



Supplement of

Bacterial community composition changes independently of soil edaphic parameters following localized permafrost disturbance

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Supplementary Material

Methods

PMA Treatment

Soils A, F, Core 1 95-100, Core 2 95-100, and Core 3 95-100 were subsampled. Each sample was weighed to 0.50 g and suspended in 4.5 ml of filter sterilized and autoclaved 1×PBS (pH 7.4; 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, and 1.8 mM KH₂PO₄). A serial dilution of this 1:10 (w/v) soil:PBS solution was performed to obtain a 1:1000 (w/v) soil:PBS dilution at a final volume of 1 ml. This solution was mixed with 0.04 mM propidium monoazide (PMA) prepared by dissolving 1 mg PMA in 98 µl dH₂O to create a 20 mM stock solution, of which 2 µl were added to the soil solution (Biotium Inc., CA, USA). The soil solution was then incubated in the dark for 10 min with vigorous hand shaking every 2 min. PMA was cross-linked to DNA with a 500 W halogen lamp (Globe Electric, Quebec, Canada) by placing the samples 20 cm from the light source for 10 min on ice. After light exposure, samples were filtered onto a 47 mm diameter 0.22 µm pore size polysulfone (Supor) filter (Pall Corporation, NY, USA). Filter papers were cut into 1/3 segments and DNA from each segment was then extracted using a MOBio PowerSoil DNA extraction kit (Qiagen, Germany), following manufacturer's instructions. Non-PMA samples were extracted from a 0.5 g solid sample without dilution, PMA treatment, or crosslinking.

Results

Table S1. Physical description of frozen permafrost, active layer, and disturbance gradient soils. Standard error of each value was reported underneath the mean of each edaphic parameter (n=4).

	Series	Material Type	pH (aq)	% SOM (w/w)	Water Content (w/w)	DNA Concentration (ug/mg)
Core 1	Core 1 15	Peat	6.42	0.667	82%	106.31
			0.03	0.038	3%	7.18
	Core 1 30	Peat	5.187	0.638	77%	148.93
			0.079	0.068	2%	16.74
	Core 1 45	Peat	5.473	0.088	59%	29.04
			0.106	0.02	10%	0.50
Core 2	Core 1 75	Silt	5.627	0.365	64%	25.19
			0.085	0.073	8%	2.41
	Core 1 95	Silt	6.173	0.382	62%	2.77
			0.032	0.085	3%	0.36
	Core 2 30	Peat	5.553	0.804	83%	14.99
			0.237	0.009	1%	3.74
Core 3	Core 2 45	Peat	5.933	0.722	85%	9.46
			0.059	0.105	4%	0.61
	Core 2 75	Peat	5.733	0.703	73%	17.94
			0.003	0.097	5%	2.73
	Core 2 95	Peat	5.703	0.837	79%	13.73
			0.022	0.006	2%	1.44
Disturbance Gradient	Core 3 80	Silt	6.13	0.173	59%	102.84
			0.06	0.054	2%	3.20
	Core 3 95	Silt	5.84	0.615	88%	37.75
			0.075	0.047	1%	9.35
	A	Peat	6.25	57%	76%	374.62
			0.03	6%	3%	50.58
Disturbance Gradient	B	Peat	6.04	44%	67%	140.36
			0.84	21%	10%	26.01
	C	Peat	5.49	73%	77%	140.76
			0.18	5%	3%	9.20
	D	Peat	5.53	85%	89%	78.13
			0.09	6%	1%	25.64
Disturbance Gradient	E	Silt	5.76	33%	62%	36.52
			0.07	6%	4%	24.52
	F	Silt	6.33	32%	70%	39.05
			0.26	10%	6%	22.96

Table S2. Soil chemistry including dissolved metals and nutrient levels in frozen active layer samples, permafrost samples, and the surface soil disturbance gradient.

