SUPPLEMENTARY MATERIAL

A differential metabarcoding approach to describe taxonomy profiles of Bacteria and Archaea in the Saltern of Margherita di Savoia (Italy)

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Table S1. List of primers for PCR and probes for CARD-FISH.

Name	**Sequence (5’-3’)**	**Ref.**
Eub338 I	GCTGCCTCCCGTAGGAGT	23,24
Eub338 II	GCAGCCACCCGTAGGTGT	23,24
Eub338 III	GCTGCCACCCGTAGGTGT	23,24
Arch915	GTGCTCCCCCGCCAATTCCT	25
B-V5*	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG/ATTAGATACCCYGGTAGTCC	28,29
A-V6*	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG/ACGAGCTGACGACARCCATG	28,29
Arch_349F*	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG/GYGCASCAGKCGMGAAW	30
Arch_806R*	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG/GGACTACVSGGGTATCTAAT	30

* The first part of the primer, before the slash, corresponds to the Nextera transposase sequence, required by the Illumina protocol.

Symbols for degenerate nucleotides are according to the IUPAC nomenclature: Y~~:~~ C/T; R~~:~~ A/G; S~~:~~ G/C; K~~:~~ G/T; M~~:~~ A/C; W ~~:~~A/T; V ~~:~~A/C/G.

Table S2. Cell counts (cells/ml) obtained from the different waters of the saltern of MdS. Ponds are indicated by conventional names, with salinity reported as salt percentage (w/v). Three different counts were carried out for each pond. B/T, A/T and (B+A)/T are the relative abundances of bacterial (B), archaeal (A) and living prokaryotic cells (B + A), respectively, compared to total cells (T) as counted by DAPI staining.

	DAPI	***Bacteria***	***Archaea***	**DAPI dev**	***Bacteria* dev**	***Archaea* dev**	**B/T (%)**	**A/T (%)**	**B/ (B+A) (%)**	**A/ (B+A) (%)**	**(B+A)/T (%)**
Alma Dannata (4.9%)	**2.70E+05**	**5.04E+04**	**1.46E+04**	**3.74E+04**	**5.80E+03**	**3.43E+03**	**18.7**	**5.4**	**77.5**	**22.5**	**24.1**
Polmone (5.2%)	**3.11E+05**	**2.70E+05**	**3.51E+04**	**1.12E+04**	**1.48E+04**	**3.85E+03**	**86.8**	**11.3**	**88.5**	**11.5**	**98.1**
Zero (8.4%)	**2.09E+05**	**1.93E+05**	**1.31E+04**	**1.04E+04**	**7.39E+03**	**1.77E+03**	**92.3**	**6.3**	**93.6**	**6.4**	**98.6**
Fine (13.1%)	**3.10E+05**	**1.94E+05**	**1.08E+05**	**1.76E+04**	**2.05E+04**	**2.92E+03**	**62.6**	**34.8**	**64.2**	**35.8**	**97.4**
Paradiso (14.5%)	**5.06E+05**	**3.60E+05**	**1.26E+05**	**3.10E+04**	**7.93E+03**	**1.24E+04**	**71.1**	**24.9**	**74.1**	**25.9**	**96.0**
Inizio (24.1%)	**2.80E+05**	**1.07E+05**	**1.58E+05**	**1.04E+04**	**1.02E+04**	**1.36E+04**	**38.2**	**56.4**	**40.4**	**59.6**	**94.6**
Armellina (30.6%)	**3.42E+05**	**1.81E+05**	**1.38E+05**	**2.00E+04**	**2.09E+04**	**9.73E+03**	**52.9**	**40.4**	**56.7**	**43.3**	**93.3**
Cappella (34.6%)	**2.47E+05**	**1.24E+05**	**9.69E+04**	**1.20E+04**	**1.06E+04**	**1.04E+04**	**50.2**	**39.2**	**56.1**	**43.9**	**89.4**
Imperatrice (36.0%)	**1.84E+05**	**4.70E+04**	**9.60E+04**	**1.16E+04**	**5.32E+03**	**1.04E+04**	**25.5**	**52.2**	**32.9**	**67.1**	**77.7**

Table S3. Summary of pairwise Dunn tests. Pond names and salinities are listed in columns and rows. The inferred adjusted p-values are shown. Significant p-values are in bold and starred (*≤ 0.05, **≤ 0.01, ***≤0.001).

***Bacteria*:**

Pond (Salinity %)	**Fine (13.1%)**	**Paradiso (14.5%)**	**Inizio (24.1%)**	**Armellina (30.6%)**	**Cappella (34.6%)**	**Imperatrice (36.0%)**	**Alma Dannata (4.9%)**	**Polmone (5.2%)**
Paradiso (14.5%)	0.4387							
Inizio (24.1%)	0.1085	0.1400						
Armellina (30.6%)	**0.0448***	0.0614	0.3217					
Cappella (34.6%)	**0.0059****	**0.0090****	0.0992	0.2053				
Imperatrice (36.0%)	**0.0118***	**0.0175***	0.1518	0.2858	0.2858			
Alma Dannata (4.9%)	0.2202	0.2685	0.3217	0.1773	**0.0402***	0.0679		
Polmone (5.2%)	0.2685	0.2202	**0.0320***	**0.0103***	**0.0009*****	**0.0020****	0.0825	
Zero (8.4%)	0.1400	0.1085	**0.0103***	**0.0027****	**0.0002*****	**0.0004*****	**0.0320***	0.3217

***Archaea*:**

Pond (Salinity %)	**Fine (13.1%)**	**Paradiso (14.5%)**	**Inizio (24.1%)**	**Armellina (30.6%)**	**Cappella (34.6%)**
Paradiso (14.5%)	0.2456				
Inizio (24.1%)	**0.0234***	0.0968			
Armellina (30.6%)	**0.0004*****	**0.0037****	0.0843		
Cappella (34.6%)	**0.0109***	0.0541	0.3798	0.1422	
Imperatrice (36.0%)	**0.0234***	0.0968	0.5000	0.0843	0.3798

Table S4. Pearson's product moment correlation coefficient (PC) between ASVs and salinity.

