

# SUPPLEMENTARY MATERIALS

## Community Vertical Composition of the Laguna Negra Hypersaline Microbial Mat, Puna Region (Argentinean Andes)

Flavia Jaquelina Boidi <sup>1,\*</sup>, Estela Cecilia Mlewski <sup>2</sup>, Guillermo César Fernández <sup>3</sup>, María Regina Flores <sup>4</sup>,  
Emmanuelle Gérard <sup>5</sup>, María Eugenia Farías <sup>6</sup> and Fernando Javier Gomez <sup>3,\*</sup>

<sup>1</sup> Laboratorio de Biología Molecular, Hospital SAMCo Rafaela “Dr. Jaime Ferré”, 737 Lisandro de la Torre, Rafaela 2300, Argentina; flajboidi@gmail.com

<sup>2</sup> Instituto Multidisciplinario de Biología Vegetal (IMBIV), Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, CONICET, 1666 Vélez Sarsfield Av., Córdoba 5000, Argentina; ecmlewski@imbiv.unc.edu.ar

<sup>3</sup> Centro de Investigaciones en Ciencias de la Tierra (CICTERRA), Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, CONICET, 1699 Vélez Sarsfield Av., Córdoba 5000, Argentina; guillefernandez.unc@gmail.com (G.C.F.); fjgomez@unc.edu.ar (F.J.G.)

<sup>4</sup> Department of Research and Development, PROMICOL B.V., 12 De Asselen Kuil, Geleen 6161, Netherlands; acm\_regy@hotmail.com

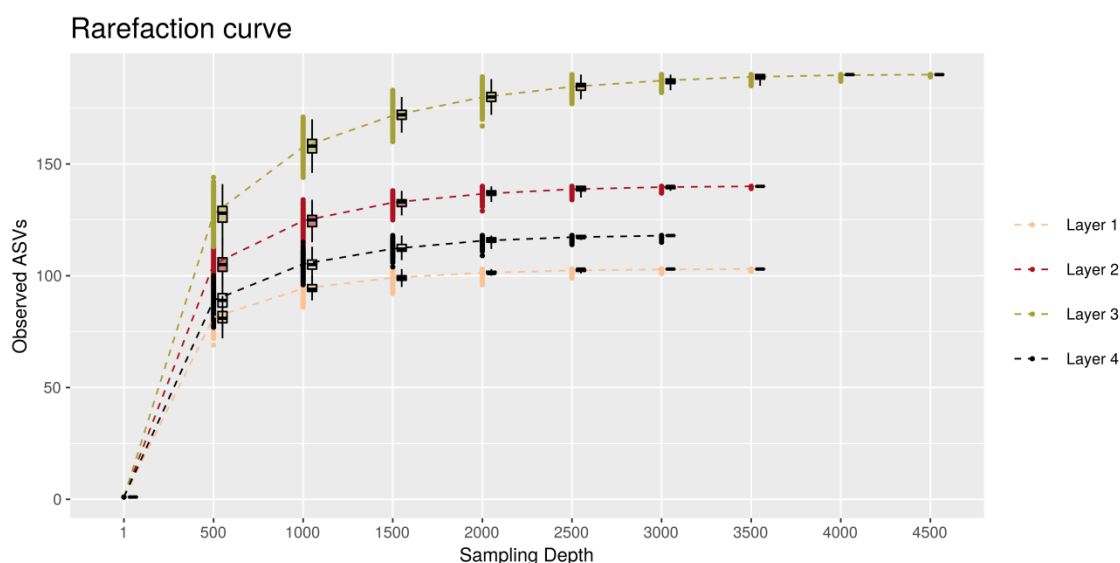
<sup>5</sup> Université de Paris Cité, Institut de physique du globe de Paris, CNRS, F-75005 Paris, France; emgerard@ipgp.fr

<sup>6</sup> Planta Piloto de Procesos Industriales y Microbiológicos (PROIMI), Centro Científico Tecnológico – CONICET, Belgrano Av. & Pasaje Caseros, San Miguel de Tucumán 4000, Argentina; mefarías2009@gmail.com

