Title page

Types of contribution: Research article

Title: Sea-crossing bridge construction interference reduced soil microbial biomass and diversity in mangrove ecosystems

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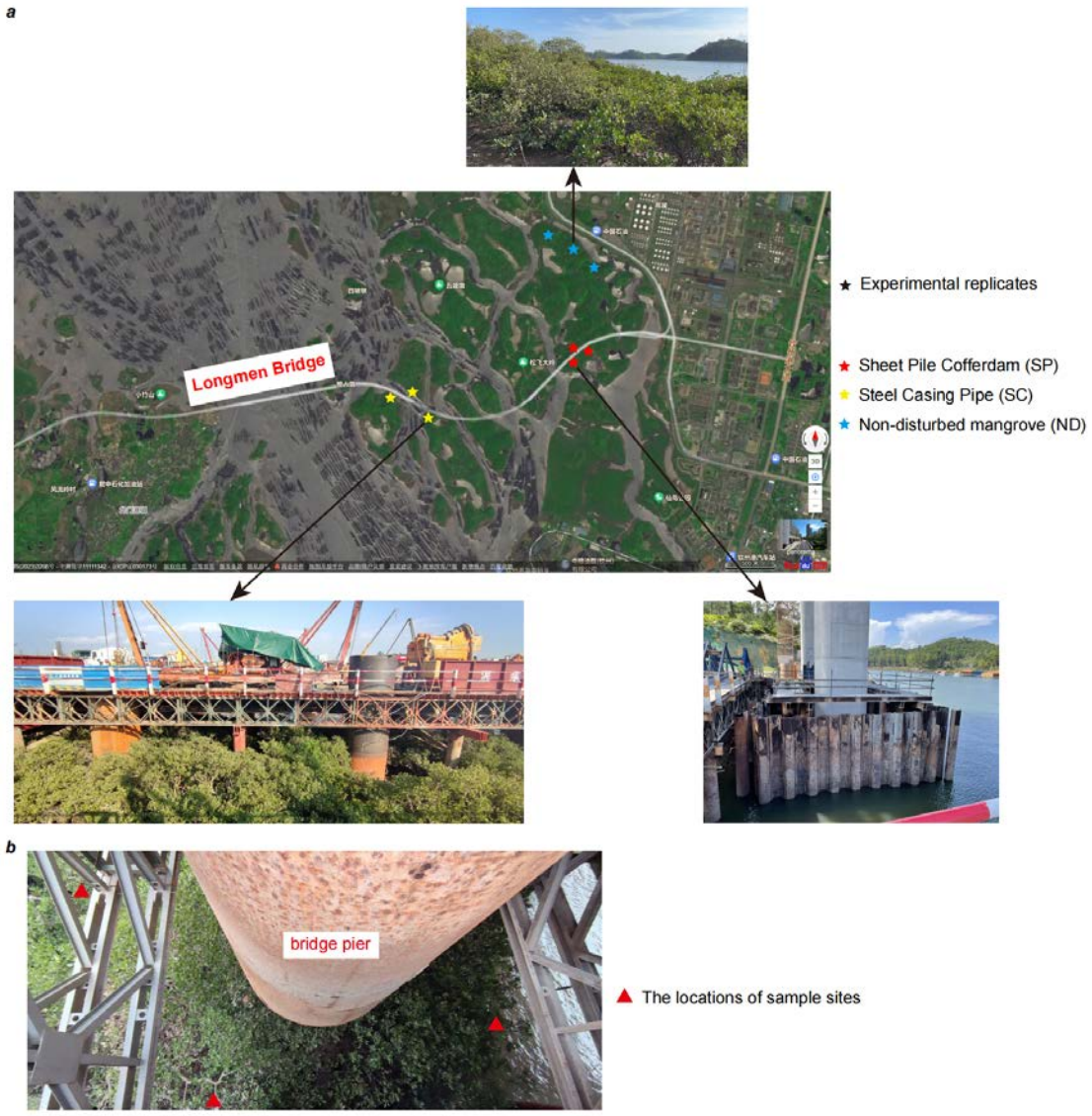

Figure S1. The geographic location of each experimental replicate and the snapshots of the habitats. The map was generated using the Baidu Maps (https://map.baidu.com) (***a***). An example shows the sample sites' geographic location within the habitats (***b***). ND: non-disturbed mangroves; SC: Steel Casing Pipe; SP: Sheet Pile Cofferdam.

