

*“Human subtlety will never devise an invention more beautiful,
more simple or more direct than does Nature,
because in her inventions,
nothing is lacking and nothing is superfluous.”*
Leonardo da Vinci (1452–1519)

SUPPLEMENTARY INFORMATION FOR

The perennially ice-covered Lake Enigma, Antarctica supports unique microbial communities

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Supplementary Text

Geochemical analyses of water column.

By analyzing the chemical composition of two surface ice and 12 water samples (Supplementary Table 2), a slight enrichment in HCO_3^- and Mg^{2+} was recognized in the DP#2-NEAR water sample, whereas the DP#4–9m water sample showed a relatively higher SO_4^{2-} content compared to the other water samples (33% of total anions with respect to an overall average value of 21%). Differently, ice samples taken at the depth of 20 cm at the sampling sites DP#2 and DP#4 were enriched in Na^+ (ca 93 % of total cations) and depleted in Cl^- (ca. 52% of total anions), showing a slight enrichment in HCO_3^- with respect to water samples (on average, ca. 33% and 6% of total anions, respectively). The predominance of main cations and anions in ice samples was $\text{Na}^+ > \text{K}^+ > \text{Mg}^{2+} > \text{Ca}^{2+}$ and $\text{Cl}^- > \text{HCO}_3^- + \text{CO}_3^{2-} > \text{SO}_4^{2-}$. The observed compositions were in agreement with those reported for superficial glacier meltwaters, spanning from Na-Cl to Na- HCO_3 (Webster-Brown et al., 2010; Abollino et al. 2004, Gragnani and Torcini 1992, Borghini et al. 2004, Gragnani et al 1998, Legrand 1987), indicating that the surface of Lake Enigma is ultimately influenced by supraglacial meltwater (Supplementary Fig. 4B, C). The strong correlation between Na^+ and Cl^- contents and their reciprocal balance (median Na/Cl molar ratio in lake waters: 0.98) indicated a common source of these ions, possibly related to NaCl salt dissolution. Due to the extreme local aridity, precipitation and accumulation of salts in soils has been recognized in the Antarctic Northern Foothills and fluctuations in relative humidity have been proposed to produce a salt-separation allowing the most deliquescent salts (e.g. CaCl_2 , MgCl_2 , and much of NaCl) to percolate toward groundwaters and the least deliquescent salts (e.g. sodium sulfates) to remain in the soil (e.g. Wilson, 1981). Accordingly, the presence of mirabilite ($\text{Na}_2\text{SO}_4 \times 10\text{H}_2\text{O}$) and thenardite (Na_2SO_4) has been reported in the study area (e.g. French and Guglielmin, 2000; Azzaro et al., 2022). Alternatively, a freezing/sublimation process (i.e. freeze-concentration; Thompson and Nelson, 1956; Lyons et al., 2004; Liu et al., 2014;

Porcino et al., 2020) and the possible involvement of bacterial sulfate reduction and subsequent reoxidation within meltwater ponds (Socki et al., 2012) has been proposed to explain the formation of the mirabilite-mounds in the area. During freezing, calcium carbonate is indeed first precipitated, followed by sodium sulphate, sodium chloride and, eventually, Mg and K chlorides (Thompson and Nelson, 1956; Schmidt et al., 1991). These salts may then be scavenged by melt- and ground-waters. Furthermore, the interaction with local bedrock and sediments, although kinetically limited by the low temperature conditions, represented a further potential origin of solutes in the lake water.

Supplementary Table 1. Lake Enigma locations identified as candidates for drilling activities.

ID	Latitude	Longitude	Depth ice	Depth Max
ESI	-74°43.087'	164°00.532'	-	0.1m
DP#2	-74°42.835'	164°01.203'	6.3m	9.3m
DP#4	-74°42.956'	164°01.037'	8.4m	22.5m
DP#C22	-74°43.012'	164°01.363'	12m	22m
DP#1	-74°42.999'	164°01.393'	6m	6m
DP#3	-74°43.033'	164°01.177'	12m	12m
DP#C21	-74°43.012'	164°01.369'	9m	9m

ESI= Enigma Surface Ice, used as a reference point corresponding to the influx of meltwater from the Amorphous Glacier.

Supplementary Table 2. Chemical data of Lake Enigma ice and water samples

Depth m	ID	pH	El. Cond. (mS/cm)	HCO ₃ ⁻ mg L ⁻¹	CO ₃ ²⁻ mg L ⁻¹	F ⁻ mg L ⁻¹	Cl ⁻ mg L ⁻¹	Br ⁻ mg L ⁻¹	NO ₃ ⁻ mg L ⁻¹	SO ₄ ²⁻ mg L ⁻¹	Ca ²⁺ mg L ⁻¹	Mg ²⁺ mg L ⁻¹	Na ⁺ mg L ⁻¹	K ⁺ mg L ⁻¹	NH ₄ ⁺ mg L ⁻¹	As ⁵⁺ mg L ⁻¹	B mg L ⁻¹	Li ⁺ mg L ⁻¹	Rb mg L ⁻¹	TDS mg L ⁻¹
Drilling point DP#2																				
0.2	DP#2-ICE	8.17	166	28.1	0.21	0.4	24	<0.1	0.8	8	0.3	0.3	29	1.9	0.05	0.4	19	2.8	<1	93
7.8	DP#2-7.8m	9.85	622	15.0	5.32	1.3	182	0.3	0.2	40	21	7	91	7.7	<0.01	1.1	37	6.0	<1	371
9.3	DP#2-NEAR	8.17	634	61.0	0.45	0.9	142	0.24	<0.1	33	19	12	78	7.7	0.01	1.1	52	6.5	<1	354
Drilling point DP#4																				
0.2	DP#4-ICE	7.90	163	26.0	0.10	0.4	25	<0.1	<0.1	8	0.4	0.3	29	2.2	0.07	0.4	22	3.5	1.9	91
9	DP#4-9m	8.80	594	28.0	0.89	2.7	104	0.23	0.4	83	20	7	86	7.7	0.01	1.0	40	5.7	<1	340
11	DP#4-11m	9.56	1,454	37.0	6.73	1.9	322	0.56	0.2	154	53	15	213	15.7	0.03	1.9	82	12.6	1.1	819
12	DP#4-12m	9.14	1,732	36.6	2.56	3.3	341	0.44	2.2	157	52	15	217	15.2	0.01	2.0	84	13.6	1.2	843
13	DP#4-13m	8.89	2,129	67.1	2.61	2.8	564	0.91	<0.1	145	83	23	331	23.0	0.01	2.7	103	18.2	1.5	1,242
21	DP#4-21m	8.86	2,722	65.0	2.36	4.4	525	0.75	0.1	278	92	26	386	33.0	<0.01	2.6	146	23.3	1.7	1,413
22	DP#4-22m	7.36	3,261	107.4	0.12	5.1	753	0.98	0.8	285	117	36	462	35.0	0.03	3.5	197	34.4	6.8	1,802
22.5	DP#4-22.5m	7.64	3,187	99.0	0.22	4.5	710	0.94	0.8	288	118	37	474	34.0	<0.01	2.3	170	28.7	2.1	1,766
Drilling point DP#C22																				
12	DP#C22-12m	8.94	1,717	54.0	2.36	4.5	458	0.54	0.3	141	64	18	255	19.4	0.01	2.2	90	15.0	1.2	1,017
17	DP#C22-17m	8.58	2,156	66.0	1.26	4.3	473	0.51	0.6	191	77	21	313	23.6	0.01	2.7	109	18.8	1.5	1,172
22.5	DP#C22-NEAR	7.91	2,406	80.0	0.33	4.7	552	0.56	1.1	201	88	27	363	25.0	<0.01	2.5	126	21.0	1.5	1,343
Average of Enigma Lake deep water (≥ 17 m)																				
		8.1	2,746	83.5	0.86	4.6	602.6	0.75	0.7	248.6	98.4	29.6	399.6	30.2	0.01	2.7	150	25.2	2.7	1499

Supplementary Table 3. Isotopic data of Lake Enigma ice and water samples

Depth	ID sample	$\delta^{18}\text{O}$ (‰ V-SMOW)	$\delta^2\text{H}$ (‰ V-SMOW)	D-excess
Drilling point DP#2				
0.2	DP#2-ICE	-18.36	-146.03	0.85
7.8	DP#2-7.8m	-25.91	-201.31	5.97
Drilling point DP#4				
9	DP#4-9m	-24.57	-193.49	3.07
11	DP#4-11m	-25.82	-199.91	6.65
11.5	DP#4-11.5m	-26.03	-202.96	5.28
12	DP#4-12m	-26.21	-201.28	8.40
13	DP#4-13m	-27.33	-208.87	9.77
21	DP#4-21m	-27.3	-209.68	8.72
22.5	DP#4-22.5m	-27.61	-211.97	8.91
24	DP#4-NEAR	-26.69	-206.11	7.41
Drilling point DP#C22				
12	DP#C22-12.0	-27.24	-208.6	9.32
17	DP#C22-17.0	-27.41	-208.22	11.06
22.5	DP#C22-NEAR	-27.64	-211.96	9.16

Supplementary Table 4. Crustal and seawater components of Lake Enigma waters.

