CHARACTERIZING THE SOIL PROPERTIES AND MICROBIAL DIVERSITY ASSOCIATED TO THE ENDANGERED *BUDDLEJA SESSILIFOLIA*

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Abstract. *Buddleja sessilifolia* is a plant species with extremely small populations (PSESP) in southwest China. The trial of transplantation failed in our *Buddleja* outdoor garden, and only succeeded in the flowerpots of greenhouse. In this study, we conducted metagenomic analyses on microbial diversity in bulk soil from wild habitat of *B. sessilifolia*, the *Buddleja* garden and the *B. sessilifolia* pots of greenhouse, and meanwhile analyzed soil physicochemical properties. We aimed to uncover the related soil factors which may influence the transplantation of *B. sessilifolia*. Our results showed the bulk soil from the wild habitat of *B. sessilifolia* and the *B. sessilifolia* pots had significantly richer total potassium content than that from the *Buddleja* garden. Bacterial order Rhizobiales and archaeal class Nitrososphaeria showed a higher diversity in both the wild habitat and the pot than the garden. Furthermore, soil total potassium content was the most correlated to bacterial community structure of the bulk soil, while soil total nitrogen content and total phosphorus content were most important to archaeal and fungal community structures. We accordingly inferred that *B. sessilifolia* had a high demand on potassium and microbial Rhizobiales and Nitrososphaeria in soil for its survival. Our findings will contribute to the *ex-situ* conservation of *B. sessilifolia*.
Keywords: *soil microbiome, metagenome, PSESP, Buddleja, ex-situ conservation, Scrophulariaceae*

Introduction

Buddleja sessilifolia B. S. Sun ex S.Y. Pao is a shrub species of the genus *Buddleja* belonging to the family Scrophulariaceae. It was originally described in 1983 by *Flora Yunnanica*, and was later treated as a synonym of *B. colvilei* Hook. f. et Thoms. (Chang et al., 1992). Recently, on the basis of molecular evidence and careful morphological examination, *B. sessilifolia* was retained as an independent species (Ge et al., 2018). This species was mainly restricted in Gaoligong Mt. of Yunnan in China with high altitudes (2600 m~3150 m) and cold temperatures (Ge et al., 2018), and has been evaluated as a Plant Species with Extremely Small Populations (PSESP) in 2021 by Yunnan Provincial Government (http://www.yn.gov.cn/hdjl/yjzh/202104/t20210414_220452.html). Therefore, the rescuing conservation on this species is imperative and *ex-situ* conservation is one of the effective ways. In the past years, the wild plants of

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B. sessilifolia and the seedlings were tried to transplant in the *Buddleja* outdoor garden of Kunming Botanic Garden, Yunnan, China, but all of them unfortunately died in one year since plantation. However, some seedlings of *B. sessilifolia* transplanted in the pots of greenhouse in Kunming Botanic Garden successfully survived. Thus, it is worthy uncovering the possible soil factors beneficial to the successful transplantation of *B. sessilifolia*.

Soil microbiome has been considered as an important driver for plant growth and health by assisting in the acquisition of nutrients, supporting abiotic stress tolerance and disease-resistance (Berendsen et al., 2012; Bulgarelli et al., 2013; Jamir et al., 2019; Saleem et al., 2019; Muneer et al., 2020; Qu et al., 2020; Gao et al., 2021; Ding and Li, 2022). For example, the diseased Chili pepper could recruit beneficial bacteria such as *Enterobacter*, *Klebsiella*, *Citrobacter*, and *Pseudomonas* to facilitate host or its offspring survival (Gao et al., 2021). The rhizobacterium *Pseudomonas protegens* and *P. stutzeri* utilization can promote the available nitrogen content and increase the seeds yield of wheat (Fox et al., 2016). Phosphate-solubilizing bacteria have a synergistic effect on *Agave angustifolia* plant growth promotion, especially *Pseudomonas luteola* and *Bacillus* sp. (Bautista-Cruz et al., 2019). Mycorrhizal fungi can improve the uptake of mineral nutrients of the associated plants by extending hyphae (van der Heijden et al., 1998). On the other hand, the microbial diversities between bulk and rhizosphere soil were proven similar in some cases (Cui et al., 2018; Praeg et al., 2019), although the differences between them were detected in other cases (Liu et al., 2018; Essel et al., 2019; Goss-Souza et al., 2020). Besides soil microbiome, soil physicochemical properties are well known to be very important for the plant growth (Bathke et al., 1992; Kekane et al., 2015), and microbial structures were closely linked to variation in soil physicochemistry among sites (Wakelin et al., 2016; Mechiah et al., 2021).

In this study we aimed to uncover the potential soil factors which can contribute to the successful transplantation of *B. sessilifolia*. We investigated the microbial diversities including bacteria, archaea and fungi in bulk soil from the wild habitat of *B. sessilifolia*, the successful transplantation site (the *B. sessilifolia* in the greenhouse) and the failed transplantation site (the *Buddleja* garden), as well as the soil physicochemical properties.

Materials and Methods

Sampling

The wild populations of *Buddleja sessilifolia* were investigated in Gongshan County, Nujiang Lisu Autonomous Prefecture of Yunnan Province of China with 2600~3150 m elevation in August 2018. Eight bulk soil samples of four individuals were collected from two *in-situ* populations (abbreviated as D and K) and meanwhile some living plants and seeds of *B. sessilifolia* were collected (*Table 1*). The wild plants and cultivated seedlings were transplanted in a specialized outdoor garden for *ex-situ* conservation of *Buddleja* species as well as in some flowerpots of a greenhouse, both of which are located at Kunming Botanic Garden in Kunming City of Yunnan Province of China with ca. 1950 m elevation (*Table 1*). The specialized garden has been established for over 15 years and receives intensive care. All the wild plants and cultivated seedlings of *B. sessilifolia* in the *Buddleja* garden died in one year since the plantation, but those in the flowerpots of greenhouse survived.

