

Supplementary Information

Microbial processes in the weathering crust aquifer of a temperate glacier

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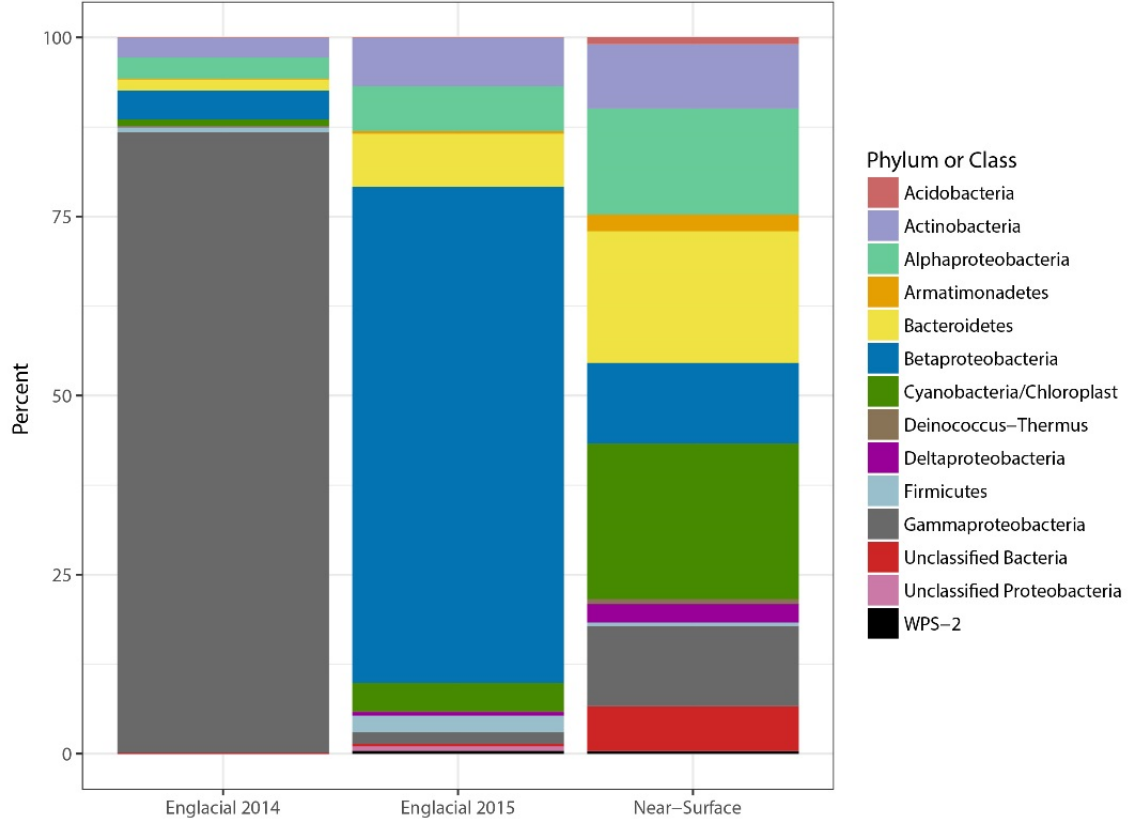
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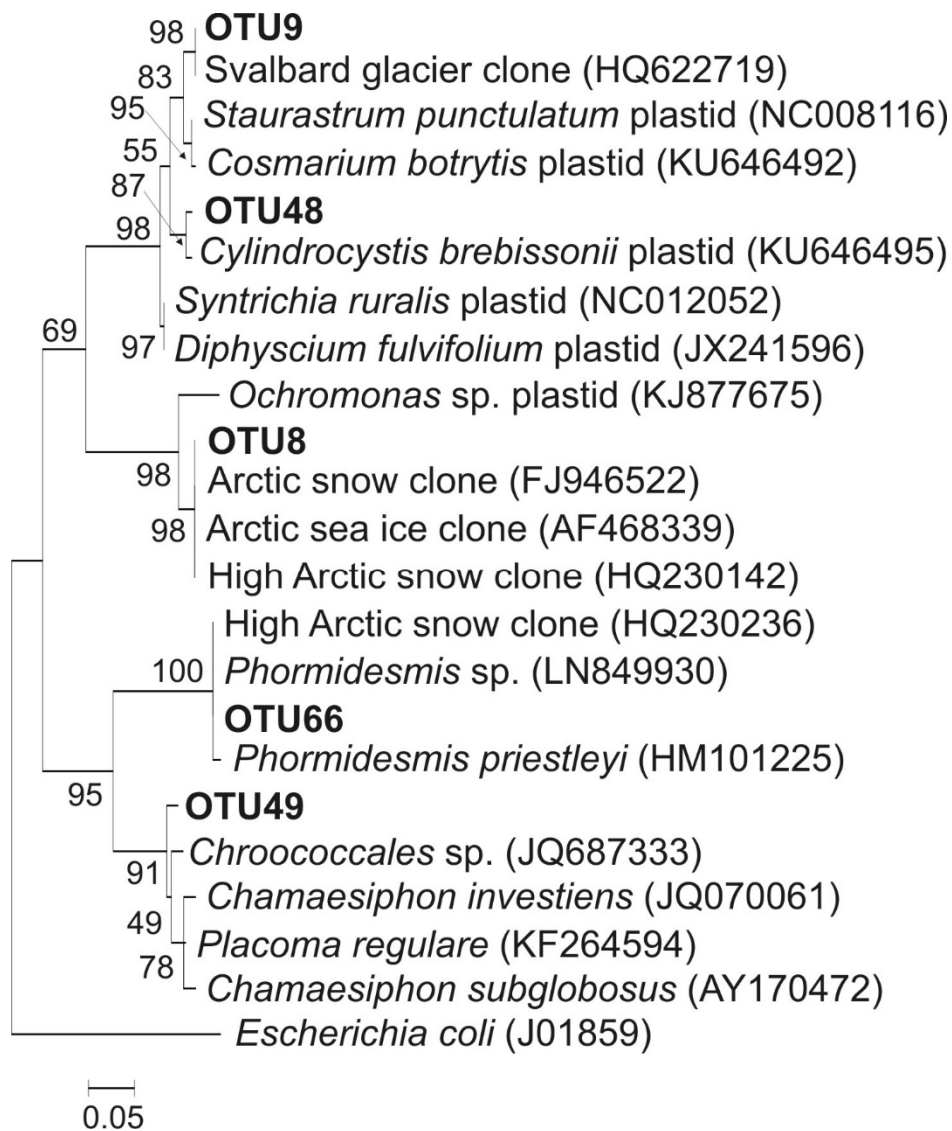
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Supplementary Figure 1. The relative abundance of bacterial phyla within samples obtained from the deepest boreholes ('Englacial 2014' and 'Englacial 2015') with those in near-surface ice and water. All sequences are classified at the phylum level with the exception of the Proteobacteria, which are designated by class.



