INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18F–033/2018/20–7–1632–1640
DOI: 10.17957/IJAB/15.0689
http://www.fspublishers.org

Full Length Article

Impact of Ecological Factors on the Diversity and Community Assemblage of the Bacteria Harbored in the Rhizosphere of *Hippophae rhamnoides*

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Abstract

There is concerted understanding that soil bacterial communities have the main influence in directing the structure of soil environment yet little is acknowledged about their ecological distribution. We assessed the distribution pattern for the unique community structure and rich diversity of bacteria including *Frankia* and the connection between environmental factors and ecological distance. Bacterial communities in *Hippophae rhamnoides* roots were evaluated along 6 geographically distributed sites in Tibet-Sichuan signifying various soil characteristics. Using Illumina MiSeq sequencing the V3-V4 region of bacterial 16S rRNA genes, *Actinobacteria*, *Proteobacteria*, and *Cyanobacteria* were found the dominant phyla across all samples, while in *Actinobacteria*, the *Fankia* was abundant and total 5 *Frankia* operational taxonomic units (OTUs) were classified based on 97% sequence similarity, respectively. pH and altitude correlated to (OTUs) richness in bacteria however in *Frankia*, it was with Shannon's diversity indicating its significant effect on community. In addition, the geographic distance and soil characters significantly contributed to the variation in the bacteria and *Frankia* community and diversity. This is the leading and most comprehensive research that has tested ecological distribution of bacterial assemblage. Our findings support that the environmental factors have direct influence on ecological distribution, shaping bacterial community and diversity of Tibetan and non-Tibetan region, China. © 2018 Friends Science Publishers

Keywords: Bacterial diversity; Community assembly; *Frankia*; Geographic distance; *Hippophae rhamnoides* L

Introduction

The nitrogen-fixing trees play an important role in nitrogen (N) balancing and recycling within natural ecosystems (Trujillo *et al.*, 2010; Pasternak *et al.*, 2013; Kucho *et al.*, 2014) and this nitrogen-fixing ability is due to the nodule that is formed by the symbiotic nitrogen-fixing bacteria and the roots (Mirza *et al.*, 2009). Root nodule-based nitrogen fixing associations are ecologically vital communications that provide huge natural involvements in the universal N cycle. The nodules in non-leguminous plants are formed by *Frankia* (*Actinobacteria*) that is different to leguminous nodules formed by *Rhizobium* (*Proteobacteria*) (Anderson *et al.*, 2013; Walker *et al.*, 2014). Sea-buckthorn (*Hippophae rhamnoides* L.) is a small non-leguminous nitrogen-fixing tree growing globally. It has two kinds of symbioses including the nodule and mycorrhiza (Bosco *et al.*, 1992; Mishra *et al.*, 2010) and performs rich root nodulation and symbiotic nitrogen-fixation that is vital for degraded ecosystem restoration and improving soil conditions, has a very good flexibility in drought, cold and barren environs (Wang *et al.*, 2010; Jia *et al.*, 2012). Hence, it is a type of perfect pioneer species for vegetation restoration in the Loess Plateau. However, until now, the diversity and community assembly of rhizospheric bacteria harbored in the sea-buckthorn are not clarified at large

To cite this paper: Zhang, J., F. Nasir, L. Tian, A. Bahadur, A. Batool, L. Ma, X. Zhou, S. Zhao and C. Tian, 2018. Impact of ecological factors on the diversity and community assemblage of the bacteria harbored in the rhizosphere of *Hippophae rhamnoides*. *Int. J. Agric. Biol.*, 20: 1632–1640

geographic scale. Evidence to date designates that in the habitats bacterial community and nitrogen-fixation *Frankia* may be appreciable in China.

Effect of geographical distance with or without involvement of environmental effects recommends that current microbial distributions can be affected by past events (speciation, extinction and dispersal limitation) (Oakley *et al.*, 2010; Martiny *et al.*, 2011). Soil microbes are abundant, genetically diverse and functionally key organisms (van der Heijden *et al.*, 2008) and hence exploring their bio-geographical trends and what operate them is crucial to sustain ecosystems under climate and eco-environmental changes. Under different scales, the natural selection theory and competitive inhibition theory play an important role in determining microbial community structure (Hazard *et al.*, 2013; Penczykowski *et al.*, 2016). Compared with most soil microbial groups susceptible to environmental variation, the root-associated microbes are also sensitive to the selective pressure by the host plant (Anderson *et al.*, 2013; Horn *et al.*, 2014). Several remarkable investigations have acknowledged numerous edaphic factor that influence soil bacterial community compositions. Ecological factors have been notorious to impact the soil bacteria (Lauber *et al.*, 2009).

