegetation coverage region, soil samples were collected from a depth of 15-20 cm. And the soil samples from non-vegetation coverage region in A, B and C sampling sites were marked as ACK, BCK and CCK, respectively. Finally, we conserved all soil samples at -80°C for application.

Controlled experiments were finished in green house in Changchun, Jilin Province (43°59'N, 125°23'E). Wild soybean breeds (Williams 82) and cultivated soybean breeds (ZYQ95) used controlled experiment were the same as those in field samples. These plants' growth conditions were at temperature of 25°C, regular watering, field moisture capacity at 20%, and without chemical fertilizers. We planted germinated wild soybean and germinated cultivated soybean in controlled experimental plots. The soil samples

from wild soybean rhizosphere and cultivated soybean rhizosphere were marked as PW and PZ, respectively. The soil samples from unplanted plots were marked as PCK. Soil samples collecting and conserving methods were the same as those field soil samples.

DNA Extraction and Illumina MiSeq

Soil DNA was extracted from soil samples (0.5 g wet weight) using the FastDNA™ SPIN Kit for Soil (MPBio, Santa Ana, CA, USA). DNA purity and concentration were measured using electrophoresis and the NanoDrop2000™ spectrophotometer (Thermo Scientific, Wilmington, DE, USA). DNA of the sample soil was sent to Shanghai Pesano Biotechnology Co., Ltd. for Illumina MiSeq system sequencing. The fungal ITS primer used for the amplified sequence was ITS5F (5'-GGAAGTAAAAGTCGTAACAA GG-3'), ITS1R (5'-GCTGCGTTCTTCATCGATGC-3'). The mixture subjected to PCR was prepared using the methods presented by Wang *et al.* (2016). And then, using a Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany), the PCR products were pooled and purified. Samples whose sizes were between 150-450 bp were selected for further analysis. Sequencing libraries were produced using a TruSeq® DNA PCR Free Sample Preparation Kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions, and index codes were appended. The library quality was evaluated by Agilent Bioanalyzer 2100 system and Qubit® 2.0 Fluorometer (Thermo Scientific). Samples were sequenced on an Illumina MiSeq platform. Quantitative Insights in to Microbial Ecology, v1.8 (<http://qiime.org/>) (Caporaso *et al.*, 2010) was used to remove the sequences shorter than 150 bp or longer than 500 bp, and the sequences less than 20% in mass fractions. Then, we used the UCLUST (Edgar, 2010) sequence comparison tool in QIIME software to do the merging and OTU partitioning on the sequence obtained, according to 97% sequence similarity. We use the Unite database to carry out species annotation of ITS sequences. QIIME software was used to calculate the diversity index (Shannon diversity index, Simpson diversity index) and richness index (Chao1 estimator of richness, ACE estimator of richness) (Shannon, 1938; Simpson, 1949; Chao, 1984, 1993).

Statistical Analysis

Soil physicochemical, fungal alpha diversity and relative abundance of fungi at different taxonomic levels for each group of soil samples were analyzed by analysis of variance (ANOVA). The correlation between the dominant genus and the arbuscular mycorrhizal fungi (AMF) genus was studied by correlation analysis (Pearson's analysis). Analysis of variance and correlation analysis were done using SPSS24.0 software and $p < 0.05$ indicates significant difference. Principal component analysis (PCA) was used to identify differences in fungal community composition.

The correspondence between environmental factors and fungal communities was got verified by Redundancy analysis (RDA). PCA analysis and RDA analysis were performed using the "R" package (R v.3.4.3).

Results

Rhizospheric Fungal Diversity

In order to compare the diversity of different samples, all samples in the OTU abundance matrix were first uniformly resampled at a minimum sequencing depth of 90%. The coverage of diversity for all sample captures exceeded 99% (Table 1), which indicates that the diversity of the entire fungal population was sufficiently captured at this sequencing depth in study. The α -diversity index (Simpson's diversity, Chao 1 richness, ACE, Shannon's diversity) of the 12 groups of samples showed in Table 1, indicated that there was no significant difference in the α -diversity index of soil samples (AW, AZ, BW, BZ, CW and CZ) under field conditions. There was no significant difference in the α -diversity index of soil samples (PW, PC) under controlled conditions. However, the α -diversity index of soil samples (PW, PZ) and soil samples (AW, AZ, BW, BZ, CW and CZ) under field conditions were significantly different ($p < 0.05$).

