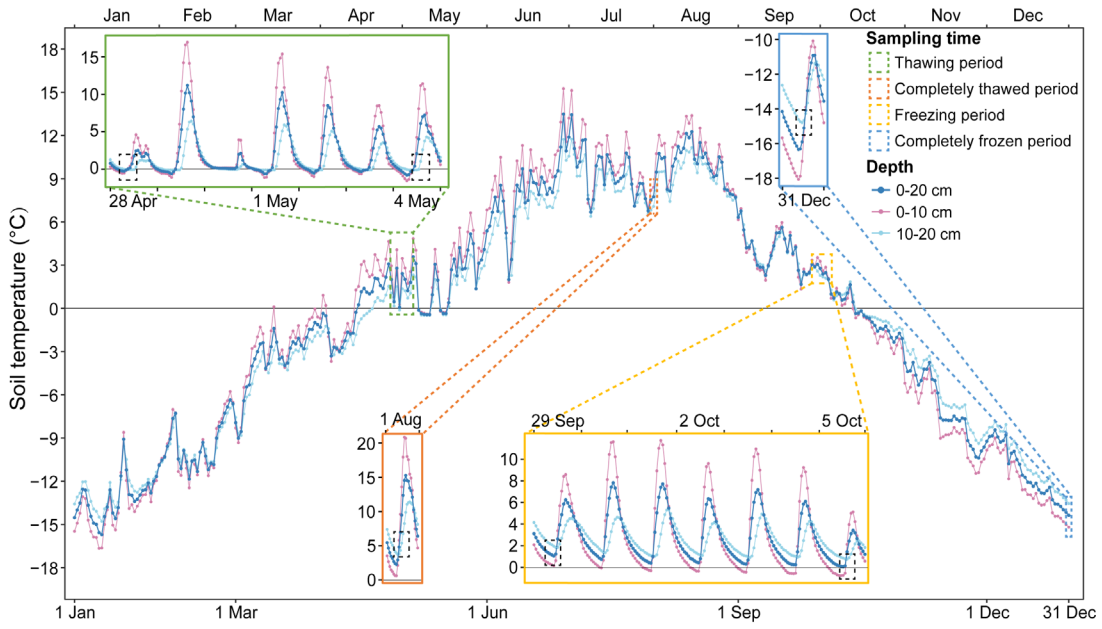
Supplementary Information

Supplementary Notes

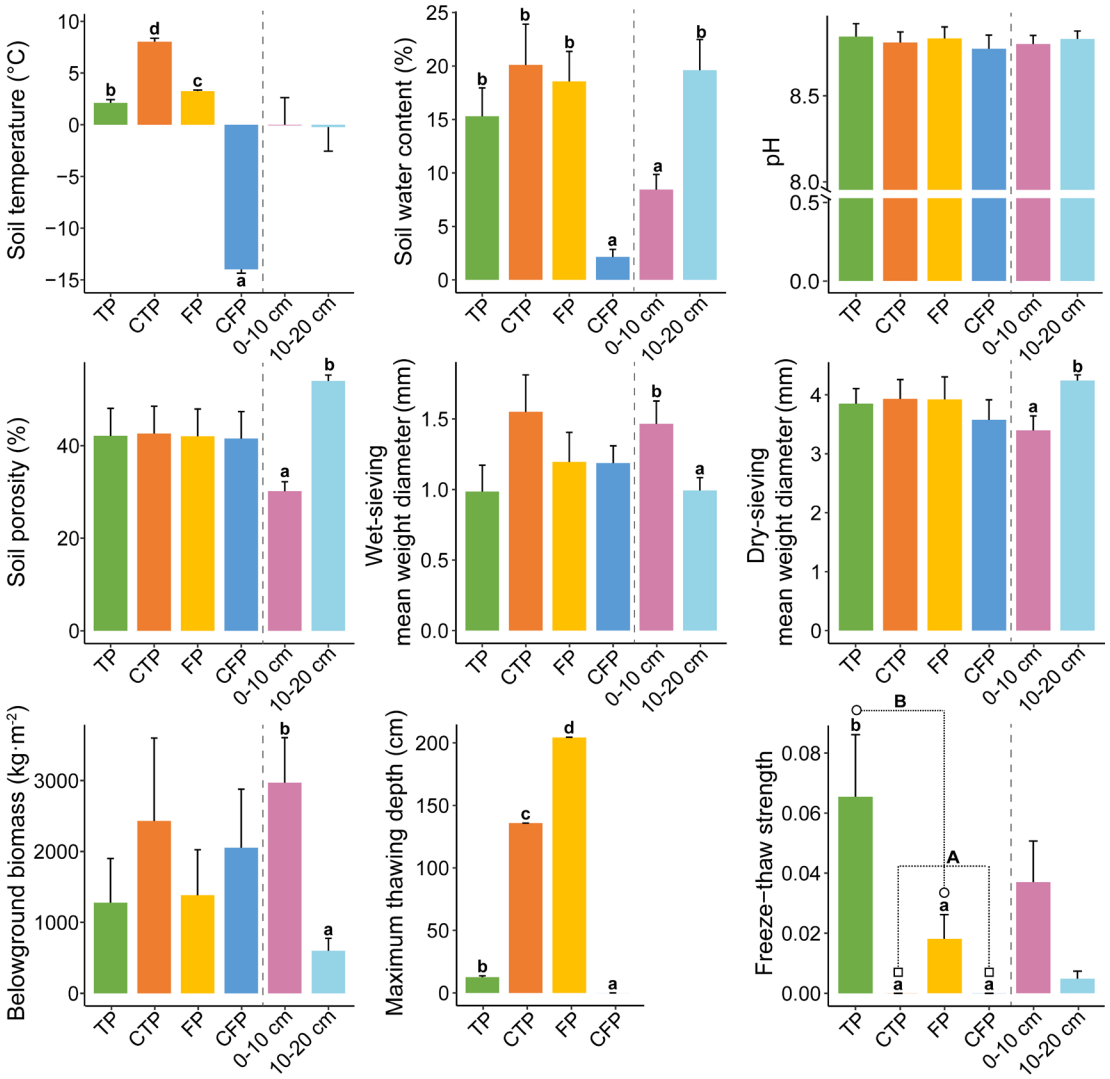
Properties of freeze-thaw processes and environmental variables