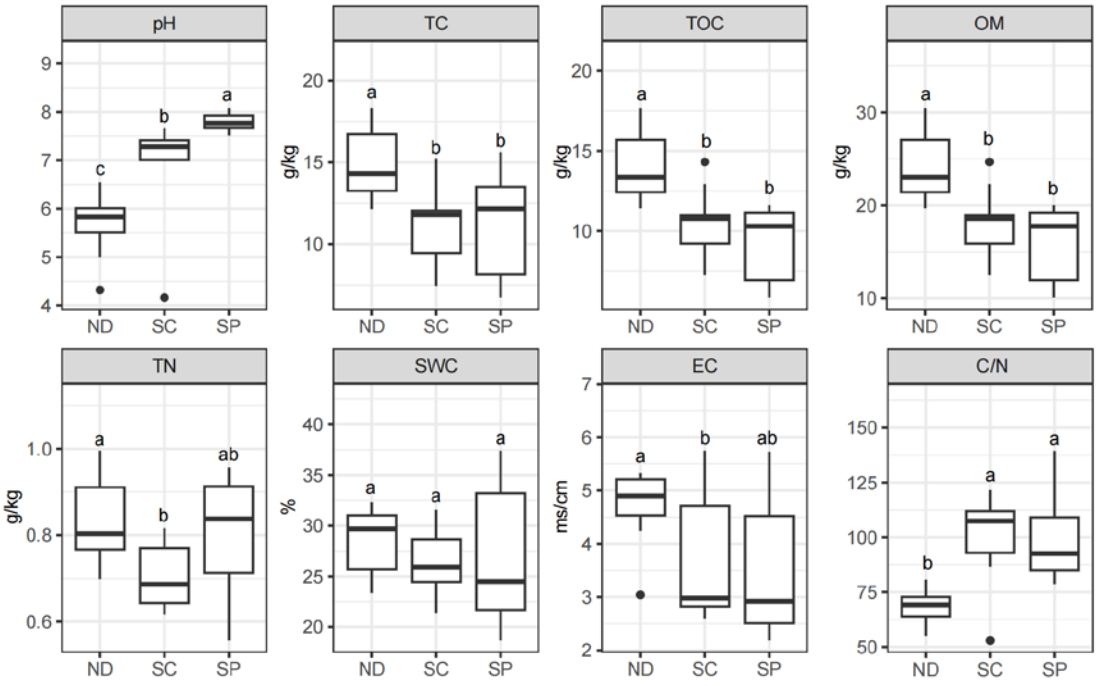

Figure S2. Difference in soil properties. Significant levels were examined by the Wilcoxon tests. Different lowercase letters in the panels represent significant differences. SWC: soil water content; EC: electrical conductivity; TC: total carbon; TN: total nitrogen; TOC: soil total organic carbon; OM: soil organic matter. For abbreviation, please refer to the legend of Figure S1.

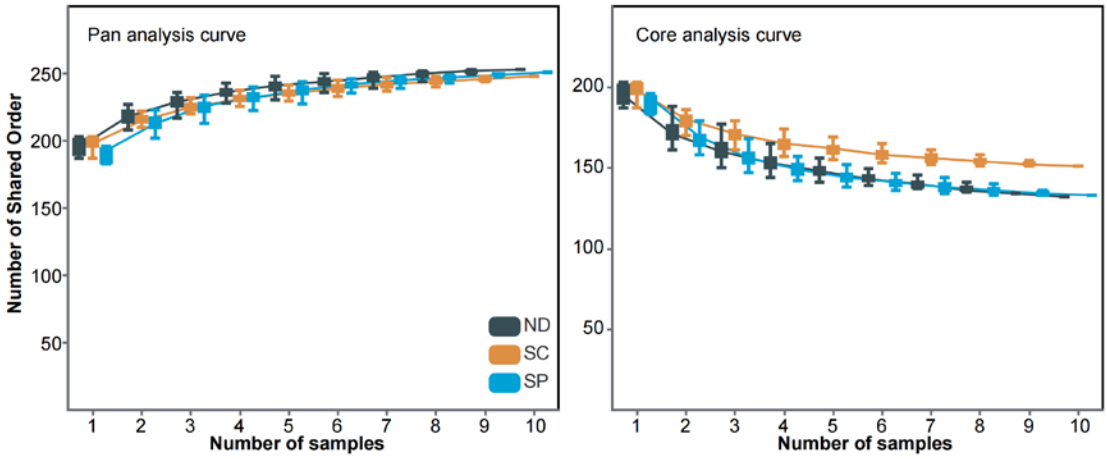

Figure S3. The pan-core analysis curves evaluate whether the sample size is adequate and evaluate the total species richness and the number of core species in the environment. For abbreviation, please refer to the legend of Figure S1.

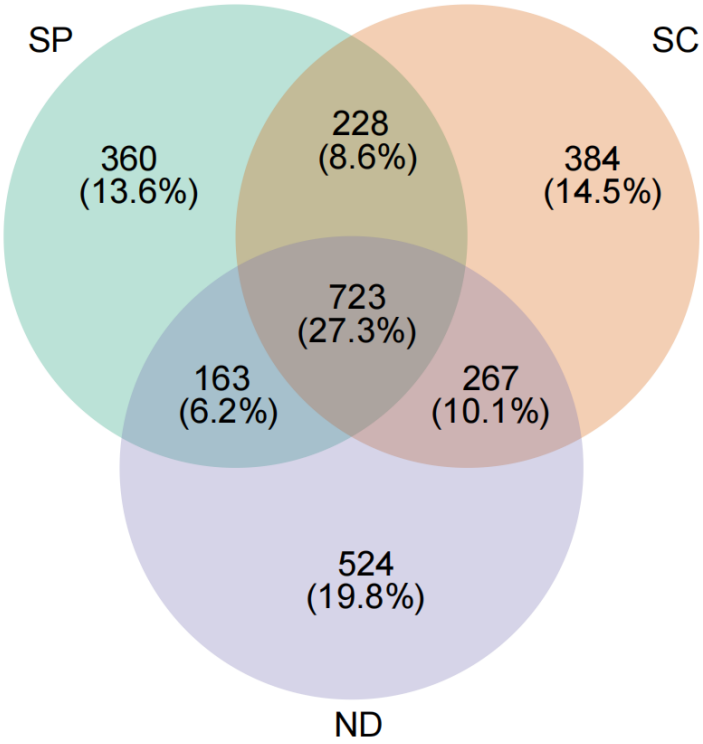

Figure S4. Venn plot shows the shared and unique OTUs between different habitats. For abbreviation, please refer to the legend of Figure S1.

