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Research Article 研究报告

The rhizosphere bacterial community dynamics of the halophyte *Lycium ruthenicum* in different growth stages

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Abstract: [Objective] *Lycium ruthenicum* is a halophyte and used to improve saline lands in northwest China. However, little is known about the bacterial community structural dynamics with growth stage. **[Methods]** We investigated the dynamics of rhizosphere bacterial community structure in three growth stages using Illumina MiSeq high-throughput sequencing. **[Results]** We obtained a total of 317467 16S rDNA reads, corresponding to 7028 bacterial/archaeal operational taxonomic units. The alpha diversity was higher in the rhizosphere than in bulk soil. The diversity and richness of rhizosphere bacteria were much lower in senescence stage than that in vegetative and flowering/fruiting stages. The relative abundances of Proteobacteria and Acidobacteria gradually decreased, whereas the abundance of Cyanobacteria increased along with growth cycle. The phylum Firmicutes abundance was significantly higher in senescence stage than in other stages. The abundant genera composition also changed with growth stage. Seventeen genera (i.e. *Corynebacterium*, *Acidovorax*, *Elizabethkingia*, *Albirhodobacter* and *Pseudomonas*) were abundant at vegetative stage; Sixteen bacterial genera were enriched in flowering/fruiting stage, including *Rhodoligotrophos*, *Geminicoccus*, *Gracilimonas* and *Thioprofundum*. Four bacterial genera, *Exiguobacterium*, *Citrobacter*, *Acinetobacter* and *Pseudomonas*, were abundant in senescence stage. In vegetative and flowering/fruiting stages, the rhizosphere bacterial community was of high similarity, and the similarities between rhizosphere communities were higher than that between rhizosphere and bulk soil communities. However, in senescence stage, the rhizosphere bacterial community composition was more different from the communities in previous stages, but turned to be more similar with that of bulk soil. **[Conclusion]** The rhizosphere bacterial community diversity and composition were changing with growth stage, and great difference was found between senescence stage and previous two stages. Plant growth stage had important effects on structuring the rhizosphere bacterial community of *L. ruthenicum*.

Keywords: halophyte, rhizosphere soil, bacterial community, growth stage dynamics

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Salinization is an important land degradation problem, and high salinity limits plant growth. Due to natural processes such as mineral weathering, dust and precipitation or artificial processes such as irrigation[1], approximately 50% of the world's arable land is estimated to be affected by salinization by 2050[2–3]. In dry regions, salts may accumulate, leading to saline soils and increasing the difficulty for plants to absorb soil moisture. Halophytes are salt-tolerant plants that can grow in saline soil, such as that found in saline semi-deserts, mangrove swamps, marshes, sloughs and seashores. Dominant halophytes play significant role in carbon sequestration, nutrient mineralization, nutrient cycling and improving micro-environment[4].

The rhizosphere represents one of the most diverse habitats on the planet[5]. Rhizosphere microbiomes receive carbon metabolites from the plant through root exudates[6]. In turn, they convert nutrients into more usable forms for assimilation by plants[7] or secreted growth regulators, such as growth-promoting hormones and volatile organic compounds to promote plant growth[8–9]. Some beneficial microbes enhance pathogen resistance, water retention, and the drought and salt resistance ability of plants[10–11].

Rhizosphere microbial community structures are influenced by various factors, among which plant species, soil properties, and growth stage are the most important[12–13]. Plenty of researches have demonstrate that the rhizosphere bacterial community composition is plant-specific, and different plant species tend to shape distinct rhizobacterial community[4,14–15]. The rhizosphere bacteria associated with halophytes are mostly salt-tolerant or halophilic, which is distinct from non-halophytic plants[16–18]. A large amount of halophilic bacteria have been identified or isolated from halophytic plants, such as species or strains belonging to genera *Halomonas*, *Halobacillus*, *Brachybacterium*, *Brevibacterium*, *Bacillus*, *Cronobacter*, *Zhihengliuella*, *Stenotrophomonas*, *Alkalimonas*, *Staphylococcus*, *Methylibium*, *Marinococcus*, *Oceanobacillus*, *Nesterenkonia* and *Virgibacillus*[16–21].

Considerable studies have evidenced that developmental stage of the plant plays a critical role in deciding the rhizobacterial community structure[22–26]. In different plant growth stages, root physiology, and the quality and quantity of root exudates vary, consequently influencing the rhizosphere soil microenvironment and exerting selective pressure on root-associated microorganisms[27–28].