Base on the monitoring data of soil temperature, we found that soils of the active layer were completely frozen for 105 days (from 1 January to 31 January and from 19 October to 31 December) and completely thawed for 138 days (from 14 May to 28 September), and undergo diurnal freeze-thaw cycles for 122 days (from 1 February to 13 May in the thawing period and from 29 September to 18 October in the freezing period). We found that soil temperature, soil water content, freeze-thaw strength, and maximum thawing depth were significantly different by periods (Supplementary Fig. S2). Notably, freeze-thaw strength was noticeably greatest in the thawing period, and was obviously greater during the freeze-thaw stage than the non-freeze-thaw stage. In addition, 0-10 cm layer had significantly lower soil water content, soil porosity (Por), and dry-sieving mean weight diameter (DMWD), but higher belowground biomass (BGB) and wet-sieving mean weight diameter (WMWD) compared to 10-20 cm layer (Supplementary Fig. S2).

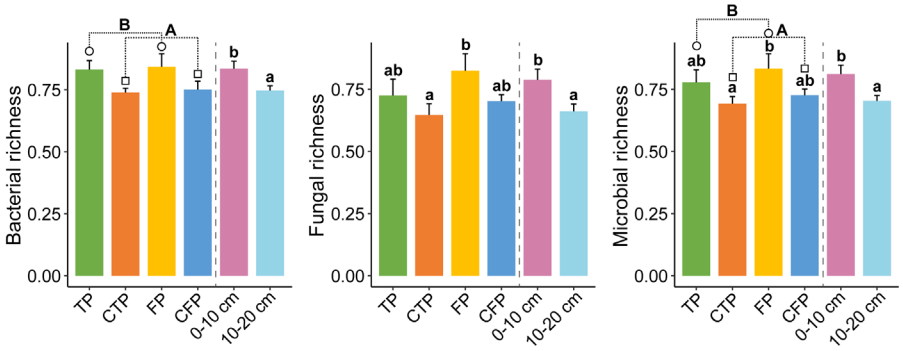
Supplementary Figures



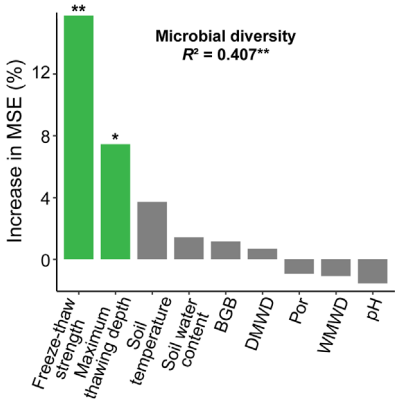
Supplementary Figure 1 Soil temperature at the sampling site across an entire year and the settings of sampling times. Variations of mean daily soil temperature at the depths of 0-20 cm, 0-10 cm, and 10-20 cm in the active layer of permafrost over a year in 2013, and mean hourly soil temperature of sampling times during the periods of diurnal and seasonal freeze-thaw processes.



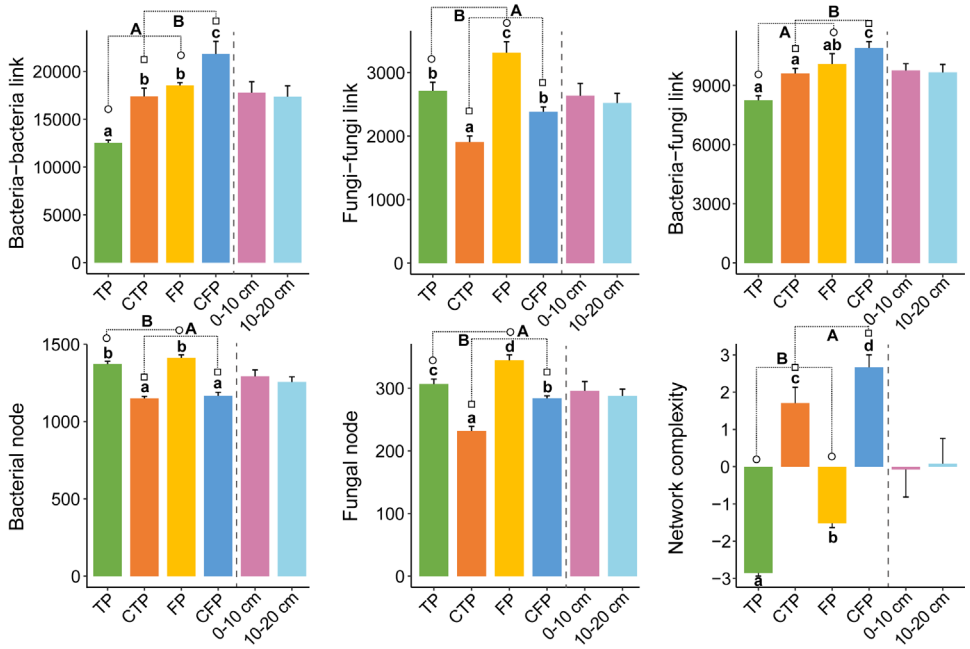
Supplementary Figure 2 Environmental variables during four periods of seasonal freeze-thaw processes and at two depths. Differences in soil temperature, water content, pH, porosity, wet-sieving mean weight diameter, dry-sieving mean weight diameter, belowground biomass, freeze-thaw strength, and maximum thawing depth among four periods and between two depths (mean ± se). TP, CTP, FP, and CFP represent the thawing, completely thawed, freezing, and completely frozen periods, respectively. Lowercase letters represent the significance of differences among four periods or between two depths, and uppercase letters represent the significance of differences between the freeze-thaw and non-freeze-thaw stages.



