

# Supporting Information

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**Table S1.** Interactive effects of afforestation and depth in top and deep soil layers. The chi-square and P-values were obtained for the interactions of afforestation and depth based on a generalized linear mixed effects model by using lme4 package. Target variables included soil physicochemical properties, alpha diversity, soil functions, dominant phyla, and families.

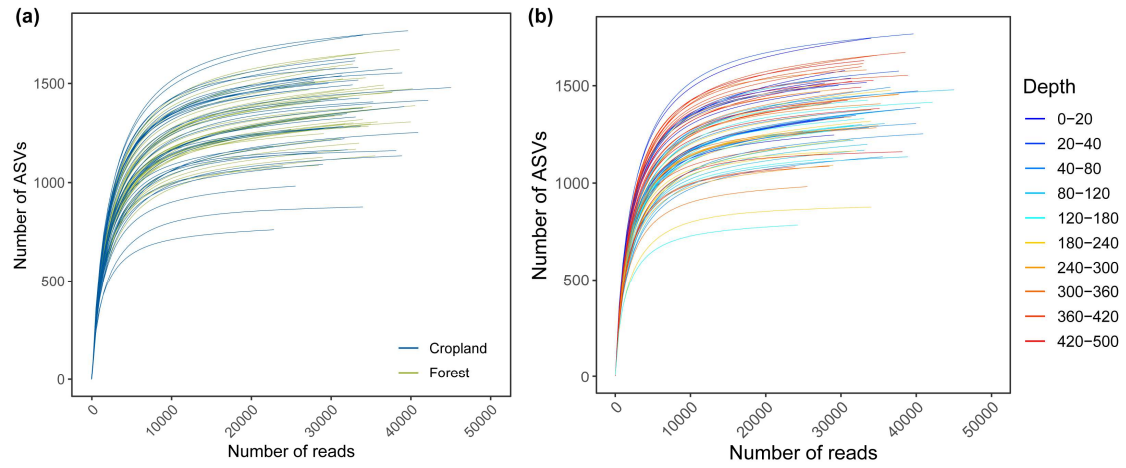
variable	Top soils (0-120 cm)		Deep soils (120-500 cm)	
	<i>Chisq</i>	<i>P.value</i>	<i>Chisq</i>	<i>P.value</i>
SOC	26.5836	<b>&lt;0.0001</b>	6.7963	0.2362
TN	28.6466	<b>&lt;0.0001</b>	3.4446	0.6318
C/N	6.6517	0.0839	2.1346	0.8302
OP	2.2584	0.5205	1.7675	0.8803
NH <sub>4</sub> <sup>+</sup>	7.784	0.0507	4.7339	0.4492
NO <sub>3</sub> <sup>-</sup>	14.3954	<b>0.0024</b>	14.1796	<b>0.0145</b>
pH	0.9899	0.8037	2.0167	0.8468
Moisture	24.168	<b>&lt;0.0001</b>	28.6598	<b>&lt;0.0001</b>
Observed	3.0712	0.3808	1.6873	0.8905
Shannon	3.0496	0.3840	0.7005	0.9829
C <sub>min</sub>	8.2608	0.0409	10.7395	0.0568
N <sub>min</sub>	2.4786	0.4792	13.407	<b>0.0198</b>
Vector length	6.5455	0.0879	31.4628	<b>&lt;0.0001</b>
Vector angle	4.9277	0.1772	3.2801	0.6569
Multifunctionality	4.0492	0.2562	7.2639	0.2017
p_GAL15	9.5803	<b>0.0225</b>	5.6336	0.3435
p_Nitrospirota	3.8679	0.2761	4.5276	0.4762
p_Gemmatimonadota	4.271	0.2337	6.1278	0.2940
p_Verrucomicrobiota	6.0426	0.1096	3.3727	0.6427
p_Proteobacteria	0.3404	0.9523	4.7085	0.4525
p_Bacteroidota	1.9389	0.5852	3.7514	0.5857
p_Firmicutes	2.3522	0.5026	12.377	<b>0.0300</b>
p_Acidobacteriota	5.0429	0.1687	10.5415	0.0613
p_Actinobacteriota	16.1223	<b>0.0011</b>	6.4447	0.2653
p_Chloroflexi	2.4735	0.4801	1.118	0.9525
f_Gaiellaceae	4.2466	0.2360	2.1248	0.8316
f_GAL15	9.5803	<b>0.0225</b>	5.6336	0.3435
f_S085	6.4744	0.0907	4.7038	0.4531
f_JG30.KF.CM45	13.9775	<b>0.0029</b>	1.5532	0.9069
f_KD4.96	1.989	0.5747	2.0442	0.8430
f_Gitt.GS.136	2.5591	0.4647	9.9547	0.0765
f_P2.11E	9.605	0.0222	2.4822	0.7792
f_Pedosphaeraceae	7.8602	<b>0.0490</b>	3.6682	0.5981
f_Chthoniobacteraceae	5.6676	0.1290	3.2788	0.6571
f_Nitrospiraceae	3.8679	0.2761	4.5276	0.4762
f_Gemmatimonadaceae	4.2699	0.2338	6.0934	0.2972