Exploring the environmental factors that affect the microbial community's variation across various scales is a main objective of ecology and biogeography. Numerous researchers have shown that present environmental factors, such as soil pH, trophic status, rhizosphere, and metalloid contamination explain and shape variation in microbial community structure. In odd, and since these great effects by local factors, interact with geographic distance and microbial community structure that is still remained controversial and elusive (Martiny *et al.*, 2006). Several studies have recognized several edaphic variables that affect the soil fungal and bacterial community structures, and several of them are linked to operating distributions. Soil pH has also been reported to clearly affect the bio-geographical patterns of soil bacterial community (Lauber *et al.*, 2009). Soil microbes govern a diversity of ecological and biogeochemical processes such as phosphorus (P), N, potassium (K), and carbon cycling, and eventually deliver precarious ecological services including greenhouse gas mitigation and soil fertility (Green *et al.*, 2008). Soil bacteria form a symbiotic association with plant roots. Through their influences on plants, soil bacteria maintain different ecosystem stability, practices, and functions in the Tibetan Plateau.

The Tibetan Plateau considered as roof of the world is known as "the third pole", that is one of the most sensitive regions to global climate change in the world and also rich with natural treasure (Qiu, 2008). As of its unique geographic history, recent decades have witnessed severe climatic impacts which led this area into a susceptive region (Zhang *et al.*, 2013). More recently, substantial development has been made to maintain the vegetation ecology and aboveground flora in response to variations in environmental conditions (Yang *et al.*, 2014). Due to the unique geologic settings and formation of Tibetan Plateau, greater attention has been paid to track below-ground microbial distribution responding to environmental changes (Yu *et al.*, 2015). However, we still acknowledge little information about how bacterial communities might reflect to changes in key ecological and environmental services (Fierer *et al.*, 2010), which restrict our ability to calculate the ecosystem level replies by the Tibetan Plateau and to future climatic changes (Hawkes *et al.*, 2011).

Here, we nominated the *H. rhamnoides* mostly distributed along the Tibet-Sichuan highway from high to low altitudes, as a host plant to assess the rhizospheric bacteria by using Illumina MiSeq Sequencing. There has been no such study at this level. Here we determine the diversity and community assembly of the rhizospheric bacteria including symbiotic nitrogen-fixing *Frankia* under selective pressure of the ecological factors to answer the following main questions: (1) Do distributional patterns govern diversity of the rhizospheric bacteria community including symbiotic nitrogen-fixing *Frankia*? (2) How do the ecological distance influence the community assembly and diversity?

Materials and Methods

Locations and Sampling Procedures

The study was performed in the population of *H. rhamnoides* L. and the sampling sites were distributed along the Tibet-Sichuan highway from high to low altitudes (Table 1). Six study locations along a 3000 km geographical transect, located in central and northeastern (two provinces: Shanxi and Liaoning) and Tibetan Autonomous Region (three provinces: Sichuan, Gansu, and Qinghai), China (Fig. 1).

Twenty samples were collected at intervals of several miles (3–5 samples per site) from the six sites, respectively. At each site, 5–10 individual plants were collected on each transect, with the stipulation that the sites were at least 20 m apart to avoid clonal growth. Approximately 5~10 centimeters of soil was removed, and the soils at a depth of approximately 20~30 centimeters were collected and homogenized in the field. Soil and plant samples were collected in September 2014. Soil samples for molecular and physiochemical analysis (Table S1) were packed in polyethylene bags, and immediately stored in ice boxes and then transferred to the lab and stored at -20^{o}C until further processing. The root samples were collected and used for DNA extraction and bacterial diversity analysis.

Physiochemical Data Measurements

All replicates were operated for soil chemistry analysis. The total N and soil organic C contents were determined by dry combustion on ground samples (100-mesh) with an

elemental analyzer (Vario EL III, Elementar Analysensysteme GmbH, Germany). Soil pH was measured with a glass electrode using a soil-to-water ratio of 1:2.5. Available N, P, and K contents were determined according to the reference of Luo *et al.* (2018).

