Bacterial Composition and Diversity Associated with Rhizosphere of the Chinese Medicinal Herb Dendrobium

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Abstract

Background: *Dendrobium* is a precious herbal belongs to Orchid and widely used as health care traditional Chinese medicine in Asia. Although orchids are mycorrhizal plants, most researches still focus on endophytes, and there is still large unknown in rhizosphere microorganisms. In order to investigate the rhizosphere microbial community of different Dendrobium species during the maturity stage, we used high-throughput sequencing to analyze microbial community in rhizosphere soil during maturity stage of three kinds of *Dendrobium* species.

Results: In our study, a total of 240,320 sequences and 11,179 OTUs were obtained from these three *Dendrobium* species. According to the analysis of OTU annotation results, different *Dendrobium* rhizosphere soil bacteria include 2 kingdoms, 63 phyla, 72 classes, 159 orders, 309 families, 850 genera and 663 species. Among all sequences, the dominant bacterial phyla (relative abundance > 1%) were Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria, Firmicutes, Verrucomicrobia, Planctomycetes, Chloroflexi, Gemmatimonadetes. We analyzed the environmental factors of the growth of *Dendrobium* and found that the environmental factor that affects the rhizosphere soil microorganisms of *Dendrobium* is the soil factor. Among them, soil factors most closely related to the influence of *Dendrobium* rhizosphere soil microorganisms include total nitrogen, available phosphorus, ammonium nitrogen and pH value.

Conclusions: We found that the rhizosphere bacterial communities of the three kinds of *Dendrobium* have significant differences, and the main species of rhizosphere microorganisms of *Dendrobium* are concentrated in the Proteobacteria, Actinobacteria, Bacteroidetes. Moreover, the smaller the level of bacterial, the greater the difference among *Dendrobium* species. Soil is the most important environmental factor affecting the bacterial communities in the rhizosphere soil of *Dendrobium*. These results fill the gap in the rhizosphere microbial community of *Dendrobium* and provide a theoretical basis for the subsequent mining of microbial functions and the study of biological fertilizers.

1. Background

Rhizosphere soil refers to the narrow zone of soil affected by root exudations, containing up to 10^{11} microbial cells and over 30,000 prokaryotic species [1–3]. Rhizosphere microbes are significantly different from non-rhizosphere microbes in terms of species, number, and activity [4]. There are abundant microbial resources in rhizosphere soil, which is 10-1000 times that in non-rhizosphere soil [5]. Proteobacteria is the dominant population in the rhizosphere bacterial community, and the highest content is in Pseudomonadaceae [6]. In addition, the contents of the Firmicutes and Acidobacteria are also high [7]. Through high-throughput sequencing, Lundberg et al. found that *Arabidopsis thaliana* had the highest relative abundance of Proteobacteria in the rhizosphere bacterial community, with Pseudomonadaceae being the main population, followed by Bacteroides and Actinomyces [8]. The interaction between rhizosphere microbes and plants can realize material circulation and energy flow. Changes in its community structure and abundance can affect plant growth and development, flowering

and fruiting, and its interaction with phytophagous insects, which is of great significance for plant growth and yield [9, 10].

Dendrobium is the second largest genus in Orchidaceae, with more than 1400 species in the world. There are 74 species and 2 varieties of *Dendrobium* in Orchidaceae in China, most of which are precious medicinal plants. *Dendrobium* has many functions, such as benefiting stomach and promoting body fluid, clearing heat and nourishing yin, relieving inflammation and relieving pain, clearing eyesight, and enhancing immunity [11]. Currently, artificial cultivation using non-symbiotic tissue culture does not meet market demand because of slow growth and low survival rates [12]. Therefore, the development of an effective method for propagating these endangered species for both conservation and commercial production is needed. There are three kinds of medicinal *Dendrobium* in Ta-pieh Mountain area, which are *Dendrobium huoshanense*, *Dendrobium officinale* and *Dendrobium moniliforme* [13]. All of them are perennial herbs of *Dendrobium* in Orchidaceae. They mainly contain polysaccharides, alkaloids and bibenzyls. They have antitumor, immunomodulatory, antioxidant, vasodilator and hypoglycemic effects [14].

At present, the research in *Dendrobium* mainly focuses on the endophytic bacteria. Indeed, there is a complex relationship between *Dendrobium* and its endophytes. Endophytic fungi can provide nutrients for *Dendrobium* plants [12, 15]. Previous studies have shown that Sphiugomouas and Mycobacterium bacteria isolated from the roots of *D. moschatum* (Buch. - ham) SW. could significantly improve the seed germination rate of *D. moschatum* (Buch. - ham) SW [16]; and there are many studies using protocorm as material to successfully isolated and obtained effective fungi that promote seed germination [17–19]. However, few reports are available regarding *Dendrobium* and soil microbial communities. There are abundant bacteria, fungi and actinomycetes in the rhizosphere of medicinal plants. Rhizosphere microbes of medicinal plants are inseparable from the growth, reproduction and metabolic activities of medicinal plants, not only can it promote the absorption of soil nutrients by medicinal plants, but it can also improve the yield and quality of medicinal plants, however it can also cause continuous cropping obstacles for medicinal plants. So, studying *Dendrobium* medicinal plants and their rhizosphere microbes is of great significance to clarify how rhizosphere microbes can improve the yield and quality of medicinal plants.

However, the traditional culturable methods only account for 0.1–1% of the environmental microorganisms, which cannot fully reflect the real situation of the environmental microbial community. In recent years, with the rapid development of new generation sequencing technology, based on 16 s rRNA sequence amplification and Illumina Miseq high-throughput sequencing technology, huge data information can be obtained. Through bioinformatics means, the overall microbial community composition can be obtained. With the advantages of high-throughput, low price and short operation cycle, it has been widely used in the study of microbial community structure. Especially in the field of medicinal plants, such as *Panax ginseng* [20], *Panax notoginseng* [21], *Ajuga bracteosa* [22], *Origanum vulgare* [23], *Lilium davidii* [24] and other plants are widely used.

Therefore, the goal of our work reported here was to characterize the rhizosphere microbial community of different *Dendrobium* species during the maturity stage. Our specific objective was to describe taxa associated with each *Dendrobium* species and determine which the environmental factors were related to microbial diversity and community composition. Specifically, we hypothesized that: (1) the dominant genus bacteria of *Dendrobium* and its comparison with other medicinal plants; (2) rhizosphere community composition will differ between three different *Dendrobium*; and (3) examine the relationship between environmental factors and composition of microbial communities. A better understanding of the difference of soil microbial communities in *Dendrobium* and the main influencing factors that affect the rhizosphere soil microbes of the *Dendrobium*, could further provide a theoretical basis for analyze microbial functions and development of *Dendrobium* bio-fertilizer.