f_Vicinamibacteraceae	0.6001	0.8964	5.5451	0.3530
f_Paenibacillaceae	2.4098	0.4918	31.8597	< <b>0.0001</b>
f_Bryobacteraceae	4.9313	0.1769	1.8656	0.8674
f_Solibacteraceae	18.2596	<b>0.0004</b>	8.5728	0.1274
f_Polyangiaceae	2.8483	0.4156	2.9266	0.7113
f_BIrii41	4.6985	0.1953	2.0648	0.8401
f_Haliangiaceae	1.1558	0.7636	6.7615	0.2390
f_Methyloligellaceae	7.4773	0.0581	1.6091	0.9001
f_Beijerinckiaceae	0.5461	0.9086	6.5017	0.2604
f_Xanthobacteraceae	4.7836	0.1883	7.0563	0.2165
f_Hyphomicrobiaceae	5.0557	0.1678	3.3891	0.6402
f_Rhizobiales_Incertae_Sedis	3.4186	0.3315	2.5139	0.7744
f_Rhizobiaceae	1.9124	0.5908	8.8052	0.1171
f_Hyphomonadaceae	0.5841	0.9001	4.6285	0.4629
f_Sphingomonadaceae	0.491	0.9209	2.038	0.8439
f_Caulobacteraceae	4.2417	0.2365	5.1226	0.4011
f_Rhodobacteraceae	8.5997	<b>0.0351</b>	5.9658	0.3096
f_Dongiaceae	6.0632	0.1086	1.8575	0.8685
f_Reyranellaceae	6.8721	0.0761	3.1533	0.6764
f_Solimonadaceae	5.6451	0.1302	3.3436	0.6472
f_Hymenobacteraceae	2.7456	0.4325	7.4252	0.1909
f_Microscillaceae	2.3306	0.5067	3.8728	0.5679
f_Chitinophagaceae	4.7719	0.1893	2.0611	0.8406
f_Blastocatellaceae	3.9887	0.2627	5.5244	0.3553
f_Pyrinomonadaceae	11.5749	<b>0.0090</b>	4.0932	0.5361
f_0319.7L14	8.8271	<b>0.0317</b>	20.0395	<b>0.0012</b>
f_MB.A2.108	4.282	0.2326	12.2323	<b>0.0317</b>
f_Rubrobacteriaceae	4.6391	0.2002	4.919	0.4258
f_Illumatobacteraceae	3.8126	0.2824	1.5556	0.9066
f_IMCC26256	7.8027	0.0503	2.4826	0.7791
f_Streptomycetaceae	9.8772	<b>0.0196</b>	4.0909	0.5364
f_Nocardiodaceae	15.3536	<b>0.0015</b>	2.3437	0.7998
f_Pseudonocardiaceae	4.671	0.1975	10.7637	0.0563
f_Dietziaceae	3.0118	0.3898	2.6229	0.7579
f_Mycobacteriaceae	2.2963	0.5132	15.5643	<b>0.0082</b>
f_Micrococcaceae	7.7261	0.0520	5.5912	0.3480
f_Micromonosporaceae	2.2197	0.5281	5.1293	0.4003
f_Geodermatophilaceae	1.252	0.7406	3.0377	0.6942

**Table S2.** Mantel tests of soil physicochemical properties against bacterial community composition (ASV table), phylogenetic turnover ( $\beta$ -nearest taxon index,  $\beta$ NTI) and Bray-Curtis-based Raup-Crick ( $RC_{\text{bray}}$ ) of bacterial communities in top and deep soil layers.