DNA Extraction and Sequencing

The nodules from different host plants were combined and cut into pieces before being ground into powder with liquid N. Afterwards, DNA was extracted using a Fast DNA SPIN kit (Catalog No. 6560-220, Germany). The DNA concentration and purity were analyzed by NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, USA) and the qualified DNA was used for the following PCR.

The V3-V4 hypervariable regions of the 16S rRNA for bacteria (341F "CCTACGGGNGGCWGCAG" and 785R "GACTACHVGGGTATCTAATCC") were targeted for two rounds of PCR amplification before sequencing. The reaction parameters were as follows: an initial denaturation for 4 min at 94°C, followed by 35 amplification cycles of denaturation (94°C for 50 s), annealing (54°C for 50 s), and extension (72°C for 50 s), and a final extension at 72°C for 10 min. The PCR products were checked by agarose (Invitrogen, California, USA) gel electrophoresis. Then the 2×300 bp paired-end sequencing of PCR amplicons were performed on a MiSeq platform (Illumina, San Diego, CA, USA) at Beijing Ori-Gene Science and Technology Co., LTD. The raw paired sequence was combined by FLASH to get the clean sequence (Magoc and Salzberg, 2011). Then the clean sequence was filtered by mothur to delete the sequences with score<20, with mismatched primer longer than 4 bp, shorter than 200 bp and longer than 500 bp (Schloss *et al.*, 2009). Then UCHIME was used to delete chimera referred to Gold database (Edgar *et al.*, 2011). The qualified and filtered sequences were used for taxonomy assignment using RDP classifier based on SILVA database (Wang *et al.*, 2007) with minimal 80 confidence estimates. The operational taxonomic units (OTUs) at the 97% similarity level were classified based on USEARCH (Edgar, 2013), and singletons were removed. Random re-sampling was performed with the least sequences of all samples. Alpha diversities include Shannon and abundance indexed was performed by mothur. UniFrac distance was calculated by FastTree (Price *et al.*, 2009). Rarefaction curves were estimated based on an OTU across different soil samples (Fig. S1). Total bacterial communities were characterized by Illumina MiSeq sequencing of rhizosphere soil from six sites surveyed by using three to five replicates. Across all 20 samples, a total of 192,272, ranging from 2×300 bp, raw reads of 16S rDNA V3-V4 bacterial sequences were obtained. After removing the low quality reads, a total of 178,535 valid reads were obtained with an average of 8927 effective reads and 5206 least reads per sample. The reads were clustered according to 97% similarity level.

Statistical Analysis

The operational taxonomic units (OTUs) at the 97% similarity level were classified based on USEARCH (Edgar, 2013), and singletons were removed. The taxonomic assignment was conducted by Ribosomal Database Project (RDP) classifier (Wang *et al.*, 2007) with minimal 50 confidence estimates. Random re-sampling was performed with the least sequences of all samples. Detrended correspondence analysis (DCA) was used to explore changes in overall bacterial community composition. Partial Mantel tests based on the Bray-Curtis distances were performed to correlate microbial communities with environmental parameters. Principal components analysis (PCA) was used to collapse soil geochemical parameters into vectors. Redundancy analysis (RDA) was chosen to examine the correlation between the bacterial distribution and environmental factors. All of the analyses were conducted by functions in the VEGAN package (V.2.0-3) in R software.

The Shannon-Weaver index was used to evaluate microbial diversity, and Simpson's evenness was used to evaluate microbial community evenness. The differences in diversity across sampling sites were evaluated by analysis of variance (ANOVA) followed by a least significant difference (LSD) test. The phylogenetic trees were constructed by using Neighbor-Joining method with MEGA 5.1 software, and *Escherichia coli* (HF584703) was selected as the out-group.

To estimate the effects of environmental factors on bacterial diversity and richness, structural equation models were fitted to our data with the IBM SPSS Amos 22. We used soil pH, organic matter, organic C and N, available N, P, K, and geographical distance as explanatory variables. We evaluated the significance of the model fitness according to the $\chi 2$ value, P value, root mean square error of approximation (RMSEA), and goodness-of-fit index (GFI). In addition, the variation partition analysis, to explore the effects of geographic distance and environmental variables on the community assembly of bacterial OTUs was carried out by RDA analysis in vegan 2.3-3 package of R 3.2.1 software.

