

Supplemental Materials

Supplemental Methods: Molecular methods used on our soil microbial analyses.

Supplemental Tables :

Supplemental Table S1 The difference of soil properties between O horizon and A horizon regardless of forest types

Supplemental Table S2 The soil properties in different forest types regardless of soil depth

Supplemental Table S3 The relationship between soil bacterial alpha diversity and soil pH, Ca and Mg

Supplemental Table S4 The relationship between soil properties and the more abundant bacteria in Sc, Pi and Sc_Pi

Supplemental Table S5 Correlation between soil properties and the more abundant bacteria in Ci and Ci_Pi

Supplemental Figure:

Supplemental Figure S1 Soil bacterial community heatmap analysis of the top 50 genera. Samples with letter “O” means soil from O horizon; Samples with letter “A” means soil from A horizon.

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Soil microbial community analysis

Genomic DNA was extracted from soil samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer’s instruction. The quality of extracted DNA was checked by 1% agarose gel electrophoresis and spectrophotometry (optical density at 260 nm/280 nm ratio). All extracted DNA samples were stored at -20°C for further analysis. The V3–V4 hypervariable regions of the bacterial 16S were subjected to high-throughput sequencing by Majorbio BioTech Co., Ltd. (Shanghai, China) using the Illumina Miseq PE300 sequencing platform (Illumina, Inc., CA, USA). The V3-V4 region of the bacteria 16S rRNA gene were amplified with the universal primers of the forward primer 515F 5′-barcode- GTGCCAGCMGCCGCGG)-3′ and 907R 5′-CCGTCAATTCMTTTRAGTTT-3′, along with a unique eight-base barcode assigned to each sample. These primers contained a set of 8-nucleotide barcodes sequence unique to each sample. PCR reactions were performed in triplicate 20 µL mixture containing 4 µL of

5 × FastPfu Buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.4 µL of FastPfu Polymerase, and 10 ng of template DNA.

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor™ -ST (Promega, U.S.). Purified amplicons were pooled in equimolar concentrations for downstream Illumina sequencing and paired-end sequences were generated (2 × 300) according to the standard protocols.

Bioinformatic analysis

Raw fastq files were demultiplexed and performed with the QIIME package (Quantitative Insights Into Microbial Ecology) (v1.2.1) (Caporaso et al. 2010) with the following criteria: (i) 300 basepair (bp) reads were truncated with an average quality scores <20 over a 50 bp range and truncated reads shorter than 50bp were discarded. (ii) in the process of exact barcode matching, two nucleotide base mismatches, however were allowed, however reads containing ambiguous characters were removed. Only sequences overlapping longer than 10 bp were assembled according to their overlap sequence.

Chimeric sequences were identified and removed using Usearch (version 8.0.1623). Operational Units (OTUs) were clustered using a threshold of 97% identity using UPARSE (version 7.1 <http://drive5.com/uparse/>) (Edgar, 2013). The taxonomy of each 16S rRNA gene sequence was analyzed using QIIME against the Silva119 16S rRNA database (Cole et al., 2009) using confidence threshold of 90%. The taxonomy of each ITS sequence was analyzed using BLAST against the UNITE database.

Reference:

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Supplemental Tables

Supplemental Table S1 The soil properties in different forest types regardless of soil depth

	pH	SOC g·kg ⁻¹	TN mg·kg ⁻¹	TP g·kg ⁻¹	TK g·kg ⁻¹	NH ₄ -N mg·kg ⁻¹	NO ₃ -N mg·kg ⁻¹	Ca cmol·kg ⁻¹	Mg cmol·kg ⁻¹	SMBC mg·kg ⁻¹	SMBN mg·kg ⁻¹
Ci	4.50±0.04a	39.35±7.43	2.82±0.49	0.55±0.02a	7.92±0.12a	22.06±6.31ab	35.44±8.33a	1.90±0.31ab	2.13±0.40ab	50.63±8.65ab	11.99±2.96ab
Pi	4.07±0.04b	47.31±12.23	2.58±0.62	0.39±0.03b	7.79±0.11a	22.78±6.48ab	11.59±2.42b	1.21±0.22bc	1.10±0.23c	50.33±16.07ab	20.45±7.50a
Sc	4.09±0.04b	40.31±9.24	2.19±0.55	0.20±0.02c	2.53±0.07c	35.63±8.77ab	18.15±5.49b	0.90±0.20c	1.15±0.28bc	29.34±3.35b	3.27±0.88b
Ci_Pi	4.50±0.01a	41.71±10.96	2.74±0.65	0.42±0.03b	7.91±0.10a	14.72±4.41b	20.94±5.30ab	2.10±0.45a	2.38±1.33a	75.56±9.34a	21.04±7.94a
Sc_Pi	3.99±0.07b	52.37±12.81	2.80±0.75	0.25±0.03c	3.02±0.04b	42.80±12.52a	19.60±5.70ab	1.03±0.23c	1.24±0.30 bc	39.32±8.32b	3.50±0.74b

The values are means ±SE (n = 8). Results followed by different letters are statistically significant at the 0.05 level among different forest types.

