

## Aberystwyth University

### *Possible interactions between bacterial diversity, microbial activity and supraglacial hydrology of cryoconite holes in Svalbard*

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# Supplementary Data

**Supplementary Data S1 (Text).** Detailed methodology used for real-time PCR, T-RFLP analysis and clone library construction.

**Supplementary Data S2 (Figure).** Detrended correspondence analysis (DCA) of T-RFLP relative abundance profiles from a) 2006 and b) 2007. Coloured convex hulls indicate glacier groups. Distinct community structures are observed between glaciers, in particular, AB (red) appears to be considerably different from ML (blue) and VB (green).

**Supplementary Data S3 (Figure).** Mean linear correlation coefficient of log-transformed T-RFLP abundance data for all pairwise comparisons of all the holes on each glacier was lowest for AB

**Supplementary Data S4 (Table).** Distance matrices (metres) for the holes sampled on each glacier.

**Supplementary Data S5 (Table).** Summary of physiological measurements obtained from cryoconite holes in 2006 and used in CCA (Figure 4). Results of statistical analyses of differences between glaciers are also shown using ANOVA or Kruskal-Wallis (K-W) non-parametric tests.

**Supplementary Data S6 (Table).** Table of data (eigenvalues etc.) relating to the CCA presented as Figure 4.

**•Supplementary Data S7 (Figure).** Analysis of cryoconite dust particles. A) Size distribution of cryoconite ash from the three glaciers. B) Cryoconite granules from AB706 under plane polarising light showing the mineral particles aggregated into cryoconite granules by dark coloured material x10; C) Cross-polarising light micrograph showing a sharp-edged lithic clast x25, AB703; D) A mica particle showing sharp edges also indicative of local origin, ML706 x63

**•Supplementary Data S8 (Table).** Sequence similarity (% identity) of clones identified as 54 OTUs discrete at the 97% identity cut-off threshold along with nearest SEQMATCH hit and predicted T-RF length.

**Supplementary Data S9 (Table).** Chi-squared analysis of clone abundance on different glaciers by OTU, phylum and class.

**Supplementary Data S1 (Text).** Detailed methodology used for Quantitative (real-time) PCR, T-RFLP analysis and clone library construction.

### **1. Real-time PCR estimation of bacterial and archaeal abundance**

DNA (23 ng in 5  $\mu$ l) extracted from the sediment of 12 cryoconites was used as template in real-time PCR reactions using a SYBR Green I Real-Time PCR mix (ABgene, Ltd. Epsom, Surrey, UK). Primer pairs 27F (5'-AGAGTTTGATCCTGGCTCAG) and 1389R (5'-ACGGGCGGTGTGTACAAG) were used for bacteria and either Arch021F (5'-TTCCGGTTGATCCYGCCGGA)-Arch958R (5'-YCCGGCGTTGAMTCCAATT) or *mcr*AF (5'-TTCCGGTGGATCDCARAGRGC) – *mcr*AR (5'-GBARGTCGWAWCCGTAGAATCC) for archaea and methanogenic archaea respectively (each primer at a concentration of 300 nM). Four replicate PCR reactions were conducted for each DNA sample in a Bio-Rad iQ cycler with 15 min at 94°C to activate the hotstart *Taq* polymerase and 30 cycles of 90 sec at 94°C, 54°C and 105 sec at 72°C and a final extension of 30 min at 72°C. Melt curve analyses were conducted on samples after amplification and iQCycler software used to derive  $C_t$  values for each reaction. All reactions were conducted in a single run to allow comparisons.

Real-time PCR with bacterial 16S primers demonstrated the ubiquity and abundance of bacteria in cryoconites (Mean threshold cycle,  $C_t$  19.3 $\pm$  1.80 SD), with lower  $C_t$  values being observed for samples from VB glacier than AB and ML (ANOVA;  $F=6.19$ ,  $P=0.004$ ) for bacteria, as shown in Table 1. However, primers targeting the Archaea and methanogen 16S rRNA gene did not yield any specific product, with  $C_t$  values above 30 and 35 respectively (with no distinct PCR product by melt curve analysis or gel electrophoresis; data not shown). Subsequent attempts to amplify archaeal using the Archaea-specific primers and conditions suggested by Lyimo *et al.* (Arch07 [5'-TTGATCCTGCCAGAGGYA] - Arch1406 [5'-ACGGGCGGTGTGTGCAAG];; Arch021F [5'-TTCCGGTTGATCCYGCCGGA] -Arch1406) were also unsuccessful in amplifying archaeal DNA from cryoconite sediment. Even when nested PCR was tested, cloning and sequencing of the resulting PCR products did not reveal any archaeal 16S rRNA genes (data not shown).

### **2. Terminal Restriction Fragment Length Polymorphism (T-RFLP)**

PCR amplification of DNA extracted from all 36 of the sampled cryoconites was conducted with primers 27F (5' tagged with fluorophore Cy5) and 1389R (without fluorophore). Reactions (25  $\mu$ L) contained 2  $\mu$ l (9 ng) DNA, 300 nM of each primer, 2.75 mM MgCl<sub>2</sub>, 32  $\mu$ g BSA and 200  $\mu$ M of each dNTP in PCR buffer (Colourless Go*Taq* flexi) and Go*Taq* DNA polymerase, according to the manufacturer's instructions (Promega, Inc., Madison, Wisconsin, USA). The amplification conditions were as follows: 10 min at 94 °C, followed by 30 cycles of one min each at 94°C, 53°C and 72°C, followed by 10 min at 72°C.

In order to eliminate residual *Taq* polymerase activity, PCR products were subsequently treated for one hour at 37°C with *Exo*I nuclease (1U per reaction, Fermentas UK, York, UK; to degrade unbound primers) and shrimp alkaline phosphatase (0.025U per reaction, Fermentas UK; to dephosphorylate dNTPs). This treatment prevented both the filling-in of sticky ends and addition of "plus A" artefacts during subsequent restriction digestion.

## Supplementary Data S1 (Text) continued

Restriction digestion of PCR products was conducted in 50 µl volumes with 10 µl of DNA and 30U of *Hae*III (Promega) incubated for five hours at 37°C. Digested DNA was purified using MinElute cleanup columns (Qiagen, Inc, Crawley, West Sussex, UK), eluted into 10 µL water and quantified using a NanoDrop spectrophotometer (Labtech International). Fifty nanograms of DNA were mixed with 40 µl SLS buffer (Beckman-Coulter, High Wycombe, Bucks, UK) and labelled size standard (DNA size Standard Kit -600 [608095]; bands from 50 bp to 600 bp; Beckman-Coulter) and loaded onto a Beckman-Coulter CEQ-8000 capillary electrophoresis system for separation using the Frag-4 program.