Sample	Meq L ⁻¹								
	Cl ⁻	Na ⁺	Ca ²⁺	Mg ⁺	K ⁺	HCO ₃ ⁻	SO ₄ ²⁻	Br ⁻	F ⁻
Lake water average ^a	16,445	16,817	4,690	2,322	607	1,283	5,510	6.3	225
Seawater component ^b	16,445	14,216	608	3,207	312	66	1,694	25.2	2
Non-seawater crustal weathering component ^c	0	2,691	4,082	-882 ^d	295	1,217	3,816	-19.1 ^e	223

^a Average values of the deepest waters (≥ 17 m) of Lake Enigma.

^b Calculated using Cl⁻ concentrations and ratios of each species to Cl⁻ in seawater in $\mu\text{eq L}^{-1}$; Na⁺ 0.859; K⁺ 0.019; Mg²⁺ 0.195; Ca²⁺ 0.037; SO₄²⁻ 0.103; HCO₃⁻ 0.04; Br⁻ 0.016; F⁻ 0.00013 (Holland, 1978).

^c Calculated by subtracting the seawater component from the average LEW solute concentration for each ion.

^d Negative values indicate the potential for ion exchange of Mg²⁺ with other cations on minerals present in Lake Enigma sediments.

^e Negative values of this highly conservative anion indicate that at least three-quarters of ions calculated as components of seawater originate from freeze-dried or evaporitic sea salt.

Supplementary Table 5. General characteristics of DNA extracted from surface ice, water and microbial mat samples of Lake Enigma

Sample ID	Depth, m	Filtered volume, ml	Type of sample	Concentration, $\text{ng } \mu\text{l}^{-1}$	Total extracted DNA, $\text{ng } 50\mu\text{l}^{-1}$	Extracted DNA, ng/ml water	DNA copy number, $\text{e}^{+5} \text{ ml}^{-1*}$
Drilling point DP#2							
DP#2-Ice	0.2	2,300	Melted Ice	30.7	1,535	0.67	131
DP#2-7.8m	7.8	800	Water	46.6	2,330	2.91	5.67
DP#2-NEAR	9.3	900	Water	13.4	670	0.74	1.44
DP#2-MAT	Bottom	250 mg	Microbial mat	21.2	1,060	4,240**	8,260 g^{-1}
Drilling point DP#4							
DP#4-Ice	0.2	780	Melted Ice	20.8	1,040	1.33	2.59
DP#4-9m	9	900	Water	177.1	8,855	9.84	19.18
DP#4-11m	11	3,100	Water	187.7	9,385	3.03	5.91
DP#4-12m	12	2,800	Water	164.8	8,240	2.94	5.73
DP#4-13m	13	900	Water	136.3	6,815	7.57	14.75
DP#4-15m	15	900	Water	140.0	7,000	7.78	15.16
DP#4-15rep	15	2,800	Water	433.4	21,670	7.74	15.09
DP#4-17m	17	900	Water	82.3	4,115	4.57	8.91
DP#4-20m	20	3,500	Water	50.3	2,515	0.72	1.4
DP#4-21.3m	21.3	900	Water	138.1	6,905	7.67	14.95
DP#4-21.5m	21.5	900	Water	255.6	12,780	14.20	27.68
DP#4-22.5m	22.5	900	Water	81.6	4,080	4.53	8.83
DP#4-21.5F	21.5***	900	0.1 μm Filtrate	35.42	1,771	1.97	3.84
DP#4-22.5F	22.5***	900	0.1 μm Filtrate	41.46	2,073	2.30	4.48
DP#4-MAT	23	250 mg	Microbial mat	28.1	1,405	5,620**	10,950 g^{-1}

* Average of 5 Mbp genome size was taken in consideration and DNA copy number was calculated using online calculator <https://nebiocalculator.neb.com/#!/dsdnaamt>;

** Values were calculated for 1 g of microbial mat sample

*** After filtration of DP#4-21.5m and DP#4-22.5m water samples using 0.22 μm Sterivex filter, the penetrant was further filtered using 0.1 μm filter

Supplementary Table 6. Statistics and Alpha diversity indices.

Sample ID	Number of sequences*	Number of ASVs [†]	Coverage [‡]	Inverse Simpson Diversity Index [‡]	Shannon Diversity Index [‡]	Chao1 Richness Estimator [‡]
DP#2 drilling point						
DP#2-Ice	20,896	34	100	23.05	3.28	34
DP#2-7.8m	28,131	79	100	59.29	4.20	79
DP#2-NEAR	19,222	89	99.9	48.46	4.09	90.5
DP#2-MAT	61,606	245	99.9	116.59	4.98	245
DP#4 drilling point						
DP#4-9m	16,485	50	99.9	29.93	3.59	50
DP#4-12m	38,481	100	100	51.55	4.23	100
DP#4-13m	18,375	81	100	43.78	4.03	81
DP#4-15m	30,590	102	99.9	63.75	4.30	105
DP#4-17m	21,872	99	100	43.84	4.20	99
DP#4 20m	24,034	74	100	48.04	4.05	74
DP#4-21.3m	35,700	117	99.9	76.34	4.53	117
DP#4 21.3F	23,244	62	100	35.17	3.80	62
DP#4 22.5m	72,227	178	99.9	70.59	4.56	178
DP#4 22.5F	76,343	164	100	62.06	4.47	164
DP#4-MAT	64,221	278	100	75.90	4.84	278
Total sequences	551,427					

*Sequences remaining after quality filtering, and removal of chimeric sequences.

[†] ASVs passed quality filtering.

[‡] R version 4.1.1 with the DADA2 v1.16.0 package was used to process reads, assigning taxonomic value based on the SILVA database v138 training set (Callahan et al., 2016; Davis et al., 2023).

[‡] After filtering the water sample through a 0.22 µm filter, the penetrant was filtered through a 0.1 µm and the remaining material was used for DNA extraction and sequencing.

Supplementary Table 7. One-Way ANOSIM test obtained using ASV abundance table and Bray-Curtis index.

Samples were clustered in five different groups according to their origin: ICE (DP2-Ice), SUP (DP2-7.8m, DP4-9m, DP4-12m, DP4-13m, DP4-15m, DP4-17m; NEAR (DP2-9m), DEEP (DP4-20m, DP4-21.3m, DP4-21.3m-0.10µm, DP4-21.5m, DP4-22.3m-0.10µm); MAT (microbial mats, pinnacles) (DP2-Mat; DP4-Mat).