2. Methods

2.1 Plant Material

Dendrobium plants were artificially cultivated in the greenhouse of Anhui Tongjisheng Biotechnology Company, Lu'an, China. The original source was collected by the company from the wild after obtaining local permission. The growth of protocorm-like bodies protocols and the condition of planting were described by our previous study [25]. From the grown plants of the three species of *Dendrobium*, two-year-old *D. huoshanense*, *D. moniliforme* and *D. officinale* were selected to provide eight replicates of each sample. The voucher specimens were authenticated by Maoyun Yu and deposited at Jiangsu Key Laboratory of Crop Genetics and Physiology in Yangzhou University, Yangzhou, China (Voucher number: 20A01, 20A02 and 20A03).

2.2 Soil sampling

In order to obtain rhizosphere soil, plants were removed from flowerpots and large soil aggregates were removed by hand; soil firmly attached to roots was collected with sterile brushes and regarded as rhizosphere soil. The rhizosphere soil was sampled and sieved to remove plant debris. A part of soil samples was put into sterile centrifuge tubes, frozen in liquid nitrogen immediately, and then stored at −80 °C until the soil microbial composition was analyzed. The other part was air dried for chemical analysis.

2.3 Soil properties and climate factors

Soil pH was measured with a soil/deionized water ratio of 1/2.5 [26]. Soil total nitrogen (TN) was determined by Kjeldahl method [27]. Soil ammonium nitrogen (AN) was extracted using 2 M KCl solution followed by the method of detection using colorimetric-indophenol blue [26]. Available phosphorus (AP) of soil samples was extracted with ammonium fluoride (NH_4F, 0.03 M) and hydrochloric acid (HCl, 0.025 M), and measured by UV–Vis spectrophotometer [28]. Available potassium (AK) of soil samples was determined by the extraction with CH_3COONH_4 (1 M), and measured by a flame photometer [29]. There were four replicates for each sample and each indicator. The climate data has been used in our

previous experiments, and the collection method has been reported in the previous study [30]. The measurement results of all environmental factors are shown in Table 1.

Table 1
Environmental factors for the growth of three *Dendrobium* species

Environmental factors		Dh	Do	Dm
Soil factors	pH value	5.67	5.29	5.26
	Total nitrogen (TN): $mg \cdot g^{-1}$	0.52	0.41	0.35
	Ammonium nitrogen (AN): $mg \cdot g^{-1}$	0.03	0.02	0.01
	Available phosphorus (AP): $mg \cdot g^{-1}$	0.004	0.001	0.001
	Available potassium (AK): $mg \cdot g^{-1}$	0.02	0.01	0.01
Climate factors	Temperature: ℃	18.39		
	Relative humidity (RH): %	73.81		
	Solar radiation (SR): $W \cdot m^3$	16.5		
	Sunshine duration (SD): h	10.59		
	Maximum monthly average temperature (MaxT): ℃	34.43		
	Minimum monthly average temperature (MinT): ℃	8.38		
	Maximum monthly average solar radiation (MaxSR): $W \cdot m^3$	174.48		
	Maximum monthly average relative humidity (MaxRH): %	0.89		
	Minimum monthly average relative humidity (MinRH): %	0.34		

2.4 DNA extraction, PCR amplification and 16S rRNA sequencing

According to the manufacturer's instructions, total DNA was extracted from 250 mg of each sample using a using the DNeasy Power Soil Kit (Qiagen, Valencia, CA, USA). DNA concentration was measured using Qubit® dsDNA Assay Kit in Qubit® 2.0 Flurometer (Life Technologies, CA, USA). The purity of DNA was expressed as the ratio of absorbance at 260 nm and 280 nm (A_{260}/A_{280}) using a Nanodrop® spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). DNA degradation degree and potential contamination was monitored on 1% agarose gels. For each pot we obtained a single DNA sample, which was used for 16S rRNA sequencing.

For bacterial diversity analysis, V4 hypervariable regions of 16S rRNA genes were amplified with universal primers 515 F (5'-GTGCCAGCMGCCGCGG-3') and 806 R (5'-GGACTACHVGGGTWTCTAAT-3') [31], and then PCR products were sequenced by IonS5^{TM}XL sequencing technique platform. Quality filtering on the raw reads were performed under specific filtering conditions to obtain the high-quality clean reads according to the Cutadapt [32] (V1.9.1, http://cutadapt.readthedocs.io/en/stable/) quality-controlled process. Chimeric sequences were removed by comparing with the Silva database (https://www.arb-silva.de/) [33] using UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) [34] to detect chimera sequences, and then the chimera sequences were removed. Operational taxonomic units (OTUs, cutoff 97% sequence identity for 16S rRNA) were clustered by UPARSE software (Uparse v7.0.1001, http://drive5.com/uparse/) [35]. The phylogenetic taxonomy was assigned according to the Ribosomal Database Project (RDP) classifier at an 80% confidence threshold (Version 2.2) [36] using the Silva databases for bacteria. Alpha- and Beta-diversity analyses were calculated in QIIME software (Version 1.7.0). Alpha-diversity was described for each sample using the metrics observed species (OTU numbers), Chao1, Shannon and Simpson index, ACE and Good-coverage were generated to compare the level of bacterial OTU diversity. Beta-diversity was estimated by computing weighed/unweighed UniFrac and Bray-curtis distances followed by principal coordinate analysis (PCoA).

2.5 Statistical analysis

Statistical analysis of $p < 0.05$ was performed using SPSS 19.0 (IBM, USA). The WGCNA software package, stat and ggplot2 packages in R software (Version 2.15.3) were used for PCoA analysis. The Spearman correlation index between species and environmental factors was calculated, and its significance was tested by the corr.test in the psych package of R, and then the visualization was performed by the pheatmap function in the pheatmap package.