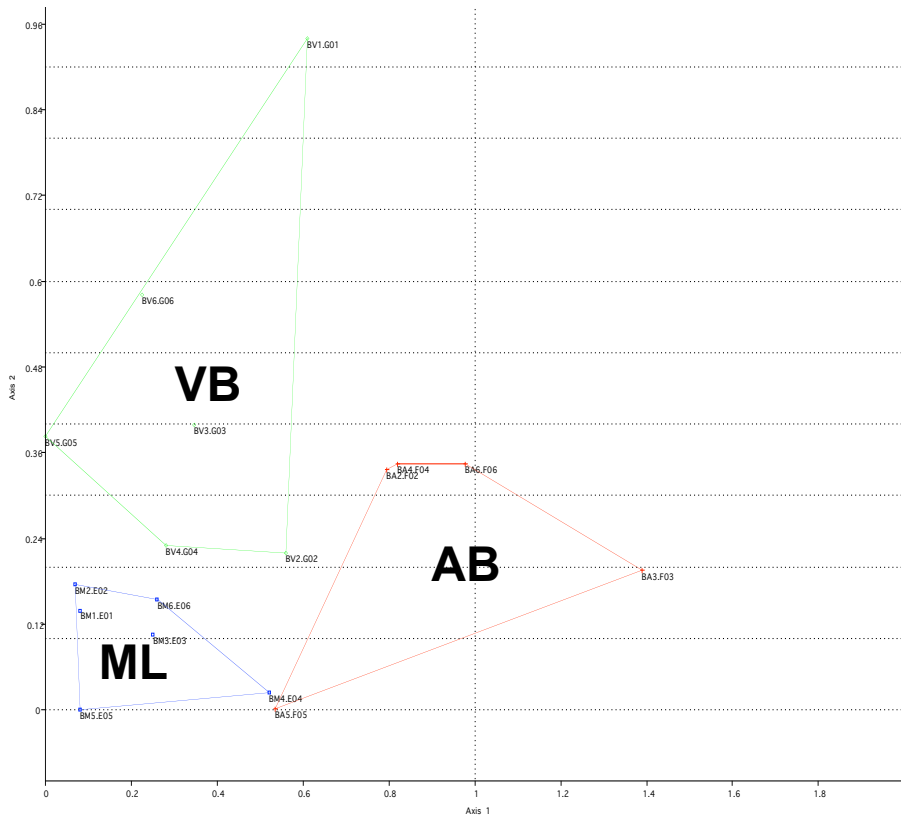
Raw data were analysed using the Fragments module of Beckman-Coulter's CEQ-8000 Genetic Analysis System. Samples were filtered to exclude peaks less than 100 relative fluorescence units or with a slope threshold less than 10% and a relative peak height threshold of 20% before sizing by Local Southern size calling with reference to the 600 bp size standard. Samples showing more than 5% current change per minute or missing more than 3 size standards were excluded from analysis using the *AFLP* routine of the software. A binary presence/absence matrix and a peak height matrix were exported to Microsoft Excel. Subsequently, peak height data was converted to relative abundance with each peak height as percentage of the total sample peak height.

## 2. Clone library construction and sequencing

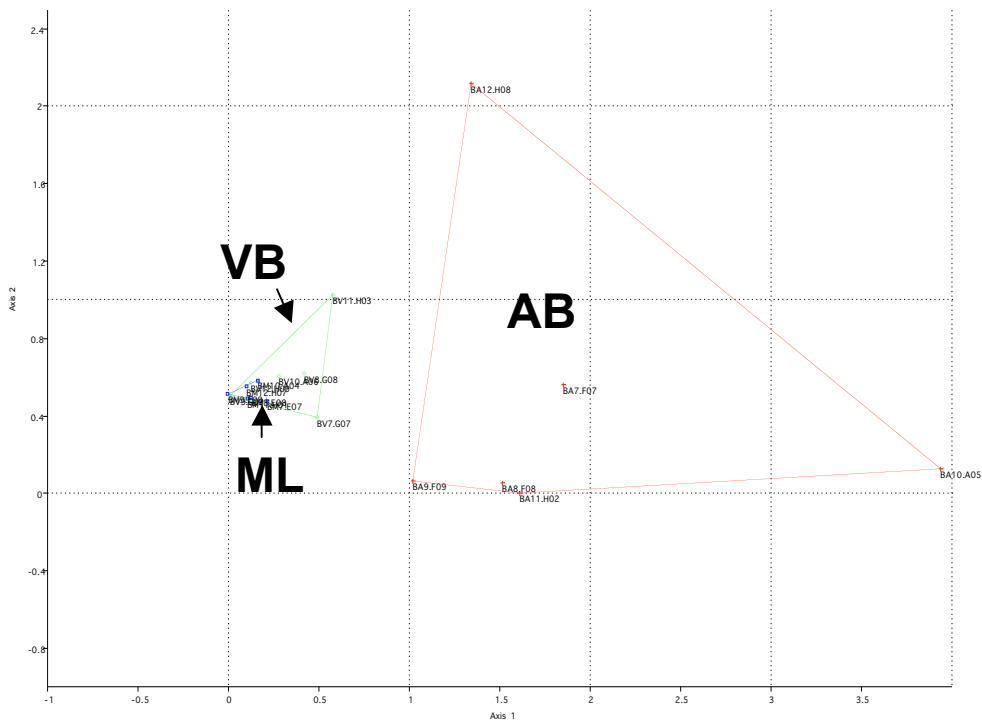
Three clone libraries, one for each glacier, were constructed using the 27F-1389R PCR products obtained from the qPCR experiment (see above and [Table 1](#)). For each glacier, 16 PCR products (four replicates for four samples; two holes per year) were pooled, cleaned to remove residual primers and nucleotides using QIAQuick columns (Qiagen, Ltd. Crawley, Sussex UK) and concentrated using a SpeedVac (Thermo Savant, Reading, UK). Ligation into the T-tailed pGEM-T Easy vector (Promega UK, Ltd. Southampton, UK.) was conducted according to the manufacturer's instructions with 3 µl of PCR product, and transformed into High efficiency JM109 *Escherichia coli* chemically-competent cells (Promega UK, Ltd. Southampton, UK.) Following overnight selection on LB-carbenicillin:X-Gal:IPTG plates, white colonies per glacier were picked and incubated in Terrific Broth supplemented with carbenicillin (100 µg l<sup>-1</sup>) PCR was conducted directly from 2 µl of bacterial cells with primers M13F (5'-GTAAAACGACGGCCAGT) and M13R (5'-CAGGAAACAGCTATGAC). The resulting PCR products (32 per glacier) were purified using Montage Seq96 sequencing reaction plates (Millipore, Inc. Bedford, MA. USA) and sequenced (Macrogen, Inc., Seoul, Korea) using the 27F primer. Potentially chimeric sequences using Bellerophon (Huber et al., 2004) and Pintail (Ashelford et al., 2005). Sequence trace files were imported into *myRDP* (Cole et al., 2007) and trimmed using the Phred and Lucy components, before being individually aligned and classified against the ribosomal database using the RNACAD and the RDP classifier components.

