

Supplementary material for

Abundant Species Govern the Altitude Patterns of Bacterial Community in Natural and Disturbed Subalpine Forest Soils

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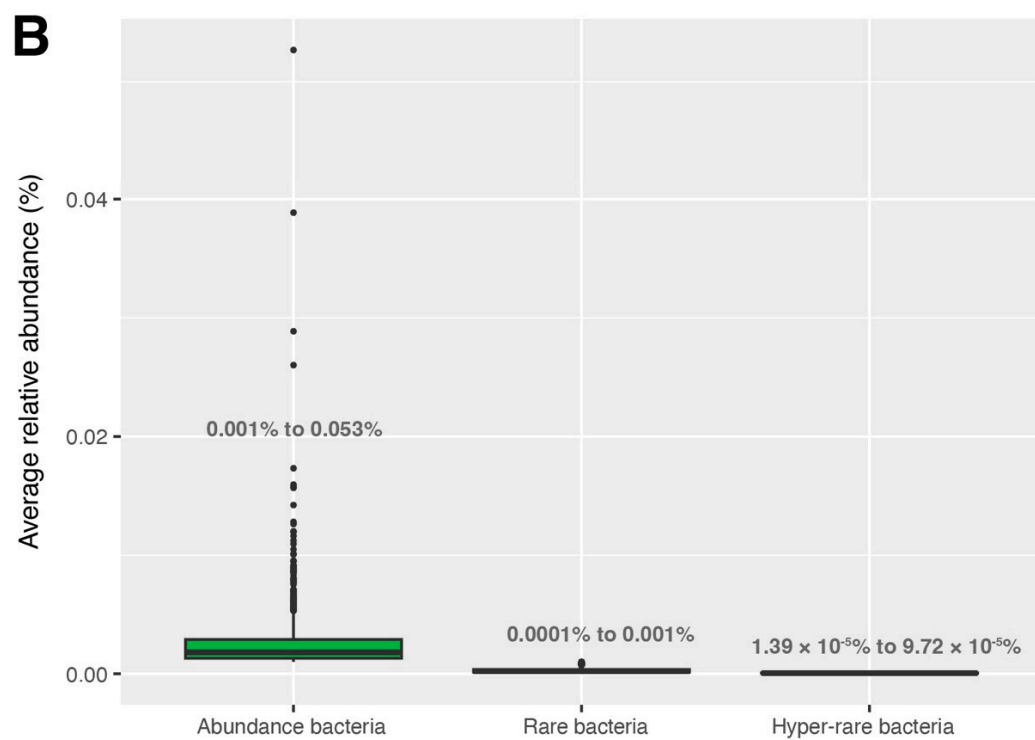
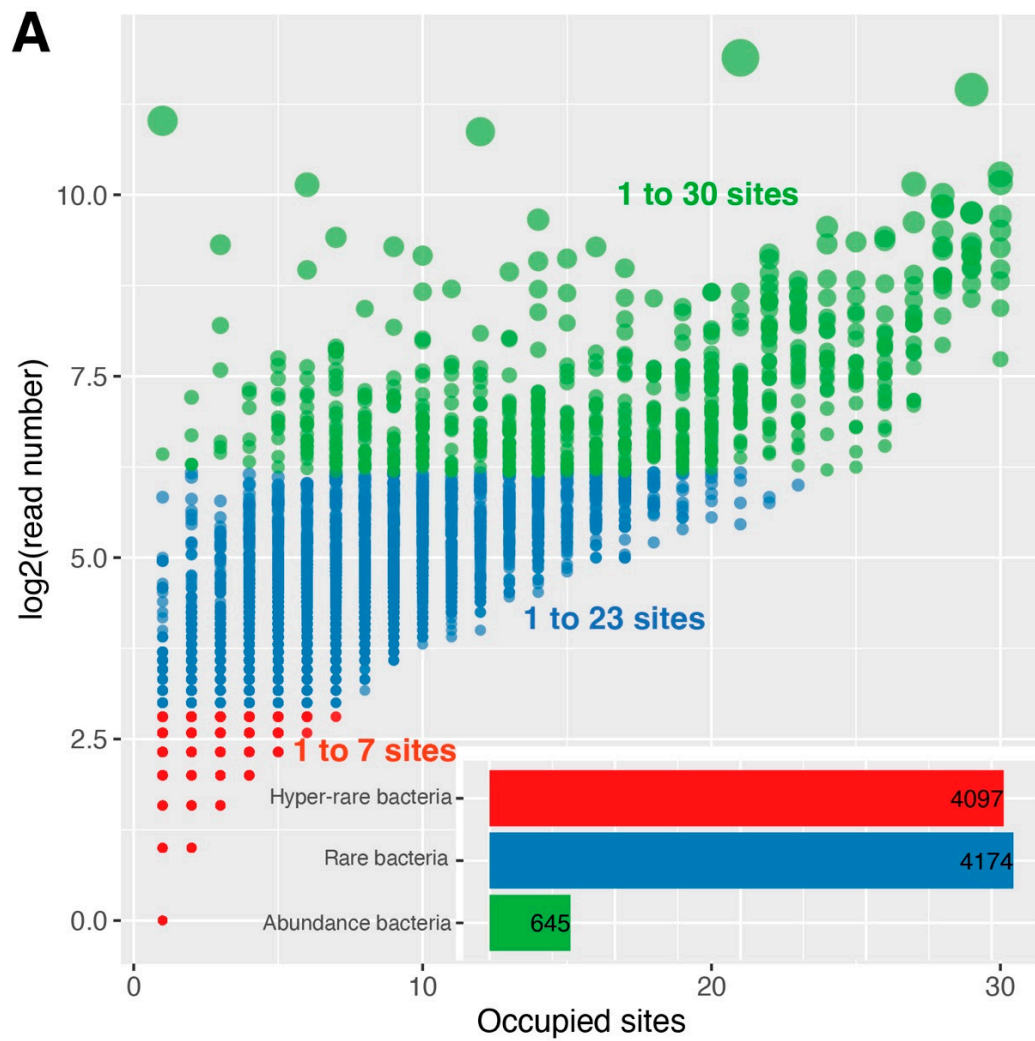


Figure S1 The distributions of amplicon sequence variants (ASVs) with varying average relative abundances in 30 soil samples (A), and the average relative abundances (RB) of abundant (RB \geq 0.001%), rare ($0.0001\% \leq$ RB $<$ 0.001%) and hyper-rare (RB $<$ 0.0001%) bacteria (B).

