Temporal variation in glacier snowpack bacterial communities mediated by nitrogen

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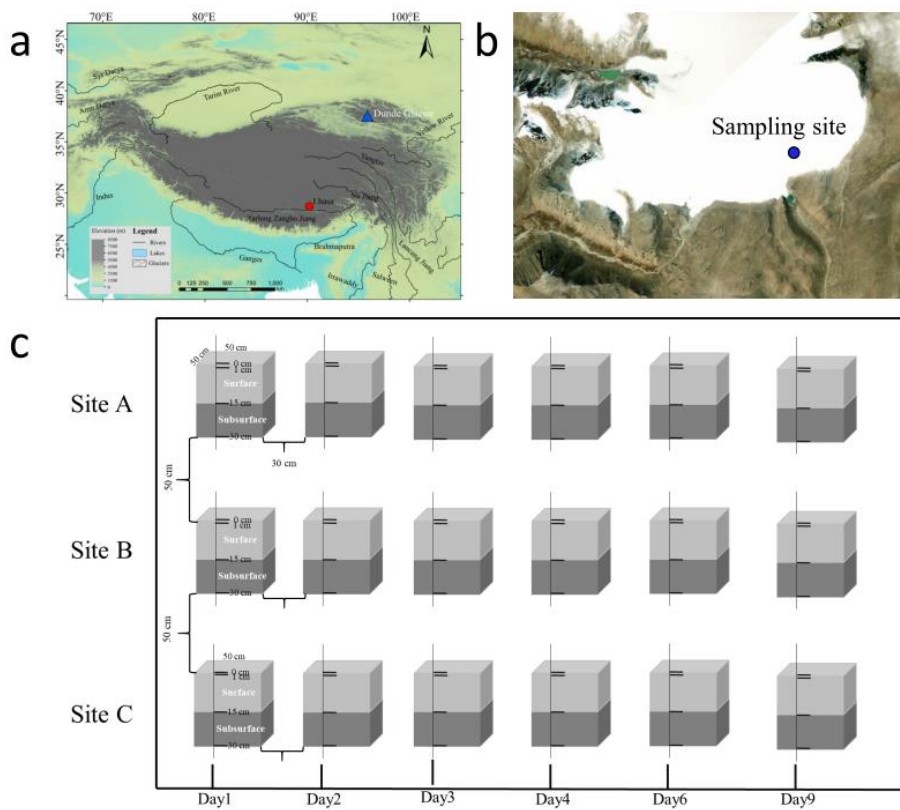
Fig S1 Geographic location of the Dunde glacier (a and b) and schematic of sampling design (c).