Bacteria:

ASV	Pearson Correlation	P-value	taxonomy
aa1f4fd53f406a3613fcf5661e0b8035	0.97	0.000	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*
99c793755e7c20d3416d3e113d63475e	0.96	0.000	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*
da3ae34876ceb295b8846f57e0bf98fc	0.96	0.000	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*
b7c04eaa669a35ed42a78381dfc78138	0.96	0.000	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*
2c815d6b32f92298b7578702b0dd1510	0.96	0.000	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*
be1f4e92c0ef762f0a1b05758e0a4bf9	0.95	0.000	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*;s__uncultured *Salinibacter*
3a3161d5e80646e5938102d9d23acf5f	0.95	0.000	d__*Bacteria*;p__*Bacteroidota*;c__*Bacteroidia*;o__*Chitinophagales*;f__uncultured *Chitinophagales*;g__uncultured *Chitinophagales*;s__uncultured *Chitinophagales*
34b2ada6eb11c95621549b76a674c1c1	0.94	0.000	d__*Bacteria*;p__*Bacteroidota*;c__*Bacteroidia*;o__*Chitinophagales*;f__uncultured *Chitinophagales*;g__uncultured *Chitinophagales*;s__uncultured *Chitinophagales*
2119951188cda0bdd84b64568e593a4a	0.94	0.000	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*
c47adbe8321dce0f73d55e94e3e72780	0.93	0.000	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*;s__*Salinibacter_ruber*
e5936cfb1715b6b9b7e14c4f953f94d3	0.92	0.000	d__*Bacteria*;p__*Bacteroidota*;c__*Bacteroidia*;o__*Chitinophagales*;f__uncultured *Chitinophagales*;g__uncultured *Chitinophagales*;s__uncultured *Chitinophagales*
b1189c366efdb76647929e59bc67f874	0.92	0.000	d__*Bacteria*;p__*Bacteroidota*;c__*Bacteroidia*;o__*Chitinophagales*;f__uncultured *Chitinophagales*;g__uncultured *Chitinophagales*;s__uncultured *Chitinophagales*
57f64b6d2bae0c9eeed6063e20153acd	0.91	0.001	d__*Bacteria*;p__*Bacteroidota*;c__*Bacteroidia*;o__*Chitinophagales*;f__uncultured *Chitinophagales*;g__uncultured *Chitinophagales*;s__uncultured *Chitinophagales*
ac3da6eb5e5938d0b631004834859280	0.88	0.002	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*;s__uncultured *Salinibacter*
23c128e062a85e3f9edad481d971abb5	0.86	0.003	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*;s__uncultured *Salinibacter*
c2962efec2da9314edd38d7167b6aaea	0.86	0.003	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*
0d88996a0249a67e3208cb890f339219	0.81	0.009	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*;s__uncultured *Salinibacter*
2cd0e6986ba98bb71bc7cc679fd6e2c6	0.80	0.010	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*
49ef03099317acbf97e22e34a253fcb2	0.79	0.011	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*
3092c2440ff9a90461054bf40642f489	0.79	0.011	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*;s__uncultured *Salinibacter*
00a224b16122e353591ac23cc72d6e7d	0.78	0.013	d__*Bacteria*;p__*Proteobacteria*;c__*Gammaproteobacteria*;o__*Nitrococcales*;f__*Nitrococcaceae*;g__uncultured *Nitrococcaceae*;s__uncultured *Nitrococcaceae*
eaed4b8971f40866bd1f31e062f579f9	0.77	0.016	d__*Bacteria*;p__*Bacteroidota*;c__*Rhodothermia*;o__*Rhodothermales*;f__*Rhodothermaceae*;g__*Salinibacter*;s__uncultured *Salinibacter*
038e1e6407a6fbf106d92d05eb71621d	0.74	0.022	d__*Bacteria*;p__*Proteobacteria*;c__*Alphaproteobacteria*;o__*Rhodobacterales*;f__*Rhodobacteraceae*;g__*Sediminimonas*;s__uncultured *Sediminimonas*