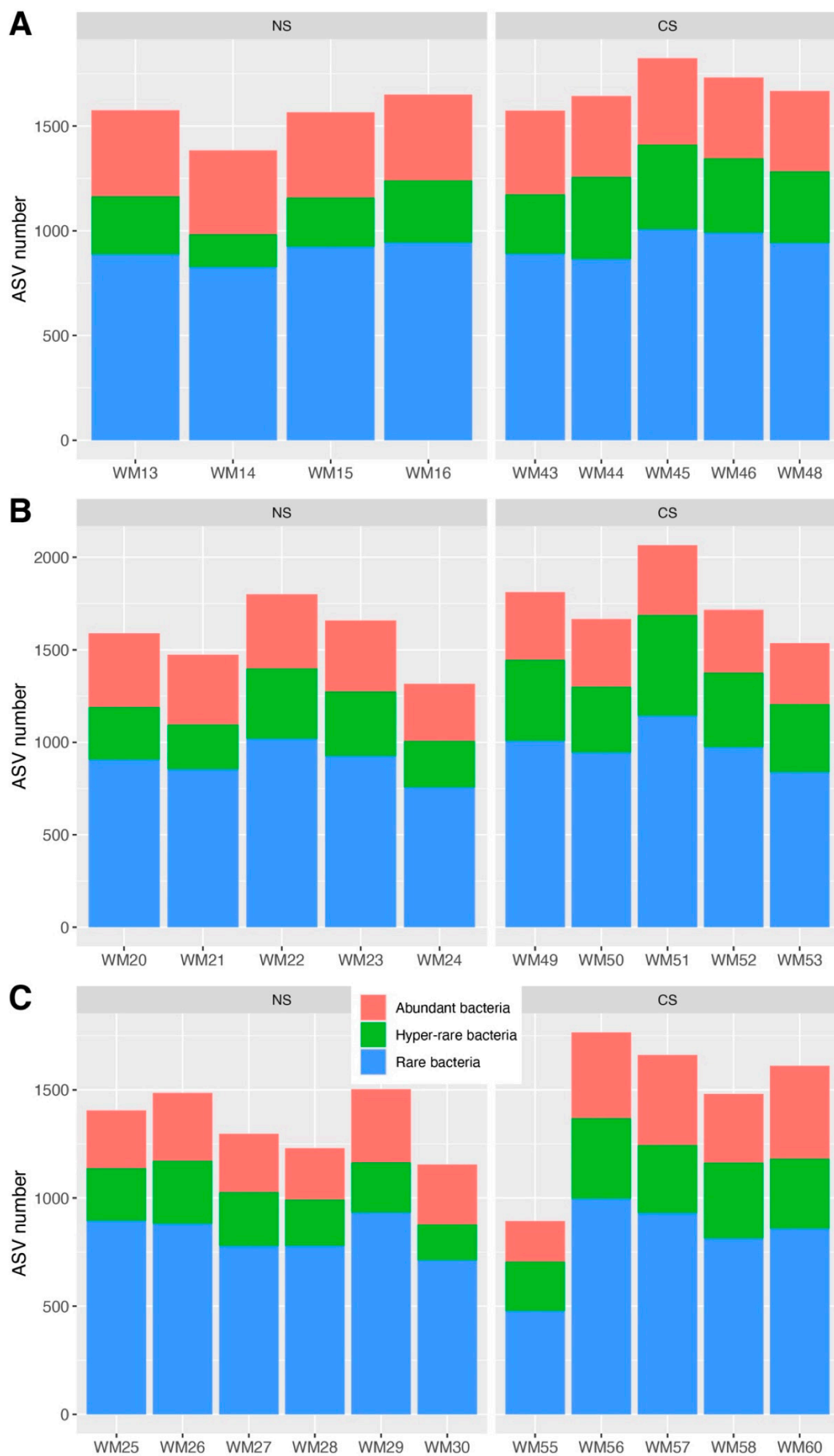


Figure S2 The ASV numbers of abundant ($RB \geq 0.001\%$), rare ($0.0001\% \leq RB < 0.001\%$) and hyper-rare ($RB < 0.0001\%$) bacteria in soils sampled from 2900 m (A), 3102 m (B) and 3194 m (C). ASV: amplicon sequence variant; RB: average relative abundance; NS: natural soils; CS: cut slope soils.

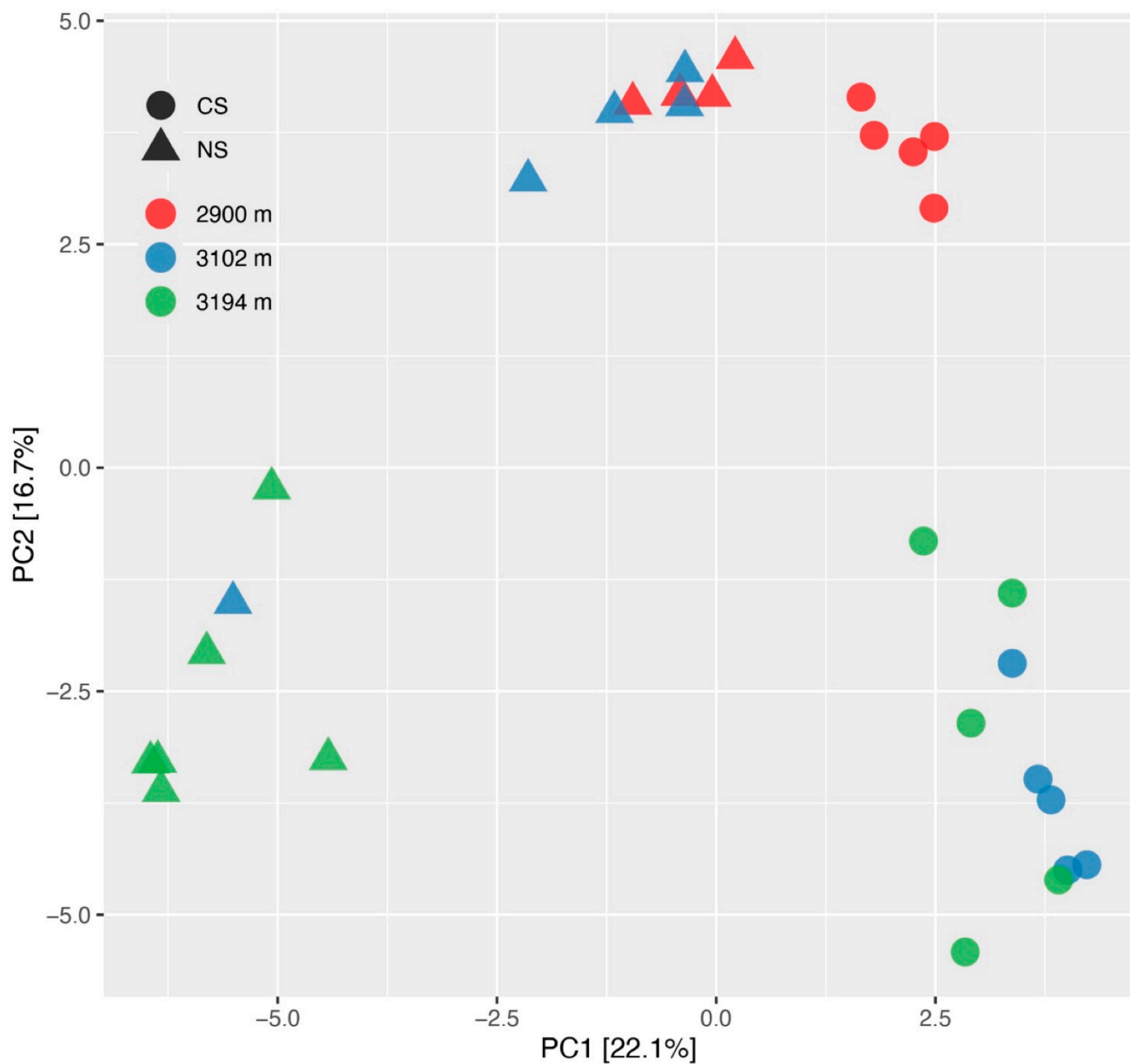


Figure S3 A principal component analysis (PCA) showing the overall difference in soil properties including soil pH, moisture content (MC), soil temperature (ST), conductivity (CD), total organic carbon (TOC), total nitrogen (TN), ammonium nitrogen (NH_4^+ -N), nitrate nitrogen (NO_3^- -N), total phosphorus (TP) and soil available phosphorus (SAP). NS: natural soils; CS: cut slope soils.