NO₃⁻

Slope = 0.0859
Intercept = -0.0019
r = 0.9999
P < 0.001

NH₄⁺

Slope = 0.5278
Intercept = 0.0345
r = 0.9989
P = 0.001

TN

Slope = 74.03
Intercept = 1.4350
r = 0.9997
P < 0.001
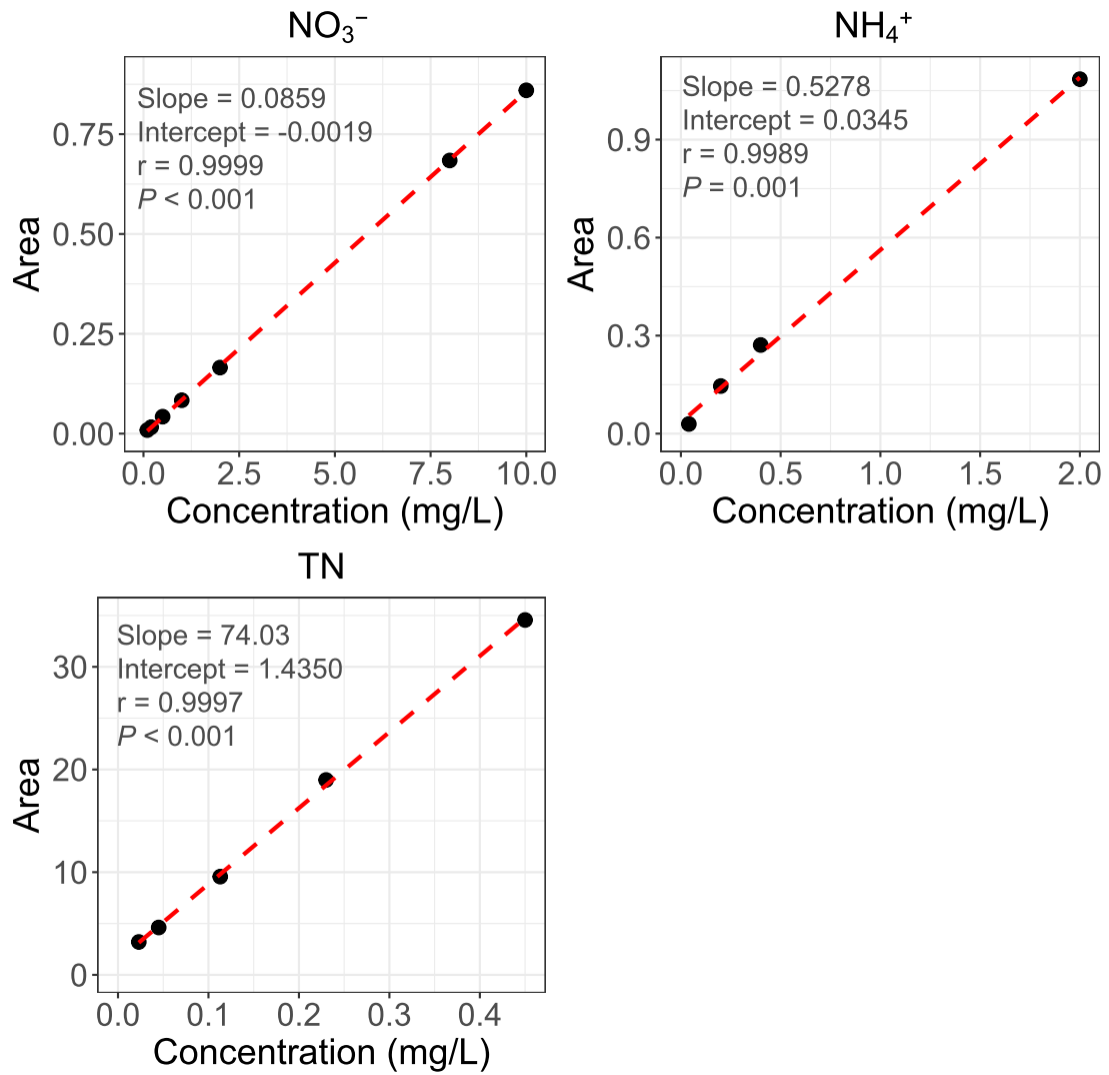
Fig S2 Standard curve of NO_3^-, NH_4^+ ions, and TN. The x-axis is the concentration of the standard sample; the y-axis represents the peak area. Significance is based on Pearson correlations.
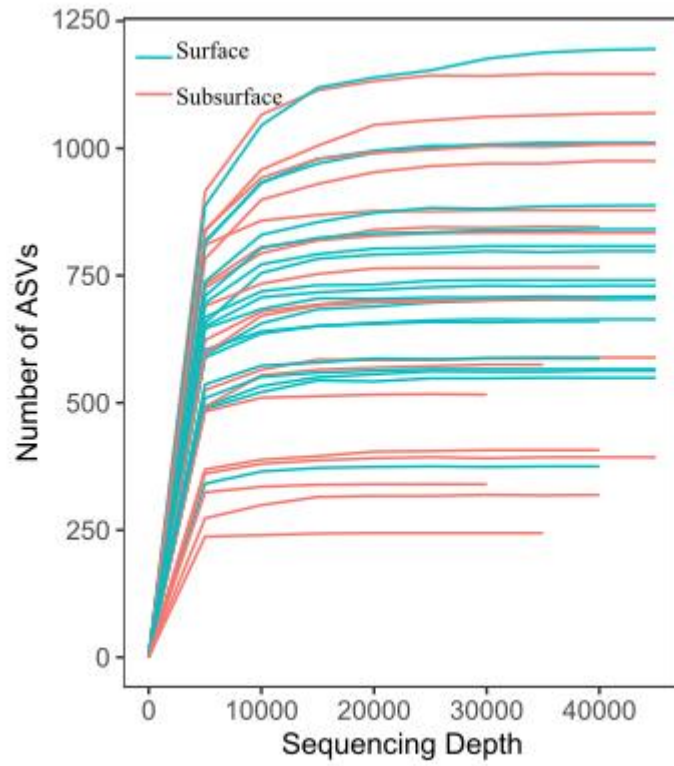
Fig S3 Rarefaction curves for each sample, relating the number of ASVs detected as a function of the sequencing effort, indicating that existing diversity was likely covered by the sequencing effort.
