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Permafrost thawing exhibits a greater influence on bacterial richness and community structure than permafrost age in Arctic permafrost soils

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Table S1 The individual and interactive effects of permafrost thawing status and age on the soil bacterial richness.

Source	Type III Sum of Squares	d.f.	Mean Square	F	*P* value
Corrected Model	189108	11	17192	4.3	**0.001**
Intercept	3789453	1	3789452	942.3	**< 0.001**
Permafrost thawing status	81055	2	40527	10.1	**< 0.001**
Permafrost age	50181	3	16727	4.2	**.013**
Permafrost thawing status× permafrost age	27544	6	4591	1.1	.361
Error	132707	33	4021		
Total	4323958	45			
Corrected Total	321816	44			

Analysis is based on two-way ANOVA, significance at $P < 0.05$ is in bold.

Table S2 The bacterial richness by permafrost thawing status.

	Mean±S.D.	Active	Transition	Permanently frozen
Active	354 ±17	-	-	-
Transition	287±16	**0.011**	-	-
Frozen	248±18	**<0.001**	0.221	-

S.D. standard deviation, significance is based on the Tukey's post hoc test, statistically significant differences (at $P < 0.05$ level) are in bold. Active, transition, and frozen are different permafrost thawing status.

Table S3 The bacterial richness by permafrost age.

	Mean±S.D. (%)	Young	Medium	Old	Ancient
Young	358±21	-	-	-	-
Medium	268±19	**0.001**	-	-	-
Old	272±18	**0.002**	0.995	-	-
Ancient	287±18	**0.009**	0.836	0.931	-

S.D. standard deviation, significance is based on the Tukey's post hoc tests, statistically significant differences (at $P < 0.05$ level) are in bold. Young, medium, old, and ancient are permafrost soil ages.

Table S4 The influence of permafrost thawing status on bacterial richness for soils of different ages.

Soil age	Variation	Sum of Squares	d.f.	Mean Square	F	Sig.
Young	Between Groups	70837	2	35418	41.644	**<0.001**
	Within Groups	5954	7	851		
	Total	76790	9			
Medium	Between Groups	5431	2	2715	0.898	0.445
	Within Groups	24184	8	3023		
	Total	29615	10			
Old	Between Groups	9255	2	4628	0.798	0.480
	Within Groups	52192	9	5799		
	Total	61447	11			
Ancient	Between Groups	15218	2	7609	1.359	0.305
	Within Groups	50378	9	5598		
	Total	65596	11			

Analysis is based on two-way ANOVA, significant influences (at $P < 0.05$ level) are in bold. Young, medium, old, and ancient are permafrost soil ages.

Table S5 The permafrost soil bacterial richness of the various permafrost layer in the young permafrost.

	Mean±S.D. (%)	Active	Transition	Permanently frozen
Active	471 ±37	-	-	-
Transition	355±22	**0.002**	-	-
Frozen	248±19	**<0.001**	**0.009**	-

Significance is based on the Tukey's post hoc test, statistically significant differences (at P=0.05 level) are in bold. Active, transition, and frozen are different permafrost thawing status.

Table S6 The richness of bacteria phyla by permafrost thawing status (*Proteobacteria* at the Class level).

		Mean ± S.D.	Active	Transition	Frozen
Firmicutes	Active	87±15	-	-	-
	Transition	47±15	**0.013**	-	-
	Frozen	49±8	**0.042**	0.986	-
Actinobacteria	Active	128±14	-		
	Transition	106±10	0.062	**0.021**	
	Frozen	71±2	**0.002**	-	
Chloroflexi	Active	36±4	-	-	
	Transition	29±3	0.095	-	
	Frozen	16±6	**0.002**	**0.02**	
Alphaproteobacteria	Active	35±6	-	-	
	Transition	19±7	0.016	-	
	Frozen	16±4	0.016	0.774	
Deltaproteobacteria	Active	25±8	-	-	
	Transition	12±2	**0.028**	-	
	Frozen	11±2	**0.049**	0.976	

S.D. standard deviation, dominant phyla are defined with relative abundance >1% across all samples. Significance is based on the Tukey's post hoc tests, statistically significant differences (at P=0.05 level) are in bold. S.D.: Standard deviation. Active, transition, and frozen are different permafrost thawing status.