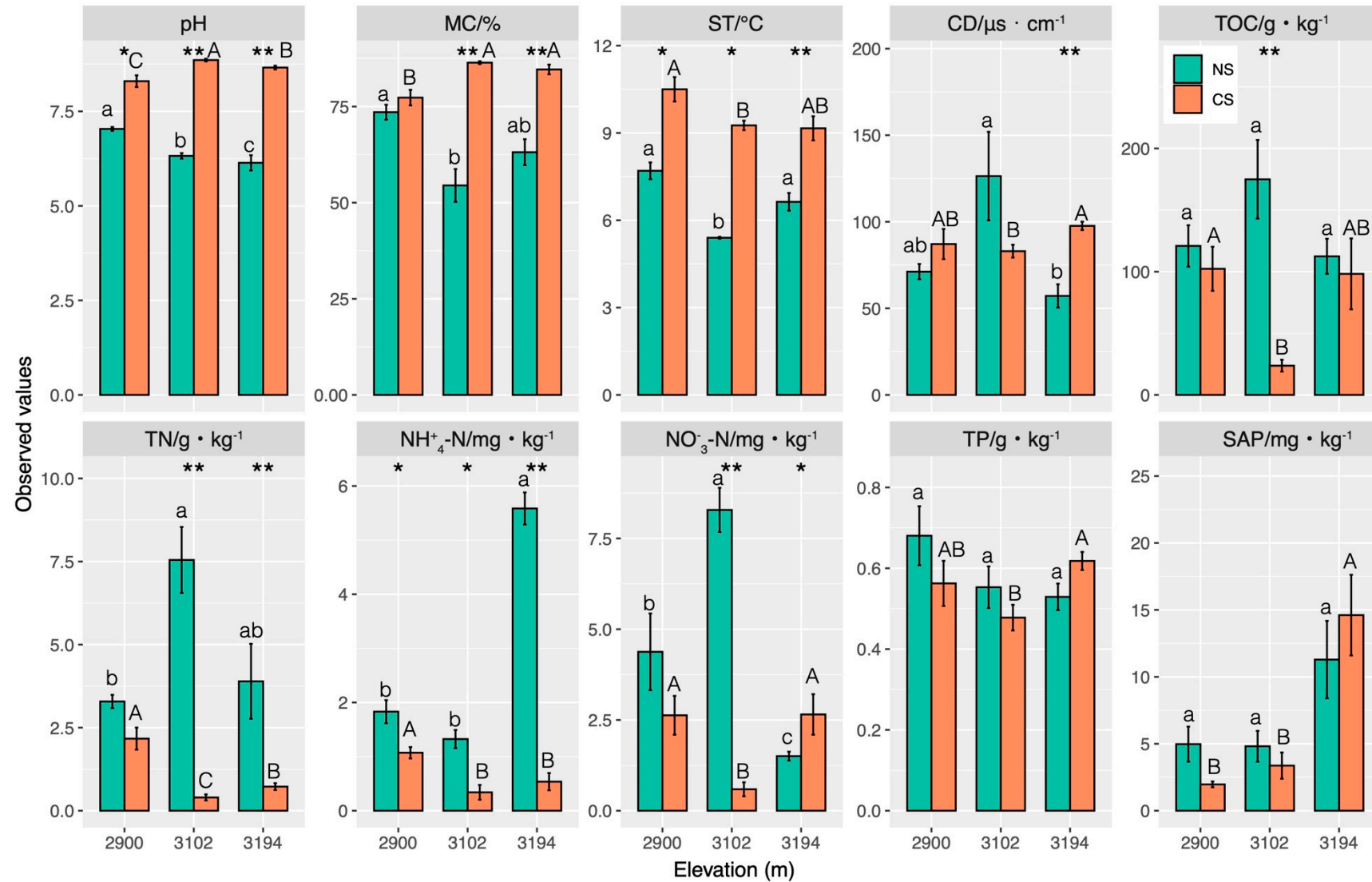


Figure S4 Soil properties in natural (NS) and cut slope (CS) soils [1]. Asterisks represent the significant differences between NS and CS at an altitude (* $p < 0.05$, ** $p < 0.01$; Wilcoxon rank sum test). Lowercase and uppercase letters on the top of bars denote the significance of differences among three altitudes within NS and CS, respectively, and it indicates no significant difference ($p > 0.05$; Wilcoxon rank sum test) between two groups if they share one same letter. MC: moisture content; ST: soil temperature; CD: conductivity; TOC: total organic carbon; TN: total nitrogen; $\text{NH}_4^+\text{-N}$: ammonium nitrogen; $\text{NO}_3^+\text{-N}$: nitrate nitrogen; TP: total phosphorus; SAP: soil available phosphorus.



Figure S5 A heatmap showing the Spearman correlations between soil properties and the Shannon-Wiener indices of total, abundant (RB $\geq 0.001\%$), rare ($0.0001\% \leq \text{RB} < 0.001\%$) and hyper-rare (RB $< 0.0001\%$) bacteria. The ρ in the legend represents the correlation coefficient. MC: moisture content; ST: soil temperature; CD: conductivity; TOC: total organic carbon; TN: total nitrogen; NH₄⁺-N: ammonium nitrogen; NO₃⁻-N: nitrate nitrogen; TP: total phosphorus; SAP: soil available phosphorus. RB: average relative abundance; NS: natural soils; CS: cut slope soils. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

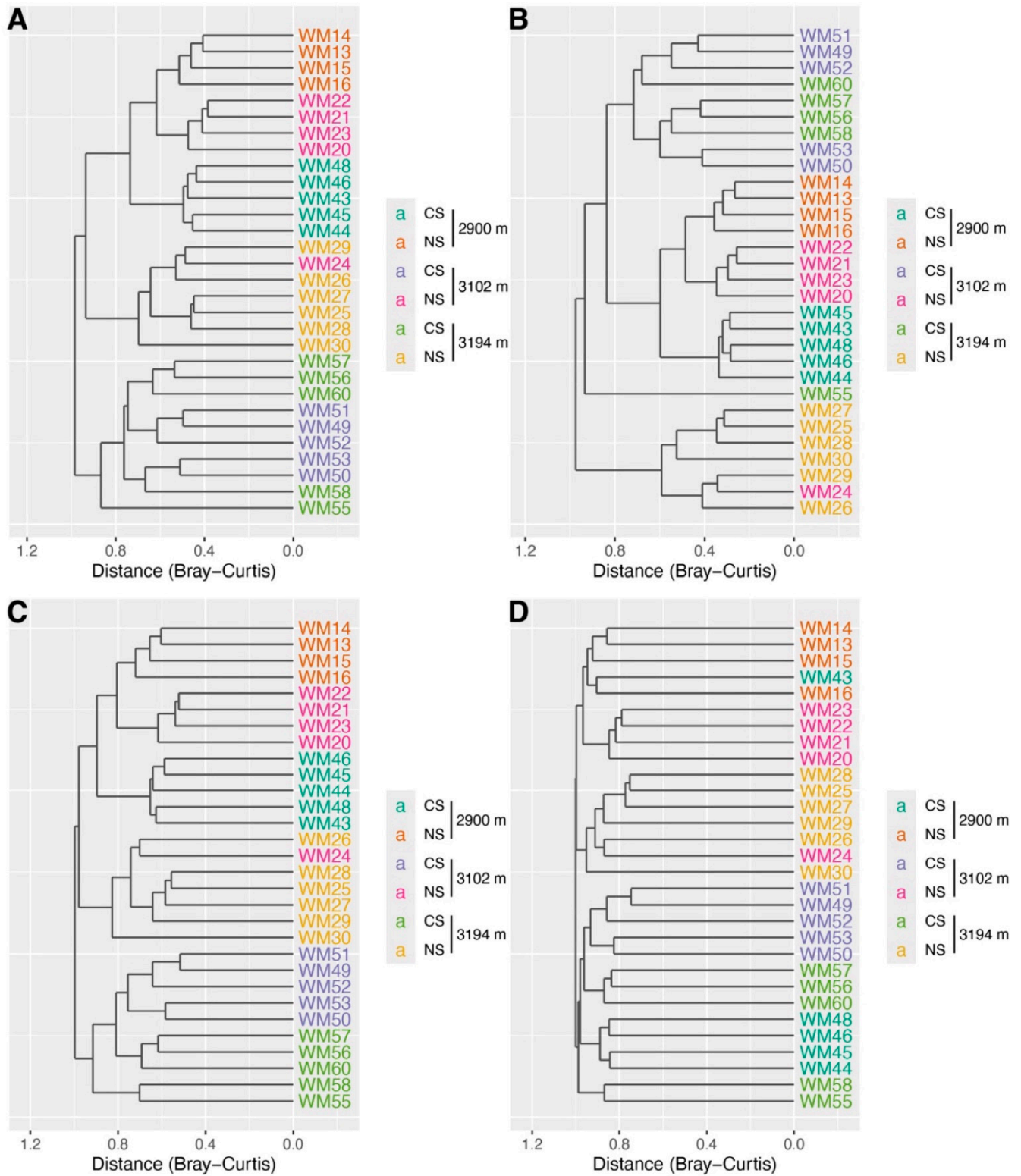


Figure S6 Bray-Curtis distance-based clustering analysis for total (A), abundant (RB $\geq 0.001\%$) (B), rare ($0.0001\% \leq \text{RB} < 0.001\%$) (C) and hyper-rare (RB $< 0.0001\%$) (D) bacterial communities. RB: average relative abundance; NS: natural soils; CS: cut slope soils.

