

Supplementary Material

Temperature response of aerobic methane-oxidizing bacteria in lake sediments from King George Island, Maritime Antarctica

Diego M. Roldán, Daniel Carrizo, Laura Sánchez-García and Rodolfo Javier Menes

Supplementary Tables

Table S1 Data and physicochemical characteristics of the Antarctic freshwater lake sediment C-2020. DO, dissolved oxygen. TOC and TN, total organic carbon and total nitrogen respectively (percentages relative to total dry weight). C/N, ratio of TOC over TN (dimensionless)

Table S2 Identification of microcosm samples prepared from Antarctic freshwater lake sediment C-2020 (different temperatures and incubation periods) used for 16S rRNA gene amplicon sequencing and PLFA-SIP analysis. Samples C25, C26 and C27 were the original sediment (not incubated)

Table S3 Relative abundance of MOB ASVs (16S rRNA gene, *Bacteria* domain) from microcosm samples of the Antarctic freshwater lake sediment C-2020 (different temperatures and incubation periods). The size of the blue bars indicates the relative abundance of each ASV respect the total of known methanotrophic bacteria

Table S4 Alpha diversity indices from microcosm samples of the Antarctic freshwater lake sediment C-2020 (different temperatures and incubation periods, each condition included 3 vials with $^{12}\text{CH}_4$ and 3 vials with $^{13}\text{CH}_4$ and $^{12}\text{CH}_4$) based on 16S rRNA gene analysis of total *Bacteria* and MOB. Different letters indicate significant differences for each sample at different temperatures (Wilcoxon rank sum test, $p < 0.05$)

Table S5 Differential abundance analysis with DESeq2 at the level of the last taxonomic rank reached (*'best_hit'*) from microcosm samples of the Antarctic freshwater lake sediment C-2020 (different temperatures and incubation periods, each condition included 3 vials with $^{12}\text{CH}_4$ and 3 vials with $^{13}\text{CH}_4$ and $^{12}\text{CH}_4$). Bacterial taxa that were statistically different between time zero (T_0 , not incubated) and the different conditions tested (p -value < 0.01) are shown. Bacterial groups that were significantly enriched in the tested condition showed a positive log2FoldChange (> 0), while those that were significantly depleted showed a negative one (< 0). ASVs associated with potential known MOB are indicated in grey

Table S6 Comparison of microbial taxonomy of MOB ASVs based on SILVA and EzBioCloud from microcosm samples of the Antarctic freshwater lake sediment C-2020

Supplementary Figures

Fig. S1 Schematic representation of the workflow for preparing the microcosm experiments for assessing the effect of temperature on the biologically active MOB community of the Antarctic freshwater lake sediment C-2020

Fig. S2 Heatmap of the mean percentages of relative abundance of the 28 phyla of *Bacteria* based on 16S rRNA gene sequence analysis from microcosm samples of the Antarctic freshwater lake sediment C-2020 (different temperatures and incubation periods)

Table S1 Data and physicochemical characteristics of the Antarctic freshwater lake sediment C-2020. DO, dissolved oxygen. TOC, total organic carbon. TN, total nitrogen. Percentages are relative to total dry weight. C/N, ratio of TOC over TN (dimensionless)

Depth (m)	3.0
DO (mg.L ⁻¹)	12.98
pH	7.20
Conductivity (µS.cm ⁻¹)	215
TOC (% dw)	5.01
TN (% dw)	0.55
C/N	9.11

Table S2 Identification of microcosm samples prepared from Antarctic freshwater lake sediment C-2020 (different temperatures and incubation periods) used for 16S rRNA gene amplicon sequencing and PLFA-SIP analysis. Samples C1, C2 and C3 are the original sediment (not incubated)