Results

Amplification of Rhizospheric Bacterial Communities and *Frankia* by using Illumina MiSeq Sequencing

Based on the current classification criteria, *Frankia* (nitrogen-fixing bacteria) is the only genus identified in the (phylum *Actinobacteria*) family *Frankiaceae* and we found a total of 38,279 valid reads, with an average of 1914 effective reads and 432 least reads per sample. The cluster for the OTUs of *Frankia* was carried out based on 97% similarity level, and total 5 OTUs was produced including OTU1, OTU2, OTU5, OTU3, and OTU4 with the relative

abundance of 99.30%, 0.68%, 0.11%, 0.01% and 0.01%, respectively (Fig. 1).

Diversity and Community Assembly of the Rhizospheric Bacteria and *Frankia*

Of all the OTUs, effective bacterial sequences in the 20 samples were consigned and 29 phyla were obtained containing seven candidates and two unidentified phyla. The dominant phyla across all soil samples were *Actinobacteria*, *Proteobacteria*, and *Cyanobacteria*, as shown in Fig. 2. Across the samples from the Tibetan highest region (XZ), *Proteobacteria* was the dominant phylum of the total 16S rRNA gene reads sequences. The relative abundance of the *Actinobacteria* was found in higher percentage in Tibetan regions including Qinghai (QH), Gansu (GS), Sichuan (SC), and Tibetan highest region (XZ) than non-Tibetan regions such as Liaoning (LN) and Shanxi (SX), respectively that might be due to different altitude (Fig. 2 and Table S1). However, LN and SX contained higher percentage of Candidate_division_TM7 (Fig. 2).

For bacteria, the distributional pattern of the Shannon's diversity matched with OTUs richness whereas in *Frankia*, the Shannon's diversity pattern was quite different than that of OTUs richness (Fig. 3). The bacterial diversity between Tibetan and non-Tibetan regions was not significantly different while the diversity of *Frankia* in XZ was significantly lower (Fig. 3A and B).

Environmental factors such as AN (available nitrogen), pH, and altitude showed positive correlation while TN (total nitrogen) and AK (available potassium) showed the negative correlation with OTUs richness in bacteria. Though TN had significantly positive correlation while OM, AN, and AK showed the negative association with Shannon diversity index respectively, in bacteria (Table 1). Moreover, organic matter (OM) significantly correlated with Shannon diversity index and OTUs richness of *Frankia*, while the AP, pH and altitude were only correlated to the Shannon diversity index and TN and AK to OTUs richness in *Frankia* (Table 1).

Ecological Factors Contribution to the Community Assembly of the Rhizospheric Bacteria and *Frankia*

We used the weighted and un-weighted Unifrac distance metrics to estimate β-diversity. RDA was performed to expose what ecological factors shifted bacterial structure and *Frankia* in the rhizosphere. Statistical analysis revealed that ecological variables were different across sampling sites (Fig. 4). The results of these analysis showed that the dominated factors acting on bacterial group cluster were pH and altitude. The pH value showed a negative correlation to the bacterial distributional pattern from XZ to SC, GS, QH, LN and SX, while the altitude showed a positive correlation. In addition, AN, TN, OM and AP correlated at a certain degree.

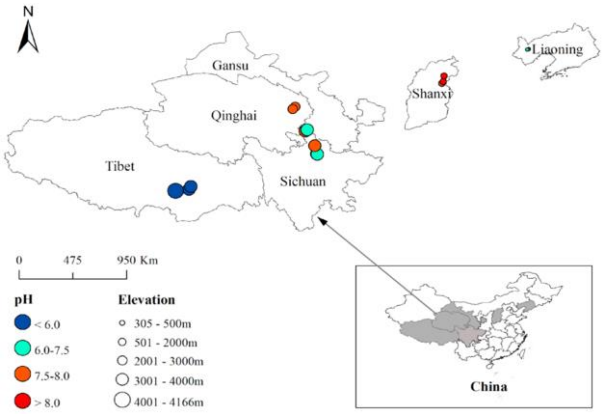

Fig. 1: Site location, soil types and altitude of the 20 *Hippophae rhamnoides* populations selected for study. The sites in Tibet (XZ), Qinghai (QH), Gansu (GS) and Sichuan (SC) represent the regions located in the Tibetan Plateau, while Shanxi (SX) and Liaoning (LN) represent non-Tibetan Plateau regions.