P-values uncorrected anosim test (Bray-Curtis similarity)					
	ICE	SUP	NEAR	PNCL	DEEP
ICE		0.0367	0.3327	0.3314	0.0469
SUP	0.0367		0.0356	0.0360	0.1274
NEAR	0.3327	0.0356		0.3399	0.0490
MAT	0.3314	0.0360	0.3399		0.0522
DEEP	0.0469	0.1274	0.0490	0.0522	

Supplementary Table 8. Results of the linear vector fitting of the environmental community predictors on the bacterial diversity nMDS. Number of permutations: 9999.

env_fitting	NMDS1	NMDS2	r2	Pr(>r)
Depth	0.64268	-0.76614	0.1051	0.5215
T	-1.00000	0.00131	0.3977	0.0434
PH	0.99976	0.02195	0.4528	0.0233
Conductivity	0.91854	-0.39532	0.0335	0.8407
HCO ₃ ⁻	-0.32182	-0.94680	0.0424	0.7925
F ⁻	0.97075	0.24008	0.0723	0.6611
Cl ⁻	0.82115	-0.57071	0.0475	0.7793
Br ⁻	0.97244	0.23316	0.2273	0.2086
NO ₃ ⁻	-0.32201	0.94674	0.0756	0.5680
SO ₄ ²⁻	0.94274	0.33354	0.0445	0.7859
Ca ²⁺	0.85627	-0.51653	0.0417	0.8037
Mg ²⁺	0.37799	-0.92581	0.0416	0.7982
Na ⁺	0.89363	-0.44880	0.0342	0.8421
K ⁺	0.56834	-0.82280	0.0333	0.8442
NH ₄ ⁺	-0.88619	0.46331	0.1388	0.4260
As ⁵⁺	0.99357	0.11322	0.1503	0.3885
B	0.79213	-0.61035	0.0121	0.9393
Li ⁺	0.95252	-0.30447	0.0144	0.9300
Rb ⁺	0.44465	0.89570	0.0227	0.8793

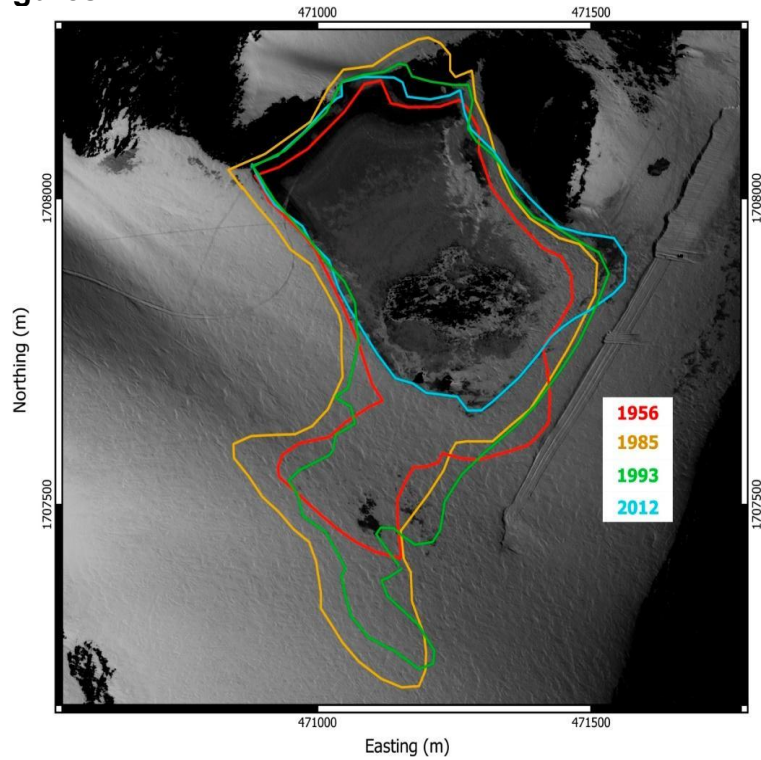
Supplementary Table 9. Similarity percentage analysis (SIMPER) between Lake Enigma samples came from different sub-habitats, and relative abundance of the key player phylum in shaping microbial biodiversity among them.

Taxon	Cryptophyceae	Bacteroidota	Actinobacteriota	Proteobacteria	Patescibacteria	Chloroflexi	Stramenopiles	Verrucomicrobiota	Planctomycetota
Av. dissim	9.58	6.63	5.29	4.66	4.41	2.97	2.35	2.22	2.13
Contrib. %	20.92	14.48	11.55	10.18	9.63	6.50	5.14	4.85	4.65
Cumulative %	20.92	35.40	46.95	57.13	66.76	73.26	78.39	83.24	87.89
ICE_DP2-Ice	0.00	28.54	3.63	42.55	0.00	0.00	25.28	0.00	0.00
SUP_DP2-7.8m	27.82	35.63	18.38	11.67	4.16	0.00	0.00	0.98	0.00
NEAR_DP2-Near	1.72	20.54	7.70	9.04	35.05	0.39	0.00	8.14	6.24
MAT_DP2-Pncl	0.31	10.57	14.95	16.89	4.21	14.67	0.23	8.05	1.72
SUP_DP4-9m	18.32	34.62	22.82	15.80	1.10	0.00	0.00	1.31	0.00
SUP_DP4-12m	45.85	26.17	13.76	7.06	2.99	0.00	0.00	1.53	0.19
SUP_DP4-13m	40.09	33.49	5.84	18.18	1.68	0.00	0.00	0.71	0.00
SUP_DP4-15m	29.67	39.92	17.16	7.77	1.01	0.00	0.00	1.83	0.21
SUP_DP4-17m	26.66	37.76	15.02	15.64	0.94	0.00	0.00	0.00	0.28
DEEP_DP4-20m	17.75	36.20	27.16	11.49	0.94	0.00	0.00	1.30	3.00
DEEP_DP4-21.3m	20.33	25.15	25.37	8.75	0.93	1.10	0.21	4.84	10.23
DEEP_DP4-21.3m 0.10µm	10.89	10.70	32.88	18.44	5.55	7.52	0.00	3.65	2.93
DEEP_DP4-21.5m	17.82	28.00	24.76	9.68	4.03	1.80	0.00	1.42	11.09
DEEP_DP4-21.5m 0.10µm	17.24	27.60	19.31	20.22	6.43	1.80	0.00	3.12	1.83
MAT_DP4-Pncl	0.14	7.78	19.63	14.88	12.59	15.78	1.31	11.44	1.84

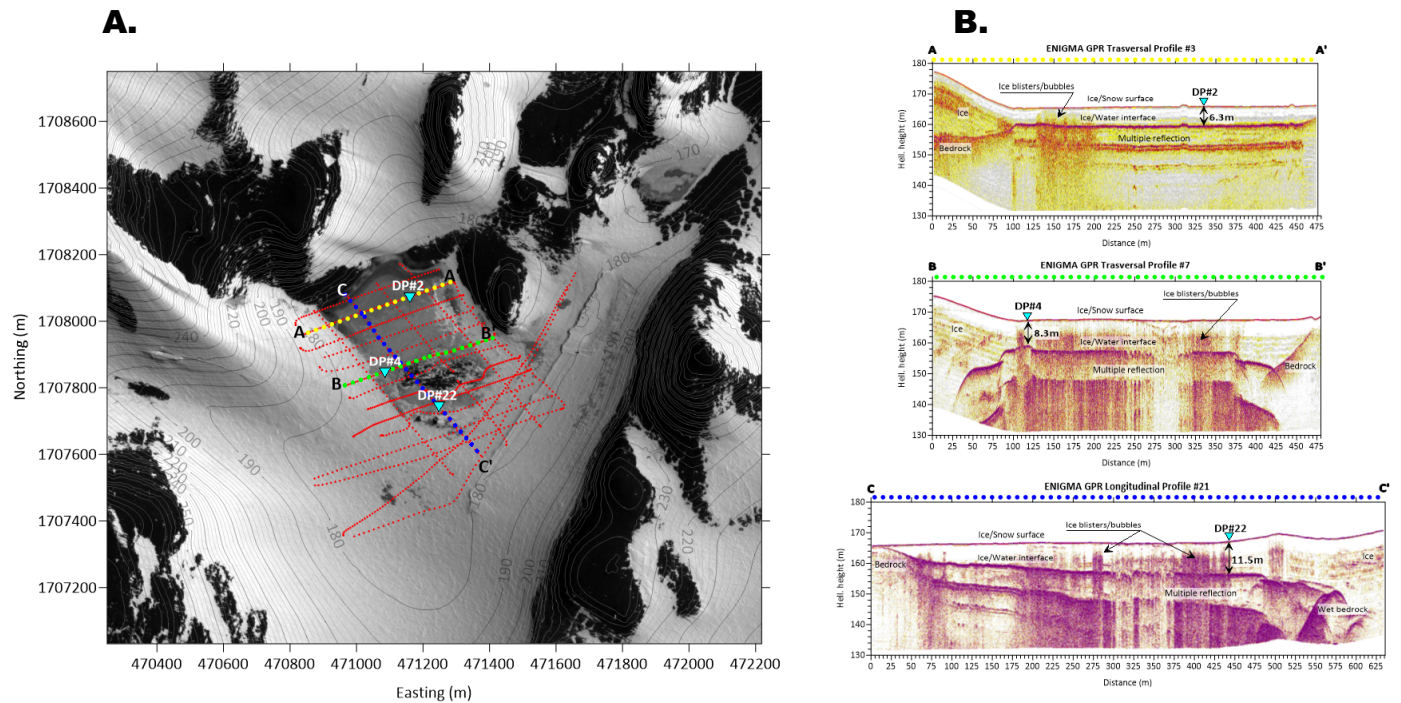
Supplementary Table 10. Significance of Mantel's test variables.