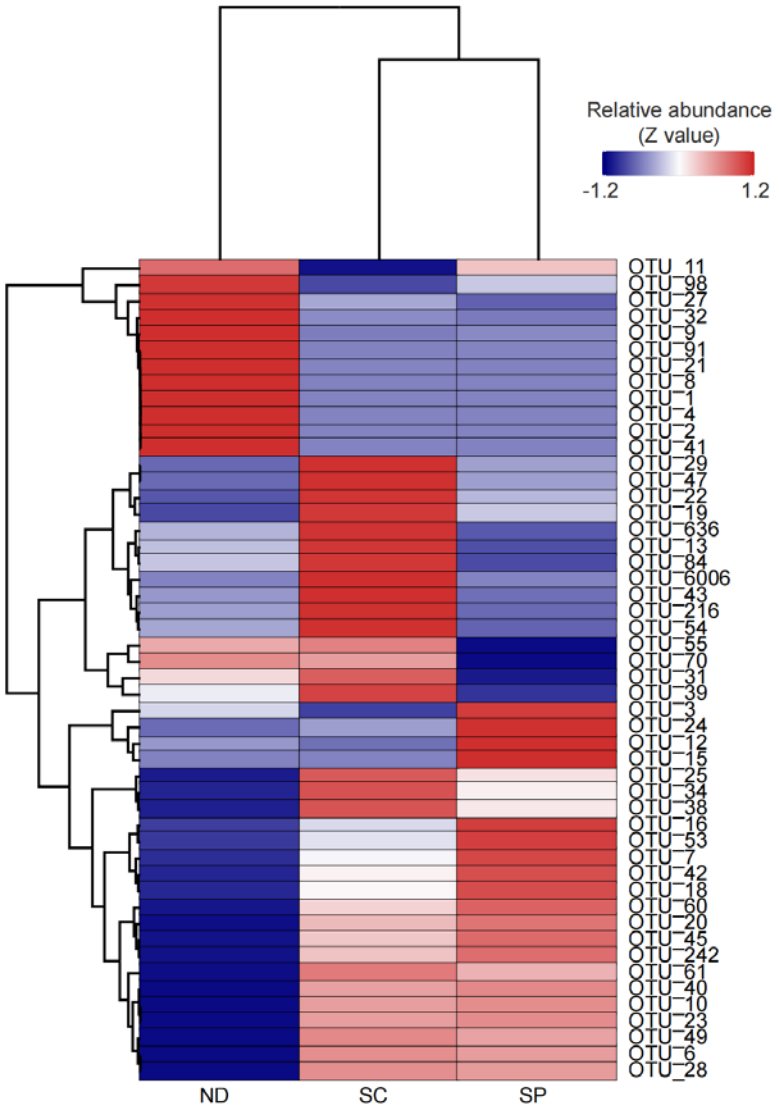

Figure S5. Species abundance cluster analysis represents the community composition and abundance of soil samples at the OTU level. For abbreviation, please refer to the legend of Figure S1.

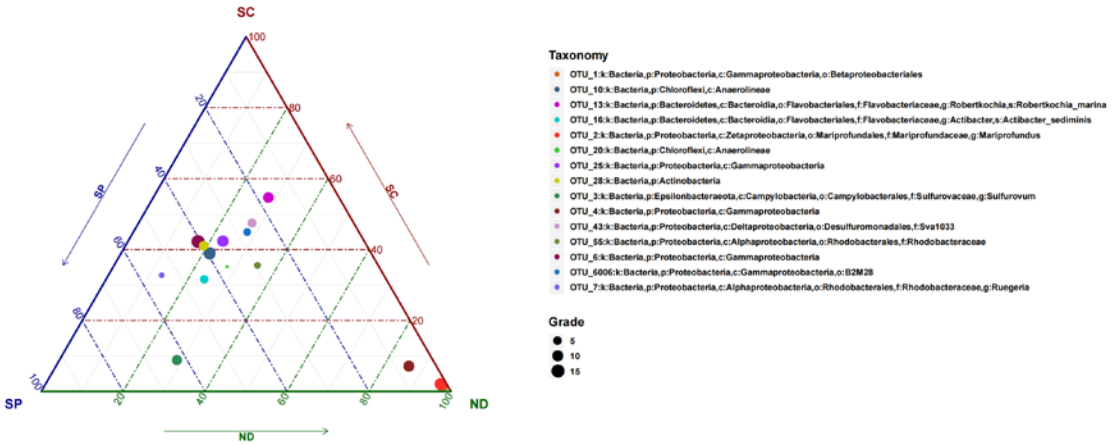

Figure S6. Ternary plot showing the differences in dominant species among different habitats. We only select those OTUs that rank in the top 15 in average abundance at each classification level for display. The circle represents the species, and the size of the circle is proportional to the relative abundance. The closer the circle is to a vertex, the higher the content of this species in this habitat. For abbreviation, please refer to the legend of Figure S1.