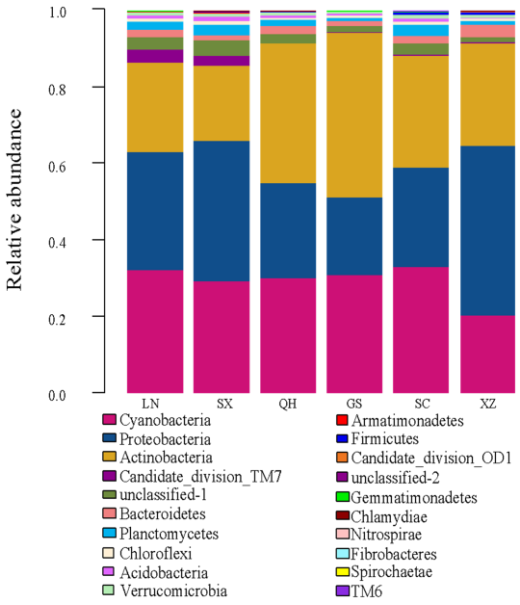

Fig. 2: The relative abundance of dominant phyla of bacteria harbored in the rhizosphere of *Hippophae rhamnoides* from Tibetan and non-Tibetan Plateau regions

The linkages between the geographical distance and community dissimilarity of bacteria and *Frankia* were examined, (Fig. 5A and B) which exhibited very strong and significant ($P< 0.01$) correlations with r values of 0.64 and 0.21, respectively. Linear regression analysis was conducted to investigate the effect of the individual environmental factor on the relative abundance of the dominant bacterial phyla (*Cyanobacteria*, *Proteobacteria*, and *Actinobacteria*) in soil samples (Fig. 2).

Table 1: Partial Mantel test analyses for the correlation between environmental factors and both bacteria and *Frankia*, the association was calculated by Shannon's diversity index and OTUs richness

Environmental factors (unit)	Bacteria		*Frankia*	
	OTUs richness	Shannon diversity index	OTUs richness	Shannon diversity index
SOM[a] (g.kg^{-1})	- 0.01	**-0.29***	**-0.49***	**0.31***
TN (mg.kg^{-1})	**- 0.14***[b]	**0.33***	**0.62***	-0.19
AN (mg.kg^{-1})	**0.11***	**-0.42***	-0.12	0.33
AP (mg.kg^{-1})	0.09	0.10	0.18	**0.32***
AK (mg.kg^{-1})	**-0.34***	**-0.58***	**0.26***	0.14
Soil pH	**0.81***	0.08	0.12	**0.22***
Altitude (m)	**0.12***	0.04	0.19	**0.71***

[a]Abbreviations: SOM, Soil organic matter; TN, Total Nitrogen; AP, Available Phosphorus; AK, Available Potassium
[b]Significant differences (P<0.05) indicated by bold letters . **P<0.01, *P<0.05

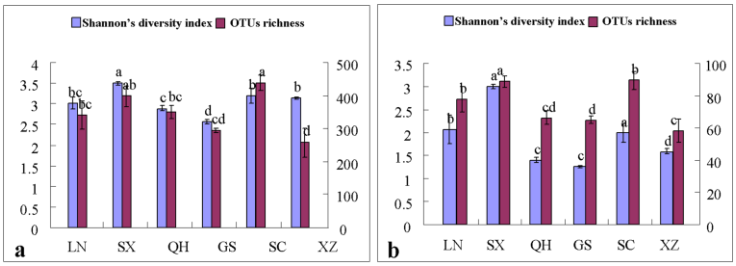

Fig. 3: Significant difference analysis patterns for Shannon's diversity index and OTUs richness across different samples for bacteria (A) and *Frankia* (B), respectively. Liaoning (LN), Shanxi (SX), Qinghai (QH), Gansu (GS), Sichuan (SC) and Tibet (XZ) represent the samples locations

