

Supplementary Information

Bacterial Microbiome of the Rhizosphere: from Structure to Functions

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Supplementary Table 1. Summary table of response ratios from the original meta-analysis as well as from trim and fill test. Response ratio with 95% CIs were represented.

	Original Meta analysis			Trim and fill test		
	RR	CI Low	CI High	RR	CI Low	CI High
Shannon's diversity index	-0.0092	-0.0148	-0.0037	-0.0079	-0.0135	-0.0022
Pielou's evenness	-0.0005	-0.0035	0.0025	0.0002	-0.0028	0.0032
Heterotrophic strategy	0.0638	0.0522	0.0754	0.0416	0.0265	0.0567
Nitrification	-0.3546	-0.4001	-0.3092	-0.3257	-0.3724	-0.2790
Denitrification	0.1292	0.0657	0.1927	0.1195	0.0556	0.1834
Chitinolysis	0.2729	0.2033	0.3426	0.2603	0.1905	0.3302
Respiration of sulfur compounds	-0.2816	-0.3409	-0.2223	-0.2390	-0.2997	-0.1782
Cellulolysis	0.2078	0.1410	0.2745	0.1770	0.1090	0.2449
Ureolysis	0.5318	0.4661	0.5974	0.5346	0.4690	0.6003

Supplementary Table 2. Major properties of bacterial networks in bulk soil and the rhizosphere.

		edges	nodes	average. Degree	modularity
Global	Bulk	3608	559	12.91	0.52
	Rhizosphere	2210	534	8.28	0.60
Gramineae	Bulk	3240	526	12.32	0.56
	Rhizosphere	2284	482	9.48	0.58
Leguminosae	Bulk	3111	492	12.65	0.70
	Rhizosphere	2579	475	10.86	0.74
Solanaceae	Bulk	3970	486	16.34	0.59
	Rhizosphere	4070	507	16.06	0.57
Cucurbitaceae	Bulk	2298	339	13.56	0.71
	Rhizosphere	1621	354	9.16	0.71
Grass	Bulk	4822	468	20.61	0.70
	Rhizosphere	4271	473	18.06	0.72
Forbs	Bulk	3088	404	15.29	0.60
	Rhizosphere	3797	437	17.38	0.63
Trees	Bulk	2857	473	12.08	0.60
	Rhizosphere	4018	491	16.37	0.63

Supplementary Table 3. The proportions of removed sequences and unknow sequences during the processing of sequencing data.

	Sequences proportion (%)
Singleton	0.0001
ASVs presented only in one sample	0.20
Archaea	0.88
Eukaryota	0.43
Chloroplast	0.90
Mitochondria	0.45
Unknown	14

Supplementary Table 4. Heterogeneity statistics of categorical group analyses on bacterial alpha diversity. Total heterogeneity (Q_T) was partitioned into within-group heterogeneity (Q_W) and between-group heterogeneity (Q_B). A significance of Q_B indicates that the effect sizes are significantly different between the levels of the categorical group. A two-sided p value <0.05 was considered to be statistically significant.

Response variables	Categorical variables	Q_T	Q_B	Q_W	p value (Q_B)
Observed species richness	Ecosystem type	533.19	15.47	517.71	0.001
	Crop family	379.67	17.88	361.79	0.003
	Crop management	449.20	3.35	445.85	0.083
	Fertilizer type in cropland	149.42	2.54	146.88	0.138
	Group of herbaceous plants	88.82	1.24	87.58	0.817
	Grass photosynthetic pathway	62.56	8.03	54.53	0.009
	Forest leaf traits	59.22	2.25	56.97	0.166
	Forest classification	62.90	5.41	57.48	0.063
Shannon's diversity index	Ecosystem type	986.88	55.65	931.23	0.001
	Crop family	698.67	68.79	629.89	0.001
	Crop management	758.99	5.85	753.14	0.094
	Fertilizer type in cropland	170.86	4.95	165.92	0.056
	Group of herbaceous plants	93.86	6.75	87.11	0.065
	Grass photosynthetic pathway	55.76	4.44	51.32	0.027
	Forest leaf traits	60.41	0.52	59.89	0.502
	Forest classification	62.86	3.22	59.64	0.101
Pielou's evenness	Ecosystem type	551.10	59.58	1381.36	0.001
	Crop family	1194.48	141.3	1053.16	0.001
	Crop management	1267.26	1.76	1265.50	0.422
	Fertilizer type in cropland	368.17	12.58	355.59	0.053
	Group of herbaceous plants	187.12	19.43	167.69	0.007
	Grass photosynthetic pathway	189.28	5.94	183.34	0.082
	Forest leaf traits	30.20	0.27	29.94	0.540
	Forest classification	33.64	0.46	33.18	0.398
Faith's phylogenetic diversity	Ecosystem type	610.56	13.30	597.26	0.010
	Crop family	446.93	18.32	428.61	0.016
	Crop management	478.99	2.65	476.35	0.155
	Fertilizer type in cropland	141.22	3.41	137.81	0.079
	Group of herbaceous plants	52.47	2.48	49.99	0.186
	Grass photosynthetic pathway	55.24	2.32	52.92	0.144
	Forest leaf traits	67.11	7.29	59.82	0.055
	Forest classification	66.32	1.35	64.98	0.289

Ecosystem type: cropland, forest and grassland; Crop family: Gramineae, Leguminosae, Solanaceae, Cucurbitaceae; Crop management: dryland, paddyland; Fertilizer type in cropland: chemical fertilizer and organic fertilizer; Group of herbaceous plants: grasses, forbs and shrubs; Grass photosynthetic pathway: C3, C4; Forest classification: trees, shrubs; Forest leaf traits: broad leaved, coniferous.

Supplementary Table 5. Heterogeneity statistics of categorical group analyses on bacterial alpha diversity depending on experiment management, sequencing platform and target regions of primer pairs. Total heterogeneity (Q_T) was partitioned into within-group heterogeneity (Q_W) and between-group heterogeneity (Q_B). A significance of Q_B indicates that the effect sizes are significantly different between the levels of the categorical group. A two-sided p value <0.05 was considered to be statistically significant.

Response variables		Categorical variables	Q_T	Q_B	Q_W	p value (Q_B)
Observed species richness	Total	Sequencing platform	573.93	26.06	547.87	0.001
		Target regions	476.51	6.47	470.04	0.053
		Experimental management	583.67	2.91	580.76	0.121
	Cropland	Target regions	342.10	1.41	340.69	0.259
	Grassland	Target regions	84.02	11.18	72.84	0.003
	Forest	Target regions	56.61	0.17	56.44	0.722
Shannon's diversity index	Total	Sequencing platform	964.54	57.98	906.56	0.001
		Target regions	817.25	27.40	789.85	0.001
		Experimental management	604.79	13.26	591.54	0.398
	Cropland	Target regions	604.79	13.26	591.54	0.077
	Grassland	Target regions	82.40	0.13	82.27	0.002
	Forest	Target regions	54.89	2.08	52.81	0.183
Pielou's evenness	Total	Sequencing platform	1340.7	53.75	1286.95	0.060
		Target regions	1121.1	41.33	1079.80	0.001
		Experimental management	1408.7	0.12	1408.58	0.814
	Cropland	Target regions	988.51	30.78	957.73	0.001
	Grassland	Target regions	230.20	0.41	229.79	0.659
	Forest	Target regions	39.98	4.26	35.72	0.008
Faith's phylogenetic diversity	Total	Sequencing platform	633.77	38.33	595.44	0.001
		Target regions	541.27	2.57	538.70	0.152
		Experimental management	647.69	2.78	644.90	0.132
	Cropland	Target regions	418.55	0.57	417.98	0.502
	Grassland	Target regions	71.37	2.65	68.72	0.112
	Forest	Target regions	59.82	0.12	59.70	0.767

Ecosystem type: cropland, forest and grassland; Sequencing platform: Illumina and Ion S5; Target regions: V3~V4 and V4~V5; Experiment management: field and greenhouse.