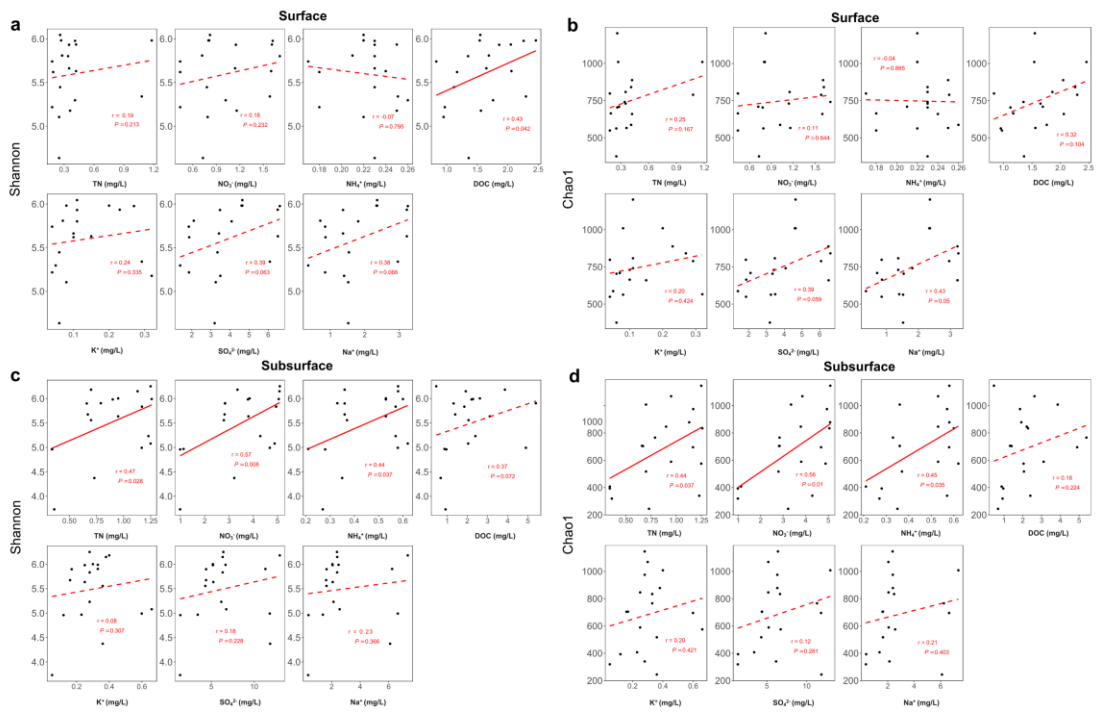
Fig S4 The influence of environmental factors on bacterial diversity. Correlations of Shannon (a, c) and Chao1 (b, d) diversity index with environmental factors in the surface and subsurface layers. Each dot represents an individual sample. The solid and dashed lines indicate significant and nonsignificant changes respectively. Significance is based on Pearson correlations.
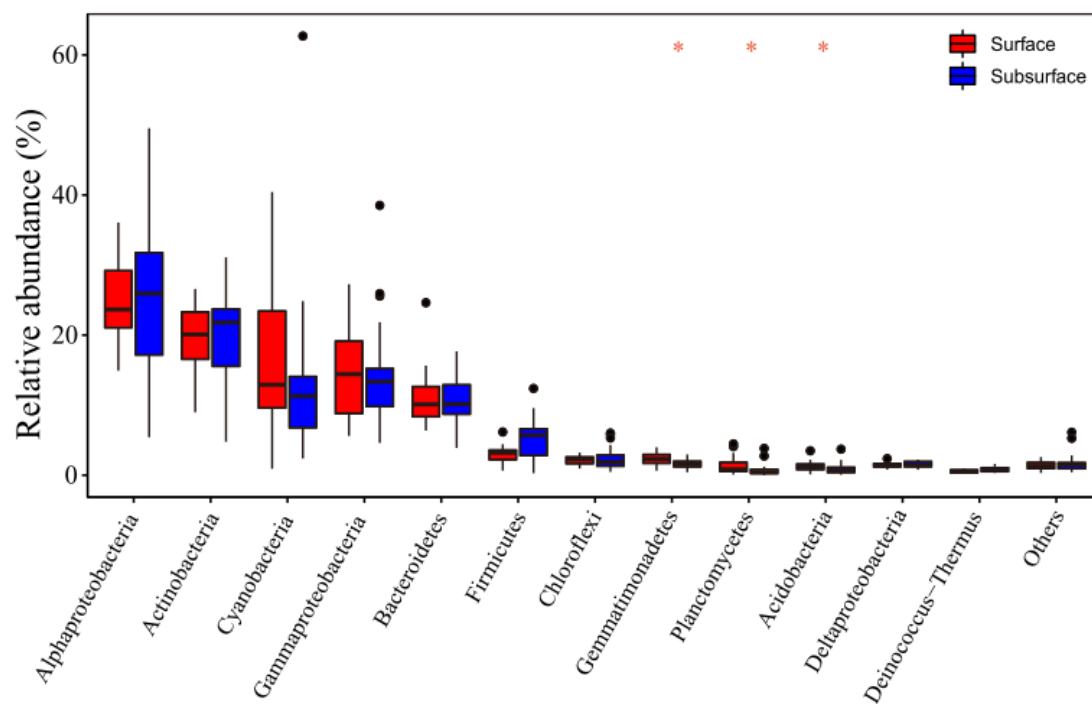
Fig S5 Relative abundances of the dominant (average relative abundance > 1%) bacterial phyla (Proteobacteria