Sample ID	Temperature (°C)	Incubation time (days)	Substrate
A1	5	20	¹² CH ₄
A2	5	20	¹² CH ₄
A3	5	20	¹² CH ₄
A4	5	40	¹² CH ₄
A5	5	40	¹² CH ₄
A6	5	40	¹² CH ₄
A7	5	20	¹³ CH ₄ / ¹² CH ₄
A8	5	20	¹³ CH ₄ / ¹² CH ₄
A9	5	20	¹³ CH ₄ / ¹² CH ₄
A10	5	40	¹³ CH ₄ / ¹² CH ₄
A11	5	40	¹³ CH ₄ / ¹² CH ₄
A12	5	40	¹³ CH ₄ / ¹² CH ₄
B13	20	20	¹² CH ₄
B14	20	20	¹² CH ₄
B15	20	20	¹² CH ₄
B16	20	40	¹² CH ₄
B17	20	40	¹² CH ₄
B18	20	40	¹² CH ₄
B19	20	20	¹³ CH ₄ / ¹² CH ₄
B20	20	20	¹³ CH ₄ / ¹² CH ₄
B21	20	20	¹³ CH ₄ / ¹² CH ₄
B22	20	40	¹³ CH ₄ / ¹² CH ₄
B23	20	40	¹³ CH ₄ / ¹² CH ₄
B24	20	40	¹³ CH ₄ / ¹² CH ₄
C1	-	0	-
C2	-	0	-
C3	-	0	-

Table S4 Alpha diversity indices from microcosm samples of the Antarctic freshwater lake sediment C-2020 (different temperatures and incubation periods, each condition included 3 vials with ¹²CH₄ and 3 vials with ¹³CH₄ and ¹²CH₄) based on 16S rRNA gene analysis of total *Bacteria* and MOB. Different letters indicate significant differences for each sample at different temperatures (Wilcoxon rank sum test, *p* < 0.05)

Total <i>Bacteria</i> community									
Sample	Observed			Shannon			Simpson		
	Median	Average	Standard deviation	Median	Average	Standard deviation	Median	Average	Standard deviation
Not incubated	931.0	953.0	39.9	5.19	5.07 ^{abc}	0.22	0.98	0.98 ^{abc}	0.01
5 °C 20 days	928.5	908.8	46.6	5.12	5.11 ^a	0.13	0.98	0.98 ^b	0.00
5 °C 40 days	937.0	922.3	36.9	4.82	4.85 ^b	0.06	0.97	0.97 ^c	0.00
20 °C 20 days	954.0	956.5	24.2	4.83	4.87 ^c	0.07	0.97	0.97 ^c	0.00
20 °C 40 days	927.5	927.2	19.8	3.96	3.97 ^d	0.16	0.91	0.91 ^d	0.01
MOB community									
Sample	Observed			Shannon			Simpson		
	Median	Average	Standard deviation	Median	Average	Standard deviation	Median	Average	Standard deviation
Not incubated	27.0	27.0	2.0	2.06	2.10 ^a	0.07	0.81	0.80 ^a	0.02
5 °C 20 days	28.0	27.8	2.9	1.50	1.51 ^b	0.10	0.65	0.65 ^b	0.04
5 °C 40 days	30.5	29.8	1.6	1.60	1.59 ^b	0.08	0.72	0.72 ^c	0.02
20 °C 20 days	28.0	28.2	2.3	1.75	1.74 ^c	0.14	0.75	0.73 ^c	0.06
20 °C 40 days	27.0	27.2	1.6	1.64	1.62 ^{bc}	0.32	0.72	0.69 ^{abc}	0.13

Table S5 Differential abundance analysis with DESeq2 at the level of the last taxonomic rank reached (*'best_hit'*) from microcosm samples of the Antarctic freshwater lake sediment C-2020 (different temperatures and incubation periods, each condition included 3 vials with ¹²CH₄ and 3 vials with ¹³CH₄ and ¹²CH₄). Bacterial taxa that were statistically different between time zero (T₀, not incubated) and the different conditions tested (*p*-value < 0.01) are shown. Bacterial groups that were significantly enriched in the tested condition showed a positive log2FoldChange (> 0), while those that were significantly depleted showed a negative one (< 0). ASVs associated with potential known MOB are indicated in grey