Lycium ruthenicum is a halophyte that mostly occurs in saline deserts and sands across Europe, Central Asia, the southern part of Russia, and Northwest China (Flora of China). It is capable of migrating and accumulating salt from outside the crown to under the crown or in the rhizosphere soil, which consequently reduces the salt concentration of surrounding soils[29]. They are therefore used as pioneer plants to improve barren hills and saline lands. We have investigated the diversity and structure of rhizobacterial community to gain some insights of the composition of rhizosphere bacterial community and the rhizosphere effect on saline habitat[30]. However, the dynamics of rhizosphere bacterial community with growth stage is not clear. In this study, the rhizosphere bacterial community diversity and structure of *L. ruthenicum* was investigated over three growth stages (vegetative, flowering/fruiting and senescence) using the Illumina MiSeq sequencing platform. We aim to explore the effect of growth stage on the bacterial community.

1 Materials and methods

1.1 Study areas and sample collection

The study area was located at the Ebinur Lake Wetland Nature Reserve at the Western margin of the Gurbantunggut Desert, Xinjiang Uygur Autonomous Region, China. The Reserve has a typical continental climate and is dry and windy,

with an annual average precipitation of 105 mm and an evaporation of 1315 mm. The soils are mainly gray desert, gray-brown desert, and sandy soils. The soil in the Reserve is highly salinized and alkalized, and the average electrical conductivity (EC) and pH value of the 0–10 cm soil layer are 5.41 mS/cm and 8.77, respectively, with an average water content of 7.19%[31].

The soil was sampled during three growth stages (vegetative, flowering/fruiting and senescence) of *L. ruthenicum*. The rhizosphere soil was collected and processed following the protocol of Edwards et al.[32]. Three to four healthy individuals were selected and sampled in each of these stages from the same population. In total, 22 samples, including 11 rhizosphere and 11 bulk soils, were collected. The bulk soils were collected from the 0–40 cm soil layer at least 20–50 cm away from the plant. Roots were discarded, and the remaining soil was processed in the same manner as the rhizosphere soils.

1.2 DNA extraction, amplification, and sequencing

Total genomic DNA of each sample was extracted using the E.Z.N.A™ Mag-Bind Soil DNA Kit (OMEGA). Extracted DNA was detected by 1.0% agarose gel and quantified using a Nanodrop 2000 spectrophotometer (Nanodrop Technologies, Wilmington DE). The PCRs were performed with two rounds of amplification. The first round amplified with barcode-fused primers. The 16S rDNA V3–V4 region was amplified with the forward primer 341F [ccctacacgacgctcttccgatctg (barcode) cctacgggnggcwgcag] and reverse primer 805R (gactggagttccttggcacccgagaattccagactachvgggtatctaatcc); Amplification reactions were performed in 30 μL volumes containing 15 μL of 2×*Taq* Master Mix (Thermo), 1 μL of each primer (10 μmol/L), and 20 ng of template; the procedure began at 94 °C for 3 min; followed by 5 cycles of 94 °C for 30 s, 45 °C for 20 s, and 65 °C for 30 s; then 20 cycles of 94 °C for 20 s, 55 °C for 20 s, and 72 °C for 30 s; and a final extension for 10 min at 72 °C. The second round of amplification was conducted using Illumina bridge PCR-compatible primers, and PCRs were performed using 5 cycles of 95 °C for 30 s, 95 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s, and a final extension for 5 min with the same reaction mix as the first round. PCR products were visualized using electrophoresis on 1.5% agarose gels and purified using VAHTS™ DNA Clean Beads (Vazyme, Nanjing, China). The PCR products of all replicates from each stage and each soil type were pooled together as one sample for sequencing, thus each of the six sequencing sample (one rhizosphere and one bulk sample at each of the three growth stage) contained all replicates. Finally, 10 ng of DNA from each sequencing sample was sequenced with the Illumina MiSeq platform by the Sangon Technology Co., Ltd. (Shanghai, China).

1.3 Sequence preprocessing and operational taxonomic unit (OTU) assignment

Raw sequence data were first quality controlled as in previous study[33]. Bases with quality scores<20 were removed from raw reads, paired-end reads were merged into sequences based on overlapping regions with PEAR[34], and the maximum mismatch rate of overlapping areas was constrained to 0.1. Sequences were removed when they contained the ambiguous base Ns or were shorter than 200 bp. Chimeric sequences were identified by UCHIME and discarded. Sequences were assigned to OTUs at a 97% similarity level. Taxonomies of representative OTUs were annotated according to their RDP classifier and BLAST against the Silva and NCBI databases[35]. OTUs with an RDP classification threshold below 0.8 or with identity and coverage lower than 90% were denoted unclassified. All sequencing data in this study were deposited in the NCBI with accession number SAMN06650232–SAMN06650237.