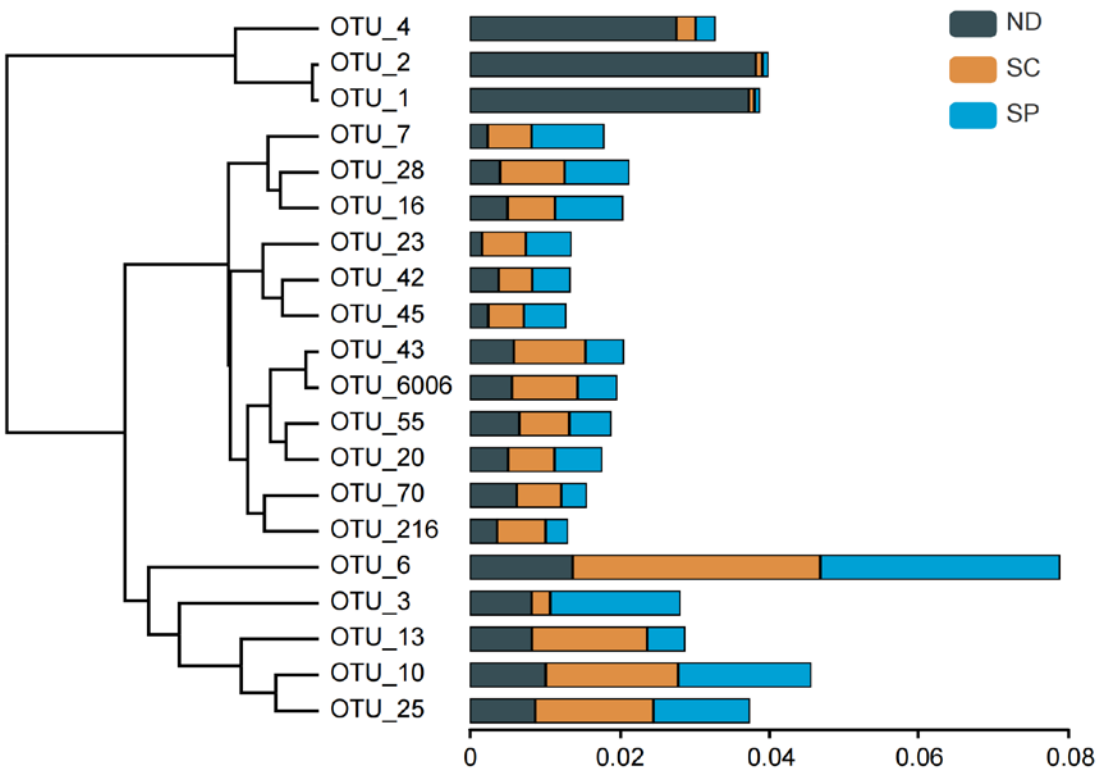

Figure S7. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) showing the average relative abundance of the top 20 OTUs among habitats. For abbreviation, please refer to the legend of Figure S1.

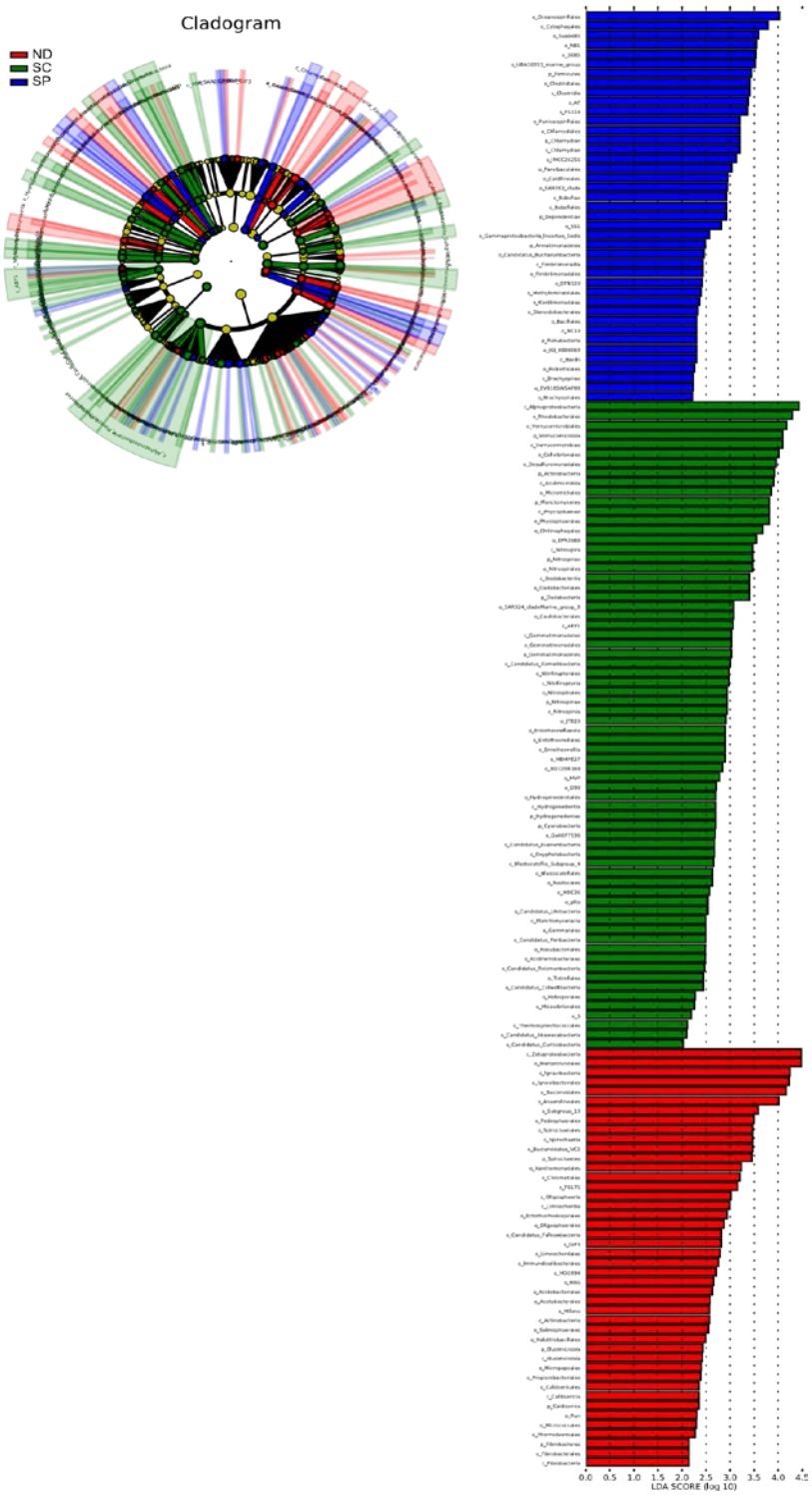

Figure S8. Linear discriminant analysis Effect Size (LEfSe) analysis explores the biomarkers for each habitat. We first used the non-parametric Kruskal-Wallis rank sum to detect the species with significant differences in abundance between different habitats. Then, we used the Wilcoxon rank sum to test the consistency of differences in subgroups between different habitats. Finally, we used linear regression analysis (LDA, the default setting is 2) to estimate the magnitude of the differential effect of each species' abundance. The length of the histogram represents the impact size (LDA Score) of the different species. For abbreviation, please refer to the legend of Figure S1.

