Online Supporting information for

The vertical patterns of soil bacterial and fungal communities along a soil depth gradient in a natural *Picea crassifolia* forest of Qinghai Province, China

Lei Hu [1†], Xin Wang [1†], Xiaoyan Song [1], Di Dai [1], Keyu Chen [1], Luming Ding [1], Abraham Allan Degen [2], Changting Wang [1*]

Supplementary tables:

Table S1. The ANOVA for soil physicochemical properties and microbial community characteristics along soil depths

Variables		Soil depths (fixed factor)	
		F	***P***
Soil properties	Soil pH	26.999	< 0.001
	Soil bulk density	0.172	0.914
	SOC	9.874	< 0.001
	TN	13.052	< 0.001
	TP	2.939	0.058
	C/N	3.995	0.022
	C/P	9.191	0.001
	N/P	14.23	< 0.001
Relative abundance	Acidobacteria	0.069	0.976
	Verrucomicrobia	4.814	0.011
	Proteobacteria	0.906	0.456
	Ascomycota	0.351	0.789
	Basidiomycota	0.148	0.930
α diversity	Bacterial OTU	18.088	< 0.001
	Bacterial Chao1	10.127	< 0.001
	Bacterial PD	13.231	< 0.001
	Bacterial Shannon-Wiener	8.566	0.001
	Fungal OTU	3.363	0.039
	Fungal Chao1	4.492	0.014
	Fungal PD	0.573	0.639
	Fungal Shannon-Wiener	1.121	0.364
Potential functions	Endophyte	2.242	0.115
	Arbuscular Mycorrhizal	0.736	0.543
	Ectomycorrhizal	0.799	0.509
	Undefined Saprotroph	0.598	0.624

Table S2. The characteristics of soils and roots along the soil depths

Soil depth	Soil pH	Soil bulk density (g cm^{-3})	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	C/N	C/P	N/P
0-10 cm	5.93±0.39^{c}	0.83±0.31	116.8±13.60^{a}	8.42±0.09^{a}	0.60±0.08 b	14.1±0.06^{b}	195.5±29.20^{a}	14.1±1.71^{a}
10-20 cm	6.56±0.14^{b}	0.86±0.31	102.0±12.35^{ab}	6.00±0.36^{b}	0.71±0.12^{a}	14.9±0.93^{ab}	149.8±42.92^{b}	8.76±2.06^{b}
20-30 cm	6.62±0.03^{b}	0.90±0.32	94.4±20.01^{b}	6.06±1.76^{b}	0.73±0.06^{a}	15.2±0.39^{a}	132.3±39.44^{bc}	8.52±3.13^{bc}
30-50 cm	7.06±0.16^{a}	0.95±0.32	67.6±17.24^{c}	3.83±1.80^{c}	0.72±0.06^{a}	15.3±0.83^{a}	93.5±20.57^{c}	5.28±2.38^{c}

Means with different superscript letters within a column differ from each other ($P < 0.05$). SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; C/N, the ratio of SOC/TN; C/P, the ratio of SOC/TP; N/P, the ratio of TN/TP.

Figure S1

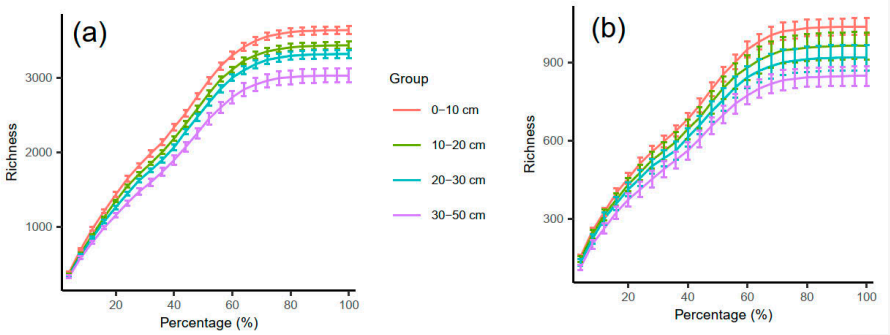

Figure S1 The rarefaction curves of soil bacteria (a) and fungi (b).

Soil bacteria and fungi analysis using Illumina MiSeq sequencing

The V4-V5 region of bacterial 16S rRNA and the ITS region of fungal rRNA were amplified via polymerase chain reaction (PCR) with the primers 515F (5'-GTGCCAGCMGCCGCGG-3') and 909R (5'-CCCCGYCAATTCMTTTRAGT-3') for bacteria (Tamaki et al., 2011), and ITS3 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') for fungi. The PCR mixture (25 μL) was prepared with 10× Ex Taq buffer (2.5 μL), 25 mM $MgCl_2$ (2 μL), 2.5 mM dNTPs (2 μL), 10 nM forward primer and reverse primer (each 1 μL), 0.5 UExTaq polymerase (TaKaRa, Dalian, China) (0.2 μL), 10 ng·μL^{-1} soil genomic DNA (5 μL), and ddH_2O (11.3 μL). The PCR amplification used a Bio-Rad C1000 Touch Thermal Cycler system (Bio-Rad, Hercules, CA, USA). The procedures for amplification of bacteria samples were as follows: denaturation at 95 °C, 35 cycles of denaturation at 94 °C, 50 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The procedures for amplification of fungal samples were as follows: 95 °C for 5 min, 35 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 45 s, and 72 °C for 10 min. The purified amplicons were pooled equally and sequenced on an Illumina MiSeq platform according to standard protocols.

Processing of sequenced data

The MiSeq sequenced data were processed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline version 1.7.0 (http://qiime.org/tutorials/tutorial.html). The original sequences were sorted based on the unique sample barcodes and trimmed for sequence quality (length > 300 bp, average base quality score > 30) using the QIIME pipeline [1]. Chimaera sequences were removed using the UCHIME algorithm [2]. The sequences were clustered using the complete-linkage clustering method incorporated in the QIIME pipeline [3]. Sequences were clustered into operational taxonomic units (OTUs) based on a similarity threshold of 97%. Taxonomy was assigned using the Ribosomal Database Project Classifier. The original sequence data are available at the European Nucleotide Archive by accessing PRJEB11649 (http://www.ebi.ac.uk/ena/data/view/ PRJEB11649).

Commented [1]: Here, we have revised the reference numbers

References

Commented [M2]: Citations and References in Supplementary files are permitted provided that they also appear in the reference list (manuscript).

1. Segata, N.; Izard, J.; Waldron, L.; Gevers, D.; Miropolsky, L.; Garrett, W.S.; Huttenhower, C. Metagenomic biomarker discovery and explanation. *Genome Biol.* **2011**, *12*, 60. https://doi.org/10.1186/gb-2011-12-6-r60.
2. Edgar, R.C.; Haas, B.J.; Clemente, J.C.; Quince, C.; Knight, R. Uchime improves sensitivity and speed of chimera detection. Bioinformatics. **2011,** 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.

3. Caporaso, J.G.; Lauber, C.L.; Walters, W.A.; Berg, D.L.; Huntley, J.; Fierer, N.; Owens, S.M.; Betley, J.; Fraser, L.; Bauer, M. Ultra-high-throughput microbial community analysis on the illumina hiseq and miseq platforms. ISME J. **2011**, 6, 1621–1624. https://doi.org/10.1038/ismej.2012.08.