0869c07ab138130212f6a46f19029879	0.74	0.024	d__*Bacteria;*p__*Desulfobacterota;*c__*Desulfuromonadia;*o__*Bradymonadales;*f__*Bradymonadaceae;*g__*Bradymonadaceae;*s__uncultured *Bradymonadaceae*
d4d4a7b2415a45986d9a4418acbff493	0.73	0.025	d__*Bacteria;*p__*Desulfobacterota;*c__*Desulfuromonadia;*o__*Bradymonadales;*f__*Bradymonadaceae;*g__*Bradymonadaceae;*s__uncultured *Bradymonadaceae*
fc3010d03af677da6c45d7ca8c5f6d1e	0.71	0.032	d__*Bacteria;*p__*Bacteroidota;*c__*Bacteroidia;*o__*Chitinophagales;*f__uncultured *Chitinophagales;*g__uncultured *Chitinophagales;*s__uncultured *Chitinophagales*
0ec4c750eed1d8de856d341ea479b427	0.71	0.034	d__*Bacteria;*p__*Bacteroidota;*c__*Rhodothermia;*o__*Rhodothermales;*f__*Rhodothermaceae;*g__*Salinibacter;*s__uncultured *Salinibacter*
1c45f7a1f3d3061ed0f29a04908f348b	0.70	0.036	d__*Bacteria;*p__*Desulfobacterota;*c__*Desulfuromonadia;*o__*Bradymonadales;*f__*Bradymonadaceae;*g__*Bradymonadaceae;*s__uncultured *Bradymonadaceae*
a4436f7093a0e7cafbb276baddf40e3a	0.69	0.039	d__*Bacteria;*p__*Bacteroidota;*c__*Rhodothermia;*o__*Rhodothermales;*f__*Rhodothermaceae;*g__*Salinibacter*
7512cf96f90d72ea620a31db72947a13	0.69	0.040	d__*Bacteria;*p__*Bacteroidota;*c__*Rhodothermia;*o__*Rhodothermales;*f__*Rhodothermaceae;*g__*Salinibacter*
00cd9e7bb10f47a21b0e61c54fa18bc6	0.69	0.040	d__*Bacteria;*p__*Bacteroidota;*c__*Bacteroidia;*o__*Chitinophagales;*f__uncultured *Chitinophagales;*g__uncultured *Chitinophagales;*s__uncultured *Chitinophagales*
f36dec1f57e22cf290c271114e3fc2e2	0.68	0.045	d__*Bacteria;*p__*Proteobacteria;*c__*Alphaproteobacteria;*o__*Kiloniellales;*f__*Fodinicurvataceae;*g__*Rhodovibrio*
1a99e469d7cc0789a1247729c7bc2166	0.67	0.049	d__*Bacteria;*p__*Bacteroidota;*c__*Rhodothermia;*o__*Rhodothermales;*f__*Rhodothermaceae;*g__*Salinibacter;*s__uncultured *Salinibacter*
4748d319246942d0f227de0f249b89fb	-0.67	0.050	d__*Bacteria;*p__*Actinobacteriota;*c__*Actinobacteria;*o__*Micrococcales;*f__*Microbacteriaceae;*g__*Candidatus_Aquiluna;*s__uncultured *Candidatus_Aquiluna*
8abac3ecc5613a43e2db3016c8231957	-0.68	0.044	d__*Bacteria;*p__*Proteobacteria;*c__*Gammaproteobacteria;*o__*Oceanospirillales;*f__*Litoricolaceae;*g__*Litoricola;*s__uncultured *Litoricola*
5aecc6ad4f49b8ed8b0fe71d086294c2	-0.68	0.044	d__*Bacteria;*p__*Actinobacteriota;*c__*Actinobacteria;*o__*Micrococcales;*f__*Microbacteriaceae*
7988d516d5c461335aec989e6e9f6678	-0.69	0.041	d__*Bacteria;*p__*Bacteroidota;*c__*Bacteroidia;*o__*Flavobacteriales;*f__*Flavobacteriaceae;*g__*Psychroflexus;*s__uncultured *Psychroflexus*
487af4ed0c1c3666a40833f83e10ecae	-0.69	0.040	d__*Bacteria;*p__*Proteobacteria;*c__*Alphaproteobacteria;*o__*Rhodobacterales;*f__*Rhodobacteraceae*
459af99af321115ac4423547dde3f5f5	-0.71	0.034	d__*Bacteria;*p__*Actinobacteriota;*c__*Actinobacteria;*o__*Micrococcales;*f__*Microbacteriaceae;*g__*ML602J-51;*s__uncultured *ML602J-51*
4ba2d372a67c34ba6b9e53c2f8a0dd60	-0.73	0.027	d__*Bacteria;*p__*Actinobacteriota;*c__*Actinobacteria;*o__*Micrococcales;*f__*Microbacteriaceae;*g__*Candidatus_Aquiluna*
76357b935a80b43cb75d98be6bb3e020	-0.73	0.026	d__*Bacteria;*p__*Proteobacteria;*c__*Alphaproteobacteria;*o__*Rhodobacterales;*f__*Rhodobacteraceae;*g__*Marivita*
685246984508b28064b88baeac9f3864	-0.76	0.017	d__*Bacteria;*p__*Proteobacteria;*c__*Gammaproteobacteria;*o__*Alteromonadales;*f__*Alteromonadaceae;*g__*Alteromonas;*s__*Alteromonas_mediterranea*
d96b8ae4478e8036ffcac7d0ac078c3c	-0.77	0.015	d__*Bacteria;*p__*Proteobacteria;*c__*Gammaproteobacteria;*o__*Alteromonadales;*f__*Alteromonadaceae;*g__*Alteromonas*
2e69b4b8c8c5e5230d457c617a80f4ab	-0.78	0.013	d__*Bacteria;*p__*Firmicutes;*c__*Bacilli;*o__*Izemoplasmatales;*f__*Izemoplasmataceae;*g__*Izimaplasma;*s__uncultured *Izimaplasma*
8c20b9b4a39902fcc1ae0a4e34ee0a4e	-0.80	0.010	d__*Bacteria;*p__*Bacteroidota;*c__*Bacteroidia;*o__*Flavobacteriales;*f__*Cryomorphaceae;*g__*Phaeocystidibacter*

***Archaea*:**

ASV	**Pearson Correlation**	**P-value**	**taxonomy**
915aa73cc4f1ba83cef2cb31f5ea1062	0.97	0.002	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Halomicrobiaceae*;g__*Halapricum*
ca018f9f87f1b2613964b7d79af0a2df	0.96	0.002	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Haloplanus*;s__uncultured *Haloplanus*
a49a8bd91ebd61f0794acf3da2568aec	0.96	0.003	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Haloplanus*;s__uncultured *Haloplanus*
76df539101391133a38d72abdcbb0c23	0.95	0.004	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Halobellus*;s__uncultured *Halobellus*
d049749b5278d20888a6dbcaec51f835	0.93	0.007	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Halobellus*
49d8cc73ead043049db3c342ae42a699	0.93	0.008	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Halorubrum*
262e1c5b89a9bc1a77cffc791875dd1e	0.92	0.010	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Haloplanus*;s__uncultured *Haloplanus*
703bfc369b52686be14ed09c24df7d55	0.91	0.011	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Haloplanus*;s__*Haloplanus_sp.*
6aa1a4ae0da8f76dca7a29e8f9c70857	0.91	0.013	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Haloplanus*
17b7647f7de8c21fec9b6c2aa14e6c77	0.90	0.015	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Haloplanus*;s__uncultured *Haloplanus*
7ebdc6af4f353477b542f6983f5f9e5c	0.90	0.015	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Halomicrobiaceae*;g__*Halapricum*;s__uncultured *Halapricum*
d5c885c41d53a91bf7a1321b7cab09c7	0.89	0.016	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Halobellus*;s__uncultured *Halobellus*
4a350830e87730f00ddf6bd30fbb2799	0.89	0.016	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Halomicrobiaceae*;g__*Natronomonas*;s__uncultured *Natronomonas*
0c6add50ff5e77902aabde9d4654e17b	0.89	0.017	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Haloquadratum*
5c17f84513f90bc63667b88ec91dd999	0.88	0.020	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Halobacterales*;g__uncultured *Halobacterales*;s__uncultured *Halobacterales*
6d9d52cabf2578b1abcd85e3d713307d	0.88	0.020	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Haloplanus*
783146ad4e5f9ec18b63385b193c65ee	0.87	0.024	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Halobacterales*;g__uncultured *Halobacterales*
12174d7f1ced3b549478e28255017a14	0.87	0.024	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Haloplanus*;s__uncultured *Haloplanus*
ecc9b026cd2680fa18587576ee4c34f1	0.87	0.024	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Halococcaceae*;g__*Halococcus*;s__uncultured *Halococcus*
fd3c0dc24923533308a5e34c5a80690c	0.87	0.025	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Halobacterales*;g__uncultured *Halobacterales*
16c452e96f771a7a9fa218a2d487521f	0.87	0.026	d__*Archaea*;p__*Nanohaloarchaeota*;c__*Nanosalinia*;o__*Nanosalinales*;f__*Nanosalinaceae*;g__*Nanosalinaceae*;s__uncultured *Nanosalinaceae*
3dabb665c3a7f8a544b5dc4d7118b4ce	0.85	0.034	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Halobellus*;s__uncultured *Halobellus*
73f9bdf2fa500fced5dd37dc7217ed18	0.85	0.034	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Haloplanus*;s__uncultured *Haloplanus*
8c6fee6a317e640e294ed4d99daf276d	0.84	0.035	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*
1be7b1c781cac2b29578d55cc251dce8	0.84	0.035	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Halomicrobiaceae*;g__*Halapricum*;s__uncultured *Halapricum*
6bc26dd7410b686f291f8100eb57c5f8	0.84	0.035	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Haloquadratum*
d1035bc334303ce23721677da8f722f6	0.83	0.039	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Halorubrum*;s__uncultured *Halorubrum*