Taxonomic Classification and Relative Abundance of Fungi

A total of 2,847,365 mass sequences were obtained from 47 samples, and 27041-67010 sequences were obtained in per sample (average = 49093). Reading length was at 200-450 bp. A total of 10080 different OTUs were obtained by classifying all sequences at 97% similarity levels, with an average of 3992 OTUs per sample. From Fig. 1, the dominant fungal species in all soil samples were *Ascomycota*, *Basidiomycota* and *Zygomycota*. Their relative abundance percentage in all samples was 34.84-92.53, 1.15-34.73, 1.45-56.30, respectively. *Glomeromycota* was a relatively small gate in all samples and its relative percentage was 0.03-11.51 in all samples. In addition, *Rozellomycota* and *Chytridiomycota* were only found in field soil samples. Their relative abundance percentages were 0.18-17.82 and 0.01-0.81, respectively. *Neocallimastigomycota* only appeared in samples under controlled soil samples, and its relative abundance percentage was 0.04-0.53.

Further analysis was performed on the relative abundance data of genus fungi, and a total of 387 fungal genera were detected. The relative abundances of *Mortierella*, *Acremonium* and *Penicillium* in all samples were more than 0.05, and these were dominant genera. Their relative abundance percentages were 0.50-56.30, 0.05-4.78, and 0.05-5.66, respectively. We analyzed the correlation analysis of 8 fungal genera and the three dominant genera of

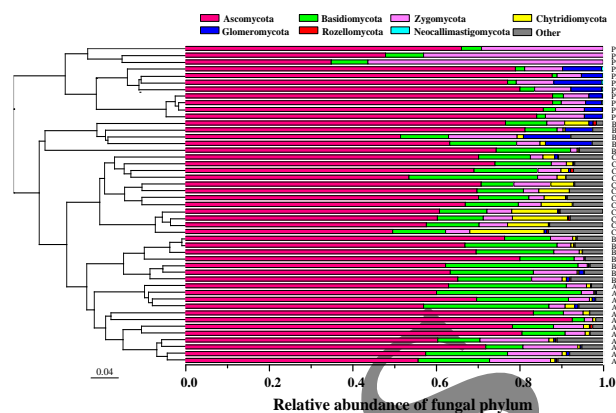


Fig. 1: Phylogenetic relationships of fungal communities shown with the relative abundances of different fungal phyla

Glomeromycota phyla, as shown in Table 2. It was found that there were obviously positive correlation between *Mortierella* and all samples; there were obviously negative correlation between *Penicillium* and all samples. Furthermore, *Paraglomus* and *Rhizophagus* of *Glomeromycota* were significantly positively correlated with all samples. *Funneliformis* was positively correlated with all samples.

The relative abundance of *Mortierella*, *Penicillium*, *Paraglomus*, *Funneliformis*, and *Rhizophagus* were shown in Fig. 2. The relative abundance of *Mortierella* in soil samples AW and CW were significantly higher than that in samples AZ and CZ (Fig. 2A). The relative abundance of sample PCK was significantly higher than other soil samples. The relative abundance of *Penicillium* in sample AZ was significantly higher than that in sample AW, and there was no significant difference in the other samples (Fig. 2A). *Paraglomus* was detected in sample AW, BW, CW, PW, PZ, and the relative abundance of *Paraglomus* in sample PW was significantly greater than that in sample PC (Fig. 2B). *Funneliformis* was detected in samples AW and PZ. *Rhizophagus* was detected in samples PW and PZ and the relative abundance of *Funneliformis* and *Rhizophagus* in sample PZ were significantly higher than that in soil sample PW (Fig. 2B).