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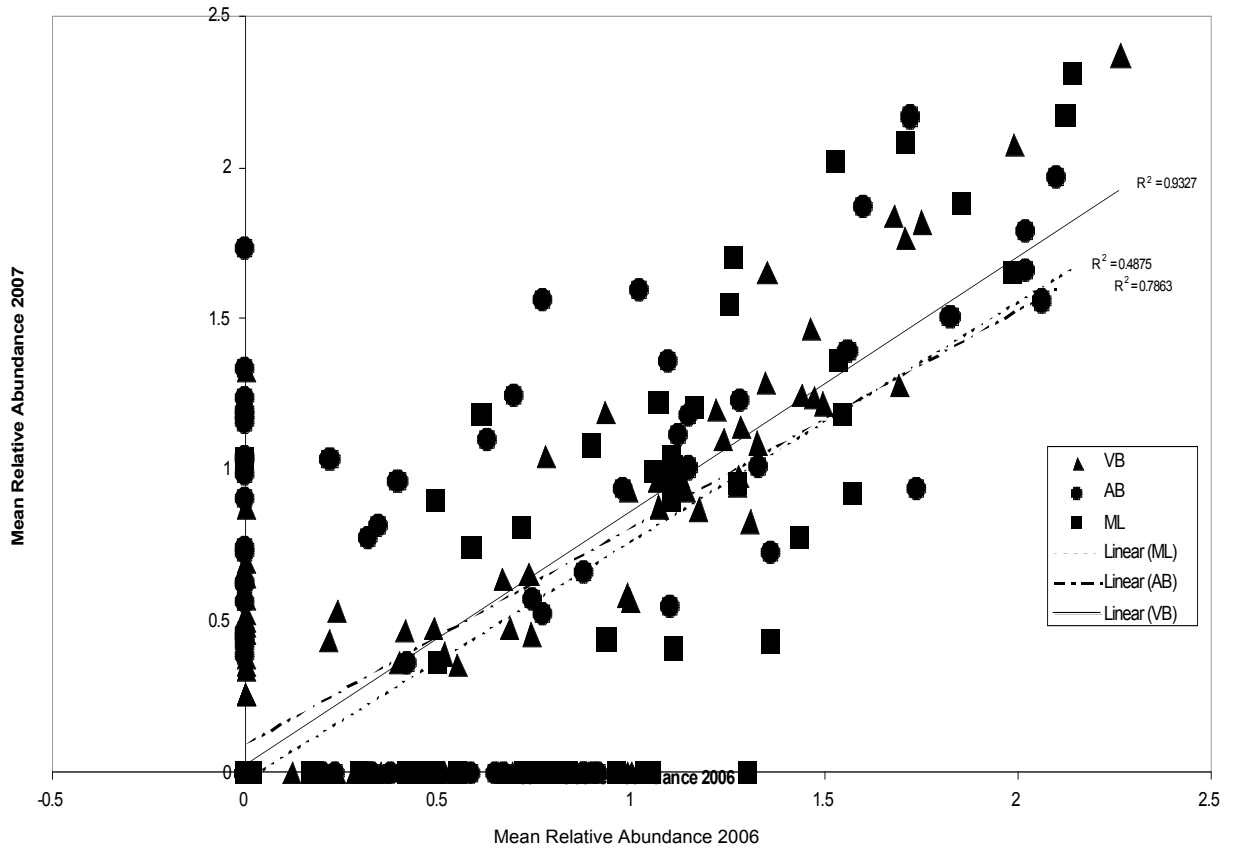


a) DCA of 2006 T-RFLP relative abundances



b) DCA of 2007 T-RFLP relative abundances

**Supplementary Data S2 (Figure).** Detrended correspondence analysis (DCA) of T-RFLP relative abundance profiles from a) 2006 and b) 2007. Coloured convex hulls indicate glacier groups. Distinct community structures are observed between glaciers, in particular, AB (red) appears to be considerably different from ML (blue) and VB (green).



**Supplementary Data S3 (Figure).** Mean linear correlation coefficient of log-transformed T-RFLP abundance data for all pairwise comparisons of all the holes on each glacier was lowest for AB

- **Supplementary Data S4 (Table).** Distance matrices (metres) for the holes sampled on each glacier.

	ML701	ML702	ML703	ML704	ML705	ML706
ML701	0					
ML702	408	0				
ML703	466	61	0			
ML704	651	243	185	0		
ML705	598	193	133	61	0	
ML706	655	250	190	39	57	0

	AB702	AB703	AB704	AB705	AB706
AB702	0				
AB703	37	0			
AB704	23	58	0		
AB705	205	242	185	0	
AB706	334	371	315	130	0

	VB706	VB705	VB704	VB703	VB702	VB701
VB706	0					
VB705	154	0				
VB704	319	166	0			
VB703	625	476	310	0		
VB702	813	662	495	193	0	
VB701	1043	890	724	427	234	0

**Supplementary Data S5 (Table).** Summary of physiological measurements obtained from cryoconite holes in 2006. Only those samples listed in bold font were used for CCA analysis (Figure 4).. Results of statistical analyses of differences between glaciers are also shown using ANOVA or Kruskal-Wallis (K-W) and Mann-Whitney non-parametric tests. Different letters following glacier means indicate those means which are significantly different.