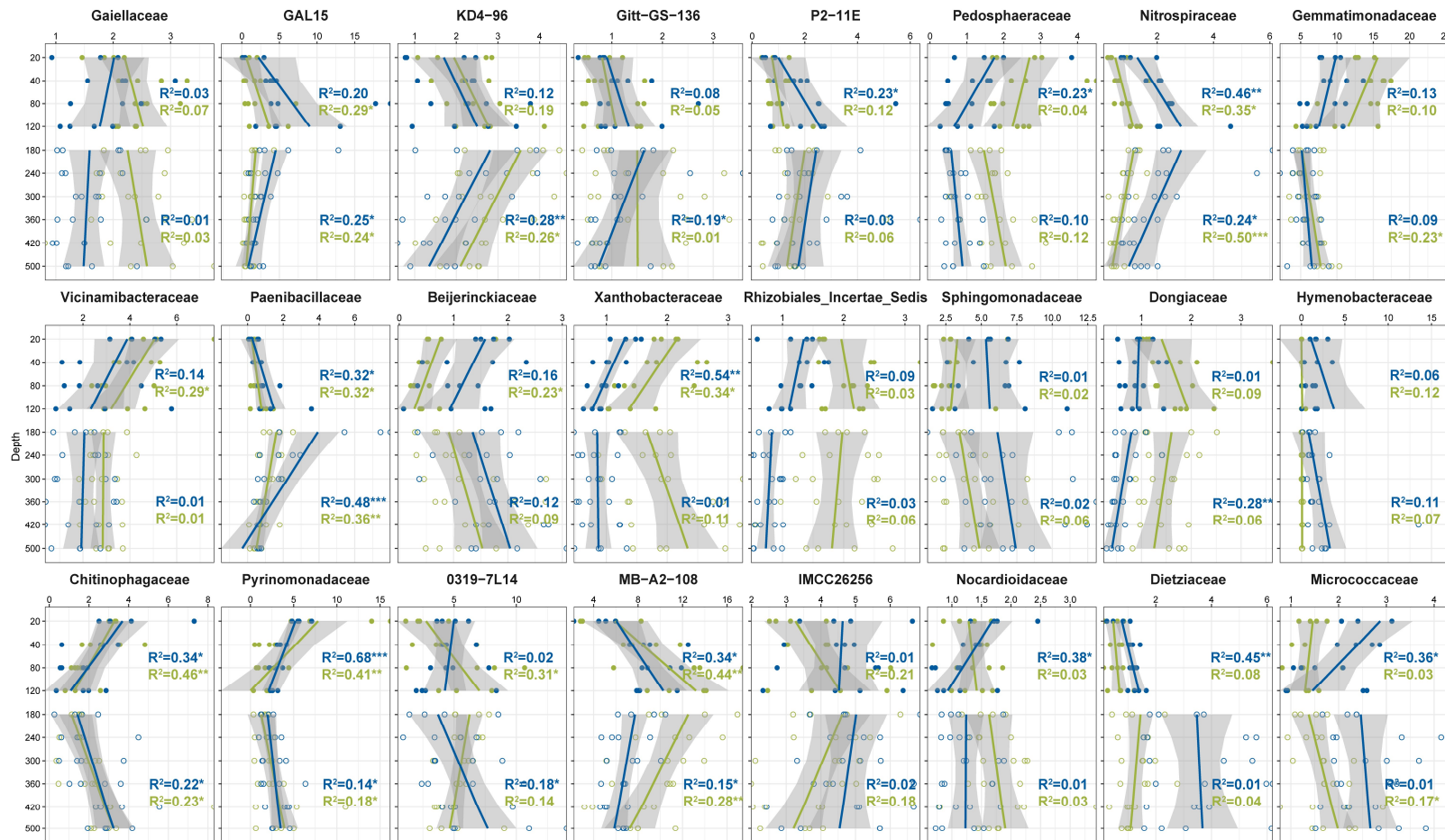
	ASV		$\beta$ NTI		$RC_{\text{bray}}$	
	Top	Deep	Top	Deep	Top	Deep
SOC	<b>0.451***</b>	0.010	0.092	-0.011	<b>0.402***</b>	0.041
TN	<b>0.429***</b>	-0.017	0.057	0.020	<b>0.378**</b>	0.023
OP	0.140	0.048	-0.083	0.071	<b>0.141*</b>	0.088
C/N	<b>0.190*</b>	-0.053	<b>0.263**</b>	-0.010	<b>0.161*</b>	0.003
NH <sub>4</sub> <sup>+</sup>	<b>0.253**</b>	<b>0.191**</b>	0.147	<b>0.158**</b>	<b>0.132*</b>	<b>0.206***</b>
NO <sub>3</sub> <sup>-</sup>	<b>0.433***</b>	<b>0.367**</b>	0.141	<b>0.284***</b>	<b>0.420***</b>	<b>0.329***</b>
pH	<b>0.173**</b>	-0.070	0.082	-0.058	0.090	0.008
Moisture	0.036	<b>0.639***</b>	0.113	<b>0.353***</b>	<b>0.188*</b>	<b>0.632***</b>