Effect of Environmental Factors on Rhizosphere Fungal Community

In order to explore the rhizosphere fungal community structure of all soil samples, principal component analysis (PCA) was done on the rhizosphere fungal, as shown in Fig. 3. The results showed that the variation interpretation rate along the PCA1 axis was 5.22%, and the variation interpretation rate along the PCA2 axis was 4.86%. From Fig. 3, along the PCA1 axis, the distances between field soil samples AW, BW, CW and PW were large, which indicated that in different growing environment,

Table 1: Sequencing data of fungal ITS rRNA and diversity index of fungal community

Soil simple	Sequences	Chao1	ACE	Shannon	Simpson	Coverage(%)
AW	34375±1749	517.86±51.12 ^a	514.50±47.69 ^a	6.80±0.44 ^a	0.97±0.01a	99.8%
AZ	34635±718	392.37±87.89 ^a	391.43±87.82 ^a	6.30±0.60 ^a	0.96±0.03a	99.9%
ACK	30881±3845	332.36±16.43 ^a	332.51±16.32 ^a	6.10±0.28 ^a	0.96±0.01a	99.9%
BW	32631±2244	374.73±112.18 ^a	375.07±111.46 ^a	5.96±1.06 ^a	0.95±0.03a	99.7%
BZ	41804±10191	481.37±76.64 ^a	483.25±75.52 ^a	6.05±0.29 ^a	0.95±0.01a	99.8%
BCK	52612±5523	201.25±29.46 ^{ab}	202.15±29.01 ^{ab}	5.08±1.32 ^{ab}	0.87±0.15ab	99.9%
CW	56764±4257	600.72±51.51 ^a	602.47±50.94 ^a	6.70±0.15 ^a	0.96±0.01a	99.9%
CZ	57440±6534	435.61±29.07 ^a	435.50±29.10 ^a	6.58±0.29 ^a	0.97±0.01a	99.9%
CCK	56982±2111	490.13±91.59 ^a	490.66±92.04 ^a	7.36±0.48 ^a	0.98±0.01a	99.9%
PW	53098±5007	155.36±12.16 ^{ab}	156.88±12.31 ^{ab}	4.14±0.19 ^{ab}	0.86±0.03ab	99.9%
PZ	55059±3815	135.66±10.84 ^{ab}	137.43±13.19 ^{ab}	3.88±0.48 ^{ab}	0.86±0.07ab	99.9%
PCK	61023±2639	101.67±10.28 ^b	102.34±10.47 ^b	3.21±0.23 ^b	0.77±0.01b	99.9%

Mean ± standard deviation. Different letters within the same column indicate significant difference between soil samples tested by ANOVA ($p < 0.05$)

Table 2: Correlations between relative abundance of genus level fungi and all sample

Genuses	<i>r</i>	<i>P</i>
Mortierella(M)	0.370*	0.010
Acremonium(M)	-0.181	0.224
Penicillium(M)	-0.502**	<0.001
Paraglomus(G)	0.471**	0.001
Funneliformis(G)	0.368*	0.011
Rhizophagus(G)	0.445**	0.002
Glomus(G)	-0.058	0.700
Septoglomus(G)	0.045	0.765
Redeckera(G)	0.229	0.122
Pacispora(G)	-0.153	0.303
Claroideoglomus (G)	0.114	0.447

M, dominant genus; G, genus of *Glomeromycota*. * ($p < 0.05$); ** ($p < 0.01$)

the similarity of wild soybean rhizosphere fungal community were different. Similarly, the distances between controlled soil samples AZ, BZ, CZ and PZ were large as well, which indicated that in different growing environment, the similarity of cultivated soybean rhizosphere fungal community were also different.

Based on the results of correlation analysis between soil physicochemical properties (Table 3) and soil fungal communities in each soil sample (Fig. 4), we performed a model redundancy analysis (RDA) on the rhizosphere fungal communities and their associated environmental factors in various soil samples, as shown in Fig. 4. The results showed that the total phosphorus, available potassium, soil organic matter, pH, available phosphorus, and total nitrogen were significantly correlated with the rhizosphere fungal communities in all soil samples. The RDA map showed that the influence of environmental factors on rhizospheric fungal communities varied along the RDA1 axis, and its variation interpretation rate was 4.02%. All environmental factors mutated along the RDA2 axis of the rhizosphere fungal community and the variation interpretation rate of 2.52%. The total variation interpretation rate is 6.54%. The effect of available phosphorus on AW, AZ, PW, PZ, BW, BZ, CZ and CW decreased in order. And the effects of total phosphorus and pH on BW, BZ, AW, AZ, CW, CZ, PZ and PW decreased