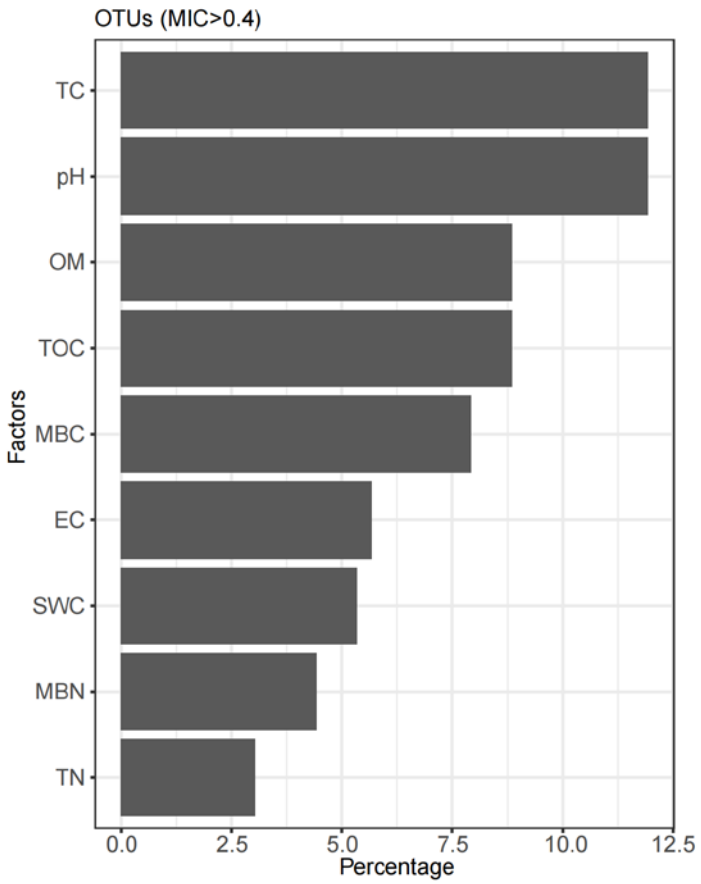

Figure S9. The proportion of OTUs that are significantly (MIC > 0.4) correlated with each environmental factor in the number of all OTUs. Maximum information coefficient (MIC) was used in calculating the correlation between environmental factors and OTUs. SWC: soil water content; EC: electrical conductivity; TC: total carbon; TN: total nitrogen; TOC: soil total organic carbon; SOM: soil organic matter; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen.

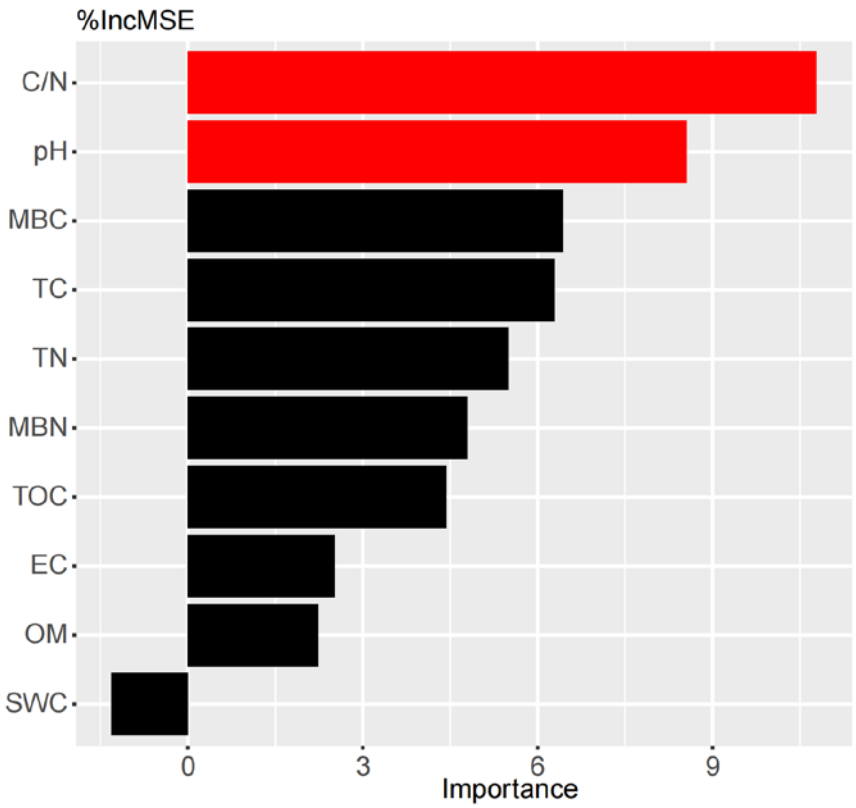

Figure S10. Random Forest analysis reveals the key drivers of microbial richness. Significant and insignificant predictors were marked in red and black, respectively. SWC: soil water content; EC: electrical conductivity; TC: total carbon; TN: total nitrogen; TOC: soil total organic carbon; SOM: soil organic matter; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen.