Treatment	P	K	S	Mg	Ca	Fe	Cu	Mn	Zn	Na	NO3-N	NH4-N	TN	TC
	(%)						(mg/kg)				(mg/kg)		(w/w%)	
DC1 AL_1	0.10	0.18	0.18	0.57	2.10	2.13	760.61	1913.42	88.14	260.00	0.35	0.73	0.95	29.66
DC1 AL_2	0.10	0.09	0.53	0.23	2.57	1.71	249.87	2161.04	71.75	190.78	0.13	0.58	1.66	35.53
DC1 AL_3	0.08	0.16	0.13	0.43	0.93	2.06	87.29	645.10	66.28	234.79	5.85	10.97	0.59	9.93
DC1 P_1	0.07	0.12	0.12	0.47	0.84	2.34	42.64	344.53	72.83	220.38	0.09	39.26	0.53	9.01
DC1 P_2	0.09	0.14	0.19	0.47	1.31	2.59	44.99	501.93	89.99	212.79	0.24	27.05	0.75	13.52
DC4 AL_2	0.12	0.16	0.20	0.43	1.28	3.05	99.49	1000.20	116.73	187.93	0.44	12.74	0.92	19.93
DC4 AL_3	0.12	0.20	0.22	0.48	1.24	2.30	67.59	786.22	88.33	266.63	0.41	23.14	1.18	19.84
DC4 P_1	0.12	0.06	0.51	0.20	2.40	1.99	45.15	1693.89	66.17	150.24	0.37	1.14	1.90	37.75
DC4 P_2	0.07	0.18	0.25	0.38	1.91	1.60	61.74	677.83	80.87	176.52	0.28	0.95	1.07	27.26
DC3 P_1	0.10	0.04	0.57	0.12	2.48	1.53	62.05	897.00	49.05	195.00	0.17	6.83	2.04	37.23
DC3 P_2	0.08	0.02	1.12	0.10	2.87	1.50	44.97	858.00	54.11	172.16	0.29	12.92	2.43	41.56
A	0.08	0.23	0.16	0.56	2.43	2.30	33.91	769.77	108.20	214.24	0.82	19.23	6.12	1.01
B	0.07	0.24	0.10	0.63	1.31	2.91	36.18	1487.96	118.79	196.60	0.76	15.23	1.54	0.56
C	0.15	0.10	0.35	0.25	2.35	3.38	32.50	4533.75	128.38	169.81	0.25	4.78	1.05	1.76
D	0.16	0.04	0.49	0.15	2.50	2.16	39.00	1248.00	65.00	136.81	1.55	1.35	0.14	2.70
E	0.07	0.17	0.19	0.53	1.24	2.80	48.13	816.61	111.49	188.01	0.72	13.95	1.40	0.79
F	0.08	0.14	0.17	0.44	1.11	2.36	33.05	639.07	89.42	251.25	1.27	1.28	0.31	0.75
Error	±0.02	±0.03	±0.03	±0.05	±0.03	±0.0025	±1	±7	±6	±0.1	±0.01	±0.20	±1.19	±0.1

Table S3. α -diversity metrics of frozen active layer, permafrost, and disturbance gradient soils.

Series	Coverage	Diversity		Richness		Evenness		DNA Content	
		Invsimpson	Shannon	Chao1	Richness	Simpson	Shannon	Concentration (ng/uL)	Standard Deviation
Core 1 15	0.924412	189.61	6.20	2884.41	1552.00	0.12	0.84	26.58	1.80
Core 1 30	0.930006	76.86	5.51	1232.44	1039.00	0.07	0.79	37.23	4.19
Core 1 45	0.979709	36.68	4.55	1269.60	570.00	0.06	0.72	7.26	0.12
Core 1 75	0.979167	27.42	4.46	1008.00	600.00	0.05	0.70	6.30	0.60
Core 1 95	0.978103	26.80	4.26	430.58	393.00	0.07	0.71	0.69	0.09
Core 3 80	0.967037	41.82	4.48	571.28	456.00	0.09	0.73	3.75	0.93
Core 3 95	0.969964	35.32	4.63	1064.23	682.00	0.05	0.71	2.36	0.15
Core 2 30	0.965359	84.04	5.47	1215.38	871.00	0.10	0.81	4.48	0.68
Core 2 45	0.967889	63.00	5.19	1261.78	829.00	0.08	0.77	3.43	0.36
Core 2 80	0.959523	60.05	5.00	1110.76	714.00	0.08	0.76	25.71	0.80
Core 2 95	0.961462	42.39	4.79	738.61	636.00	0.07	0.74	9.44	2.34
A	0.958626	153.05	6.07	1064.00	1064.00	0.14	0.87	93.66	12.65
B	0.9238	110.12	5.84	2686.24	1433.00	0.08	0.80	35.09	6.50
C	0.946705	87.23	5.56	1947.19	1127.00	0.08	0.79	35.19	2.30
D	0.94213	86.87	5.60	2722.53	1263.00	0.07	0.78	19.53	6.41
E	0.89999	186.53	6.15	1649.87	1400.00	0.13	0.85	9.13	6.13
F	0.89436	138.37	6.08	4494.70	1773.00	0.08	0.81	9.76	5.74