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Table 1. *The locality information of the collected soil samples*

Group	Samples	Locality	Longitude	Latitude	Altitude
WILD	D12, D16	On the border to Myanmar, Danzhu village, Gongshan County	98°35'51"	27°37'56"	2850 m
	K13, K15	43 kilometers from Gongshan to Dulongjiang, Gongshan County	98°29'52"	27°46'48"	3140 m
GARDEN	YY1, YY2, YY3, ZYZ1, ZYZ2, ZYZ3, ZYY1, ZYY2, ZYY3, MMH1, MMH2, MMH3	The *Buddleja* outdoor garden in Kunming Botanic Garden, Kunming City	102°44'30.35"	25°8'29.64"	1970 m
POT	WB_1, WB_2, WB_3	The greenhouse in Kunming Botanic Garden, Kunming City	102°44'27.68"	25°8'45.41"	1940 m

Notes: WILD means soil from wild habitat of *B. sessilifolia*, GARDEN means soil from the Buddleja outdoor garden of Kunming Botanic Garden, POT means soil from pots in the greenhouse of Kunming Botanic Garden. YY refers to the bulk soil near to *B. caryopteridifolia*, ZY refers to soil near to *B. crispa*, ZYZ refers to soil near to *B. crispa* × *officinalis*, MMH refers to soil near to *B. officinalis*, WB refers to soil from the different pots in the greenhouse, D and K refer to soil from the wild plants of *B. sessilifolia* in different populations

In year 2021, the bulk soil samples were collected from the two transplanted sites. Because of the death of *B. sessilifolia* in the specialized garden, the bulk soil samples were taken from the spots near to other conserved *Buddleja* species which have different wild habitats. They are *B. crispa* (abbreviated as ZY) which originally distributes in the dry-hot and dry-warm valleys with 1600~3600 m elevation, *B. caryopteridifolia* (YY) which originally distributes in the dry-hot valleys with 1000~2300 m elevation, *B. officinalis* (MMH) which widely distributes in the subtropical areas of China with 200~2800 m and *B. crispa* × *officinalis* (ZYZ) which is only recorded in Xishan mountain of Kunming City with ca. 2000 m elevation. A total of 24 bulk soil samples were taken from the spots near to 12 individuals of the abovementioned four species. Besides, six bulk soil samples were collected near to three individuals of *B. sessilifolia* (abbreviated as WB_1, WB_2 and WB_3) which were planted in the flowerpots of greenhouse. All bulk soil samples were sieved at a mesh size of 2 mm for excluding the undecomposed litter and small stones and sealed in separate polyethylene bags. The sieved soil samples taken near to each individual of plants were subsequently manually mixed in new polyethylene bags and stored in 50 ml sterile tubes, resulting in one composite soil sample per spot. The samples were then stored in a dry-ice box, and transported to the laboratory, and finally stored in -80 ℃ ultra-low temperature freezer. Additionally, a ca. 100 g of soil was dried at 40 ℃ for the analysis of soil physicochemical properties. The following soil variables were measured by Yunnan Sanbiao Agro-forestry Technology Co., Ltd.: pH, organic matter, organic carbon, total N, total P, total K, available N, available P, available K.

DNA extraction, library construction, and Illumina MiSeq sequencing

For each sample, total microbial DNA was extracted from 0.5~1 g per soil using a DNeasy PowerSoil Kit (QIAGEN, Inc., Netherlands) according to the manufacturer's protocols. The quantity and quality of extracted DNAs were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA)

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and agarose gel electrophoresis, respectively, to ensure that they were available for amplicon sequencing.

The sequencing libraries were prepared according to the Illumina Metagenomic sequencing library protocols for the 16S rRNA gene for bacteria and archaea, and the internal transcribed spacer 1 (ITS1) region for fungi. The primers 338F (ACTCCTACGGGAGGCAGCA) and 806R (GGACTACHVGGGTWTCTAAT) were used for amplifying the bacterial 16S rRNA gene V3–V4 regions, ITS5F (GGAAGTAAAAGTCGTAACAAGG) and ITS1R (GCTGCGTTCTTCATCGATGC) were used for the fungal ITS1, and ARC349F (GYGCASCAGKCGMGAAW) and ARC806R (GGACTACVSGGGTATCTAAT) were used for the archaea 16S rRNA gene. Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR components contained 5 μl of buffer (5×), 0.25 μl of Fast pfu DNA Polymerase (5U/μl), 2 μl (2.5 mM) of dNTPs, 1 μl (10 uM) of each Forward and Reverse primer, 1 μl of DNA Template, and 14.75 μl of ddH_2O. Thermal cycling consisted of initial denaturation at 98 °C for 5 min, followed by 25 cycles consisting of denaturation at 98 °C for 30 s, annealing at 53 °C for 30 s, and extension at 72 °C for 45 s, with a final extension of 5 min at 72 °C. PCR amplicons were purified with Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2-250 bp sequencing was performed using the Illlumina MiSeq platform with MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

Sequence analysis

Microbiome bioinformatics were performed with Vsearch pipeline (Rognes et al., 2016). Briefly, primers of raw sequences were cut with Cutadapt (Martin, 2011). Sequences were then quality filtered, denoised, merged and chimera removed using the following commands: *fastq_filter*, *derep_fulllength*, *fastq_mergepairs*, *cluster_size*, *uchime_denovo* implemented in Vsearch (Rognes et al., 2016). Non-singleton operational taxonomic units (OTUs) were aligned with mafft (Katoh et al., 2002) and used to construct a phylogeny with fasttree2 (Price et al., 2009). Alpha-diversity metrics (Observed species, Shannon (Shannon 1948b, a)), beta diversity metrics (Bray-Curtis dissimilarity (Bray and Curtis, 1957)) were estimated using the diversity plugin with rarefied samples. Taxonomy was assigned to OTUs using the classify-sklearn naïve Bayes taxonomy classifier in feature-classifier plugin (Bokulich et al., 2013) against the databases of SILVA Release 132 (Quast et al., 2013) for bacteria/archaea, and UNITE Release 8.0 (Kõljalg et al., 2013) for fungi.