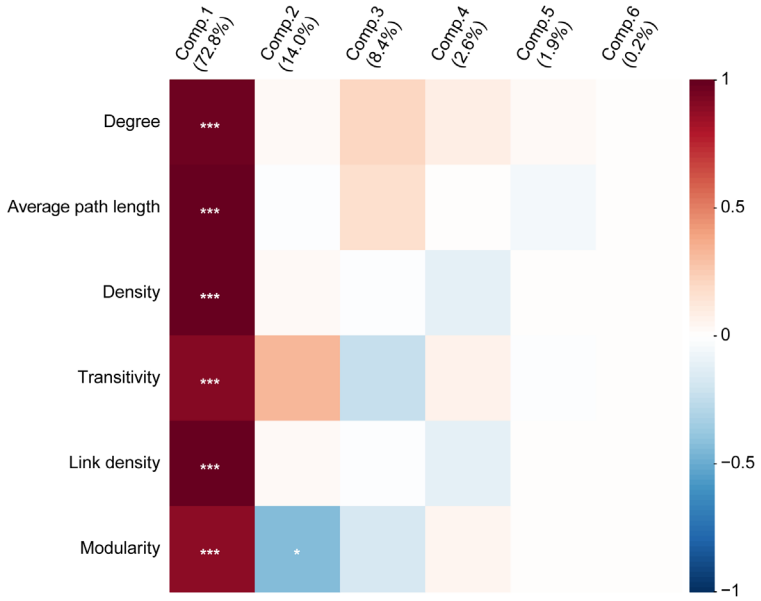
Supplementary Figure 3 Microbial richness during four periods of seasonal freeze-thaw processes and at two depths. Differences in bacterial, fungal, and microbial richness among four periods and between two depths (mean ± se). TP, CTP, FP, and CFP represent the thawing, completely thawed, freezing, and completely frozen periods, respectively. Lowercase letters represent the significance of differences among four periods or between two depths, and uppercase letters represent the significance of differences between the freeze-thaw and non-freeze-thaw stages.



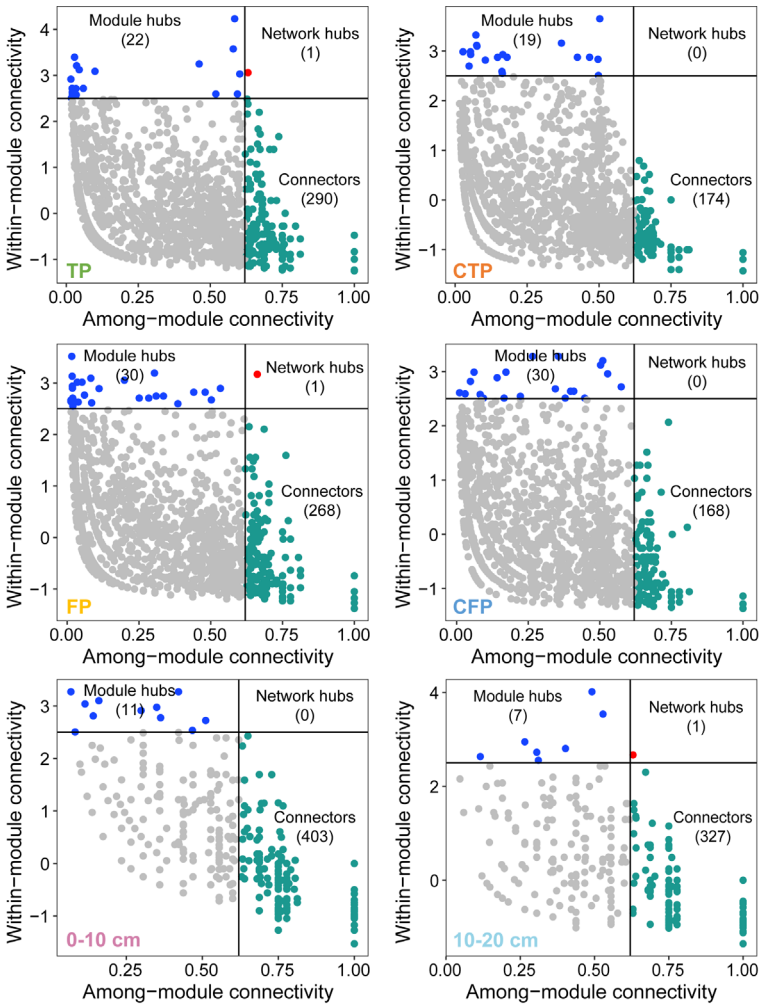
Supplementary Figure 4 Variables affecting microbial diversity tested by random forest model. Relative importance of the variables affecting microbial diversity. Por, soil porosity; WMWD, wet-sieving mean weight diameter; DMWD, dry-sieving mean weight diameter; BGB, belowground biomass. R^2 indicate the explained variation of the model. Asterisks indicate the statistical significance (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).



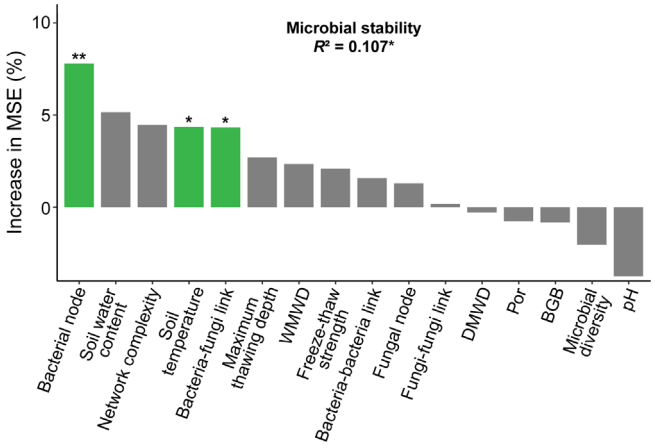
Supplementary Figure 5 Nodes, links, and complexity in microbial co-occurrence networks during four periods of seasonal freeze-thaw processes and at two depths. Differences in bacteria-bacteria links, fungi-fungi links, bacteria-fungi links, bacteria nodes, fungi nodes, and network complexity among four periods and between two depths (mean ± se). TP, CTP, FP, and CFP represent the thawing, completely thawed, freezing, and completely frozen periods, respectively. Lowercase letters represent the significance of differences among four periods or between two depths, and uppercase letters represent the significance of differences between the freeze-thaw and non-freeze-thaw stages.