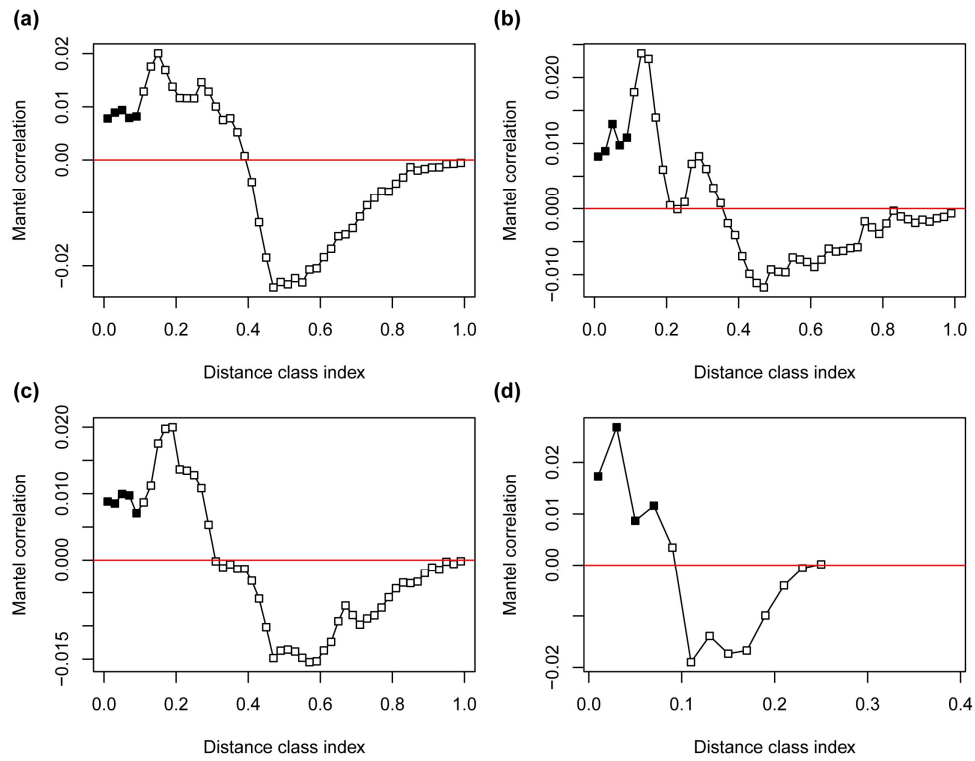


**Figure S1.** Rarefaction curves showing the saturation of ASV saturation. Rarefaction curves are labeled with (a) afforestation and (b) depth.