Bioinformatics and statistical analysis

Sequence data analyses were mainly performed using QIIME2 (Bolyen et al., 2019) and R (R Core Team, 2018) with packages *ape* (Paradis and Schliep, 2018), *ggplot2* (Wickham, 2009), *vegan* (Oksanen et al., 2019). OTU-level alpha diversity indices, such as Observed species and Shannon diversity index were calculated using the OTU tables in QIIME2, and visualized as box plots. Beta diversity analysis was performed to investigate the structural variation of microbial communities across samples using Bray-Curtis metrics (Bray and Curtis, 1957) and visualized via principal coordinate analysis (PCoA). The significance of differentiation of microbiota structure among groups was assessed by PERMANOVA (Permutational multivariate analysis of variance)

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(McArdle and Anderson, 2001) using QIIME2. The taxonomy compositions were visualized using QIIME2 via *qiime taxa barplot*. The most significant factors shaping the structures of the microbial communities were determined by a Redundancy Analysis (RDA) and a Monte Carlo permutation test using the Hellinger transferred data of microbial taxa at generic level and the data of soil variables. The correlations between microbial alpha diversity (bacteria, archaea, and fungi) and soil variables were analyzed using Pearson correlation coefficients in R. The significance of differentiation of the soil physicochemical variables among groups were assessed using Welch Two Sample t-test in R as well as the differences among the dominant bacterial and archaeal taxa.

Results

Soil physicochemical properties in different sampling sites of Buddleja sessilifolia

The bulk soil from the *B. sessilifolia* pots in the greenhouse (POT) tended to be more fertile than the bulk soil from the wild habitat (WILD) of *B. sessilifolia* and the Buddleja outdoor garden (GARDEN) (*Tables 2, 3*), and the bulk soil from WILD is barren in organic matter, total nitrogen, total phosphorus (*Tables 2, 3*). However, the bulk soil from both WILD and POT had significantly richer total potassium content (K) than that from GARDEN (*Table 3*), and the content of available N, available P, and available K in bulk soil from both WILD and POT also tended to be more than that from GARDEN (*Tables 2, 3*).

Table 2. *The values of soil variables in different sampling spots*

Group	Sample	pH	Organic (g/kg)	Total_N (g/kg)	Total_P (g/kg)	Total_K (g/kg)	available_N (mg/kg)	available_P (mg/kg)	available_K (mg/kg)
GARDEN	YY1	6.47	91.47	3.92	1.64	1.92	282.31	14.24	152.4
	YY2	6.34	76.06	3.23	1.57	1.82	238.56	5.84	101.6
	YY3	5.39	47.5	2.01	1.37	1.2	168.4	10.14	49.77
	ZYZ1	6.31	66.98	2.55	1.63	1.28	205.54	8.58	77.08
	ZYZ2	6.18	60.66	2.52	1.69	1.24	195.64	2.72	64.18
	ZYZ3	5.83	56.78	2.42	1.61	1.37	193.99	7.99	86.75
	ZYY1	6.26	57.51	2.21	1.61	1.83	184.91	6.43	91.06
	ZYY2	6.65	73.57	3.01	1.69	1.36	224.53	6.43	59.27
	ZYY3	6	79.55	3.26	1.56	1.27	236.91	8.19	54.51
	MMH1	7	62.62	3.14	1.37	2.89	231.13	6.43	132.1
	MMH2	6.45	28.24	1.32	1.56	1.27	98.23	5.65	51.03
	MMH3	6.5	42.73	1.75	1.55	1.48	146.11	5.25	71.22
POT	WB-1	7.58	198.49	5.84	1.89	6.11	255.07	24.2	405.9
	WB-2	7.6	189.3	5.73	1.83	5.62	351.65	28.89	363.9
	WB-3	7.68	168.5	5.4	1.83	6.43	321.94	20.3	422.2
WILD	D12	5.22	5.4	0.1	0.1	6.6	44.13	3.96	35.92
	D16	4.24	46.1	1.6	0.2	5.4	324.99	12.45	139.12
	K7	4.71	54	1.9	0.2	4.6	373.13	13.77	139.12
	K15	4.96	24.8	0.7	0.1	4.3	176.54	2.45	61.2

Notes: WILD, bulk soil from wild habitat of *B. sessilifolia*; GARDEN, bulk soil from the Buddleja outdoor garden of Kunming Botanic Garden; POT, bulk soil from pots in the greenhouse of Kunming Botanic Garden; YY, bulk soil near to *B. caryopteridifolia*; ZY, bulk soil near to *B. crispa*; ZYZ, bulk soil near to *B. crispa* × officinalis; MMH, bulk soil near to *B. officinalis*; WB, bulk soil from the different pots in greenhouse; D and K, bulk soil from the wild plants of *B. sessilifolia* in different populations

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Table 3. *Welch Two Sample t-test of the selected physicochemical variables among the bulk soil from the WILD habitat of Buddleja sessilifolia, GARDEN and POTs*

	TN			TP			TK			AN			AP			AK		
	t	**df**	**p-value**	**t**	**df**	**p-value**	**t**	**df**	**p-value**	**t**	**df**	**p-value**	**t**	**df**	**p-value**	**t**	**df**	**p-value**
WILD vs GARDEN	-3.310	4.701	**0.023**	-33.958	9.952	**0.000**	6.849	3.459	**0.004**	0.384	3.213	0.725	0.277	3.512	0.797	0.398	3.778	0.712
WILD vs POT	-10.564	3.590	**0.001**	-48.407	4.884	**0.000**	-1.466	4.121	0.215	-0.999	3.819	0.377	-4.281	4.987	**0.008**	-9.542	4.793	**0.000**
POT vs GARDEN	12.186	11.586	**0.000**	7.692	11.089	**0.000**	16.327	0.588	**0.000**	3.429	3.040	**0.041**	6.544	2.469	**0.013**	15.937	3.294	**0.000**

Notes: WILD, bulk soil from wild habitat of *B. sessilifolia*; GARDEN, bulk soil from the Buddleja outdoor garden of Kunming Botanic Garden; POT, bulk soil from pots in the greenhouse of Kunming Botanic Garden

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Microbial alpha diversity and community composition

A total of 4126596 high-quality microbial sequences were identified from all soil samples including 974685 bacterial, 1432566 archaeal and 1719345 fungal sequences (*Table 4*). The bacterial, archaeal and fungal sequences were clustered into 25969, 15020 and 8055 OTUs, respectively (*Table 4*). The alpha diversity (Shannon index and Observed species index) of the microbiome of bulk soil showed the difference among the sample sites (*Fig. 1*). For bacterial communities, the bulk soil from the WILD and POT had similar Shannon index and Observed species index, but significantly higher than that from the GARDEN. For archaeal communities, the Shannon index showed no significantly differences among the three kinds of bulk soil, but the Observed species index of the bulk soil from WILD was significantly higher than those from other two sites. For fungal communities, the Shannon index and Observed species index were significantly higher in the bulk soil from WILD than those from GARDEN and POT, and the latter two showed no significant difference.