0ee966c0882ec6969d0d5d3fb94003b3	0.83	0.039	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__*Haloquadratum*
f816cc791191aa39bf671e1d70e32164	0.83	0.041	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Halomicrobiaceae*;g__uncultured *Halomicrobiaceae*;s__*Halomicroarcula_sp.*
f290beb60494ab94f9ce57ca7512676d	0.82	0.046	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Halomicrobiaceae*;g__*Halapricum*;s__uncultured *Halapricum*
d5668ef5271e6d386f749812b4c0b2fc	0.82	0.048	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Halomicrobiaceae*;g__*Halapricum*;s__uncultured *Halapricum*
477af6b666b5e847cd81f9afd6be6c1b	0.81	0.050	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Halomicrobiaceae*;g__*Halomicroarcula*;s__*Halomicroarcula_sp.*
a98eda4127fec71276cd970ec62264f9	-0.82	0.048	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
08332dbb947a584cd23c6da4de658f62	-0.82	0.048	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
ae51480ebc650b47691282f0269a3411	-0.82	0.043	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
586d364926de38c880d1163850dd9a81	-0.84	0.038	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*SCGC_AAA286-E23*;g__*SCGC_AAA286-E23*;s__uncultured *SCGC_AAA286-E23*
c2984c7c61260e1930788bf6e31b3788	-0.84	0.036	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*SCGC_AAA286-E23*;g__*SCGC_AAA286-E23*
35a745929095d7e1b41b30e4e8cc3160	-0.85	0.030	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
c24193857c7d70e01c9f12e163768429	-0.86	0.030	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
45f3c2b14b687d59cb0cfddeb6c25dbe	-0.86	0.029	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
5a830c437b8f3d8a9bb64de8affc1a57	-0.86	0.029	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*SCGC_AAA286-E23*;g__*SCGC_AAA286-E23*
eb50e33ff310a4bdc5fdc80f8ddae09e	-0.86	0.028	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*SCGC_AAA286-E23*;g__*SCGC_AAA286-E23*
63d5522ff64f6b3c4d4bb647f844c845	-0.86	0.028	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
f006c8e87e1cb5ed303f4f604f1e051b	-0.86	0.027	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
5de53397fd9b23517077d90786daa91e	-0.86	0.026	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
b5e3b86102d272a518768e5947dd887d	-0.87	0.026	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
ca25dfcc580d5ae810f07bb4a0fab561	-0.87	0.024	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*SCGC_AAA286-E23*;g__*SCGC_AAA286-E23*;s__uncultured *SCGC_AAA286-E23*
f78424c30233f2b7760e4ea79c59f2ec	-0.87	0.023	d__*Archaea*;p__*Halobacterota*;c__*Halobacteria*;o__*Halobacterales*;f__*Haloferacaceae*;g__uncultured *Haloferacaceae*;s__uncultured *Haloferacaceae*
c1b719c6514a7431e5381fcfceef56cb	-0.87	0.023	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
76dcf6baead4e9cbd880e256b547f6d1	-0.88	0.021	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*GW2011*;g__*AR15*;s__uncultured *AR15*
387e4d114e2484968674765833607afa	-0.88	0.021	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
7a992d24b6fe6764300ee609db1a14a0	-0.88	0.020	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*
32a01c7ee8c009c4e5014a52103ef45f	-0.88	0.020	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
c94f6c522b9bcb2a9165b8b6462118c0	-0.89	0.018	d__*Archaea*;p__*Nanoarchaeota*;c__*Nanoarchaeia*;o__*Woesearchaeales*;f__*Woesearchaeales*;g__*Woesearchaeales*;s__uncultured *Woesearchaeales*
132cc77567d2d5dca75da0d2ca7ed2c1	-0.89	0.018	*d__Archaea;p__Aenigmarchaeota;c__Deep_Sea_Euryarchaeotic_Group(DSEG);o__Deep_Sea_Euryarchaeotic_Group(DSEG);f__Deep_Sea_Euryarchaeotic_Group(DSEG);g__Deep_Sea_Euryarchaeotic_Group(DSEG);s__uncultured Deep_Sea_Euryarchaeotic_Group(DSEG)*