Table S7 The bacterial community structure differences identified among various permafrost thawing status and soil ages.

Source	d.f.	SS	MS	Pseudo-F	P(perm)	Unique permutations
Soil age	3	11653	3884.2	2.2297	**0.001**	999
Thawing status	2	10890	5445.1	3.1258	**0.001**	998
Thawing status × soil age	6	16106	2684.3	1.5409	**0.001**	994
Residual	33	57486	1742			
Total	44	97193				

Significance is based on PERMANOVA.

Table S8 Bacterial community structure comparison among different thawing status by PERMANOVA post-hoc analysis.

	Active	Transition	Frozen
Active	-	-	-
Transition	**0.006**	-	-
Frozen	**0.001**	**0.007**	-

P-values are shown, significant P-values are shown in bold. Active, transition, and frozen are different permafrost thawing status.

Table S9 Bacterial community structure comparison among different permafrost ages by PERMANOVA post-hoc analysis.

	Young	Medium	Old	Ancient
Young	-	-	-	-
Medium	**0.004**	-	-	-
Old	**0.001**	0.207	-	-
Ancient	**0.001**	**0.024**	0.109	-

P-values are shown, significant P-values are shown in bold. Young, medium, old, and ancient are permafrost soil ages.

Table S10 The community structure differences of the soils with same age but at different permafrost thawing status.

	Source	d.f.	SS	MS	Pseudo-F	P(perm)	Unique permutations
Young	Thawing status	2	9526	4763	3.45	**0.002**	749
	Residual	7	9658	1380			
	Total	9	19184				
Medium	Thawing status	2	6671	3336	1.66	**0.027**	926
	Residual	8	16079	2010			
	Total	10	22750				
Old	Thawing status	2	4807	2404	1.3	0.124	914
	Residual	9	16699	1855			
	Total	11	21506				
Ancient	Thawing status	2	5853	2927	1.75	**0.016**	914
	Residual	9	15050	1672			
	Total	11	20904				

Significance is based on PERMANOVA. Young, medium, old, and ancient are permafrost soil ages.

Table S11 The community structure differences of the soils with same thawing status but at different permafrost ages.

	Source	d.f.	SS	MS	Pseudo-F	P(perm)	Unique permutations
Active	Soil age	3	10650	3550.2	2.2246	**0.001**	999
	Residual	11	17554	1595.8			
	Total	14	28205				
Transition	Soil age	3	9873.4	3291.1	1.9473	**0.001**	999
	Residual	11	20281	1690.1			
	Total	14	30154				
Frozen	Soil age	3	8186.2	2728.7	1.3886	**0.043**	998
	Residual	11	19651	1965.1			
	Total	14	27837				

Significance is based on PERMANOVA.Active, transition, and frozen are different permafrost thawing status.