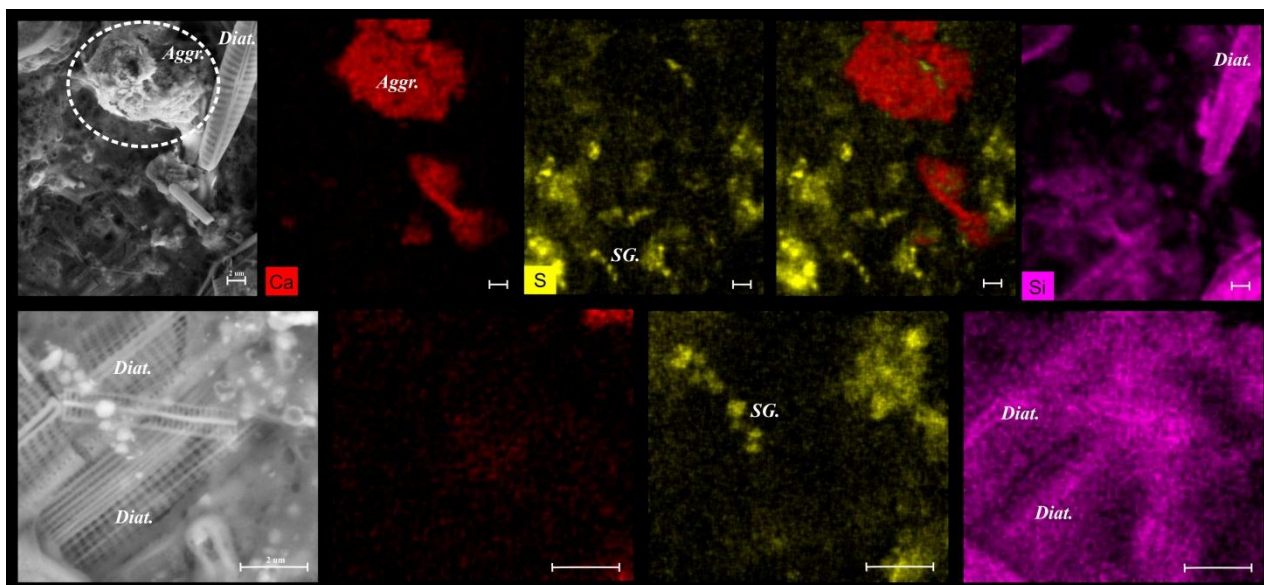
\* Correspondence: flajboidi@gmail.com (F.J.B.); fjgomez@unc.edu.ar (F.J.G.)

### SUPPLEMENTARY FIGURES

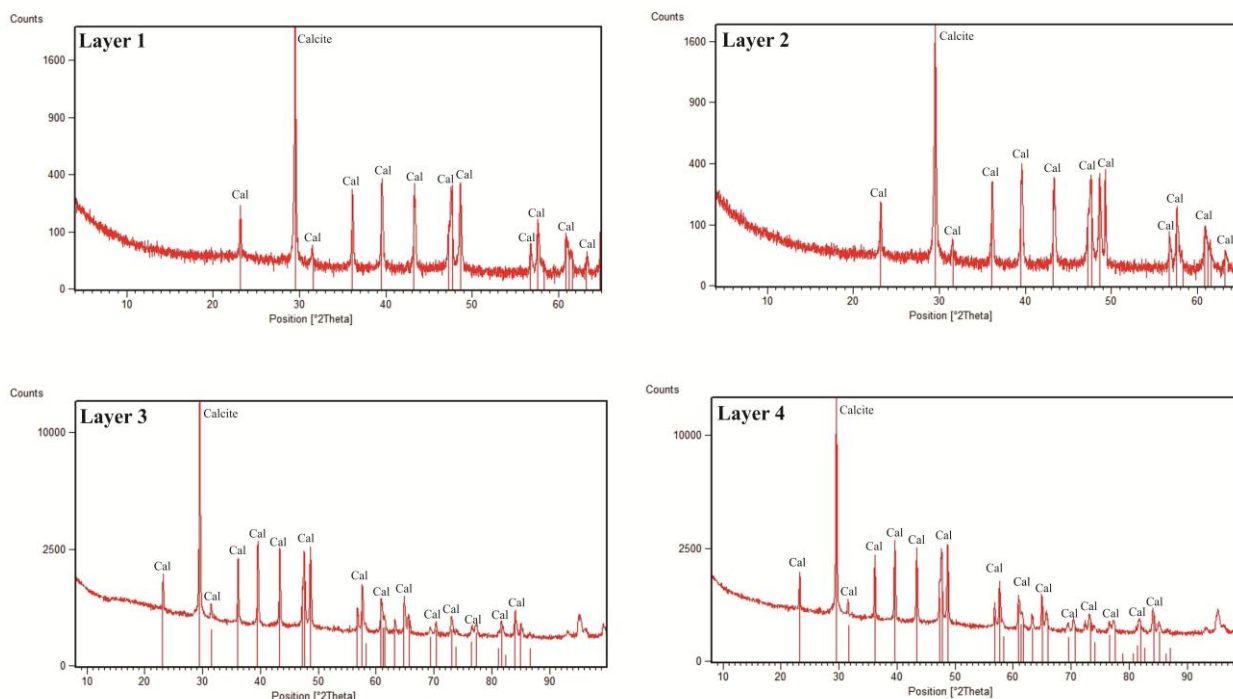
**Supplementary Figure S1.** Observed ASVs rarefaction curve. Rarefaction curves were obtained by multiple repeated sub-sampling (1000 times). For each sampling depth the 1000 calculated value are plotted as circles and the data dispersion is showed in boxplots.