is further classified at the class-level) across the surface and subsurface layers. Statistical significance is indicated by $^{*}P < 0.05$ based on Wilcoxon rank-sum test.
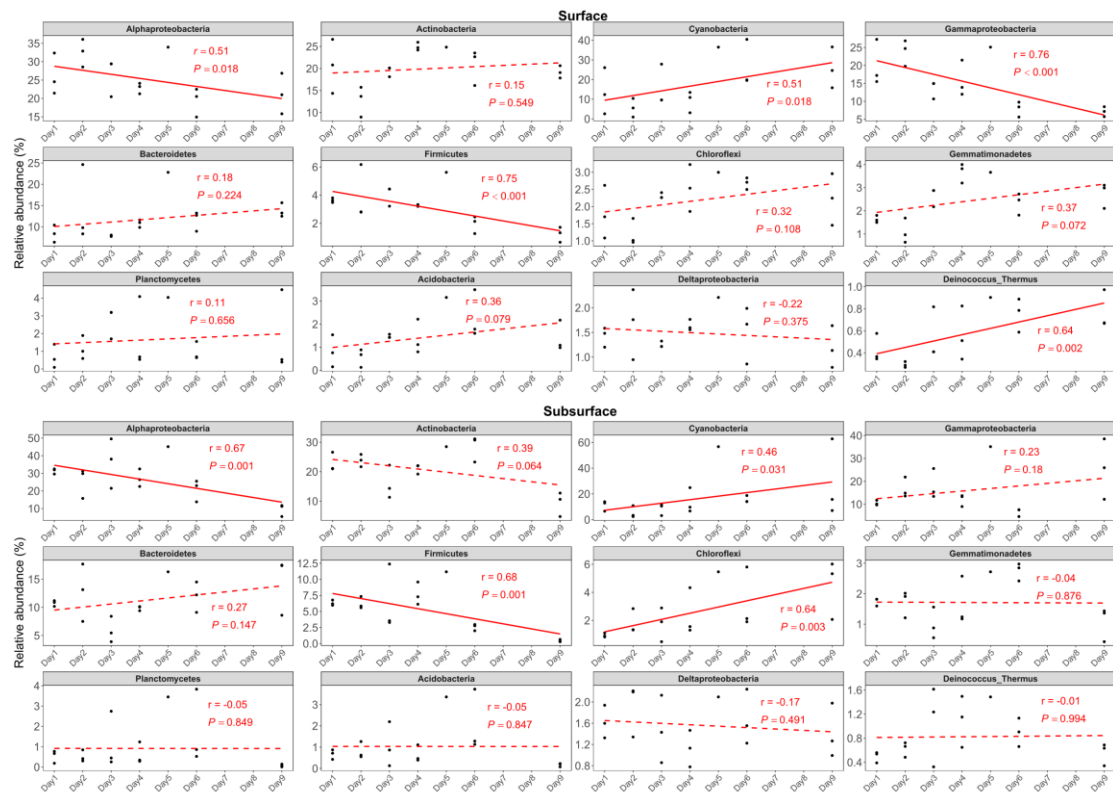
Fig S6 Temporal changes of the dominant bacterial phyla in the surface and subsurface snow layers. Each dot represents an individual sample. The solid and dashed lines indicate significant and nonsignificant changes, respectively. Significance is based on Pearson correlations.
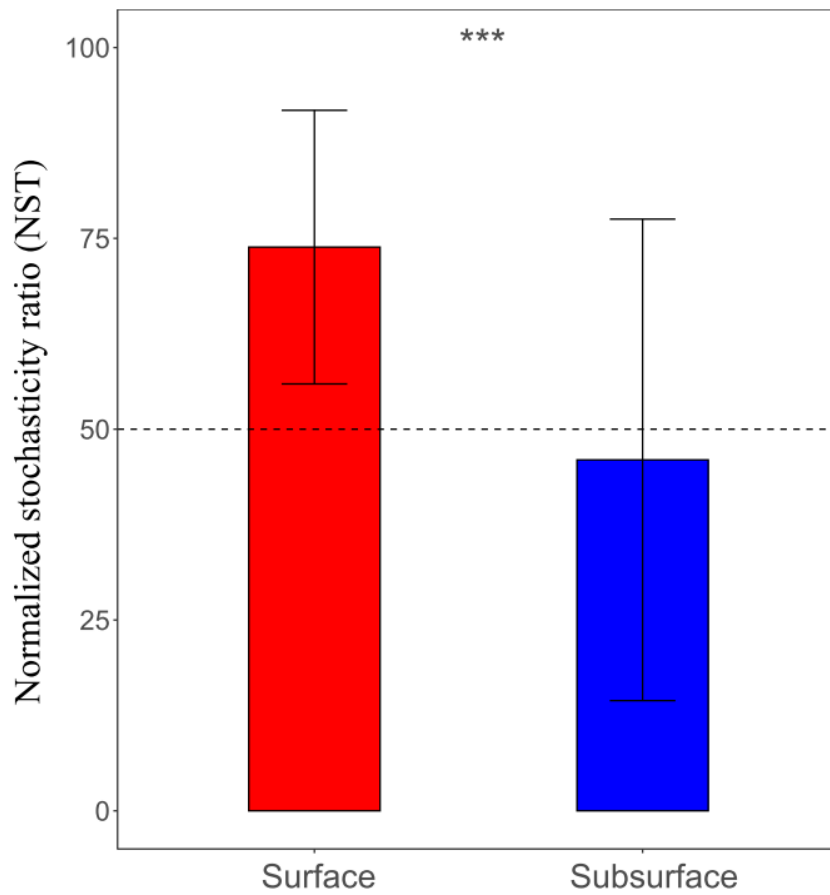