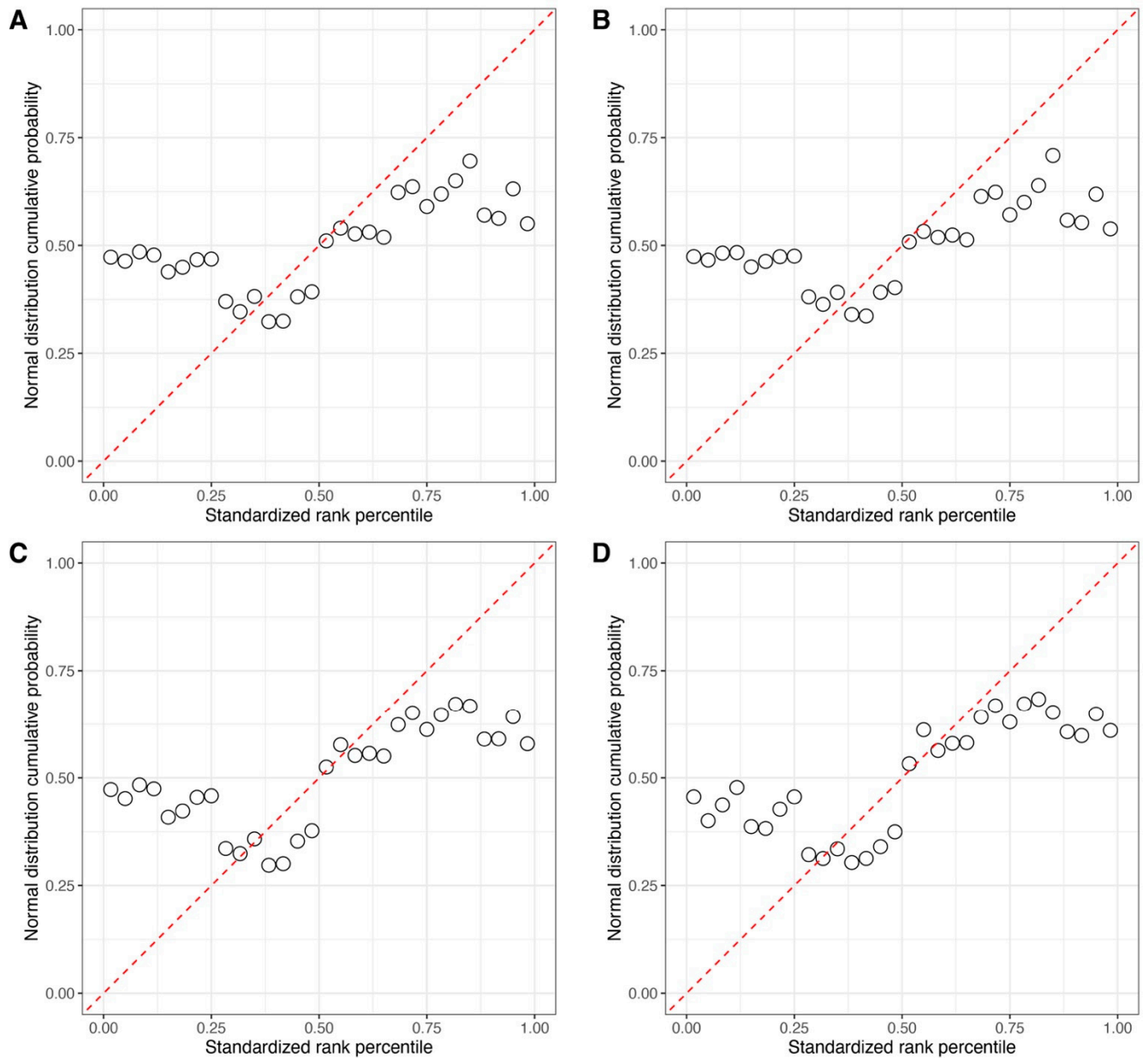


Figure S7 The Sheppard Plot along with the Bray-Curtis distance-based non-metric multidimensional scaling (NMDS) analysis shown in **Figure 2**. **A:** total soil bacterial community; **B:** abundant (RB $\geq 0.001\%$) soil bacterial community; **C:** rare ($0.0001\% \leq \text{RB} < 0.001\%$) soil bacterial community; **D:** hyper-rare (RB $< 0.0001\%$) soil bacterial community. RB: average relative abundance.

