

# Isolation of Rhizosheath and Analysis of Microbial Community Structure Around Roots of *Stipa Grandis*

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## Research Article

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# Abstract

**Background** The root zone microbial structure is particularly complex for plants with rhizosheaths, which may play an important role in the future agricultural sustainable development. However, one of the important reasons for restricting our study of rhizosheath microbial structure is that there is no definite method for rhizosheath separation. The aim of this study was to explore the isolation methods of rhizosheath and the diversity characteristics of microorganisms around the rhizosphere. In this study, we isolated the rhizosheath of *Stipa grandis*, a dominant species in desert steppe, and the microorganisms in the roots, root epidermis, rhizosheath, rhizosphere soil were extracted and sequenced by 16SrRNA and ITS.

**Results:** The bacterial alpha diversity index was in the order rhizosphere soil > rhizosheath > root epidermis > endophytic, and the fungal alpha diversity index was rhizosphere soil and rhizosheath > root epidermis and endophytic. There were significant differences in bacterial community structure between the root epidermis and endophytic, rhizosheath, rhizosphere soil. Different from bacterial community structure, the community structure of root epidermis fungi was similar to endophytic, but significantly different from rhizosheath and rhizosphere soil. Our method is feasible for separating plant rhizosheath and root epidermis.

**Conclusions** We suggest that the root epidermis can act as the interface between the host plant root and the external soil environment. We will have to re-examine the biological and ecological significance of root sheath and microorganisms in rhizosheath, as well as the mechanism of its close relationship with plant root epidermis. This study will provide theoretical and technical guidance for the isolation of plant rhizosheath and the study of microorganisms in it.

## Introduction

The close interaction between plants and rhizosphere microorganisms has prompted people to regard plants as a superorganism. Research on plant root – rhizosphere soil microorganisms aims to identify correlations between plant – soil – microorganisms and reveal their important roles in the ecosystem. However, the relationship between the root and the soil around the root of some plants is more complex, and these plants are plants with rhizosheaths. For example, barley [1], wheat [2], corn [3], rushes [4, 5], etc. even some scholars have proposed that there is a rhizosheath on the fine roots of some leguminous plants [6, 7]. However, McCully [8] suggest that this needs further research and confirmation. It has been more than 100 years [9] since the rhizosheath was first described, initiating study of its structure [10], formation, function and genetic characteristics. However, little is known about it even to this day.

Rhizosheath is particularly obvious in the root structure of *Gramineae* in arid areas. Price [11] and Young [12] believe that the rhizosheath plays an important role in increasing the drought resistance of plants, and existing research supports this view [13–15]. This understanding is of great significance for improving agricultural sustainability in the context of future climate change, limited resources and a growing global population. Some scholars believe that the rhizosheath plants may play an important role in the second green revolution and future agricultural sustainable development [16, 17].

The research on the structure and function of plant rhizosphere microorganisms has always been a hot topic, although most microorganisms in the environment have not been cultured, with the rapid development of high-throughput sequencing technology, the structure of microorganisms in the environment is gradually becoming known. Previous studies have shown that there are some differences in the composition of plant root

microorganisms and rhizosphere and non-rhizosphere soil microorganisms, which directly affect the normal growth of host plants. For example, some high concentrations of molecules released by rhizosphere microorganisms inhibit the elongation of primary roots and promote the formation of lateral roots and root hairs [18]. Some rhizosphere bacteria or fungi produce auxin, which directly interferes with auxin signal transduction [19]. Plant rhizosphere microorganisms play an important role in improving crop yield and resistance. Recent reviews have shown that rhizosphere microorganisms and drought resistant crops interact through several different mechanisms to respond to climate change[18]. Different plant species or genotypes can select different rhizosphere microbial communities by producing different secretions from their roots. Although there is still uncertainty about global climate change, there are signs that global temperature will continue to rise, and that drought frequency and duration will change in many locations. Plants respond to this stress through their own regulation. A recent review by Vries [20] found little evidence for a coupling relationship between the drought tolerance mechanism of microorganisms and the functional characteristics of plant drought resistance, highlighting the need for further research. However, there are few reports on the structure and function of microorganisms in plant rhizosphere. Therefore, it will be a challenge to study the microorganisms around the rhizosphere plants.

York et al. [21] summarized and defined the generation process and semantics of "rhizosphere", and considered that rhizosphere is a mixture of soil particles adhered by mucus (the secretion of plant roots or microorganisms) [1, 9]. The epidermal cell layer attached to the rhizosphere was not a part of the rhizosphere, and they called the combination of the epidermal cell layer and the rhizosphere as "rhizosphere" [21]. The purpose of our study is to isolate root sheath and root epidermis and extract microbial DNA from them. The difficulty of rhizosphere separation is the separation of rhizosphere and root epidermis. And there is no standard method to isolate microorganisms from rhizosphere. In this study, using *Stipa grandis* as experimental material, we isolated the roots, root epidermis, rhizosphere and rhizosphere soil of *Stipa grandis*, extracted microbial DNA from roots, root epidermis, rhizosphere and rhizosphere soil, and sequenced 16S rRNA and ITS. The purpose of our research is to explore the isolation methods of rhizosphere and root epidermis, and analyze the similarities and differences of microbial communities among the root, root epidermis, rhizosphere and rhizosphere soil of *Stipa grandis*, so as to provide new methods and suggestions for the future research of rhizosphere plants.