ASV	Last taxonomic rank reached	5 °C				20 °C			
		20 days		40 days		20 days		40 days	
		log2FoldChange	p-value	log2FoldChange	p-value	log2FoldChange	p-value	log2FoldChange	p-value
ASV212	Blvii28 wastewater-sludge group							-2.01	1.79E-06
ASV49	Blvii28 wastewater-sludge group					-0.84	2.38E-04	-1.92	2.16E-15
ASV55	Blvii28 wastewater-sludge group							-1.33	7.29E-06
ASV16	BSV13							-1.82	3.21E-15
ASV21	BSV13	1.59	1.26E-08	1.10	8.66E-05	1.00	3.32E-04		
ASV232	BSV13							-2.29	1.89E-05
ASV415	BSV13	2.81	7.69E-05						
ASV58	BSV13							-1.51	1.25E-06
ASV95	BSV13							-1.93	4.35E-08
ASV141	<i>c __Alphaproteobacteria</i>							1.81	4.00E-07
ASV2	<i>c __Alphaproteobacteria</i>					1.56	6.07E-11	2.14	2.75E-19
ASV36	<i>c __Alphaproteobacteria</i>			4.99	2.63E-06	1.28	1.12E-05		
ASV36	<i>c __Alphaproteobacteria</i>							1.87	1.35E-10
ASV477	<i>c __Alphaproteobacteria</i>							3.08	3.60E-04
ASV53	<i>c __Alphaproteobacteria</i>							1.08	1.07E-03
ASV68	<i>c __Alphaproteobacteria</i>							1.34	2.82E-04
ASV960	<i>c __Alphaproteobacteria</i>							4.35	9.90E-04
ASV56	<i>c __Firmicutes</i>							0.63	3.87E-04
ASV281	<i>c __Gammaproteobacteria</i>	3.36	2.34E-06						
ASV420	<i>c __Gammaproteobacteria</i>			5.68	5.52E-06			4.41	8.03E-04
ASV71	<i>c __LCP-89</i>							-1.13	1.46E-05
ASV252	<i>Caldisericum</i>							-1.32	4.77E-04
ASV60	<i>Caldisericum</i>							-1.87	1.26E-11
ASV62	<i>Caldisericum</i>							-1.69	1.26E-11
ASV255	' <i>Candidatus</i> Paracaedibacter'					1.65	2.72E-04		
ASV413	' <i>Candidatus</i> Paracaedibacter'			4.78	8.91E-06				
ASV184	' <i>Candidatus</i> Planktophila'							2.18	2.42E-06
ASV210	' <i>Candidatus</i> Planktophila'							2.29	2.51E-06
ASV253	' <i>Candidatus</i> Planktophila'	2.51	5.23E-05	2.66	1.68E-05				
ASV3	' <i>Candidatus</i> Planktophila'			1.37	2.99E-21	1.33	2.77E-04	2.72	1.15E-13
ASV716	' <i>Candidatus</i> Planktophila'							4.80	2.15E-04
ASV727	' <i>Candidatus</i> Planktophila'							4.25	8.31E-04
ASV189	' <i>Candidatus</i> Solibacter'	1.79	1.89E-04	1.72	3.27E-04				
ASV565	<i>Clostridium</i> sensu stricto 12			-2.93	3.57E-04	-2.11	4.52E-04	-2.41	1.30E-04
ASV4	<i>Clostridium</i> sensu stricto 13							0.52	6.73E-04
ASV10	<i>Crenothrix</i>			2.55	3.73E-05			3.37	6.60E-08
ASV17	<i>Crenothrix</i>	2.50	7.83E-07	4.47	8.91E-19			2.54	2.07E-06
ASV342	<i>Crenothrix</i>			4.69	3.82E-04				
ASV546	<i>Crenothrix</i>	-2.68	2.79E-04			-2.15	3.89E-04		
ASV105	<i>Cryptanaerobacter</i>					-1.26	4.78E-05	-1.69	1.12E-07
ASV192	<i>Cryptanaerobacter</i>							-1.92	1.79E-05
ASV226	<i>f __Anaerolineaceae</i>							1.39	1.01E-03
ASV123	<i>f __Bacteroidetes</i> vadinHA17							-1.27	4.09E-04
ASV128	<i>f __Bacteroidetes</i> vadinHA17							-1.90	9.67E-07
ASV157	<i>f __Bacteroidetes</i> vadinHA17							-1.78	4.05E-05
ASV20	<i>f __Bacteroidetes</i> vadinHA17							-1.34	3.33E-07