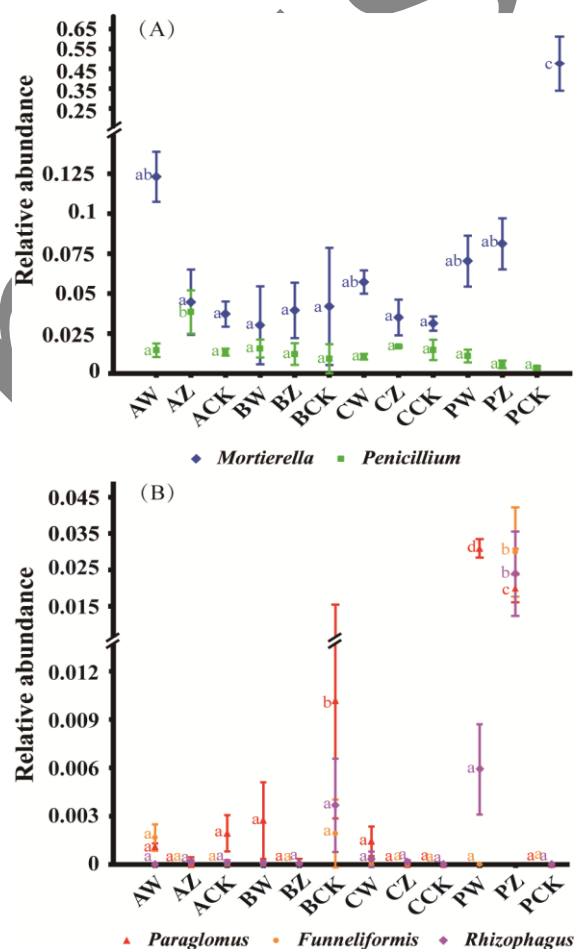


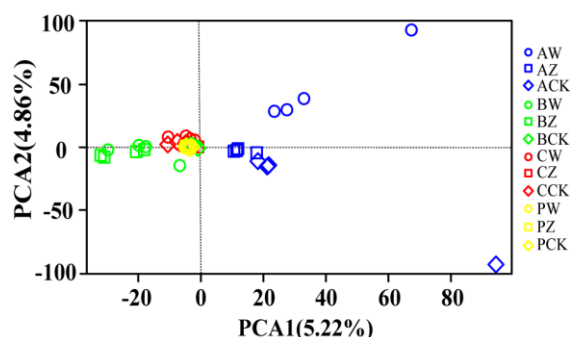
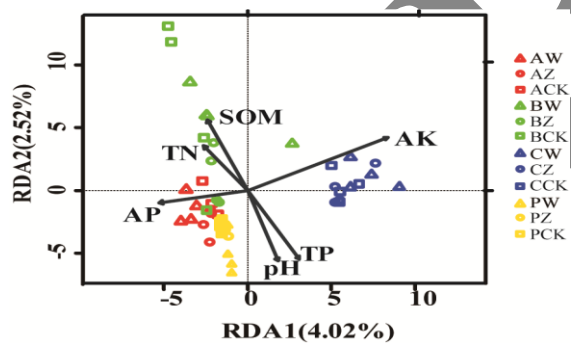
Fig. 2: Relative abundance of *Mortierella*, *Penicillium*, *Paraglomus*, *Funneliformis*, and *Rhizophagus* in various soil samples. Different letters of the same color represent significant differences between soil samples tested by ANOVA ($p < 0.05$)

in order too. In addition, the effects of organic matter and total nitrogen on BW, BZ, AW, AZ, CW, CZ, PZ and PW decreased in that order as well.

