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SHORT COMMUNICATION

Bacterial Diversity in Soybean Rhizosphere Soil at Seedling and Mature Stages

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Abstract

Changes in the structural diversity of bacterial communities in soybean rhizospheres play important roles in plant growth and crop productivity. However, there are only a few studies on different soybean growth stages. Here, we investigated the changes in the bacterial community of soybean rhizosphere soil at two stages using Illumina high-throughput sequencing. The results showed that the bacterial abundance and diversity in the seeding stage were higher than those in the mature stage and that the diversity changed significantly. *Actinobacteria*, *Acidobacteria*, and *Proteobacteria* were the dominant bacteria in the soybean rhizosphere soil. Additionally, changes in *Actinobacteria* and *Proteobacteria* abundances showed opposite trends.

Key words: soybean, growth stages, rhizosphere soil, bacterial community, Illumina high-throughput sequencing

Rhizosphere microbes are important components of soil ecosystems. These organisms are closely related to soil fertility and are essential indicators for evaluating soil health. Soil microbial community and diversity can reflect changes in the soil environmental quality and reveal differences in microbial ecological functions, which are critical for maintaining soil quality and ecosystem stability (Gertini 2005). Rhizosphere microorganisms can absorb the hydrogen released by nitrogen-fixing nodules of leguminous plants lacking hydrogenase. Experiments have shown that bacteria remove hydrogen (Mclearn and Dong 2002), and these bacteria were beneficial to the growth of plants (Dong et al. 2003; Abdellatif et al 2017). Therefore, studying the rhizosphere microbial diversity of legumes will improve our understanding of microbes that promote the growth of legumes.

Accordingly, in this study, Illumina high-throughput sequencing was used to investigate the microbial communities of rhizosphere soil samples. The soybean field was located in Xianyang, Shaanxi, NW China (107°38'–109°10' E, 34°11'–35°32' N), which is a typical warm temperate continental monsoon climate. The soil is dominated by ash-calcium soil. The soybean rhizosphere soil samples, numbered as S, were collected on June 24, 2016, when after more than half of the cotyledons were breaking out, and also the soil samples, numbered as M, were collected on September 30, 2016. At this time, the leaves and beans were dehydrated, demonstrating the inherent traits of the varieties.

The five-point sampling method was used to collect the rhizosphere soil samples according to the shake-off method of Riley et al. (1969). The soil sample was divided into two portions after filtering through a 2-mm sieve. One portion was stored in an –80°C refrigerator for soil microbial diversity analysis and was sequenced using an Illumina HiSeq 2500 at Biomarker Technologies (Beijing, China). The primers used for sequencing were 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Zhang et al. 2015); the other was naturally air-dried and sieved for soil physical and chemical properties analysis in a reference to Soil Agrochemical Analysis (Bao 2000).

Some physical and chemical properties of soil in different time are shown in Table I. There were significant differences in pH, available potassium, and soil

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Table I
Changes in basic physical and chemical properties of soils at different sampling times.

	S	M
Organic matter (g/kg)	1.40 ± 0.05a	1.51 ± 0.02a
Total nitrogen (g/kg)	2.05 ± 0.02a	2.13 ± 0.07a
pH	7.30 ± 0.04a	7.55 ± 0.03b
Available phosphorus (mg/kg)	7.54 ± 0.04a	7.59 ± 0.09a
Available potassium (mg/kg)	86.09 ± 0.93a	88.18 ± 0.40b
Soil temperature (°C)	18.48 ± 0.13a	23.65 ± 0.09 b
Moisture content (%)	30.58 ± 0.91a	31.27 ± 0.83a

temperature, whereas other indicators did not differ at the two sampling times. The contents of organic matter, total nitrogen, available phosphorus, and potassium, as well as the moisture in M soil samples were higher than those in S soil samples, and the pH and temperature increased in the M phase.