Supplementary Table 6. Heterogeneity statistics of categorical group analyses on dormancy potential and heterotrophic strategy. Total heterogeneity (Q_T) was partitioned into within-group heterogeneity (Q_W) and between-group heterogeneity (Q_B). A significance of Q_B indicates that the effect sizes are significantly different between the levels of the categorical group. A two-sided p value <0.05 was considered to be statistically significant.

Response variables	Categorical variables	Q_T	Q_B	Q_W	p value (Q_B)
Toxin-Antitoxin	Ecosystem type	789.32	0.93	788.38	0.748
	Crop family	721.74	628.5	93.15	0.001
	Crop management	595.82	63.05	532.77	0.001
	Fertilizer type in cropland	178.91	0.60	178.31	0.519
	Group of herbaceous plants	95.34	5.09	90.25	0.102
	Grass photosynthetic pathway	111.96	24.02	87.94	0.001
	Forest leaf traits	92.99	4.46	88.53	0.135
	Forest classification	97.14	0.56	96.58	0.552
Sporulation	Ecosystem type	755.56	3.21	752.36	0.335
	Crop family	582.76	39.69	543.07	0.001
	Crop management	574.89	5.52	569.37	0.062
	Fertilizer type in cropland	171.97	3.30	168.66	0.103
	Group of herbaceous plants	110.76	8.85	101.91	0.061
	Grass photosynthetic pathway	64.98	0.02	64.98	0.958
	Forest leaf traits	77.53	2.52	75.01	0.184
	Forest classification	79.82	0.026	79.80	0.879
Heterotrophic-strategy	Ecosystem type	335.0	3.51	331.50	0.038
	Crop family	204.7	4.02	200.78	0.137
	Crop management	243.1	3.96	239.23	0.007
	Fertilizer type in cropland	322.8	8.78	314.02	0.059
	Group of herbaceous plants	156.1	7.30	148.83	0.040
	Grass photosynthetic pathway	120.5	8.73	111.77	0.015
	Forest leaf traits	66.98	0.00	66.98	0.952
	Forest classification	70.79	0.02	70.77	0.900

Ecosystem type: cropland, forest and grassland; Crop family: Gramineae, Leguminosae, Solanaceae, Cucurbitaceae; Crop management: dryland, paddyland; Fertilizer type in cropland: chemical fertilizer and organic fertilizer; Group of herbaceous plants: grasses, forbs and shrubs; Grass photosynthetic pathway: C3, C4; Forest classification: trees, shrubs; Forest leaf traits: broad leaved, coniferous.

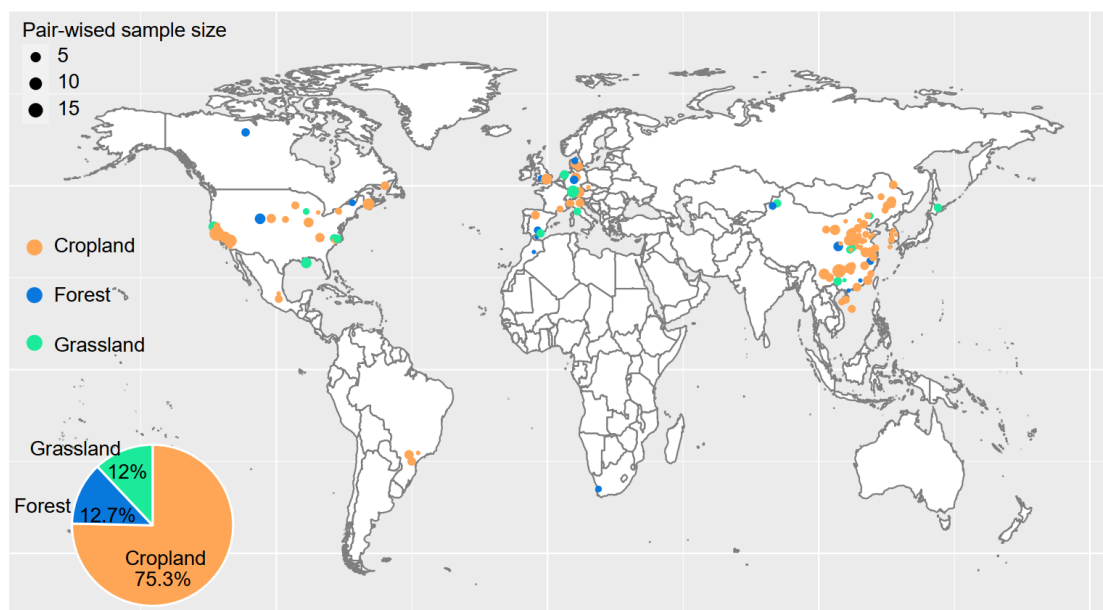
Supplementary Table 7. Heterogeneity statistics of categorical group analyses on bacterial community function. Total heterogeneity (Q_T) was partitioned into within-group heterogeneity (Q_W) and between-group heterogeneity (Q_B). A significance of Q_B indicates that the effect sizes are significantly different between the levels of the categorical group. A two-sided p value <0.05 was considered to be statistically significant.

Response variables	Categorical variables	Q_T	Q_B	Q_W	p value (Q_B)
Nitrification	Ecosystem type	793.90	7.29	786.61	0.078
	Crop family	594.28	41.02	553.26	0.001
	Crop management	619.33	13.54	605.79	0.007
	Fertilizer type in cropland	185.29	2.46	182.83	0.206
	Group of herbaceous plants	88.64	0.13	88.50	0.924
	Grass photosynthetic pathway	71.28	1.59	69.69	0.217
	Forest leaf traits	75.86	1.58	74.28	0.239
	Forest classification	64.50	0.03	64.47	0.858
Denitrification	Ecosystem type	644.64	11.31	633.33	0.010
	Crop family	425.25	3.26	421.99	0.049
	Crop management	470.37	1.10	469.27	0.353
	Fertilizer type in cropland	178.03	0.47	177.56	0.531
	Group of herbaceous plants	116.34	13.19	103.15	0.004
	Grass photosynthetic pathway	77.07	6.22	70.85	0.024
	Forest leaf traits	65.48	0.30	65.18	0.577
	Forest classification	71.04	4.44	66.60	0.039
Methanol oxidation	Ecosystem type	670.32	0.40	669.92	0.888
	Crop family	586.63	54.13	532.50	0.001
	Crop management	559.41	36.89	522.52	0.001
	Fertilizer type in cropland	202.48	6.98	195.50	0.031
	Group of herbaceous plants	69.54	5.66	63.88	0.068
	Grass photosynthetic pathway	57.47	6.52	50.95	0.024
	Forest leaf traits	22.69	3.10	19.59	0.076
	Forest classification	25.96	0.03	25.93	0.884
Nitrogen fixation	Ecosystem type	717.65	2.66	714.99	0.335
	Crop family	512.82	34.86	477.96	0.001
	Crop management	527.67	2.94	524.73	0.162
	Fertilizer type in cropland	178.19	0.47	177.72	0.531
	Group of herbaceous plants	130.15	15.69	114.46	0.002
	Grass photosynthetic pathway	62.39	0.74	61.65	0.412
	Forest leaf traits	71.28	1.63	69.65	0.209
	Forest classification	77.27	2.75	74.52	0.110
Chitinolysis	Ecosystem type	503.30	3.25	500.05	0.488
	Crop family	320.91	20.76	300.15	0.001
	Crop management	347.66	0.56	347.10	0.466
	Fertilizer type in cropland	104.52	2.11	102.41	0.131