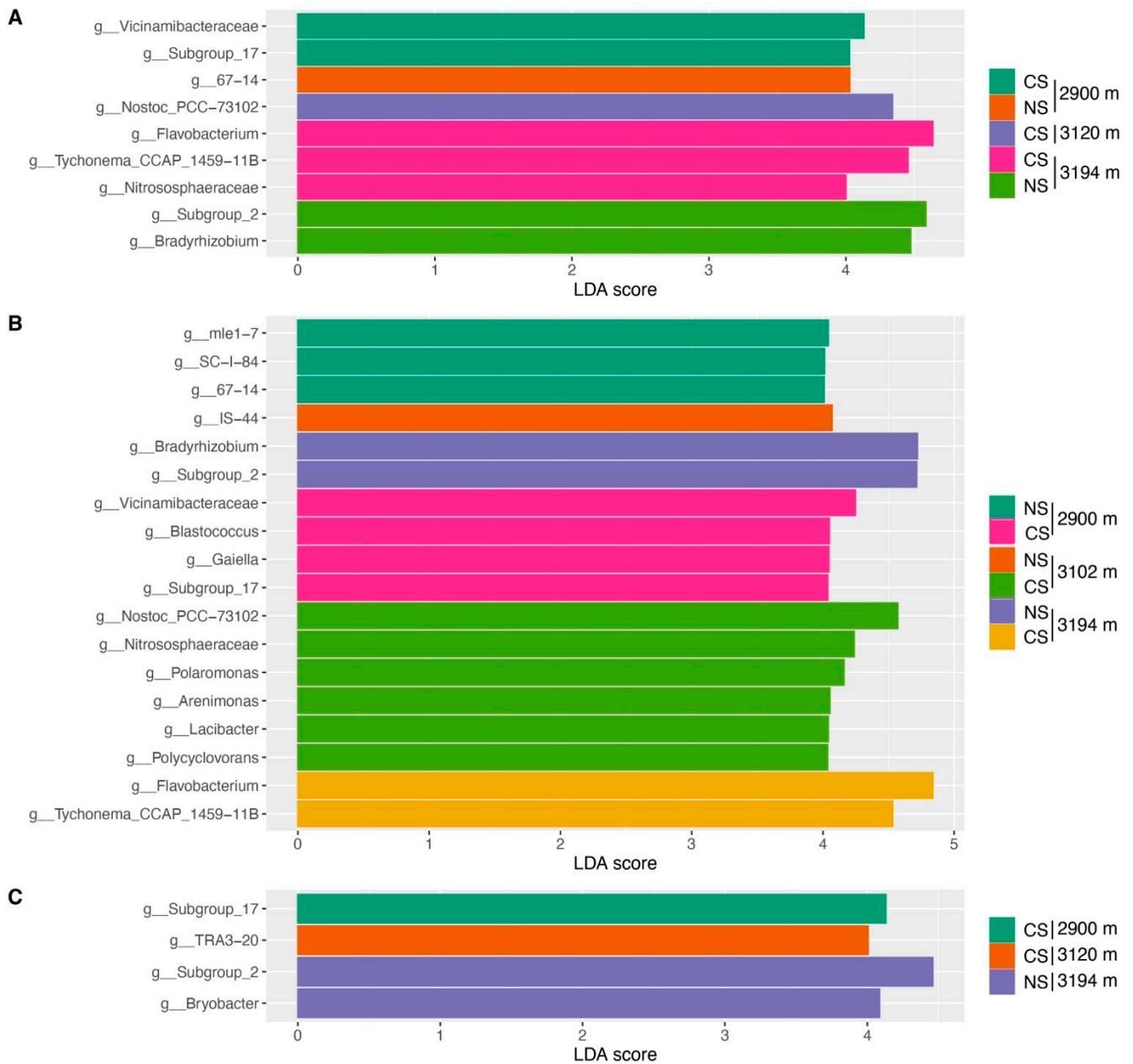


Figure S8 Differential genera identified by the LEfSe (linear discriminant analysis effect size) analysis ($p \leq 0.01$ and LDA score ≥ 4 ; no significant genus was identified under this criterion for hyper-rare bacterial communities) for total (A), abundant (RB $\geq 0.001\%$) (B), and rare ($0.0001\% \leq \text{RB} < 0.001\%$) (C) bacterial communities. RB: average relative abundance; NS: natural soils; CS: cut slope soils.

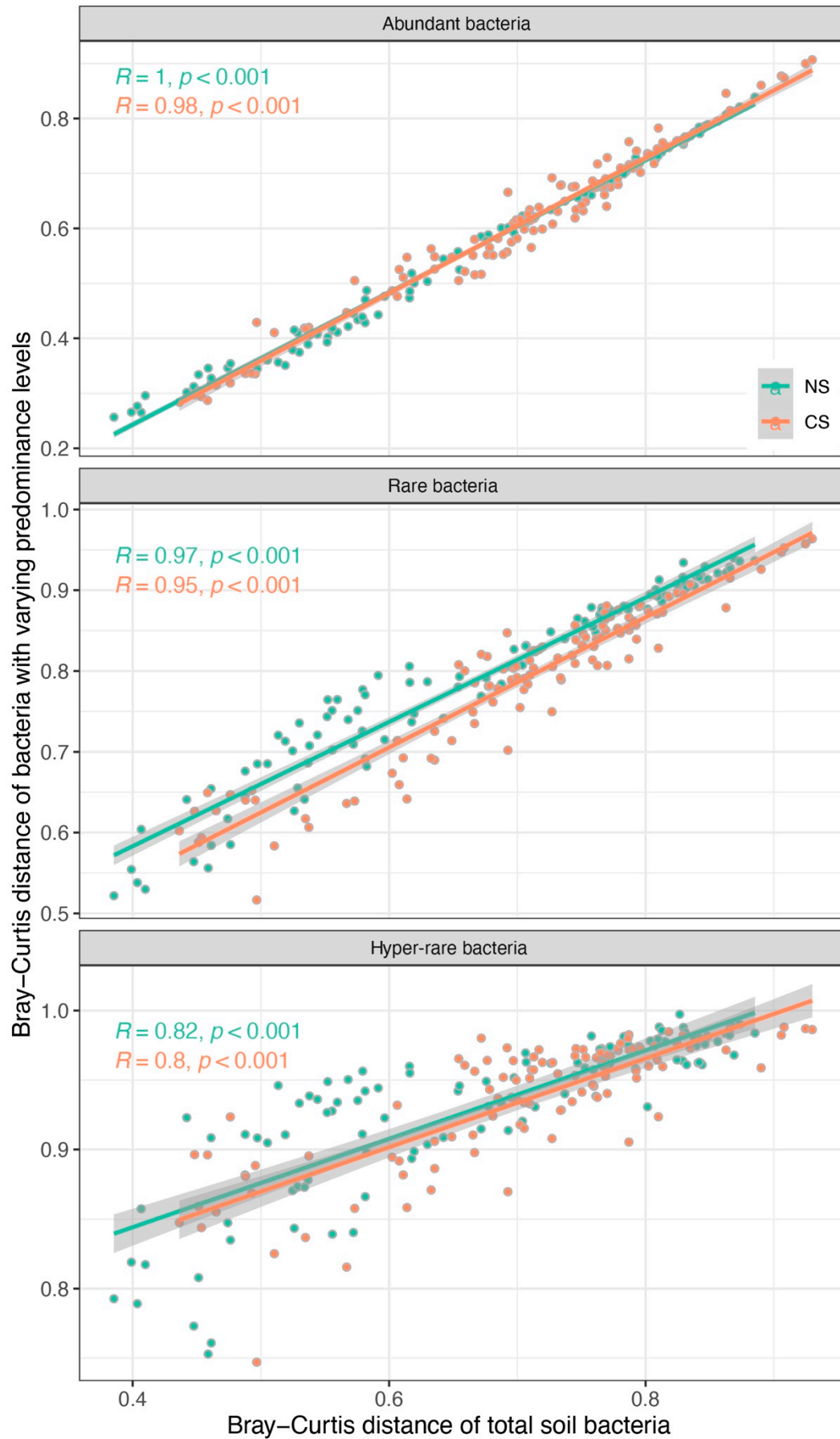


Figure S9 The relationships between the Bray-Curtis distances of total bacterial communities and those of abundant ($RB \geq 0.001\%$), rare ($0.0001\% \leq RB < 0.001\%$) and hyper-rare ($RB < 0.0001\%$) ones. R values and p -values represent Pearson correlation coefficients and associated significance, respectively. RB: average relative abundance; NS: natural soils; CS: cut slope soils.