Supplementary Figure 2. Phylogenetic analysis of 16S rRNA gene sequences (positions 533 to 786, *Escherichia coli* numbering) related to phototrophic cyanobacteria and algal plastids. Genbank accession numbers are listed in parentheses. The scale bar represents 0.05 substitutions per site and bootstrap values are shown as a percentage of 1000 replications.

Supplementary Table 1 Year, volume, and borehole properties for the samples collected in this study.

Sample [†]	Internal designation	Year of sampling	Sample volume (L)	Borehole	Diameter (cm)	Depth (m)
BH1a (0.8µm)	En1_8µm	2014	10	1	30	10
BH1a (3µm)	En1_3µm	2014	10	1	30	10
BH1b	En2	2014	50	1	30	10
BH1c (0.2µm)	Mat-En-3 (0.2µm)	2014	404	1	30	10
BH1c (0.8µm)	Mat-En-3 (0.8µm)	2014	404	1	30	10
BH1c (3µm)	Mat-En-3 (3µm)	2014	404	1	30	10
BH2a (0.2µm)	Mat-En-6 (0.2µm)	2014	27	2	30	15
BH2a (0.8µm)	Mat-En-6 (0.8µm)	2014	27	2	30	15
BH2a (3µm)	Mat-En-6 (3µm)	2014	27	2	30	15
BH2b	Mat-En-11	2014	40	2	30	15
BH2c	Mat-En-12	2014	30	2	30	15
BH2d (0.2µm)	Mat-En-18 (0.2µm)	2014	176	2	30	30
BH2d (0.8µm)	Mat-En-18 (0.8µm)	2014	176	2	30	30
BH2d (3µm)	Mat-En-18 (3µm)	2014	176	2	30	30
BH3	Mat-En-8	2014	40	3	30	4
BH4a	Mat-En-13	2014	28	4	10	4
BH4b	Mat-En-19	2014	20	4	10	4
BH5	En15	2014	50	5	30	7
Supraglacial	Sp2	2014	61	-	-	-
BH6a	Val 3 filter #3	2015	20	Val2	30	4.4 - 5.2
BH6b	V3F6	2015	29	Val2	30	5.3 - 5.5
BH6c	Val4	2015	20	Val2	30	8.3 - 9.8
BH7a	Val 5 filter #3	2015	30	Val3	30	1.4 - 4.4
BH7b	Val 5 filter #6	2015	30	Val3	30	4.4 - 6.8
BH7c	Val 6 filter #3	2015	60	Val3	30	11.1 - 15.4
BH8	B2D	2015	60	2	10	5
BH9	B3_15	2015	ND	3	10	4
BH10a	B4D	2015	60	4	10	4
BH10b	B4R2	2015	25	4	10	4
BH10c	B4R3	2015	60	4	10	4
BH10d	B4R4	2015	25	4	10	4

[†] Ten boreholes, designated BH1 to BH10, were sampled. Multiple samples obtained from the same borehole are indicated by letters (e.g., BH1a, BH1b, and BH1c). Names followed by parentheses (e.g., 0.2µm) specify filter pore sizes for samples that were size-fractionated.

ND = no data available

Supplementary Table 2 Concentration of major ions and elements (ppb) in melt water from discrete ice depths (BH6 and BH7) and the WCA (sample BH10b). The limit of detection is listed parenthetically below each analyte.