Fig S7 Normalized stochasticity (NST) ratios estimated for the bacterial community in the surface and subsurface layers. Bar plots shows the comparison of NST between the surface and subsurface bacterial communities. Stars indicate significance at $P < 0.001$. The contribution of stochasticity was significantly higher in the surface layer than in the subsurface layer. The contribution of stochasticity was higher and lower than 50% in the surface and the subsurface layer indicating that the community assembly processes are dominated by stochasticity and determinism, respectively.
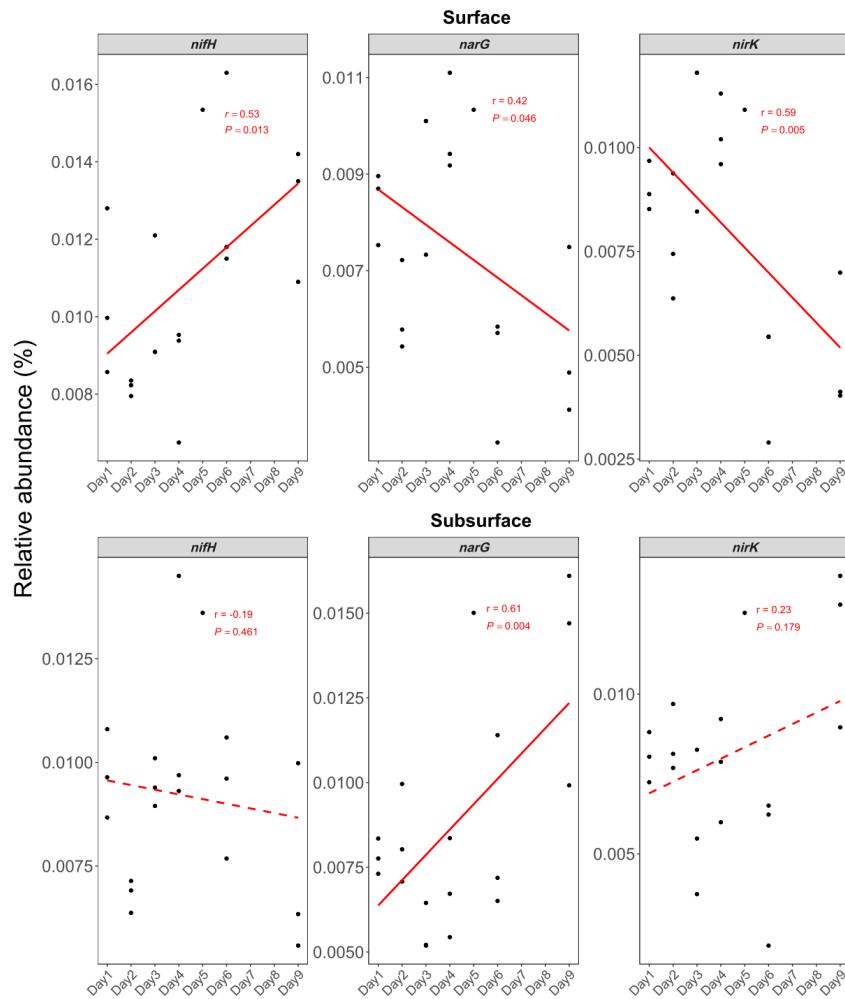
Fig S8. The temporal changes of the relative abundance of nitrogen-cycle related genes in the surface and subsurface layers. The solid and dashed lines indicate significant and nonsignificant changes (based on Pearson correlations), respectively. For the surface layer, the relative abundance of *nifH* gene significantly increases with time, while the relative abundance of *narG* and *nirK* genes significantly decreases with time. In the subsurface layer, the relative abundance of *nifH* gene exhibits no significant change with time, while the relative abundance of *narG* and *nirK* genes significantly and nonsignificantly increase with time, respectively.