Table 3: Basic information of soil samples and soil physicochemical properties

Soil sample	SOM (g·kg ⁻¹)	TN (g·kg ⁻¹)	TP (g·kg ⁻¹)	AP (mg·kg ⁻¹)	AK (mg·kg ⁻¹)	pH
AW	57.28±8.48c	3.13±0.54b	0.21±0.19a	38.24±2.94bc	129.25±2.48b	5.29±0.87cde
AZ	40.62±11.24bc	2.25±0.19a	0.30±0.22ab	40.93±1.44c	103.76±3.05b	4.52±0.05ab
ACK	10.21±9.25a	1.14±0.13a	0.16±0.01a	6.81±1.19a	46.05±2.13a	4.50±0.06ab
BW	140.21±19.90e	6.28±1.01c	0.33±0.03ab	29.47±1.50b	186.84±1.75c	4.28±0.04a
BZ	221.23±1.85f	11.17±2.34d	0.48±0.01bc	52.23±17.43d	255.48±14.11d	4.22±0.04a
BCK	77.36±3.74d	3.01±0.93b	0.14±0.01a	4.18±1.35a	206.45±75.15c	5.03±0.07bcd
CW	40.71±15.05bc	1.68±0.17ab	0.56±0.03cd	2.88±0.71a	320.42±4.20e	4.77±0.03abc
CZ	45.50±5.41bc	1.68±0.06ab	0.71±0.03de	4.43±1.27a	277.21±4.88d	5.56±0.06de
CCK	47.67±7.49bc	1.85±0.04ab	0.63±0.03cde	2.94±1.15a	313.91±5.17e	5.69±0.02e
PW	32.25±3.28b	1.02±0.28a	0.77±0.13ef	8.28±0.53a	98.49±6.96b	6.36±0.39f
PZ	32.46±3.02b	1.19±0.19a	0.91±0.16f	10.35±1.62a	103.14±7.90b	6.58±0.23f
PCK	28.88±1.29b	1.02±0.19a	1.26±0.02g	33.9±2.61bc	102.68±6.48b	7.11±0.40g

Mean ± standard deviation. Different letters within the same column indicate significant difference between soil samples tested by ANOVA ($P < 0.05$). SOM, soil organic matter; TN, total nitrogen; TP, the total phosphorus; AP, available phosphorus; AK, available potassium

**Fig. 3:** Principal Component Analysis of Soil Fungal Communities in Soil Samples (PCA)**Fig. 4:** Redundancy analysis of soil fungi and environmental factors in soil samples (RDA). SOM, soil organic matter; TN, total nitrogen; TP, the total phosphorus; AP, available phosphorus; AK, available potassium

Discussion

Differences in Composition and Diversity of Fungi in the Rhizosphere

This study explored the rhizosphere fungal structure of wild soybean and cultivated soybean. *Ascomycota*, *Basidiomycota* and *Zygomycota* were dominant fungi in wild and cultivated soybeans at the phylum level.

Mortierella, *Acremonium* and *Penicillium* were the dominant genera of wild soybean and cultivated soybean at the genus level. Studies had shown that *Ascomycota*, *Basidiomycota* and *Zygomycota* were dominant phyla in many plants rhizosphere. These fungi had a large number of phylogenetic branches and occupied large components in the fungal community (Zhou *et al.*, 2017a). By analyzing the diversity index of wild and cultivated soybeans rhizosphere, it was found that there was no significant difference in the number and types of rhizospheric fungi between wild and cultivated soybean under the same growth environment. But, the number and types of wild soybean and cultivated soybean rhizosphere fungi were significantly different in different growth environments. In other words, the growth environment had a great influence on the number and types of wild soybean and cultivated soybean rhizosphere fungi, which has the similar pattern to the study on wide and cultivated rice by Yuan (Yuan *et al.*, 2010).

Effects of AMF and Dominant Fungi on Wild and Cultivated Soybeans at Genus Level

Among the dominant fungi *Mortierella* and *Penicillium* were significantly associated with wild and cultivated soybeans. The number of *Mortierella* in wild soybeans rhizosphere is higher than that in cultivated soybeans rhizosphere. *Mortierella* could decompose chitin and hemicellulose, which can provide nitrogen source and carbon source to plants (Lähn *et al.*, 2002; Deacon *et al.*, 2006). In this study, total nitrogen and organic matter in wild soybean soil were lower than those in cultivated soybean (Table 1), so more *Mortierella* will provide more nitrogen and sugar for wild soybeans. Many species of *Penicillium* are plant pathogens (Sillero *et al.*, 2006; Zitnickanderson and Nelson, 2014). The number of *Penicillium* in cultivated soybean rhizosphere was higher than wild soybean. This indicated that wild soybean had better disease resistance, which caused pathogenic bacteria could not accumulate in the wild soybean rhizosphere. While, a large accumulation of pathogenic bacteria accumulated in cultivated soybean rhizosphere due to continuous cropping or other reasons.