The coverage of each sample was greater than 99%, demonstrating that the sequencing results could reflect the real situation of the sample. The ACE ($P = 0.453$) index and the Chao1 ($P = 0.909$) index were higher at the S stage than at the M stage, indicating that the S stage soil samples were rich in microorganisms; however, the difference was not significant. The Shannon index of the S stage was larger than that of the M stage, and the P values between the S and M stages were the Shannon index ($P = 0.00074$) and Simpson index ($P = 0.0037$), respectively, indicating that the diversity of the bacteria were higher in the S stage.

As shown in Fig. 1, ten phyla including *Actinobacteria*, *Acidobacteria*, *Proteobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, *Chloroflexi*, *Verrucomicrobia*, *Nitrospirae*, *Planctomycetes*, and *Armatimonadetes* were obtained from the six soil samples.

The dominant phyla at the S and M stages were *Actinobacteria*, *Acidobacteria*, and *Proteobacteria*. At the S stage, *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* accounted for 27.7%, 27.2%, and 14.3%, respectively; at the M stage, *Proteobacteria*, *Actinobacteria*, and *Acidobacteria* accounted for 17.0%, 38.1%, and 21.8%, respectively. Compared with the soil samples collected at the S stage, the number of *Actinobacteria* increased by 165.6%, and the numbers of *Acidobacteria* and *Proteobacteria* decreased by 20.1% and 38.6%, respectively.

Bacteria are important soil microbes, and their types and quantities have direct effects on soil biochemical properties and soil nutrients. The Illumina high-throughput sequencing is widely used in the study of soil microbial diversity owing to its rapid, convenient, and highly accurate results. In this study, we analyzed the community and diversity of bacteria in rhizosphere soil from two different growth stages. We found that

Table II
Alpha diversity of samples.

	S	M
ACE	1,590.75 ± 6.14a	1582.79 ± 12.11a
Chao1	1594.44 ± 5.52a	1593.50 ± 9.42a
Shannon	6.31 ± 0.0100a	5.70 ± 0.0919b
Simpson	0.0047 ± 0.00a	0.0152 ± 0.0024b
Coverage	0.9993	0.9983

microbial abundance and diversity at the S stage were higher than those at the M stage. Many factors influence microbial diversity (Lupwayi et al. 2001; Schutter and Dick 2002; Lipson 2007; Peralta et al. 2013). Rasche et al. (2011) suggested that humidity and temperature are the main environmental factors affecting soil microbes in temperate forests, similar to the results of the present study. The appropriate soil temperature affects the availability of nutrients in the soil and the growth environment of microorganisms, further stim-