Table 4. *The number of high-quality sequences and OTUs of Bacteria, Archaea, Fungi*

Group	Sample ID	Bacteria		Archaea		Fungi	
		Sequences	OTUs	Sequences	OTUs	Sequences	OTUs
GARDEN	YY1	34841	4389	103167	2514	101739	811
	YY2	42116	4255	75641	2449	116700	811
	YY3	49612	4479	97016	3289	102179	691
	ZYZ1	56323	4439	79504	2492	115702	897
	ZYZ2	52352	4219	72682	2058	111987	604
	ZYZ3	50672	4472	84901	3094	117662	846
	ZYY1	55597	3856	94738	2423	108471	620
	ZYY2	48925	4428	96067	2510	95158	978
	ZYY3	58066	3927	72953	2256	116117	695
	MMH1	38580	4151	93044	2559	114010	956
	MMH2	59115	3959	88085	2618	108544	879
	MMH3	39791	4582	79641	2644	97366	838
POT	WB_1	51566	4813	93247	2576	102691	834
	WB_2	51222	4876	88546	2708	105450	825
	WB_3	60211	5145	101111	2542	110831	878
WILD	D12	67380	4445	25876	277	23168	848
	D16	57388	4449	22496	1369	22011	1134
	K13	48488	5299	33297	918	21349	1386
	K15	52440	5282	30554	622	28210	1346
Total		974685	—	1432566	—	1719345	—

Notes: WILD, bulk soil from wild habitat of *B. sessilifolia*; GARDEN, bulk soil from the Buddleja outdoor garden of Kunming Botanic Garden; POT, bulk soil from pots in the greenhouse of Kunming Botanic Garden; YY, bulk soil near to *B. caryopteridifolia*; ZY, bulk soil near to *B. crispa*; ZYZ, bulk soil near to *B. crispa* × *officinalis*; MMH, bulk soil near to *B. officinalis*; WB, bulk soil from the different pots in greenhouse; D and K, bulk soil from the wild plants of *B. sessilifolia* in different populations

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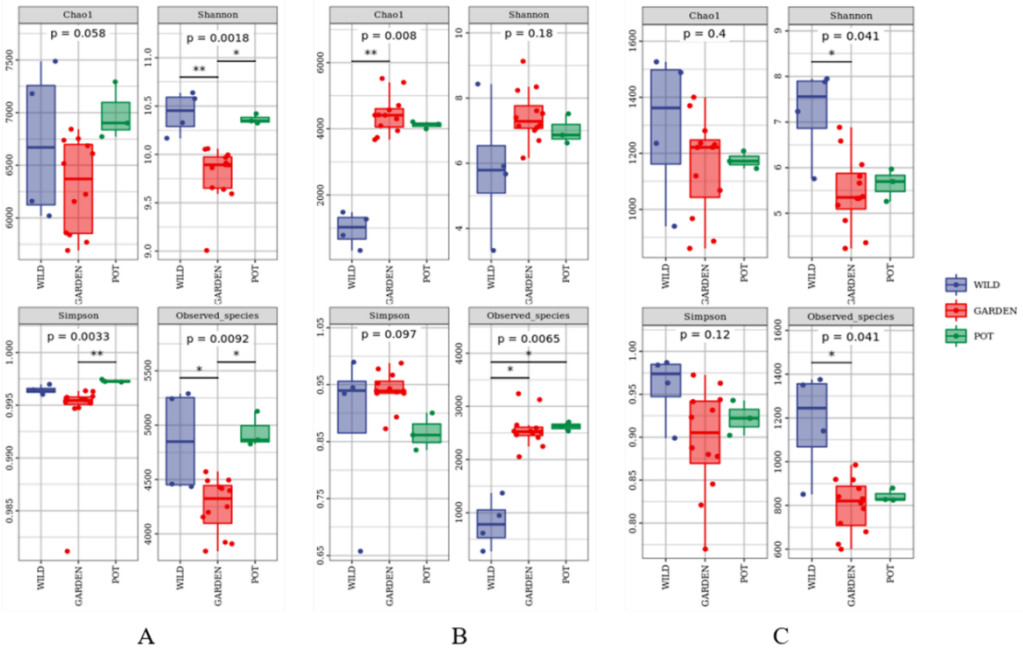

Figure 1. *Differences in bacterial (A), archaeal (B) and fungal (C) alpha diversity of the bulk soils from different sampling sites showed by Shannon Index and Observed Species Index. The p values in the charts were calculated by Kruskal-Wallis test. The Dunn's test was also made and the significant difference was indicated by ** (P<0.01) and * (P < 0.05). WILD, bulk soil from wild habitat of B. sessilifolia; GARDEN, bulk soil from the Buddleja outdoor garden of Kunming Botanic Garden; POT, bulk soil from pots in the greenhouse of Kunming Botanic Garden*