Table S4. The taxonomic assignment of indicator OTUs associated with the frozen active layer.

OTU ID	Phylum	Class	Order	Family	Genus	Species
OTU5	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_in certae_sedis			
OTU29	Proteobacteria	Betaproteobact eria				
OTU31	Proteobacteria	Deltaproteobac teria	Desulfuromonadales	Geobacterace ae	Geobacter	
OTU33	Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellac eae	Nakamure lla	Nakamurella_flavid a
OTU36	Acidobacteria	Acidobacteria_ Gp6	Gp6			
OTU39	Proteobacteria	Betaproteobact eria				
OTU42	Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobact eraceae	Solirubrob acter	Solirubrobacter_tai baiensis
OTU44	Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobia ceae	Ilumatoba cter	
OTU48	Proteobacteria	Alphaproteoba cteria	Rhizobiales			
OTU63	Proteobacteria	Betaproteobact eria				
OTU64	Actinobacteria	Actinobacteria	Actinomycetales	Kineosporiace ae		
OTU70	Proteobacteria	Betaproteobact eria	Burkholderiales			
OTU76	Proteobacteria	Betaproteobact eria				
OTU78	Acidobacteria	Acidobacteria_ Gp4	Gp4			
OTU101	Acidobacteria	Acidobacteria_ Gp4	Gp4			
OTU109	Actinobacteria	Actinobacteria	Gaiellales	Gaiellaceae	Gaiella	Gaiella_occulta
OTU132	Acidobacteria	Acidobacteria_ Gp4	Gp4			
OTU133	Acidobacteria	Acidobacteria_ Gp4				
OTU144						
OTU152	Actinobacteria	Actinobacteria	Actinomycetales			
OTU153	Acidobacteria	Acidobacteria_ Gp3	Gp3			
OTU158						
OTU159	Proteobacteria	Betaproteobact eria				
OTU167	Acidobacteria	Acidobacteria_ Gp6	Gp6			
OTU168	Actinobacteria	Actinobacteria				
OTU171	Verrucomicrobia	Opitutae	Opitales	Opitutaceae		
OTU181	Acidobacteria	Acidobacteria_ Gp4	Aridibacter			
OTU190	Actinobacteria	Actinobacteria	Solirubrobacterales			
OTU197						
OTU203	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_in certae_sedis			
OTU208	Proteobacteria	Deltaproteobac teria	Myxococcales			
OTU221	Acidobacteria	Acidobacteria_ Gp6	Gp6			
OTU222	Verrucomicrobia	Subdivision3	Subdivision3_genera_inc ertae_sedis			
OTU224	Acidobacteria	Acidobacteria_ Gn3				