## Materials And Methods

### Overview of the research site

The experiment was carried out in Maodeng pasture (116.03°E-116.50°E, 44.80 ° N-44.82°N), Xilinhot City, Inner Mongolia, China. The area has a temperate arid continental climate, with an elevation of 1055 m. The annual average temperature is 0–1°C, the frost-free period is 90–115 d, and the accumulated temperature greater than or equal to 0°C is 1800°C.

### Test Sample Collection

*Stipa grandis*, a typical gramineous plant in desert steppe, is a constructive species. Samples were taken from Maodeng pasture (*Stipa grandis* steppes) in Xilinhot City, Inner Mongolia, in July 2019. We designed 5 replicates and randomly sampled at 5 sites 50 m apart. Three *Stipa grandis* were collected from each site as one sample of replicates, and the depth of excavation is 20 cm. The mixed samples of plant and soil were put into plastic bags and immediately placed into ice boxes, and brought back to the laboratory for root, root epidermis, rhizosphere and

rhizosphere soil separation. The isolation of root, root epidermis, rhizosheath and rhizosphere soil and the extraction of microbial DNA were carried out at the Sino-Dutch joint laboratory.

## Rhizosphere, Rhizosheath, Root Epidermis And Root Harvesting

Phosphate buffer is needed before separating plant roots, root epidermis, rhizosheath, rhizosphere soil. Phosphate buffer plays an important role in maintaining microbial activity and does not change the composition of the sampled microorganisms. Phosphate buffer: PB, per litre: 6.33 g  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ , 10.96 g  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  and 200  $\mu\text{L}$  Silwet L-77.

The harvesting protocol closely followed the procedures described previously [22, 23], with minor modifications, as described here. In Bulgarelli and Lundberg's study, *Arabidopsis thaliana* was taken as the model plant, but the root system of *Arabidopsis thaliana* did not have rhizosheath structure. Therefore, we made minor modifications to the harvesting method according to the definitions of rhizosheath [1, 9, 24] and rhizosphere soil, as described here. Wear sterile gloves and sterilize the workspace with 70% EtOH. The forceps and scissors used in the experiment were wiped and disinfected with 70% EtOH. Select 3 *Stipa grandis* plants from each sampling point, remove large pieces of soil that is easy to fall off, knead and shake with gloved hands, and pat the roots to obtain rhizosphere soil. The soil that is not shaken off and adheres to the root surface is defined as rhizosheath soil (Fig. 1). Place the root with rhizosphere soil in a 50 mL tube containing 25 mL phosphate buffer. Vortex max 15 s, and filter rhizosphere through a 100  $\mu\text{m}$  nylon mesh cell strainer into an empty 50 mL tube. After centrifuging at 4000 $\times$ g for 15 minutes, pour out the supernatant. The soil samples left in the 50 ml tube are rhizosheath soil samples. Remove the root hairs on the filtered root samples with tweezers, and carefully separate the filtered roots and outer epidermal cell layer with tweezers. Place them in a 15 ml tube filled with buffer solution, vortex for 30 s, and wash them 3 times. Place the root and outer epidermal cell layer on sterile filter paper and air dry the root and outer epidermal cell layer sample naturally, air dry in about 6 hours.

## Sequencing Process (Novogene Company)

### (1) Extraction and PCR amplification of microbial genomic DNA

Microbial DNA from rhizosheath, root epidermis and rhizosphere soil samples was extracted with MOBIO DNeasy PowerSoil kit 12888-100. Microbial DNA from the root was extracted with FastDNATM Spin Kit for Soil. Agarose gel electrophoresis was used to detect the purity and concentration of DNA. An appropriate sample of DNA was applied to the centrifuge tube and diluted with sterile water to 1ng/ L. Using diluted genomic DNA as a template, according to the selection of the sequencing region, specific primers with Barcode, Phusion® High-Fidelity PCR Master Mix with GC Buffer from New England Biolabs company, and high fidelity enzyme were used for PCR to ensure the efficiency and accuracy of amplification. The corresponding regions of primers were: 16S V4 region primers (515F and 806R) – identification of bacterial diversity. ITS1 region primers (ITS5-1737F and ITS2-2043R) –identification of fungal diversity.

### (2) Mixing and purification of PCR products

The PCR product was detected by electrophoresis with 2% agarose gel. According to the concentration of PCR product, the samples were mixed equally, and then the PCR products were detected by agarose gel electrophoresis with 2% agarose gel. The gel recovery kit provided by Qiagen company was used to recover the target band.

### (3) Library construction and sequencing

Library construction used TruSeq® DNA PCR-Free Sample Preparation Kit (Building Database Kit). The library was quantified by qubit and Q-PCR, and after the library was qualified, it was sequenced by novaseq6000.