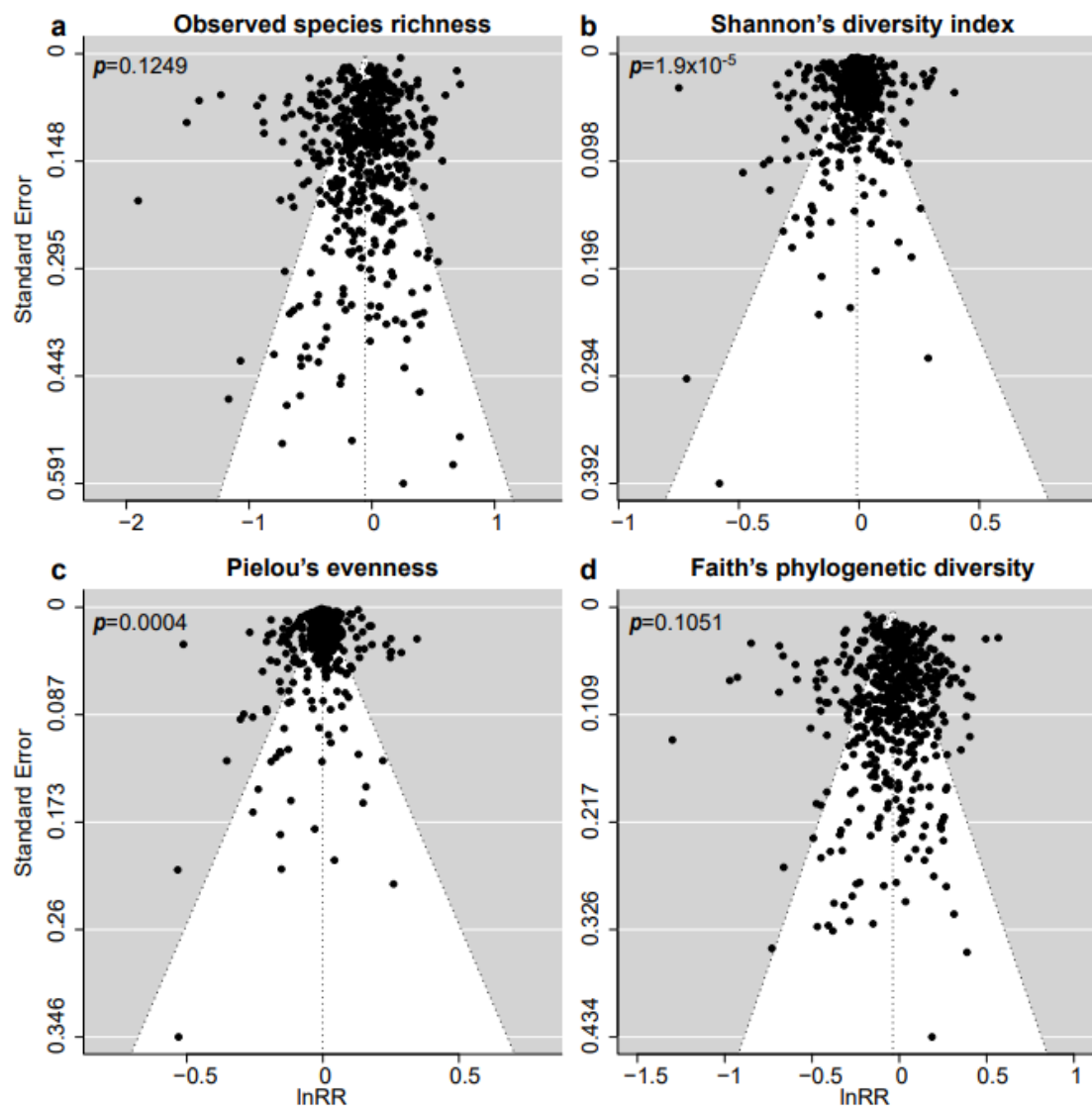
	Group of herbaceous plants	100.16	1.15	99.01	0.606
	Grass photosynthetic pathway	71.00	0.24	70.76	0.664
	Forest leaf traits	36.29	5.57	30.72	0.03
	Forest classification	35.36	0.03	35.33	0.89
Ligninolysis	Ecosystem type	416.44	7.48	408.96	0.110
	Crop family	262.24	0.57	261.67	0.952
	Crop management	370.18	26.05	344.13	0.001
	Fertilizer type in cropland	89.00	0.61	88.39	0.496
	Group of herbaceous plants	34.80	1.06	33.74	0.611
	Grass photosynthetic pathway	30.83	2.09	28.74	0.152
	Forest leaf traits	8.98	0.68	8.30	0.411
	Forest classification	13.12	2.83	10.29	0.126
Respiration of sulfur compounds	Ecosystem type	586.07	5.18	580.89	0.161
	Crop family	434.56	34.29	400.27	0.001
	Crop management	453.29	38.29	415.00	0.001
	Fertilizer type in cropland	131.04	0.05	130.99	0.803
	Group of herbaceous plants	81.81	78.96	2.85	0.594
	Grass photosynthetic pathway	58.66	8.60	50.06	0.013
	Forest leaf traits	31.82	2.05	29.78	0.182
	Forest classification	33.98	0.52	33.46	0.506
Xylanolysis	Ecosystem type	344.04	1.50	342.54	0.504
	Crop family	285.25	4.12	281.13	0.367
	Crop management	287.39	0.52	286.87	0.460
	Fertilizer type in cropland	115.36	114.6	0.80	0.415
	Group of herbaceous plants	43.90	6.01	37.89	0.086
	Grass photosynthetic pathway	22.57	0.07	22.50	0.790
	Forest leaf traits	16.80	0.01	16.79	0.958
	Forest classification	17.49	0.09	17.40	0.770
Methylotrophy	Ecosystem type	608.29	12.24	596.05	0.016
	Crop family	614.66	103.45	511.21	0.001
	Crop management	513.85	32.20	481.65	0.001
	Fertilizer type in cropland	145.04	1.53	143.51	0.287
	Group of herbaceous plants	66.73	3.18	63.5	0.217
	Grass photosynthetic pathway	41.61	0.13	41.48	0.702
	Forest leaf traits	52.64	1.05	51.59	0.383
	Forest classification	54.32	0.29	54.03	0.630
Cellulolysis	Ecosystem type	517.17	6.11	511.06	0.063
	Crop family	401.63	4.26	397.38	0.323
	Crop management	379.47	3.91	375.56	0.070
	Fertilizer type in cropland	115.97	0.37	115.60	0.539
	Group of herbaceous plants	106.31	9.14	97.17	0.032
	Grass photosynthetic pathway	67.29	0.01	67.28	0.967
	Forest leaf traits	38.47	1.12	37.35	0.270
	Forest classification	41.47	0.27	41.20	0.547