3. Results

3.1 α- and β-diversity of soil microbial communities

The rhizosphere soil alpha diversity indexes of different *Dendrobium* species are different (Fig. 1). The Good's coverage index of *Dendrobium* library was greater than 98.5%, indicating that the sequencing results reflect the real situation of the bacterial population in the sample. We found that Dh had the highest Shannon diversity index (10.29), while Do had the lowest (9.16), which indicated that Dh rhizosphere soil has the highest diversity of bacterial communities. The index of ACE and Chao1 have the same trend, Dh is the highest, respectively 5225 and 4877, indicating that Dh samples have the highest community richness. Further analysis revealed that the Shannon index and Simpson index of soil bacteria of Dh was significantly different from Do, and Do was significantly different from Dm.

Principal coordinate analysis (PCoA) was performed at the operational taxonomic unit (OTU) level (Fig. 2). The analysis of PCoA using the weighted UniFrac distance, indicated a distinct pattern in the rhizosphere bacterial communities associated with the two axes explaining 29.58% and 19.62% of the

total variation in the *Dendrobium* rhizosphere soil. Using the unweighted UniFrac distance, the two axes explaining 17.64% and 11.79% of the total variation in the *Dendrobium* rhizosphere soil. Bacterial communities associated with the rhizosphere soil were clustered in three regions according to a PCoA using the unweighted UniFrac distance, corresponding to Dh, Do, and Dm. However, no clustering was detected using the weighted UniFrac distance from different *Dendrobium* species.

3.2 Taxonomic classification and abundance

Rarefaction curves for bacterial communities suggested that changes in OTU density within the different *Dendrobium* species was sufficiently captured, and the sequencing was relatively comprehensive in covering the microbial communities (Fig. 3a). After quality filtering and processing according to a 97% similarity, a total of 240,320 sequences were obtained from these three *Dendrobium* species, respectively: 80,101 for Dh, 80,109 for Do and 80,110 for Dm. The OTUs common to the different *Dendrobium* species are presented in Fig. 3b as a venn diagram to assess the relationships among the bacterial communities. 5609 OTUs were shared by Dh, Do and Dm; 1041 OTUs were shared by Dm and Do; 926 OTUs were shared by Dh and Do; and 971 OTUs were shared by Dm and Dh. The numbers of OTUs unique to each species were as follows: 1007 for Dm, 909 for Do, 716 for Dh. A total of 11,179 OTUs were detected in all samples (Fig. 3b).

According to the analysis of OTU annotation results, different *Dendrobium* rhizosphere soil bacteria include 2 kingdoms, 63 phyla, 72 classes, 159 orders, 309 families, 850 genera and 663 species (Fig. 4). Among all sequences, the dominant bacterial phyla (relative abundance > 1%) were Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria, Firmicutes, Verrucomicrobia, Planctomycetes, Chloroflexi, Gemmatimonadetes, and with contributions of 45.41%, 17.58%, 9.94%, 7.77%, 3.93%, 3.03%, 2.88%, 1.98% and 1.14%, respectively. The difference at the phylum level of different *Dendrobium* species is not large, but there were more and more obvious differences in the level of the class afterwards. At the order level, the relative abundance of Alteromonadeles on Dh and Dm was low, while the relative abundance on Do was relatively high. Pseudoalteromonadaceae at family level and Pseudoalteromonas at genus level also have the same relative abundance pattern. At the species level, although the top ten species of bacteria in the rhizosphere soils of the three *Dendrobium* species were the same, the relative expression abundances were very different.

3.3 LEfSe analysis based on the relative abundance of different *Dendrobium* species

To further elucidate the possible interactions between identified bacterial dependencies in rhizosphere soil samples, linear discriminant analysis (LDA) effect size (LEfSe) method was used for quantitative analysis of biomarkers in different species. We detected significant differences in the abundance of bacterial biomarkers from different groups and identified a total of 13 biomarkers from all rhizosphere soil samples as shown in the branching diagram (Fig. 5b). The significant taxa in the Dh were affiliated with diverse phylogenetic groups, including the family Sphingomonadaceae, and order

Sphingomoadales. In the Dm, significantly abundant taxa were the genus Flavobacterium, family Flavobacteriaceae and order Flavobacteriales. In the Do, the significant taxa belonged to the phylum Actinobacteria, class unidentified Actinobacteria and Acdobacteriia, order Pseudonocardiales and Acidobacteriales, family Pseudonocardiaceae and unidentified Acidobacteriales, genus Crossiella, were abundant.

3.4 Relationship between ecological factors and bacterial communities

To obtain further evidence for this relationship between environmental factors and bacterial communities, the contributions of soil and climate factors to variations in the soil bacterial community were quantified by performing variance partitioning canonical correspondence analysis (Fig. 4a). Soil factors and climate factors were significantly related to variation in bacterial communities based on forward model selection ($p < 0.05$). Moreover, soil factors explained a higher proportion (25.31%) of the variation than climate factors (5.3%). It can be seen from the results that soil factors are considered to be one of the most important environmental factors affecting the rhizosphere microorganisms of *Dendrobium*.

Environmental factors influenced the percentage abundance of soil bacterial communities. The correlation of bacterial genus with environmental factors was analyzed by Spearman's rank correlation (Fig. 4b). Among these environmental factors, soil factors are the most important factors affecting the rhizosphere microorganisms of *Dendrobium*, especially TN, AP, AN and pH value. However, among climate factors, the most influential factors are only concentrated in the three factors including solar radiation, maximum monthly average relative humidity and temperature. Moreover, relative humidity has no significant effect on any bacterial genus.

4. Discussion

Rhizosphere microorganisms can co-exist with plant roots, colonize and maintain in roots, and play an important role in promoting plant growth and development [37]. Among them, the utilization rate and sensitivity of bacteria to root exudates are far higher than that of fungi, and bacteria are the most active and dominant microorganisms in rhizosphere [38, 39]. In addition, the number and species of rhizosphere microorganisms have a direct impact on soil biochemical activity and nutrient transformation. Under the influence of various complex factors of natural conditions, there are great differences in rhizosphere microbial flora of different plants and even different genotypes of the same plant [40, 41]. So, in this study, bacterial communities associated with the rhizosphere of three different *Dendrobium* species were characterized by high-throughput sequencing during the maturity stage.