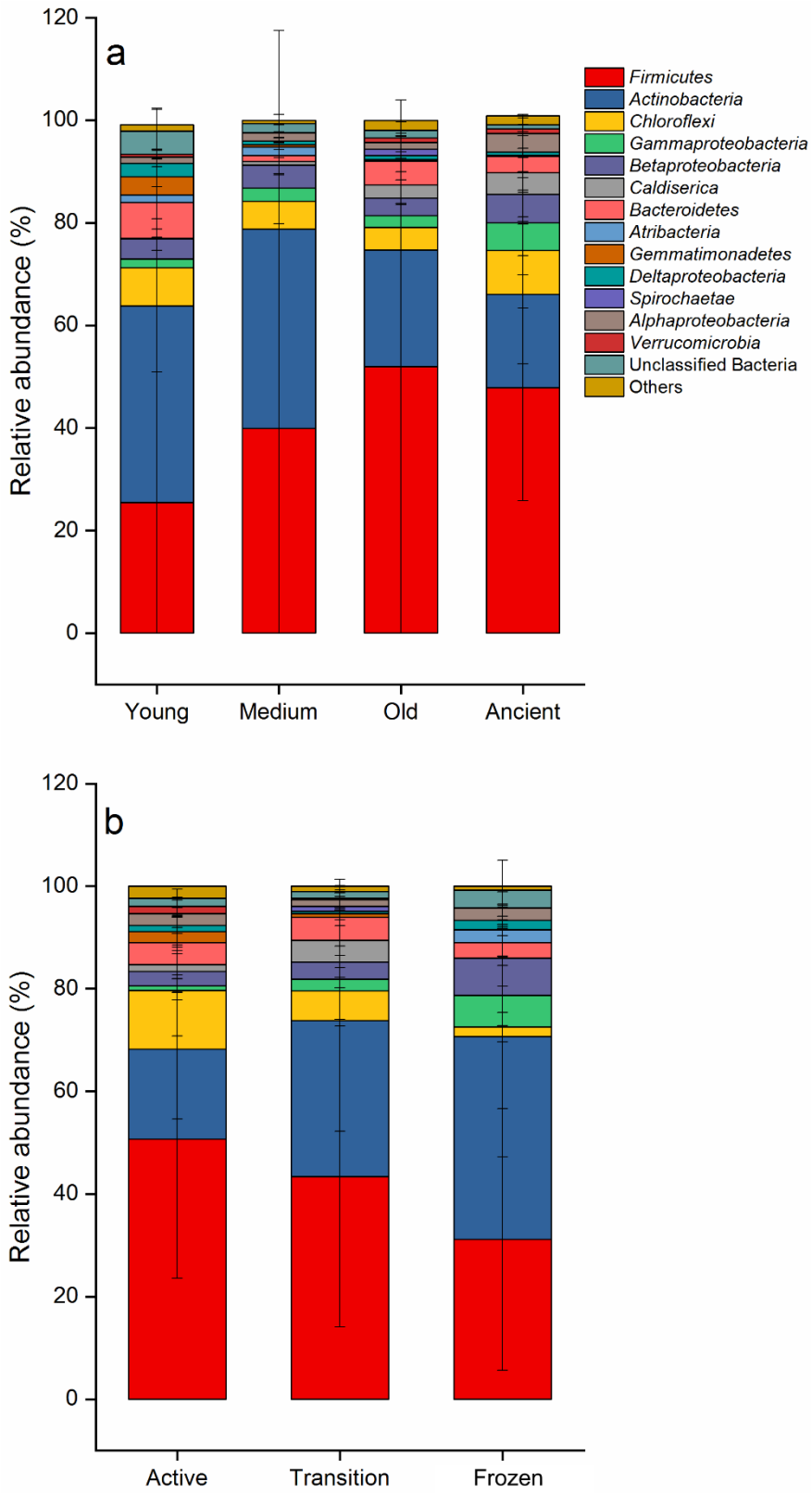

Fig. S1The relative abundance of the dominant bacteria with the permafrost age (a) and thawing status (b). *Proteobacteria* is at the class level. Young, medium, old, and ancient are permafrost soil ages, active, transition, and frozen are different permafrost thawing status.