**Supplementary Figure S2.** EDS maps where major elements distribution is visualized. Note Ca as part of aggregates (*Aggr.*), S forming globules (*SG.*) and Si as part of diatoms (*Diat.*).



**Supplementary Figure S3.** XRD spectra showing calcite pattern with red lines in all layers.



## SUPPLEMENTARY TABLES

**Supplementary Table S1.** Descriptive statistics of Observed ASVs analysis. Values were obtained using a sub-sampling quota of 3000 reads per sample. The sub-sampling routine was performed for 1000 iterations.

Observed ASVs					
Layer	N	Mean	Standard Deviation	Standard Error	Confident Intervals (95%)
1	1000	102.830	0.4065283	0.012855554	0.02522699
2	1000	139.675	0.5794055	0.018322410	0.03595482
3	1000	187.319	1.5848504	0.050117370	0.09834739
4	1000	117.941	0.2521559	0.007973869	0.01564745

### Non-parametric statistical analysis of Observed ASVs

(Kruskal-Wallis test,  $H_3 = 3862.3082$ ,  $p\text{-value} < 0.0001$ ).

Dunn's test: Layer 1 vs Layer 2 ( $p\text{-value} < 0.0001$ ), Layer 1 vs Layer 3 ( $p\text{-value} < 0.0001$ ), Layer 1 vs Layer 4 ( $p\text{-value} < 0.0001$ ), Layer 2 vs Layer 3 ( $p\text{-value} < 0.0001$ ), Layer 2 vs Layer 4 ( $p\text{-value} < 0.0001$ ), Layer 3 vs Layer 4 ( $p\text{-value} < 0.0001$ ).

**Supplementary Table S2.** Descriptive statistics of Shannon index analysis. Values were obtained using a sub-sampling quota of 3000 reads per sample. The sub-sampling routine was performed for 1000 iterations

Shannon					
Layer	N	Mean	Standard Deviation	Standard Error	Confident Intervals
1	1000	5.451751	0.01325192	0.0004190624	0.0008223434
2	1000	5.887495	0.01467669	0.0004641178	0.0009107576
3	1000	6.488320	0.01911901	0.0006045961	0.0011864240
4	1000	5.452609	0.01176552	0.0003720584	0.0007301056

### Non-parametric statistical analysis of Shannon index

(Kruskal-Wallis test,  $H_3 = 3374.5$ ,  $p\text{-value} < 0.0001$ ).

Dunn's test: Layer 1 vs Layer 2 ( $p\text{-value} < 0.0001$ ), Layer 1 vs Layer 3 ( $p\text{-value} < 0.0001$ ), Layer 1 vs Layer 4 ( $p\text{-value} < 0.0001$ ), Layer 2 vs Layer 3 ( $p\text{-value} < 0.0001$ ), Layer 2 vs Layer 4 ( $p\text{-value} < 0.0001$ ), Layer 3 vs Layer 4 ( $p\text{-value} < 0.0001$ ).

**Supplementary Table S3.** Descriptive statistics of Simpson index analysis. Values were obtained using a sub-sampling quota of 3000 reads per sample. The sub-sampling routine was performed for 1000 iterations.