ASV241	<i>f__Bacteroidetes vadinHA17</i>							-1.67	9.77E-04
ASV29	<i>f__Bacteroidetes vadinHA17</i>							-1.45	1.45E-07
ASV32	<i>f__Bacteroidetes vadinHA17</i>							-1.06	2.05E-05
ASV50	<i>f__Bacteroidetes vadinHA17</i>							-1.55	1.05E-06
ASV73	<i>f__Bacteroidetes vadinHA17</i>							-0.92	8.55E-05
ASV92	<i>f__Bacteroidetes vadinHA17</i>							-0.89	1.00E-03
ASV1	<i>f__Chitinophagaceae</i>				1.00	6.21E-05		3.84	1.85E-53
ASV671	<i>f__Chitinophagaceae</i>							5.14	4.84E-05
ASV793	<i>f__Comamonadaceae</i>		4.99	2.49E-04					
ASV946	<i>f__Comamonadaceae</i>							-3.63	4.11E-04
ASV131	<i>f__Dysgonomonadaceae</i>							-1.53	3.45E-06
ASV323	<i>f__Dysgonomonadaceae</i>							-2.76	1.66E-05
ASV72	<i>f__Dysgonomonadaceae</i>							-1.89	3.81E-11
ASV224	<i>f__env.OPS 17</i>	3.23	2.72E-05	3.62	2.29E-06				
ASV28	<i>f__env.OPS 17</i>	3.83	4.03E-14	3.11	8.08E-10				
ASV34	<i>f__env.OPS 17</i>	2.29	8.19E-12	2.33	3.03E-12				
ASV90	<i>f__env.OPS 17</i>	2.52	1.06E-09	2.03	9.42E-07				
ASV117	<i>f__Gallionellaceae</i>					1.63	2.80E-06	2.25	6.62E-11
ASV132	<i>f__Gallionellaceae</i>					1.64	7.31E-05	2.14	1.85E-07
ASV139	<i>f__Gallionellaceae</i>							1.69	3.48E-04
ASV146	<i>f__Gallionellaceae</i>					2.21	4.68E-05	2.93	6.00E-08
ASV211	<i>f__Gallionellaceae</i>							3.01	6.29E-08
ASV143	<i>f__Lentimicrobiaceae</i>					-1.26	3.69E-04	-2.71	1.35E-11
ASV153	<i>f__Lentimicrobiaceae</i>							-1.27	9.81E-05
ASV272	<i>f__Lentimicrobiaceae</i>	1.97	1.99E-04			2.13	1.35E-04		
ASV303	<i>f__Lentimicrobiaceae</i>							-1.84	5.68E-04
ASV35	<i>f__Lentimicrobiaceae</i>							-1.38	6.81E-07
ASV177	<i>f__Methyloiligellaceae</i>							1.65	1.37E-04
ASV133	<i>f__Micropepsaceae</i>							1.79	1.18E-06
ASV148	<i>f__Micropepsaceae</i>							1.86	1.56E-05
ASV75	<i>f__Micropepsaceae</i>							1.40	6.88E-04
ASV113	<i>f__NS11-12 marine group</i>	2.98	1.27E-07	2.74	1.17E-06				
ASV453	<i>f__Paludibacteraceae</i>							-2.31	1.20E-04
ASV112	<i>f__Parvibaculaceae</i>					1.31	2.92E-05	1.77	1.25E-08
ASV23	<i>f__Pedosphaeraceae</i>							0.91	8.85E-04
ASV436	<i>f__Pseudomonadaceae</i>							-2.26	3.19E-04
ASV180	<i>f__Rhizobiales Incertae Sedis</i>	-1.24	3.61E-05	-1.15	9.16E-05	-1.45	5.44E-06	-1.80	5.22E-08
ASV431	<i>f__SB-5</i>							-2.60	5.59E-05
ASV106	<i>Ferriphaselus</i>					1.90	4.93E-07	2.46	5.69E-11
ASV64	<i>Ferruginibacter</i>			1.72	1.72E-09	1.92	1.65E-05	2.06	3.72E-06
ASV70	<i>Leptolinea</i>							1.43	8.27E-06
ASV80	<i>Leptolinea</i>			1.31	3.92E-04	1.46	1.26E-06	2.02	1.26E-11
ASV40	<i>Longivirga</i>							2.40	6.08E-15
ASV389	<i>Magnetospirillum</i>			-1.86	3.48E-04			-2.18	7.22E-05
ASV44	<i>Methylobacter</i>							3.75	1.04E-06
ASV5	<i>Methylobacter</i>	3.36	3.61E-71	4.08	6.61E-105	1.54	7.05E-05	1.65	1.98E-05
ASV9	<i>Methylobacter</i>	3.21	3.41E-20	4.84	6.82E-44			2.39	1.26E-06
ASV914	<i>Methylobacter</i>			4.91	2.95E-04				
ASV107	<i>Nitrospira</i>					-1.07	3.36E-05	-2.05	4.37E-13
ASV317	<i>Nitrospira</i>							-1.75	8.07E-04
ASV665	<i>Nitrospira</i>							-2.68	9.11E-04
ASV98	<i>o__Aminicenantales</i>							-1.05	2.93E-04
ASV121	<i>o__Burkholderiales</i>							2.57	3.84E-06
ASV125	<i>o__Burkholderiales</i>					2.15	3.06E-06	2.93	1.36E-10
ASV130	<i>o__Burkholderiales</i>							2.86	3.50E-07