4.1 Bacterial communities in rhizosphere soil of *Dendrobium*

It can be seen from the results that the dominant phylum in the rhizosphere soil of *Dendrobium* are Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria, Firmicutes, Verrucomicrobia, Planctomycetes, Chloroflexi and Gemmatimonadetes. This indicates that the richness and diversity of the rhizosphere microbial community of *Dendrobium*. However, the main dominant bacteria of *Dendrobium* are still concentrated in Proteobacteria, Actinobacteria and Bacteroidetes. Lundberg et al. found that the relative abundance of Proteobacteria, Bacteroidetes, Actinobacteria, Acidobacteria, Firmicutes and Gemmatimonadetes in Arabidopsis rhizosphere soil were relatively high, which were the dominant bacteria in Arabidopsis rhizosphere soil [8]. Ling et al. studied the rhizosphere microorganisms of watermelon, and found that Acidobacteria, Actinomycetes, Bacteroidetes, Cyanobacteria, Firmicutes and Proteobacteria were the main leading bacteria [42]. Davide et al. found that Actinobacteria, Bacteroidetes and Proteobacteria were dominant in barley rhizosphere soil [43]. Although there are differences in the bacterial communities of different plant rhizosphere soils, the dominant phylum of Actinobacteria, Bacteroidetes and Proteobacteria are the common dominant phylum of *Dendrobium* and the above-mentioned plants, indicating that these phyla may be the common dominant phylum of all plant rhizosphere bacterial communities. Proteobacteria predominates in all ecosystems, especially in soil systems [44, 45], due to the fact that Proteobacteria contains a large level of physiological, morphological and metabolic diversity, and that Proteobacteria is of great significance to the C and N cycles [46]. Proteobacteria reproduce fast, have good adaptability to unstable carbon sources, and are widely distributed in the global soil environment [47]. In general, the abundance of Proteobacteria or Acidobacteria in soil samples is the most abundant. These bacterial groups rich in rhizosphere microorganisms of *Dendrobium* are also found to be the dominant communities of other plant rhizosphere microbiomes [48, 49].

4.2 Differences of bacterial communities in rhizosphere soils of three different *Dendrobium* species

According to the diversity index of alpha and beta, there were significant differences among the three *Dendrobium* species. The relative abundance of Acidobacteriales, Pseudonocardiaceae, Pseudoalteromonas and Peseudomonadales in *Dendrobium officinale* were higher than those in the other two species. Acidobacteriales is the dominant bacteria in the common plant rhizosphere bacterial community. Because Acidobacteriales can degrade complex root exudates such as cellulose and lignin, it plays a major role in the plant rhizosphere carbon cycle [50]. Pseudoalteromonas sp. secretes a variety of extracellular active substances, including proteins, polysaccharides, brominated compounds, extracellular enzymes, extracellular toxins, antibiotics and so on [51]. These substances have antibacterial, algicidal, bactericidal and cellulose degrading activities [52, 53]. Peseudomonadales is an important group of biocontrol microorganisms, and is also one of the most widely distributed microorganisms in nature. Its rapid reproduction, strong colonization ability and simple nutrition requirements have been widely studied for its inhibition of plant diseases and promotion of plant growth. In the existing studies, Pseudonocardiaceae is mainly associated with cellulose degradation and antibiotic synthesis [54-56]. It

can be seen from the above that the function of the bacterial community in the rhizosphere of *Dendrobium officinale* may be stronger than that of the other two kinds of *Dendrobium*. In *Dendrobium moniliforme*, the relative abundance of Bacteroidetes is relatively high, and Bacteroidetes is a poor nutrient bacterium, which is suitable for growth in the environment with less absorbable nutrients such as organic matter and available nitrogen [57].

In bacterial species, we found that Lysobacter soli have a high relative abundance in *Dendrobium moniliforme*, this fungus has previously been isolated from the soil where ginseng grown [58], and has been found in other plants to promote plant activity [59]. Psychrobacter ibarius has been found in *Dendrobium officinale*. Some researchers have isolated this bacterium from the root plane of *Angelica sinensis* [60]. This is a fungus related to polysaccharide synthesis. This is consistent with our previous research results [13]. Our previous research also found that among these three kinds of *Dendrobium*, the polysaccharides of two-year-old *Dendrobium* are higher than the other two kinds of two-year-old *Dendrobium*.

4.3 Soil is the most important environmental factor affecting the bacterial communities in the rhizosphere soil of *Dendrobium*

Due to the differences of research scale and microbial groups, there are no environmental factors that can fully explain the distribution pattern of all soil microorganisms. For different space-time scales and different research objects, the environmental factors that affect the distribution of microorganisms are also different, and there is no single natural factor that affects the microbial community. Various environmental factors work together to make a choice for the spatial distribution of microorganisms [61]. Studies have shown that soil properties, vegetation types and meteorological factors have varying degrees of influence on soil microbial diversity. Therefore, this study selected a total of 14 factors of soil factors and climate factors, and analyzed their correlation with the rhizosphere bacterial community of *Dendrobium*, so as to determine the most important environmental factors affecting the rhizosphere bacterial community of *Dendrobium*.

Among the climatic factors, it has been reported that temperature and precipitation have a driving effect on soil microbial composition [62-64]. In this study, the bacterial community of *Dendrobium* was affected by the maximum monthly average relative humidity and temperature, which was consistent with previous studies [65]. These data indicate that temperature and relative humidity are the key climatic factors affecting the bacterial composition of *Dendrobium*. Research has shown that the increase of nitrogen input had consistent effects on the richness, diversity and composition of soil bacterial community in two consecutive crop seasons. In our soil factors, total nitrogen explained most of the major variations in bacterial communities, consistent with previous studies [66]. These results indicate that TN is an important soil factor for bacterial community formation. In addition, we also found that solar radiation is also the main climate affecting factor, which was also reported in *Panax notoginseng* [67]. Zarraonaindia

studies showed that as a potential microbial pool of plant-related microorganisms, soil microorganisms have a great impact on grape root related microorganisms [68]. Soil type is an important factor affecting the rhizosphere microbial community, and the main reason for the influence is the difference of soil microbial community [69]. Therefore, in our previous analysis, we also found that the most important environmental factor affecting the bacterial community of *Dendrobium* is also soil factor, accounting for 25.31%. Total nitrogen, available phosphorus and available potassium in soil are important indexes to measure soil fertility, and they are also the main sources of nutrients absorbed by plants. Previous studies have shown that plant roots can affect the community structure of root microorganisms by changing the physical and chemical properties of soil. For example, plant existence can change the structure of rhizosphere soil, water holding capacity, nutrients [70] and form environmental conditions that are conducive to the growth of some strains or not conducive to the growth of certain strains, thus changing the community structure of soil microorganisms.