OTU237	Proteobacteria	Betaproteobact eria				
OTU240	Acidobacteria	Acidobacteria_ Gp3	Gp3			
OTU256	Acidobacteria	Acidobacteria_ Gp16	Gp16			
OTU273	Actinobacteria	Actinobacteria	Acidimicrobiales	Iamiaceae	Aquihabit ans	Aquihabitans_dacc hungensis
OTU279	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiracea e	Nitrospira	
OTU288	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_in certae sedis			
OTU301	Bacteroidetes	Sphingobacteri ia	Sphingobacteriales	Chitinophaga ceae		
OTU310	Actinobacteria	Actinobacteria				
OTU321						
OTU322	Proteobacteria	Deltaproteobac teria	Myxococcales			
OTU327	Proteobacteria	Betaproteobact eria				
OTU331	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	Opitutus_terrae
OTU353	Actinobacteria	Actinobacteria	Solirubrobacterales			
OTU361	Planctomycetes	Planctomyceti a	Planctomycetales	Planctomycet aceae		
OTU367	Acidobacteria	Acidobacteria_ Gp6	Gp6			
OTU369						
OTU383	Actinobacteria	Actinobacteria	Actinomycetales			
OTU390	Acidobacteria	Acidobacteria_ Gp6	Gp6			
OTU430	Actinobacteria	Actinobacteria				
OTU444	Actinobacteria	Actinobacteria				
OTU459	Proteobacteria	Deltaproteobac teria	Myxococcales			
OTU471	Bacteroidetes	Sphingobacteri ia	Sphingobacteriales	Chitinophaga ceae		
OTU481	Proteobacteria	Betaproteobact eria				
OTU497						
OTU514						
OTU569	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Alterococ cus	Alterococcus_agaro lyticus
OTU600	Planctomycetes	Planctomyceti a	Planctomycetales	Planctomycet aceae		
OTU614						
OTU636	Proteobacteria	Betaproteobact eria	Rhodocyclales	Rhodocyclace ae		
OTU659	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_in certae sedis			
OTU682						
OTU686		candidate_divisio n WPS-I				
OTU692						
OTU724	Actinobacteria	Actinobacteria				
OTU745						
OTU772	Proteobacteria	Gammaproteo bacteria				
OTU868	Actinobacteria	Actinobacteria	Gaiellales	Gaiellaceae	Gaiella	Gaiella occulta
OTU885	Acidobacteria	Acidobacteria_ Gp6	Gp6			
OTU943	Proteobacteria	Betaproteobact eria				

OTU964	Acidobacteria	Acidobacteria_ Gp16				
OTU987						
OTU1031	Actinobacteria	Actinobacteria	Acidimicrobiales	lamiaceae	Aquihabitans	Aquihabitans_daec hungensis
OTU1180						
OTU1227						
OTU1291						
OTU1301	Acidobacteria	Acidobacteria_ Gp6	Gp6			
OTU1390	Acidobacteria	Acidobacteria_ Gp6	Gp6			
OTU1740	Acidobacteria	Acidobacteria_ Gp3	Candidatus Solibacter			
OTU1744	Acidobacteria	Acidobacteria_ Gp6	Gp6			
OTU1997	Proteobacteria	Betaproteobact eria	Rhodocyclales	Rhodocyclace ae		
OTU2159	Acidobacteria	Acidobacteria_ Gp6	Gp6			
OTU2283	Acidobacteria	Acidobacteria_ Gp6	Gp6			
OTU2393	Acidobacteria	Acidobacteria_ Gp6	Gp6			
OTU2972	Verrucomicrobia	Spartobacteria	Spartobacteria_genera_in certae sedis			
OTU4020	Proteobacteria	Alphaproteoba cteria	Rhizobiales			
OTU4207	Acidobacteria	Acidobacteria_ Gp4	Gp4			
OTU4526	Proteobacteria					
OTU4972						
OTU5097	Verrucomicrobia	Subdivision3	Subdivision3_genera_in certae sedis			
OTU5141	Acidobacteria	Acidobacteria_ Gp6	Gp6			

Table S5. The taxonomic assignment of indicator OTUs associated with the frozen permafrost.