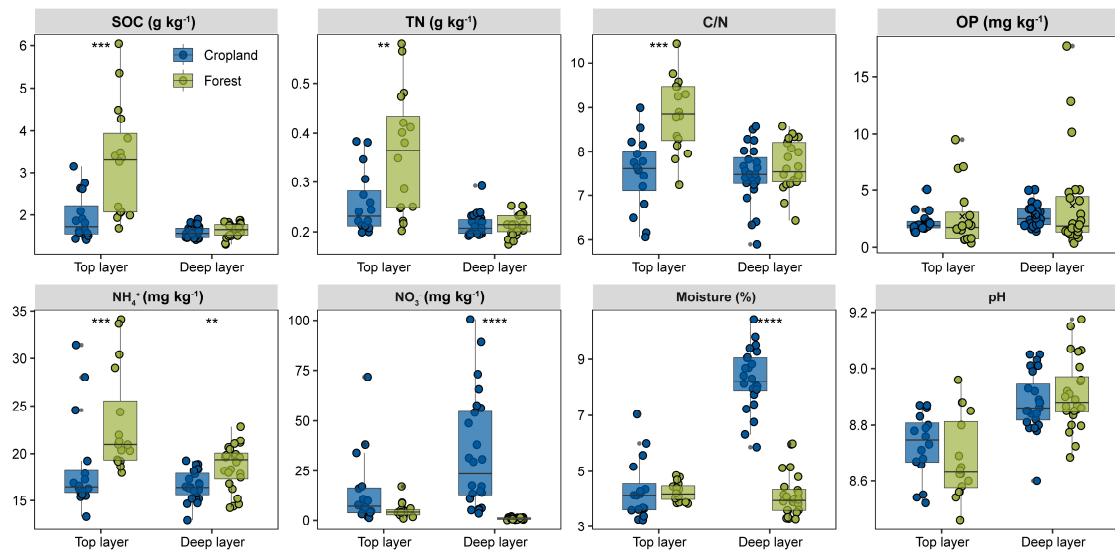




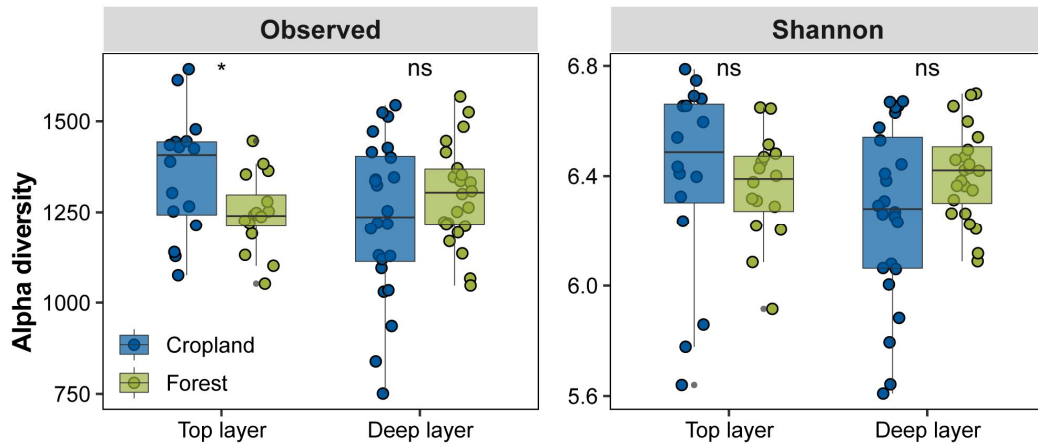
**Figure S3** Variation in dominant families (relative abundance greater than 1%) with depth. The lines denote the least-squares linear regressions across soil depth, with their 95% confidence intervals. The response patterns of most measured parameters to afforestation (cropland [blue] vs. forest [light green]) were markedly different between top (0-120 cm) and deep (120-500 cm) layer. R<sup>2</sup> is explained variance. Asterisks denote \* P < 0.05, \*\* P < 0.01, and \*\*\* P < 0.001.



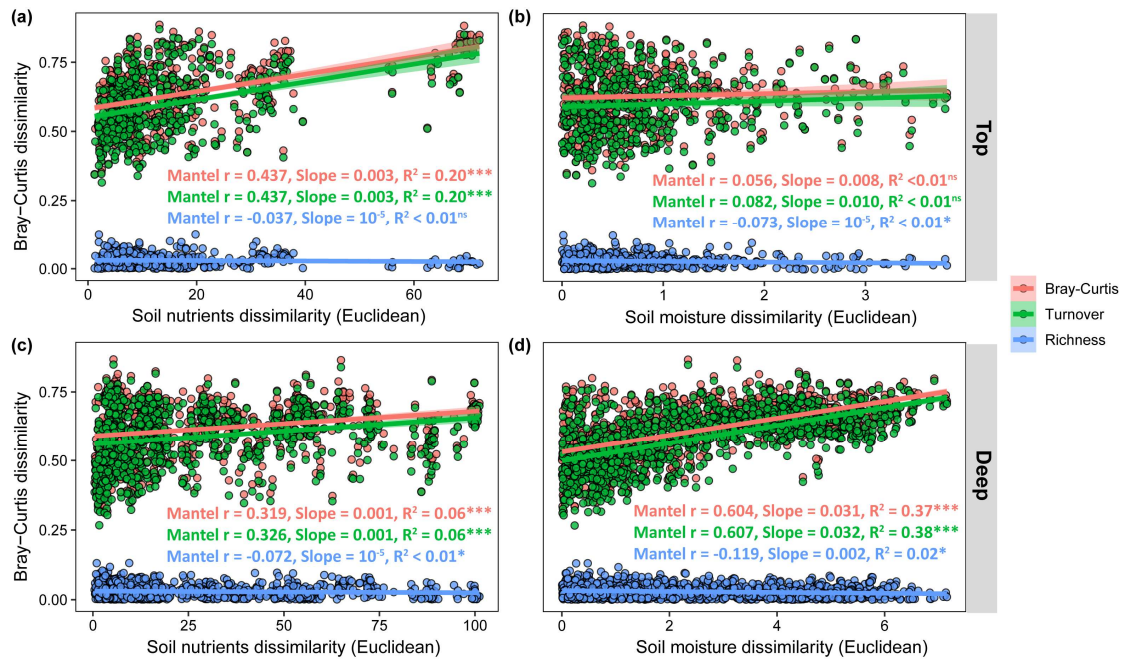
**Figure S4.** Phylogenetic Mantel correlogram for: top layer in cropland **(a)**, deep layer in cropland **(b)**, top layer in forest **(c)**, and deep layer in forest **(d)**. The plots show significant phylogenetic signal across relatively short phylogenetic distances. Solid circles denote significant correlations ( $P < 0.05$ ).



**Figure S5.** Soil physicochemical properties affected by afforestation and soil depth (top vs. deep layers). Significant differences were based on the Wilcoxon test. Asterisks denote \* P < 0.05, \*\* P < 0.01 and \*\*\* P < 0.001. Legend: SOC, soil organic carbon; TN, soil total nitrogen; C/N, ratio of carbon to nitrogen; OP, available phosphorus; NH<sub>4</sub><sup>+</sup>, ammonium; NO<sub>3</sub><sup>-</sup>, nitrate; pH, soil pH; and Moisture, soil moisture.