### Data analysis based on the Analysis Platform of Novogene company

#### (1) Sequencing data processing

According to the barcode sequence and PCR amplification primer sequence, each sample data was separated from the offline data. After the barcode and primer sequences were cut off, the reads of each sample were spliced with FLASH (V1.2.7, <http://ccb.jhu.edu/software/FLASH/>) [25], and the splicing sequence was the original tags data (Raw Tags). The raw tags obtained by splicing need to be filtered [26] strictly to obtain high-quality tags data (Clean Tags). Following Qiime's tags quality control process (V1.9.1, [http://qiime.org/scripts/split\\_libraries\\_fastq.html](http://qiime.org/scripts/split_libraries_fastq.html)) [27], the effective data were obtained (Effective Tags).

#### (2) OTU clustering and species annotation

Uparse software (Uparse v7.0.1001, <http://www.drive5.com/uparse/>) [28] was used to cluster all effective tags of all samples. By default, the sequences were clustered into OTUs (Operational Taxonomic Units) with 97% consistency. At the same time, the representative sequences of OTUs were selected. According to the algorithm principle, the sequence with the highest frequency in OTUs was selected as the representative sequence of OTUs. The OTUs sequences were annotated and analyzed using the Mothur method and the SSUrRNA database [29] of silva132 (<http://www.arb-silva.de/>) [30] (the threshold value was set at 0.8 ~ 1). The taxonomic information was obtained and the community composition of each sample was counted at the phylum level. The fast multi sequence alignment was carried out using MUSCLE [31] (Version 3.8.31, <http://www.drive5.com/muscle/>) software, and the phylogenetic relationships of all OTUs representative sequences were obtained. Finally, the data for each sample was homogenized, and the least amount of data in the sample was taken as the standard. The subsequent alpha diversity analysis and beta diversity analysis were based on the homogenized data.

Alpha diversity analysis: Qiime software (version 1.9.1) was used to calculate observed OTUs, Chao1, Shannon, Simpson, ACE and goods coverage index, and the dilution curve was drawn using R software (version 2.15.3), and alpha diversity index was analyzed using R software.

Beta diversity analysis: The UniFrac distance was calculated with Qiime software (version 1.9.1), and the UPGMA sample clustering tree was constructed. NMDS were drawn using R software (version 2.15.3). Vegan software package of R software was used for NMDS analysis. AMOVA analysis used the amova function in Mothur software.

## Results

### Distribution characteristics of bacterial and fungal communities

Bacterial OUTs (Fig. 2, a): A total of 8760 OTUs were generated by OTUs clustering at 97% similarity level. Among them, 3787 OTUs were common in roots, root epidermis, rhizosheath, and rhizosphere soil. There were 188, 347, 576 and 711 unique OTUs in roots, root epidermis, rhizosheath, and rhizosphere soil, respectively, accounting for 3.68%, 5.98%, 8.48% and 10.06% of their respective total number of OTUs. Fungal OUTs (Fig. 2, b): A total of 1423 OTUs were generated by OTUs clustering at 97% similarity level. Among them, 318 OTUs were common in roots, root

epidermis, rhizosheath soil and rhizosphere soil. The number of unique OTUs in roots, root epidermis, rhizosheath soil and rhizosphere soil were 11, 19, 164 and 262, respectively, accounting for 2.28%, 3.84%, 15.96% and 22.13% of their respective total numbers of OTUs. The dilution curves of OTUs tended to be gradual, indicating that the measured data could accurately reflect the information on the plant fungal community (Fig. 2, c, d).

Table 1 shows that the number of Observed\_species, Chao1 index and ACE number index were highest in rhizosphere soil, followed by rhizosheath soil, and were smallest in the root system, and there were significant differences between them. There was no significant difference in the Shannon index between rhizosphere soil and rhizosheath soil, which were both significantly higher than the Shannon index in root epidermis and roots, but the Shannon index of bacteria in root epidermis was significantly higher than in roots. The Simpson index of bacteria in rhizosphere soil, rhizosheath soil and root epidermis had no significant differences, but were significantly higher than those in the root system. The goods\_coverage index of the samples was higher than 98%.

The observed\_species, Shannon, Simpson, Chao1, ACE, PD\_whole\_tree index of fungi in rhizosphere soil were the highest, followed by rhizosheath soil, but there was no significant difference between the two groups. The observed\_species, Shannon, Simpson, Chao1, ACE, index of rhizosphere soil and rhizosheath soil fungi were significantly larger than the root epidermis and root. There was no significant difference in observed\_species, Shannon, Simpson, Chao1, ACE index between roots and root epidermis. The good\_coverage indexes of the samples were all higher than 99%.