OTU ID	Phylum	Class	Order	Family	Genus	Species
OTU4						
OTU6	Actinobacteria	Actinobacteria				
OTU13	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	Smithella	Smithella propionica
OTU17						
OTU28	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	Nitrosospira	Nitrosospira multiformis
OTU32	Firmicutes	Clostridia	Clostridiales	Clostridiaceae 1	Clostridium_sensu_stricto	Clostridium_estertheticum_subsp. laramiense
OTU40	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae		
OTU56	Bacteroidetes					
OTU67	Bacteroidetes					
OTU71	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Halomonas	
OTU80	Bacteroidetes	Bacteroidia	Bacteroidales			
OTU81	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	
OTU82	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae		
OTU84						
OTU87	Firmicutes	Bacilli	Bacillales	Bacillaceae_1		
OTU95						
OTU99	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ercella	Ercella succinigenes
OTU100	Firmicutes	Clostridia	Clostridiales	Peptococcaceae 1	Desulfosporosinus	
OTU104	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae		
OTU110	Firmicutes	Clostridia	Clostridiales			
OTU128	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae		
OTU135	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Saccharofermentans	Saccharofermentans acetigenes
OTU136	Bacteroidetes					
OTU138	Proteobacteria					
OTU149						
OTU151						
OTU176	Bacteroidetes					
OTU195						
OTU199	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	Syntrophus	
OTU202	Actinobacteria	Actinobacteria				
OTU204	Firmicutes	Clostridia	Clostridiales	Peptococcaceae 2		
OTU206						
OTU211	Bacteroidetes	Bacteroidia	Bacteroidales			
OTU233						
OTU269						

OTU313	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiacae	Beijerinckia	Beijerinckia_indica_subsp._lacticoenes
OTU328	Armatimonadetes	Armatimonadetes_gp2				
OTU330	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae		
OTU337	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	Bacillus	Bacillus_psychrosaccharolyticus
OTU366						
OTU370	Firmicutes	Bacilli	Bacillales	Paenibacillaceae_1		
OTU372	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae		
OTU376	Firmicutes	Bacilli	Bacillales	Planococcaceae	Sporosarcina	Sporosarcina_psychrophila
OTU379	Ignavibacteriae	Ignavibacteria	Ignavibacteriales	Ignavibacteriaceae		
OTU381						
OTU385	Armatimonadetes	Armatimonadetes_gp2				
OTU407						
OTU414	Firmicutes					
OTU425	Firmicutes	Clostridia	Clostridiales			
OTU491						
OTU564						
OTU771	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ercella	Ercella_succinigenes
OTU857	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Psychrosinus	
OTU896						
OTU1016						
OTU1468						
OTU2041						
OTU2176						
OTU2334	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto	

Table S6. α -diversity metrics of bacterial communities in viable and total assemblages of disturbance gradient soils and permafrost soils.

Group	Observed OTUs	Chao1	Inverse Simpson Diversity	Simpson Evenness	Shannon Diversity	Shannon Evenness	Heip's	Faith's Phylogenetic Diversity	PD/OTU	NRI	NTI	NRI/NTI
A	708	1624.019231	144.083243	0.203507	5.837234	0.889491	0.483493	140.08771	0.197863997	19.01354	7.249364	2.62279
A_PMA	614	1475.038462	129.692308	0.211225	5.658511	0.881389	0.466104	123.81563	0.201654121	14.174452	8.404254	1.68658
F	527	1103.111111	106.826001	0.202706	5.436953	0.867525	0.434869	111.76609	0.212079867	17.069337	7.788324	2.19166
F_PMA	485	935.575	75.639784	0.155958	5.212326	0.842853	0.377108	109.5806	0.225939381	13.554406	5.685879	2.38387
Core_1_95cm	247	467.676471	24.726249	0.100106	4.150408	0.753334	0.253902	61.64078	0.249557814	4.85515	4.330423	1.12117
Core_1_PMA	186	209.522727	26.216063	0.140947	4.008001	0.766972	0.292091	48.39722	0.260200108	6.881259	4.773885	1.44144
Core_2_95cm	310	743.15625	36.95431	0.119207	4.486186	0.782032	0.253902	91.27496	0.294435355	4.373524	4.095833	1.0678
Core_2_PMA	172	172	26.721017	0.155355	4.106583	0.797783	0.34935	52.64157	0.30605564	4.119986	3.377273	1.21992
Core_3_95cm	332	609.471698	32.046067	0.096524	4.459681	0.76823	0.258187	82.58873	0.248761235	5.222707	4.569107	1.14305
Core_3_PMA	210	249.487179	27.768285	0.13223	4.149855	0.776093	0.298683	61.26225	0.291725	5.724337	4.178047	1.3701

Figure S1. Geochemical profiles of Core 1, Core 2, Core 3, and Core 4. Parameters included pH, gravimetric water content, and LOI. Core 4 was not used for later analyses due to the absence of substantial soil organic matter from 70-110 cm in depth.

