

Supplementary information

Variation in Root-associated Microbial Communities among Three Different Plant Species in Natural Desert Ecosystem

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S1.1 DNA extraction, PCR, and Illumina sequencing

The DNeasy Power Soil DNA Isolation Kit (Qiagen, Inc., Netherlands) was used to extract total microbial DNA from 0.5 g of BS and RS, following the provided instructions. For root samples, 0.4 g was first ground with liquid nitrogen, and then the DNeasy Plant Maxi Kit (Qiagen, Inc., Netherlands) was applied to extract total microbial DNA. The quantity of the obtained DNA was determined using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and its quality was assessed through agarose gel electrophoresis. The PCR (Polymerase Chain Reaction) products were purified using agarose gel electrophoresis with a 2% concentration. Post-detection, the qualified PCR products underwent purification utilizing magnetic beads and were subsequently quantified using a microplate reader. Based on the PCR product concentration, the same amount of samples were mixed. After fully mixing, the PCR products underwent further detection via 2% agarose gel electrophoresis. Subsequently, the bands were retrieved utilizing the glue recovery kit supplied (made in Qiagen, Netherlands). The library was built using the TruSeq® DNA PCR-Free Sample Preparation Kit, followed by quantification using Qubit and Q-PCR. Following library qualification, NovaSeq 6000 was used for on-machine sequencing.

According to the Barcode sequence and PCR amplified primer sequence, each sample data was separated from the dismounting data. After the Barcode sequence and primer sequence were cut off, the reads of each sample were spliced using FLASH (v1.2.11) software [1]. The spliced sequences were Raw Tags data. Then FASTP (v0.23.1) software is used to process the Raw Tags obtained by splicing through strict filtering to obtain high-quality Clean Tags data [2]. The Tags obtained after the above processing need to be processed to remove the chimeric sequence. The Tags sequence is compared with the species annotation database through VSEARCH (v2.16.0) software to detect the chimeric sequence, and the chimeric sequence is finally removed to obtain the final Effective Tags [3].

Additional sequence filtering was performed using the QIIME-II software (v202202) [4]. Operational taxonomic units (OTUs) were generated using the **UPARSE software** (v7.0.1001) with a 97% similarity threshold [5]. Bacterial OTUs were classified with an RDP classifier (confidence threshold of 70%) based on the Mothur method and SSUrRNA database of Silva database (version 138.1) (Set threshold to 0.8~1) [6-7]. Fungal OTUs were classified based **on the UNITE database** [8] were utilized. To ensure bacterial and fungal (RE, RS, and BS) sequence uniformity among all samples, the minimum number (174 and 29166) of sequences {dataset\$sample_sums () %>% range} were used as the depth to filter other samples to generate in a filtered OTU table {This result removed plant sequences ("mitochondria", "chloroplasts") in fungal results, however,

bacteria do not remove plant sequences}. The raw sequencing data are available at the National Center for Biotechnology Information (NCBI) Short Read Archive, BioProjectID PRJNA1024038.

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S1.2 Measurement of soil and root physicochemical properties

To measure soil and root organic carbon (SOC and ROC), total nitrogen (TN), available nitrogen (AN), and available potassium (AK), the following methods were used: the $K_2Cr_2O_7$ - H_2SO_4 oxidation method, the Kjeldahl Nitrogen Analyzer (K1160, Jinan Hanon Instruments Co. Ltd., China), the alkali hydrolyzable method, and the NH_4OAc extraction method [9-11]. Before measuring total phosphorus (TP) and total potassium (TK) with the Inductively Coupled Plasma-Optical Emission Spectrometer (iCAP 6300, Thermo Elemental, USA), the samples were digested in concentrated HNO_3 [12]. To determine the amount of accessible phosphorus (AP), a continuous-flow autoanalyzer (Autoanalyzer 3, Bran and Luebbe, Germany) with HCl/NH_4F was used to perform colorimetric analysis using the ascorbic acid molybdate technique [10, 11]. The pH of the soil {soil/water ratio of 1:2.5 (w/v)} and its electrical conductivity (EC) {soil/water ratio of 1:5 (w/v)} were measured using a pH and EC meter (PHSJ-6 L and DDSJ-319 L, INESA Scientific Instrument Co. Ltd. in China) manufactured, respectively.

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Table S1 Plant growth characteristics

Species	Height (cm)	Crown width (cm ²)
<i>A. sparsifolia</i>	46.25±3.22c	2705.17±531.59c
<i>T. ramosissima</i>	202.08±10.74b	41745.08±5967.57b
<i>C. caput-medusae</i>	248.92±14.53a	74213.67±7566.51a

Note: Different lowercase letters (a, b, and c) indicate that the different desert plants have significant differences (LSD test, $P < 0.05$).