5. Conclusions

Overall, the chemical properties of rhizosphere soil of *Dendrobium* and the climatic characteristics of *Dendrobium* cultivation were firstly analyzed. Then, combined with high-throughput sequencing technology, the rhizosphere soil microbial communities of different species of *Dendrobium* were studied, and the rhizosphere microorganisms and environmental driving factors of different species of *Dendrobium* were discussed. The main conclusions are as follows: the dominant bacteria in rhizosphere soil of *Dendrobium* are as follows: Proteobacteria, Bacteroidetes, Actinobacteria, and Acidobacteria. These are the dominant bacteria in the rhizosphere bacterial community. However, there are some differences in the bacterial communities among different *Dendrobium* species, and the smaller the bacterial level is, the greater the difference is. We found that there are Psychrobacter ibarius in *Dendrobium officinale*, which is a kind of fungi related to the accumulation of polysaccharides. Soil factors and climate factors jointly affect the species diversity and community composition of rhizosphere soil microorganisms of *Dendrobium*. And soil is considered to be the most important factor that affects the rhizosphere soil microbes of *Dendrobium*. Among these soil factors, total nitrogen, available phosphorus, ammonium nitrogen and pH value have the greatest impact on the rhizosphere soil microbes of *Dendrobium*.

Abbreviations

AN: Ammonium nitrogen; AP: Available phosphorus; AK: Available potassium; OTUs: Operational taxonomic units; RDP: Ribosomal Database Project; PCoA: Principal coordinate analysis.

Declarations

Ethics approval and consent to participate

Dendrobium huoshanense, *Dendrobium officinale* and *Dendrobium moniliforme* used in this study were cultivated by Maoyun Yu's greenhouse from Anhui Tongjisheng Biotechnology Company, Lu'an, China. No permits were required for the collection of the samples.

Consent to publish

Not applicable.

Availability of data and materials

Raw amplicon sequence data related to this study were deposited in the NCBI Sequence Read Archive (NCBI SRA) under Bioprojects PRJNA638443.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

Conception and design of the research: YY; acquisition of data: MZ and LL; analysis and interpretation of data: XS; statistical analysis: MZ; drafting the manuscript: YY and MZ; revision of manuscript for important intellectual content: YY. All authors read and approved the final manuscript.

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Figures

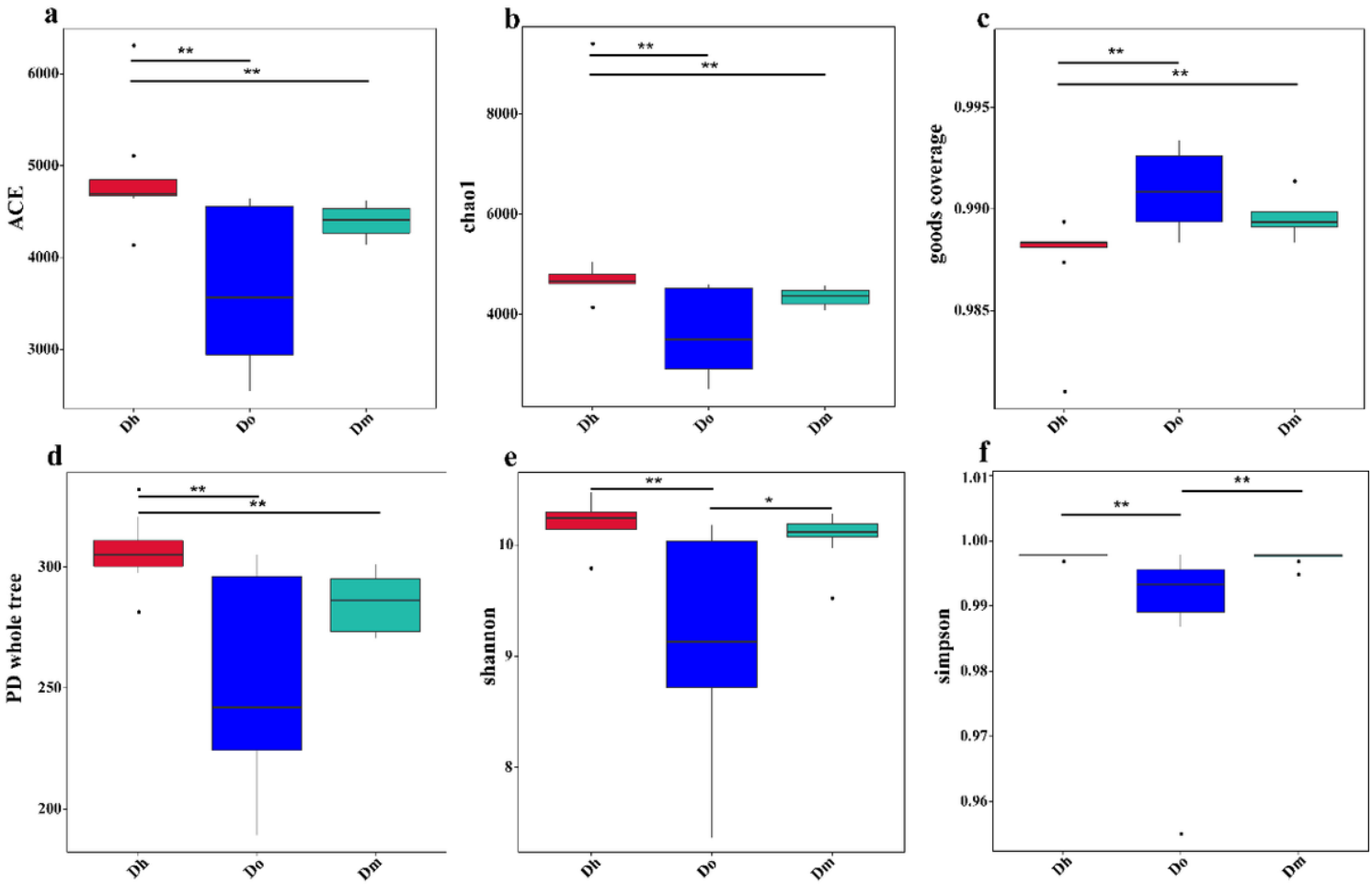

Figure 1

α-diversity indexes of three Dendrobium species. a. ACE; b. chao1; c. goods coverage; d. PD whole tree; e. shannon; f. simpson.