Simpson					
Layer	N	Mean	Standard Deviation	Standard Error	Confident Intervals
1	1000	0.9591646	0.0005086771	1.608578e-05	3.156580e-05
2	1000	0.9608285	0.0007163063	2.265160e-05	4.445017e-05
3	1000	0.9803524	0.0004251427	1.344419e-05	2.638210e-05
4	1000	0.9434626	0.0007591020	2.400491e-05	4.710583e-05

### Non-parametric statistical analysis of Simpson index

(Kruskal-Wallis test,  $H_3 = 3707.6696$ ,  $p\text{-value} < 0.0001$ ).

Dunn's test: Layer 1 vs Layer 2 ( $p\text{-value} < 0.0001$ ), Layer 1 vs Layer 3 ( $p\text{-value} < 0.0001$ ), Layer 1 vs Layer 4 ( $p\text{-value} < 0.0001$ ), Layer 2 vs Layer 3 ( $p\text{-value} < 0.0001$ ), Layer 2 vs Layer 4 ( $p\text{-value} < 0.0001$ ), Layer 3 vs Layer 4 ( $p\text{-value} < 0.0001$ ).

**Supplementary Table S4.** Descriptive statistics of Dominance index analysis. Values were obtained using a sub-sampling quota of 3000 reads per sample. The sub-sampling routine was performed for 1000 iterations.

Dominance					
Layer	N	Mean	Standard Deviation	Standard Error	Confident Intervals
1	1000	0.04083544	0.0005086771	1.608578e-05	3.156580e-05
2	1000	0.03917145	0.0007163063	2.265160e-05	4.445017e-05
3	1000	0.01964765	0.0004251427	1.344419e-05	2.638210e-05
4	1000	0.05653737	0.0007591020	2.400491e-05	4.710583e-05

### Non-parametric statistical analysis of Dominance index

(Kruskal-Wallis test,  $H_3 = 3707.7$ ,  $p\text{-value} < 0.0001$ ).

Dunn's test: Layer 1 vs Layer 2 ( $p\text{-value} < 0.0001$ ), Layer 1 vs Layer 3 ( $p\text{-value} < 0.0001$ ), Layer 1 vs Layer 4 ( $p\text{-value} < 0.0001$ ), Layer 2 vs Layer 3 ( $p\text{-value} < 0.0001$ ), Layer 2 vs Layer 4 ( $p\text{-value} < 0.0001$ ), Layer 3 vs Layer 4 ( $p\text{-value} < 0.0001$ ).

**Supplementary Table S5.** Descriptive statistics of Pielou\_e (J) index analysis. Values were obtained using a sub-sampling quota of 58000 reads per sample. The sub-sampling routine was performed for 1000 iterations.

Pielou_e (J)					
Layer	N	Mean	Standard Deviation	Standard Error	Confident Intervals
1	1000	0.8156294	0.002054605	6.497231e-05	0.0001274979
2	1000	0.8262090	0.002079192	6.574983e-05	0.0001290236
3	1000	0.8594608	0.002544751	8.047209e-05	0.0001579137
4	1000	0.7923095	0.001698435	5.370922e-05	0.0001053958

### Non-parametric statistical analysis of Pielou\_e (J) index

(Kruskal-Wallis test,  $H_3 = 3749$ ,  $p\text{-value} < 0.0001$ ).

Dunn's test: Layer 1 vs Layer 2 ( $p\text{-value} < 0.0001$ ), Layer 1 vs Layer 3 ( $p\text{-value} < 0.0001$ ), Layer 1 vs Layer 4 ( $p\text{-value} < 0.0001$ ), Layer 2 vs Layer 3 ( $p\text{-value} < 0.0001$ ), Layer 2 vs Layer 4 ( $p\text{-value} < 0.0001$ ), Layer 3 vs Layer 4 ( $p\text{-value} < 0.0001$ ).

**Supplementary Table S6.** Descriptive statistics of Chao1 index analysis. Values were obtained using a sub-sampling quota of 3000 reads per sample. The sub-sampling routine was performed for 1000 iterations.

Chao1					
Layer	N	Mean	Standard Deviation	Standard Error	Confident Intervals
1	1000	103.0906	0.6621615	0.02093939	0.04109023
2	1000	140.2106	0.8781844	0.02777063	0.05449546
3	1000	191.0313	2.8453931	0.08997923	0.17656997
4	1000	118.0717	0.3468058	0.01096696	0.02152092

### Non-parametric statistical analysis of Chao 1 index

(Kruskal-Wallis test,  $H_3 = 3749.1$ ,  $p\text{-value} < 0.0001$ ).