Table S2 Bare soil physical and chemical properties

Site	pH	EC ($\mu\text{s}\cdot\text{cm}^{-1}$)	TN ($\text{g}\cdot\text{kg}^{-1}$)	TP ($\text{g}\cdot\text{kg}^{-1}$)	TK ($\text{g}\cdot\text{kg}^{-1}$)
CL	8.46 \pm 0.07b	323.50 \pm 22.99b	0.15 \pm 0.01a	0.57 \pm 0.01a	19.54 \pm 0.10a
TLF	8.63 \pm 0.01a	480.50 \pm 12.24a	0.10 \pm 0.001c	0.54 \pm 0.01b	18.51 \pm 0.13b
MSW	8.51 \pm 0.05ab	505.00 \pm 34.66a	0.13 \pm 0.003b	0.54 \pm 0.01b	18.88 \pm 0.21b

Site	SOC ($\text{g}\cdot\text{kg}^{-1}$)	AN ($\text{mg}\cdot\text{kg}^{-1}$)	AP ($\text{mg}\cdot\text{kg}^{-1}$)	AK ($\text{mg}\cdot\text{kg}^{-1}$)
CL	1.72 \pm 0.09a	4.40 \pm 0.30a	0.54 \pm 0.03b	57.50 \pm 1.04c
TLF	1.24 \pm 0.04b	4.20 \pm 0.13a	1.77 \pm 0.30a	118.00 \pm 2.48b
MSW	1.42 \pm 0.02b	4.95 \pm 0.30a	1.98 \pm 0.29a	144.50 \pm 2.02a

Note: Different lowercase letters (a, b, and c) indicate that the different sites have significant differences (LSD test, $P < 0.05$). **EC**, electrical conductivity; **SOC**, soil organic carbon; **TN**, total nitrogen; **TP**, total phosphorus; **TK**, total potassium; **AN**, available nitrogen; **AP**, available phosphorus; **AK**, available potassium.

Figure legends

Figure. S1 Sequence number [A (number of sequence) of the bacteria] and [B (number of sequence) of the fungi] of root endosphere (RE), rhizosphere soil (RS), and bulk soil (BS) bacteria and fungi in three desert plants.

Figure. S2 Alpha diversity {[A, B, and C (Chao1), [D, E, and F (Shannon)], [G, H, and I (Pielou_e)], and [J, K, and L (Simpson)] indexes} of root endosphere (RE), rhizosphere soil (RS), and bulk soil (BS) fungi in three desert plants. Different lowercase letters (a and b) indicate significant differences among species at the $p < 0.05$ level and the ns indicate no significant differences among species at the $p > 0.05$ level (ANOVA and Duncan's test).

Figure. S3 Beta diversity {[A, B, and C (Bray–Curtis)] and [D, E, and F (nonmetric multidimensional scaling)]} of root endosphere (RE), rhizosphere soil (RS), and bulk soil (BS) fungi of three desert plants. ** $p < 0.01$.

Figure. S4 Co-occurrence network {[A, D, and G (Network characteristics)] of the *A. sparsifolia*, [B, E, and H (Network characteristics)] of the *T. ramosissima*, and [C, F, and I (Network characteristics)] of the *C. caput-medusae*} of root endosphere (RE), rhizosphere soil (RS), and bulk soil (BS) fungi of three desert plants.