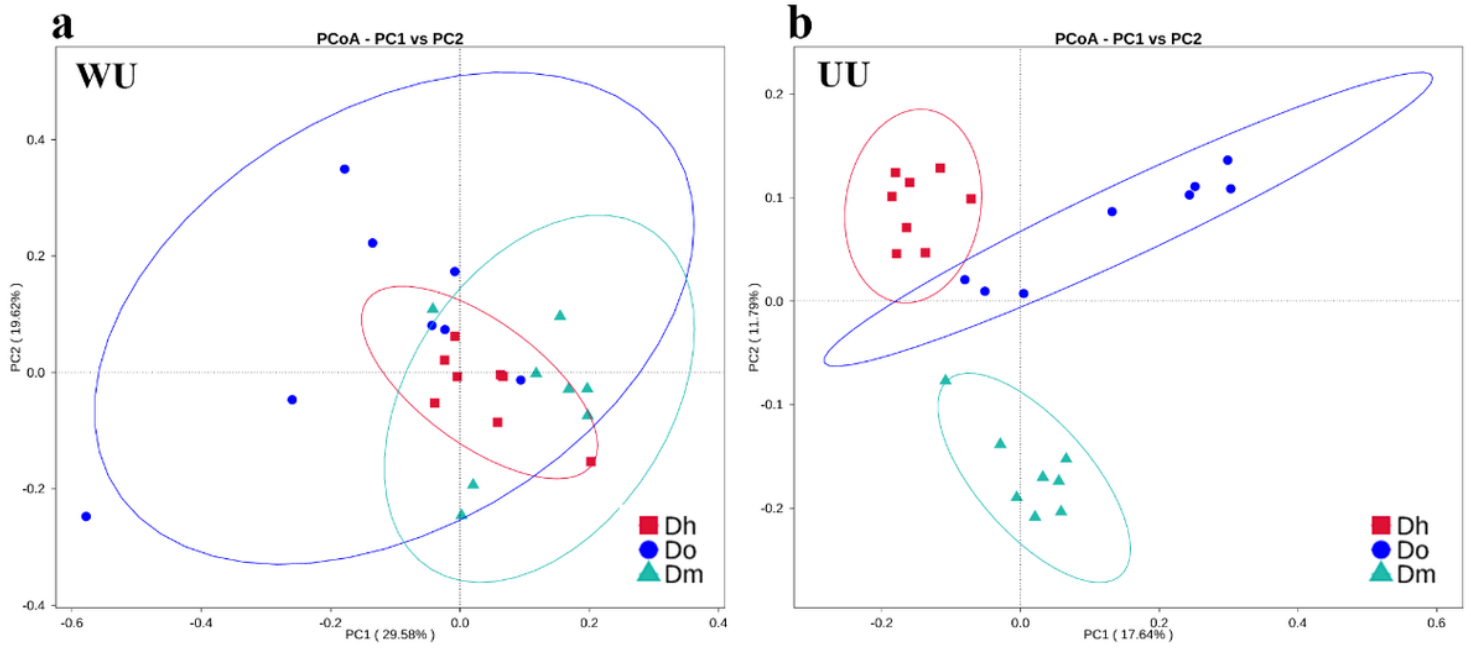

Figure 2

PCoA analysis of β-diversity based on the weighted UniFrac (WU) and unweighted UniFrac (UU) distances. a: WU distance; b: UU distance. Symbols with different colors represent different species: red square: Dendrobium huoshanense; blue circle: Dendrobium officinale; green triangle: Dendrobium moniliforme.

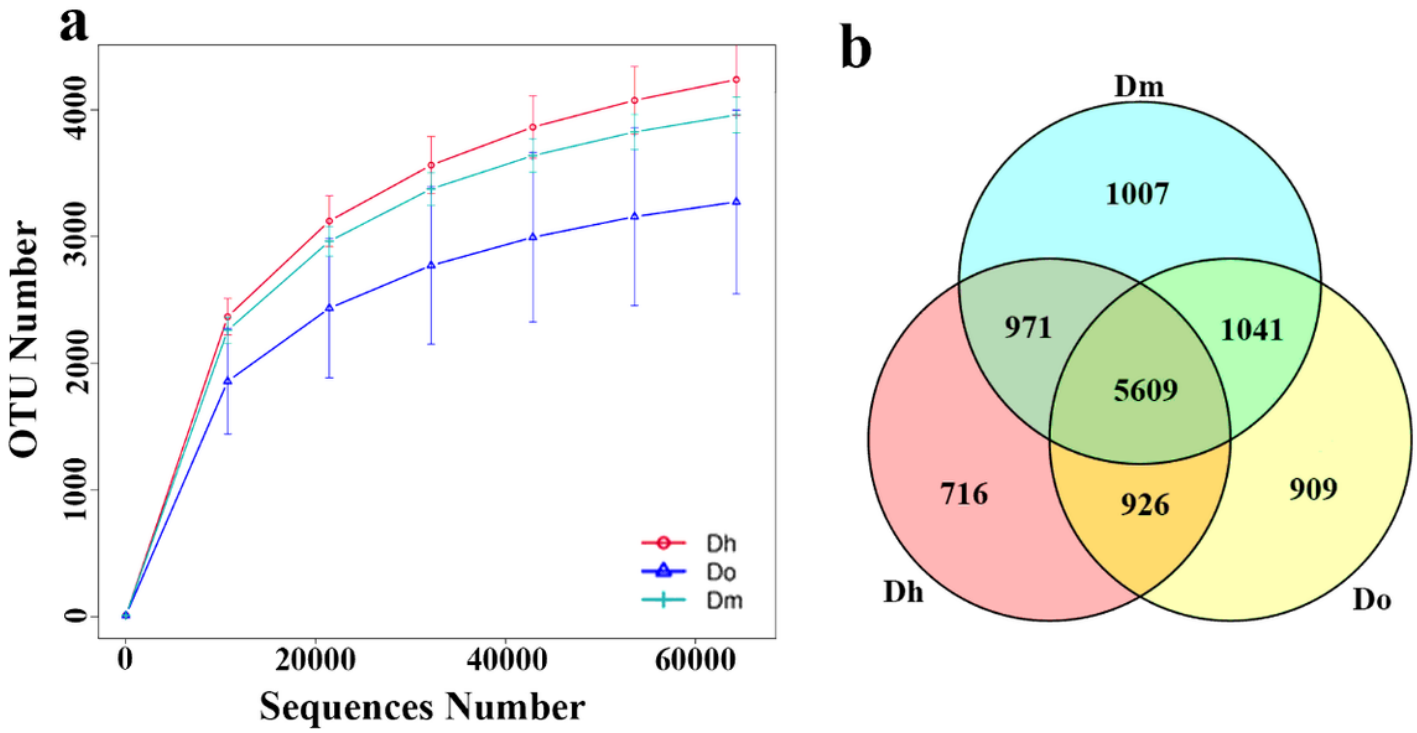

Figure 3

(a) Rarefaction curves depicting the number of sequences against the number of operational taxonomic units (OTUs) identified from three Dendrobium species. (b) Venn diagram representing bacterial operational taxonomic units (OTUs) associated with the rhizosphere of Dendrobium.