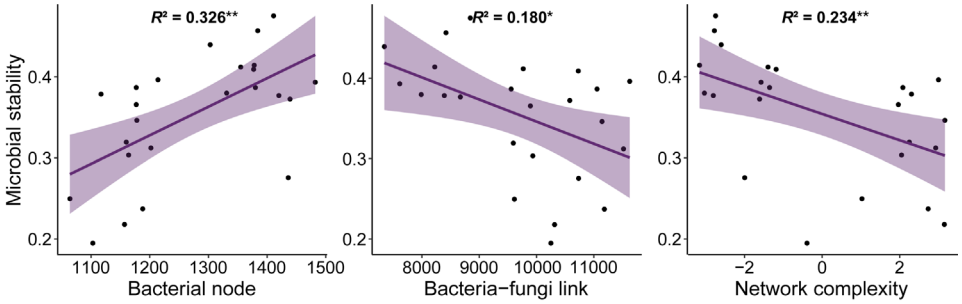
Supplementary Figure 6 Spearman correlations between multiple network properties and different components (Comp.) from the principal coordinate analysis. Noted that average path length and modularity, implying the network sparsity, are calculated the inverse of these variables before calculating the complexity. Comp.1 represents network complexity. Asterisks indicate the statistical significance (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).



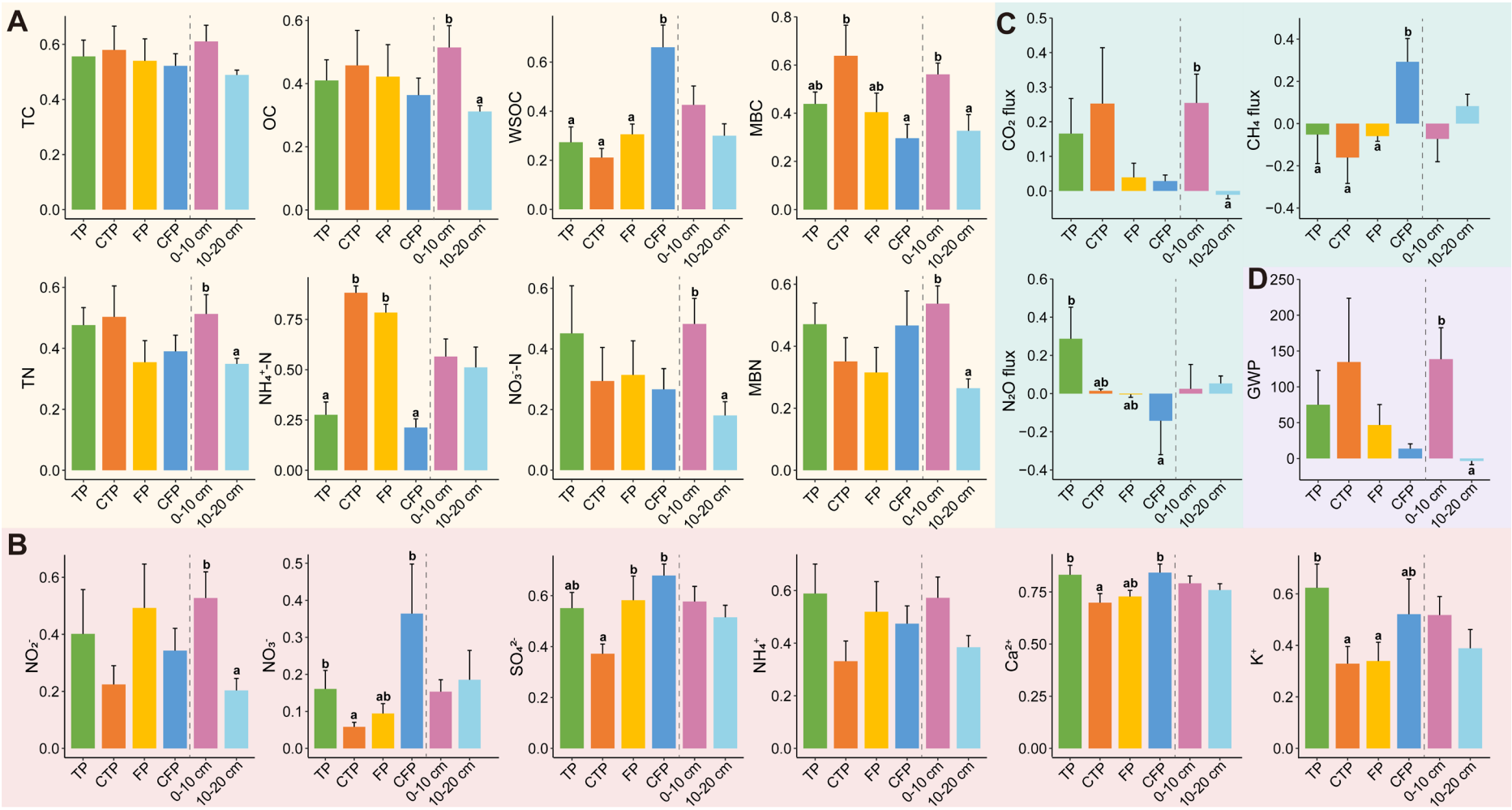
Supplementary Figure 7 Identifications of keystone taxa based on their topological roles in microbial co-occurrence networks during four periods of seasonal freeze-thaw processes and at two depths. Differences in keystone taxa among four periods and between two depths. Module hubs are identified as Zi ≥ 2.5, Pi < 0.62, connectors are identified as Zi < 2.5, Pi ≥ 0.62, and network hubs are identified as Zi ≥ 2.5, Pi ≥ 0.62. TP, CTP, FP, and CFP represent the thawing, completely thawed, freezing, and completely frozen periods, respectively. Zi, within-module connectivity; Pi, among-module connectivity.



Supplementary Figure 8 Variables affecting microbial stability tested by random forest model. Relative importance of the variables affecting microbial stability. Por, soil porosity; WMWD, wet-sieving mean weight diameter; DMWD, dry-sieving mean weight diameter; BGB, belowground biomass. R^2 indicate the explained variation of the model. Asterisks indicate the statistical significance (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).