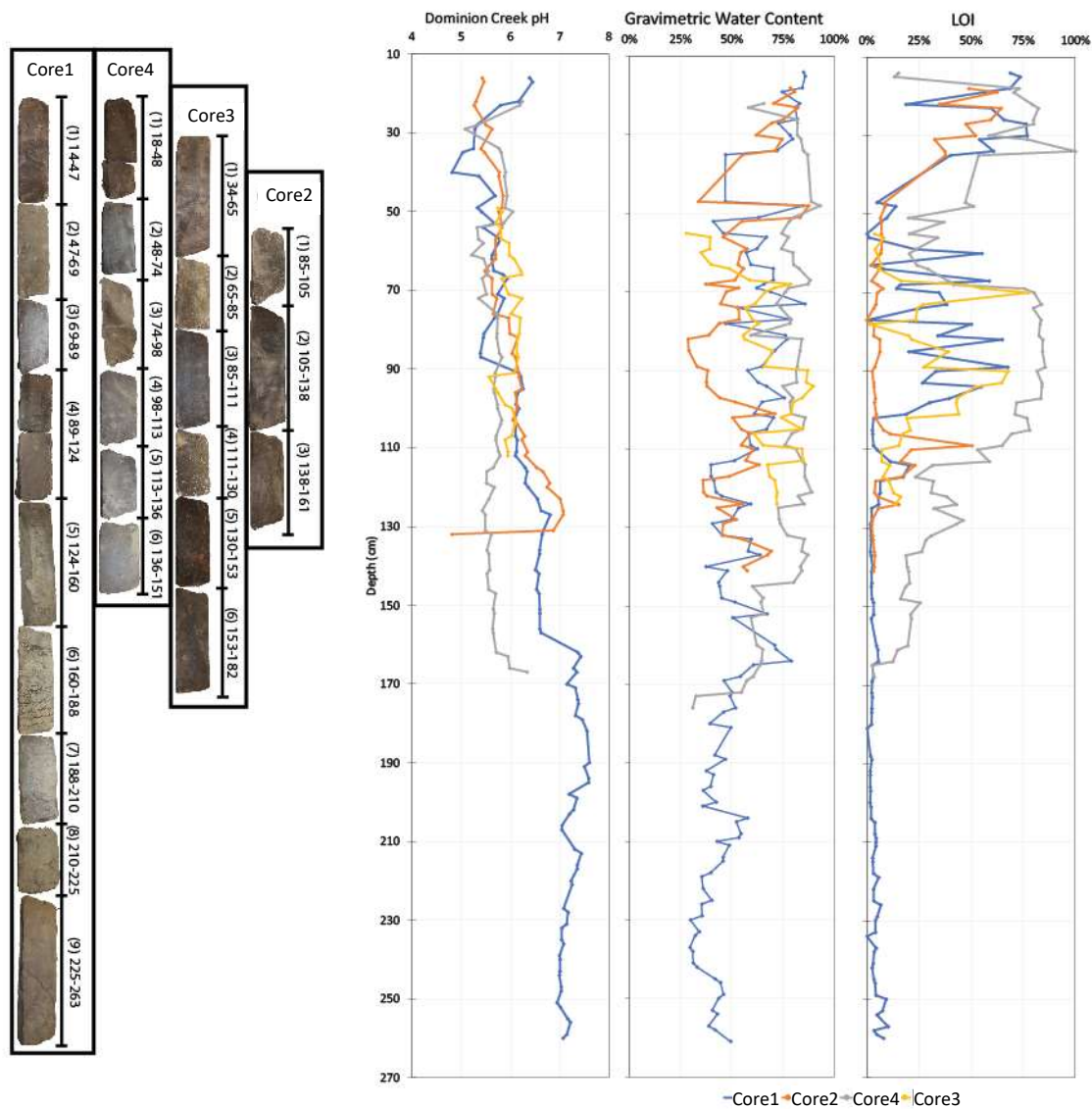


Figure S2. Taxa that are more abundant in active layer soils than permafrost, with FDR multiple test correction analysis ($p < 0.05$). Only taxonomic groups which were $>1\%$ in any sample were included in analysis, and only significantly different groups are shown as assessed using a two-tailed Mann-Whitney U test ($p < 0.05$).