In the bacterial community, the most dominant bacterial phylum in the bulk soil from WILD, POT, and GARDEN was the same, namely Proteobacteria (45.9%, 45.4%, and 29.9%, respectively). However, the OTU numbers of Proteobacteria showed different among them, which both the former two sites (WILD: 45.9%, POT: 45.4%) had much more OTU numbers than GARDEN (29.9%; *Fig. 2-A*). In the phylum Proteobacteria, the most dominant order was Rhizobiales in all kinds of soil, and the OTU numbers of Rhizobiales in the bulk soil from WILD and POT tended to be higher than that from GARDEN (13.3%, 16.8% and 11.3%, respectively; *Fig. 2-B*, *Table 5*). In the archaeal community, the two most dominant phyla (*Fig. 2-C*) in the bulk soil from WILD, POT, GARDEN were all Thaumarchaeota (69.7%, 43.7% and 21.2%, respectively) and Euryarchaeota (16.1%, 14.5% and 12.9%, respectively). In the phylum Thaumarchaeota, the most dominant class was Nitrososphaeria in all kinds of soil, but the OTU number of Nitrososphaeria in the bulk soil from WILD and POT tended to be higher than that from GARDEN (65.7% in WILD, 43.7% in POT and 21.0% in GARDEN; *Fig. 2-D*, *Table 5*). In the fungal community, the most dominant phylum in the bulk soils from WILD, POT, and GARDEN was all Ascomycota (32.1%, 65.9% and 48.3% respectively; *Fig. 2-E*), while the second dominant phylum was different among them. Basidiomycota (26.5% and 36.9%, respectively) is the second dominant phylum in the bulk soil from WILD and GARDEN, but Mortierellomycota is the second dominant group in the soil from POT with a much low percentage (5.7%). Agaricomycetes is the most dominant class in the bulk soil from WILD and GARDEN (22.2% and 24.9%, respectively), while Sordariomycetes is the most abundant one in that from POT (27.5%; *Fig. 2-F*).

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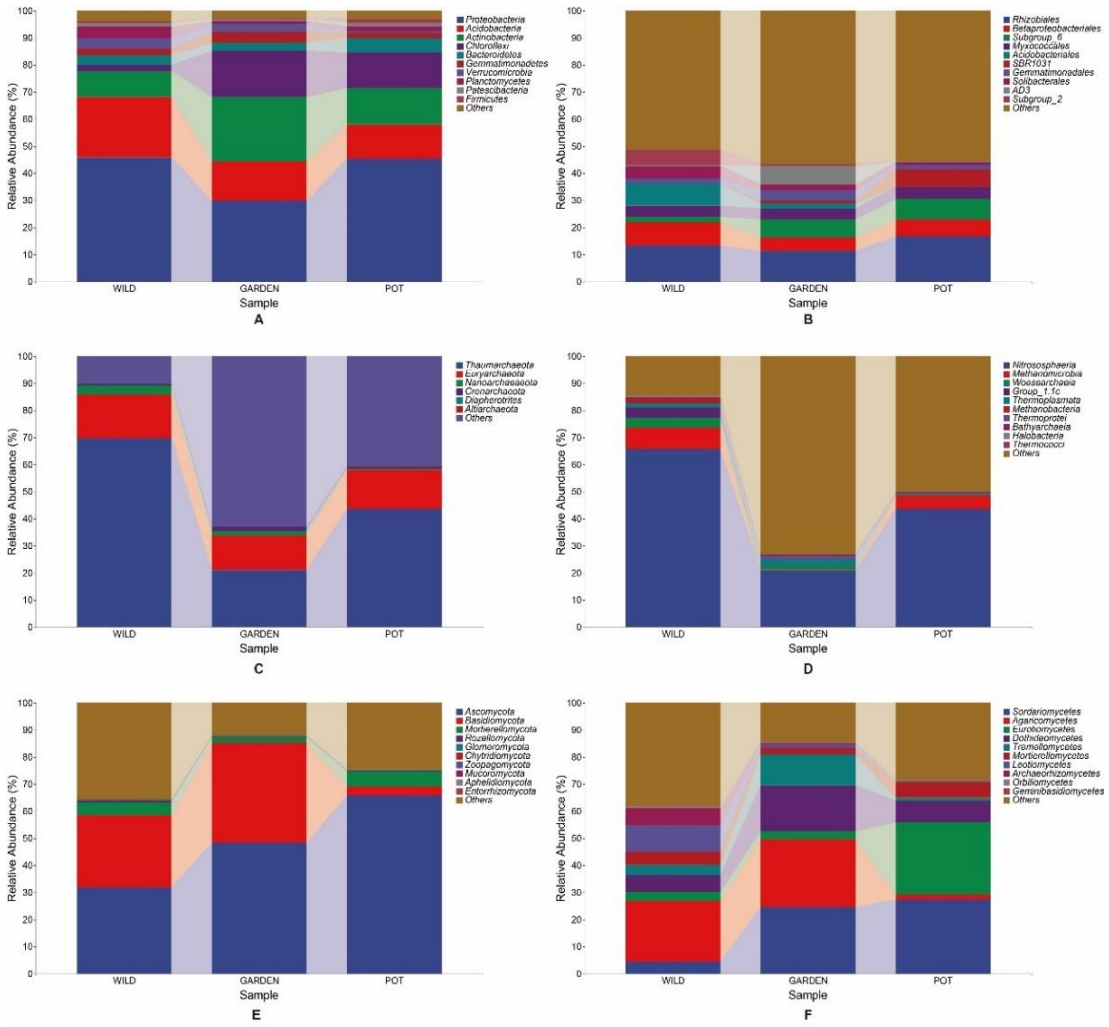

Figure 2. *Relative abundance (%) of the dominant bacterial (A. phylum, B. order), archaeal (C. phylum, D. class) and fungal (E. phylum, F. class) taxa in the bulk soils from the wild habitat, garden and pot in the greenhouse. WILD, bulk soil from wild habitat of B. sessilifolia; GARDEN, bulk soil from the Buddleja outdoor garden of Kunming Botanic Garden; POT, bulk soil from pots in the greenhouse of Kunming Botanic Garden*

Table 5. *Welch Two Sample t-test of the dominant Rhizobiales and Nitrososphaeria among the bulk soil from the WILD habitat of Buddleja sessilifolia, GARDEN and POTs*

	Rhizobiales			**Nitrososphaeria**		
	t	**df**	**p-value**	**t**	**df**	**p-value**
WILD vs GARDEN	1.471	6.268	0.190	2.403	3.115	0.092
WILD vs POT	-0.987	5.121	0.368	1.180	3.179	0.319
POT vs GARDEN	2.123	4.311	0.096	5.547	4.980	**0.003**