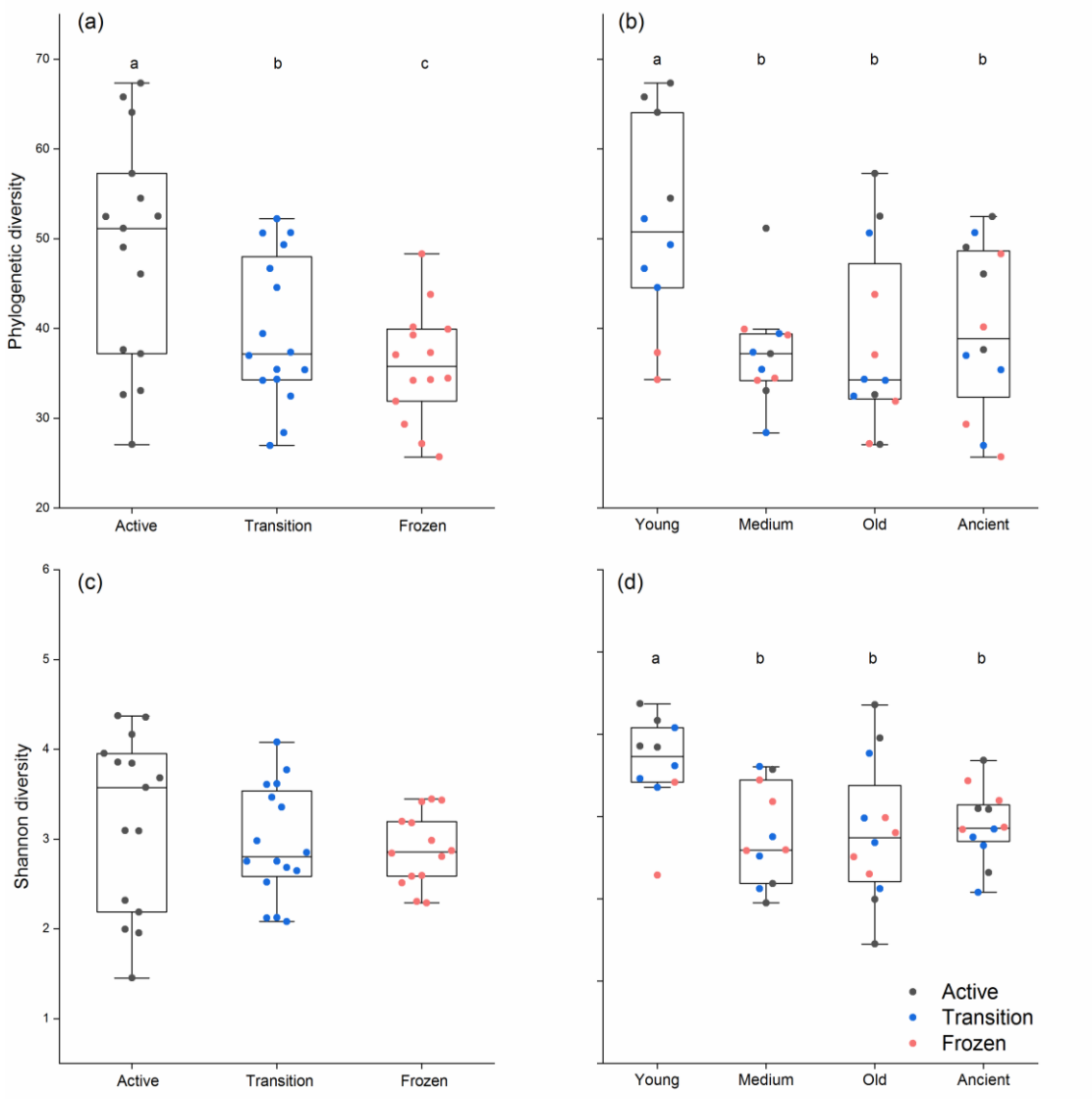

Fig. S2 Bacterial Phylogenetic diversity (a and b) and Shannon diversity (c and d) with the permafrost thawing status (a and c) and age (b and d). Different letters indicate significant difference at $P < 0.05$. Young, medium, old, and ancient are permafrost soil ages, active, transition, and permanently frozen are permafrost thawing statuses