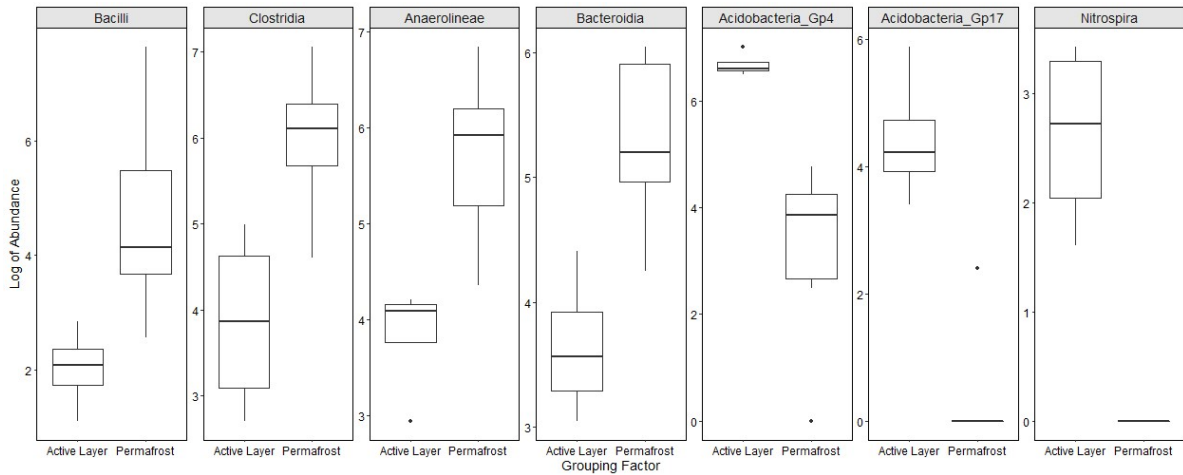


Figure S3. Rarefaction curves present coverage of most samples with the exception of the most disturbed surface soils. Each curve represents the subsampled richness level at each level of sequencing intensity from 0 to 10,000 sequences.