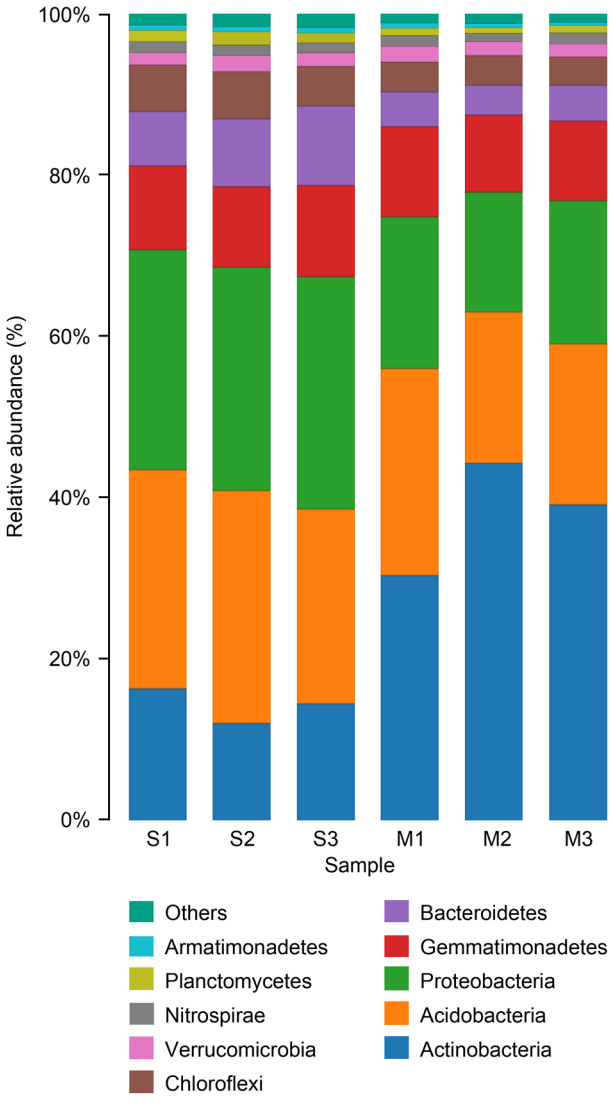

Fig. 1. Microbial communities at the phylum level.

ulating the activity of soil microbes and function of soil microbial communities (Luo et al. 2001; Melillo et al. 2002). Suitable water can significantly change the microbial community and activity. A certain degree of humidity in the soil plays an important role in cultivating strong seedlings and improving the ability to resist drought in the later period. In June, the temperature and rainfall in Xianyang were appropriate, which led to increases in the number of nodules, the emission of hydrogen gas during nitrogen fixation, and enhancement of the number of hydrogen-oxidizing bacteria (Dong and Layzell 2001; Dong et al. 2003). This caused soil microbial diversity and abundance to be high in June. In September, the soil temperature rose significantly to 23°C, which was beneficial for microorganism growth; however, the diversity was lower in the M stage than in the S stage. The reason for this result may be that the appropriate temperature enhanced microbial metabolism, changing some specific flora into the main flora. These dominant bacteria had a competitive advantage over other microbiota, resulting in the proliferation of a few dominant phyla, whereas others were inhibited or eliminated during competition. At the same time, there was heavy rain in Xianyang in September 2016. Unger et al. (2009) also found that continuous flooding conditions reduced soil microbial biomass and affected the soil microbial community, causing microbial diversity and abundance to decrease.

In this study, we found that compared with the S stage, the number of *Actinobacteria* increased, and both *Proteobacteria* and *Acidobacteria* decreased in the M stage. At the M stage, the nitrogen fixation capacity of soybean nodules is weakened, and the hydrogen concentration is lowered. Studies have shown that certain microorganisms in *Actinobacteria* (Constant et al. 2008; Constant et al. 2010) consume atmospheric tropospheric trace hydrogen at 0.553 ppmv. This may increase the number of *Pseudonocardia* and *Nocardioides*. Whether these two genera can absorb atmospheric trace hydrogen is worthy of further study. Li et al. (2018) found that in the original rhizosphere soil samples of *Medicago sativa* and the hydrogen-treated soil samples, changes in the abundances of *Actinobacteria* and *Proteobacteria* showed opposite trends. In this study, we found that soil samples from the rhizospheres of soybeans also showed similar trends. Further studies are needed to determine whether this trend exists in other legumes or other crops. Many studies have shown that the microbial community is related to the growth stage. For example, Duineveld et al. (2001) found that the microbial community structures of rhizosphere soils of young plants and mature plants differed. Additionally, Farina et al. (2012) found that the microbial community structure of canola differed accordingly to growth stage, with the greatest abundance during the rosette period. Xu et al. (2009) also found that the bacterial diversity in soybean rhizosphere increased primarily and then decreased; the increase was observed beginning from the flowering period, reached a maximum during the drum period, and was lowest during the maturity period.

In summary, the soybean growth period was found to have an important influence on the bacterial community in rhizosphere soil, and bacterial diversity was mainly affected by soil temperature and humidity. Changes in the abundances of *Acidobacteria* and *Proteobacteria* showed opposite trends. Our findings provided a theoretical basis for understanding the diversity and changes in the bacterial community in soybean rhizosphere soil.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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