1.4 Statistical analysis

Alpha diversity was estimated using the

vegan package in R software. Chao1 was used to estimate richness, and Shannon and Simpson indices were used to estimate diversity. Weighted UniFrac distances between samples, based on OTU abundance, were analyzed using the *vegan* package, and based on which hierarchical clustering maps were used to visualize the relationships and similarities between samples. Pearson's correlation analysis between soil properties and OTUs was performed using the SPSS program.

2 Results

2.1 Soil properties

The total organic carbon (TOC), soil organic matter (SOM) and total organic nitrogen (TON) content of rhizosphere soils were higher than those of bulk soils, whereas their EC and pH values were lower than those of bulk soils. The TOC, SOM and TON were significantly different among the three growth stages in both the rhizosphere and bulk soils. TOC, SOM and TON contents decreased from vegetative to flowering/fruiting, and increased in senescence stage (Table 1).

2.2 Diversity of microbial community

In total, 317467 reads were obtained from samples. After quality control and OTU assignment, 7028 bacteria/archaeal OTUs were obtained. Rarefaction curves (97% identity) in all samples almost approached the plateau (Figure 1), indicating a reasonable representation of bacterial community diversity. The alpha diversity and richness was higher in rhizosphere soils than that in bulk soils. The rhizosphere bacterial/archaeal community diversity peaked in flowering/fruiting stage, and decreased dramatically in senescence stage. For bulk soil, the community diversity in vegetative stage was similar to that in flowering/fruiting stage, and both higher than that in senescence stage (Table 2).

Table 1. Chemical characteristics of rhizosphere and bulk soils associated with *L. ruthenicum* in the three growth stages

Soil type	Growth stage	TOC/(g/kg)	SOM/(g/kg)	TON/(g/kg)	pH	EC/(mS/cm)
Bulk	Vegetative	6.46±1.05	11.13±1.82	0.22±0.17	8.78±0.15	7.13±1.06
	Flowering/fruiting	5.99±1.28	10.32±2.20	0.14±0.05	8.93±0.61	6.37±0.72
	Senescence	6.71±0.61	11.55±1.05	0.52±0.23	8.90±0.09	6.26±1.89
Rhizosphere	Vegetative	12.71	21.92	0.85	8.23	5.66
	Flowering/fruiting	8.26	14.24	0.65	8.25	1.94
	Senescence	10.25	17.68	0.76	8.09	1.96

Mean and standard deviation values were showed, number of samples (n) of bulk soil was 3–4, while for rhizosphere soil n=1 because rhizosphere soil quantity was too low to test for each individual, thus the rhizosphere soil of all individuals at each stage were mixed together and tested.

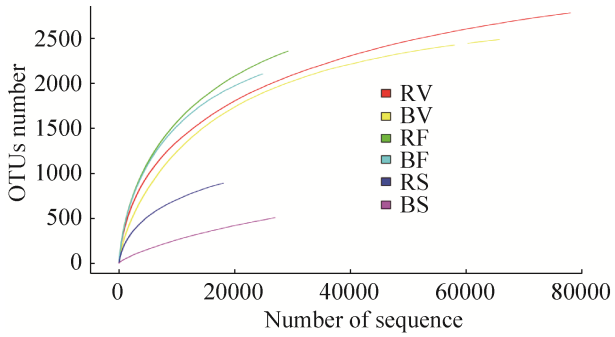

Figure 1. Rarefaction curves for bacterial OTUs clustering at 97 % sequence similarity.

2.3 Bacterial communities composition

Three Archaea phyla (Euryarchaeota, Thaumarchaeota and Crenarchaeota) were detected but accounted for only a very small proportion of the entire microbial community. These three phyla accounted for 0.30% and 0.63% of the total sequences in rhizosphere and bulk soils, respectively (Table 3).

Table 2. Alpha diversity indices and numbers of OTUs in rhizosphere and bulk soil microbial communities in different growth stages.