Table 1  
Alpha diversity of bacteria and fungi in roots, rhizosheath, rhizosphere and non-rhizosphere soil

Microorganism	Sample name	Observed_ species	Shannon	Simpson	Chao1	ACE	Goods_ coverage
Bacteria	SG.EC	2769.0 ± 143.9d	5.41 ± 0.31c	0.82 ± 0.03b	3210.4 ± 159.4d	3312.3 ± 174.8d	0.989 ± 0.001
	SG.RS	3287.4 ± 213.2c	8.87 ± 0.46b	0.99 ± 0.01a	3714.6 ± 248.7c	3798.1 ± 266.4c	0.989 ± 0.001
	SG.RH	3919.2 ± 187.9b	9.77 ± 0.28a	1.00 ± 0.00a	4392.5 ± 145.6b	4444.4 ± 156.3b	0.988 ± 0.000
	SG.SO	4278.6 ± 256.5a	10.10 ± 0.10a	1.00 ± 0.00a	4858.4 ± 512.0a	4938.6 ± 504.8a	0.986 ± 0.003
Fungi	SG.EC	230.2 ± 27.5b	2.38 ± 0.59b	0.62 ± 0.14b	264.3 ± 34.5b	278.1 ± 37.7b	0.999 ± 0.000
	SG.RS	223.8 ± 29.2b	2.61 ± 0.75b	0.68 ± 0.15b	260.8 ± 33.4b	272.6 ± 31.3b	0.999 ± 0.000
	SG.RH	532.2 ± 65.5a	5.11 ± 0.62a	0.89 ± 0.06a	597.7 ± 65.4a	613.4 ± 68.8a	0.998 ± 0.000
	SG.SO	563.0 ± 70.9a	5.64 ± 0.44a	0.93 ± 0.04a	693.9 ± 163.6a	656.6 ± 83.6a	0.998 ± 0.001

**Note:** Different lowercase letters indicate significant differences between bacterial or fungal groups ( $P \leq 0.05$ ). Observed\_ species and Goods\_ coverage represents sequencing depth index; Shannon and Simpson represent the diversity index of the bacterial community, and Chao1 and ACE represent the index of bacterial community richness; SG.EC, SG.RS, SG.RH, SG.SO refer to roots (endophytic), root epidermis, rhizosheath, and rhizosphere soil of *Stipa grandis*, respectively.

The top 10 phylum level classification of bacterial communities is shown in Fig. 3 (a). The relative abundance of *Cyanobacteria* in the root system was 46.4%, while the relative abundance of *Cyanobacteria* in rhizosphere, rhizosphere and non-rhizosphere soil was only 5.0%, 2.1% and 1.1%, respectively. *Actinobacteria* was the dominant population in root epidermis, and its relative abundance was 43.0%, which was significantly higher than that in the root system, rhizosphere soil and rhizosphere soil. The relative abundance of *Proteobacteria* in root epidermis, rhizosphere soil and rhizosphere soil was 30.9%, 31.3% and 30.2%, respectively, while that in roots was only 11.7%. The relative abundances of *Acidobacteria*, *Gemmatimonadetes*, *Bacteroidetes* and *Verrucomicrobia* in rhizosphere soil and rhizosphere soil were similar and significantly higher than those in root systems and root epidermis. The relative abundances in rhizosphere soil were 22.1%, 9.2%, 8.2% and 3.2%, respectively, and those in rhizosphere soil were 21.8%, 9.6%, 7.6% and 3.7%, respectively.

The top 10 phylum level classification of the sample fungal communities is shown in Fig. 3 (b). Different from bacterial communities, the analysis shows that *Basidiomycota* and *Ascomycota* have higher relative abundance in roots, root epidermis, rhizosphere and rhizosphere soil. The relative abundance of *Basidiomycota* in roots and root epidermis are 72.3% and 70.3%, respectively, which is significantly higher than in rhizosphere soil and rhizosphere soil. The relative abundance of *Ascomycota* in roots, rhizosphere and rhizosphere soil was 20.4%, 22.4% and 27.6%, respectively, while that in root epidermis was only 8.7%. The relative abundance of Others was in the order rhizosphere soil > rhizosphere soil > root epidermis > roots.

Results of non-metric multidimensional scaling (NMDS) analysis of bacteria in roots, root epidermis, rhizosphere and rhizosphere soil of *Stipa grandis* are shown in Fig. 3 (c). The microbial community structure of root epidermis, rhizosphere soil and rhizosphere soil are distinct and distant in the plot, while the distance between rhizosphere soil and rhizosphere soil is relatively close. The UPGMA cluster analysis of all samples also showed that the similarity of bacterial composition and relative abundance between rhizosphere soil and rhizosphere soil was high, and that there was a big difference in bacterial composition between soil, roots and root epidermis.

The results of NMDS analysis of fungi are shown in Fig. 3 (d). The fungal community structure of roots and root epidermis, and rhizosphere and rhizosphere soil were relatively close, while that of roots and root epidermis were relatively distinct from those of rhizosphere and rhizosphere soil. Through UPGMA cluster analysis of all the samples, in contrast to bacterial community clustering, rhizosphere soil and rhizosphere soil fungal communities can be clustered into one group, while root and root epidermis can be clustered into another group.

As shown in Table 2, the bacterial community composition in rhizosphere soil and rhizosphere soil of *Stipa grandis* was not significantly different ( $P > 0.05$ ). The bacterial community composition of roots and root epidermis of *Stipa grandis* was significantly different at  $P < 0.05$ , and the differences in bacterial community among other groups was extremely significantly different ( $P < 0.01$ ).

There was no significant difference in fungal communities between the roots and root epidermis of *Stipa grandis* or between rhizosphere soil and rhizosphere soil ( $P > 0.05$ ). The difference of fungal community among other groups reached an extremely significant level ( $P < 0.01$ ).