Ureolysis	Ecosystem type	433.66	9.21	424.45	0.005
	Crop family	292.23	10.27	281.96	0.026
	Crop management	283.26	16.93	266.33	0.001
	Fertilizer type in cropland	94.66	0.22	94.44	0.597
	Group of herbaceous plants	91.02	2.17	88.85	0.347
	Grass photosynthetic pathway	68.61	3.76	64.85	0.063
	Forest leaf traits	108.04	0.42	107.62	0.639
	Forest classification	115.13	4.53	110.60	0.103
Plant pathogen	Ecosystem type	466.16	14.14	452.02	0.004
	Crop family	300.24	12.49	287.75	0.621
	Crop management	343.16	5.31	337.85	0.050
	Fertilizer type in cropland	151.67	0.04	151.63	0.858
	Group of herbaceous plants	67.40	6.99	60.41	0.048
	Grass photosynthetic pathway	47.92	1.71	46.22	0.234
	Forest leaf traits	35.39	6.17	29.22	0.069
	Forest classification	32.67	0.47	32.20	0.537

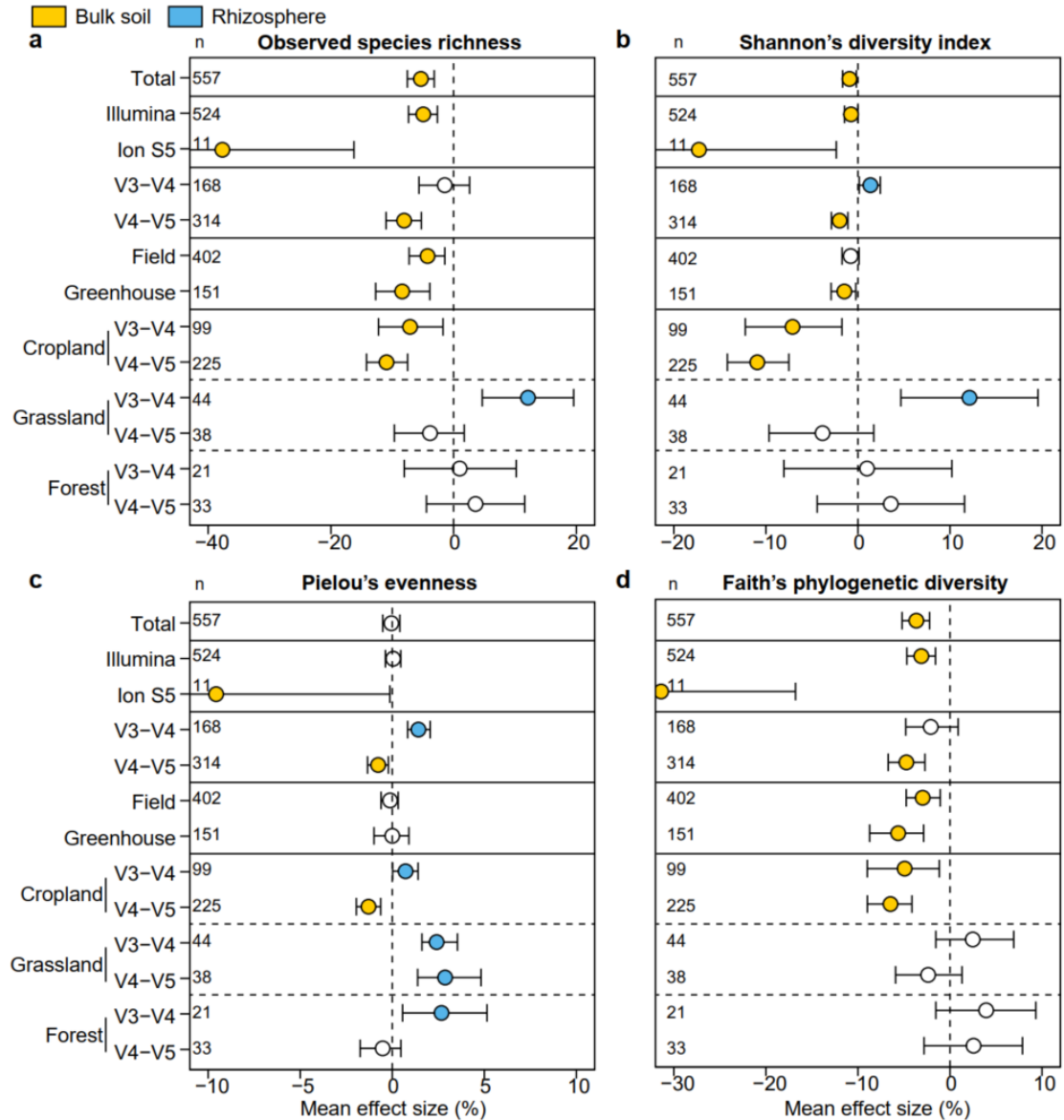
Ecosystem type: cropland, forest and grassland; Crop family: Gramineae, Leguminosae, Solanaceae, Cucurbitaceae; Crop management: dryland, paddyland; Fertilizer type in cropland: chemical fertilizer and organic fertilizer; Group of herbaceous plants: grasses, forbs and shrubs; Grass photosynthetic pathway: C3, C4; Forest classification: trees, shrubs; Forest leaf traits: broad leaved, coniferous.