Notes: WILD, bulk soil from wild habitat of *B. sessilifolia*; GARDEN, bulk soil from the Buddleja outdoor garden of Kunming Botanic Garden; POT, bulk soil from pots in the greenhouse of Kunming Botanic Garden

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Effect of soil variables on microbial alpha diversity and microbial-community structure

The Pearson correlation analysis showed that total phosphorus content (TP), total potassium content (TK), available phosphorus content (AP), available potassium content (AK), C:N ratio, C:P ratio, and TN (total nitrogen content):TP ratio were significantly correlated with bacterial alpha diversity (*Table 6*). Among them, TK and C:P ratio were the two most correlated variables, and only TP was negatively correlated. The archaeal alpha diversities were significantly affected by pH, TN, TP, TK, C:N ratio, C:P ratio, C:K ratio, and TN:TP ratio, among which, TP and C:N ratio were the two most significant, and the effect of TK, C:N ratio, C:P ratio, and TN:TP were negative (*Table 6*). The fungal alpha diversities were only significantly associated with pH, TP, C:P ratio, C:K ratio, TN:TP ratio, and the most influential variables namely C:P ratio and TN:TP ratio were also the only positively correlated ones (*Table 6*). By comparison, the significant correlations between the archaeal alpha diversity and soil variables tended to be opposite to those between the bacteria/fungal alpha diversities and soil variables (*Table 6*).

Table 6. *Pearson correlation coefficients between bacterial, archaeal and fungal alpha diversity and soil variables*

	pH	OM	OC	TN	TP	TK	AN	AP	AK	C:N ratio	C:P ratio	C:K ratio	TN:TP ratio	AN:AP ratio
Bacteria														
Observed species	-0.061	0.328	0.328	0.189	-0.353	**0.629****	0.447	**0.460***	**0.500***	0.402	**0.690****	-0.426	**0.625****	0.003
Shannon	-0.126	0.291	0.291	0.162	**-0.461***	**0.720*****	0.435	0.399	0.443	**0.576****	**0.680****	-0.430	**0.610****	0.004
Archaea														
Observed species	**0.625****	0.415	0.415	**0.542***	**0.889*****	**-0.518***	0.085	0.263	0.214	**-0.685****	**-0.653****	**0.678****	**-0.582****	-0.300
Shannon	0.126	0.136	0.136	0.230	**0.462***	**-0.470***	0.204	0.175	0.034	**-0.750*****	-0.216	0.371	-0.123	-0.174
Fungi														
Observed species	**-0.482***	-0.203	-0.203	-0.298	**-0.714*****	0.400	0.308	0.008	0.002	0.208	**0.850*****	**-0.602****	**0.838*****	0.185
Shannon	-0.449	-0.201	-0.201	-0.287	**-0.642****	0.339	0.266	0.017	-0.029	0.183	**0.758*****	**-0.510***	**0.747*****	0.088

Note: OM, organic matter content; OC, organic-carbon content; TN, total nitrogen content; TP, total phosphorus content; TK, total potassium content; AN, available nitrogen content; AP, available phosphorus content; AK, available potassium content; C:N, the ratio of OC to TN; C:P, the ratio of OC to TP; C:K, the ratio of OC to TK; TN:TP, the ratio of TN to TN; AN:AP, the ratio of AN to AP. **, P<0.001; **, P < 0.01; *, P < 0.05

The PCoA and PERMANOVA found that the structures of the bacterial, archaeal and fungal communities differed significantly among the bulk soil from the WILD habitat, GARDEN and POT, respectively (*Fig. 3, Table 7*). Both the RDA and Permutation test indicated all soil variables except the ratio of AN (available nitrogen content): AP (available phosphorus content) concurrently affected the structures of the bacterial, archaeal and fungal communities (*Fig. 4, Table 8*). Among them, pH, TN, TP, TK, C:N, C:K significantly influenced all microbial community structures. TK was the most correlated soil variable with bacterial community structures. TN was the most related with the archaeal and fungal community structures as well as the highly correlated TP and OM.

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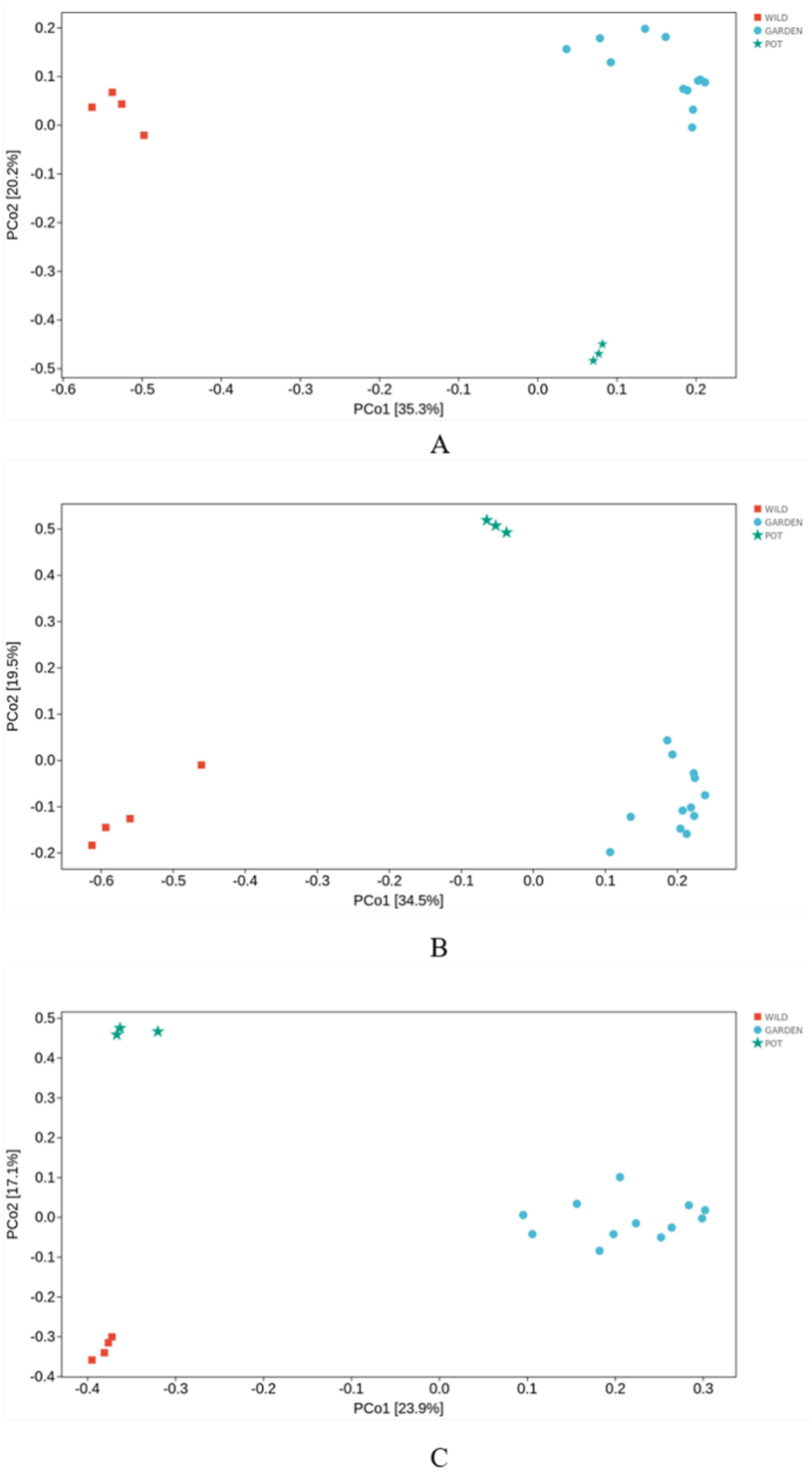