Table 2  
Significance test table of difference in bacterial and fungal community structure between groups

Microorganism	vs_group	SS	df	MS	Fs	p-value
Bacteria	SG.EC- SG.RH- SG.RS- SG.SO	2.18148(0.234448)	3(16)	0.727159(0.014653)	49.6253	< 0.001**
	SG.EC- SG.RS	1.12673(0.0835403)	1(8)	1.12673(0.0104425)	107.898	0.013*
	SG.RS- SG.SO	0.305587(0.11343)	1(8)	0.305587(0.0141787)	21.5525	0.003**
	SG.RH- SG.RS	0.286011(0.154591)	1(8)	0.286011(0.0193239)	14.8009	0.005**
	SG.EC- SG.RH	1.28529(0.121018)	1(8)	1.28529(0.0151273)	84.9651	0.007**
	SG.RH- SG.SO	0.0135499(0.150908)	1(8)	0.0135499(0.0188635)	0.718314	0.672
	SG.EC- SG.SO	1.34579(0.0798571)	1(8)	1.34579(0.00998214)	134.82	0.006**
Fungi	SG.EC- SG.RH- SG.RS- SG.SO	6.63178(6.53519)	3(16)	2.21059(0.408449)	5.41216	< 0.001**
	SG.EC- SG.RS	0.407402(2.75986)	1(8)	0.407402(0.344982)	1.18094	0.309
	SG.RS- SG.SO	3.31283(3.25717)	1(8)	3.31283(0.407146)	8.1367	0.006**
	SG.RH- SG.RS	2.17638(3.47909)	1(8)	2.17638(0.434886)	5.00449	0.002**
	SG.EC- SG.RH	2.86233(3.27802)	1(8)	2.86233(0.409753)	6.98551	0.006**
	SG.RH- SG.SO	0.429087(3.77533)	1(8)	0.429087(0.471917)	0.909244	0.541
	SG.EC- SG.SO	4.07552(3.0561)	1(8)	4.07552(0.382013)	10.6685	0.008**

Note: \* Indicates significant difference between groups ( $P \leq 0.05$ ); \*\* indicates extremely significant difference between groups ( $P \leq 0.01$ ). SS stands for total variance, also known as the sum of squares of deviation; df is degrees of freedom; MS is the mean square (difference), i.e. SS / DF; FS is the F test value; P-value is the p value, with a value less than 0.05 indicating significant difference between groups. The values corresponding to the residual items are in brackets. SG.EC, SG.RS, SG.RH, SG.SO refer to roots (endophytic), root epidermis, rhizosphere soil, and rhizosphere soil of *Stipa grandis*, respectively.

## Discussion

Rhizoplane refers to the outer surface of plant roots and any closely attached soil or debris particles, which was proposed by Clark [32]. However, some scholars after him have different views, that is, only the soil attached to the

root can be regarded as the rhizosphere, while the root epidermis washed by soil particles is called rhizoplane [33–35]. York et al. [21] suggest that it was incorrect to call root epidermis as rhizoplane, which would greatly reduce the spatial range of rhizosphere and he agreed with Clark's [32] definition of rhizoplane. Based on this theory, we separated the rhizosheath, root epidermis and rhizosphere soil of *Stipa grandis*, a species with strong drought resistance in semi desert grassland. The results showed that there were significant differences in the composition of bacterial and fungal communities in root epidermis and rhizosheath, which could be inferred that they played different but important roles in plant growth. We agree with Clark [32] and York et al [21] that the combination of rhizosheath and root epidermis is called rhizoplane and belongs to part of rhizosphere. The unique environment of the internal roots of plants is called the inner boundary of roots [36], which is also recognized by York et al [21]. Our results showed that the bacterial and fungal community structure of root was significantly different from that of rhizosheath soil and rhizosphere soil, and although there was no significant difference in fungal community between root and root epidermis, there were differences in bacterial community. Both sides of root epidermis are root system and rhizosheath, which can be used as a compartment to separate root system from soil. In terms of rhizosphere microbial ecology, Philippot [37] mentioned that rhizosphere is the interface between plant roots and soil, and the rhizosphere environment is complex and dynamic. The interaction between various microorganisms affects plant growth and tolerance to biotic and abiotic stresses. However, we suggest that for plants with rhizosheath, the outer root epidermis is the interface between the root system and the external soil.

In recent years, research on the structure and function of plant roots, rhizosphere microbial communities has gradually increased [38–40]. Research has not only focused on changes in microbial diversity, but also included study of several different interactions and mechanisms between plant and soil microorganisms. For example, a recent review mentioned that the interaction of underground root soil microorganisms is very important for the growth and health of aboveground plants, and discussed the overall view of root soil rhizome microbial interaction realized by the progress of omics and bioinformatics technology, and the potential strategies for managing complex rhizosphere interaction to improve crop yield [41–43]. Studies on roots, rhizosheath, soil and microorganisms and the mechanisms of their interaction are rare. The main reasons may include: (1) Not all plants have a rhizosheath, and its function may be underestimated; (2) there is no standard method for separation of the rhizosheath from rhizoplane. The method of this study isolated roots, root epidermis, rhizosheath and rhizosphere soil, which seemed to clarify the relationship between rhizosheath, root epidermis and microbial differences in roots. This shows that the method of separating plant rhizosheath in this study is feasible, which will provide guidance for the extraction of other plant rhizosheath microorganisms and the study of interaction with plants.