Figure S1

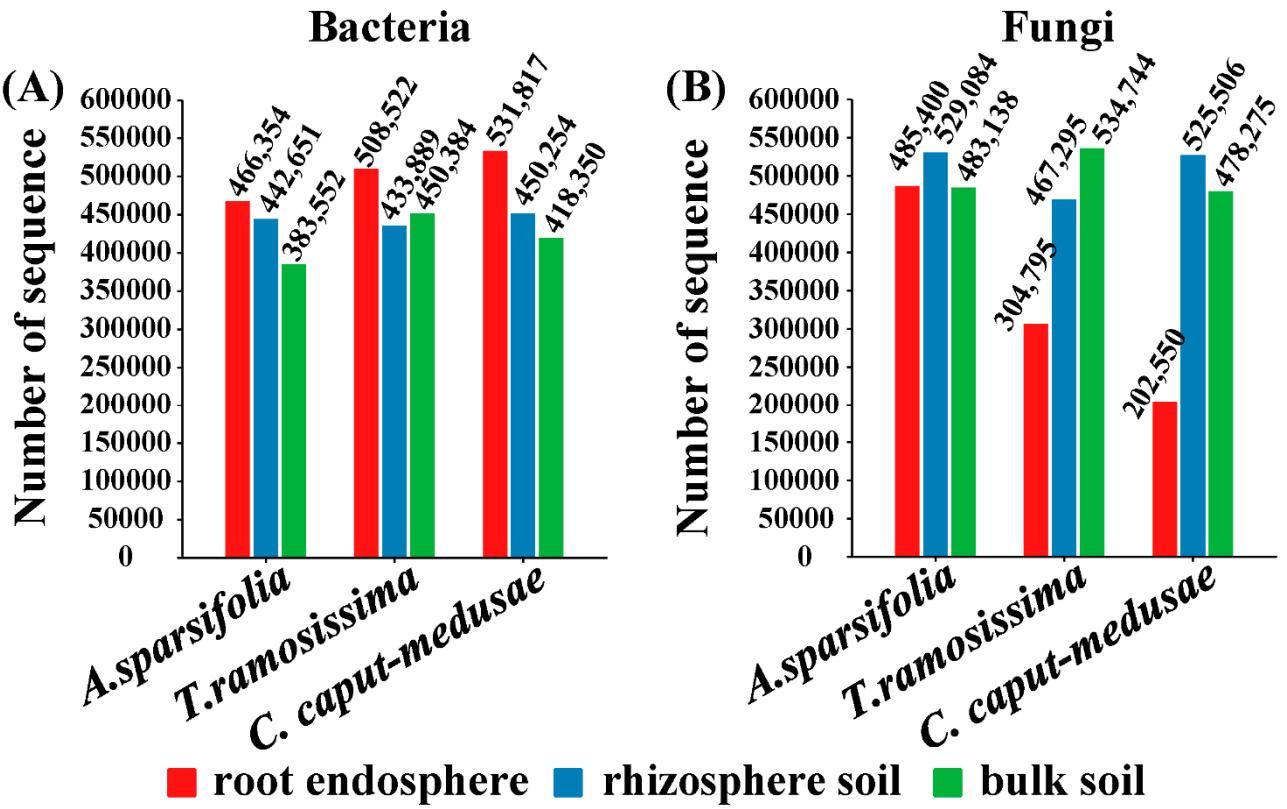


Figure S2

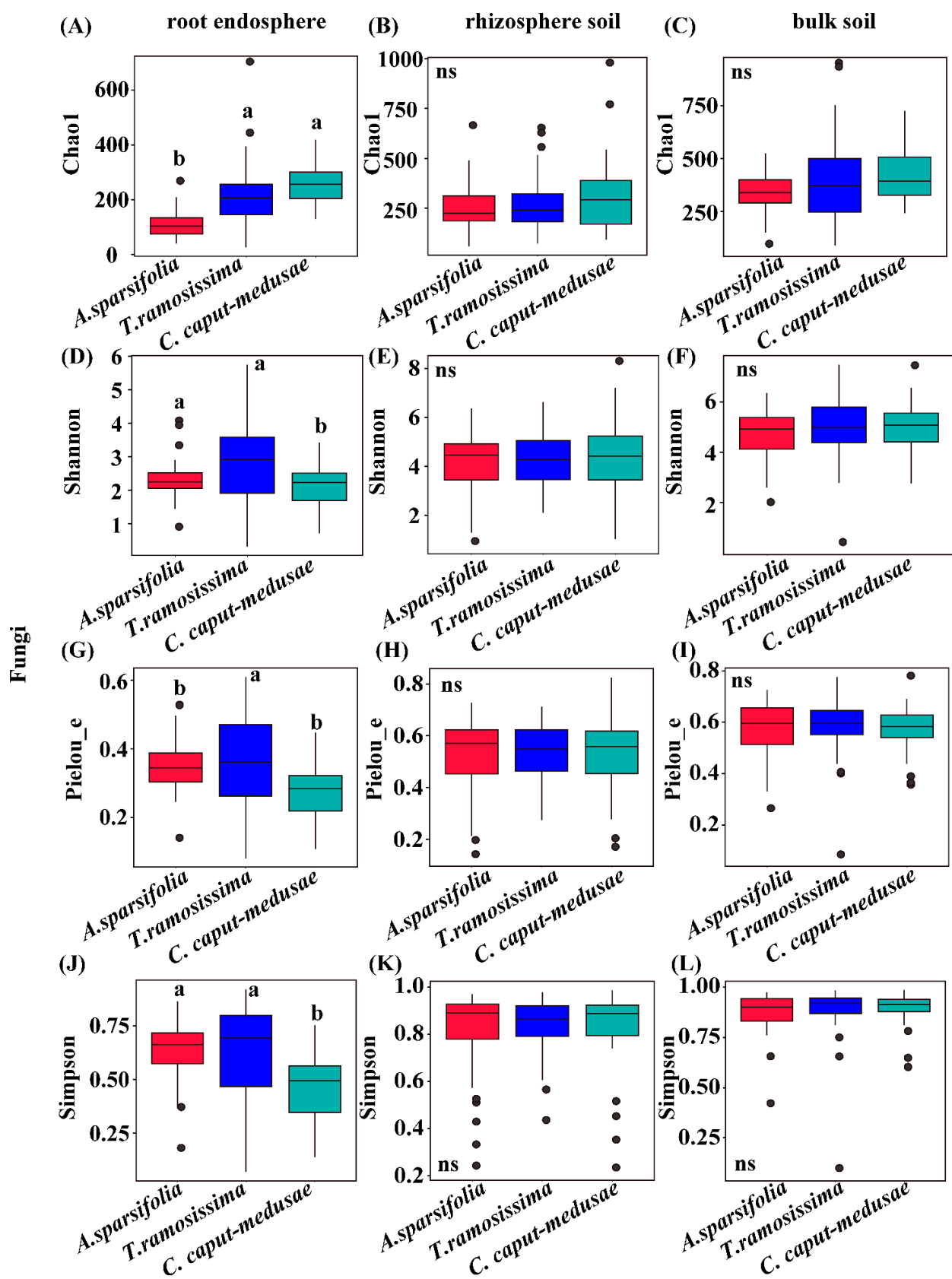