Dunn's test: Layer 1 vs Layer 2 ( $p\text{-value} < 0.0001$ ), Layer 1 vs Layer 3 ( $p\text{-value} < 0.0001$ ), Layer 1 vs Layer 4 ( $p\text{-value} < 0.0001$ ), Layer 2 vs Layer 3 ( $p\text{-value} < 0.0001$ ), Layer 2 vs Layer 4 ( $p\text{-value} < 0.0001$ ), Layer 3 vs Layer 4 ( $p\text{-value} < 0.0001$ ).

**Supplementary Table S7.** Most abundant ASVs (>0.02 relative abundance) per layer classified at the lowest possible taxonomic level. Known genera are highlighted in bold.

ASV	Layer	Relative abundance	Silva predicted taxonomy		NCBI best BLAST match (Accession Number)	Identity	Isolation source	Reference
01	1	0.00	Bacteria	Phylum Halanaerobiaeota	Uncultured <i>Halanaerobium</i> sp. (FM879119.1)	100 %	Hyperhaline lake sediment, Salar de Ascotán, Chile	[53]
	2	0.03		Class Halanaerobiia				
	3	0.05		Order Halanaerobiales				
	4	0.19		Family Halanaerobiaceae Genus <b><i>Halanaerobium</i></b> Species Unknown				
02	1	0.00	Bacteria	Phylum Thermotogae	Uncultured organism clone SBYH_2623 (JN455679.1)	98.8 %	Hypersaline microbial mat, Guerrero Negro, Mexico	[41]
	2	0.02		Class Thermotogae				
	3	0.04		Order Petrotogales				
	4	0.03		Family Petrotogaceae Genus SC103 Species Uncultured organism				
03	1	0.04	Bacteria	Phylum Proteobacteria	Uncultured bacterium clone ProA23S_19 (GQ915826.1)	95.6 %	Marine water, Gulf of Mexico, USA	[54]
	2	0.00		Class Alphaproteobacteria				
	3	0.00		Order Rickettsiales				
	4	0.00		Family Unknown Genus Unknown Species Unknown				
04	1	0.01	Bacteria	Phylum Patescibacteria	Uncultured organism clone SBXZ_5580 (JN436702.1)	99.2 %	Hypersaline microbial mat, Guerrero Negro, Mexico	[41]
	2	0.04		Class Gracilibacteria				
	3	0.00		Order Absconditabacteriales (SR1)				
	4	0.00		Family Unknown Genus Unknown Species Unknown				
05	1	0.03	Bacteria	Phylum Verrucomicrobia	Uncultured bacterium clone 59_1854298 (LT720392.1)	100 %	Marine water, Rio de Janeiro, Brazil	[55]
	2	0.00		Class Verrucomicrobiae				
	3	0.01		Order Opitutales				
	4	0.00		Family Puniceicoccaceae Genus <b><i>Lentimonas</i></b> Species Uncultured Verrucomicrobia				
06	1	0.00	Bacteria	Phylum Proteobacteria	Uncultured organism clone SBYH_6277 (JN459997.1)	99.6 %	Hypersaline microbial mat, Guerrero Negro, Mexico	[41]
	2	0.01		Class Deltaproteobacteria				
	3	0.07		Order Oligoflexales				
	4	0.03		Family 0319-6G20 Genus Unknown Species Unknown				

**Supplementary Table S7.** Most abundant ASVs (>0.02 relative abundance) per layer classified at the lowest possible taxonomic level. Known genera are highlighted in bold. (continued)