35a52acdec8f4e30ac7a5a8feccaaacf	-0.89	0.017	*d__Archaea;p__Nanoarchaeota;c__Nanoarchaeia;o__Woesearchaeales;f__Woesearchaeales;g__Woesearchaeales*
68a4981c5d14f3506f29b7ba4c21982f	-0.89	0.017	d__*Archaea;*p__*Iainarchaeota;*c__*Iainarchaeia;*o__*Iainarchaeales;*f__*Iainarchaeales;*g__*Candidatus_Iainarchaeum;*s__*metagenome*
d6019e724d7b2c7d3b38b40e3feeaa8e	-0.90	0.015	d__*Archaea;*p__*Halobacterota;*c__*Halobacteria;*o__*Halobacterales;*f__*Halomicrobiaceae;*g__*Natronomonas;*s__uncultured *Natronomonas*
9d21d400740fe36476bac8600e4c40eb	-0.90	0.015	d__*Archaea;*p__*Nanoarchaeota;*c__*Nanoarchaeia;*o__*Woesearchaeales;*f__*Woesearchaeales;*g__*Woesearchaeales;*s__uncultured *Woesearchaeales*
b64d79c9eb23ec53164ff0644c23c070	-0.90	0.015	d__*Archaea;*p__*Nanoarchaeota;*c__*Nanoarchaeia;*o__*Woesearchaeales;*f__*Woesearchaeales;*g__*Woesearchaeales;*s__uncultured *Woesearchaeales*
cf1f41d9e9acd422b3b7ef006024d907	-0.90	0.013	d__*Archaea;*p__*Nanoarchaeota;*c__*Nanoarchaeia;*o__*Woesearchaeales;*f__*Woesearchaeales;*g__*Woesearchaeales;*s__uncultured *Woesearchaeales*
bbf4959fc31c8226839b8ae97721f961	-0.91	0.013	d__*Archaea;*p__*Nanoarchaeota;*c__*Nanoarchaeia;*o__*Woesearchaeales;*f__*Woesearchaeales;*g__*Woesearchaeales;*s__uncultured *Woesearchaeales*
a7e3012a77db9e26760d6dbc8374d1aa	-0.91	0.012	d__*Archaea;*p__*Nanoarchaeota;*c__*Nanoarchaeia;*o__*Woesearchaeales;*f__*Woesearchaeales;*g__*Woesearchaeales;*s__uncultured *Woesearchaeales*
b4707fb5c3fde18a710f6699af1dbdf4	-0.91	0.012	d__*Archaea;*p__*Halobacterota;*c__*Halobacteria;*o__*Halobacterales;*f__*Haloferacaceae;*g__*Haloterrigena;*s__*Haloterrigena_hispanica*
82e84c861f76ea436ab9b6170397faba	-0.91	0.012	d__*Archaea;*p__*Nanoarchaeota;*c__*Nanoarchaeia;*o__*Woesearchaeales;*f__*Woesearchaeales;*g__*Woesearchaeales;*s__uncultured *Woesearchaeales*
5b3ee5898068da70c958aeb81dd10a12	-0.91	0.012	d__*Archaea;*p__*Nanoarchaeota;*c__*Nanoarchaeia;*o__*Woesearchaeales;*f__*Woesearchaeales;*g__*Woesearchaeales;*s__uncultured *Woesearchaeales*
4a6e347dc8cf80daa333e2332bd8e47f	-0.92	0.010	d__*Archaea;*p__*Halobacterota;*c__*Halobacteria;*o__*Halobacterales;*f__*Halomicrobiaceae;*g__*Natronomonas;*s__uncultured *Natronomonas*
8828cb9fe570a42d894c3ae297ebd706	-0.93	0.006	d__*Archaea;*p__*Halobacterota;*c__*Halobacteria;*o__*Halobacterales;*f__*Haloferacaceae;*g__uncultured *Haloferacaceae;*s__uncultured *Haloferacaceae*

Table S5. Relative abundances of bacterial and archaeal genera in the ponds of the MdS saltern obtained by metabarcoding analysis. Ponds are indicated by conventional names, with salinity reported as salt percentage (w/v). Groups with relative abundances < 1.0 % were joined as ‘Others’. ASVs which could not be resolved at the genus level were reported with the notation g_unclassified followed by the name of the closest known parental rank.

***Bacteria*:**

Phylum	**Genus**	**Alma Dannata (4.9%)**	**Polmone (5.2%)**	**Zero (8.4%)**	**Fine (13.1%)**	**Paradiso (14.5%)**	**Inizio (24.1%)**	**Armellina (30.6%)**	**Cappella (34.6%)**	**Imperatrice (36.0%)**
*Actinobacteriota**	*Candidatus_Aquiluna*	19.33%	15.09%	9.27%	22.74%	25.98%	1.72%			
	ML602J-51	1.87%	1.48%							
	Pontimonas				2.10%	2.23%				
*Bacteroidota***	g_uncultured *Chitinophagales*						6.49%	15.90%	12.21%	11.41%
	Brumimicrobium			4.05%						
	Owenweeksia		1.36%		1.70%	1.56%				
	g_uncultured *Cryomorphaceae*	1.09%	2.23%							
	NS3a_marine_group	6.69%	2.08%							
	NS5_marine_group	3.59%								
	Psychroflexus			3.56%	18.17%	14.09%	4.21%			
	ML602M-17			1.52%	1.62%					
	Balneola		1.88%	1.34%						
	Gracilimonas			4.12%	3.20%	2.01%				
	g_uncultured *Balneolaceae*		2.09%	1.82%	3.14%	2.41%				
	Salinibacter						52.85%	79.00%	86.86%	88.18%
	Peredibacter		1.43%							
*Bdellovibrionota****	*Candidatus_Izimaplasma*	4.45%	4.46%	1.40%						
Firmicutes	*Izemoplasmatales*		3.86%							
	Cohaesibacter	1.42%								
Proteobacteria	*Donghicola*		3.69%							
	Marivita			1.35%						
	Roseovarius			4.86%	3.23%	3.55%				
	WDS1C4				3.00%	3.09%				
	AEGEAN-169_marine_group	2.05%	1.29%							
	Clade_III	14.10%	3.41%	1.03%						
	Alteromonas	1.04%	1.14%							
	Idiomarina			2.43%						
	Pseudoalteromonas	7.65%		7.71%						
	g_uncultured *Alcaligenaceae*		14.70%							
	Halioglobus	1.65%								
	Luminiphilus		1.97%							
	g_uncultured Unknown_Family				2.73%	3.12%				
	Spiribacter					2.24%	12.47%			
	Methylophaga				2.25%	1.11%				
	Cobetia	2.26%								
	Halomonas			3.87%	5.34%	7.35%				
	Litoricola	11.39%	8.55%	1.04%		1.23%				
	Salinivibrio			8.30%	5.22%	1.62%				

Verrucomicrobiota	g_uncultured *Simkaniaceae*		1.58%							
	Lentimonas			1.55%						
	Others	6.47%	13.41%	29.43%	6.15%	6.34%	3.60%	2.88%	0.93%	0.41%
	Unclassified	14.96%	14.32%	11.35%	19.43%	22.09%	18.66%	2.23%		

*~~)~~ also known as *Actinobacteria*; **~~)~~ also known as *Bacteroidetes*; ***~~)~~ also known as *Tenericutes*.