Figure 3. *Principal coordinates analysis (PCoA) of bacteria (A), archaeal (B) and fungal (C) community structures in the bulk soils from the WILD habitat (tetragon), GARDEN (asterisk), POT (circle). WILD, bulk soil from wild habitat of B. sessilifolia; GARDEN, bulk soil from the Buddleja outdoor garden of Kunming Botanic Garden; POT, bulk soil from pots in the greenhouse of Kunming Botanic Garden*

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Table 7. *The assessment on the significance of differentiation of microbiota structure among groups by PERMANOVA*

Bacteria						
Group1	**Group2**	**Sample size**	**Permutations**	**pseudo-F**	**p-value**	**q-value**
all	-	19	999	9.388098	0.001	-
WILD	GARDEN	16	999	11.18632	0.001	0.003
WILD	POT	7	999	8.840944	0.032	0.032
GARDEN	POT	15	999	7.429365	0.002	0.003
Archaea						
Group1	**Group2**	**Sample size**	**Permutations**	**pseudo-F**	**p-value**	**q-value**
all	-	19	999	8.785917	0.001	-
WILD	GARDEN	16	999	10.40054	0.001	0.0015
WILD	POT	7	999	5.311272	0.033	0.033
GARDEN	POT	15	999	8.930574	0.001	0.0015
Fungi						
Group1	**Group2**	**Sample size**	**Permutations**	**pseudo-F**	**p-value**	**q-value**
all	-	19	999	5.342811	0.001	-
WILD	GARDEN	16	999	5.21793	0.001	0.003
WILD	POT	7	999	4.234046	0.033	0.033
GARDEN	POT	15	999	6.10904	0.002	0.003

Notes: WILD, bulk soil from wild habitat of *B. sessilifolia*; GARDEN, bulk soil from the Buddleja outdoor garden of Kunming Botanic Garden; POT, bulk soil from pots in the greenhouse of Kunming Botanic Garden

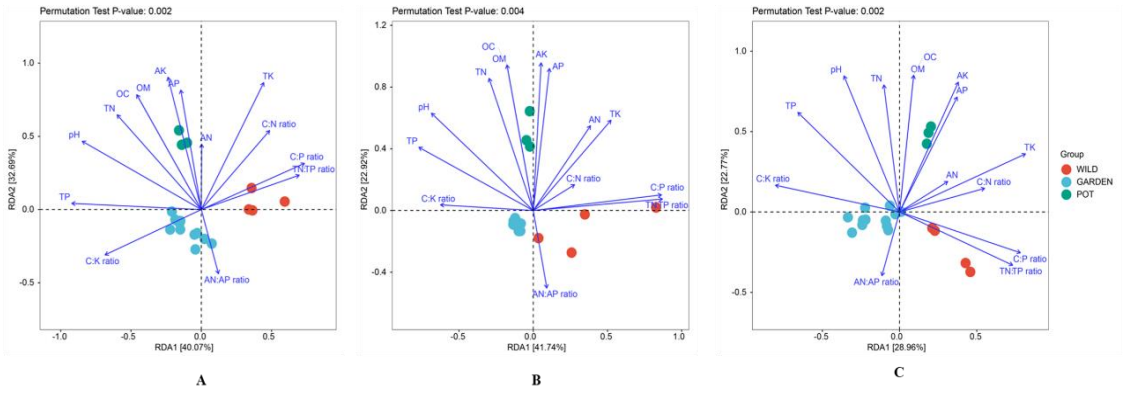

Figure 4. *Redundancy Analysis (RDA) used to identify the relationships of the bacterial (A), archaeal (B), and fungal (C) communities (circles) from the WILD habitat, GARDEN, POT with soil variables. pH, pH; OM, organic matter content; OC, organic-carbon content; TN, total nitrogen content; TP, total phosphorus content; TK, total potassium content; AN, available nitrogen content; AP, available phosphorus content; AK, available potassium content; C:N, the ratio of OC to TN; C:P, the ratio of OC to TP; C:K, the ratio of OC to TK; TN:TP, the ratio of TN to TN; AN:AP, the ratio of AN to AP. WILD, bulk soil from wild habitat of B. sessilifolia; GARDEN, bulk soil from the Buddleja outdoor garden of Kunming Botanic Garden; POT, bulk soil from pots in the greenhouse of Kunming Botanic Garden. The permutation tests were shown above the plates*

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Table 8. *The tests on the relationships of bacterial, archaeal and fungal community compositions with soil variables by Permutation test*