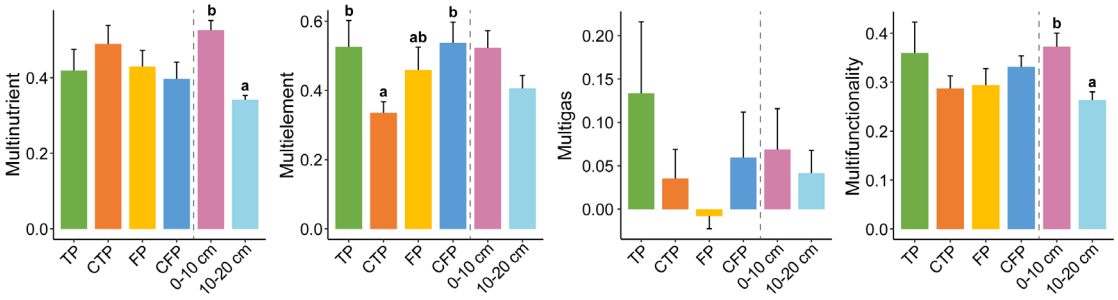


Supplementary Figure 9 Relationships of microbial stability with node, link, and complexity of networks. The linear relationships of microbial stability with bacterial node, bacterial-fungal link, and network complexity. (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).

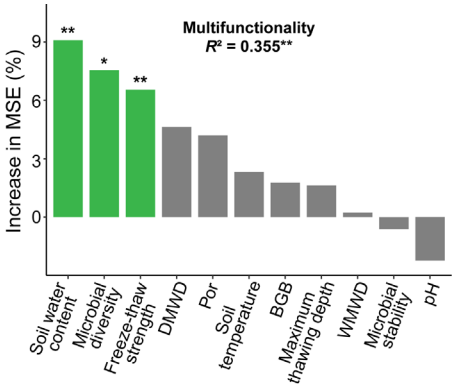


Supplementary Figure 10 Individual soil functions and GWP during four periods of seasonal freeze-thaw processes and at two depths. (A) Differences in individual functions of multinutrient among four periods and between two depths (mean ± se), including TC, OC, WSOC, MBC, TN, NH_4^+-N, NO_3^--N, and MBN. (B) Differences in individual functions of multielement among four periods and between two depths (mean ± se), including NO_2^-, NO_3^-,

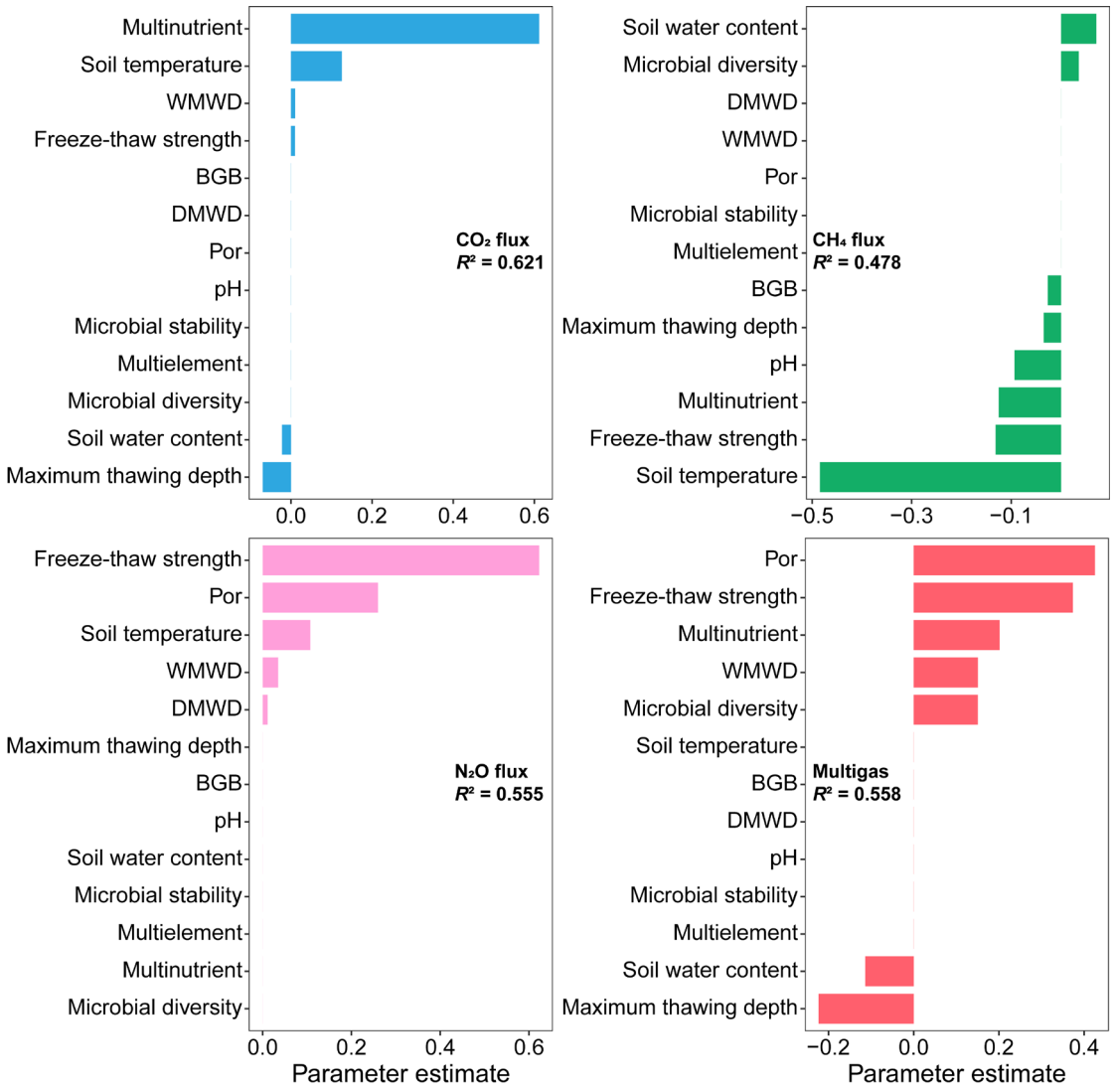
SO_4^{2-}, NH_4^+, Ca^{2+}, and K^+. (C) Differences in individual functions of multigas among four periods and between two depths (mean ± se), including CO_2, CH_4, and N_2O fluxes. (D) Differences in GWP among four periods and between two depths (mean ± se). TC, total carbon; OC, organic carbon; WSOC, water soluble organic carbon; MBC, microbial biomass carbon; TN, total nitrogen; MBN, microbial biomass nitrogen; GWP, global warming potential. TP, CTP, FP, and CFP represent the thawing, completely thawed, freezing, and completely frozen periods, respectively. Lowercase letters represent the significance of differences among four periods or between two depths, and uppercase letters represent the significance of differences between the freeze-thaw and non-freeze-thaw stages.