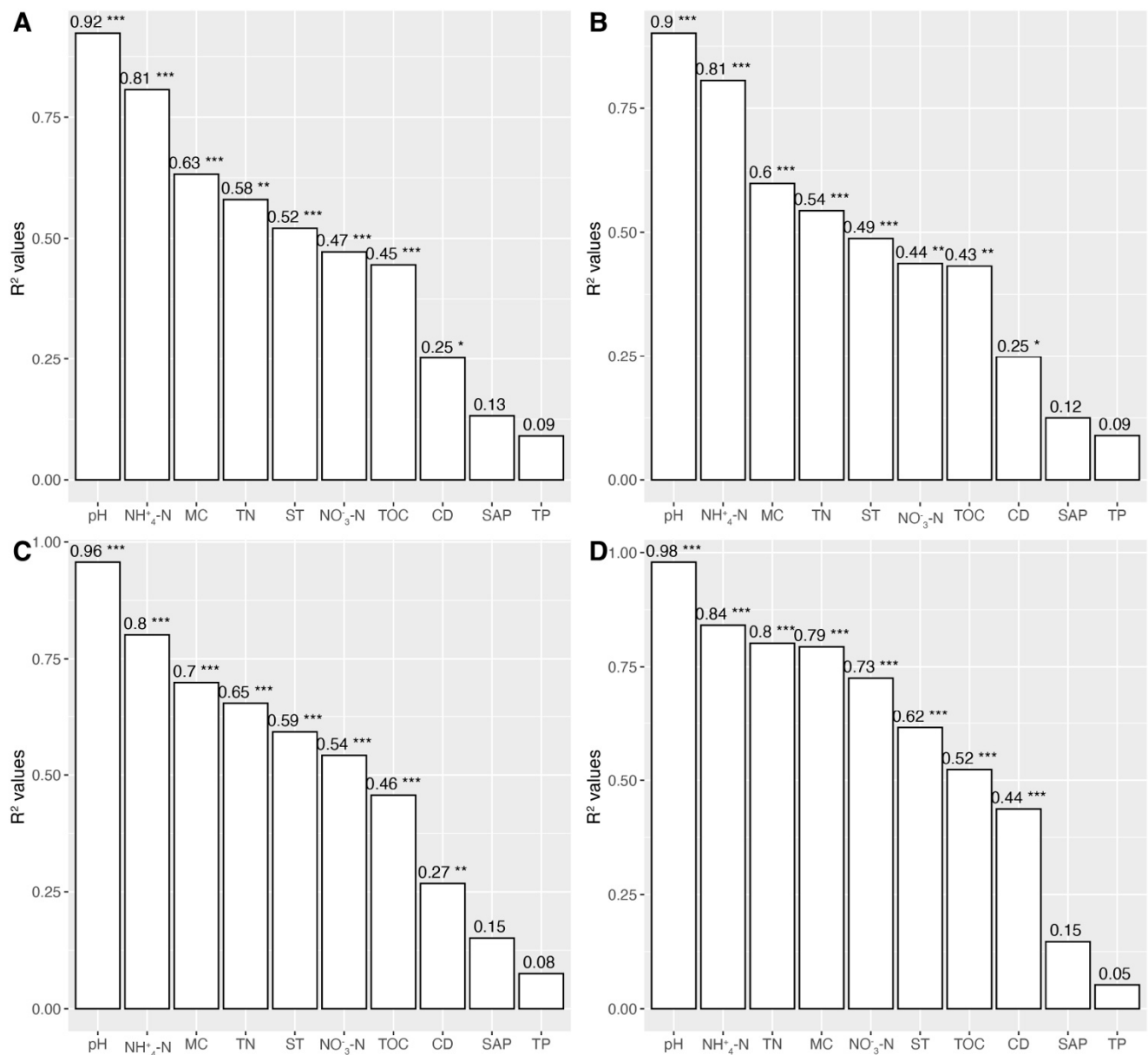


Figure S10 The relative contributions of individual soil property to the community differences of total (A), abundant (RB ≥ 0.001%) (B), rare (0.0001% ≤ RB < 0.001%) (C) and hyper-rare (RB < 0.0001%) (D) bacteria. R^2 values are calculated using the *envfit()* function of the vegan R package, and a larger R^2 means a larger contribution if $p < 0.05$. This figure is a supplement for **Figure 4**. MC: moisture content; ST: soil temperature; CD: conductivity; TOC: total organic carbon; TN: total nitrogen; NH₄⁺-N: ammonium nitrogen; NO₃⁻-N: nitrate nitrogen; TP: total phosphorus; SAP: soil available phosphorus. RB: average relative abundance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

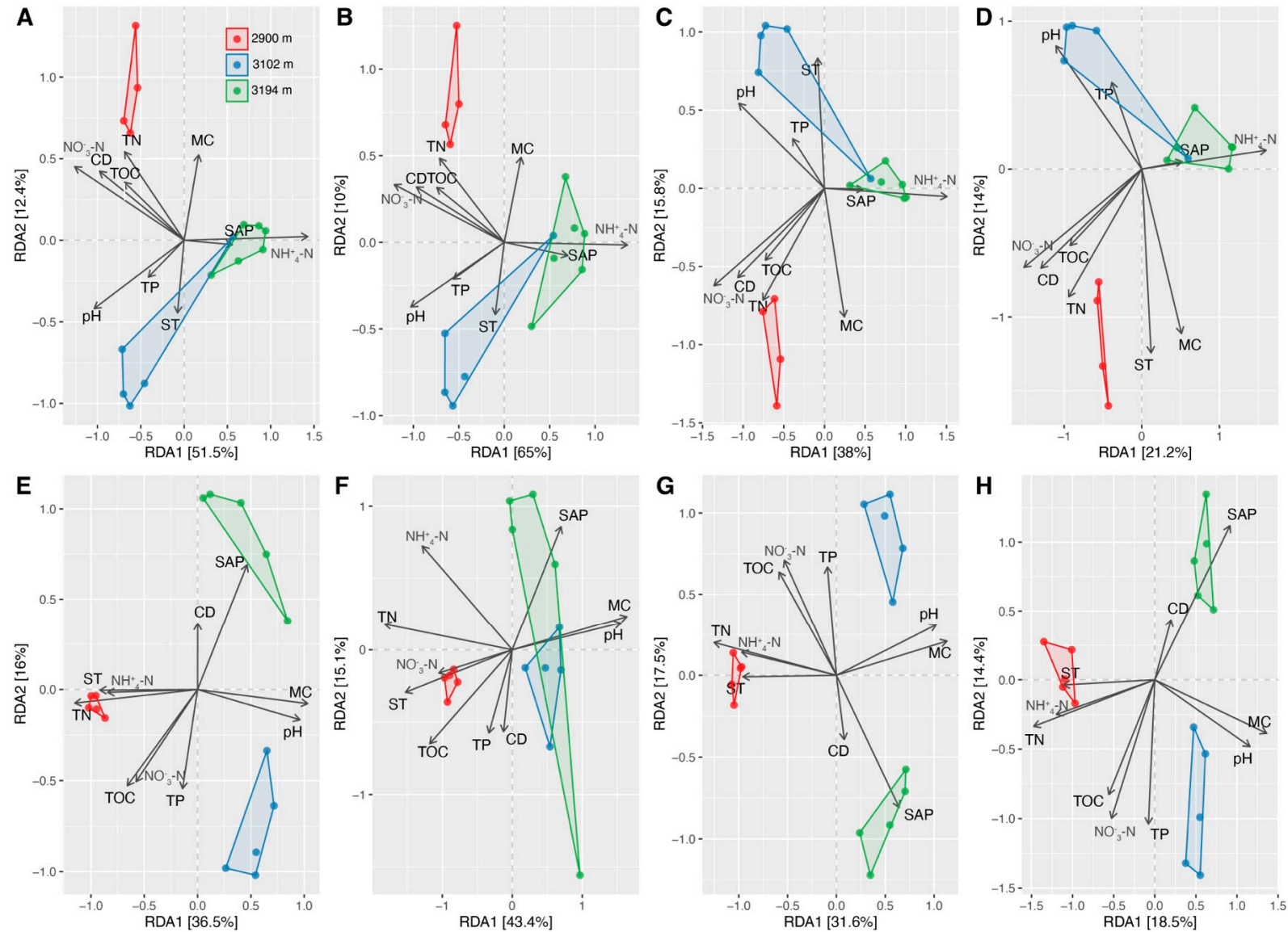


Figure S11 Bray-Curtis distance-based redundancy analysis (RDA) for the communities of total (A), abundant ($RB \geq 0.001\%$) (B), rare ($0.0001\% \leq RB < 0.001\%$) (C) and hyper-rare ($RB < 0.0001\%$) (D) bacteria in natural soils (NS), and for the communities of total (E), abundant (F), rare (G) and hyper-rare (H) bacteria in cut slope soils (CS). MC: moisture content; ST: soil temperature; CD: conductivity; TOC: total organic carbon; TN: total nitrogen; NH₄⁺-N: ammonium nitrogen; NO₃-N: nitrate nitrogen; TP: total phosphorus; SAP: soil available phosphorus. RB: average relative abundance.