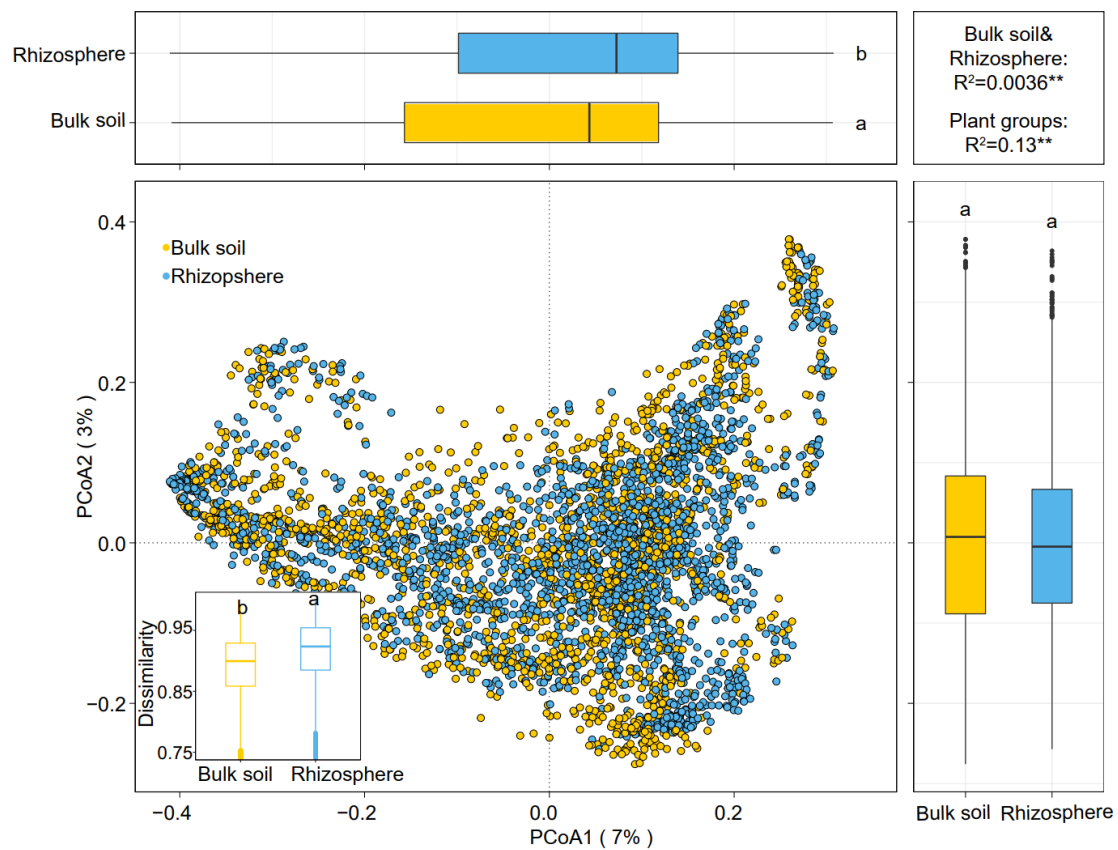
Supplementary Figure 1. Map of sampling sites from 122 independent studies with 557 pairs from rhizosphere and bulk soils for data merging. The locations of sampling sites are marked by circles on map. Circle colors represented different ecosystems. Orange, green, and blue in the pie diagram represent cropland, grassland and forest, respectively. Circle size represents the pair-wise sample size of the bulk soils and rhizosphere.



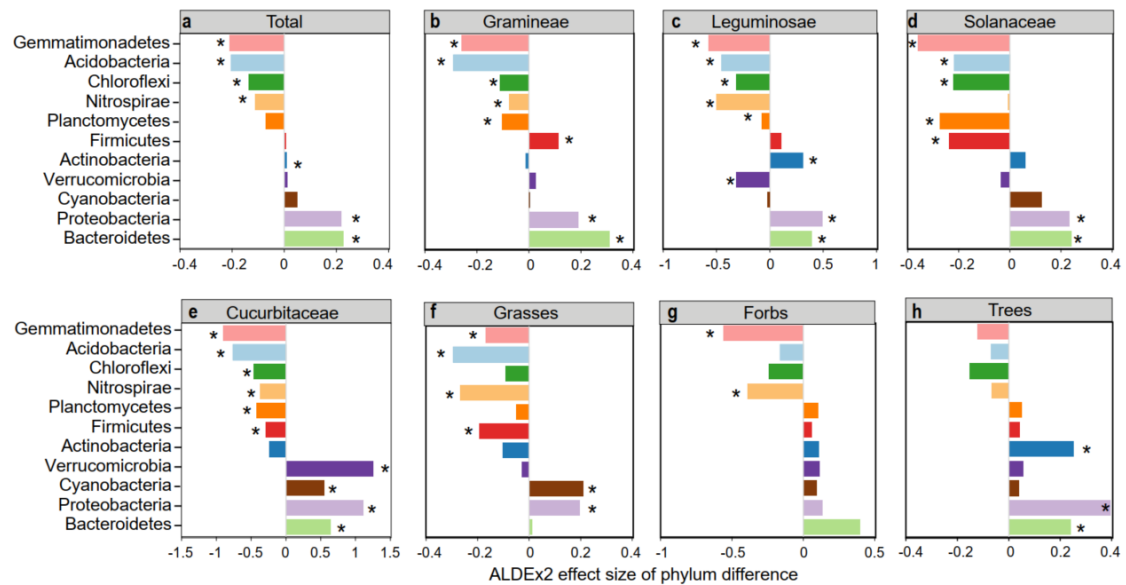
Supplementary Figure 2. Funnel plots for bacterial diversity between bulk soils and rhizosphere. **A**, Funnel plot of observed species richness; **b**, Funnel plot of Shannon's diversity index; **c**, Funnel plot of Pielou's evenness; **d**, Funnel plot of Faith's phylogenetic diversity. The top of each panel contains the results of publication bias tests using Egger's regression. A two-sided p value indicating statistical significance of the funnel plot asymmetry test. Asymmetry test showed a symmetric distribution ($p > 0.05$) in observed species richness (a) and Faith's phylogenetic diversity (d). Asymmetry test showed an asymmetric distribution ($p < 0.05$) in Shannon's diversity index (b) and Pielou's evenness (c). lnRR: natural log-transformed response ratio.



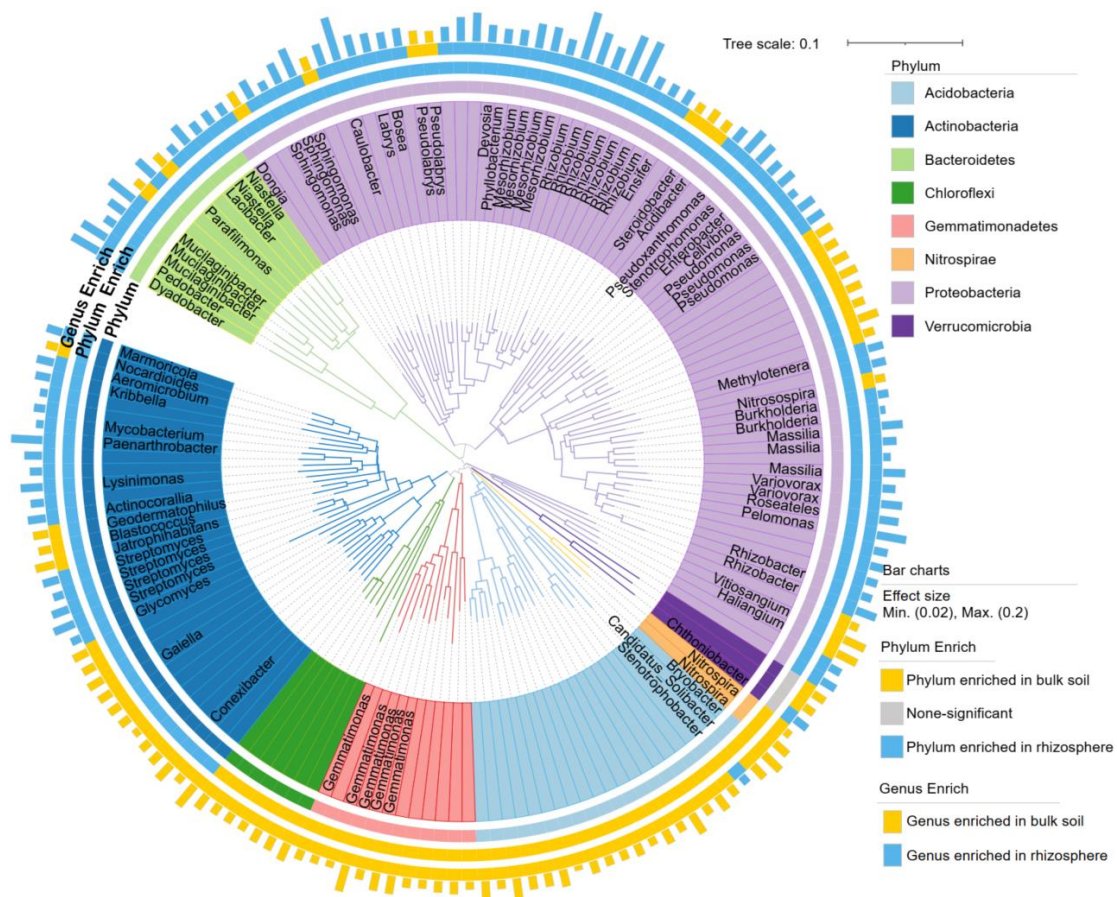
Supplementary Figure 3. Diversity of bacterial communities between bulk soils and rhizosphere under different experiment management, sequencing platform and target regions. **a**, observed species richness; **b**, Shannon's diversity index; **c**, Pielou's evenness; and **d**, Faith's phylogenetic diversity. All dots represent the percentage change in effect size between rhizosphere and bulk soils bacterial diversity at 95% confidence intervals (Cis). Mean values < 0 denote a higher diversity in the bacterial community of bulk soils (yellow dots; depletion in rhizosphere), whereas mean values > 0 reflect a significantly higher diversity in the rhizosphere bacterial community (blue dots). The intersection of error bars with the zero line indicates absence of significant difference between bacterial communities in rhizosphere and bulk soils (open dots). Sample size is showed by number of data pairs for each group. Experiment management: field and greenhouse; Sequencing platform: Illumina and Ion S5; Target regions: Bacterial V3~V4, V4~V5.