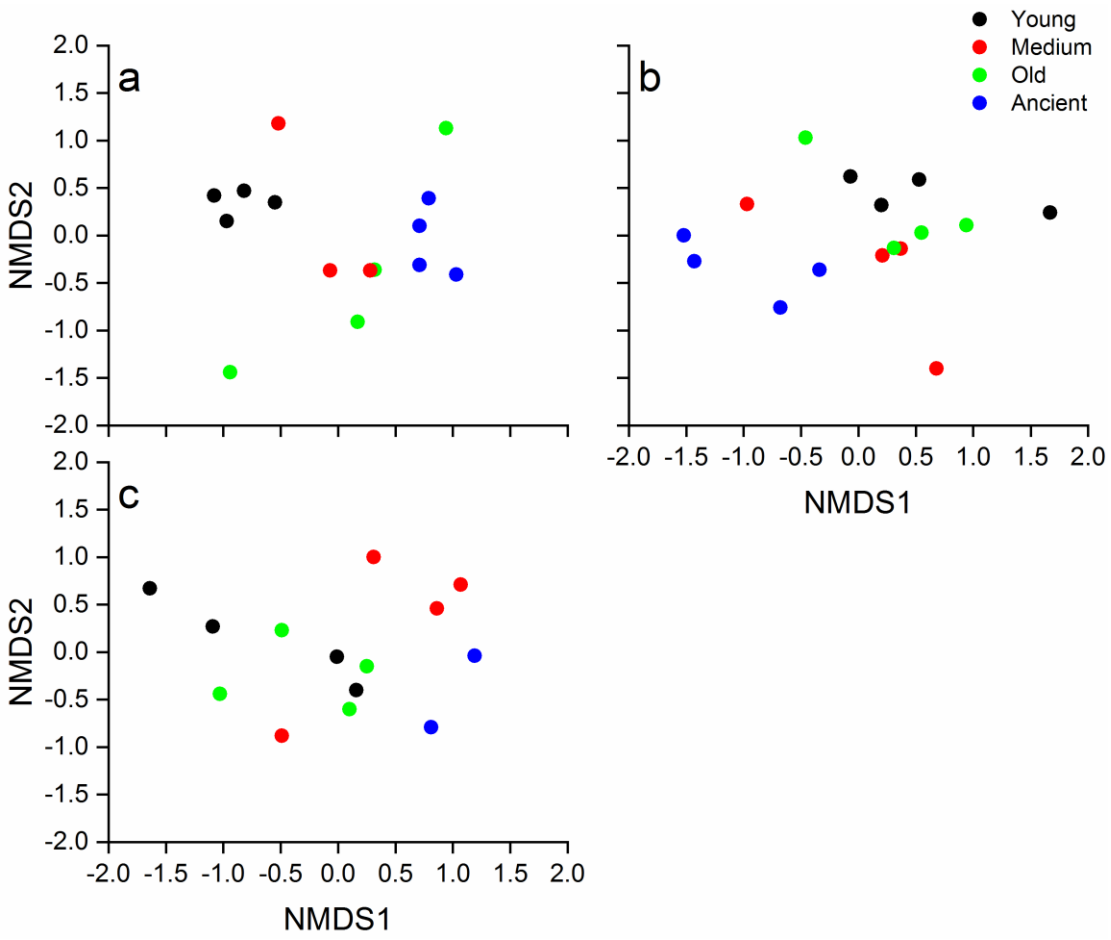

Fig. S3 NMDS ordination plot showing the community structure for permafrost soil bacteria with different permafrost ages but at the same thawing status. (a), the active layer of permafrost; (b), the transition layer of permafrost, and (c), the permanently frozen layer of the permafrost. Young, medium, old, and ancient are permafrost soil ages.

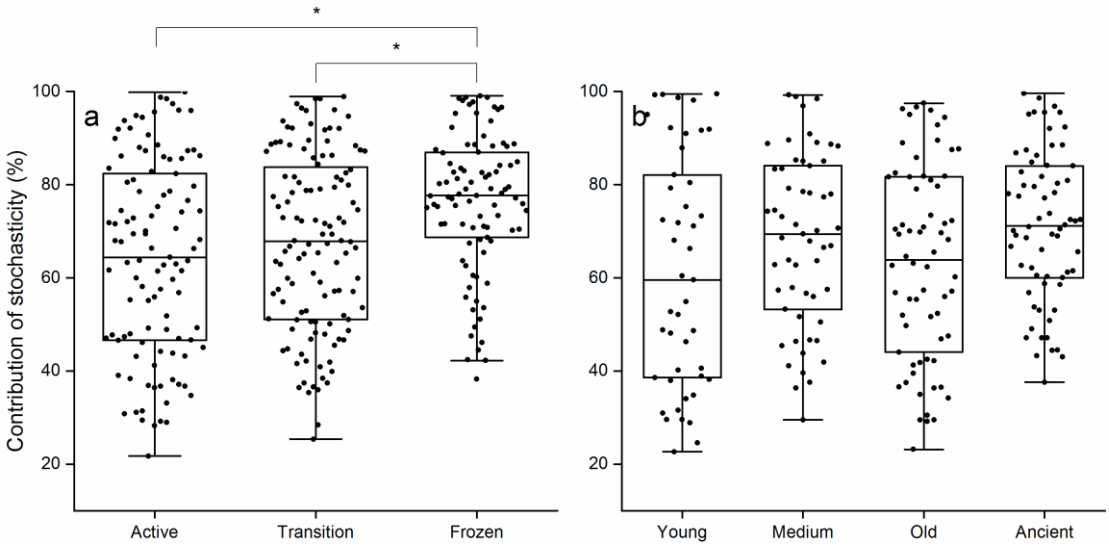

Fig. S4 Contribution of stochasticity on the bacterial community assembly for different permafrost thawing status (a) and soil ages (b). Contribution of stochasticity was based on the modified stochasticity index. * indicates significance at $P < 0.05$. Young, medium, old, and ancient are permafrost soil ages. Active, transition, and frozen are different permafrost thawing status.