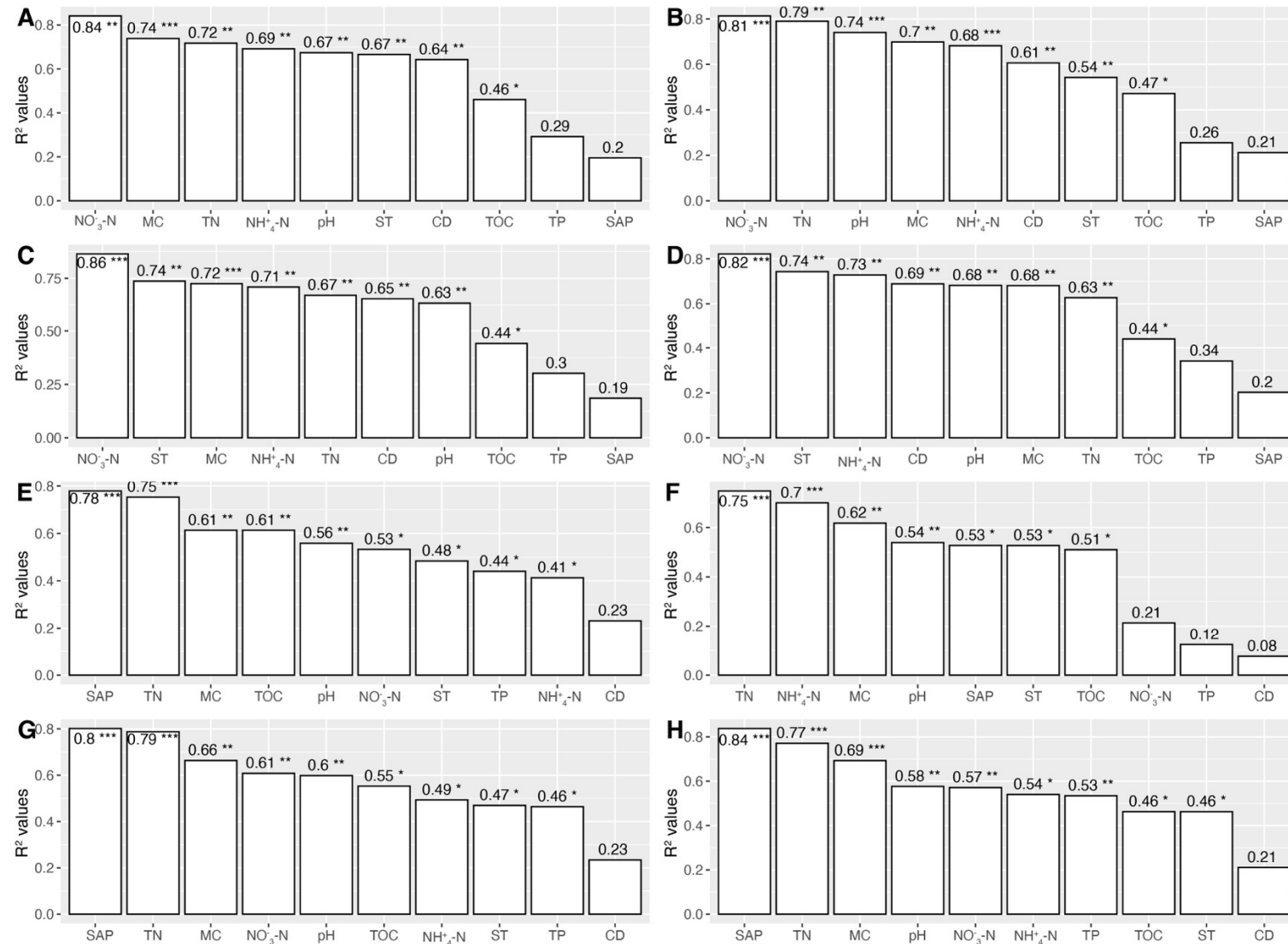


Figure S12 The relative contributions of individual soil property to the community differences of total (A), abundant (RB ≥ 0.001%) (B), rare (0.0001% ≤ RB < 0.001%) (C) and hyper-rare (RB < 0.0001%) (D) bacteria in natural soils (NS), and the community differences of total (E), abundant (F), rare (G) and hyper-rare (H) bacteria in cut slope soils (CS). R² values are calculated using the *envfit()* function of the vegan R package, and a larger R² means a larger contribution if $p < 0.05$. This figure is a supplement for **Figure S11**. MC: moisture content; ST: soil temperature; CD: conductivity; TOC: total organic carbon; TN: total nitrogen; NH₄⁺-N: ammonium nitrogen; NO₃-N: nitrate nitrogen; TP: total phosphorus; SAP: soil available phosphorus. RB: average relative abundance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.



Figure S13 A heatmap showing the Spearman correlations between soil properties and the functional redundancy of total, abundant (RB $\geq 0.001\%$), rare ($0.0001\% \leq \text{RB} < 0.001\%$) and hyper-rare (RB $< 0.0001\%$) bacteria. The ρ in the legend represents the correlation coefficient. MC: moisture content; ST: soil temperature; CD: conductivity; TOC: total organic carbon; TN: total nitrogen; NH₄⁺-N: ammonium nitrogen; NO₃⁻-N: nitrate nitrogen; TP: total phosphorus; SAP: soil available phosphorus. RB: average relative abundance; NS: natural soils; CS: cut slope soils. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table S1 Shapiro-Wilk test of normality for soil properties. The statistic and *p*-values are generated by *stats::shapiro.test()* function in R. If the *p*-value is greater than 0.05 (highlighted in the table), then it conforms to the normality hypothesis.

Variables	NS		CS		2900 m		3102 m		3194 m	
	Statistic	<i>P</i>	Statistic	<i>P</i>	Statistic	<i>P</i>	Statistic	<i>P</i>	Statistic	<i>P</i>
pH	0.926	0.234	0.841	0.013	0.828	0.042	0.730	0.002	0.799	0.009
MC	0.908	0.129	0.875	0.039	0.895	0.223	0.787	0.010	0.88	0.112
ST	0.904	0.108	0.9443	0.439	0.943	0.619	0.737	0.002	0.921	0.328
CD	0.767	0.001	0.973	0.904	0.931	0.490	0.749	0.003	0.887	0.129
TOC	0.893	0.074	0.905	0.115	0.957	0.766	0.857	0.070	0.983	0.978
TN	0.818	0.006	0.851	0.018	0.893	0.212	0.808	0.018	0.7316	0.001
NH ₄ ⁺ -N	0.806	0.004	0.864	0.027	0.929	0.476	0.898	0.208	0.769	0.004
NO ₃ -N	0.837	0.0116	0.908	0.127	0.916	0.364	0.775	0.007	0.741	0.002
TP	0.974	0.909	0.956	0.625	0.966	0.861	0.964	0.830	0.931	0.422
SAP	0.821	0.007	0.773	0.002	0.797	0.019	0.916	0.328	0.967	0.853

MC: moisture content; ST: soil temperature; CD: conductivity; TOC: total organic carbon; TN: total nitrogen; NH₄⁺-N: ammonium nitrogen; NO₃-N: nitrate nitrogen; TP: total phosphorus; SAP: soil available phosphorus. NS: natural soils; CS: cut slope soils.

Table S2 Bartlett test of homogeneity of variances for soil properties. The statistic and *p*-values are generated by *stats::bartlett.test()* function in R. If the *p*-value is greater than 0.05 (highlighted in the table), then it conforms to the homogeneity of variance hypothesis.

Variables	NS		CS		2900 m		3102 m		3194 m	
	Statistic	<i>P</i>	Statistic	<i>P</i>	Statistic	<i>P</i>	Statistic	<i>P</i>	Statistic	<i>P</i>
pH	8.193	0.017	8.483	0.014	3.454	0.063	1.677	0.195	6.321	0.012
MC	2.091	0.352	7.522	0.023	0.070	0.792	12.449	0.0004	3.725	0.054
ST	12.194	0.003	3.167	0.205	0.650	0.420	6.999	0.008	0.186	0.666
CD	10.601	0.005	5.966	0.051	1.592	0.207	8.928	0.003	3.918	0.048
TOC	2.759	0.252	8.169	0.017	0.090	0.764	8.685	0.003	1.468	0.226
TN	7.117	0.028	7.533	0.023	1.031	0.310	12.060	0.001	13.130	0.0002
NH ₄ ⁺ -N	1.958	0.376	0.614	0.736	1.099	0.295	0.151	0.698	1.810	0.179
NO ₃ ⁻ -N	10.6478	0.005	3.782	0.151	0.968	0.325	3.929	0.047	6.774	0.009
TP	1.244	0.537	3.049	0.218	0.075	0.784	0.796	0.372	0.863	0.353
SAP	5.040	0.080	16.301	0.0002	6.866	0.009	0.091	0.763	0.010	0.921

Table S3 Shapiro-Wilk test of normality, and Bartlett test of homogeneity of variances for Shannon-Wiener index. The statistic and *p*-values are generated by *stats::shapiro.test()* and *stats::bartlett.test()* functions in R, respectively. If the *p*-value is greater than 0.05 (highlighted in the table), then it conforms the hypothesis about normality or homogeneity of variance.