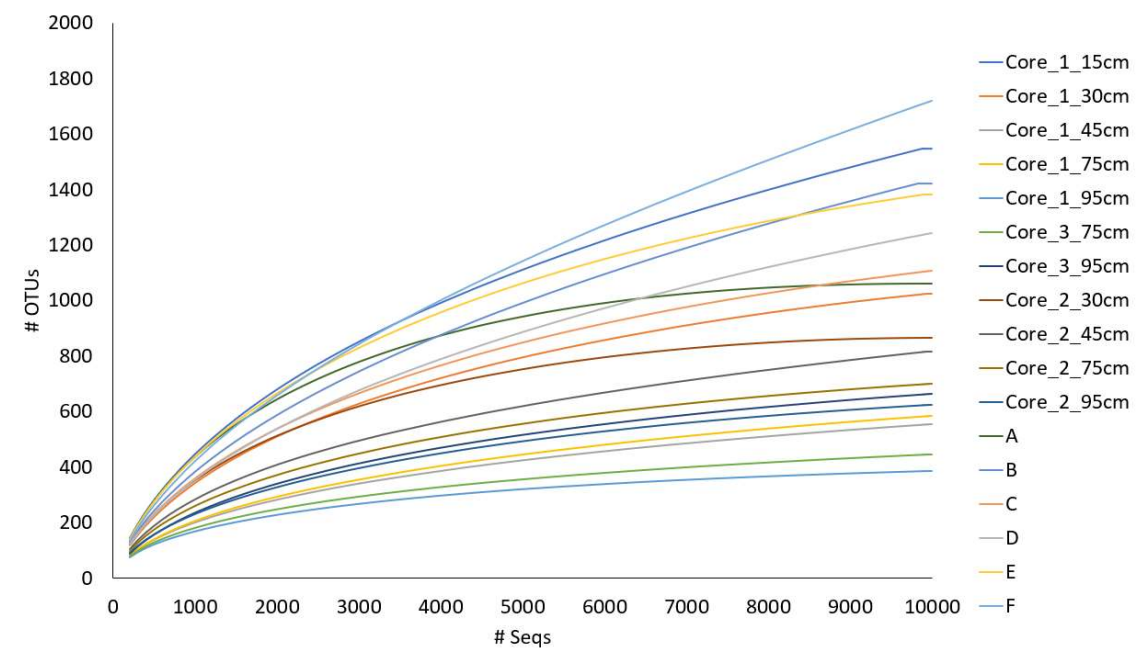


Figure S4. Jaccard distances reflect clustering patterns found in both Bray-Curtis and Unifrac distances. Stress = 0.06.

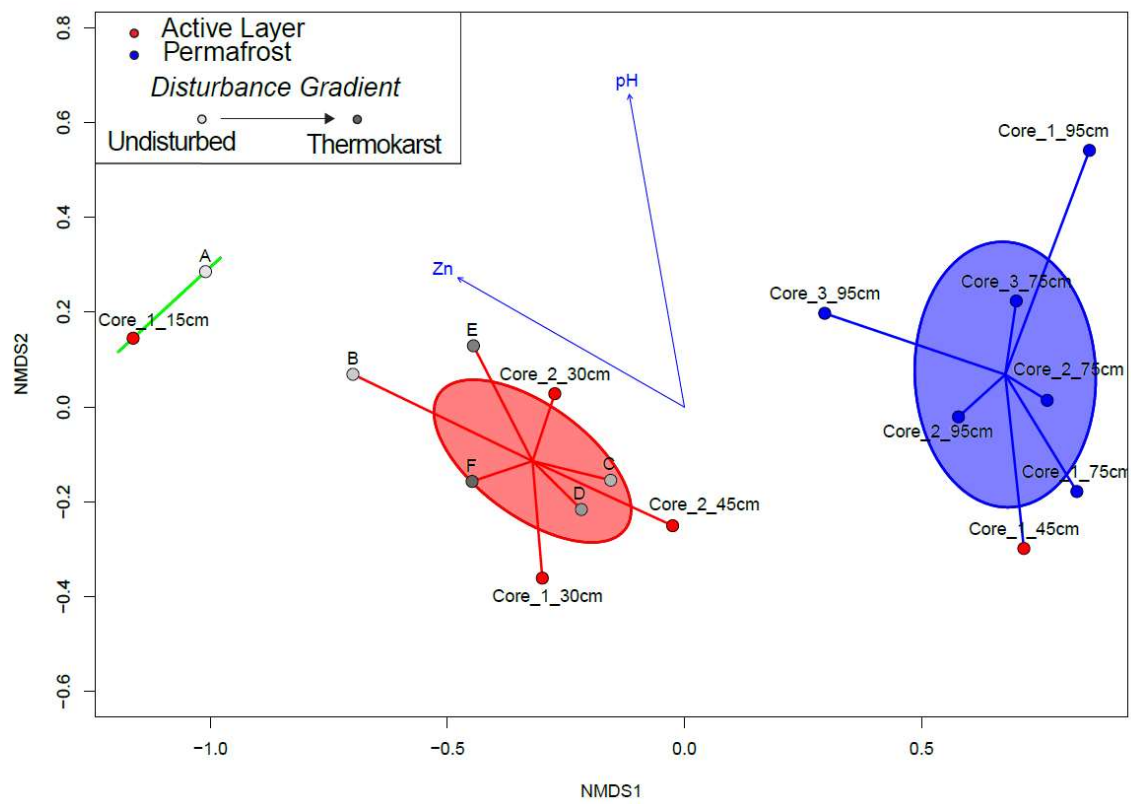


Figure S5. Similarity of viable and total microbial assemblages as visualized by nonmetric multidimensional scaling (NMDS). Ellipses encompass the standard error of sample groups A (green), F (red), viable permafrost (teal) and total permafrost assemblages (blue). Sample points correspond to sample source: A (green), F (red), and permafrost (blue). Ordinations used either (a) Bray Curtis dissimilarity matrices including OTU presence and abundance or (b) a Jaccard dissimilarity matrix assessing the presence/absence of bacterial OTUs. Stress = 0.05.