Soil type	Sample	OTU number	Shannon index	Simpson index	Chao1 index
Bulk	BV	2662	5.03	0.03	2729.24
	BF	2100	5.88	0.02	2507.88
	BS	505	1.67	0.30	1006.77
Rhizosphere	RV	2778	5.98	0.01	3241.60
	RF	2354	6.09	0.01	2939.34
	RS	888	3.83	0.11	1134.39

BV: Bulk soils in vegetative stage; BF: Bulk soils in flowering/fruiting stage; BS: Bulk soils in senescence stage. RV: Rhizosphere soils in vegetative stage; RF: Rhizosphere soils in flowering/fruiting stage; RS: Rhizosphere soils in senescence stage.

Table 3. Relative abundances of microbial phyla in rhizosphere and bulk soils at three growth stages.

Phylum	Relative abundance/%					
	RV	RF	RS	BV	BF	BS
Archaea						
Thaumarchaeota	0.01	0.01	0.02	0.09	0	0.15
Euryarchaeota	0.07	0.01	0.18	0.21	0.06	0
Crenarchaeota	0	0	0.01	0.02	0	0.10
Bacteria						
Proteobacteria	55.08	50.71	37.06	47.59	29.69	52.63
Firmicutes	3.82	3.34	33.12	17.59	4.08	45.80
Actinobacteria	8.59	17.79	5.19	15.52	29.92	0.39
Bacteroidetes	11.89	5.72	16.01	12.33	10.03	0.39
Acidobacteria	7.43	5.08	0.78	1.40	1.63	0.14
Planctomycetes	2.00	4.70	1.04	0.20	7.83	0.05
Chloroflexi	1.74	1.72	0.26	0.63	4.54	0.06
Cyanobacteria	1.30	2.73	4.46	0.16	0.02	0.03
Deinococcus-Thermus	0.03	0.05	0.06	2.11	0.49	0.01
Candidatus Saccharibacteria	1.80	1.77	0.30	0.13	0.38	0.03
Verrucomicrobia	0.69	0.62	0.27	0.78	2.05	0.04
Parcubacteria	0.40	0.41	0.12	0.06	1.14	0.02
Others*	0.97	1.44	0.13	0. 90	0.92	0.04

*: phyla with relative abundances less than 0.1% were merged. BV: Bulk soils in vegetative stage; BF: Bulk soils in flowering/fruiting stage; BS: Bulk soils in senescence stage. RV: Rhizosphere soils in vegetative stage; RF: Rhizosphere soils in flowering/fruiting stage; RS: Rhizosphere soils in senescence stage.

Forty-four bacterial phyla were detected in all samples. Proteobacteria was the most abundant phylum in rhizosphere soils (Table 3). Alphaproteobacteria and Gammaproteobacteria were the two most abundant classes. The richness of Alpha- and Deltaproteobacteria decreased in senescence stage to a value lower than those of the other two stages, while Gammaproteobacteria was enriched and became the dominant class in senescence stage. Betaproteobacteria was almost disappeared from rhizosphere communities (Figure 2). Bacteroidetes, Actinobacteria, Firmicutes and Planctomycetes were enriched in rhizosphere communities, but their relative abundances varied during the three growth stages. At the genus level, 21 genera were abundant in vegetative stage, they accounted for 48.08% of all OTUs. *Gp10*, *Thioprofundum*, *Deferrisoma*, *Haliea* and *Halomonas* were the most abundant genera; 15 genera were abundant in flowering/fruiting, accounting for 37.07% of all OTUs, and *Geminicoccus*, *Pelagibius*, *Gp10*, *Thioprofundum* and *Deferrisoma* were the five most abundant genera. In senescence stage, 9 abundant genera were found, accounting for 72.15% of all OTUs, with *Planococcus*, *Halomonas*, *Pontibacter*, *Pseudomonas*, *Salinimicrobium*, and *Streptophyta* being the most abundant (Figure 3).

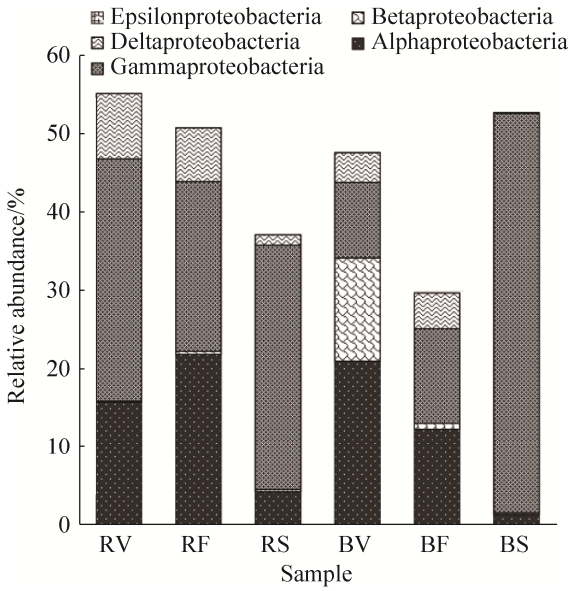

Figure 2. Relative abundance of each class of the phylum Proteobacteria in the soil samples.