	Bacteria		**Archaea**		**Fungi**	
	R^2	P	R^2	P	R^2	P
pH	0.422	**0.009**	0.615	**0.001**	0.482	**0.008**
OM	0.294	0.063	0.777	**0.001**	0.593	**0.003**
OC	0.294	0.063	0.777	**0.001**	0.593	**0.003**
TN	0.354	**0.031**	0.820	**0.001**	0.687	**0.001**
TP	0.601	**0.003**	0.769	**0.002**	0.674	**0.001**
TK	0.754	**0.001**	0.655	**0.001**	0.353	**0.032**
AN	0.221	0.135	0.438	**0.013**	0.327	**0.028**
AP	0.237	0.128	0.490	**0.012**	0.521	**0.003**
AK	0.287	0.069	0.745	**0.001**	0.418	**0.018**
C:N	0.624	**0.002**	0.678	**0.005**	0.446	**0.009**
C:P	0.555	**0.003**	0.414	**0.025**	0.292	0.059
C:K	0.479	**0.008**	0.532	**0.003**	0.549	**0.004**
TN:TP	0.431	**0.013**	0.292	0.070	0.192	0.167
AN:AP	0.027	0.807	0.020	0.856	0.207	0.147

Note: OM, organic matter content; OC, organic-carbon content; TN, total nitrogen content; TP, total phosphorus content; TK, total potassium content; AN, available nitrogen content; AP, available phosphorus content; AK, available potassium content; C:N, the ratio of OC to TN; C:P, the ratio of OC to TP; C:K, the ratio of OC to TK; TN:TP, the ratio of TN to TN; AN:AP, the ratio of AN to AP. ***, $P<0.001$; **, $P < 0.01$; *, $P < 0.05$

Discussion

Over the past decades, biodiversity conservation in China has achieved a number of successes. Since the new concept of plant species with extremely small populations (PSESP) was proposed in 2005, several national and regional-level conservation strategies and actions for conserving China's PSESP have been implemented (Sun et al., 2019), such as the conservation of *Acer yangbiense* Y. S. Chen et Q. E. Yang (Sun and Yin, 2009; Yang et al., 2015), *Magnolia sinica* (Law) Noot. (Wang et al., 2016) and *Craigia yunnanensis* W. W. Smith et W. E. Evans (Yang et al., 2016), and *Cyclobalanopsis sichourensis* Hu (Xia et al., 2016). In the latest PSESP list of 2021 proposed by Yunnan Provincial Government (http://www.yn.gov.cn/hdjl/yjzh/202104/t20210414_220452.html), our target species *Buddleja sessilifolia* has also been included for priority protection. Based on the authors' 5-year field investigation, only four populations of *B. sessilifolia* were found in the wild (WILD), which located in the cold areas of Gaoligong Mt. of Yunnan with 2600~3150 m elevation. Therefore, the rescuing conservation is urgent to be conducted for this species. However, this species was failed to transplant in the *Buddleja* garden (GARDEN) of Kunming Botanic Garden and only seedlings in the pots of greenhouse (POT) can survive. There could be many reasons to be uncovered and soil properties may play important roles in them.

By comparing the physicochemical properties of the bulk soil samples from the wild habitat of *B. sessilifolia*, the *Buddleja* outdoor garden and the pots of the greenhouse, we

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found the bulk soil from both the wild habitat and the pots had a high content of potassium (K). As we know, K is helpful to the survival of plants exposed to various biotic and abiotic stresses such as diseases, drought, salinity, cold (Cakmak, 2005; Wang et al., 2013). For example, adequate K nutrition can improve drought resistance and root longevity in *Hibiscus rosa-sinensis* (Egilla et al., 2001). A high K^+ concentration can increase *Panax ginseng*'s antioxidant levels and reduce the production of reactive oxygen species to improve plant survival under cold stress (Devi et al., 2012). K can also induce multiple mechanisms to improve the resistance of soybean against soybean cyst nematode (Gao et al., 2018). Therefore, the low K content in *Buddleja* garden may probably reduce the ability of *B. sessilifolia* to defense the cold weather or potential soil diseases, which consequently may cause the death of *B. sessilifolia* in outdoor garden.

With regards to the soil microbiomes, our analyses uncovered that the OTU number of the bacterial order Rhizobiales and the archaeal class Nitrososphaeria tended to be more in the bulk soil from both the wild habitat of *B. sessilifolia* and the pots of greenhouse than that from the *Buddleja* garden. Although these Rhizobiales members were not able to associate with legumes for nitrogen fixation, some of them were proven to promote the root growth of *Arabidopsis thaliana* (Garrido-Oter et al., 2018). Thus, it is reasonable to infer that the large number of Rhizobiales species in the soil from the wild habitat of *B. sessilifolia* and the pots of greenhouse may probably have the similar ability to promote the root growth of *B. sessilifolia*. The members of Nitrososphaeria are well known for their nitrification which oxidates ammonia to nitrite, then to nitrate (Offre et al., 2013; Kerou et al., 2016; Lu et al., 2020). It has reported that four conifer trees (*Pinus koraiensis, P. sylvestris, Picea koraiensis* and *Larix olgensis*) from the plantation forests in Northeast China can assimilate nitrate as efficiently as ammonium from soils (Zhou et al., 2021). Based on the previous evidence, *B. sessilifolia* may also have a strong capacity to uptake soil nitrates because of the larger number of Nitrososphaeria existed in the bulk soil from the wild habitat of *B. sessilifolia* and the pots. Although all these inferences still need further experimental verification, we would like to propose our findings to emphasize the importance of soil microbiota in the *ex-situ* conservation of plants.

Conclusion

This study found that the endangered plant *Buddleja sessilifolia* had a high demand on the soil potassium and the microbial Rhizobiales and Nitrososphaeria for its successful survival. Furthermore, the soil total potassium content was the most correlated to the bacterial community structure of the bulk soil, while the soil total nitrogen content and total phosphorus content were most important to the archaeal and fungal community structures. Our findings will contribute to the *ex-situ* conservation of the endangered plant *B. sessilifolia*.

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