Bergmann et al [44] found that the growth of several *Gramineae* plants in low nutrient and low moisture dune environments may be mediated by nitrogen fixing bacteria related to the rhizosheath. In addition, studies have shown that the rhizosheath can improve the drought resistance of plants [14, 15], but whether this is related to the function of some microorganisms in the rhizosheath needs further experiments to prove. It has been proved that there are nitrogen fixing bacteria in the rhizosheath, and nitrogen fixing bacteria can provide ammonia to plants in nitrogen limited soil [45–47]. There are few studies on other functions of microorganisms in root epidermis, which may be limited by experimental techniques. Therefore, our next work is to culture and verify the function of culturable microorganisms in root sheath, root epidermis and rhizosphere soil, and analyze their possible important functions by macrogenome sequencing.

## Conclusion

This study provides a feasible method to separate rhizosheath and root epidermis. This will provide the possibility for further study on the microorganism of rhizosheath and root surface. We suggest that the root epidermis can be used as a compartment to separate the root system from the soil and that it may act as the interface between the host and the external environment, with microorganisms in this interface potentially having some important functions, which we need to further study. The differences in composition and function of microorganisms inside and outside the rhizosheath prompt us to reexamine its potentially important role in plant growth. This study will provide a new method and theoretical guidance for further exploring the function and ecological significance of rhizosheath.

## Abbreviations

SG.EC:roots (endophytic); SG.RS: root epidermis; SG.RH: rhizosheath; SG.SO: rhizosphere soil..

## Declarations

### Ethics approval and consent to participate

The plant sample of *Stipa grandis* collected by us is not an endangered species. And this sample collection complies with relevant institutional, national, and international guidelines and legislation.

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### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Authors' contributions

AMZ was responsible for writing the manuscript; GDH and HLS was responsible for the design and management of the experiment. QW, HLL, assisted AMZ in sampling and determination of physiological and biochemical indexes. All authors reviewed the manuscript.

### Consent for publication

Not applicable.

### Competing interests

All authors declare that they have no competing interests.