Depth (m) or sample	Na ⁺ (0.40)	Mg ²⁺ (0.26)	Al ³⁺ (0.69)	K ⁺ (0.95)	Ca ²⁺ (0.31)	Cr ³⁺ (0.24)	Mn _(aq) (0.21)	Fe _(aq) (0.19)	Co ²⁺ (0.06)	Ni ²⁺ (0.37)	Zn ²⁺ (2.92)	Sr ²⁺ (0.25)	Ba ²⁺ (0.37)	Pb ²⁺ (0.33)	F ⁻ (112)	Cl ⁻ (90)	SO ₄ ²⁻ (41)	Br ⁻ (520)	NO ₃ ⁻ (53)	HCO ₃ ⁻ †
1.453	25.91	6.54	3.56	12.35	62.49	BD	0.47	3.09	0.79	0.48	8.90	0.33	BD	0.62	BD	BD	152.4	BD	BD	74.10
1.537	18.96	5.08	2.60	8.87	54.45	BD	0.37	1.86	0.68	BD	8.67	0.29	BD	0.47	BD	BD	98.7	BD	BD	192.68
1.557	22.94	4.90	2.87	11.65	57.10	0.25	0.41	2.37	0.67	0.46	9.45	0.30	BD	0.41	BD	BD	120.0	BD	BD	201.23
1.569	28.88	6.05	4.29	16.25	76.22	0.27	0.46	1.83	0.67	0.63	10.21	0.35	BD	0.58	BD	BD	119.8	BD	90.6	199.09
1.622	25.32	5.18	4.64	13.06	96.49	0.24	0.44	2.02	0.63	0.56	10.05	0.33	0.42	0.57	BD	BD	133.8	BD	54.2	255.81
2.499	7.72	1.59	2.33	3.54	21.38	BD	BD	0.82	0.51	BD	BD	BD	BD	BD	BD	BD	122.2	BD	BD	0.00
2.709	9.13	1.47	2.61	3.80	19.79	BD	BD	0.95	0.44	BD	BD	BD	BD	BD	BD	BD	132.7	BD	BD	0.00
3.312	20.28	6.06	4.81	6.12	110.61	BD	0.38	4.99	0.34	BD	BD	BD	0.72	BD	BD	BD	157.2	BD	101.2	188.99
4.042	15.88	6.08	2.14	3.64	71.35	BD	0.26	0.87	0.36	BD	BD	BD	0.49	BD	BD	BD	127.8	BD	BD	165.60
4.066	11.30	6.79	1.46	4.23	60.57	BD	0.31	1.41	0.38	BD	6.90	BD	0.40	BD	BD	BD	179.1	BD	BD	27.83
4.357	19.36	2.65	1.32	2.89	28.82	BD	BD	0.82	0.31	BD	BD	BD	BD	BD	BD	BD	131.3	BD	BD	65.13
5.274	10.89	1.66	1.50	4.35	47.49	BD	BD	1.15	0.34	BD	BD	BD	BD	BD	BD	BD	96.3	BD	68.4	60.08
5.501	12.31	2.03	1.52	2.79	33.09	BD	BD	0.62	0.35	BD	BD	BD	BD	BD	BD	BD	130.8	BD	BD	64.89
5.538	10.46	1.73	1.53	2.46	40.11	BD	BD	0.45	0.39	BD	BD	BD	BD	BD	BD	BD	114.9	BD	BD	89.43
5.732	10.98	2.10	2.01	2.52	41.09	BD	BD	0.75	0.33	BD	BD	BD	BD	BD	BD	BD	115.2	BD	BD	81.26
5.966	14.35	2.54	0.99	2.18	30.25	BD	BD	1.25	0.66	BD	BD	BD	BD	BD	BD	BD	112.7	BD	BD	74.68
6.332	10.36	1.76	1.19	2.00	27.71	BD	BD	1.34	0.33	BD	BD	BD	BD	BD	BD	132.23	124.1	BD	BD	0.00
6.354	9.13	1.66	1.14	2.27	33.64	BD	BD	1.45	0.30	BD	BD	BD	BD	BD	BD	220.24	126.0	BD	BD	0.00
6.428	11.21	1.45	1.28	1.35	44.34	BD	BD	2.13	0.26	BD	BD	BD	0.39	BD	BD	BD	121.9	BD	BD	0.00
6.592	7.79	2.58	1.73	1.45	31.99	BD	BD	1.28	0.28	BD	3.47	BD	0.39	BD	BD	BD	115.7	BD	BD	0.00
7.499	32.92	7.80	1.80	5.25	102.92	BD	0.53	2.08	0.31	BD	BD	0.44	BD	BD	BD	197.00	205.5	BD	59.8	0.00
8.052	22.68	10.76	8.71	7.31	87.03	BD	0.70	9.38	0.33	BD	BD	0.41	BD	BD	BD	124.06	176.6	BD	110.2	0.00
8.134	12.77	10.73	10.12	7.35	64.89	0.25	0.86	13.74	0.34	BD	2.93	0.39	BD	BD	BD	191.19	181.5	BD	85.3	0.00
8.256	36.50	12.32	3.80	6.21	94.45	BD	0.93	4.79	0.34	BD	BD	0.49	BD	BD	BD	168.60	159.9	BD	BD	64.17
WCA*	91.14	5.32	11.11	54.55	31.81	0.05	0.64	10.51	0.03	0.20	6.08	NA	0.16	0.03	18.80	62.04	44.6	5.00	10.40	253.90

BD = below the analytical limit of detection; NA = not analyzed

† HCO₃⁻ was not analytically determined and the concentration was estimated based on charge balance.