Sample code	Temp (°C)	Area (m <sup>2</sup> )	Volume (l)	% ash weight	Depth (cm)	Sediment	Meltwater	Respiration (µg C g <sup>-1</sup> h <sup>-1</sup> )	DIC (mg l <sup>-1</sup> )	pCO <sub>2</sub> (µatm)	pH
						PP (µg C l <sup>-1</sup> h <sup>-1</sup> )	PP (µg C l <sup>-1</sup> h <sup>-1</sup> )				
AB601	1.0	0.290	5.80	94.99	2.0	2.24	5.01	0.64	0.000	0.0	5.49
<b>AB602</b>	0.9	0.045	4.20	94.49	9.5	0.51	2.45	0.72	0.211	472.6	5.62
<b>AB603</b>	1.5	0.187	4.70	96.39	2.5	2.72	3.38	0.70	0.894	2046.3	5.56
<b>AB604</b>	0.5	0.104	6.20	96.27	6.0	2.99	5.29	0.75	0.401	880.3	5.68
<b>AB605</b>	1.0	0.161	16.10	95.49	10.0	0.79	8.00	0.43	0.283	614.2	5.71
<b>AB606</b>	0.2	0.127	3.80	96.59	3.0	3.38	0.53	0.26	0.727	1585.5	5.70
AB6a	0.2	0.083	1.70	ND	2.0	0.89	1.40	0.59	0.977	2358.8	5.34
AB6b	0.2	0.144	7.20	ND	5.0	1.10	1.13	0.69	0.138	335.5	5.30
AB6c	0.2	0.078	2.70	ND	3.5	0.63	1.66	1.19	0.003	6.4	5.00
AB6d	0.3	0.024	1.20	ND	5.0	0.82	8.41	0.75	0.005	12.1	4.86
AB6e	1.3	0.086	1.70	ND	2.0	0.47	10.49	0.59	0.002	4.8	5.06
AB6f	0.1	0.058	1.20	ND	2.0	4.40	6.57	0.38	0.049	115.7	5.43
AB6g	0.2	0.161	3.20	ND	2.0	1.43	4.32	0.67	0.001	3.3	5.14
AB6h	0.7	0.116	14.50	ND	12.5	2.67	9.01	0.55	0.003	6.4	5.00
AB6i	0.7	0.182	10.90	ND	6.0	5.25	7.43	0.67	0.001	3.7	5.12
AB Mean	0.6a	0.123a	5.67ab	ND	4.9	2.02a	5.01	0.64a	0.246	563.0	5.33
<b>ML601</b>	0.1	0.047	0.47	94.66	1.0	3.97	18.35	0.99	0.307	722.6	5.46
<b>ML602</b>	0.2	0.069	2.80	95.30	4.0	31.04	0.72	1.30	0.186	409.9	5.67
<b>ML603</b>	0.2	0.051	2.00	94.16	4.0	14.13	0.46	0.95	0.292	674.2	5.53
ML6a	0.1	0.023	0.70	ND	3.0	3.04	8.46	1.24	0.271	613.3	5.60
ML6b	0.1	0.023	3.30	ND	14.0	7.38	1.02	1.13	0.057	134.0	5.47
ML6c	0.2	0.055	3.30	ND	6.0	12.12	1.58	1.13	0.000	0.1	5.83
ML6d	0.1	0.064	1.30	ND	2.0	17.20	4.12	0.91	0.184	402.6	5.69
ML6e	0.2	0.032	1.30	ND	4.0	21.00	4.77	1.37	0.097	206.4	5.75
ML6f	0.1	0.044	2.60	ND	6.0	31.81	6.40	1.28	0.750	1767.7	5.45
ML6g	0.1	0.046	1.90	ND	4.0	7.02	0.38	1.44	0.000	0.4	5.60
ML Mean	0.1b	0.041b	2.06a	ND	5.6	14.22b	3.82	1.22b	0.194	446.4	5.63
<b>VB601</b>	0.1	0.175	19.30	93.36	11.0	8.43	3.10	1.33	0.206	452.6	5.68
<b>VB602</b>	0.1	0.119	3.60	91.82	3.0	10.13	2.40	1.58	0.000	0.7	5.47
<b>VB603</b>	0.2	0.043	1.70	91.36	4.0	5.43	5.28	1.42	0.000	0.6	5.53
VB6a	0.5	0.178	7.10	ND	4.0	4.24	10.85	1.41	0.000	0.8	5.46
VB6b	0.5	0.094	14.10	ND	15.0	15.51	5.45	1.40	0.001	2.7	5.19
VB6c	0.2	0.029	0.86	ND	3.0	2.24	0.05	0.92	1.745	1582.7	6.66
VB Mean	0.3ab	0.106ab	7.78b	ND	6.7	7.66b	4.52	1.34b	0.325	340.0	5.67
ANOVA (p)	ND	ND	ND	**	ND	ND	0.961	<b>&lt;0.001</b>	ND	ND	ND
ANOVA (F)	ND	ND	ND	**	ND	ND	0.04	34.65	ND	ND	ND
KW (p)	<b>0.004</b>	<b>0.006</b>	<b>0.049</b>	ND	0.674	<b>&lt;0.001</b>	ND	ND	0.512	0.4	0.077
KW (H)	11.29	10.03	6.02	ND	0.79	18.63	ND	ND	1.34	1.83	5.12

\*\*Percentage organic matter content was measured for all the cryoconite holes listed in Table 1 (ie from 2006 and 2007). Means±SD for AB, ML and VB were 3.67±1.62, 6.46±3.78 and 8.14±1.18 respectively. % organic matter was significantly lower for AB than ML and VB (p<0.001; F=10.03).

The range of the data and average values of primary production and respiration for these and other glaciers are published in Anesio et al. (2009).