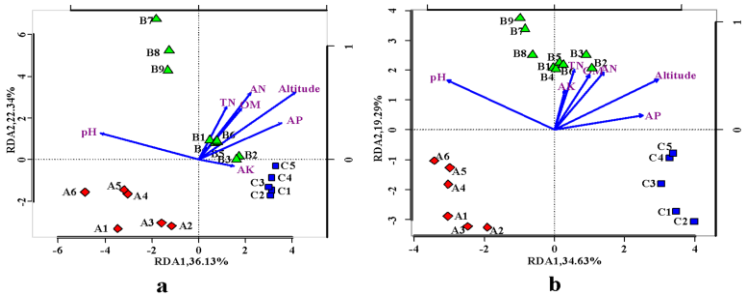

Fig. 4: Environmental factors contribution on the beta-diversity of the bacteria, based on the redundancy analysis (RDA) of weighted (A) and unweighted (B). The explanatory values of the first and second canonical axis were shown as RDA1 and RDA2 respectively. The purple words represent the environmental factors, while the black words indicate sampling locations

The altitude was negatively correlated with *Cyanobacteria* (r=0.38, P< 0.1) and *Proteobacteria* (r=0.1, P< 0.68), while it was significantly positively correlated with *Actinobacteria* (r=0.43, P< 0.04). We examined a strong positive correlation between pH and *Cyanobacteria* (r=0.77, P< 0.01), but a strong negative association with *Proteobacteria* (r=0.71, P< 0.01). The OM application rate was strongly negatively correlated with *Proteobacteria* (r= -0.44, P< 0.04), and strongly positively correlated with *Actinobacteria* (r=0.76, P< 0.01), respectively (Fig. S2).

Effect of Environmental Factors and Geographic Distance on Bacterial and *Frankia* Communities

Variation partition analysis (VPA) was performed to elucidate the contributions of geographic distance and soil characteristics to the bacterial community and *Frankia* (Fig. 6). Geographic distance contributed 7.13% and 3.71%, while soil characters contributed 60.45% and 56.79% on the community assembly of bacteria and *Frankia*, respectively which indicate vast variation in both factors. Altitude contributed equally (5.95% and 5.35%) in both bacterial and *Frankia* community however, the pH effect on bacterial community was 11.73%, and on *Frankia* was 3.60%, respectively. In addition, the nutrition factors AK and AN also had various contributions in both bacteria and *Frankia* community. Meanwhile, a considerable amount of deviation in bacterial community (32.42%) and *Frankia* (39.50%) were unexplained by measured ecological factors.

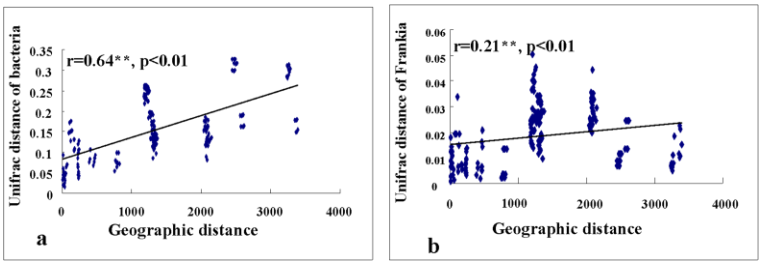

Fig. 5: The correlation between the geographic distance and community dissimilarity of bacteria (A) and *Frankia* (B) based on the weighted UniFrac distance. The r-values were correlation coefficients (**P*<0.05, ***P*<0.01)

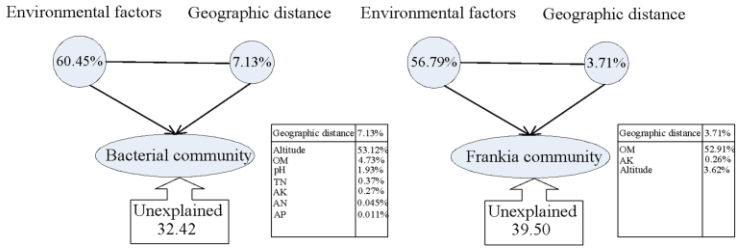

Fig. 6: Variation partition analysis (VPA) indicating the effects of the geographic distance and environmental variables on the community assembly of bacteria (A) and *Frankia* (B) based on the OTUs. Numerical values were the single explanatory values of the variable and values in italic font indicate the non-significant contribution of variables to community variation

By performing neighbor-joining phylogenetic analysis of phylum *Actinobacteria*, a total of five classes, 11 orders, 27 families, and 40 genera were obtained. A phylogenetic tree was constructed which indicated that genus *Frankia* were the dominant one. For the genus of *Frankia*, five OTUs were classified based on 97% sequence similarity, and the dominant OTU1 was identified as the typical species colonized in the host plant family of *Elaeagnus* (Fig. 3).