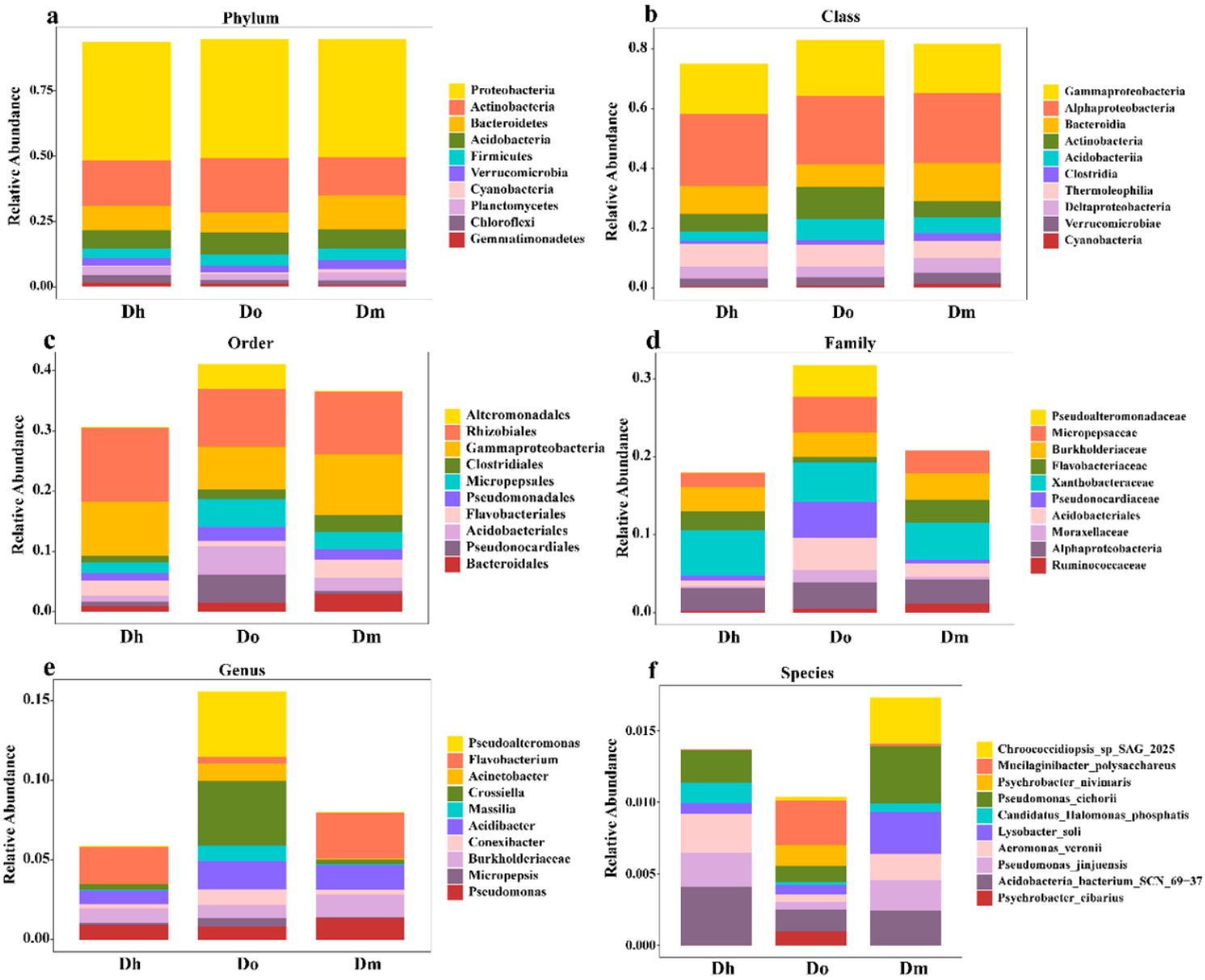

Figure 4

Top 10 relative abundances of bacterial communities classified at phylum (a), class (b), order (c), family (d), genus (e) and species (f) level in different Dendrobium species.