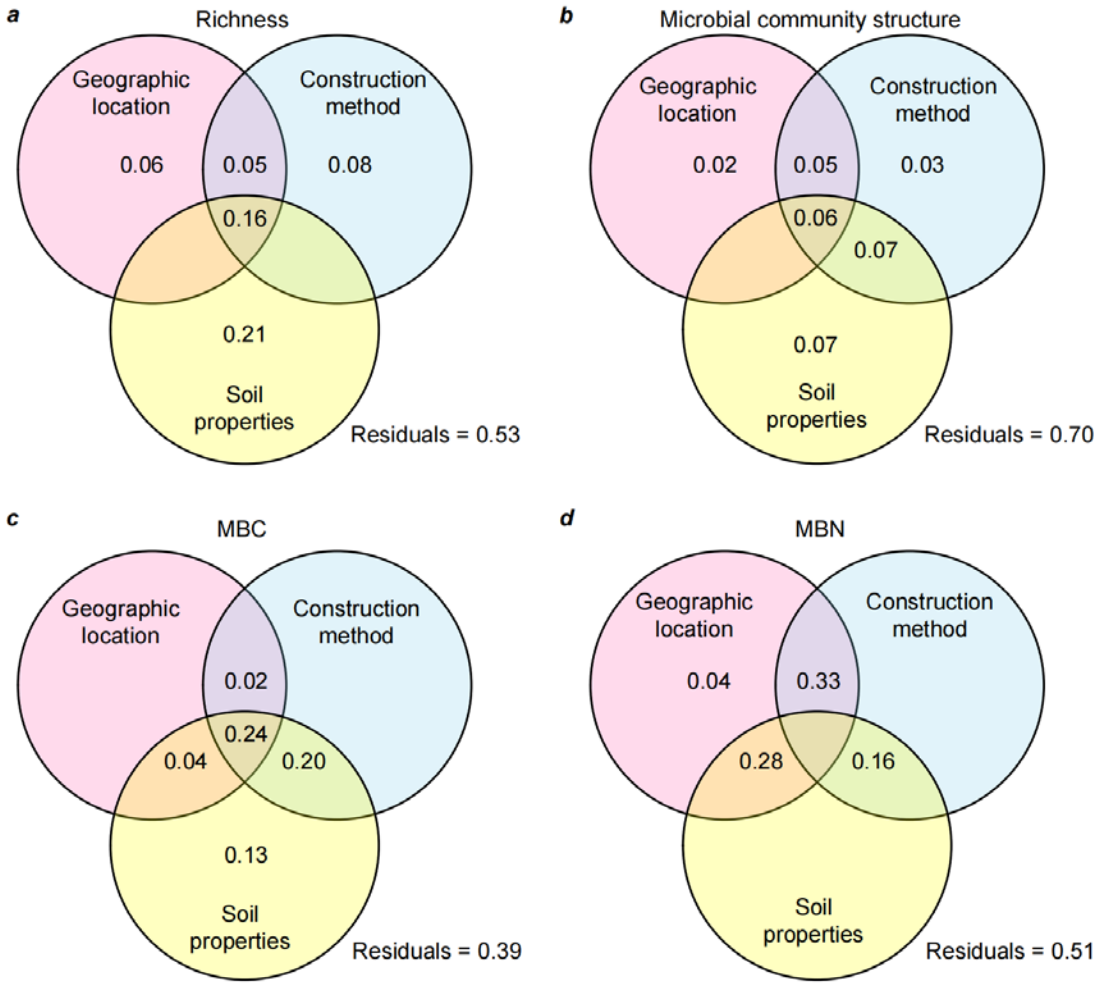

Figure S11. Variance Partitioning Analysis (VPA) revealed the effects of geographic location, construction method, and soil properties in influencing microbial richness (***a***), community structure (***b***), MBC (***c***), and MBN (***d***), respectively. Data < 0 were not shown. MBC: microbial biomass carbon; MBN: microbial biomass nitrogen.

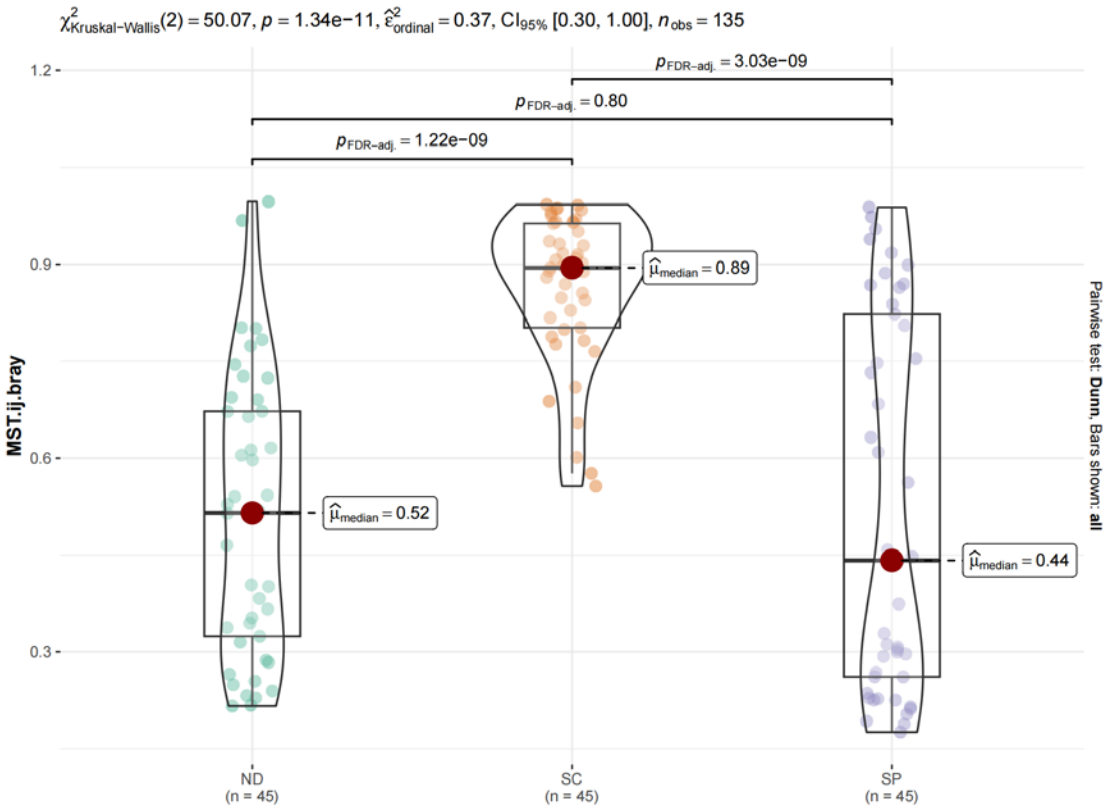

Figure S12. Difference in the distribution of Modified Stochasticity Ratio (MST) values among different habitats. For abbreviation, please refer to the legend of Figure S1.