Discussion

In present study, we mainly focused to assess the relative predictive power of various ecological factors in determining bacterial community composition including nitrogen-fixing *Frankia* along the Tibet-Sichuan highway from high to low altitudes. The main community factors were explored including geographic distance, environmental drivers and distribution patterns and below we will explain each of them regarding our hypothesis.

Bacterial Community Structure and Biodiversity Harbored in the Rhizosphere of Nitrogen-fixing *H. rhamnoides*

This study was the first region scale based, emphasizing the bacterial community and nitrogen-fixing *Frankia* of rhizosphere from *H. rhamnoides*, along the Tibet-Sichuan highway from high to low altitudes. We used Illumina MiSeq sequencing based on bacterial 16S rRNA gene. Though, the distributional pattern for the community and diversity of the rhizospheric bacteria including symbiotic nitrogen-fixing *Frankia* were mainly quantified.

The results illustrated that there was a unique rich diversity of bacteria harbored in the rhizosphere of *H. rhamnoides* from different regions and dominant three phyla were identified such as *Actinobacteria*, *Proteobacteria*, *Cyanobacteria*, respectively (Fig. 2). Our findings were contradictory to previous studies that illustrated different root-associated microbial community such as soil or plant roots microbes (Ding *et al.*, 2015; Yuan *et al.*, 2016). Chaparro *et al.* (2014) quantified the dominant phyla were *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, and *Cyanobacteria* during the different plant developmental stages and showed that the community structure did not significantly variant with respect to its evenness, diversity, and richness. Moreover, *Acidobacteria*, *Betaproteobacteria*, *Proteobacteria*, and *Actinobacteria* were found the leading taxa in the soils of German forest (Nacke *et al.*, 2011). Conclusively, these studies recommended that dispersal limitation was not much evitable in elucidating bacterial community (Finlay and Clarke, 1999; Finlay, 2002).

Another specific characteristic of the rhizospheric bacteria in the nitrogen-fixing sea buckthorn was observed that the symbiotic nitrogen-fixing bacteria were the dominant groups and the phylum of *Actinobacteria* was dominant one, accounting for approximately one-third across all identified bacterial groups. Furthermore, as expected, the nitrogen-fixing *Frankia* was dominant genus identified in the rhizosphere, which was consistent with

many reports (Jia *et al.*, 2012; Zhong *et al.*, 2013). From the phylum of *Actinobacteria* to the genus of *Frankia*, the variation on different microbial classification levels might provide us useful information on microbial distribution and evolution, especially concerning, which level of the microbial community might match with the selection pressure of the host plant and environment (Desbrosses and Stougaard, 2011).

The OTU1 took the absolute highest abundance (>99%) in all *Frankia*. However, here we found an exception that *Frankia* OTU1 was clustered into *Elaeagnus* host plants, the others were not. There was a possibility that some identified OTUs of *Frankia* could be originated from soil rather than purely host cell-colonized ones, so it might be the reason why some OTUs were out of the scope with fixed host plants as we expected.

Ecological Factors Contribution to the Bacterial Diversity and Community Assembly

The OTUs richness represents how many species were identified, while the Shannon's diversity was quantified by both species identification and its relative abundance in the community. Accordingly, in our study these two indexes showed different feedback to the ecological factors.

It is suggested from our results that the soil characters affected the microbial biodiversity, and the effect was dependent on the factor and microbial group. Different microbial groups had their own living strategies for proper ecological niches, which led to the response of microbes to the ecological factors. For example, comparing with the bacteria living in soil, *Frankia* in the rhizosphere was more sensitive to the selection pressure by the host plant that provides the carbon for its growth. In addition, in contrast to *Frankia*, the other rhizospheric bacteria maintained a balance in diversity by adjusting the relative abundance across all kinds of bacterial groups. Soil characteristics including the pH and altitude were more effective to shape the diversity of *Frankia* rather than on the total bacteria community while the N and K factors were more effective to shape the total bacterial diversity rather than structuring the *Frankia* diversity (Table 1). In contrast to our findings, C/N ratio and soil chemistry and plant developmental stages were key factors to shape *Frankia* structure and assemblages (Kato *et al.*, 2007; Põlme *et al.*, 2014).