ASV149	<i>o __Burkholderiales</i>	1.83	6.46E-05			3.36	3.99E-06
ASV152	<i>o __Burkholderiales</i>					1.51	2.16E-04
ASV167	<i>o __Burkholderiales</i>					3.07	1.42E-09
ASV576	<i>o __Burkholderiales</i>	5.87	3.51E-06				
ASV588	<i>o __Burkholderiales</i>	4.90	2.46E-04				
ASV758	<i>o __Campylobacterales</i>					-2.41	9.80E-04
ASV129	<i>o __Candidatus Kaiserbacteria</i>					2.42	2.01E-05
ASV218	<i>o __Candidatus Kaiserbacteria</i>					4.38	1.45E-10
ASV181	<i>o __Frankiales</i>					2.01	5.98E-07
ASV151	<i>o __GIF9</i>					-1.25	3.70E-06
ASV54	<i>o __GIF9</i>					-2.01	2.72E-16
ASV65	<i>o __GIF9</i>			-0.95	5.13E-07	-1.63	5.06E-16
ASV164	<i>o __GOUTB8</i>	1.58	3.31E-04	1.48	1.00E-04		
ASV122	<i>o __IMCC26256</i>					2.17	5.29E-07
ASV236	<i>o __Microtrichales</i>			-1.39	3.29E-05		
ASV236	<i>o __Microtrichales</i>					-1.21	2.80E-04
ASV19	<i>o __MSBL5</i>					-1.15	6.07E-10
ASV384	<i>o __Planctomycetales</i>					2.09	9.49E-04
ASV97	<i>o __RBG-13-54-9</i>					-1.58	1.85E-07
ASV201	<i>o __Saccharimonadales</i>					2.81	1.46E-08
ASV214	<i>o __Acidobacteriae Subgroup 2</i>					2.24	3.63E-07
ASV245	<i>Paenibacillus</i>					2.14	3.36E-05
ASV38	<i>Paludibaculum</i>					1.20	6.70E-04
ASV308	<i>Phaselicystis</i>					-1.71	2.86E-04
ASV140	<i>Phenylobacterium</i>			1.52	4.63E-04	2.02	3.08E-06
ASV280	PMMR1					2.00	3.23E-04
ASV18	<i>Polymorphobacter</i>			1.19	2.44E-04		
ASV108	<i>Pseudolabrys</i>					1.83	1.21E-05
ASV337	<i>Pseudolabrys</i>					2.48	6.87E-06
ASV27	<i>Rhodobacter</i>					1.72	2.06E-05
ASV26	<i>Rhodovastum</i>					1.56	7.43E-07
ASV89	<i>Sediminibacterium</i>					2.47	1.30E-04
ASV111	<i>Sulfuricurvum</i>	-1.65	5.84E-06	-2.43	5.81E-10	-3.10	4.42E-14
ASV395	<i>Sulfuricurvum</i>					-2.54	2.76E-05
ASV66	<i>Sulfuricurvum</i>	-1.82	4.87E-05	-2.51	4.74E-18	-3.20	5.00E-26
ASV8	<i>Sulfuricurvum</i>	-1.80	3.46E-08	-2.18	1.63E-16	-3.39	1.31E-36
ASV638	<i>Sulfuriferula</i>					5.87	2.72E-05
ASV11	<i>Sulfurimonas</i>	-1.27	1.23E-04	-1.26	5.37E-08	-2.80	4.94E-32
ASV120	<i>Sulfurimonas</i>	-1.98	2.47E-05	-2.16	8.01E-09	-2.79	7.24E-13
ASV22	<i>Sulfurimonas</i>			-1.17	3.56E-05	-2.68	5.36E-20
ASV235	<i>Sulfurimonas</i>	-1.98	7.16E-05	-2.14	7.37E-06	-3.31	4.52E-10