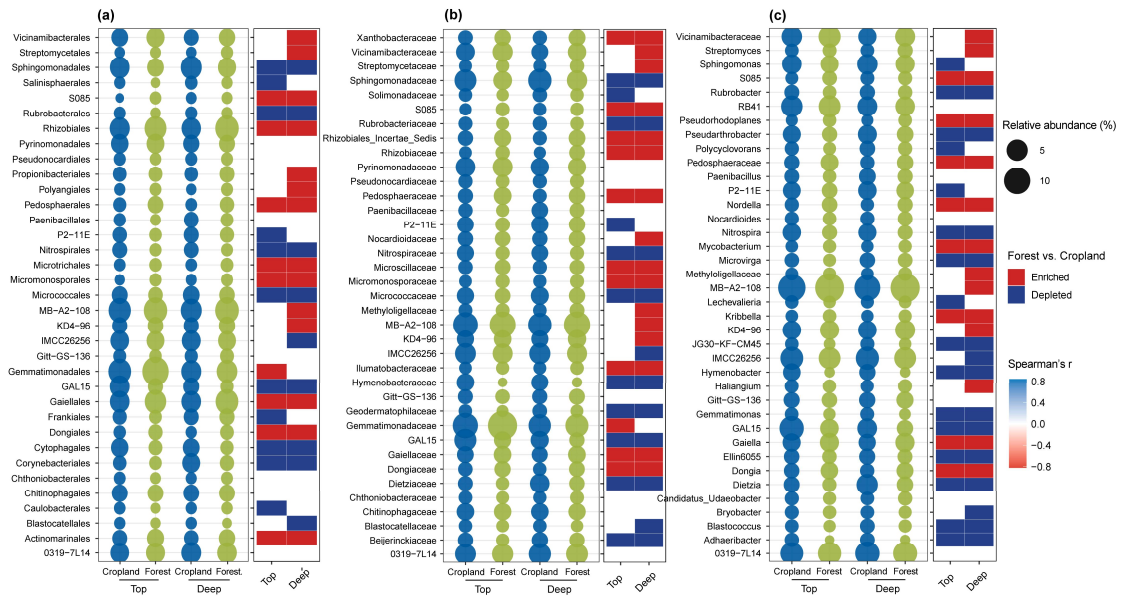


**Figure S6.** Soil bacterial alpha diversity affected by afforestation and soil depth (top vs. deep layer). Significant differences were based on the Wilcoxon test. Asterisks denote \*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$ .



**Figure S7.** Relationship of beta-diversity of microbial communities (Bray-Curtis dissimilarity) to soil nutrients (**a** and **c**) and moisture (**b** and **d**) dissimilarities (Euclidean distance) in top and deep layers after afforestation, respectively. Soil nutrients include SOC, TN, OP,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . The beta-diversity (Bray-Curtis dissimilarity) was partitioned into two components, the abundance gradient (richness) and the balanced variation (turnover), using the bray.part function of the betapart package. Asterisks denote \*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$ .