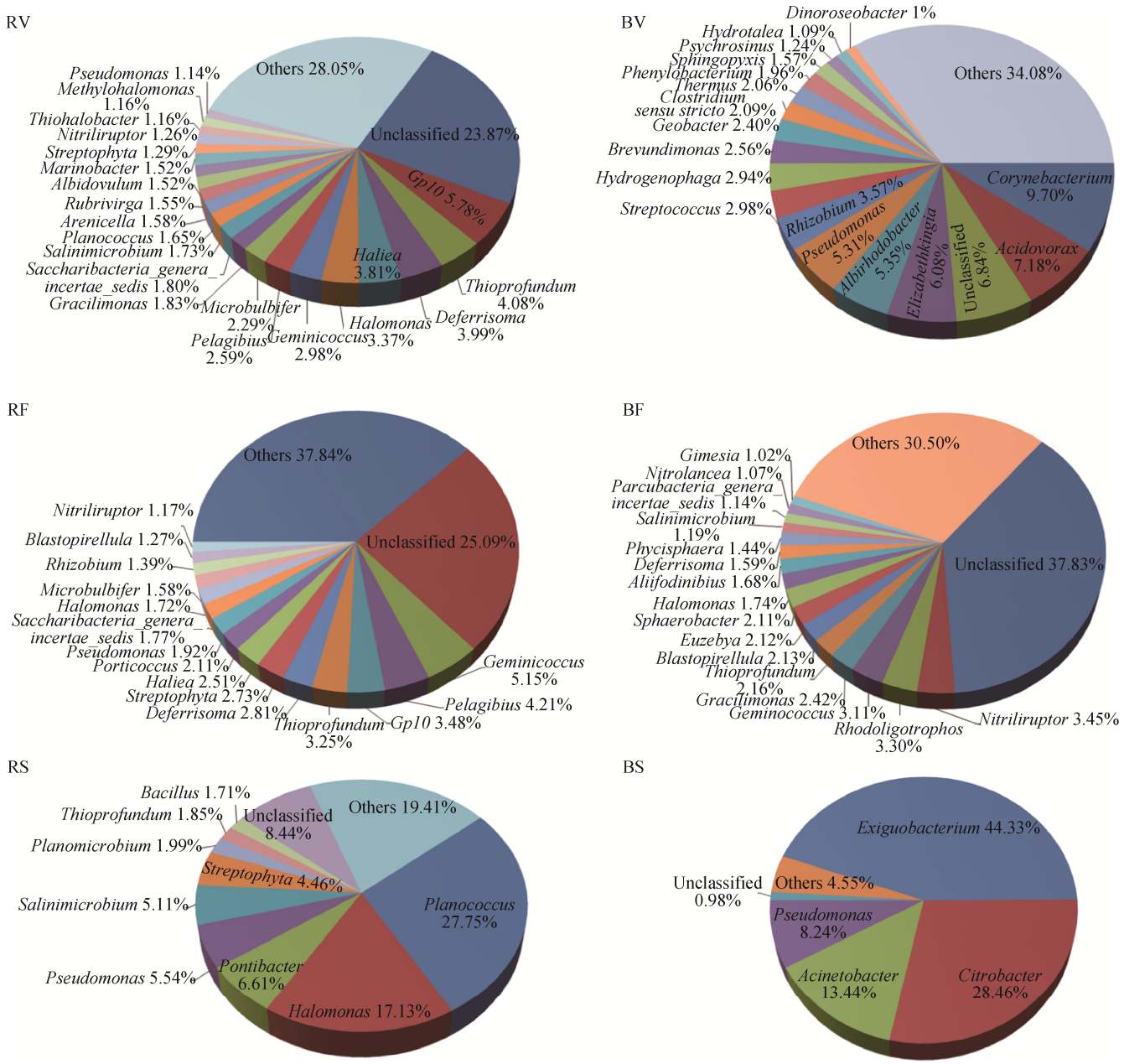

Figure 3. Pie chart of bacterial community composition at genus level in the rhizosphere and bulk soils during the three stages. Only genera with relative abundances ⩾1% are presented, and those < 1% are merged into "others".