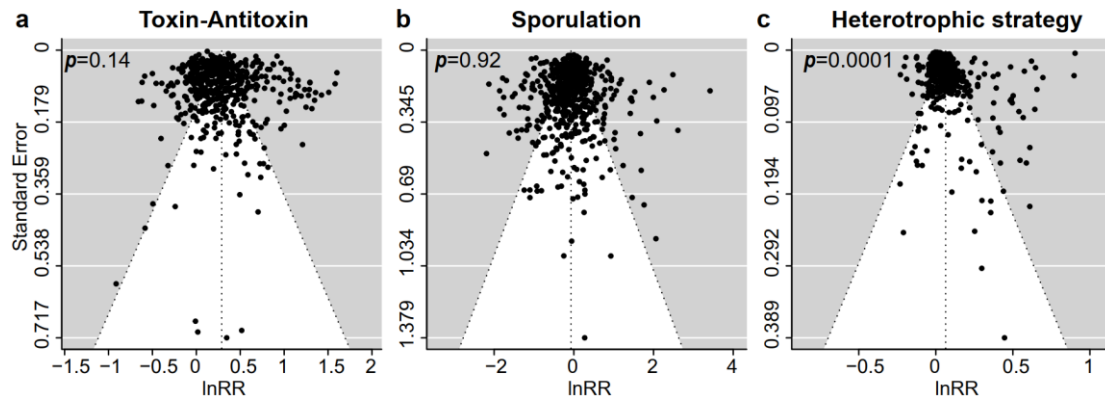
Supplementary Figure 4. Bacterial community composition of bulk soils and rhizosphere. Principal coordinate analysis (PcoA) plots depict the Bray-Curtis distance of the bacterial communities of the bulk soils and rhizosphere (bulk soils $n = 1759$ vs. rhizosphere $n = 2182$). Results from the PERMANOVA statistics tests testing the effect of the compartments (i.e., bulk soil and rhizosphere (**: $p=0.01$)) and the plant groups (**: $p=0.01$). R^2 , explained variances, are provided on the top right of panel included on the plots. Box plots on the right and top show the overall distribution of PC1 and PC2 scores within each compartment. Boxplots reflect median (center line), 25th and 75th percentile values (bounds of boxes), and ranges (whiskers) for each category. Small letters indicate significant differences between bulk soils and rhizosphere (Tukey's test for multiple comparisons). Box plots at bottom right showed the beta dissimilarity via Bray-Curtis distance between samples within the same compartment. Statistical comparison was tested using two-sided Wilcoxon's rank sum tests.



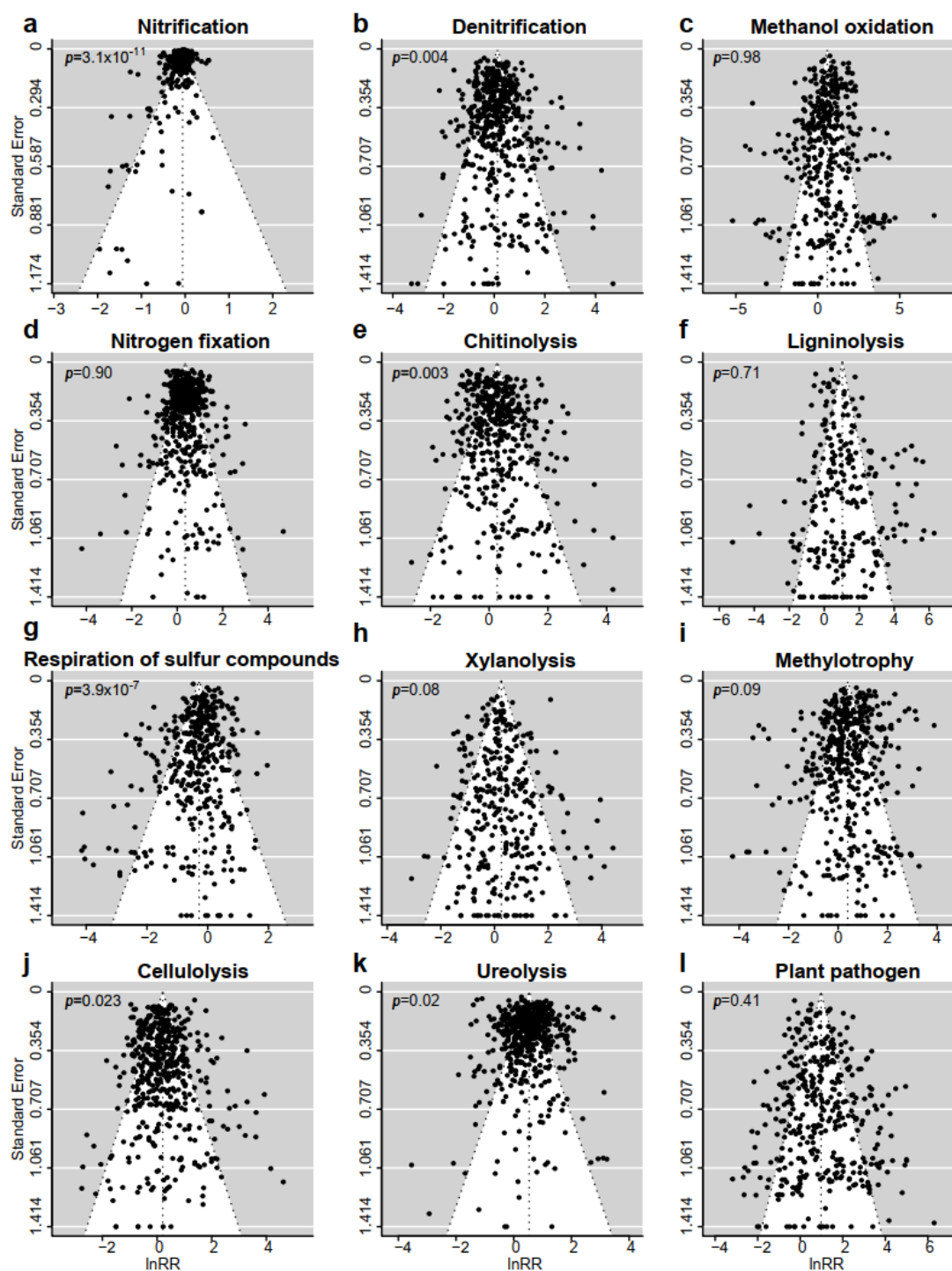
Supplementary Figure 5. The phyla differences between bacterial communities in rhizosphere and bulk soils depending on plant groups. a, total sample; b, Gramineae; c, Leguminosae; d, Solanaceae; e, Cucurbitaceae; f, grasses; g, forbs; h, trees. Positive values indicate higher relative abundance of the phylum in rhizosphere, while negative values indicate higher relative abundance of the phylum in the bulk soils. Statistical analysis performed with ALDEx2. Asterisk (*) indicated significantly differentially phylum with a false discovery rate (FDR)<0.05.