AMF played an important role in promoting plant growth and resilience. In this study, we analyzed three AMF fungi (*Paraglomus*, *Funneliformis* and *Rhizophagus*) which were significantly related to wild and cultivated soybeans. *Paraglomus* had been proved to have the effect of improving plants drought resistance (Tian *et al.*, 2013; Ortiz *et al.*, 2015; Smýkal *et al.*, 2015). *Funneliformis* and *Rhizophagus* had been proved to have the effect of improving plant nutrient absorption and enhancing the nutritional value of legume (Pellegrino and Bedini, 2014). Under field conditions, *Paraglomus* only appeared in the wild soybean rhizosphere. Under controlled conditions, *Paraglomus* appeared not only in both wild soybean rhizosphere but also in cultivated soybean rhizosphere. However, the number of *Paraglomus* in wild soybean rhizosphere was higher than that in cultivated soybean rhizosphere. The number of *Funneliformis* and *Rhizophagus* in wild soybeans rhizosphere was lower than that in cultivated soybeans rhizosphere. This means that plants in different environments had different dependencies on AMF, which was similar to the van der Boller's results (Boller *et al.*, 1998). In this study, the environmental stresses under the field conditions were much larger than under the controlled conditions. In order to adapt environmental stresses, a large amount *Paraglomus* accumulated in wild soybeans rhizospheres. Under controlled conditions, the environmental stresses were relatively small. The effects of microorganism on plants were reflected in nutrient elements conversion efficiency. Nutrient elements conversion efficiency determines the strength of the relationship between plants and microorganisms.

Effect of Environmental Factors on Rhizosphere Fungal Community Structure

By analyzing fungal community structure in wild and cultivated soybean rhizosphere, in different growing environment, community structure similarity of wild and cultivated soybeans rhizosphere fungi was different. In the same growing environment, community structure similarity of wild and cultivated soybeans rhizosphere fungi was not different. Some studies also had demonstrated that there were differences in the similarity of the rhizosphere fungal community of plants in different places (Tian *et al.*, 2017; Zhou *et al.*, 2017b). By environmental factors analysis and rhizosphere fungal communities redundancy analysis, it was found that soil physicochemical properties, total phosphorus, available phosphorus, available potassium, pH, and organic matter were significantly associated with cultivated soybean and wild soybean rhizosphere fungi. The content of available phosphorus in wild soybean soil samples was lower than that in cultivated soybean, and there was a significant negative correlation between available phosphorus and wild soybean and cultivated soybean fungal community structure. AMF had an important effect on

activating organic and inorganic phosphorus in the soil (Tawaraya *et al.*, 2006; Richardson, 2011). Especially in P absence place, AMF had the ability to activate insoluble phosphorus, which can effectively improve the absorption of phosphorus from plants rhizospheres (Boris *et al.*, 2018). This study also showed that the connection between AMF and wild soybean is closer than cultivated soybean. AMF can convert the hard to use nutrients in wild soybean rhizosphere soil into available phosphorus, and at the same time strengthen utilization of phosphorus.

Conclusion

Under different growth conditions, there were differences in the number and types of rhizosphere fungi between wild and cultivated soybean rhizosphere, and the similarity of fungal community structure was also different. However, under the same growth environment, the similarity of fungal community structure of wild and cultivated soybeans rhizosphere were the same. Both wild and cultivated soybeans are dependent on the AMF, but dependent on different genus. Wild soybean has a high dependence on *Paraglomus*, especially when the environmental stress is large. Cultivation soybean has a strong dependence on *Funneliformis* and *Rhizophagus*. But, this dependence will disappear when the environmental stress is high. So the changes in rhizosphere of wild and cultivated soybeans under different environments, especially the dependence of wild and cultivated soybeans on AMF was affected by different degrees of environmental stress. This provides a theoretical basis for future research on the application of rhizosphere fungal to improve soybean resistance.

Acknowledgments

This research was financially supported by the Special Foundation for State Major Basic Research Program of China (2016YFC0501202), Special Foundation for Basic Research Program in Soil of CAS (XDB15030103), Key Research Program of CAS (KFZD-SW-112-05-04), National Natural Science Foundation of China (41571255 and 41701332), Key Laboratory Foundation of Mollisols Agroecology (2016ZKHT-05), 135 Project of Northeast Institute of Geography and Agroecology (Y6H2043001), Jilin Provincial Natural Science Foundation (20180520048JH), and the Education Department of Jilin Province (JKH20180687KJ) and the Science and Technology Department of Changchun City, China (18DY020).