Community type	Groups	Shapiro-Wilk test		Bartlett test	
		Statistic	<i>P</i>	Statistic	<i>P</i>
Total soil bacteria	NS	0.931	0.286	1.892	0.388
	CS	0.719	0.000	15.220	0.00
	2900 m	0.960	0.802	0.102	0.750
	3102 m	0.965	0.846	0.017	0.896
	3194 m	0.853	0.047	8.409	0.004
Abundant bacteria	NS	0.814	0.006	4.075	0.130
	CS	0.775	0.002	19.760	0
	2900 m	0.938	0.564	0.370	0.543
	3102 m	0.887	0.157	0.185	0.667
	3194 m	0.860	0.057	8.882	0.003
Rare bacteria	NS	0.886	0.059	3.007	0.222
	CS	0.741	0.001	6.997	0.030
	2900 m	0.977	0.946	0.875	0.350
	3102 m	0.976	0.940	0.004	0.949
	3194 m	0.775	0.004	3.213	0.073
Hyper-rare bacteria	NS	0.947	0.476	0.498	0.780
	CS	0.940	0.387	0.581	0.748
	2900 m	0.914	0.343	1.342	0.247
	3102 m	0.945	0.605	0.112	0.737
	3194 m	0.956	0.724	0.044	0.833

Table S4 Shapiro-Wilk test of normality, and Bartlett test of homogeneity of variances for functional redundancy. The statistic and *p*-values are generated by *stats::shapiro.test()* and *stats::bartlett.test()* functions in R, respectively. If the *p*-value is greater than 0.05 (highlighted in the table), then it conforms the hypothesis about normality or homogeneity of variance.

Community type	Group	Shapiro-Wilk test		Bartlett test	
		Statistic	<i>P</i>	Statistic	<i>P</i>
Hyper-rare bacteria	NS	0.960	0.696	1.193	0.551
	CS	0.952	0.559	1.006	0.605
	2900 m	0.862	0.100	1.868	0.172
	3102 m	0.942	0.576	1.828	0.176
	3194 m	0.903	0.202	2.273	0.132
Rare bacteria	NS	0.918	0.181	2.858	0.240
	CS	0.928	0.251	1.580	0.454
	2900 m	0.874	0.135	0.120	0.729
	3102 m	0.933	0.475	1.018	0.313
	3194 m	0.957	0.736	0.1762	0.675
Abundant bacteria	NS	0.888	0.062	1.037	0.595
	CS	0.932	0.295	1.805	0.405
	2900 m	0.962	0.818	0.494	0.482
	3102 m	0.882	0.136	1.727	0.189
	3194 m	0.878	0.099	0.002	0.965
Total soil bacteria	NS	0.966	0.801	3.666	0.160
	CS	0.950	0.526	1.597	0.450
	2900 m	0.923	0.418	0.763	0.382
	3102 m	0.898	0.210	1.737	0.188
	3194 m	0.937	0.486	0.085	0.771

Table S5 Two-way and pairwise permutational multivariate analysis of variance (PERMANOVA) based on Euclidean distances for soil properties.

	Factors or comparisons	<i>F</i>	R ²	<i>P</i>
Two-way PERMANOVA	Elevation	4.099	0.155	0.002
	Soil type (i.e., NS and CS)	18.588	0.352	0.001
Pairwise PERMANOVA	2900 NS <i>vs.</i> 3102 NS	5.486	0.439	0.015
	2900 NS <i>vs.</i> 3194 NS	5.913	0.425	0.007
	3102 NS <i>vs.</i> 3194 NS	9.831	0.522	0.002
	2900 CS <i>vs.</i> 3102 CS	6.393	0.444	0.008
	2900 CS <i>vs.</i> 3194 CS	4.955	0.382	0.005
	3102 CS <i>vs.</i> 3194 CS	9.514	0.543	0.009
	2900 CS <i>vs.</i> 2900 NS	4.462	0.389	0.026
	3102 CS <i>vs.</i> 3102 NS	24.052	0.750	0.012
	3194 CS <i>vs.</i> 3194 NS	14.580	0.618	0.004

Table S6 The two-way and pairwise permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis distances for bacterial communities.

	Community type	Factors or comparisons	<i>F</i>	R ²	<i>P</i>
Two-way PERMANOVA	Hyper-rare bacteria	Elevation	1.934	0.119	0.001
		Soil type	2.734	0.084	0.001
	Rare bacteria	Elevation	3.835	0.189	0.001
		Soil type	6.832	0.169	0.001
	Abundant bacteria	Elevation	5.940	0.235	0.001
		Soil type	12.754	0.252	0.001
	Total soil bacteria	Elevation	4.720	0.212	0.001
		Soil type	9.114	0.205	0.001
Pairwise PERMANOVA	Hyper-rare bacteria	2900NS <i>vs.</i> 3102NS	1.538	0.180	0.018
		2900NS <i>vs.</i> 3194NS	1.975	0.198	0.002
		3102NS <i>vs.</i> 3194NS	1.972	0.180	0.005
		2900CS <i>vs.</i> 3102CS	2.125	0.210	0.008
		2900CS <i>vs.</i> 3194CS	2.014	0.201	0.004
		3102CS <i>vs.</i> 3194CS	1.664	0.172	0.01
		2900NS <i>vs.</i> 2900CS	1.513	0.178	0.013
		3102NS <i>vs.</i> 3102CS	2.392	0.230	0.012
		3194NS <i>vs.</i> 3194CS	2.487	0.217	0.003
	Rare bacteria	2900NS <i>vs.</i> 3102NS	2.360	0.252	0.011
		2900NS <i>vs.</i> 3194NS	4.560	0.363	0.004
		3102NS <i>vs.</i> 3194NS	3.858	0.300	0.007
		2900CS <i>vs.</i> 3102CS	4.827	0.376	0.006
		2900CS <i>vs.</i> 3194CS	4.084	0.338	0.013
		3102CS <i>vs.</i> 3194CS	2.602	0.245	0.012
		2900NS <i>vs.</i> 2900CS	2.437	0.258	0.005
		3102NS <i>vs.</i> 3102CS	5.636	0.413	0.007
		3194NS <i>vs.</i> 3194CS	5.701	0.388	0.004
	Abundant bacteria	2900NS <i>vs.</i> 3102NS	2.388	0.254	0.01
		2900NS <i>vs.</i> 3194NS	11.718	0.594	0.004
		3102NS <i>vs.</i> 3194NS	7.020	0.438	0.004
		2900CS <i>vs.</i> 3102CS	9.563	0.545	0.004
		2900CS <i>vs.</i> 3194CS	5.492	0.407	0.008
		3102CS <i>vs.</i> 3194CS	2.141	0.211	0.012
		2900NS <i>vs.</i> 2900CS	4.510	0.392	0.006
		3102NS <i>vs.</i> 3102CS	8.926	0.527	0.008
		3194NS <i>vs.</i> 3194CS	9.644	0.517	0.002
	Total soil bacteria	2900NS <i>vs.</i> 3102NS	2.343	0.251	0.02
		2900NS <i>vs.</i> 3194NS	7.203	0.474	0.005
		3102NS <i>vs.</i> 3194NS	5.058	0.360	0.005
		2900CS <i>vs.</i> 3102CS	6.426	0.445	0.009
Paired PERMANOVA	Total soil bacteria	2900CS <i>vs.</i> 3194CS	4.639	0.367	0.007
		3102CS <i>vs.</i> 3194CS	2.287	0.222	0.011
		2900NS <i>vs.</i> 2900CS	3.083	0.306	0.007
		3102NS <i>vs.</i> 3102CS	6.749	0.458	0.008

References

1. Liao H, Li C, Ai Y, Li X. Soil bacterial responses to disturbance are enlarged by altitude in a mountain ecosystem. *Journal of Soils and Sediments* **2023**, 23(11), 3820-3831.