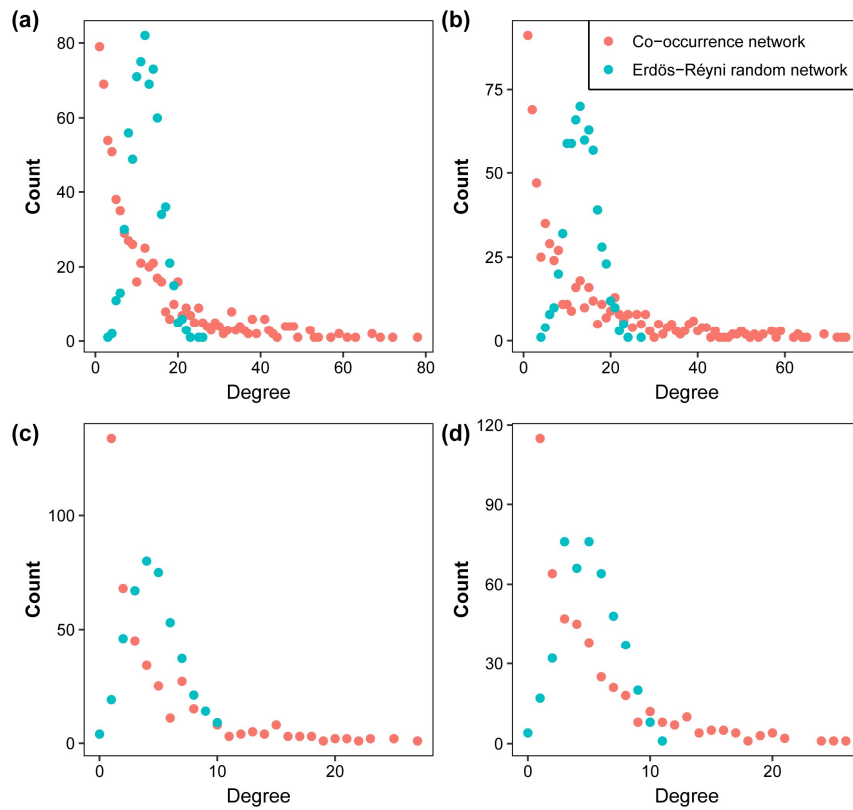




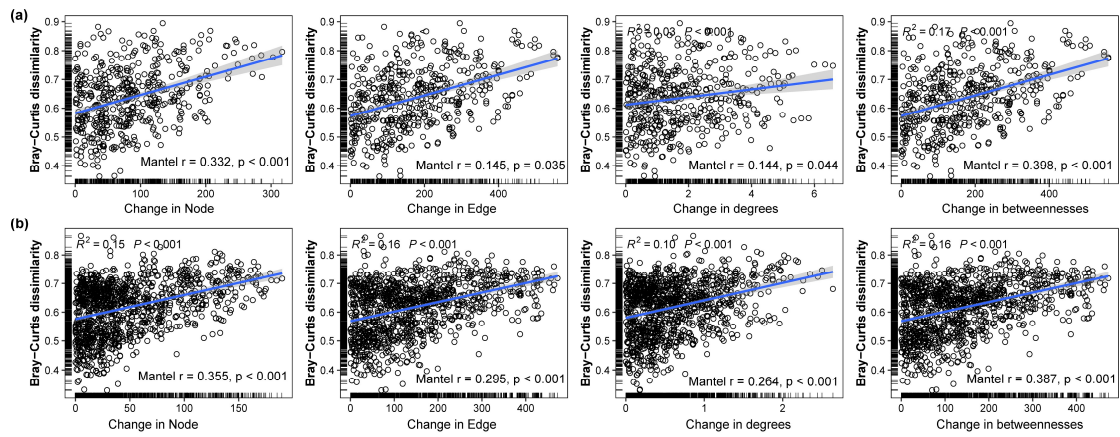
**Figure S8.** Dominant bacterial orders (a), families (b) and genera (c) affected by afforestation and soil depth (top vs. deep layer). Bubble plot showing the relative abundance of the bacterial orders (>0.5%), families (>0.5%) and genera (>0.3%). Heatmaps showing the significant changes in relative abundance of dominant taxa induced by afforestation (cropland was used as control) in top and deep soils based on the Wilcoxon test at  $P < 0.05$  level. Only taxa with significant changes are filled with color. Red or blue colors indicated that the taxa were enriched or depleted in forest, respectively.