Phylum	Physiochemical factors	Mantel's <i>r</i>	Mantel's <i>p</i>
Patescibacteria	pH	0.33	0.008
Bacteroidota	pH	0.38	0.009
Patescibacteria	B	0.31	0.016
Actinobacteriota	Temperature	0.30	0.023
Patescibacteria	NH ₄ ⁺	0.28	0.025
Bacteroidota	F ⁻	0.26	0.028
Actinobacteriota	Na ⁺	0.42	0.035
Patescibacteria	Li ⁺	0.25	0.046

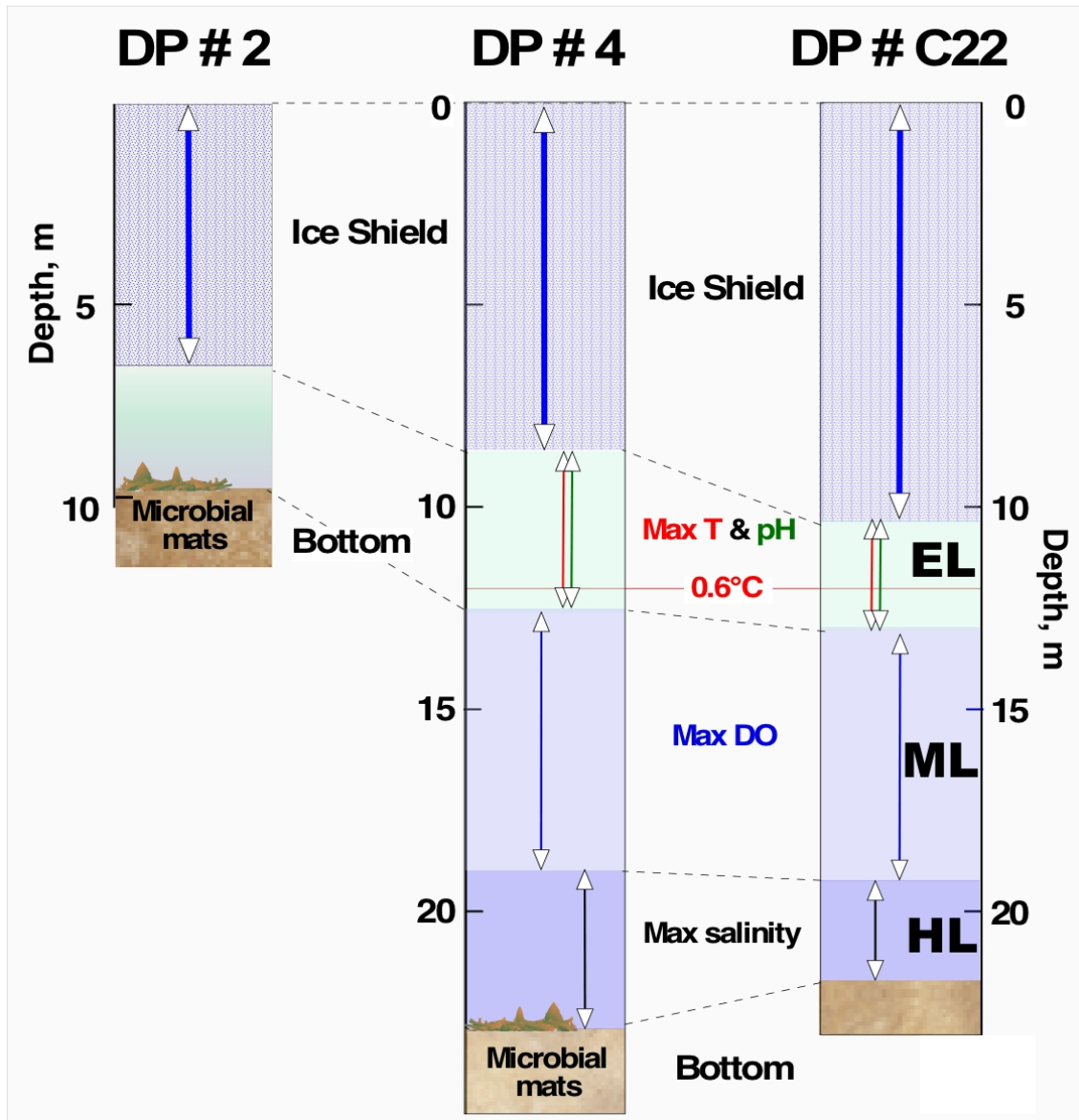
Supplementary Figures



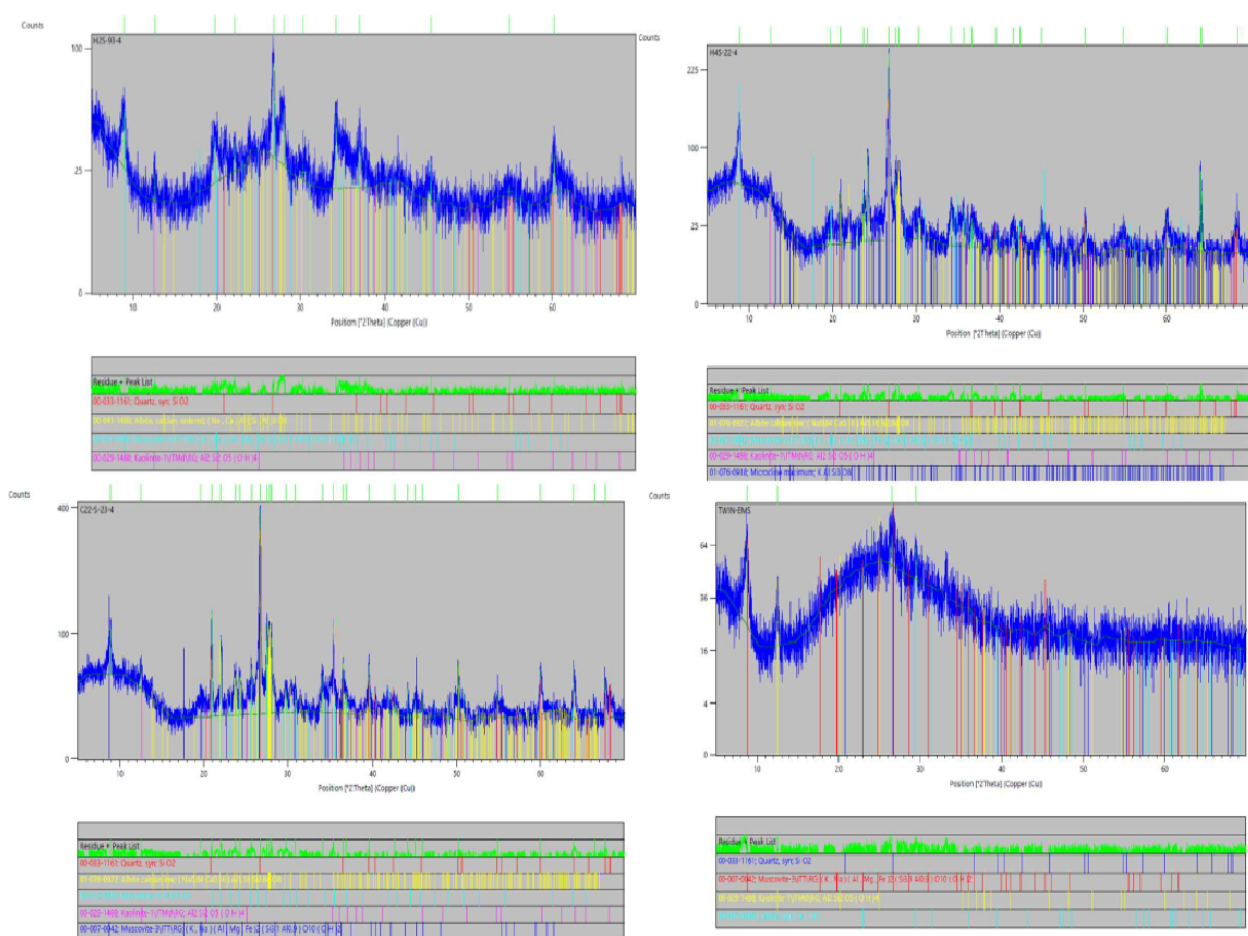
Supplementary Figure 1. Enigma Lake's surface variations from 1956 to 2012 (adapted from Urbini et al. 2019 [39]).