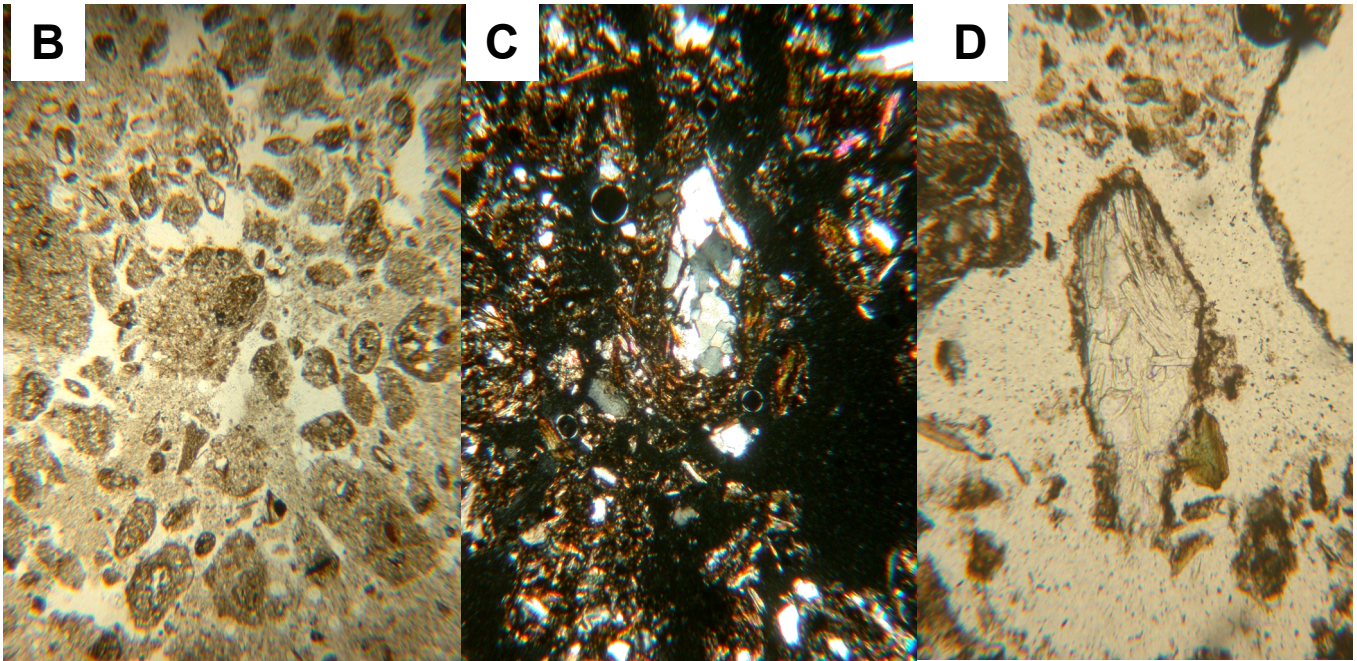
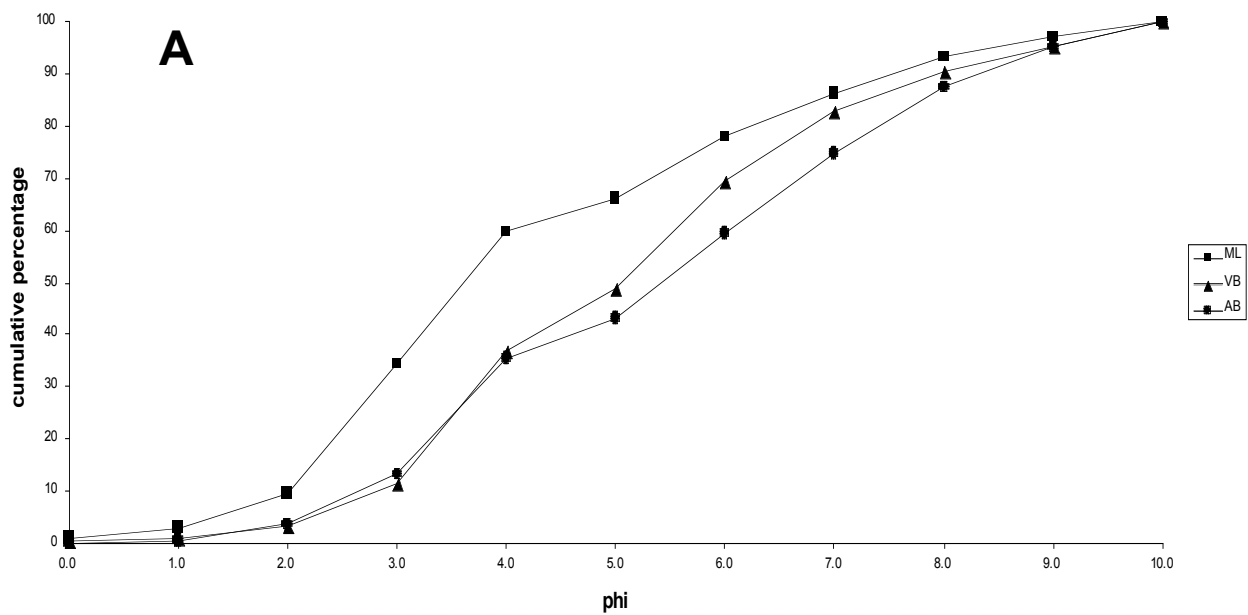
- **Supplementary Data S6 (Table).** Table of data (eigenvalues etc.) relating to the CCA presented as Figure 4.

Scores scaled by sample

Variable	Weighted mean	Weighted SD	Inflation Factor
Cryoconite temperature	0.45	0.45	6.16
Volume	5.91	5.81	2.39
% Inorganic	94.54	1.69	24.75
Primary production	7.63	8.41	11.99
Primary production (meltwater)	4.55	4.90	3.41
Respiration rate	0.95	0.40	21.42
pCO <sub>2</sub>	689.57	771.11	14.69
pH	5.59	0.13	2.64

Eigenvalues

	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6	Axis 7	Axis 8	Axis 9	Axis 10
Eigenvalues	0.286	0.161	0.104	0.08	0.068	0.053	0.049	0.028	0.123	0.083
Percentage	27.65	15.54	10.07	7.75	6.56	5.08	4.75	2.70	11.86	8.03
Cum. Percentage	27.65	43.19	53.26	61.01	67.57	72.65	77.41	80.11	91.97	100.00
Cum.Constr.Percentage	34.52	53.91	66.49	76.16	84.35	90.70	96.63	100.00		
Spec.-env. correlations	0.99	0.99	0.90	0.97	0.99	0.93	0.96	0.82		



**Supplementary Data S7 (Figure).** Analysis of cryoconite dust particles. A) Size distribution of cryoconite ash from the three glaciers ([pooled samples from ashed sediments from 2006 [AB601-606, ML601-606; VB601-606]) . B) Cryoconite granules from AB706 under plane polarising light showing the mineral particles aggregated into cryoconite granules by dark coloured material x10; C) Cross-polarising light micrograph showing a sharp-edged lithic clast x25, AB703; D) A mica particle showing sharp edges also indicative of local origin, ML706 x63

- **Supplementary Data S8 (Table).** Sequence similarity (% identity) of clones identified as 54 OTUs discrete at the 97% identity cut-off threshold along with nearest SEQMATCH hit and predicted T-RF length.