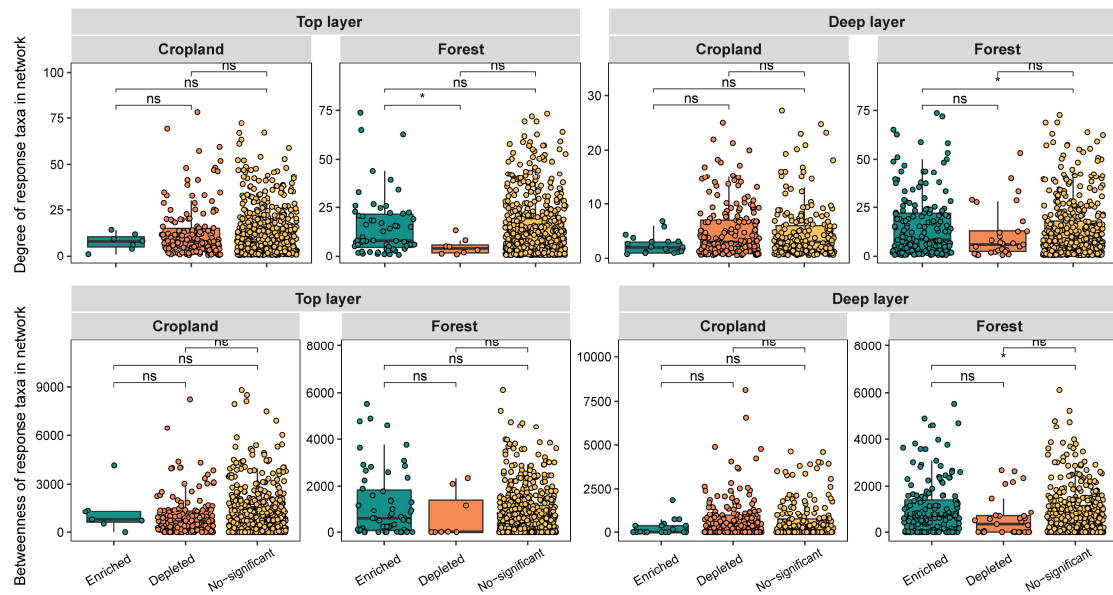




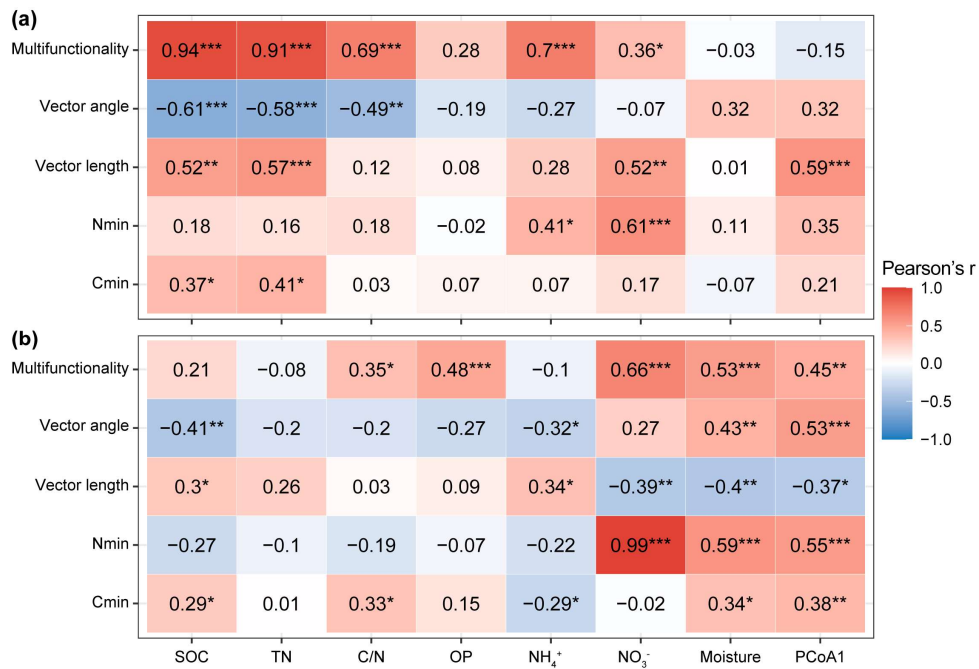
**Figure S10.** Distribution of degrees for the co-occurrence network (Red) and Erdos-Rényi random network (Blue) for soil bacteria communities in top layer in cropland **(a)**, top layer in forest **(b)**, deep layer in cropland **(c)**, and deep layer in forest **(d)**.



**Figure S11.** Relationships between potential biotic interactions (co-occurrence network properties) and beta diversity of bacterial community composition (Bray-Curtis dissimilarity) in top **(a)** and deep layers **(b)**, respectively. Network properties include node, edge, degree and betweenness. Dissimilarity distance matrix of network properties were calculated based on Euclidean distances. Linear relationships were evaluated by the Mantel test.



**Figure S12.** Node connectedness (degree) and centrality (betweenness) of bacterial networks. Nodes in each network were divided into three categories based on *DESeq2* differential abundance analysis, i.e., enriched, depleted and non-significant after afforestation. Although the number of ASVs responsive to afforestation was smaller than non-responsive ASVs in all networks, the enriched ASVs after afforestation in network had higher connectedness and centrality. Asterisk denote significant differences based on Wilcoxon tests. Asterisks denote \*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$ .



**Figure S13.** Pearson's correlations of the soil functions (soil organic carbon and nitrogen mineralization, vector length, vector angle and multifunctionality) to soil physicochemical properties and bacterial community composition in top **(a)** and deep layers **(b)**, respectively. Bacterial community diversity was quantified by using the first axis of PCoA (PCoA1) based on Bray-Curtis distance. Vector length represents relative C vs. nutrient limitation, and the vector angle denotes the relative P vs. N limitation. C<sub>min</sub> and N<sub>min</sub> denote the organic carbon and nitrogen mineralization, respectively. Legend: SOC, soil organic carbon; TN, soil total nitrogen; C/N, ratio of carbon to nitrogen; OP, available phosphorus; NH<sub>4</sub><sup>+</sup>, ammonium; NO<sub>3</sub><sup>-</sup>, nitrate; pH, soil pH; and Moisture, soil moisture. Asterisks denote \* P < 0.05, \*\* P < 0.01 and \*\*\* P < 0.001.