Supplementary Figure 2. Google map view on Lake Enigma with reference points made during the geophysical study in November-December 2019 and the positioning of the drilling points DP#2, DP#4 and DP#22 (left insertion). Right insertions are the ground radar penetration profiling of the NE-SW and E-W transects with positioning of the drilling points DP#2, DP#4 and DP#C22.

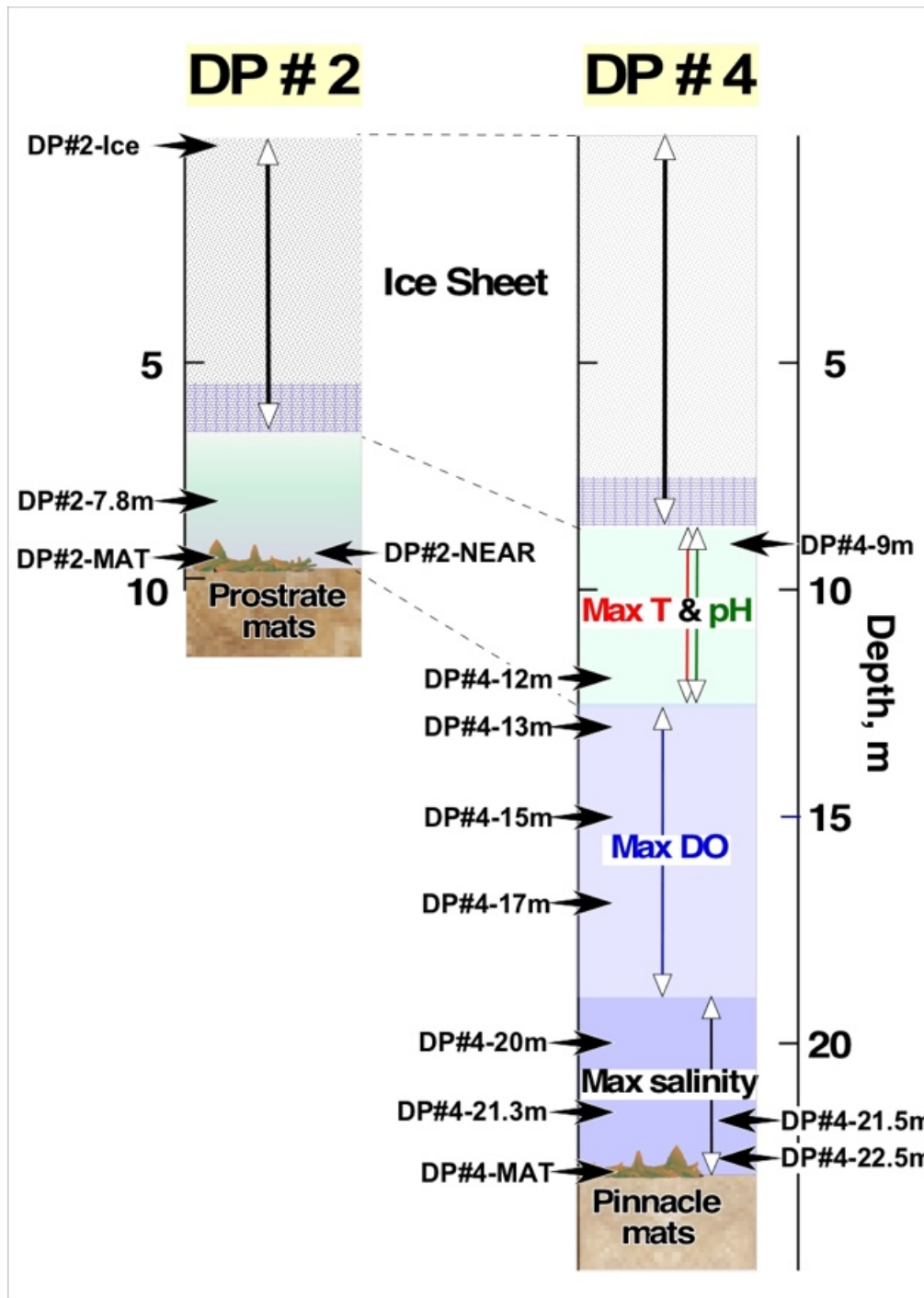


Supplementary Figure 3. Water column stratification, observed at the DP#2, DP#4 and DP#C22 drilling wells. Abbreviation used: EL, epilimnion; HL, hypolimnion; ML, metalimnion.

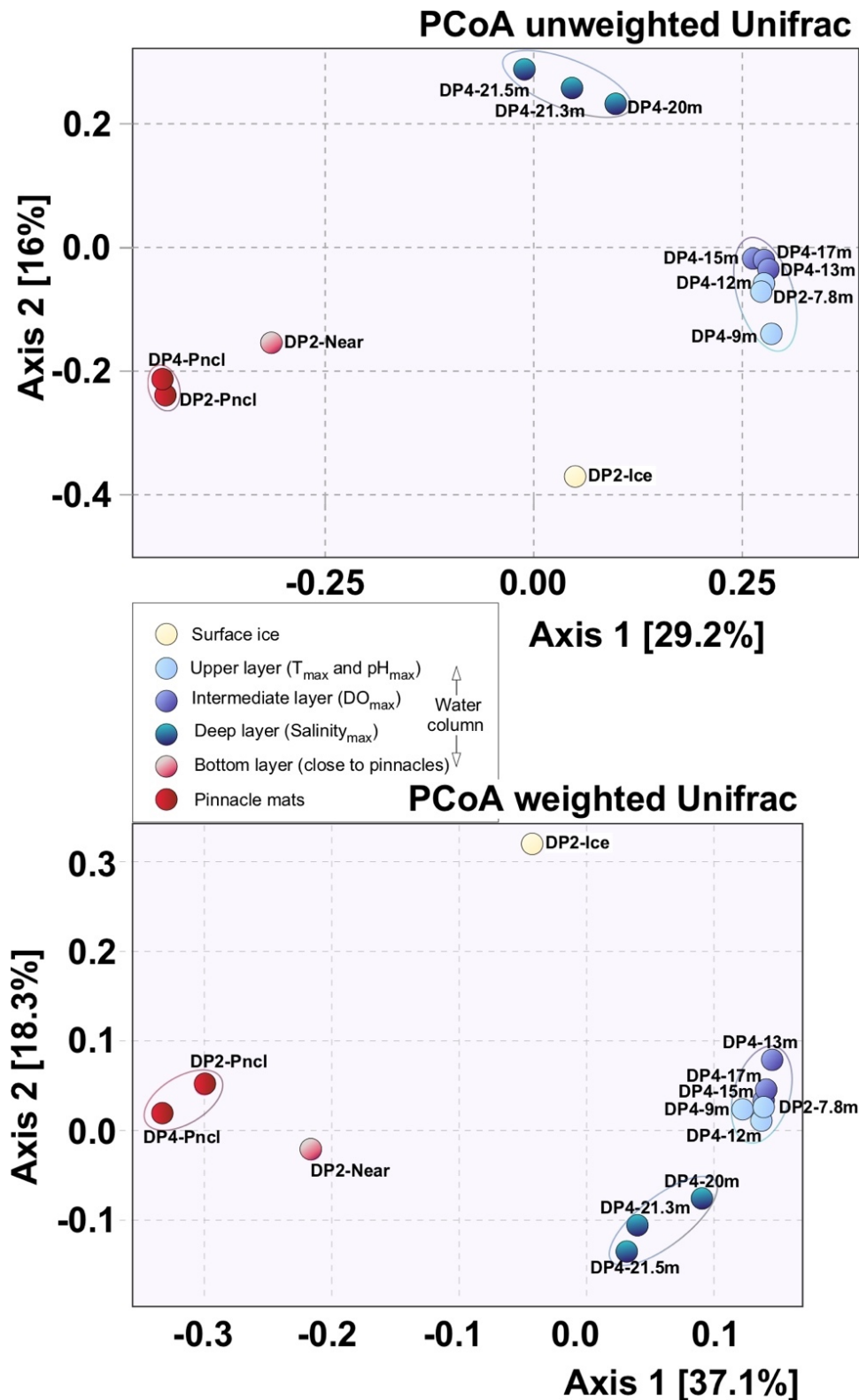


Supplementary Figure 4. X-ray diffractograms carried out on 4 sediment samples from the Enigma Lake.