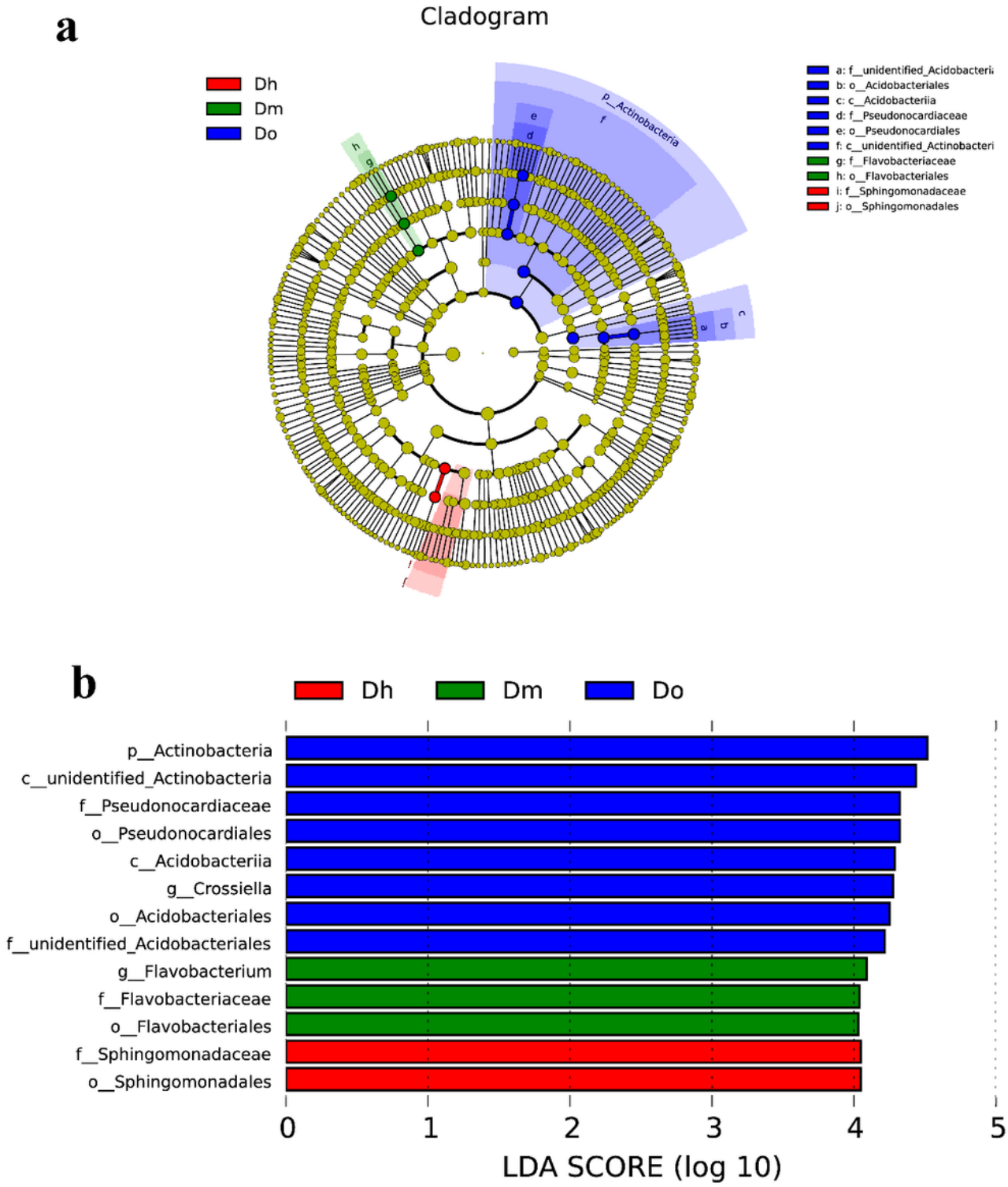

Figure 5

(LEfse) analysis of microbial abundance in Dh, Do and Dm. (a) Clustering diagram indicates phylogenetic distribution of the bacterial communities in three groups. (b) The histogram of LDA scores computed for differentially abundant microbe among different Dendrobium species identified with a threshold value of 4.0.

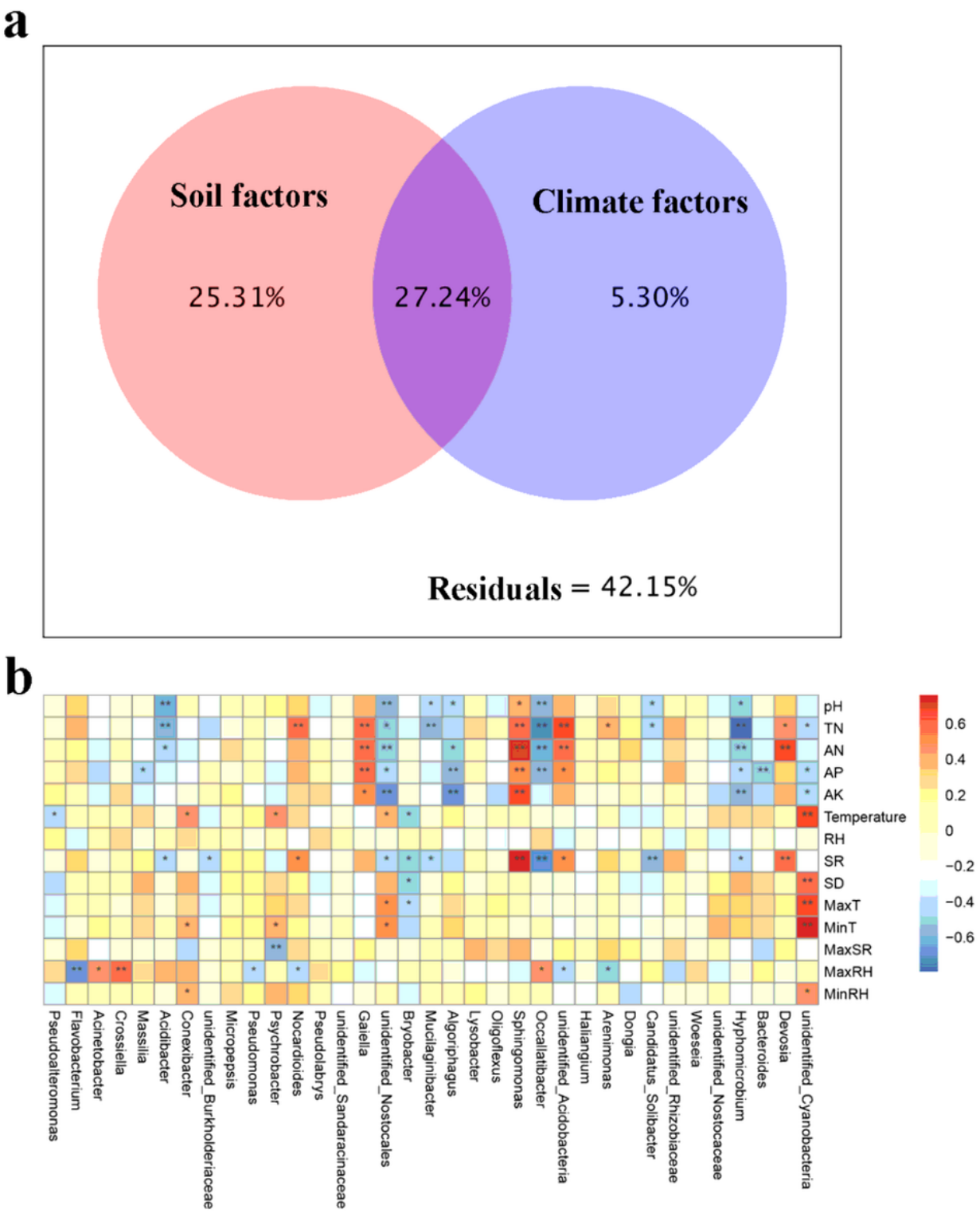

Figure 6

(a) Variance partitioning canonical correspondence analysis of bacterial communities by soil and climate factors. (b) Spearman's rank correlation coefficients and statistical significance between genus abundance relative to environmental factors.