References

- Boller, T., A. Wiemken and I.R. Sanders, 1998. Different Arbuscular Mycorrhizal Fungal Species Are Potential Determinants of Plant Community Structure. *Ecology*, 79: 2082–2091
- Boris, L., L. Tomáš and A.M. Manschadi, 2018. Arbuscular mycorrhizae modify winter wheat root morphology and alleviate phosphorus deficit stress. *Plant Soil Environ.*, 64: 47–52

- Chao, A., 1984. Nonparametric Estimation of the Number of Classes in a Population. *Scand. J. Stat.*, 11: 265–270
- Chao, A., 1993. Stopping Rules and Estimation for Recapture Debugging with Unequal Failure Rates. *Biometrika*, 80: 193–201
- Caporaso, J.G., J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello, N. Fierer, A.G. Pena, J.K. Goodrich, J.I. Gordon, G.A. Huttley, S.T. Kelley, D. Knights, J.E. Koenig, R.E. Ley, C.A. Lozupone, D. McDonald, B.D. Muegge, M. Pirrung, J. Reeder, J.R. Sevinsky, P.J. Tumbaugh, W.A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld and R. Knight, 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods*, 7: 335–336
- Deacon, L.J., E.J. Pryce-Miller, J.C. Frankland, B.W. Bainbridge, P.D. Moore and C.H. Robinson, 2006. Diversity and function of decomposer fungi from a grassland soil. *Soil Biol. Biochem.*, 38: 7–20
- Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26: 2460
- Foyer, C.H., H.M. Lam, H.T. Nguyen, K.H.M. Siddique, R.K. Varshney, T.D. Colmer, W. Cowling, H. Bramley, T.A. Mori and J.M. Hodgson, 2016. Neglecting legumes has compromised human health and sustainable food production. *Nat. Plants*, 2: 16112
- Hajjar, R. and T. Hodgson, 2007. The use of wild relatives in crop improvement: a survey of developments over the last 20 years. *Euphytica*, 156: 1–13
- Kim, M.Y. and R.L. Phillips, 2010. Whole-genome sequencing and intensive analysis of the undomesticated soybean (*Glycine soja* Sieb. and Zucc.) genome. *Proceedings National Acad. Sci. U.S. Amer.*, 107: 22032
- Lähn, K., G. Wolf, C. Ullrich-Eberius and E. Koch, 2002. Cultural characteristics and in vitro antagonistic activity of two isolates of *Mortierella alpina* Peyronel. *J. Plant Dis. Sprotection*, 109: 166–179
- Labidi, S., F.B. Jeddi, B. Tisserant, M. Yousfi, M. Sanaa, Y. Dalpé and L.H. Sahraoui, 2015. Field application of mycorrhizal bio-inoculants affects the mineral uptake of a forage legume (*Hedysarum coronarium* L.) on a highly calcareous soil. *Mycorrhiza*, 25: 297
- Ortiz, N., E. Armada, E. Duque, A. Roldán and R. Azcón, 2015. Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: effectiveness of autochthonous or allochthonous strains. *J. Plant Physiol.*, 174: 87
- Pellegrino, E. and S. Bedini, 2014. Enhancing ecosystem services in sustainable agriculture: Biofertilization and biofortification of chickpea (*Cicer arietinum* L.) by arbuscular mycorrhizal fungi. *Soil Biol. Biochem.*, 68: 429–439
- Richardson, A.E., 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant Soil*, 349: 121–156
- Shannon, C.E., 1938. A Mathematical Theory of Communication: The Bell System Technical Journal. *J. Franklin Institute*, 196: 519–520
- Simpson, E.H., 1949. Measurement of Diversity. *Nature*, 163. 163: 688–688
- Sillero, J.C., S. Fondevilla, J. Davidson, M.C. Vaz Patto, T.D. Warkentin, J. Thomas and D. Rubiales, 2006. Screening techniques and sources of resistance to rusts and mildews in grain legumes. *Euphytica*, 147: 255–272