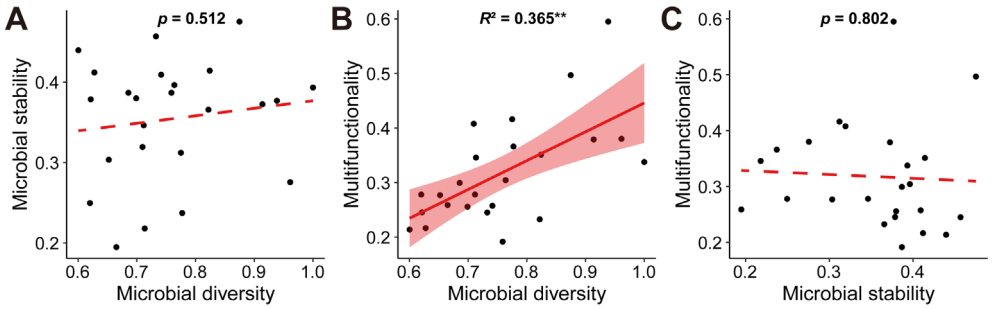
Supplementary Figure 11 Soil multifunctionality during four periods of seasonal freeze-thaw processes and at two depths. Differences in multinutrient, multielement, multigas, and multifunctionality among four periods and between two depths (mean ± se). TP, CTP, FP, and CFP represent the thawing, completely thawed, freezing, and completely frozen periods, respectively. Lowercase letters represent the significance of differences among four periods or between two depths, and uppercase letters represent the significance of differences between the freeze-thaw and non-freeze-thaw stages.



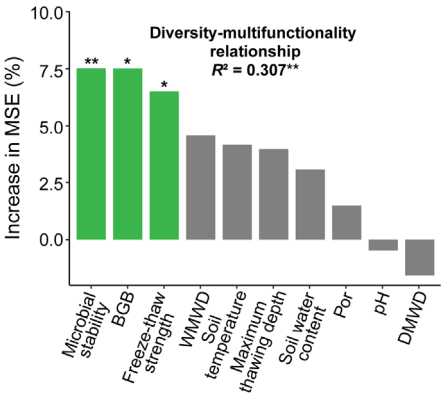
Supplementary Figure 12 Variables affecting soil multifunctionality tested by random forest model. Relative importance of the variables affecting soil multifunctionality. Por, soil porosity; WMWD, wet-sieving mean weight diameter; DMWD, dry-sieving mean weight diameter; BGB, belowground biomass. R^2 indicate the explained variation of the model. Asterisks indicate the statistical significance (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).



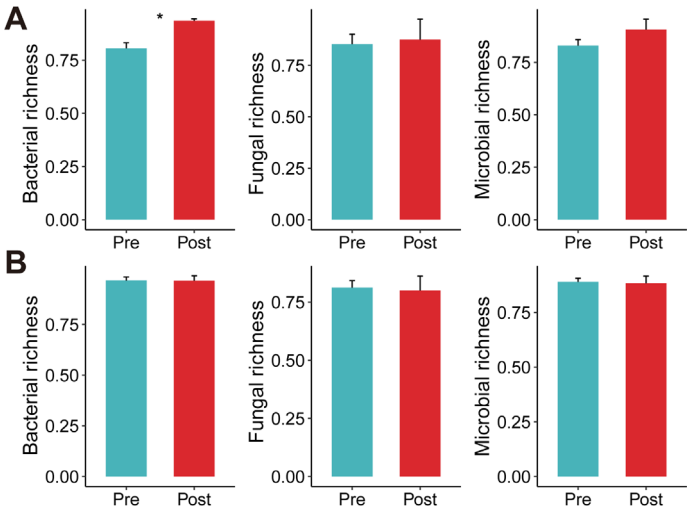
Supplementary Figure 13 Relative importance of environmental variables, microbial diversity, stability, multinutrient, and multielement on CO_2, CH_4, N_2O fluxes, and multigas estimated by generalized linear models. Estimated relative importance is the standardized regression coefficients in generalized linear models. Por, soil porosity; WMWD, wet-sieving mean weight diameter; DMWD, dry-sieving mean weight diameter; BGB, belowground biomass.



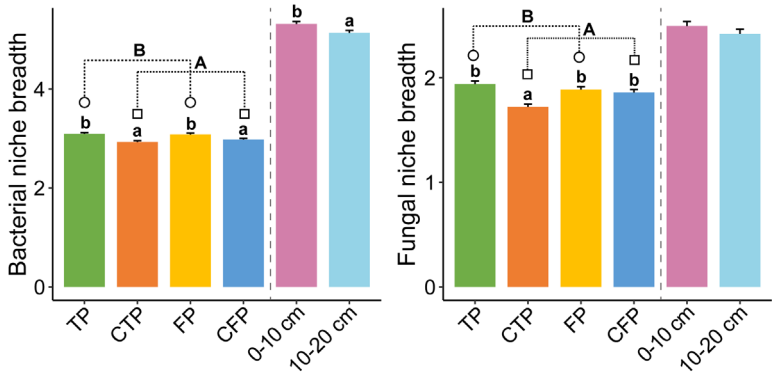
Supplementary Figure 14 Relationships among microbial diversity, stability, and soil multifunctionality. (A) The linear relationship between microbial diversity and stability. (B) The linear relationship between microbial diversity and soil multifunctionality. (C) The linear relationship between microbial stability and soil multifunctionality. Asterisks indicate the statistical significance (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).