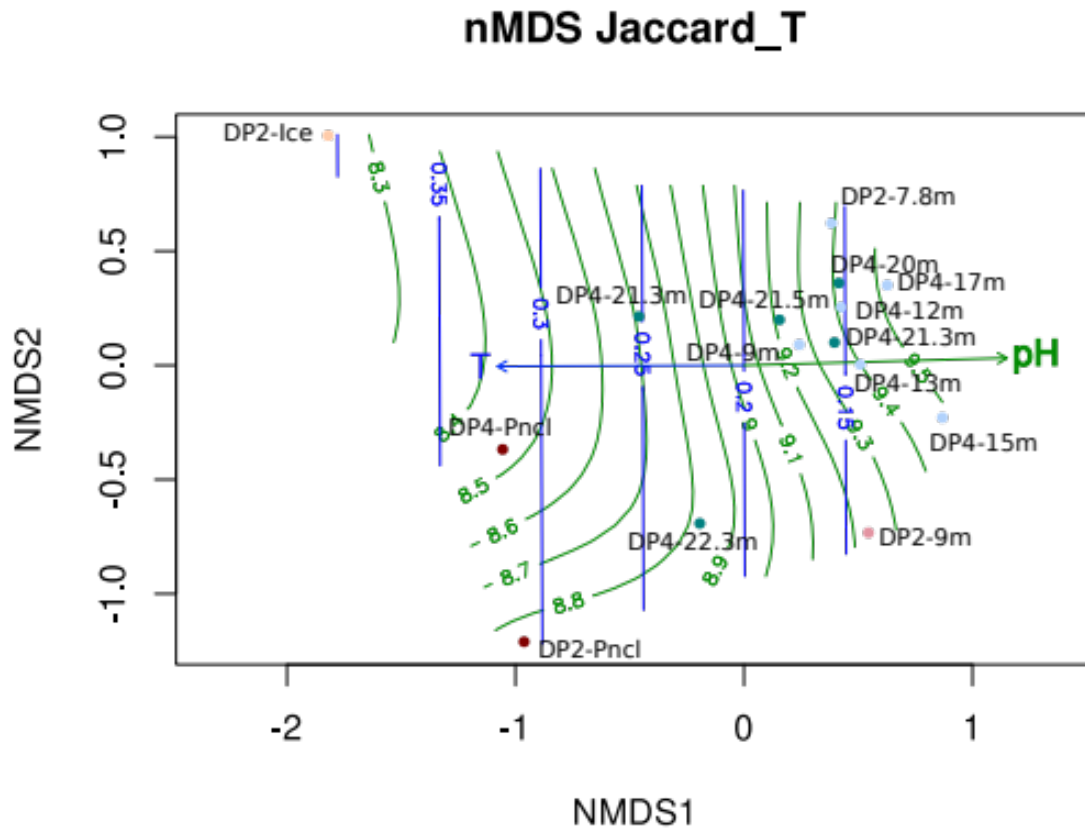
X-ray Philips PW 1050/37 diffractometer, with the X'Pert PRO Philips acquisition system, operating at 40 kV-20 mA, with a Cu anode, graphite monochromator at an interval 2θ of 5-70°. Below each diagram, the main minerals recognized by x-ray are evidenced.



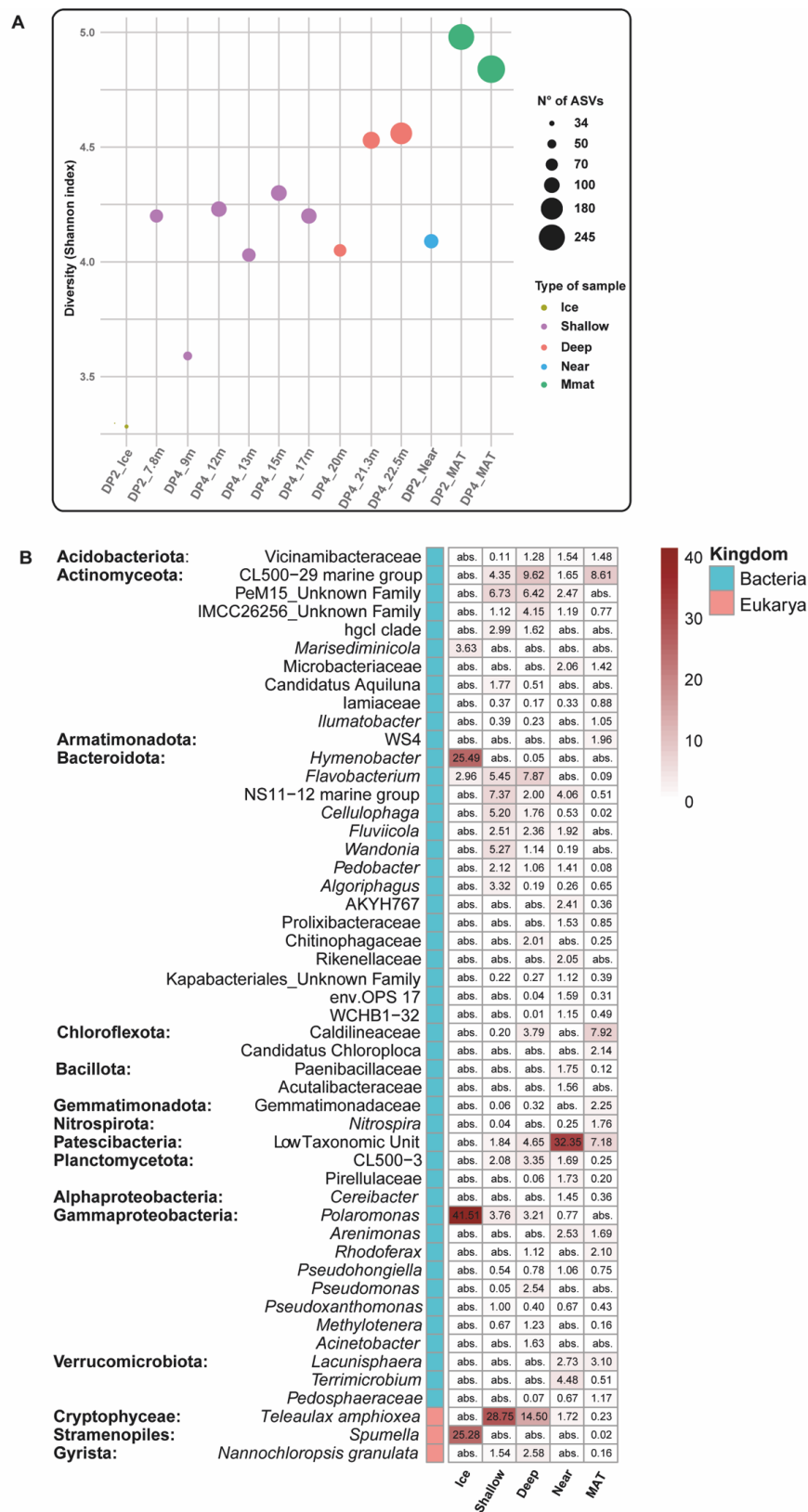
Supplementary Figure 5. Strategy for collecting biological samples from different compartments of Lake Enigma, taking into account the stratification of the water column, the thickness of the ice cover and the presence of microbial mats at drilling points DP#2 and DP#4.