ASV	Layer	Relative abundance	Silva predicted taxonomy		NCBI best BLAST match (Accession Number)	Identity	Isolation source	Reference
07	1	0.08	Bacteria	Phylum Deinococcus-Thermus	Uncultured bacterium clone PET4-24 (MG949154.1)	99.6 %	Hypersaline microbial mat, Sečovlje Salina, Slovenia	[56]
	2	0.00		Class Deinococci				
	3	0.00		Order Deinococcales				
	4	0.00		Family Trueperaceae Genus <b>Truepera</b> Species Uncultured organism				
08	1	0.05	Bacteria	Phylum Patescibacteria	Uncultured candidate division SR1 bacterium clone Elkhorn-918R-1A (JF917294.1)	98.8 %	Elkhorn slough, California, USA	[57]
	2	0.03		Class Gracilibacteria				
	3	0.00		Order Absconditabacteriales (SR1)				
	4	0.00		Family Uncultured bacterium Genus Uncultured bacterium Species Uncultured bacterium				
09	1	0.00	Bacteria	Phylum Planctomycetes	Uncultured organism clone SBZC_540 (JN508204.1)	98 %	Hypersaline microbial mat, Guerrero Negro, Mexico	[41]
	2	0.02		Class SGST604				
	3	0.00		Order Uncultured organism				
	4	0.03		Family Uncultured organism Genus Uncultured organism Species Uncultured organism				
10	1	0.00	Bacteria	Phylum Spirochaetes	Uncultured organism clone SBYO_2277 (JN466486.1)	99.6 %	Hypersaline microbial mat, Guerrero Negro, Mexico	[41]
	2	0.01		Class Leptospirae				
	3	0.03		Order Leptospirales				
	4	0.02		Family Leptospiraceae Genus Uncultured Species Uncultured organism				
11	1	0.00	Bacteria	Phylum Thermotogae	Uncultured organism clone SBXZ_2085 (JN432239.1)	98.8 %	Hypersaline microbial mat, Guerrero Negro, Mexico	[41]
	2	0.00		Class Thermotogae				
	3	0.03		Order Petrotogales				
	4	0.00		Family Petrotogaceae Genus SC103 Species Uncultured organism				
12	1	0.00	Bacteria	Phylum Patescibacteria	Uncultured eubacterium WCHA2-26 (AF050609.1)	93.2 %	Contaminated aquifer, Michigan, USA	[58]
	2	0.00		Class Gracilibacteria				
	3	0.02		Order Absconditabacteriales (SR1)				
	4	0.00		Family Unknown Genus Unknown Species Unknown				
13	1	0.00	Bacteria	Phylum Planctomycetes	Uncultured bacterium clone Kasin-B1-B10 (HE604774.1)	92 %	Hypersaline sediment, Lake Kasin, Russia	[59]
	2	0.00		Class Unknown				
	3	0.00		Order Unknown				
	4	0.02		Family Unknown Genus Unknown Species Unknown				
14	1	0.04	Bacteria	Phylum Bacteroidetes	Uncultured bacterium clone wat-80 (KC508773.1)	88.1 %	Seawater, Tateyama, Japan	[60]
	2	0.04		Class Bacteroidia				
	3	0.00		Order Unknown				
	4	0.00		Family Unknown Genus Unknown Species Unknown				
15	1	0.00	Bacteria	Phylum Patescibacteria	Uncultured bacterium clone OM_int_bact086 (KY342779.1)	99.6 %	Hypersaline intertidal microbial mats, Gulf of Oman, Arabian Sea	[61]
	2	0.01		Class Gracilibacteria				
	3	0.03		Order Candidatus Peregrinibacteria				
	4	0.02		Family Unknown Genus Unknown Species Unknown				
16	1	0.00	Bacteria	Phylum Halanaerobiaeota	Uncultured bacterium clone Kasin-B2-F08 (HE604688.1)	99.6 %	Hypersaline sediment, Lake Kasin, Russia	[59]
	2	0.00		Class Halanaerobiia				
	3	0.03		Order Halanaerobiales				
	4	0.09		Family Halanaerobiaceae Genus <b>Halanaerobium</b> Species bacterium YC-ZSS-LKJ30				

**Supplementary Table S7.** Most abundant ASVs (>0.02 relative abundance) per layer classified at the lowest possible taxonomic level. Known genera are highlighted in bold. (continued)