Table S6 Comparison of microbial taxonomy of MOB ASVs based on SILVA and EzBioCloud from microcosm samples of the Antarctic freshwater lake sediment C-2020

ASV	Last taxonomic rank (SILVA database)	Closest strain (EzBioCloud database) % 16S rRNA gene similarity GenBank accession no.
ASV10	<i>Crenothrix</i>	<i>Methylobacter psychrophilus</i> Z-0021 ^T 98.83% AF152597
ASV1045	<i>Crenothrix</i>	<i>Methylicorpusculum oleiharenae</i> XLMV4 ^T 92.99% KC963966
ASV1047	<i>Crenothrix</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 96.96% AEGW01000017
ASV1048	<i>Crenothrix</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 98.13% AEGW01000017
ASV17	<i>Crenothrix</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 94.63% AEGW01000017
ASV193	<i>Crenothrix</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 96.03% AEGW01000017
ASV251	<i>Crenothrix</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 96.73% AEGW01000017
ASV257	<i>Crenothrix</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 99.30% AEGW01000017
ASV342	<i>Crenothrix</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 95.09% AEGW01000017
ASV546	<i>Crenothrix</i>	<i>Methylosoma difficile</i> LC 2 ^T 94.63% DQ119050
ASV563	<i>Crenothrix</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 98.83% AEGW01000017
ASV615	<i>Crenothrix</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 96.03% AEGW01000017
ASV705	<i>Crenothrix</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 99.07% AEGW01000017
ASV820	<i>Crenothrix</i>	<i>Methylovulum psychrotolerans</i> Sph1 ^T 96.03% KT381578
ASV828	<i>Crenothrix</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 96.03% AEGW01000017
ASV115	<i>Methylobacter</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 100.00% AEGW01000017
ASV13	<i>Methylobacter</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 99.77% AEGW01000017
ASV229	<i>Methylobacter</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 99.53% AEGW01000017
ASV44	<i>Methylobacter</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 99.30% AEGW01000017
ASV5	<i>Methylobacter</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 99.30% AEGW01000017
ASV547	<i>Methylobacter</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 97.66% AEGW01000017
ASV600	<i>Methylobacter</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 95.79% AEGW01000017
ASV699	<i>Methylobacter</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 98.13% AEGW01000017
ASV9	<i>Methylobacter</i>	<i>Methylobacter psychrophilus</i> Z-0021 ^T 99.07% AF152597
ASV914	<i>Methylobacter</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 94.39% AEGW01000017
ASV915	<i>Methylobacter</i>	<i>Methylobacter psychrophilus</i> Z-0021 ^T 97.20% AF152597
ASV989	<i>Methylobacter</i>	<i>Methylobacter psychrophilus</i> Z-0021 ^T 96.26% AF152597
ASV1062	<i>Methylomonadaceae</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 87.85% AEGW01000017
ASV643	<i>Methylomonadaceae</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 92.52% AEGW01000017
ASV655	<i>Methylomonadaceae</i>	<i>Methylobacter psychrophilus</i> Z-0021 ^T 94.39% AF152597
ASV689	<i>Methylomonadaceae</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 88.08% AEGW01000017
ASV918	<i>Methylomonadaceae</i>	<i>Methylobacter tundripaludum</i> SV96 ^T 92.06% AEGW01000017
ASV281	<i>Gammaproteobacteria</i>	<i>Methylobacter psychrophilus</i> Z-0021 ^T 93.69% AF152597
ASV420	<i>Gammaproteobacteria</i>	<i>Methylobacter psychrophilus</i> Z-0021 ^T 91.59% AF152597