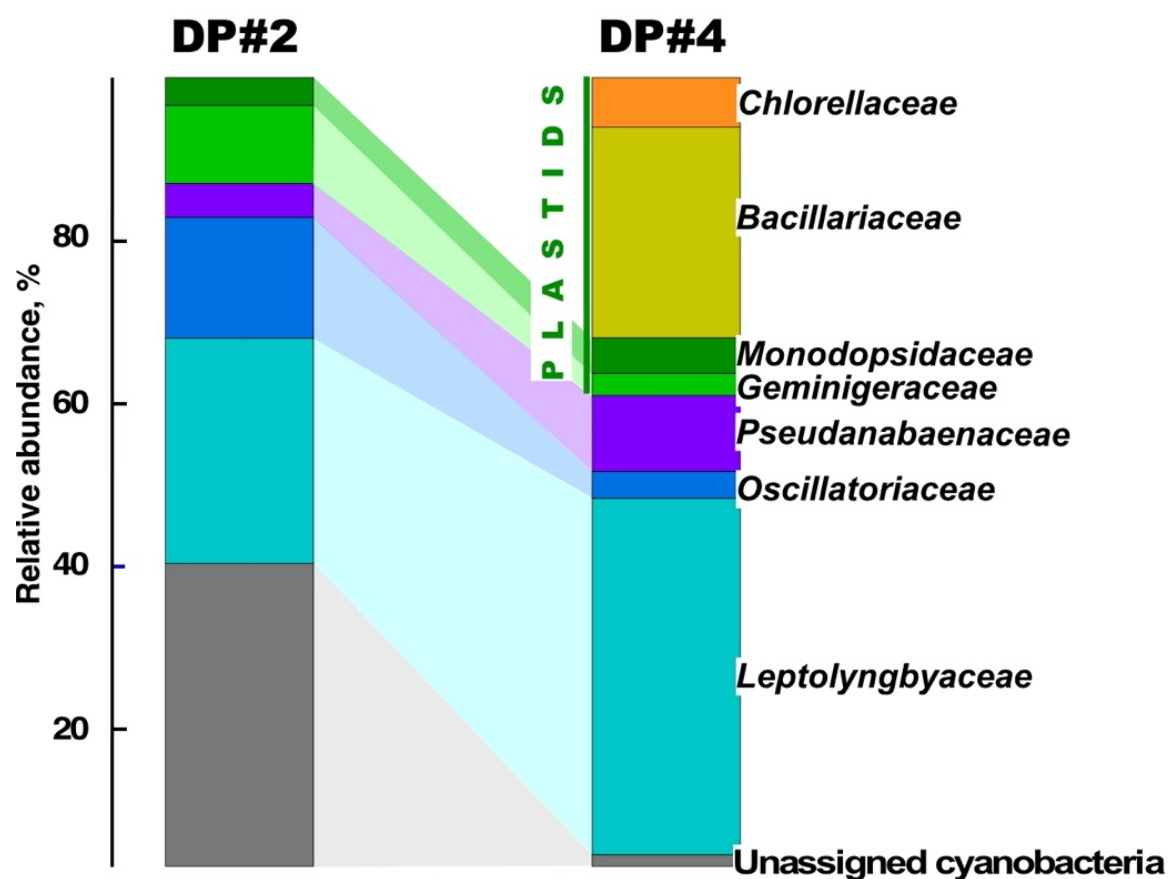
Supplementary Figure 6. Principal component analysis (PCoA) using unweighted and weighted Unifrac distance calculation for microbial community composition in different Lake Enigma habitat types. The horizontal and vertical axes represent the two selected principal components; percentages represent the magnitude of the contribution of the principal components to the difference in microbial communities; the closer the dots, the more similar the microbial community. Ellipses indicate grouping of analyzed samples.



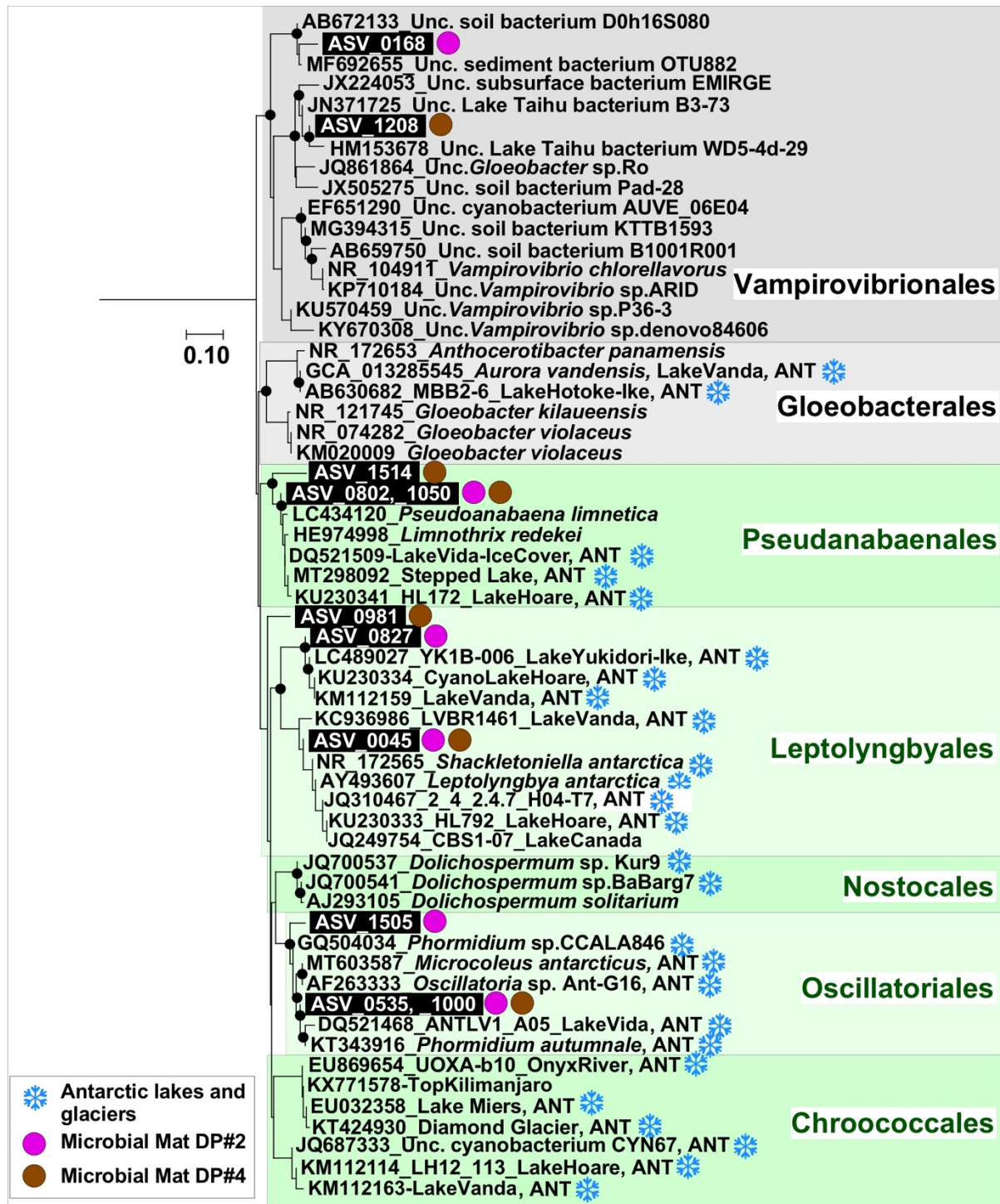
Supplementary Figure 7. Non-metric multidimensional scaling (NMDS, stress 0.02) plot of the 16S rRNA gene amplicon microbial diversity based on Jaccard dissimilarity measure overlaid with environmental vector fitting both temperature and pH values. To further explore the relationship between environmental variables and biodiversity data, the *ordisurf* function was employed. This function, employing a Generalized Additive Model (GAM), facilitated the prediction of these relationships. Notably, the results demonstrated a linear correlation with temperature, as evidenced by estimated degrees of freedom of 3.04, a total of 4.04, and an REML score of 14.68139. On the other hand, pH exhibited a non-linear correlation, characterized by estimated degrees of freedom of 1.5, a total of 2.5, and an REML score of -3.784221 (abbreviations of samples are from Supplementary Table 2).