ASV	Layer	Relative abundance	Silva predicted taxonomy		NCBI best BLAST match (Accession Number)	Identity	Isolation source	Reference
17	1	0.00	Bacteria	Phylum Epsilonbacteraeota	Uncultured organism clone SBZP_5796 (JN538963.1)	99.6 %	Hypersaline microbial mat, Guerrero Negro, Mexico	[41]
	2	0.02		Class Campylobacteria				
	3	0.00		Order Campylobacterales				
	4	0.00		Family Helicobacteraceae Genus Uncultured Species Uncultured organism				
18	1	0.07	Bacteria	Phylum Deinococcus-Thermus	Uncultured bacterium clone Hua-w/2-88 (EF632878.2)	100 %	Hypersaline water, Salar de Huasco, Chile	[62]
	2	0.00		Class Deinococci				
	3	0.00		Order Deinococcales				
	4	0.00		Family Trueperaceae Genus <b>Truepera</b> Species Uncultured bacterium				
19	1	0.00	Bacteria	Phylum Atribacteria	Uncultured organism clone SBYZ_1653 (JN496325.1)	99.2 %	Hypersaline microbial mat, Guerrero Negro, Mexico	[41]
	2	0.00		Class JS1				
	3	0.01		Order Uncultured organism				
	4	0.04		Family Uncultured organism Genus Uncultured organism Species Uncultured organism				
20	1	0.00	Bacteria	Phylum Halanaerobiaeota	<i>Halanaerobium praevalens</i> strain GSL (NR_074859.1)	100 %	Great Salt Lake sediment, Utah, USA	[63]
	2	0.02		Class Halanaerobiiia				
	3	0.00		Order Halanaerobiales				
	4	0.03		Family Halanaerobiaceae Genus <b>Halanaerobium</b> Species Unknown				
21	1	0.00	Bacteria	Phylum Chloroflexi	Uncultured bacterium clone 1109S3_C07_115 (AB785865.1)	98.4 %	Coastal microbial mats, Schiermonnikoog, Netherlands	[64]
	2	0.15		Class Chloroflexia				
	3	0.02		Order Chloroflexales				
	4	0.00		Family Chloroflexaceae Genus Candidatus <b>Chlorothrix</b> Species Unknown				
22	1	0.12	Bacteria	Phylum Bacteroidetes	Uncultured organism clone SBXZ_4804 (JN435991.1)	97.6 %	Hypersaline microbial mat, Guerrero Negro, Mexico	[41]
	2	0.00		Class Bacteroidia				
	3	0.00		Order Chitinophagales				
	4	0.00		Family Uncultured Genus Uncultured organism Species Uncultured organism				
23	1	0.00	Bacteria	Phylum Unknown	Uncultured bacterium clone 1109S3_E03_088 (AB784120.1)	95.7 %	Coastal microbial mats, Schiermonnikoog, Netherlands	[64]
	2	0.06		Class Unknown				
	3	0.01		Order Unknown				
	4	0.00		Family Unknown Genus Unknown Species Unknown				
24	1	0.03	Bacteria	Phylum Cyanobacteria	Uncultured bacterium clone cafs740 (MF439907.1)	90.8 %	Floodplain lake water, Lago Grande do Curuai, Brazil	[65]
	2	0.00		Class Sericytochromatia				
	3	0.00		Order Uncultured bacterium				
	4	0.00		Family Uncultured bacterium Genus Uncultured bacterium Species Uncultured bacterium				
25	1	0.06	Bacteria	Phylum Verrucomicrobia	Uncultured organism clone SBYO_1345 (JN465675.1)	98.8 %	Hypersaline microbial mat, Guerrero Negro, Mexico	[41]
	2	0.05		Class Verrucomicrobiae				
	3	0.01		Order Methylocaldiphilales				
	4	0.00		Family Methylocaldiphilaceae Genus Uncultured Species Uncultured				
26	1	0.06	Bacteria	Phylum Bacteroidetes	Uncultured bacterium clone 108280New.CleanUp.RefO TU12935 (MF995916.1)	96 %	Mediterranean thalassohaline lake	[66]
	2	0.00		Class Bacteroidia				
	3	0.00		Order Cytophagales				
	4	0.00		Family Flammeovirgaceae Genus <b>Flexithrix</b> Species Uncultured organism				

**Supplementary Table S7.** Most abundant ASVs (>0.02 relative abundance) per layer classified at the lowest possible taxonomic level. Known genera are highlighted in bold. (continued)

ASV	Layer	Relative abundance	Silva predicted taxonomy		NCBI best BLAST match (Accession Number)	Identity	Isolation source	Reference
<b>27</b>	1	0.00	Bacteria	Phylum Proteobacteria	Uncultured organism clone SBZP_879 (JN535274.1)	99.6 %	Hypersaline microbial mat, Guerrero Negro, Mexico	[41]
	2	0.01		Class Deltaproteobacteria				
	3	0.03		Order Oligoflexales				
	4	0.01		Family 0319-6G20 Genus Uncultured organism Species Uncultured organism				
<b>28</b>	1	0.00	Bacteria	Phylum Thermotogae	Uncultured organism clone SBXZ_2085 (JN432239.1)	99.2 %	Hypersaline microbial mat, Guerrero Negro, Mexico	[41]
	2	0.00		Class Thermotogae				
	3	0.00		Order Petrotogales				
	4	0.02		Family Petrotogaceae Genus SC103 Species Uncultured organism				