Table S1 physiochemical parameters of the snow samples in the surface and subsurface layer.

Depth	Time	Sample name[a]	DOC (mg/L)	TN (mg/L)[b]	NO_3^- (mg/L)[b]	NH_4^+ (mg/L)[b]	K^+ (mg/L)[b]	SO_4^{2-} (mg/L)[b]	Na^+ (mg/L)
Surface	Day1	D1.1s	0.97	0.18	0.44	0.18	0.04	1.85	0.85
		D1.2s	1.17	0.19	0.44	0.18	0.10	1.86	0.86
		D1.3s	0.83	0.18	0.44	0.17	0.04	1.84	0.85
	Day2	D2.1s	0.95	0.25	0.79	0.22	0.08	3.25	1.52
		D2.2s	1.12	0.26	0.78	0.23	0.06	3.34	1.54
		D2.3s	1.36	0.25	0.73	0.23	0.06	3.19	1.52
	Day3	D3.1s	2.46	1.18	0.81	0.22	0.20	4.64	2.36
		D3.2s	1.55	0.26	0.82	0.22	0.11	4.64	2.36
		D3.3s	1.54	0.27	0.80	0.22	0.08	4.60	2.34
	Day4	D4.1s	2.26	0.41	1.62	0.23	0.27	6.60	3.25
		D4.2s	2.05	0.42	1.61	0.25	0.23	6.49	3.22
		D4.3s	2.06	0.42	1.60	0.24	0.15	6.50	3.22
	Day6	D6.1s	1.35	0.34	1.71	0.23	0.11	4.08	1.81
		D6.2s	1.69	0.28	0.72	0.21	0.07	2.10	0.71
		D6.3s	1.76	0.41	1.02	0.26	0.05	1.39	0.36
	Day9	D9.1s	1.65	0.35	1.14	0.23	0.10	3.42	1.37
		D9.2s	1.86	0.35	1.15	0.23	0.11	3.47	1.37
		D9.3s	1.56	0.36	1.16	0.25	0.32	3.49	1.40
Subsurface	Day1	D1.1ss	2.21	0.90	3.78	0.53	0.25	5.18	2.06
		D1.2ss	2.62	0.95	3.85	0.53	0.30	5.18	2.05
		D1.3ss	3.11	0.95	3.80	0.53	0.25	5.23	2.07
	Day2	D2.1ss	1.85	1.17	4.94	0.57	0.28	6.32	2.38
		D2.2ss	1.92	1.13	5.08	0.58	0.38	6.32	2.39
		D2.3ss	0.46	1.25	5.09	0.58	0.28	6.40	2.37
	Day3	D3.1ss	2.28	1.26	5.05	0.60	0.33	6.66	2.48
		D3.2ss	2.04	1.25	4.88	0.62	0.66	6.74	2.56
		D3.3ss	2.40	1.23	4.29	0.57	0.28	6.16	2.13
	Day4	D4.1ss	1.32	0.66	2.84	0.36	0.17	4.39	1.64
		D4.2ss	2.06	0.70	2.79	0.37	0.36	4.30	1.64
		D4.3ss	1.40	0.67	2.82	0.36	0.16	4.38	1.61
	Day6	D6.1ss	3.86	0.70	3.35	0.36	0.40	12.98	7.34
		D6.2ss	5.39	0.79	2.84	0.33	0.33	11.31	6.24
		D6.3ss	0.68	0.73	3.22	0.35	0.36	11.86	6.10
	Day9	D9.1ss	0.92	0.36	0.99	0.27	0.05	1.36	0.35
		D9.2ss	0.87	0.34	1.13	0.21	0.23	3.41	1.38
		D9.3ss	0.96	0.34	0.99	0.28	0.12	1.38	0.37

[a.] The "s" and "ss" suffix after the name of sampling represented surface and subsurface snow layers, respectively.

[b.] The environmental parameters exhibits significant difference between the surface and subsurface snow layers.

Table S2 Topological properties comparison between empirical and random networks for the bacterial communities in surface and subsurface snow layers.

	Empirical Network		Random Network	
	Surface	Subsurface	Surface	Subsurface
Modularity	0.65	0.40	0.43±0.008	0.27±0.029
Connectedness (Con)	0.71	0.86	0.39±0.035	0.97±0.007
Transitivity (Trans)	0.45	0.49	0.05±0.010	0.23±0.010
Density (D)	0.02	0.06	0.02±0.000	0.06±0.000
Average clustering coefficient (avgCC)	0.31	0.39	0.05±0.010	0.18±0.015
Average path distance (GD)	5.51	4.72	3.44±0.055	2.73±0.048