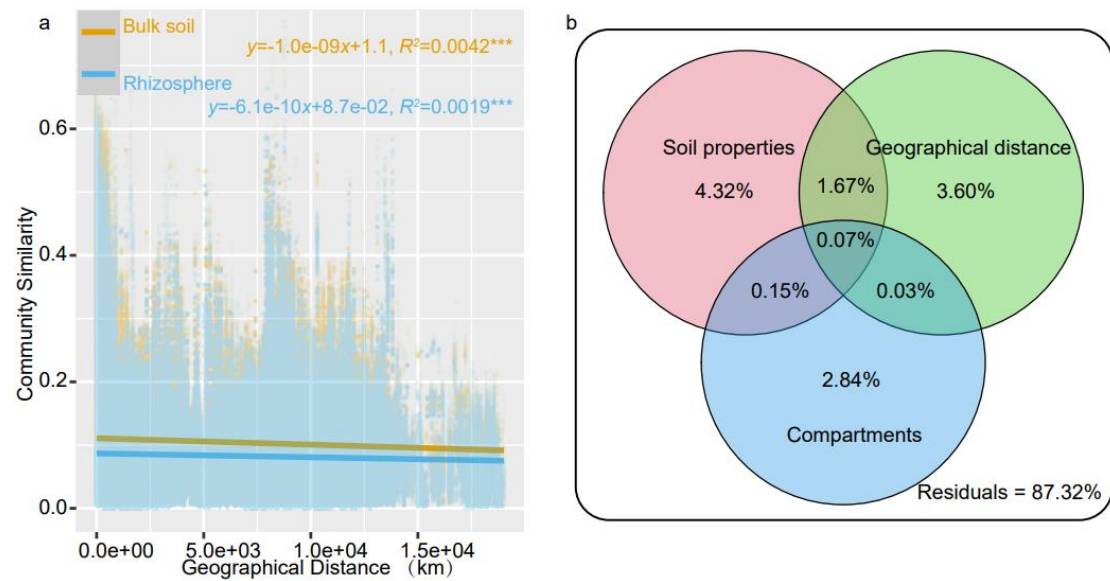
Supplementary Figure 6. Phylogeny of microorganisms with significant differences in bulk soils and rhizosphere bacterial communities. Phylogenetic tree comprising the 164 significant differences bacteria are labelled according to the genus name (exclude the unsigned genus) and the corresponding phylum is depicted in the first outer layer. The second outer layer showed the phylum enrichment between bulk soils and rhizosphere. The third outer layer represent the genus enrichment. The blue and yellow outer layer represent the ASVs with significant enrichment in the rhizosphere and bulk soils respectively. The outermost bar represents the ALDEx2 effect size between bulk soils and rhizosphere.



Supplementary Figure 9. Funnel plots for dormancy potentials and heterotrophic strategies of bacterial communities in rhizosphere and bulk soils. **a**, Funnel plot of toxin-antitoxin systems genes; **b**, Funnel plot of sporulation; **c**, Funnel plot of weighted mean ribosomal operon copy numbers. The top of each panel contains the results of publication bias tests using Egger's regression. A two-sided p value indicating statistical significance of the funnel plot asymmetry test. Asymmetry test showed a symmetric distribution ($p>0.05$) in toxin-antitoxin (**a**) and sporulation (**b**) while an asymmetric distribution ($p<0.05$) in weighted mean ribosomal operon copy numbers (**c**). lnRR: natural log-transformed response ratio.