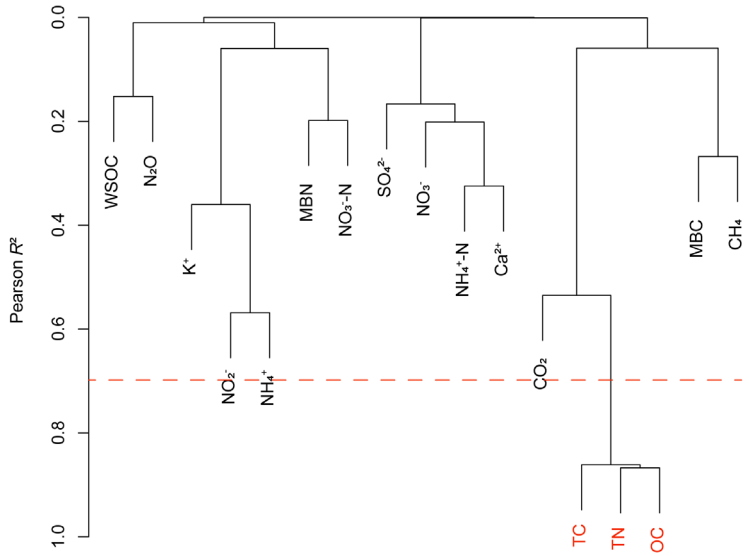
Supplementary Figure 15 Variables affecting microbial diversity-soil multifunctionality relationship tested by random forest model. Relative importance of the variables affecting microbial diversity-soil multifunctionality relationship. Por, soil porosity; WMWD, wet-sieving mean weight diameter; DMWD, dry-sieving mean weight diameter; BGB, belowground biomass. R^2 indicate the explained variation of the model. Asterisks indicate the statistical significance (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).



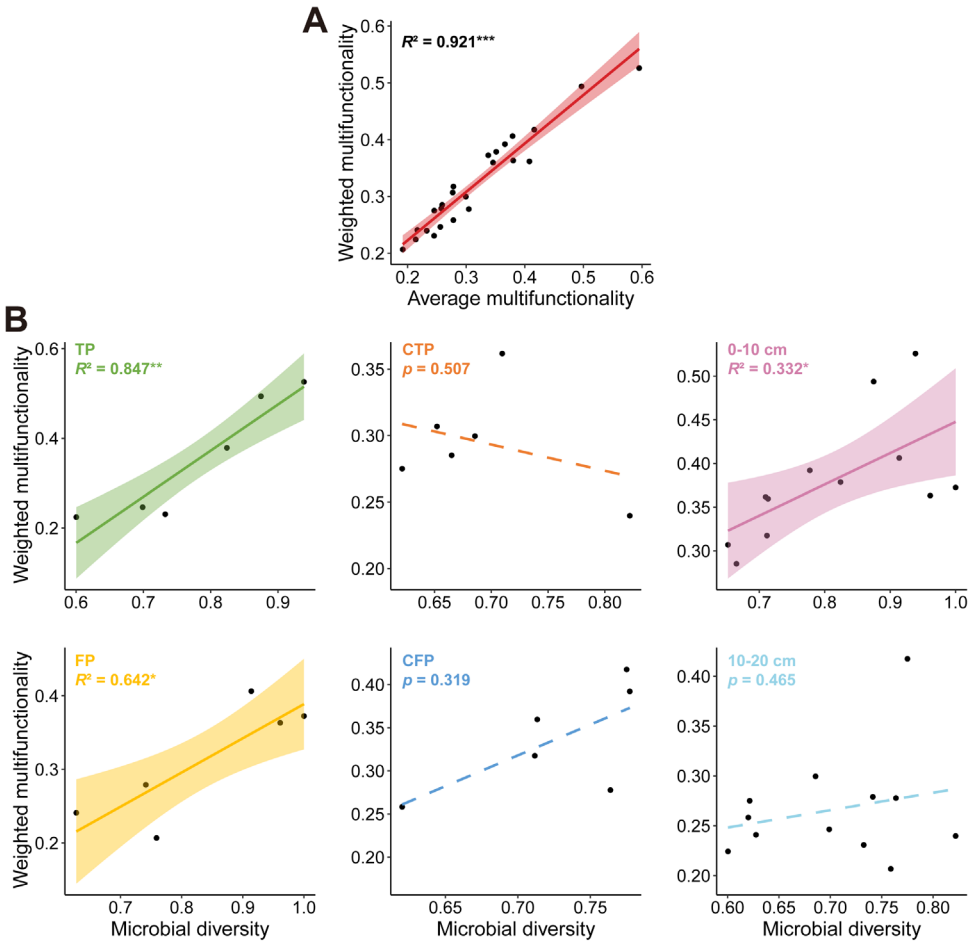
Supplementary Figure 16 Microbial richness before diurnal freeze-thaw cycles (Pre) and after diurnal freeze-thaw cycles (Post) during the thawing and freezing periods at 0-10cm depth. (A) Differences in bacterial, fungal, and microbial richness from Pre to Post during the thawing period (mean ± se). (B) Differences in bacterial, fungal, and microbial richness from Pre to Post during the freezing period (mean ± se). Asterisks indicate the statistical significance (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).



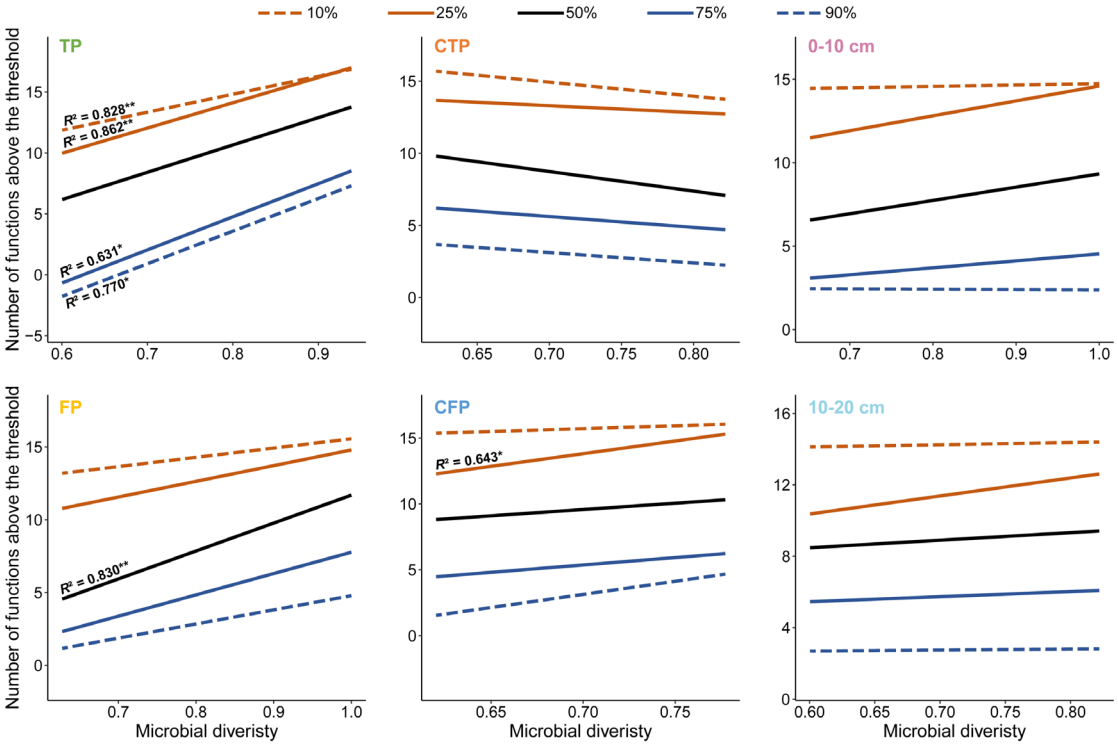
Supplementary Figure 17 Niche breadths of microbial communities during four periods of seasonal freeze-thaw processes and at two depths. Differences in bacterial and fungal niche breadths among four periods and between two depths (mean ± se). TP, CTP, FP, and CFP represent the thawing, completely thawed, freezing, and completely frozen periods, respectively. Lowercase letters represent the significance of differences among four periods or between two depths, and uppercase letters represent the significance of differences between the freeze-thaw and non-freeze-thaw stages.