OTU (97% cutoff)	Clone	Glacier	Clone	Length (bp)	Predicted T-RF (bp)	Phylum	Genus	Nearest SEQMATCH hit and GenBank Accession of hit	Similarity	Nearest SEQMATCH Hit	Habitat
1	1	AB	pbAB46	776	208	Acidobacteria	Gp3	<i>Acidobacteria</i> bacterium MPL1011; AM887761	0.995	Sphagnum peat, Tomsk	
2	2	VB	pbVB78	767	184	Proteobacteria	unclassified_Betaproteobacteria	alpha proteobacterium BAC11; EU180508	0.96	GAC filter waste treatment plant	NL
3	4	VB	pbVB85	820	174	Proteobacteria	<i>Rhodospila</i>	bacterium Ellin5134; AY234551	0.934	Soil	
4	5	ML	pbML10	756	389	Proteobacteria	<i>Bdellovibrio</i>	<i>Bdellovibrio</i> sp. HEA; AY294216	0.893	Soil	
5	6	AB	pbAB62	803	202	Proteobacteria	unclassified_Incertae sedis 5	beta proteobacterium BAC126; EU180537	0.95	GAC filter waste treatment plant	NL
6	7	AB	pbAB59	814	76	Proteobacteria	<i>Gluconacetobacter</i>	<i>Gluconacetobacter</i> sp. CC-88226; AY961985	0.924	Isolate	
6	8	AB	pbAB35	804	79	Proteobacteria	<i>Gluconacetobacter</i>	<i>Gluconacetobacter</i> sp. CC-88226; AY961985	0.946	Isolate	
7	9	VB	pbVB89	770	254	Bacteroidetes	<i>Hymenobacter</i>	<i>Hymenobacter</i> sp. 1004; EF423320	0.952	Glacier 1 of Tianshan Mountains	
8	12	VB	pbVB75	788	304	Cyanobacteria	<i>Phormidium</i>	<i>Phormidium pristleyi</i> ANT.LH66.1; AY493581	0.959	Antarctica	
8	13	AB	pbAB57	802	304	Cyanobacteria	<i>Phormidium</i>	<i>Phormidium pristleyi</i> ANT.LH66.1; AY493581	0.987	Antarctica	
8	16	ML	pbML8	621	304	Cyanobacteria	<i>Phormidium</i>	<i>Phormidium pristleyi</i> ANT.LH66.1; AY493581	0.989	Antarctica	
8	10	VB	pbVB84	815	301	Cyanobacteria	<i>Phormidium</i>	<i>Phormidium pristleyi</i> ANT.LH66.1; AY493581	0.927	Antarctica	
8	14	VB	pbVB91	819	301	Cyanobacteria	<i>Phormidium</i>	<i>Phormidium pristleyi</i> ANT.LH66.1; AY493581	0.991	Antarctica	
8	11	VB	pbVB79	798	302	Cyanobacteria	<i>Phormidium</i>	<i>Phormidium pristleyi</i> ANT.LH66.1; AY493581	0.991	Antarctica	
8	15	VB	pbVB68	812	304	Cyanobacteria	<i>Phormidium</i>	<i>Phormidium pristleyi</i> ANT.LH66.1; AY493581	0.985	Antarctica	
9	17	AB	pbAB52	805	207	Proteobacteria	<i>Thiobacillus</i>	Rhodocyclaceae bacterium FTL11; DQ451827	0.981	Lake Fryxell, Antarctica	
10	18	VB	pbVB73	803	231	Proteobacteria	<i>Sphingomonas</i>	<i>Sphingomonas desiccabilis</i> ; CPID; AJ871435	0.961	Biological soil crusts Colorado plateau	
11	19	VB	pbVB77	853	213	Actinobacteria	<i>Leifsonia</i>	uncultured actinobacterium; Blh33; AJ318140	0.972	Waste gas filter, Osnabruck	
12	25	AB	pbAB61	793	218	Actinobacteria	unclassified_Micrococci	uncultured actinobacterium; CrystalBog022E4; AY792229	0.982	Humic lake, Wisconsin	
12	24	ML	pbML13	779	218	Actinobacteria	unclassified_Micrococci	uncultured actinobacterium; CrystalBog022E4; AY792229	0.986	Humic lake, Wisconsin	
12	21	ML	pbML17	754	218	Actinobacteria	unclassified_Micrococci	uncultured actinobacterium; CrystalBog022E4; AY792229	0.985	Humic lake, Wisconsin	
12	20	ML	pbML4	753	218	Actinobacteria	unclassified_Micrococci	uncultured actinobacterium; CrystalBog022E4; AY792229	0.985	Humic lake, Wisconsin	
12	23	ML	pbML7	788	218	Actinobacteria	unclassified_Micrococci	uncultured actinobacterium; CrystalBog022E4; AY792229	0.984	Humic lake, Wisconsin	
12	22	VB	pbVB88	760	218	Actinobacteria	unclassified_Micrococci	uncultured actinobacterium; CrystalBog022E4; AY792229	0.985	Humic lake, Wisconsin	
13	26	AB	pbAB39	786	208	Actinobacteria	unclassified_Frankineae	uncultured actinobacterium; E1B-A4-114; EF016798	0.955	Soil, Yungay, Atacama	
14	27	ML	pbML15	779	57	Actinobacteria	unclassified_Frankineae	uncultured actinobacterium; FBP460; AY250884	0.961	Antarctic cryptoendolith community	
15	29	AB	pbAB64	783	227	Proteobacteria	<i>Sandarakinorhabdus</i>	uncultured alpha proteobacterium; C5; AJ867917	0.955	Nival lake, Swiss Alps	
15	28	ML	pbML6	811	228	Proteobacteria	<i>Sandarakinorhabdus</i>	uncultured alpha proteobacterium; C5; AJ867917	0.965	Nival lake, Swiss Alps	
15	30	VB	pbVB86	755	227	Proteobacteria	<i>Sandarakinorhabdus</i>	uncultured alpha proteobacterium; C5; AJ867917	0.955	Nival lake, Swiss Alps	
15	32	VB	pbVB76	780	231	Proteobacteria	<i>Sandarakinorhabdus</i>	uncultured alpha proteobacterium; C5; AJ867917	0.964	Nival lake, Swiss Alps	
15	31	VB	pbVB74	853	293	Proteobacteria	<i>Sandarakinorhabdus</i>	uncultured alpha proteobacterium; C5; AJ867917	0.958	Nival lake, Swiss Alps	
16	33	AB	pbAB51	779	171	Proteobacteria	<i>Novosphingobium</i>	uncultured alpha proteobacterium; CrystalBog022E8; AY79	0.934	Humic lake, Wisconsin	
17	34	VB	pbVB90	761	293	Proteobacteria	<i>Novosphingobium</i>	uncultured alpha proteobacterium; SW22; AJ575705	0.973	Humic lake, Germany	
18	35	VB	pbVB81	799	236	Cyanobacteria	unclassified_Cyanobacteria	uncultured Antarctic bacterium LB3-53; AF076159	0.885	Perennial ice covered antarctic lake	
19	36	VB	pbVB66	854	224	Cyanobacteria	unclassified_Cyanobacteria	uncultured Antarctic bacterium LB3-53; AF076159	0.914	Perennial ice covered antarctic lake	
20	37	ML	pbML18	712	223	unclassified	unclassified_Bacteria	uncultured bacterium; 1174-1091-13; AB128880	0.921	Deep marine sediments Nankai trough	
20	39	ML	pbML2	758	223	unclassified	unclassified_Bacteria	uncultured bacterium; 1174-1091-13; AB128880	0.941	Deep marine sediments Nankai trough	
20	38	ML	pbML25	593	277	unclassified	unclassified_Bacteria	uncultured bacterium; 1174-1091-13; AB128880	0.937	Deep marine sediments Nankai trough	
21	40	VB	pbVB94	853	234	Bacteroidetes	<i>Paludibacter</i>	uncultured bacterium; 118ds10; AY212569	0.886	Manure contaminated stream	
22	41	AB	pbAB44	820	234	Proteobacteria	unclassified_Myxococcales	uncultured bacterium; 1506; AB286567	0.958	Activated sludge, Japan	
23	42	VB	pbVB69	808	205	Proteobacteria	<i>Curvibacter</i>	uncultured bacterium; 164ds20; AY212616	0.982	Manure contaminated stream	
24	44	ML	pbML23	779	246	Cyanobacteria	GpVI	uncultured bacterium; 2ISC24; EU340169	0.868	Aquatic macrophyte, geothermal lake 1	
24	43	ML	pbML1	854	299	Cyanobacteria	GpVI	uncultured bacterium; 2ISC24; EU340169	0.906	Aquatic macrophyte, geothermal lake 1	
25	45	AB	pbAB41	790	294	Proteobacteria	<i>Novosphingobium</i>	uncultured bacterium; 2ISC34; EU340176	0.947	Aquatic macrophyte, geothermal lake 1	
26	46	ML	pbML22	801	201	Proteobacteria	unclassified_Cystobacteraceae	uncultured bacterium; AKIWR1; DQ129589	0.912	Urban aerosol US	
27	48	AB	pbAB60	811	294	Proteobacteria	<i>Sphingomonas</i>	uncultured bacterium; ANTLV1_H10; DQ521501	0.991	Perennial ice Lake Vida Antarctica	
27	47	AB	pbAB58	761	389	Proteobacteria	<i>Sphingomonas</i>	uncultured bacterium; ANTLV1_H10; DQ521501	0.993	Perennial ice Lake Vida Antarctica	