For bulk soils, Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Planctomycetes and Cyanobacteria were the most abundant phyla during the growth cycle. Acidobacteria and Chloroflexi were abundant phyla in vegetative and flowering/fruiting stage, but decreased in senescence stage. At genus level, seven genera were abundant in vegetative, for instance, *Corynebacterium*, *Acidovorax*, *Elizabethkingia*, *Albirhodobacter* and *Pseudomonas*. Sixteen genera were abundant in flowering/fruiting stage, and *Nitriliruptor*, *Rhodoligotrophos*, *Geminicoccus*, *Gracilimonas*, *Thioprofundum* were the most abundant. In senescence stage, four genera, *Exiguobacterium*, *Citrobacter*, *Acinetobacter* and *Pseudomonas* were abundant.

2.4 Changes of bacterial communities along with growth stages

In the rhizosphere communities, relative abundance of phyla Proteobacteria and Acidobacteria gradually decreased, while abundance of Cyanobacteria increased from vegetative to senescence stage. The abundance of Firmicutes was significantly higher in senescence than that in the other stages. The relative abundance of Actinobacteria, Planctomycetes and Gemmatimonadetes were higher in flowering/fruiting than in the other stages, whereas the Bacteroidetes abundance in rhizosphere soils was lowest in flowering/fruiting stage (Table 3). At the genus level, the bacterial genera *Gp10*, *Thioprofundum*, *Deferrisoma* and *Haliea* dominated in vegetative stage, while abundances of the *Planococcus*, *Halomonas*, *Pontibacter*, *Pseudomonas* and *Salinimicrobium* in senescence stage were significantly higher than those in vegetative and flowering/fruiting. In contrast, the abundances of genera *Gp10*, *Pelagibius*, *Deferrisoma* and *Geminicoccus* dramatically decreased (Figure 3).

For bulk soil communities, relative abundance of phyla Proteobacteria and Firmicutes decreased from vegetative stage to flowering/fruiting stage, then were enriched and dominated in senescence stage. Conversely, Actinobacteria, Bacteroidetes, Acidobacteria, Planctomycetes and Chloroflexi decreased dramatically in abundance to below than 0.5% (Table 3). The abundant genera number decreased during the growth cycle, twenty-one, eleven and four abundant genera were observed in vegetative, flowering/fruiting and senescence stage, respectively. The composition of abundant genera also changed with growth stage (Figure 3). The abundances of *Brevundimonas*, *Phenylobacterium*, and *Stenotrophomonas* decreased from the vegetative to senescence stage.

2.5 Similarity among samples

Hierarchical clustering based on the weighted UniFrac distance metric revealed distinct differences in microbial community structure between senescence stage and the two previous stages. The rhizosphere microbial community in vegetative and flowering/fruiting stage formed clusters that were clearly separated from the bulk soil community. However, in senescence stage, the bacterial communities of rhizosphere and bulk soils clustered together (Figure 4). These results implied that the bacterial communities, both in the rhizosphere and bulk soils, was of high similarity between vegetative and flowering/fruiting stages, but more divergent from the communities in senescence stage.

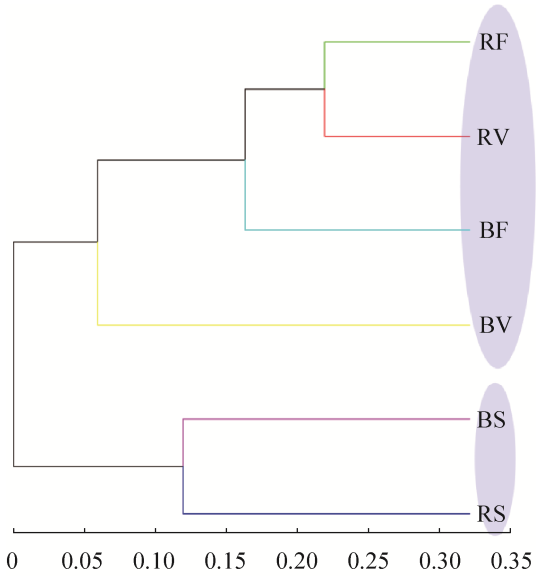

Figure 4. Hierarchical clustering trees based on the weighted UniFrac distance metric. Branch lengths represent distance (indicated by scale bar).