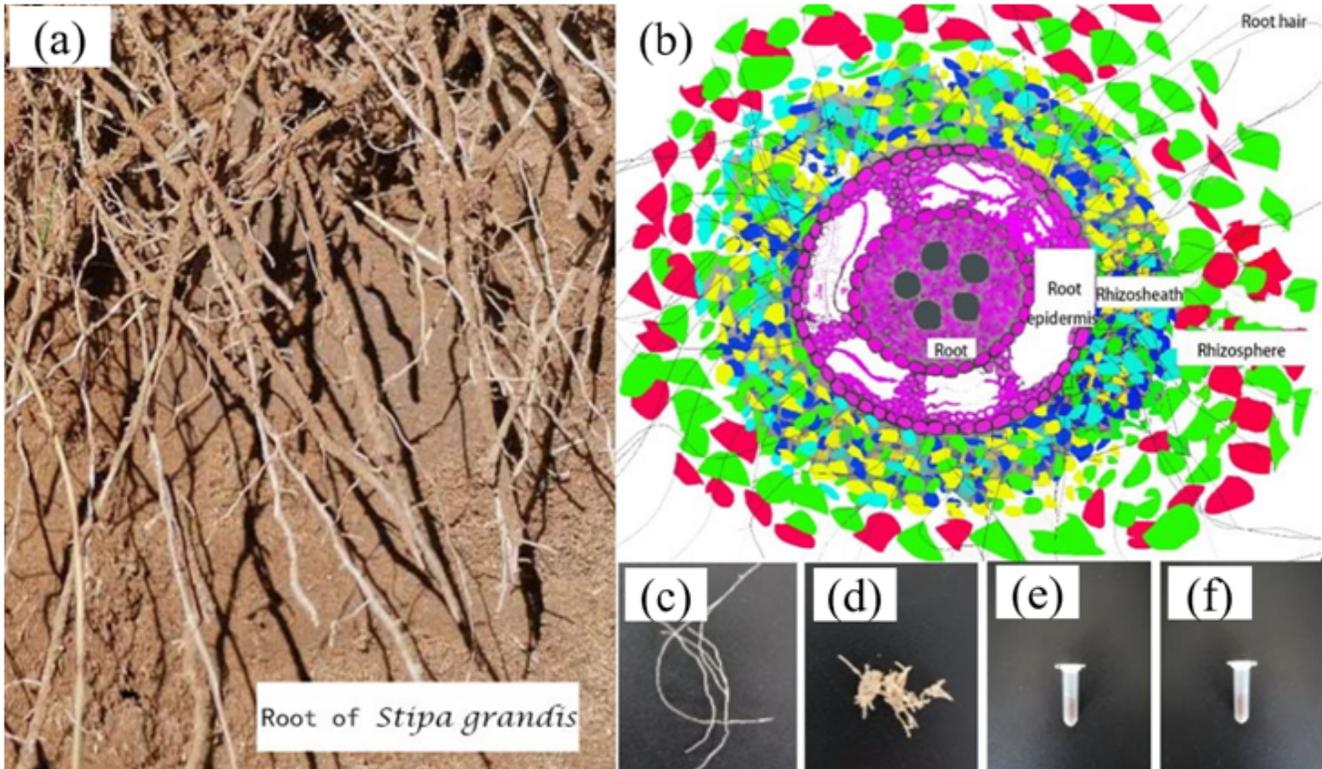
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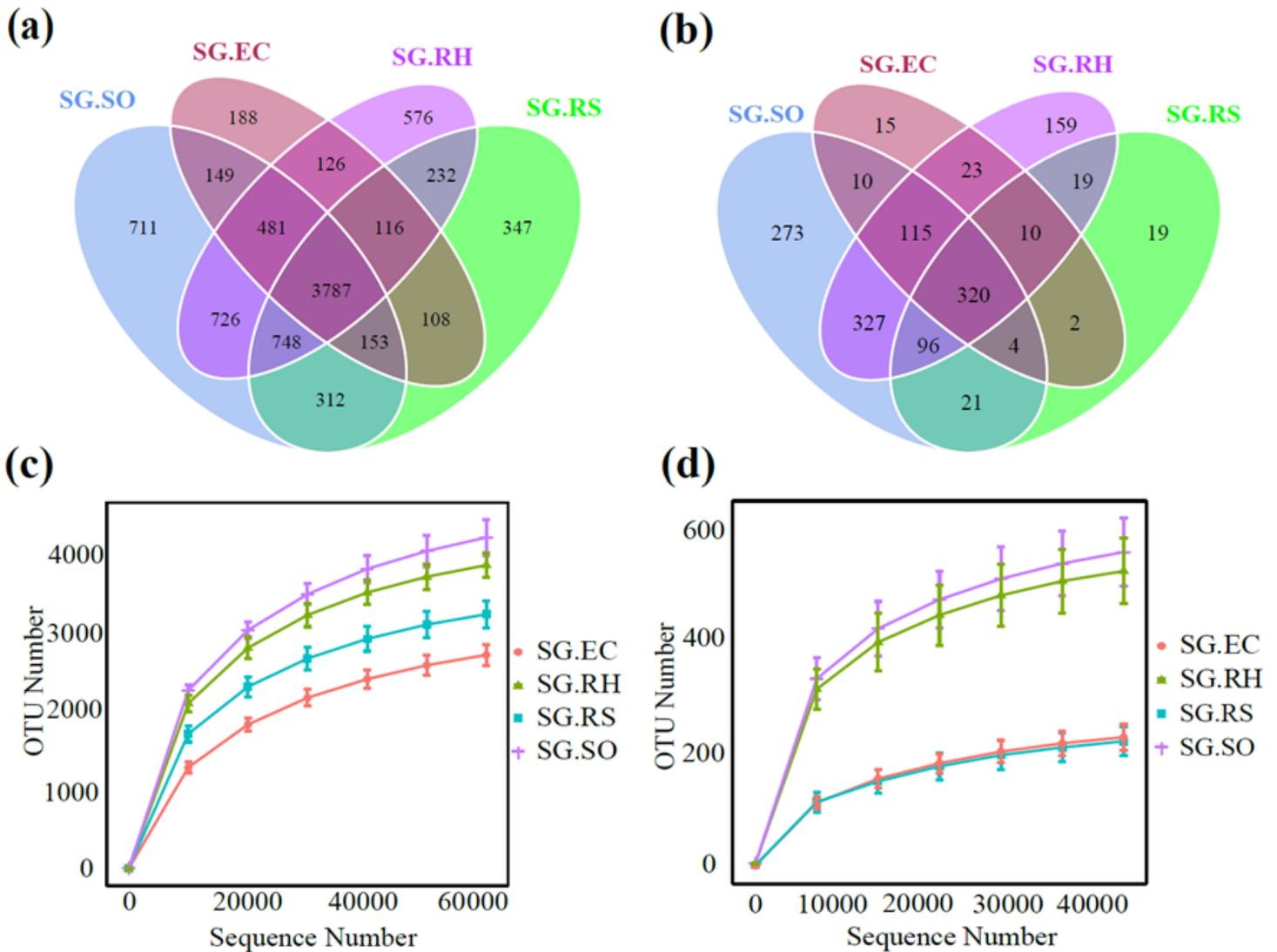
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## Figures