***Archaea*:**

Phylum	**Genus**	**Fine (13.1%)**	**Paradiso (14.5%)**	**Inizio (24.1%)**	**Armellina (30.6%)**	**Cappella (34.6%)**	**Imperatrice (36.0%)**
*Halobacterota**	g_uncultured *Halobacterales*			1.44%	1.64%	4.92%	9.97%
	g_uncultured *Haloferacaceae*	41.57%	17.41%	3.88%			
	Haloquadratum	2.18%	31.66%	40.77%	37.37%	53.40%	63.52%
	Haloplanus				1.61%	1.54%	1.31%
	Halobellus		1.09%	2.21%	4.79%	3.61%	2.77%
	Halorubrum	3.17%	5.96%	10.36%	14.47%	8.82%	5.43%
	Halonotius		6.24%	11.20%	10.99%	9.57%	3.95%
	Halomarina			1.47%	1.76%		
	Natronomonas	49.23%	25.09%	18.83%	18.78%	8.26%	3.22%
	Halomicroarcula			1.36%	1.06%	1.25%	
	Halapricum			1.43%	1.68%	4.01%	5.05%
Nanoarchaeota	*Woesearchaeales*	1.44%	1.63%				
	SCGC_AAA286-E23	1.60%					
Nanohaloarchaeota	*Candidatus_ Nanosalinarum*		3.93%				1.05%
	Nanosalinaceae		5.02%	4.08%	2.19%		1.17%
	Others	0.82%	1.96%	2.95%	2.27%	4.61%	2.56%
	Unclassified				1.38%		

*~~)~~ also known as *Euryarchaeota*.

Table S6. Relative abundances of bacterial and archaeal genera in the ponds of the MdS saltern obtained by combination of metabarcoding and CARD-FISH data. Ponds are indicated by conventional names, with salinity reported as salt percentage (w/v). Groups with relative abundances < 1.0 % were joined as 'Others'. ASVs which could not be resolved at the genus level were reported with the notation g_unclassified followed by the name of the closest known parental rank.

Domain	**Phylum**	**Genus**	**Fine (13.1%)**	**Paradiso (14.5%)**	**Inizio (24.1%)**	**Armellina (30.6%)**	**Cappella (34.6%)**	**Imperatrice (36.0%)**
Bacteria	*Actinobacteriota**	*Candidatus_Aquiluna*	14.61%	19.24%				
		Pontimonas	1.35%	1.65%				
	*Bacteroidota***	g_uncultured *Chitinophagales*			2.62%	9.02%	6.85%	3.75%
		Owenweeksia	1.09%	1.15%				
		Psychroflexus	11.67%	10.44%	1.70%			
		ML602M-17	1.04%					
		Gracilimonas	2.05%	1.49%				
		g_uncultured *Balneolaceae*	2.02%	1.79%				
		Salinibacter			21.34%	44.82%	48.76%	28.98%
	Proteobacteria	*Roseovarius*	2.08%	2.63%				
		WDS1C4	1.92%	2.29%				
		g_uncultured Unknown_Family	1.75%	2.31%				
		Spiribacter		1.66%	5.03%			
		Methylophaga	1.44%					
		Halomonas	3.43%	5.44%				
		Salinivibrio	3.35%	1.20%				
		Unclassified_B	12.48%	16.36%	7.54%	1.27%		
		Others_B	3.95%	6.42%	2.15%	1.63%	0.52%	0.13%
Archaea	*Halobacterota****	g_uncultured *Halobacterales*					2.16%	6.69%
		g_uncultured *Haloferacaceae*	14.87%	4.51%	2.32%			
		Haloquadratum		8.21%	24.31%	16.17%	23.43%	42.64%
		Halobellus			1.32%	2.07%	1.58%	1.86%
		Halorubrum	1.13%	1.54%	6.18%	6.26%	3.87%	3.64%
		Halonotius		1.62%	6.68%	4.76%	4.20%	2.65%
		Natronomonas	17.61%	6.51%	11.23%	8.12%	3.62%	2.16%
		Halapricum					1.76%	3.39%
	Nanohaloarchaeota	*Candidatus_Nanosalinarum*		1.02%				
		Nanosalinaceae		1.30%	2.43%			
		Others_A	2.16%	1.22%	5.16%	5.88%	3.25%	4.09%

*) also known as *Actinobacteria*; **) also known as *Bacteroidetes*; ***) also known as *Euryarchaeota*.