Supplemental Table S2 The difference of soil properties between O horizon and A horizon regardless of forest types

	pH	SOC g·kg ⁻¹	TN mg·kg ⁻¹	TP g·kg ⁻¹	TK g·kg ⁻¹	NH ₄ -N mg·kg ⁻¹	NO ₃ -N mg·kg ⁻¹	Ca cmol·kg ⁻¹	Mg cmol·kg ⁻¹	SMBC mg·kg ⁻¹	SMBN mg·kg ⁻¹
O horizon	4.18±0.07	72.07±2.27	4.25±0.09	0.42±0.03	5.64±0.56	47.73±4.12	35.48±2.92	2.16±0.17	2.48±0.18	61.17±8.11	21.60±3.96
A horizon	4.28±0.03	16.36±0.58	1.00±0.06	0.30±0.03	6.03±0.59	7.46±0.75	6.81±0.84	0.69±0.06	0.71±0.08	36.90±4.19	2.50±0.42
p-value	0.240	<0.001	<0.001	0.005	0.628	<0.001	<0.001	<0.001	<0.001	0.013	<0.001

The values are means ±SE (n = 20). P-values indicated the difference between O and A horizons.

Supplemental Table S3 The relationship between soil bacterial alpha diversity and soil pH, Ca and Mg

		pH	Ca	Mg
Shannon	R	0.895	0.517	0.528
	P	<0.001	0.001	<0.001
Chao	R	0.905	0.497	0.498
	P	<0.001	0.001	0.001

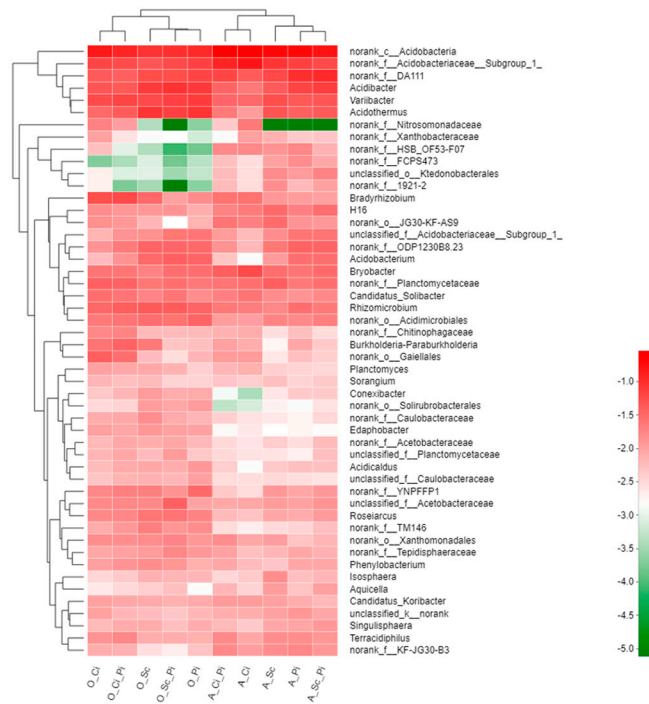
Supplemental Table S4 The relationship between soil properties and the more abundant bacteria in Sc, Pi and Sc_Pi

	pH		Ca		Mg		TP		TK		NO ₃ -N		NH ₄ -N		SOC	
	R	p	R	p	R	p	R	p	R	p	R	p	R	p	R	p
Rhodospirillales	-0.448	0.004	-0.348	0.028	-0.415	0.008	-0.495	0.001			-0.466	0.022				
Halanaerobiales	-0.733	<0.001	-0.353	0.026	-0.319	0.045	-0.633	<0.001	-0.409	0.009						
Acidibacter	-0.798	<0.001					-0.435	0.005	-0.509	0.001			0.479	0.02		
Acidothermus	-0.796	<0.001					-0.503	0.001	-0.660	<0.001			0.554	<0.001	0.345	0.029
Acidobacterium	-0.87	<0.001					-0.437	0.05	-0.485	0.02			0.512	0.001	0.371	0.019
Acidicaldu	-0.609	<0.001											0.431	0.05		
Roseiarcus	-0.559	<0.001							-0.501	0.01	0.323	0.042	0.639	<0.001	0.411	0.009
Variibacter	-0.568	<0.001							-0.376	0.017			0.417	0.007		
Rhizomicrobium	-0.524	0.001	0.331	0.037	0.423	0.006					0.443	0.004	0.513	0.001	0.409	0.009

Supplemental Table S5 Correlation between soil properties and the more abundant bacteria in Ci and Ci_Pi

[illegible]

Supplemental Figure



Supplemental Figure S1 Soil bacterial community heatmap analysis of the top 50 genera. Samples with letter “O” means soil from O horizon; Samples with letter “A” means soil from A horizon. Color printing.