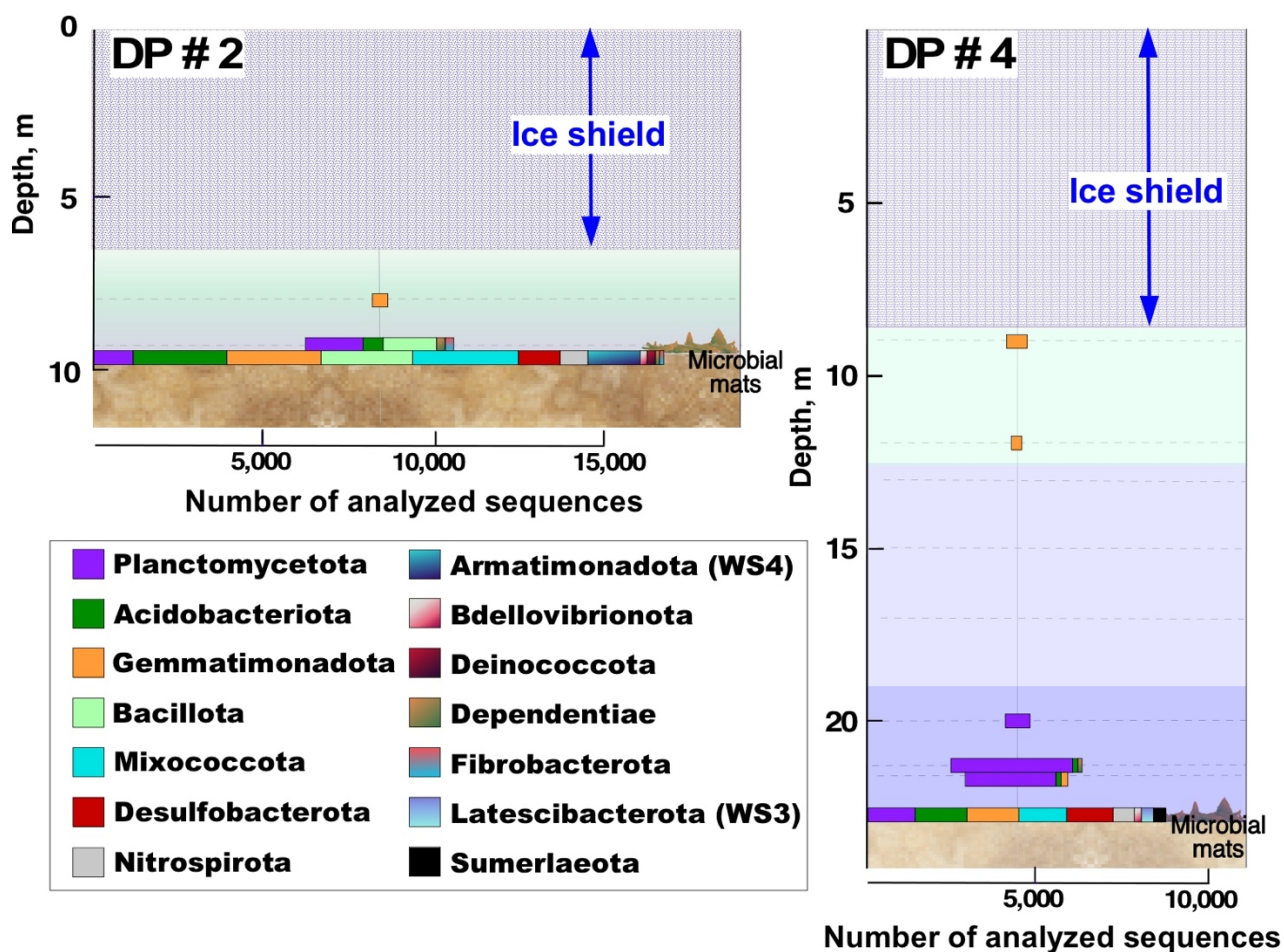
Supplementary Figure 8. Bubble chart shows the number of ASVs in each sample, colors indicate the type of sample (A) Heatmap showing the relative abundance of most representative phyla (B).



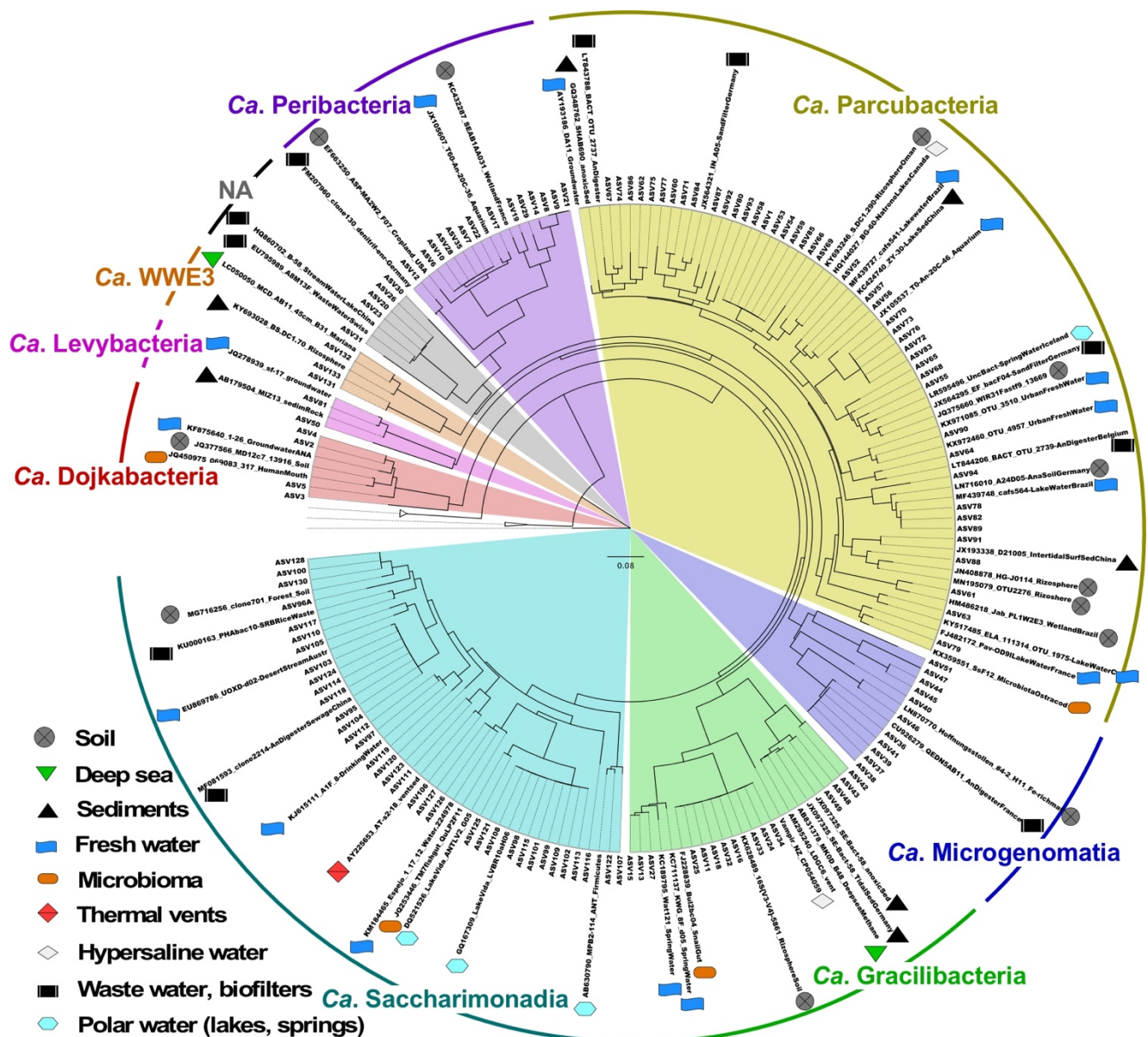
Supplementary Figure 9. Relative abundance (%) plot of cyanobacteria and unicellular algae (assigned by their plastid 16S rRNA genes) found in top layers of microbial mats collected at different drilling points.



Supplementary Figure 10. Phylogenetic position of Lake Enigma cyanobacterial ASVs within the phylum *Cyanobacteriota*. The tree was constructed based on taxonomy, assigned to ASVs using a naïve Bayesian classifier method against the Silva Database v138 (<https://www.arb-silva.de/documentation/release-138> and https://zenodo.org/record/4587955#.YgKJlb_MJH4). After alignment, the neighbor-joining algorithm of the ARB software package was used to generate the phylogenetic trees based on distance analysis for 16S rRNA. The tree was additionally inferred in the maximum likelihood framework using the MEGA v.6.0 software (Tamura et al., 2013). The robustness of inferred topologies was tested by the bootstrap resampling using the same distance model (1,000 replicates of the original dataset) and values more than 60% are shown at nodes as filled circles. Scale bar represents the average number of substitutions per site.



Supplementary Figure 11. Vertical distribution of rare bacterial taxa across the Lake Enigma habitats. Absolute numbers of Illumina-sequencing reads are shown for the bacterial groups, present in less than 1.0% of the total number of reads detected in the Lake Enigma surface ice, water column, and microbial mats, respectively.



Supplementary Figure 12. Randomized Axelerated Maximum Likelihood (RAXML) tree of 16S rRNA genes of different classes of the superphylum Patasibacteria. A phylogeny was generated using Lake Enigma's 133 distinct ASVs and reference GenBank riboclones. In the phylogenetic tree, no clear grouping was observed based on either the isolation source, trophic state of the ecosystems or the presence of oxygen.

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