27	49	AB	pbAB54	804	397	Proteobacteria	<i>Lysobacter</i>	uncultured bacterium; ANTLV_F07; DQ521521	0.986	Perennial ice Lake Vida Antarctica	
28	50	VB	pbVB93	773	209	Acidobacteria	Gp1	uncultured bacterium; AS43; AY963407	0.965	Forest soil SW China	
29	51	ML	pbML5	778	236	Acidobacteria	Gp1	uncultured bacterium; AS43; AY963407	0.961	Forest soil SW China	
30	52	VB	pbVB83	732	177	Proteobacteria	unclassified_Acetobacteraceae	uncultured bacterium; BF0002B036; AM697086	0.967	Indoor dust, Finland	
31	54	ML	pbML11	727	172	Proteobacteria	unclassified_Acetobacteraceae	uncultured bacterium; BF0002B067; AM697117	0.945	Indoor dust, Finland	
32	55	AB	pbAB34	794	76	Proteobacteria	unclassified_Acetobacteraceae	uncultured bacterium; DBS-69; EU409119	0.936	Mine drainage sediment, China	
32	56	ML	pbML12	707	178	Proteobacteria	unclassified_Acetobacteraceae	uncultured bacterium; DBS-69; EU409119	0.932	Mine drainage sediment, China	
33	58	ML	pbML26	722	252	unclassified	unclassified_Bacteria	uncultured bacterium; DC-II-8; DQ660863	0.934	Acid mine drainage, China	
33	59	VB	pbVB67	855	246	unclassified	unclassified_Bacteria	uncultured bacterium; DC-II-8; DQ660863	0.943	Acid mine drainage, China	
33	57	VB	pbVB96	827	246	unclassified	unclassified_Bacteria	uncultured bacterium; DC-II-8; DQ660863	0.943	Acid mine drainage, China	
34	61	ML	pbML20	787	70	Proteobacteria	<i>Rhodospila</i>	uncultured bacterium; ERF-C4; DQ906066	0.95	Rhizophere, Tinto river, Spain	
34	60	ML	pbML30	760	166	Proteobacteria	<i>Rhodospila</i>	uncultured bacterium; ERF-C4; DQ906066	0.943	Rhizophere, Tinto river, Spain	
35	62	AB	pbAB38	816	205	Proteobacteria	unclassified_Incertae sedis 5	uncultured bacterium; FCPO416; EF516518	0.986	Grassland soil, N California US	
36	63	AB	pbAB36	851	161	OP10	OP10_genera_incertae_sedis	uncultured bacterium; FCPO562; EF515962	0.806	Grassland soil, N California US	
37	65	VB	pbVB70	786	221	OP10	OP10_genera_incertae_sedis	uncultured bacterium; FCPT757; EF516140	0.898	Grassland soil, N California US	
38	67	AB	pbAB43	790	294	Proteobacteria	<i>Sphingomonas</i>	uncultured bacterium; FFCH11333; EU133353	0.963	Grass prairie, Oklahoma US	
38	66	AB	pbAB49	753	294	Proteobacteria	<i>Sphingomonas</i>	uncultured bacterium; FFCH11333; EU133353	0.969	Grass prairie, Oklahoma US	
39	68	ML	pbML3	703	185	Bacteroidetes	unclassified_Sphingobacteria	uncultured bacterium; FFCH2786; EU133755	0.881	Grass prairie, Oklahoma US	
40	69	VB	pbVB92	779	219	Chloroflexi	unclassified_Caldiineae	uncultured bacterium; FFCH517; EU134014	0.857	Grass prairie, Oklahoma US	
41	70	VB	pbVB95	852	161	unclassified	unclassified_Bacteria	uncultured bacterium; FFCH7771; EU135026	0.899	Grass prairie, Oklahoma US	
42	71	AB	pbAB47	776	317	Bacteroidetes	<i>Terrimonas</i>	uncultured bacterium; FFCH9723; EU133627	0.961	Grass prairie, Oklahoma US	
43	72	ML	pbML14	687	209	unclassified	unclassified_Bacteria	uncultured bacterium; IYF26; DQ984576	0.916	Mountain litterfall, Taiwan	
44	73	AB	pbAB33	775	268	Planctomycetes	unclassified_Planctomycetes	uncultured bacterium; JEG.d6; DQ228400	0.9	John Evans Glacier, Canada	
45	74	VB	pbVB80	760	227	Proteobacteria	<i>Sandarakinorhabdus</i>	uncultured bacterium; JEG.xsd4; DQ228414	0.916	John Evans Glacier, Canada	
46	75	VB	pbVB72	792	236	Acidobacteria	Gp1	uncultured bacterium; MNM13.1; DQ202227	0.929	Peatlands - Northern US	
47	79	AB	pbAB63	792	238	Proteobacteria	<i>Sphingomonas</i>	uncultured bacterium; ORCA-3N113; DQ823177	0.978	Trail, Oregon Caves US	
47	77	AB	pbAB53	754	294	Proteobacteria	<i>Sphingomonas</i>	uncultured bacterium; ORCA-3N113; DQ823177	0.972	Trail, Oregon Caves US	
47	76	AB	pbAB42	780	297	Proteobacteria	<i>Sphingomonas</i>	uncultured bacterium; ORCA-3N113; DQ823177	0.973	Trail, Oregon Caves US	
47	78	AB	pbAB45	780	298	Proteobacteria	<i>Sphingomonas</i>	uncultured bacterium; ORCA-3N113; DQ823177	0.951	Trail, Oregon Caves US	
48	81	AB	pbAB48	712	211	Proteobacteria	unclassified_Incertae sedis 5	uncultured bacterium; SRRB38; AB240512	0.993	Root base, Sosei River Japan	
49	82	VB	pbVB87	760	213	unclassified	unclassified_Bacteria	uncultured eubacterium WD294; AJ292686	0.923	PCB contaminated soil UK	
50	83	ML	pbML32	754	192	unclassified	unclassified_Bacteria	uncultured forest soil bacterium; DUNssu391 (+7C) (OTU#0	0.908	Forest soil, Germany	
51	84	AB	pbAB37	797	199	unclassified	unclassified_Bacteria	uncultured soil bacterium; C062; AF507696	0.901	Pinyon juniper soil Arizona US	
52	86	ML	pbML28	755	812	Bacteroidetes	unclassified_Sphingobacteria	uncultured Sphingobacteria bacterium; ADK-BTh02-62; EF	0.934	Acid impacted lake, Adirondack US	
52	87	ML	pbML16	755	814	Bacteroidetes	unclassified_Sphingobacteria	uncultured Sphingobacteria bacterium; ADK-BTh02-62; EF	0.931	Acid impacted lake, Adirondack US	
52	88	ML	pbML24	739	815	Bacteroidetes	unclassified_Sphingobacteria	uncultured Sphingobacteria bacterium; ADK-BTh02-62; EF	0.94	Acid impacted lake, Adirondack US	
52	89	ML	pbML27	748	824	Bacteroidetes	unclassified_Sphingobacteria	uncultured Sphingobacteria bacterium; ADK-BTh02-62; EF	0.94	Acid impacted lake, Adirondack US	
52	90	ML	pbML21	804	861	Bacteroidetes	unclassified_Sphingobacteria	uncultured Sphingobacteria bacterium; ADK-BTh02-62; EF	0.937	Acid impacted lake, Adirondack US	
52	85	ML	pbML31	805	865	Bacteroidetes	unclassified_Sphingobacteria	uncultured Sphingobacteria bacterium; ADK-BTh02-62; EF	0.94	Acid impacted lake, Adirondack US	
53	92	AB	pbAB55	732	194	Proteobacteria	<i>Rhodobacter</i>	unidentified bacterium; R-23041; AJ786815	0.967	Commercial nitrifying inoculum, Belgi	
53	91	AB	pbAB50	760	196	Proteobacteria	<i>Rhodobacter</i>	unidentified bacterium; R-23041; AJ786815	0.97	Commercial nitrifying inoculum, Belgi	
54	93	VB	pbVB65	852	188	Proteobacteria	<i>Variovorax</i>	<i>Variovorax</i> sp. KS2D-23; AB196432	0.894	Soil, S Korea	
54	94	VB	pbVB71	804	188	Proteobacteria	<i>Variovorax</i>	<i>Variovorax</i> sp. KS2D-23; AB196432	0.892	Soil, S Korea	
<b>Putative chimeras</b>											
3	3		pbML19	709	178	Proteobacteria	unclassified_Alphaproteobac	alpha proteobacterium BAC233; EU180520	0.89	GAC filter waste treatment plant	NL
31	53		pbML29	774	177	Proteobacteria	unclassified_Acetobacteraceae	uncultured bacterium; BF0002B036; AM697086	0.888	Indoor dust, Finland	
38	64		pbVB82	765	237	Proteobacteria	unclassified_Acetobacteraceae	uncultured bacterium; FCPT506; EF516502	0.88	Grassland soil, N California US	
50	80		pbAB40	752	279	Proteobacteria	<i>Sphingomonas</i>	uncultured bacterium; ORCA-3N113; DQ823177	0.899	Trail, Oregon Caves US	