Fig. S1 Schematic representation of the workflow for preparing the microcosm experiments for assessing the effect of temperature on the biologically active MOB community of the Antarctic freshwater lake sediment C-2020

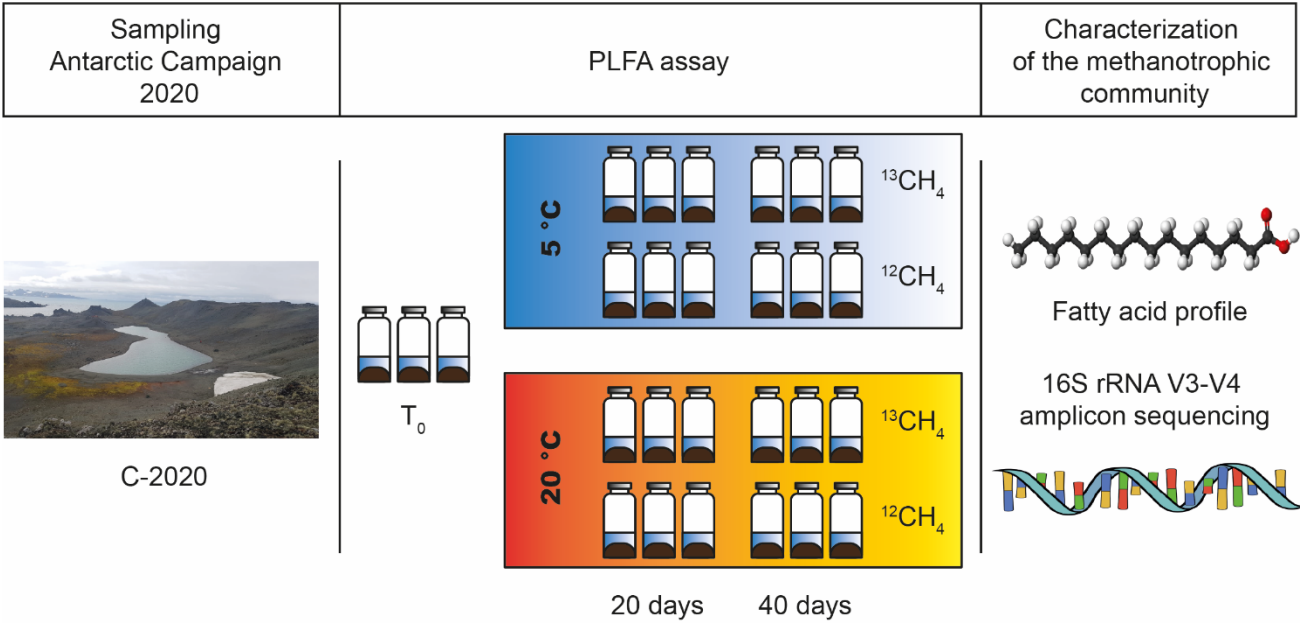


Fig. S2 Heatmap of the mean percentages of relative abundance of the 28 phyla of *Bacteria* based on 16S rRNA gene sequence analysis from microcosm samples of the Antarctic freshwater lake sediment C-2020 (different temperatures and incubation periods)