Supplementary Figure 18 Cluster analysis of the individual soil functions. The functions with red fonts had higher Pearson correlation (R^2 > 0.7), and these functions were weighted of 1/3, so that these correlated functions have a combined weight of only 1. TC, total carbon; OC, organic carbon; WSOC, water soluble organic carbon; MBC, microbial biomass carbon; TN, total nitrogen; MBN, microbial biomass nitrogen.



Supplementary Figure 19 Relationships between microbial diversity and weighted soil multifunctionality. (A) The linear regression fits between average and weighted multifunctionality. (B) Differences of the linear regression fits between microbial diversity and weighted multifunctionality among four periods and at two depths. TP, CTP, FP, and CFP represent the thawing, completely thawed, freezing, and completely frozen periods, respectively. Asterisks indicate the statistical significance (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).



Supplementary Figure 20 Relationships between soil biodiversity and multi-threshold soil functions. Differences of the linear regression fits between microbial diversity and multi-threshold soil functions among four periods and at two depths. TP, CTP, FP, and CFP represent the thawing, completely thawed, freezing, and completely frozen periods, respectively. Asterisks indicate the statistical significance (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).

Supplementary Tables

Supplementary Table 1 Differences of relative abundances of the dominant microbial phyla among four periods of seasonal freeze-thaw processes and between two depths (mean ± se).

Taxon	Phylum	Period				Depth	
		Thawing period	Completely thawed period	Freezing period	Completely frozen period	0-10 cm	10-20 cm
Bacteria	*Acidobacteria*	13.315±1.305	16.569±1.393	15.916±0.740	14.577±1.259	15.309±0.855	14.880±0.927
	Actinobacteria	10.990±1.003 ab	9.232±1.014 a	9.751±0.818 a	13.485±1.676 b	10.951±0.763	10.778±1.077
	Bacteroidetes	29.854±2.575	30.206±2.809	29.809±1.581	25.368±3.357	28.542±1.594	29.077±2.145
	Chloroflexi	3.812±0.572 a	5.422±0.410 b	4.791±0.328 ab	4.757±0.659 ab	4.485±0.355	4.906±0.401
	Firmicutes	7.462±4.998	0.875±0.170	0.998±0.157	1.265±0.304	1.502±0.400	3.798±2.592
	Gemmatimonadetes	1.938±0.185 ab	2.571±0.335 b	2.131±0.140 ab	1.797±0.181 a	1.883±0.163	2.335±0.158
	Nitrospirae	0.918±0.070	0.902±0.094	1.000±0.072	0.949±0.102	0.888±0.051	0.997±0.062
	Planctomycetes	4.293±0.394	4.888±0.402	4.234±0.240	4.691±0.376	4.847±0.239	4.206±0.235
	Proteobacteria	21.567±1.470	21.530±0.894	24.489±1.569	24.492±1.090	24.476±0.732 b	21.563±0.987 a
	Verrucomicrobia	0.409±0.051 aA	0.574±0.128 aB	0.451±0.105 aA	1.216±0.229 bB	0.711±0.168	0.613±0.096
Fungi	*Ascomycota*	68.824±7.095	69.232±2.600	72.064±2.713	79.746±2.596	73.167±2.055	71.766±3.920
	Basidiomycota	2.075±0.270 aA	3.256±0.442 abB	2.972±0.428 abA	3.836±0.450 bB	3.271±0.275	2.798±0.369
	Chytridiomycota	0.145±0.035 a	1.568±0.714 b	0.397±0.072 a	0.177±0.059 a	0.895±0.393	0.249±0.080
	Glomeromycota	1.710±0.665	1.707±0.533	4.138±1.257	3.613±0.993	3.566±0.814	2.018±0.443
	Zygomycota	21.950±8.579	14.651±3.228	12.077±2.825	8.622±2.666	10.576±1.808	18.074±4.589

Lowercase letters represent the significance of differences among four periods or between two depths, and uppercase letters represent the significance of differences between the freeze-thaw and non-freeze-thaw stages.

Supplementary Table 2 Linear regression fits between natural connectivity and proportion of removed edges in microbial co-occurrence networks during four periods of seasonal freeze-thaw processes and at two depths.

		Fitting equation	R^2	*p*
Period	Thawing period	y = -0.050x + 0.042	0.993	< 0.001
	Completely thawed period	y = -0.086x + 0.072	0.985	< 0.001
	Freezing period	y = -0.056x + 0.046	0.988	< 0.001
	Completely frozen period	y = -0.083x + 0.071	0.990	< 0.001
Depth	0-10 cm	y = -0.013x + 0.010	0.888	< 0.001
	10-20 cm	y = -0.048x + 0.034	0.967	< 0.001