- Shoresh, M., G.E. Harman and F. Mastouri, 2010. Induced Systemic Resistance and Plant Responses to Fungal Biocontrol Agents. *Annu. Rev. Phytopathol.*, 48: 21–43
- Smykal, P., C.J. Coyne, M.J. Ambrose, N. Maxted, H. Schaefer, M.W. Blair, J. Berger, S.L. Greene, M.N. Nelson and N. Besharat, 2015. Legume Crops Phylogeny and Genetic Diversity for Science and Breeding. *Critical Rev. Plant Sci.*, 34: 43–104
- Tawarayama, K., M. Naito and T. Wagatsuma, 2006. Solubilization of Insoluble Inorganic Phosphate by Hyphal Exudates of Arbuscular Mycorrhizal Fungi. *J. Plant Nutr.*, 29: 657–665
- Tian, Y.H., Y.B. Lei, Y.L. Zheng and Z.Q. Cai, 2013. Synergistic effect of colonization with arbuscular mycorrhizal fungi improves growth and drought tolerance of *Plukenetia volubilis* seedlings. *Acta Physiol. Plant.*, 35: 687–696
- Tian, L., X. Zhou, L.N. Ma, S. Xu, F. Nasir and C.J. Tian, 2017. Root-associated bacterial diversities of *Oryza rufipogon* and *Oryza sativa* and their influencing environmental factors. *Arch. Microbiol.*, 199: 563–571
- Tuo, X.Q., H.E. Li and Y.N. Zou, 2017. Alleviation of Drought Stress in White Clover after Inoculation with Arbuscular Mycorrhizal Fungi. *Notulae Botanicae Horti. Agrobotanici Cluj-Napoca*, 45: 220
- Van, P.D.R.H. and H.J.G. Krüger, 2002. Separately and simultaneously induced dark chilling and drought stress effects on photosynthesis, proline accumulation and antioxidant metabolism in soybean. *J. Plant Physiol.*, 159: 1077–1086
- Wang, P., Z. Hui, Z. Jie, D. Zhao, X. Zou, Z. Zhu, N. Jeelani, L. Xin and S. An, 2016. A Hardy Plant Facilitates Nitrogen Removal via Microbial Communities in Subsurface Flow Constructed Wetlands in Winter. *Sci. Reports*, 6: 33600
- Xue, Z.C., H.Y. Guo and J. Liu, 2011. Different response of photosynthetic apparatus between wild soybean (*Glycine soja*) and cultivated soybean (*Glycine max*) to NaCl stress. *Acta Ecol. Sin.*, 31: 3101–3109
- Yuan, Z.L., C.L. Zhang, F.C. Lin and C.P. Kubicek, 2010. Identity, Diversity, and Molecular Phylogeny of the Endophytic Mycobiota in the Roots of Rare Wild Rice (*Oryza granulata*) from a Nature Reserve in Yunnan, China. *Applied Environ. Microbiol.*, 76: 1642–1652
- Zhang, B., D.F. Wang, Y. Fan, L. Zhang and Y. Luo, 2009. Affinity Purification of Trypsin Inhibitor with Anti-*Aspergillus Flavus* Activity from Cultivated and Wild Soybean. *Mycopathologia*, 167: 163–171
- Zhang, J.F., F. Nasir, L. Tian, A. Bahadur, A. Batool, L. Ma, X. Zhou, S.X. Zhao and C.J. Tian, 2018. Impact of ecological factors on the diversity and community assemblage of the bacteria harbored in the rhizosphere of *Hippophae rhamnoides*. doi:10.17957/IJAB/15.068
- Zitnickanderson, K.K. and B.D. Nelson, Jr, 2014. Identification and pathogenicity of *Pythium* on soybean in North Dakota. *Plant Dis.*, 99: 31–38
- Zhou, Y., H. Zhu, S. Fu and Q. Yao, 2017a. Variation in Soil Microbial Community Structure Associated with Different Legume Species Is Greater than that Associated with Different Grass Species. *Frontiers Microbiol.*, 8: 1007
- Zhou, X., L. Tian, J.F. Zhang, L.N. Ma, X.J. Li and C.J. Tian, 2017b. Rhizospheric fungi and their link with the nitrogen-fixing Frankia harbored in host plant *Hippophae rhamnoides* L. *J. Basic Microbiol.*, 57

(Received 09 September 2018; Accepted 11 September 2018)