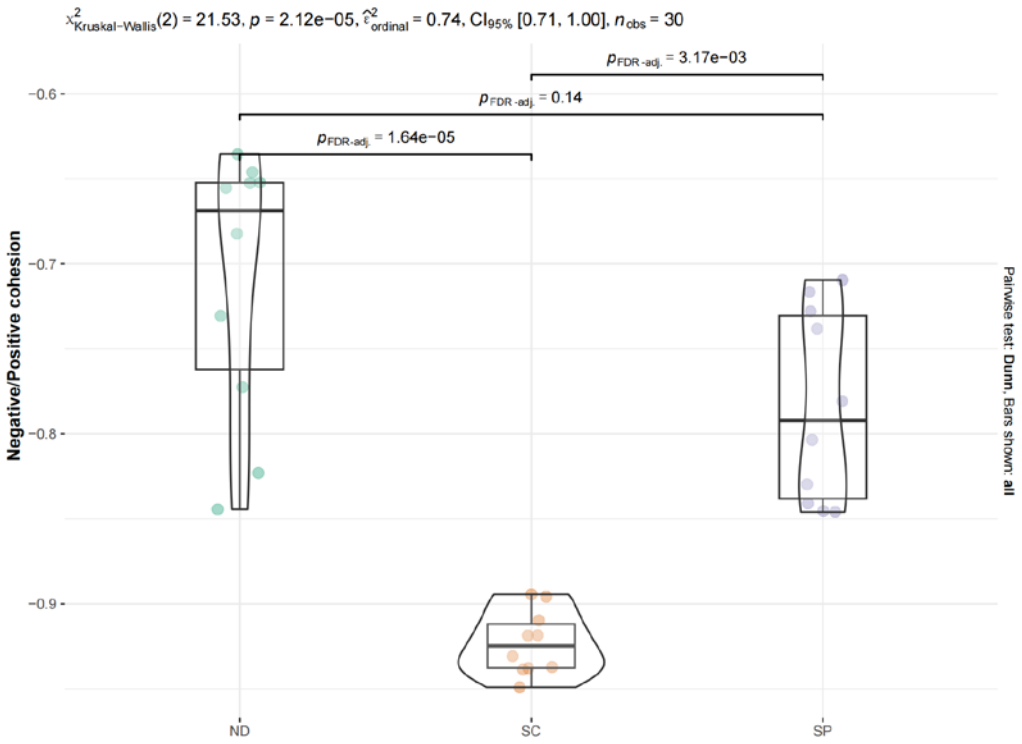

Figure S13. Difference in the distribution of Negative/Positive cohesion among different habitats. For abbreviation, please refer to the legend of Figure S1.

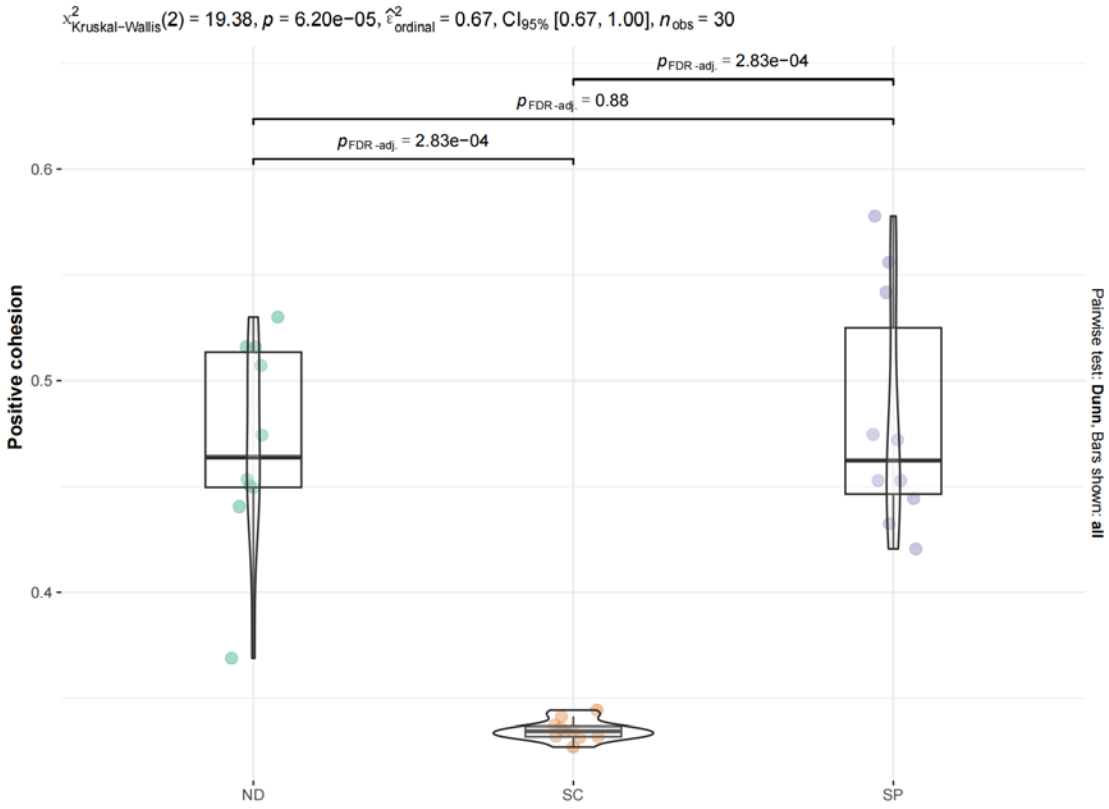

Figure S14. Difference in the distribution of Positive cohesion among different habitats. For abbreviation, please refer to the legend of Figure S1.