- **Supplementary Data S9 (Table).** Chi-squared analysis of clone abundance on different glaciers by OTU, phylum and class.

OTU (97% cutoff)	Nearest SEQMATCH hit	No. of clones				P	DF	Chi
		AB	ML	VB	Total			
<b>55</b>	uncultured bacterium; EU409119	<b>0</b>	<b>6</b>	<b>0</b>	<b>6</b>	<b>0.0025</b>	<b>2</b>	<b>12.00</b>
<b>49</b>	uncultured bacterium; DQ521521	<b>4</b>	<b>0</b>	<b>0</b>	<b>4</b>	<b>0.0183</b>	<b>2</b>	<b>8.00</b>
<b>9</b>	Hymenobacter sp.; EF423320	<b>0</b>	<b>1</b>	<b>5</b>	<b>6</b>	<b>0.0302</b>	<b>2</b>	<b>7.00</b>
<b>21</b>	uncultured actinobacterium; AY792229	<b>0</b>	<b>3</b>	<b>0</b>	<b>3</b>	<b>0.0498</b>	<b>2</b>	<b>6.00</b>
<b>28</b>	uncultured alpha proteobacterium; AJ867917	<b>3</b>	<b>0</b>	<b>0</b>	<b>3</b>	<b>0.0498</b>	<b>2</b>	<b>6.00</b>
13	Phormidium pristleyi; AY493581	1	4	1	6	<b>0.2231</b>	2	3.00
34	uncultured alpha proteobacterium; AJ575705	0	1	2	3	0.3679	2	2.00
16	Phormidium pristleyi; AY493581	1	1	3	5	0.4493	2	1.60

Phylum	No. of clones				P	DF	Chi
	AB	ML	VB	Total			
<b>Proteobacteria</b>	22	7	12	41	<b>0.0140</b>	2	8.54
<b>Bacteroidetes</b>	1	7	2	10	<b>0.0450</b>	2	6.20
Cyanobacteria	1	3	7	11	0.0784	2	5.09
unclassified_Bacteria	1	6	4	11	0.1778	2	3.45
Chloroflexi	0	0	1	1	0.3679	2	2.00
Planctomycetes	1	0	0	1	0.3679	2	2.00
Actinobacteria	2	5	2	9	0.3679	2	2.00
OP10	1	0	1	2	0.6065	2	1.00
Acidobacteria	1	1	2	4	0.7788	2	0.50

Class	No. of clones				P	DF	Chi
	AB	ML	VB	Total			
Alphaproteobacteria	16	5	8	29	<b>0.0353</b>	2	6.69
Betaproteobacteria	4	0	4	8	0.1353	2	4.00