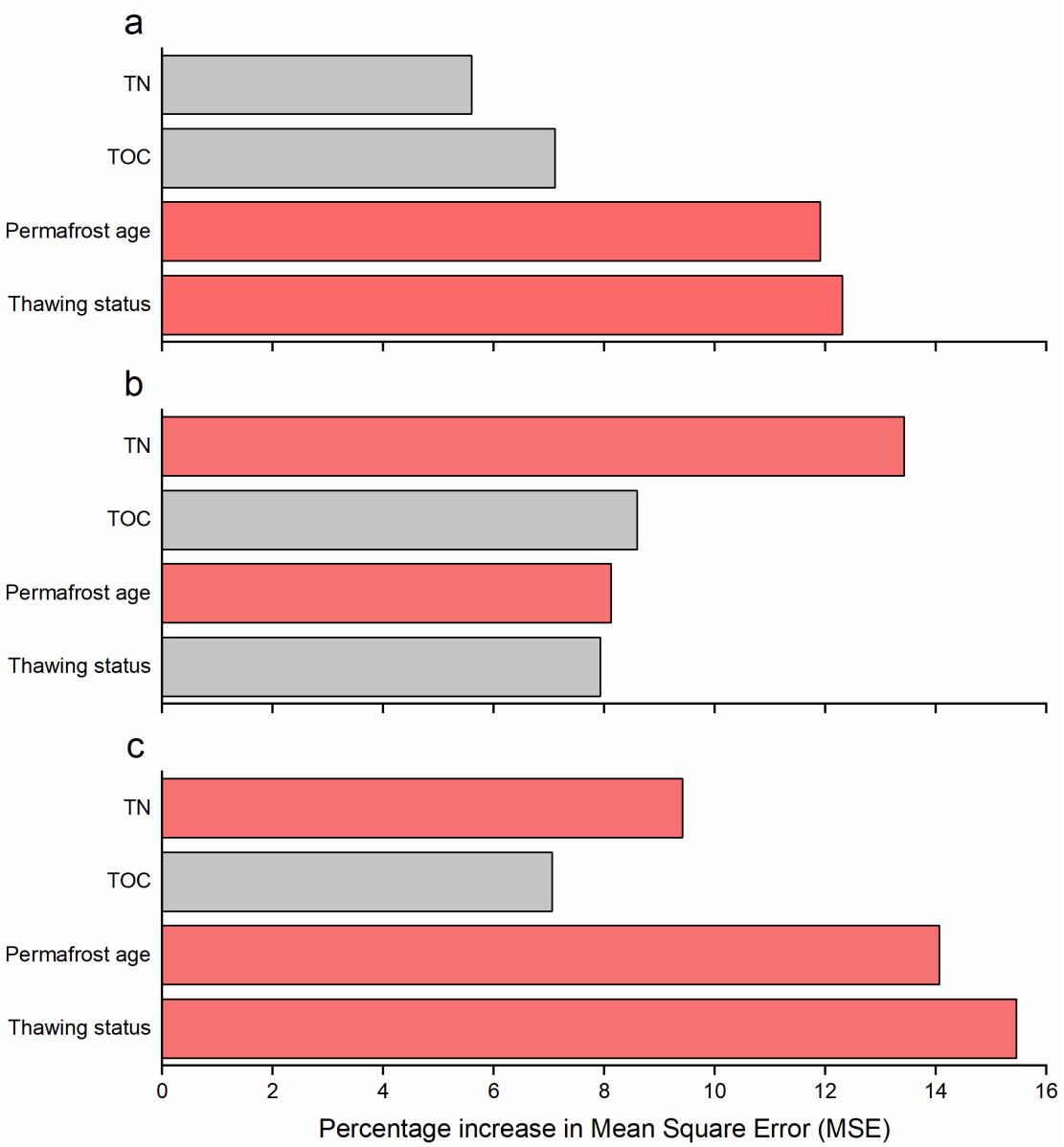

Fig. S5 Random Forest analysis results showing the contribution of permafrost age, thawing status, TOC and TN on the bacterial richness (a) and community structure. The community structure variation was assessed by the 1st (b) and 2nd (c) axis coordinates of the NMDS plot (NMDS1 and NMDS2). Significant contributions are in red, whereas non-significant contributions are in grey.