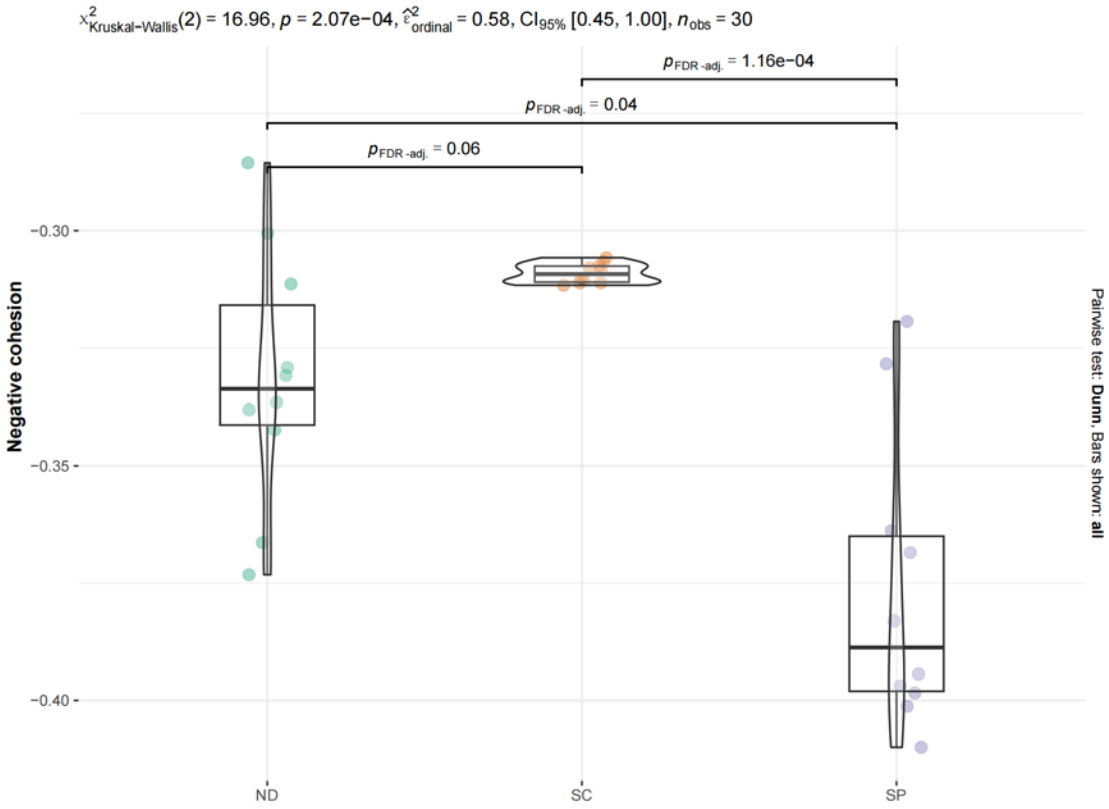

Figure S15. Difference in the distribution of Negative cohesion among different habitats. For abbreviation, please refer to the legend of Figure S1.

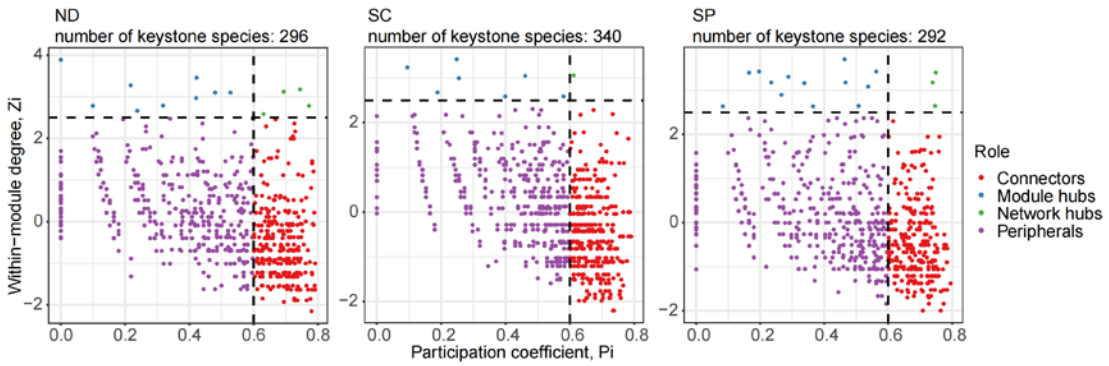

Figure S16. The universal role for each OTU in ND, SC, and SP soils, respectively. For abbreviation, please refer to the legend of Figure S1.

Table S1. Permutational Multivariate Analysis of Variance (PERMANOVA) analysis was used to examine the significant difference in microbial dissimilarities between habitats. For abbreviation, please refer to the legend of Figure S1.

test	sub-condition1 vs sub-condition2	Df	Sums of Sqs	Mean Sqs	F. Model	R^2	P-value
PERMANOVA	ND vs SC	1	0.878	0.878	6.84	0.275	< 0.001
	ND vs SP	1	0.822	0.822	4.26	0.191	< 0.001
	SC vs SP	1	0.488	0.488	3.97	0.181	< 0.001
	all_groups	2	1.46	0.729	4.93	0.267	< 0.001

Table S2. Analysis of Similarities (ANOSIM) analysis was used to examine the significant difference in microbial dissimilarities between habitats. For abbreviation, please refer to the legend of Figure S1.

test	sub-condition1 vs sub-condition2	R-value	P-value
ANOSIM	ND vs SC	0.507	< 0.001
	ND vs SP	0.383	< 0.001
	SC vs SP	0.422	< 0.001
	all_groups	0.386	< 0.001

Table S3. Multi-Response Permutation Procedure (MRPP) analysis was used to examine the significant difference in microbial dissimilarities between habitats. For abbreviation, please refer to the legend of Figure S1.

test	sub-condition1 vs sub-condition2	A	observed-delta	expected-delta	P-value
MRPP	ND vs SC	0.139	0.475	0.552	< 0.001
	ND vs SP	0.0838	0.601	0.656	< 0.001
	SC vs SP	0.0878	0.465	0.51	< 0.001
	all_groups	0.13	0.514	0.591	< 0.001

Table S4. Mantel tests examined the relationships between microbial community and soil properties based on Spearman correlations. SWC: soil water content; EC: electrical conductivity; TC: total carbon; TN: total nitrogen; TOC: soil total organic carbon; SOM: soil organic matter; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen.

Environmental factors	Statistic r	p-value
pH	0.22	0.028
TC	0.22	0.028
TOC	0.21	0.035
OM	0.21	0.034
TN	0.04	0.315
SWC	0.03	0.339
EC	-0.14	0.996
MBC	0.36	0.002
MBN	-0.07	0.760