What was the key contribution by different ecological factors to the bacterial community assemblage? The RDA analysis indicated that there was a high degree of variation across regions-based distribution (Fig. 4), and several environmental factors was surprisingly predictable to influence the rhizospheric bacteria, which we also saw in other studies (Davison *et al.*, 2015). The influence of pH and altitude on rhizospheric community structure was obvious across total bacteria and *Frankia* even we found very slight difference between them. A large body of literature witnessed the association between environmental factors and microbial structure (Fierer and Jackson, 2006) and most of these scales are evitable in governing the ecosystem processes (Lauber *et al.*, 2009). Bacterial community's diversity and structure stabilized with the pattern of soil pH gradient (Hu *et al.*, 2016; Wei *et al.*, 2017). Zhang *et al.* (2013) reported that the altitude was one of the key factors influencing the nitrogen-fixing bacterial community in alpine prairie soil. The ecological factors, which are closely correlated to altitude, might be a driving factor in the composition of the nitrogen-fixing bacterial community of Antarctic soil (Niederberger *et al.*, 2012).

Geographic distance had great significant effects on community abundance as confirmed by our results and showed correlation between the geographic distance and the Unifrac matrix for the total bacteria or *Frankia* (Fig. 5). Present study results suggest stronger influence of geographic distance on bacterial community dissimilarity because of their different environmental drivers. Zhou *et al.* (2017) demonstrated that the overwhelming effects of soil characteristics and environmental factors on the variation in soil microbial community's abundance by comparing with distance factors. Other studies have emphasized that soil with similar environmental characteristics have similar bacterial communities regardless of geographical distance (Green *et al.*, 2004; Hornerdevine *et al.*, 2004; Scheibe *et al.*, 2015). Our statements are in agreement with previous findings in other various ecosystems, where geographic distance had greater effect on bacterial community (Martiny *et al.*, 2011; Pasternak *et al.*, 2013).

VPA was used to quantify the effects of geographic distance and environmental variables on the community assemblage composition of bacteria and *Frankia* detected at OTUs for the Tibetan and non-Tibetan regions (Fig. 6A and B). These variables on VPA explained 67.58% and 60.5% of the variation for bacteria and *Frankia* community, respectively. In addition, there was 32.42 and 39.50% of the variation was unexplained respectively. There into, the geographic distance explained 7.13% and 3.71%, while soil characters explained 60.45% and 56.79% of the variations for both bacteria and *Frankia*, respectively. Hence, our results verified that rhizospheric bacterial community structures were influenced by geographic distance and soil character which was consistent with previous study (Fierer and Jackson, 2006; Kaiser *et al.*, 2016). Several vital environmental factors might elucidate the variation of microbial diversity (Sun *et al.*, 2016; Xia *et al.*, 2016). We also profiled that the altitude (5.95% and 5.35%) and pH (11.73% and 3.60%) was the crucial factors which investigated the bacterial community assembly. Soil pH contributed more to the variation, which was consistent with the findings of Lauber *et al.* (2009). More recently, studies also well recognized that soil characteristics are leading factors, which regulate bacterial community and diversity (Feng *et al.*, 2014; Scheibe *et al.*, 2015; Yuan *et al.*, 2016). Thus, based on the current data, it could be concluded that the rhizospheric bacterial community structure were

affected by geographic distance and soil characters, and the final status was quite dependent on the bacterial groups. In addition, it was speculated that the host selective pressure might function on the community structure of *Frankia* rather than the total bacteria, which needes to be confirmed in future.

Conclusion

We surmise that there were unique community structure and rich diversity of the rhizospheric bacteria, including *Frankia* by using Illumina miseq sequencing. The Shannon's diversity and OTUs richness were compared for total bacteria and *Frankia*, and N and K were confirmed to be the main nutrition factors affecting the diversity. In addition the pH and altitude functioned differently on total bacteria and *Frankia* either on Shannon's diversity or OTUs richness. Furthermore, we confirmed the association between the geographic distance and bacterial community structure, and found as the pH and altitude were the dominant factors contributing to the bacterial community assembly.

Acknowledgements

This work was financially supported by the Science Foundation of Chinese Academy of Sciences (XDB15030103), the National Natural Science Foundation of China (41401332, 41571255, 21506209), the National Basic Foundation (2016YFC0501202), and the Natural Science Foundation of Jilin Province (20140101017JC) and the 13.5 program of IGA.

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(Received 06 March 2018; Accepted 15 March 2018)