Figure S1

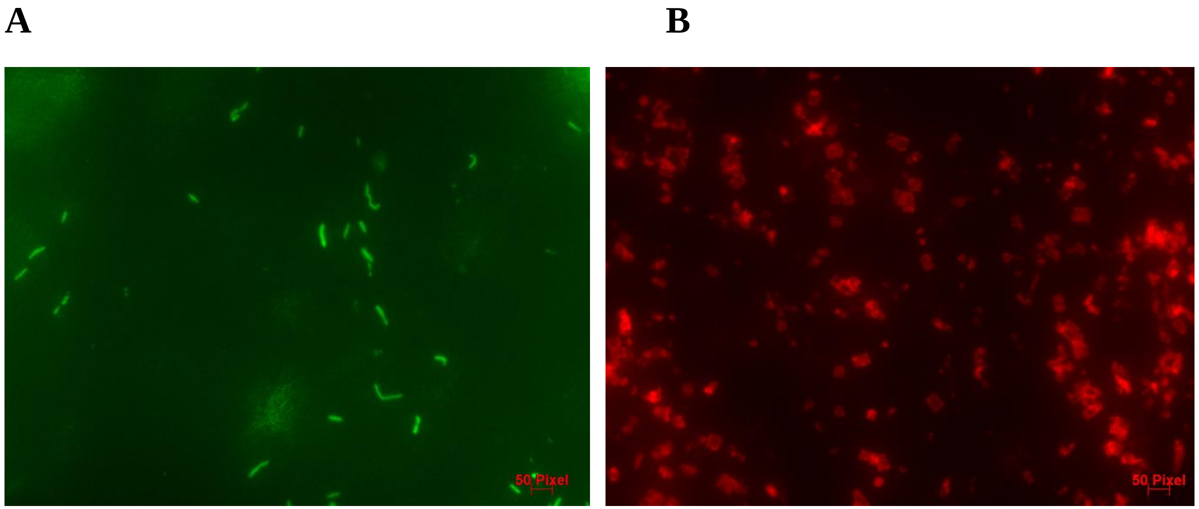
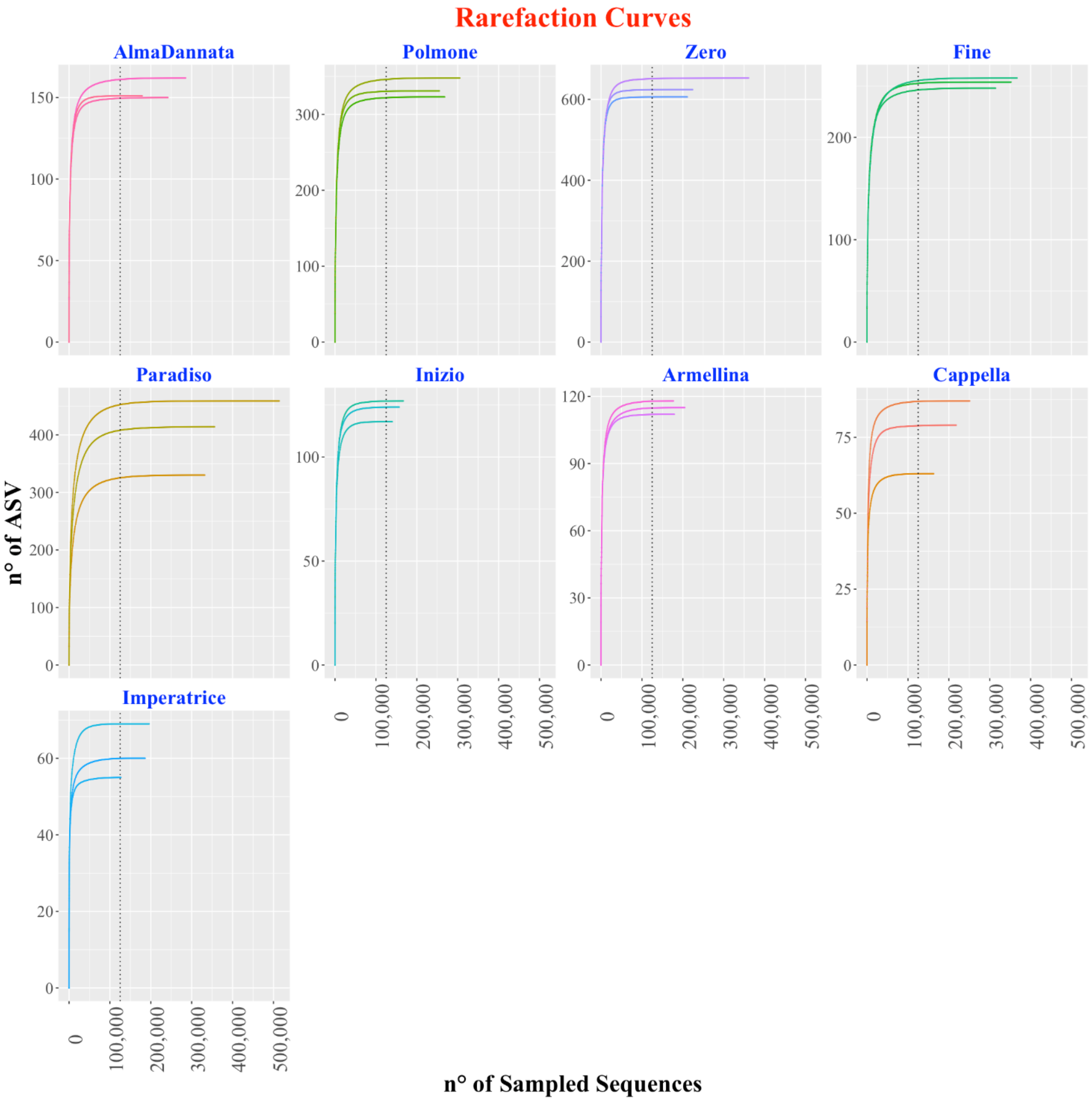


Figure S1. Detection of bacterial and archaeal cells by CARD-FISH. Prokaryotic cells (A: *Bacteria*; B: *Archaea*) in the waters of the Imperatrice pond (36.0% of salinity) as visualized by CARD-FISH analysis. Among *Bacteria*, rod-shaped cells should mostly correspond to *Salinibacter* cells according to the high abundance of the genus (about 88%); numerous square-shaped *Haloquadratum* cells are distinguishable among archaeal cells.

Figure S2

A


Rarefaction Curves
AlmaDannata
Polmone
Zero
Fine
Paradiso
Inizio
Armellina
Cappella
Imperatrice
n° of ASV
n° of Sampled Sequences
0
100,000
200,000
300,000
400,000
500,000
0
50
100
150
100
200
300
200
400
600
100
200
300
400
60
90
120
25
50
75
20
40
60