3 Discussion

3.1 Characteristics of the bacterial communities

Consistent with result of previous studies on halophytes associated bacterial communities, the enriched or abundant bacteria are classified to salt-tolerant or halophilic genera, such as, *Salinimicrobium*, *Halomonas*, *Geminicoccus*, *Pelagibius*, *Microbulbifer*, *Planococcus*, *Rubrivirga*, *Arenicella*, *Bacillus* and *Mesorhizobium*. However, the salt-tolerance ability of bacteria species found in

rhizosphere of *L. ruthenicum* are not clear and need further isolation and examination. Their enrichment in rhizosphere indicates that they are adapted to saline environments and their growth are salt dependent[36–43]. Moreover, the high abundance of these bacteria in rhizosphere implies that their reproduction and colonization in rhizosphere soil are driven by nutritional requirements as most of them are positively correlated with soil nutrients, such as TOC and TON (Figure 5). Meanwhile, some of species or strains previously identified from these genera are beneficial to plants, since they can degrade root exudates for root assimilation to help plant growth. For instance, some *Planococcus* and *Microbulbifer* members can degrade complex hydrocarbons[44]. *Mesorhizobium* can fix nitrogen[45]. *Bacillus* members are generally effective for suppressing disease, such as bacterial wilt[46]. We assume that these enriched or abundant bacterial species in rhizosphere may be also beneficial to plant growth therefore they are selected by root and colonize in rhizosphere soils. However, this hypothesis need further determination. In overall, we consider that the enrichment of halophilic bacteria in rhizosphere are deriving from rhizosphere effect and metabolism requirements of bacteria (including salt concentration and nutrients) that are different from glycophytes.

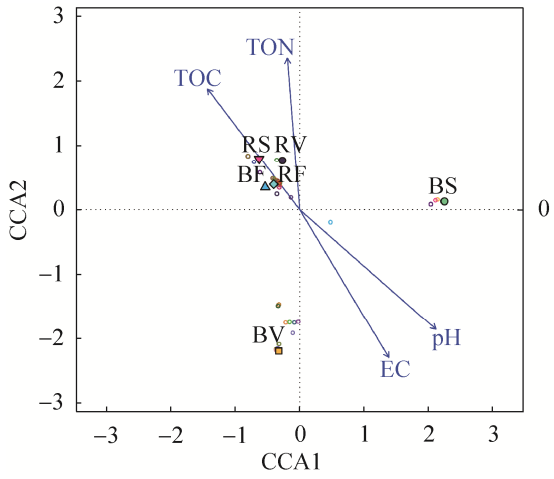

Figure 5. CCA diagram revealing the relationship of bacterial communities and soil properties.

3.2 Rhizosphere effects on soil bacterial communities

Many studies have demonstrated the rhizosphere effects on microbial communities which leads to differences of the diversity and community composition in rhizosphere compared to bulk soils[15,47–48]. In present study, we also find differences of community diversity and composition in rhizosphere and bulk soils, as consistent with our previous study[30]. Bacterial/archaeal diversity is higher in rhizosphere soils than in bulk soils. Most of the abundant phyla differed in abundance between the rhizosphere and bulk soil communities, such as the Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. Firmicutes was enriched in rhizosphere soil during the growth cycle, mainly attributed by genus *Bacillus* as reported in plant *Aster tripolium*[15], whereas the abundance of Acidobacteria was lower in rhizosphere soil than in bulk soil. These results further support that plant species have important influence on composition of soil bacterial community which may be derived by the favorable for the growth and reproduction of some bacteria in the rhizosphere due to the rich of labile organic substrates[28]. The nutrients content in rhizosphere soils are indeed much higher than those in bulk soils associated with *L. ruthenicum*.

3.3 Growth stage dynamic of bacterial communities

Growth stage dynamics are observed in the bacterial diversity and structure both in rhizosphere and bulk soils. In both compartment, the diversity peaks in flower/fruit stage but decrease dramatically in senescence stage. Further, the composition of abundant genus in flower/fruit stage is more diverse than in the senescence stage. The composition in the senescence stage differ apparently from the other two stages. As revealed by hierarchical clustering, the similarity of bacterial structure in vegetative and flowering/fruiting is higher than to that in the senescence stage, both in rhizosphere and bulk soils. Interestingly, we find that in senescence stage, the

similarity of rhizosphere community to bulk soil community is higher than to the rhizosphere communities of the former two stages, whereas the similarity of rhizosphere communities is higher than that of rhizosphere and bulk soils in vegetative and flowering/fruiting stages. These results support that the growth stage plays a critical effect on microbial community[24,27–28].