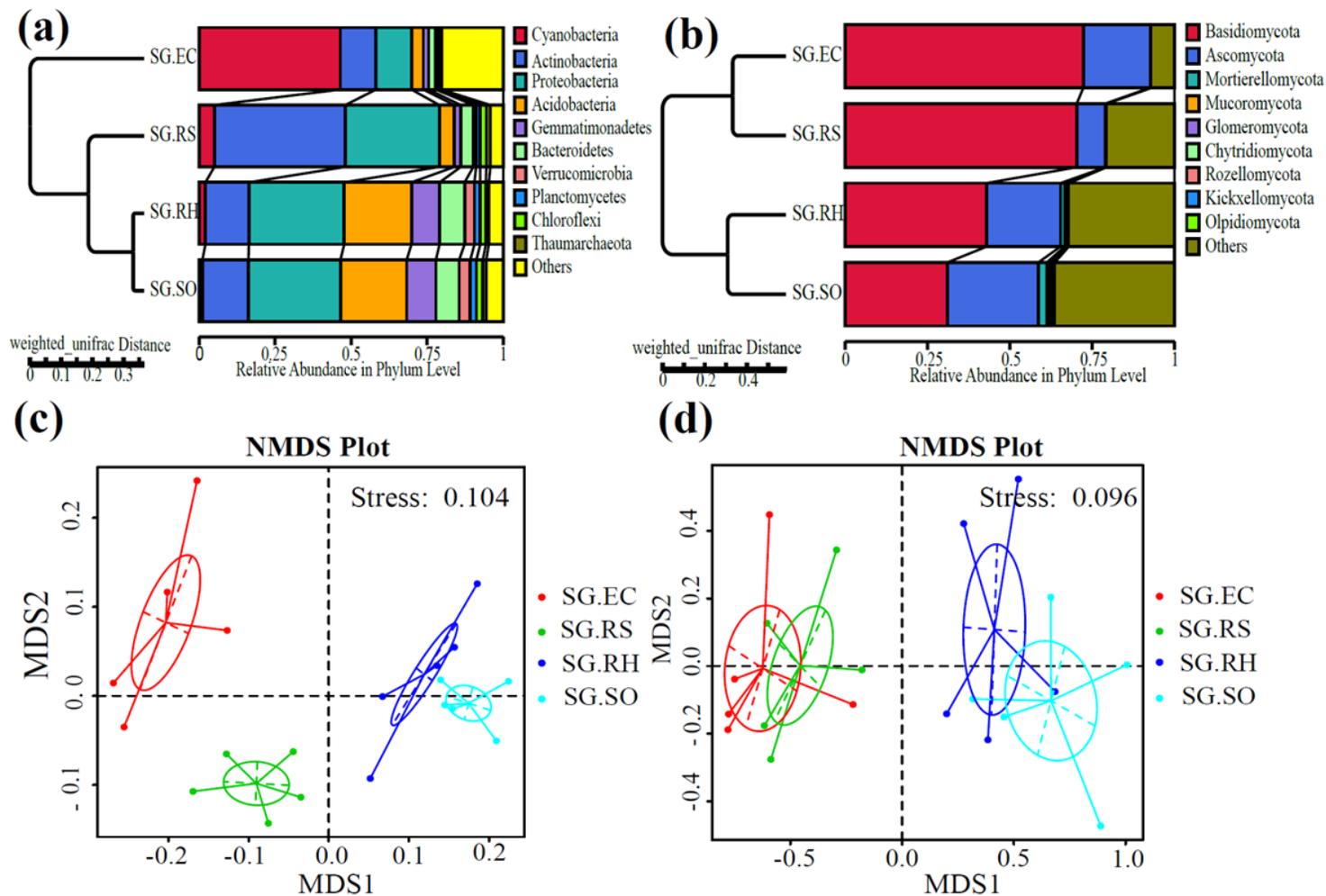
**Figure 1**

Root system of *Stipa grandis* and schematic diagram of root cross section and separation of root–root epidermis–rhizosheath–rhizosphere soil. Figure 1 (a): *Stipa grandis* root sample, Figure 1 (b): the schematic diagram of the cross section of the root system, and (c), (d), (e) and (f) in Figure 1 are root system, root epidermis, rhizosheath soil and rhizosphere soil respectively.



**Figure 2**

Venn diagrams of samples and rarefaction curves for samples. In figure 2, (a) and (b) are Venn diagrams of bacteria and fungi, and (c) and (d) are OTU dilution curves of bacteria and fungi respectively. In Fig. 2(a, b), each circle in the Venn diagram represents a group of samples. The number of overlapped parts represents the number of OTUs shared between groups, and the number without overlap represents the number of OTUs unique to the sample group. In Fig. 2 (c, d), the abscissa is the number of sequencing pieces randomly selected from a sample, and the ordinate is the number of OTUs that can be constructed based on the number of sequencing pieces to reflect the sequencing depth. SG.EC, SG.RS, SG.RH, SG.SO refer to roots(endophytic), root epidermis, rhizosphere, and rhizosphere soil of *Stipa grandis*, respectively.



**Figure 3**

UPMGA clustering tree diagram of species composition at bacteria and fungiphyla level and NMDS analysis of samples. In figure 3, (a) and (b) refer to the UPMGA cluster tree of bacteria and fungi respectively, and (c) and (d) refer to the PCA analysis of bacteria and fungi samples respectively. Both UPMGA cluster tree and PCA analysis are based on the relative abundance data of the sample species. In Fig. 3 (a, b), the length of the branch represents the distance between samples, and if the community composition of samples is similar, they are clustered into the same cluster in the cluster tree. For Fig. 3 (c, d), each point in the figure represents a sample, the distance between points indicates the degree of difference, and the samples from the same group are represented by the same color. When stress is less than 0.2, NMDS can accurately reflect the difference between samples. SG.EC, SG.RS, SG.RH, SG.SO refer to roots (endophytic), root epidermis, rhizosphere, and rhizosphere soil of *Stipa grandis*, respectively.