*The WCA sample (BH10b) was pre-concentrated by a factor of 100 before analysis. The limit of detection for Na⁺ (1.0), Mg²⁺ (1.0), Al³⁺ (0.017), K⁺ (0.2), Ca²⁺ (0.7), Cr⁺³ (0.009), Mn_(aq) (0.007), Fe_(aq) (1.3), Co⁺² (0.007), Ni⁺² (0.004), Zn⁺² (0.006), Ba⁺² (0.002), Pb⁺² (0.001), F⁻ (0.11), Cl⁻ (0.09), SO₄²⁻ (0.04), Br⁻ (0.5), and NO₃⁻ (0.05) in the WCA sample corrects for the pre-concentration factor.

Supplementary Table 3 Number of sequences, coverage, and alpha diversity in the samples.

Sample	Number of sequences	Total OTUs [†]	Good's coverage estimator	Inverse Simpson index
BH1a (0.8µm)	222906	313	0.998	22.7
BH1a (0.3µm)	126933	231	0.998	23.3
BH1b	106903	235	0.998	22.5
BH1c (0.2µm)	240477	1574	0.981	4.34
BH1c (0.8µm)	183232	1127	0.985	12.5
BH1c (3µm)	312584	1226	0.985	2.83
BH2a (0.2µm)	198979	825	0.991	3.47
BH2a (0.8µm)	242377	647	0.994	2.58
BH2a (3µm)	178910	864	0.989	3.32
BH2b	270665	1303	0.988	3.29
BH2c	277139	1151	0.989	3.68
BH2d (0.2µm)	214399	767	0.992	3.64
BH2d (0.8µm)	174365	680	0.992	3.72
BH2d (3µm)	219582	1192	0.982	7.59
BH3	108663	936	0.989	29.0
BH4a	27992	458	0.995	27.6
BH4b	98201	860	0.990	10.4
BH5	180836	1331	0.980	9.95
Supraglacial	214369	1623	0.978	20.0
BH6a	144092	3429	0.961	18.7
BH6b	33811	870	0.981	11.8
BH6c	340637	4651	0.983	4.60
BH7a	9151	202	0.996	4.06
BH7b	37750	518	0.990	5.64
BH7c	48403	648	0.987	7.09
BH8	725033	8815	0.983	3.30
BH9	285306	1444	0.982	20.0
BH10a	276258	5274	0.974	49.6
BH10b	650442	9433	0.978	4.08
BH10c	659868	10587	0.977	27.1
BH10d	465699	8991	0.974	16.4

[†] Excluding singletons and OTUs identified as potential contaminants.

Supplementary Table 4 Information on the amplified 18S rRNA gene sequences from sample BH10d.

Clone/OTU designation	Clones per OTU	Genbank accession no.	Nearest phylogenetic neighbor (Genbank accession no.)	% identity to the nearest neighbor
BH10d-clone34	4	MH037315	<i>Ancydonema nordenskioldii</i> strain CCCryo BS_0001-2000 (AF514397)	99%
BH10d-clone35	1	MH037316	Uncultured Scuticociliatia clone PR3_4E_59 (GU479975)	97%
BH10d-clone39	1	MH037317	<i>Oberwinklerozyma silvestris</i> strain CBS 11420 (KJ708366)	98%
BH10d-clone40	1	MH037318	<i>Ancydonema nordenskioldii</i> strain CCCryo BS_0001-2000 (AF514397)	91%
BH10d-clone44	7	MH037319	<i>Rotaria rotatoria</i> (DQ089736)	99%
BH10d-clone46	1	MH037320	<i>Pseudophacidium ledi</i> (AF315623)	99%
BH10d-clone55	10	MH037321	<i>Ochromonas</i> sp. CCMP1899 (EF165133)	99%