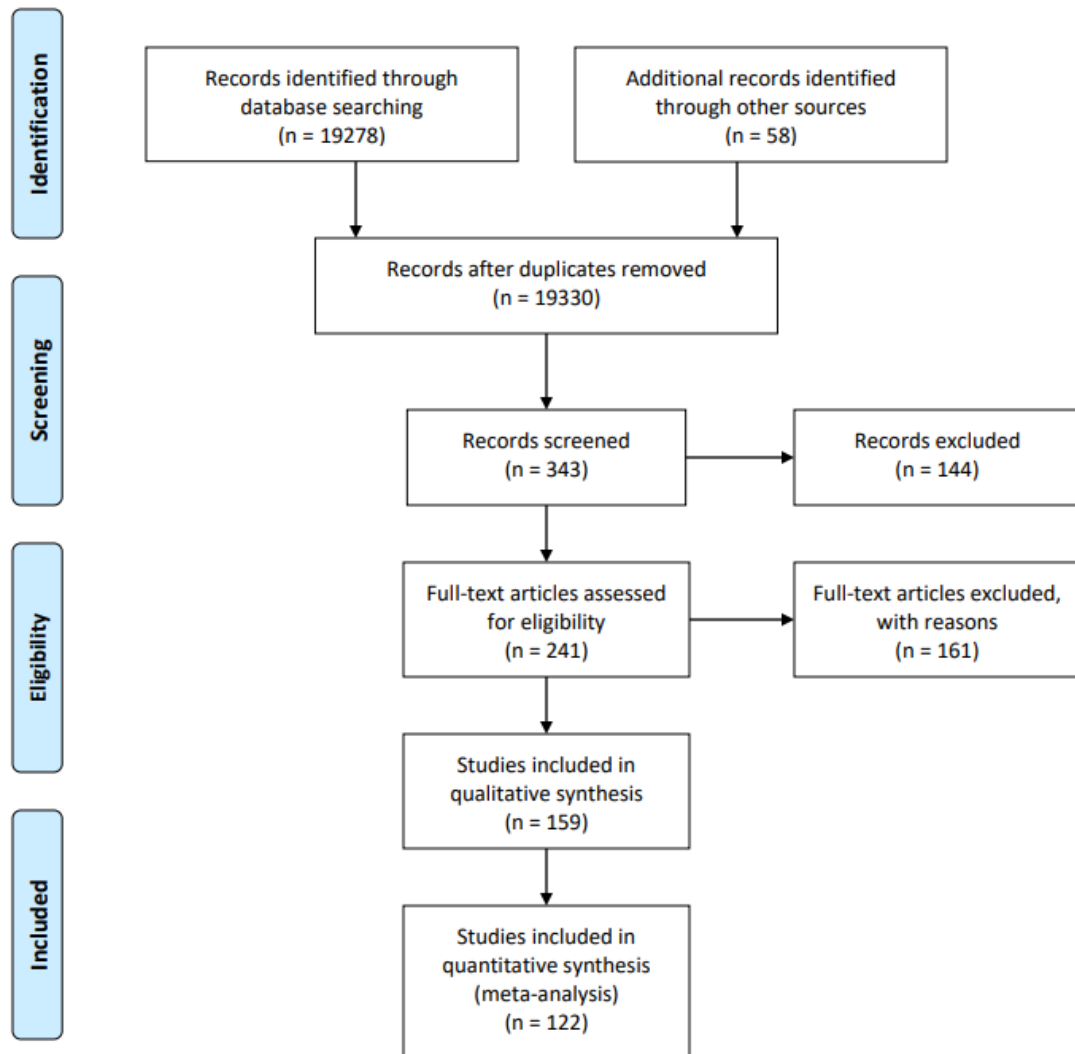


Supplementary Figure 10. Funnel plots for the functional potentials of bacterial communities in rhizosphere and bulk soils. a to l: nitrification, denitrification, methanol oxidation, nitrogen fixation, chitinolysis, ligninolysis, respiration of sulfur compounds, xylanolysis, methyлотrophy, cellulolysis, ureolysis and plant pathogen. The top of each panel contains the results of publication bias tests using Egger's regression. A two-sided p value indicating statistical significance of the funnel plot asymmetry test. Asymmetry test showed a symmetric distribution ($p > 0.05$) in methanol oxidation, nitrogen fixation, ligninolysis, xylanolysis, methyлотrophy and plant pathogen and an asymmetric distribution ($p < 0.05$) in nitrification, denitrification, chitinolysis, respiration of sulfur compounds, cellulolysis and ureolysis. lnRR: natural log-transformed response ratio.

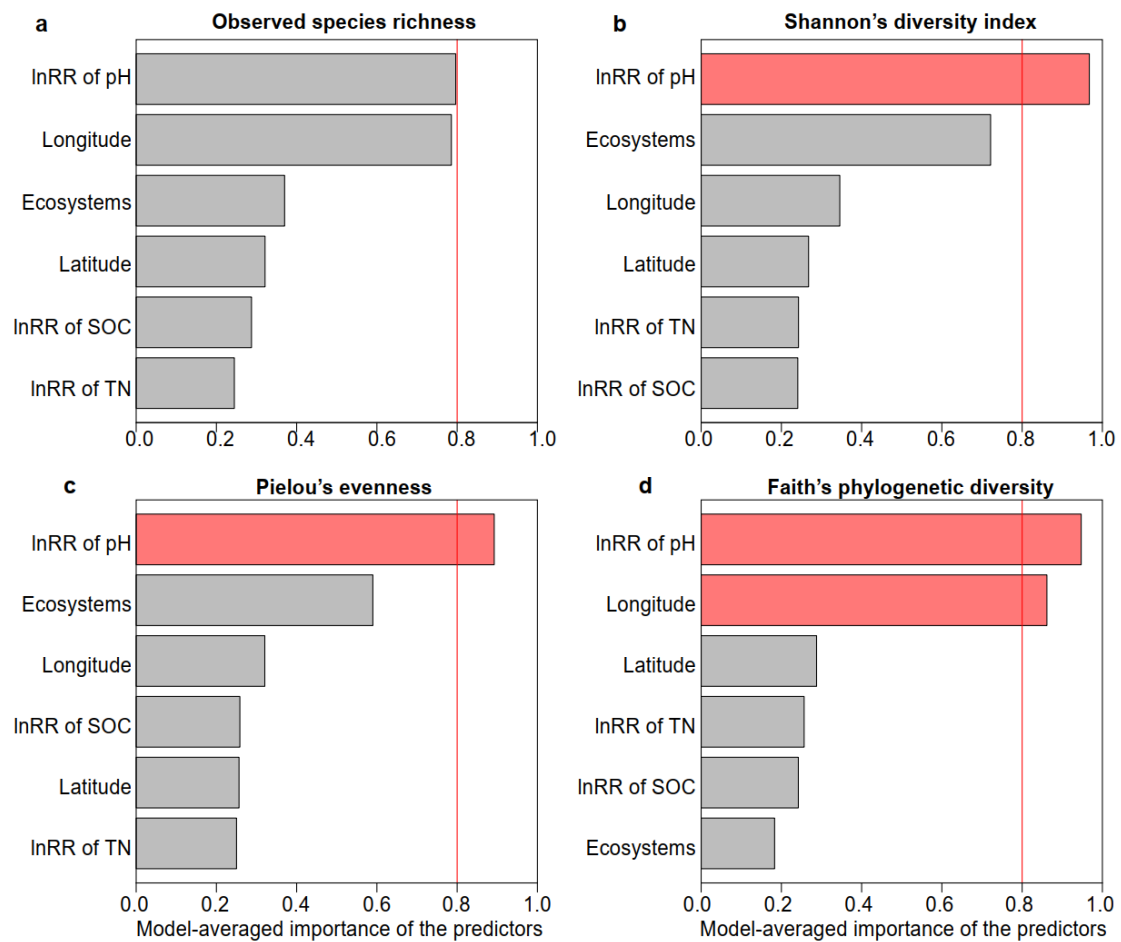


Supplementary Figure 11. Relationship between bacterial community similarity and geographical distance as well as the contributions of factors to bacterial community variation.

a, Relationship between geographic distance and bacterial community similarity (based on Bray–Curtis distances) in bulk soils and rhizosphere. The solid yellow line and solid blue line represent the fitted linear regression model in bulk soil and rhizosphere, respectively. Asterisks (***) indicate the significance at $p < 0.001$ for the regression. **b**, Variance partition analysis shows the relative contributions of geographical distance, soil properties and compartments (bulk soil vs. rhizosphere) to the community variations. The explanations were statistically significant at $p < 0.05$ presented in the Venn diagram.



Supplementary Figure 12. PRISMA flow diagram for the studies selected and included in the systematic review.



Supplementary Figure 13. Model-averaged importance of the predictors for natural log-transformed response ratios of bacterial diversity between bulk soils and rhizosphere. **a**, observed species richness; **b**, Shannon's diversity index; **c**, Pielou's evenness; and **d**, Faith's phylogenetic diversity. The importance value is estimated from the sum of Akaike weights derived from the model selection using corrected Akaike's information criteria. A cutoff of 0.8 (the red line) is set to determine the most important predictors. InRR of pH: natural log-transformed response ratio of pH; InRR of SOC: natural log-transformed response ratio of SOC; InRR of TN: natural log-transformed response ratios of TN. The model selection analysis confirmed that natural log-transformed response ratios of bacterial Shannon's diversity index, Pielou's evenness and Faith's phylogenetic diversity were best predicted by changes in soil pH.

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