Supplementary Materials

Soil microbial communities in *Pseudotsuga sinensis* forests with different degrees of rocky desertification in the karst region, southwest China

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Method 1: DNA Extraction, pyrosequencing, and bioinformatic processing

The total microbial genomic DNA in the soil samples was extracted from 0.5 g of frozen soil by a PowerSoil DNA extraction kit (MoBio, Carlsbad, USA) according to the manufacturer's recommendations. The final DNA concentration and purification were detected with a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and the DNA quality was evaluated by 1.5% agarose gel electrophoresis. Bacterial V3-V4 regions of the 18S rRNA gene and fungal ITS region were amplified by primers 338F/806R and ITS5-1737F/ITS2-2043R, respectively (Table S1). The purified, barcode-tagged amplicons from each cDNA library were merged into equimolar concentrations and paired-end sequenced (2 × 250) on an Illumina Novaseq6000 platform (Illumina, USA) by Shanghai Personal Biotechnology Co., Ltd (Shanghai, China). The Illumina sequencing raw read data deposited in the Sequence Read Archive (SRA) are available in the NCBI SRA portal with PRJNA1031341, bioproject ID. More detailed information about the PCR conditions and quality assessment is provided in Table S1.

The raw sequence data were demultiplexed using the demux plugin followed by primers cutting with the cutadapt plugin [1]. Sequences were then quality filtered, denoised, merged and chimera removed using the DADA2 plugin [2]. Non-singleton amplicon sequence variants (ASVs) were aligned with mafft [3] and used to construct a phylogeny with fasttree2 [4]. Alpha-diversity metrics (Chao1, Observed species, Shannon), and beta diversity

metrics (Bray-Curtis dissimilarity) were assessed using the diversity plugin with samples that were rarefied to 52043 sequences for bacteria and 30804 for fungi per sample. Taxonomy was assigned to ASVs using the classify-sklearn naïve Bayes taxonomy classifier in the feature-classifier plugin [5] against the Greengenes 13_8 99% OTUs reference sequences [6].

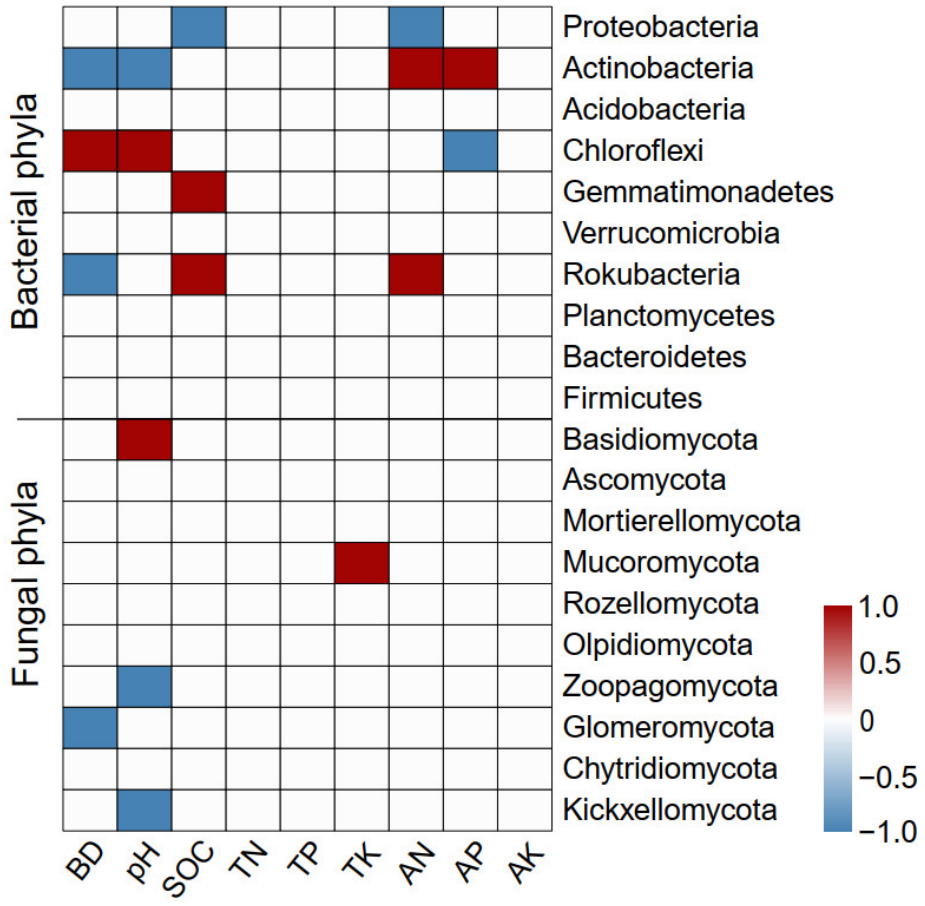

Figure S1. The correlations between the relative abundance of microbial phyla and soil properties. BD, bulk density; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; and AK, available potassium.

Table S1. Primer sets and thermal profiles used in PCR amplification.

Gene	Primer	Sequence (5′-3′)	PCR conditions
Bacterial 16S rRNA	338F	ACTCCTACGGGAGGCAGCA	3 min at 95°C followed by 30 cycles of 30 s at 95°C,30 s at 55°C, 45 s at 72°C, and 10 min at 72°C for the last cycle
	806R	GGACTACHVGGGTWTCTAAT	
Fungal ITS1	ITS5-1737F	GGAAGTAAAAGTCGTAACAAGG	2 min at 98°C followed by 30 cycles of 15 s at 98°C, 30 s at 55°C, 30 s at 72°C, and 5 min at 72°C for the last cycle
	ITS2-2043R	GCTGCGTTCTTCATCGATGC	
Reaction system	Both PCR reactions were performed in triplicate in a total volume of 20 μL, containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.4 μL of each primer (10 μM), 0.4 μL of FastPfu Polymerase, 1 μL of template DNA (approximately 10 ng), 0.2 μL of BSA, and 10.8 μL of dd H_2O. An 8 bp sequence barcode was added as a tag to distinguish the PCR products from one another.		

References

1. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J*. **2011**, *17*, 10−12.
2. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat*. *Methods* **2016**, *13*, 581−583.
3. Katoh, K.; Misawa, K.; Kuma, K.I.; Miyata, T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res*. **2002**, *30*, 3059−3066.
4. Price, M.N.; Dehal, P.S.; Arkin, A.P. FastTree 2-approximately maximum-likelihood trees for large alignments. *PloS One* **2010**, *5*, e9490.
5. Bokulich N.A.; Kaehler B.D.; Rideout J.R.; Dillon M.; Bolyen E.; Knight R.; et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2′s q2-feature-classifier plugin. *Microbiome* **2018**, *6*, 90.
6. McDonald, D.; Price, M.N.; Goodrich, J.; Nawrocki, E.P.; DeSantis, T.Z.; Probst, A.; et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J*. **2012**, *6*, 610−618.