Figure S3

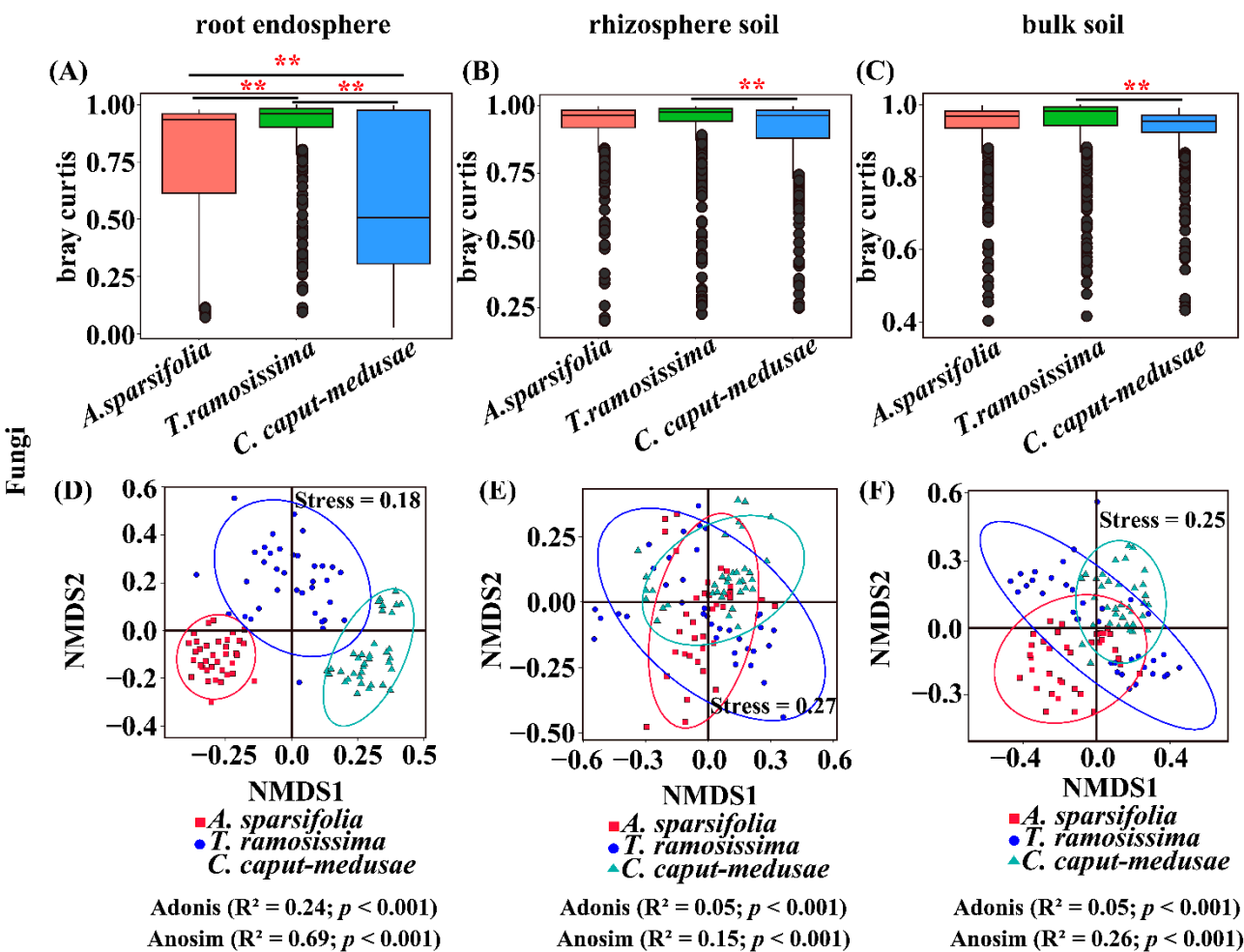


Figure S4