Some bacterial groups display similar growth stage dynamics in the rhizosphere and bulk soil, for example, the phyla Firmicutes, Actinobacteria, Planctomycetes, and the genera *Geminicoccus* and *Pelagibius*. However, most of the microbes, such as the Alpha-, Gamma-, and Deltaproteobacteria, show different temporal dynamics in the rhizosphere and the bulk soil communities. In addition, many of the abundant rhizosphere genera (e.g. genera *Streptophyta*, *Pseudomonas*, *Halomonas*, *Planococcus* and *Salinimicrobium*) displayed temporal patterns that differ from their patterns when they are present in bulk soils. These imply that even growth stage play an important role in bacterial community composition, but is not the only dominant factor influencing the microbial structure. Previous studies have revealed that the seasonal shifts and vegetation types affect soil microbial community composition by changing the soil physicochemical properties[49]. In different plant growth stages, root physiology, and the quality and quantity of root exudates vary, consequently influencing the rhizosphere soil microenvironment and exerting selective pressure on root-associated microorganisms[27–28]. We find a negative correlation between nutrients (such as SOM, TOC and TON contents) and community diversity both in rhizosphere and bulk soils. In the flowering/fruit stage, both the rhizosphere and bulk soils have the highest community diversity in spite of the lowest nutrient concentrations, which is also reported in previous research[50]. However, in the senescence stage, the community diversity decrease dramatically even though the SOM, TOC and TON contents are much higher than those in the flowering/fruit stage.

From these results, we assume that other factors may be responsible for changes of community diversity and composition. Soil temperature has been proven to be an important factors influencing soil microbial community structure[51–52]. The average temperature in senescence stage is 3.57 °C and 8.64 °C lower than that in vegetative stage flowering/fruiting stage according to our successional records of the whole year in the studying regions. We propose that the decrease in temperature might be an important factors causing decline in diversity and change of community composition in the senescence stage. Moreover, biotic factor (such as effects of fungus) might be also a possible deriving force. Previous study show that the enrichment of some pathogenic fungus can cause severe root disease of *Panax ginseng* and lead to decrease of bacterial diversity and increase of fungal diversity along with growth stages[25]. Their effects are need future research.

4 Conclusion

We investigated the growth stage dynamics of bacterial community diversity and structure of a halophytic plant *L. ruthencium* in three growth stages. The rhizosphere effect causes apparent differences in diversity and composition of rhizosphere community compared to the bulk soils. We observe clear growth stage dynamics of the bacterial community diversity and composition both in the rhizosphere and bulk soils. Specially, the community diversity and structure in the senescence stage differ dramatically from the other two growth stages. These results indicate that growth stage is an important driving forces causing changes in diversity and structure of the bacterial community associated with *L. ruthencium*. Though we get some meaningful implications regarding the growth stage dynamics of bacterial community, there are some deficiencies in present study. Firstly, there is

only one sequencing data for each sample from a mix of multiple individuals in each stage. This omits the difference of plant individuals and the sequencing accuracy tend to be easily influenced by experimental error. Secondly, the potential mechanisms and the determination factors underlying the growth stage dynamics of the bacterial community are not examined and clarified. These questions will be settled in our next research which is ongoing.

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不同生长季节黑果枸杞的根际细菌群落结构

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摘要：【目的】黑果枸杞是一种耐盐植物，是我国西北干旱区盐渍土改良的优良植物物种，其根际土壤细菌群落结构在不同生长时期的变化特征尚不清楚。【方法】本研究采用 Illumina MiSeq 高通量测序研究了黑果枸杞 3 个生长阶段的根际土壤细菌群落结构的动态变化。【结果】所有样品中共获得 317467 条序列，对应于 7028 个细菌/古细菌 OTUs。根际土壤细菌群落的 α 多样性显著高于非根际土壤。衰老期根际细菌的多样性和丰富度明显低于营养生长期和花/果期。变形菌门和酸杆菌门的相对丰度随生长时期的演变而逐渐降低，而蓝细菌门则相反。厚壁菌门的丰度在衰老期明显高于营养生长期和花/果期。优势属的组成也随生长期的演变而改变，营养生长期、花/果期、衰老期的优势属数量分别为 17、16、4，且组成也具有差异。相似性分析表明营养生长期和花/果期的根际细菌群落具有很高的相似性，衰老期根际细菌群落组成与生长期和花/果期具有很高差异，然而与非根际土壤的群落结构具有较高的相似性。【结论】根际土壤细菌群落多样性和组成随生长期的改变而表现出明显的动态变异性，表明黑果枸杞生长时期对根际土壤细菌群落结构具有重要的影响。

关键词：盐生植物，根际土壤，细菌群落，生长阶段动态

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