B

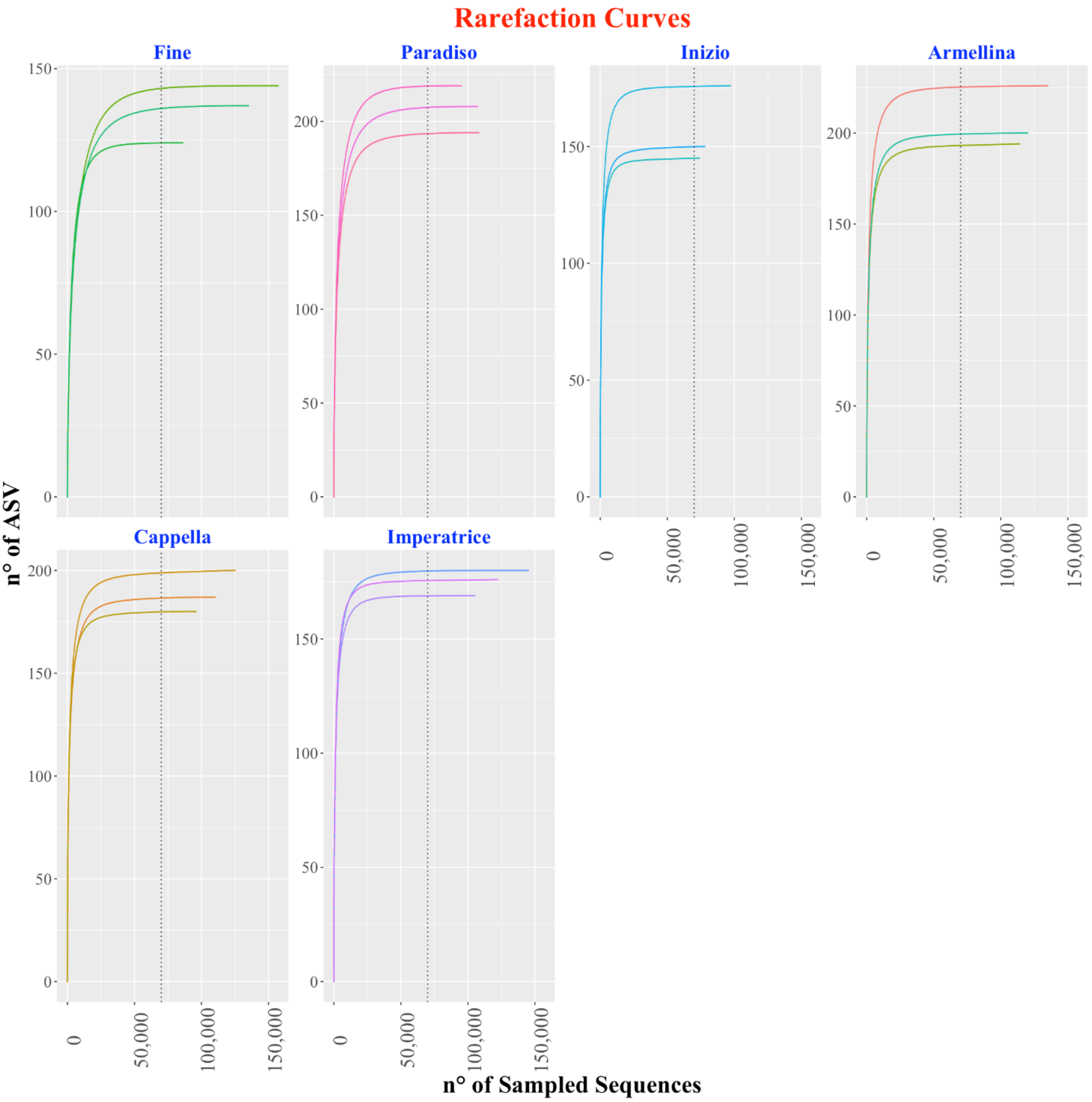
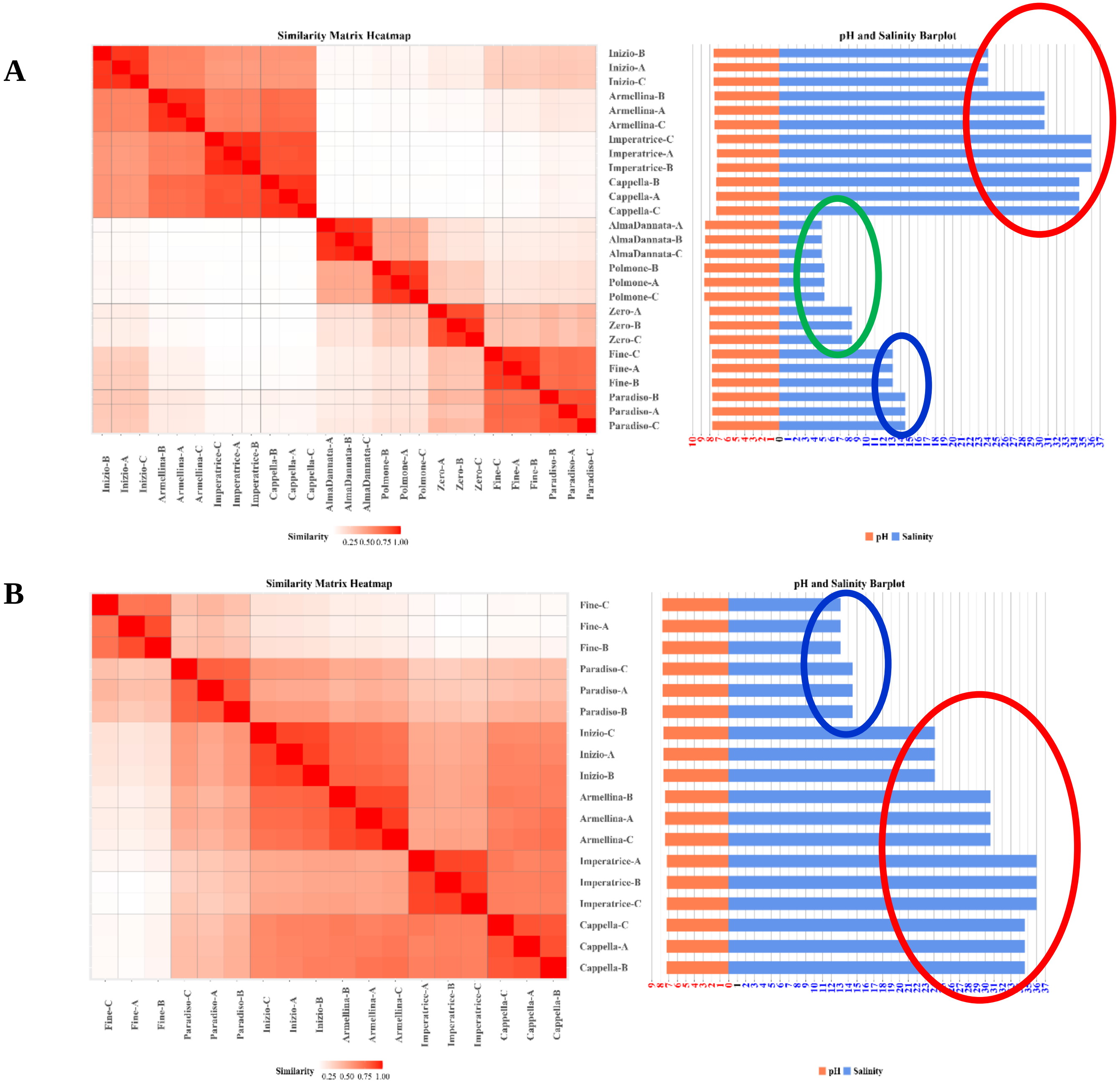


Figure S2. Rarefaction curves of 16S rRNA ASVs for the assayed ponds of the MdS saltern. A) bacterial ASVs; B) archaeal ASVs.

Figure S3



24.1-36.0% salinity

4.9-8.4% salinity

13.1-14.5% salinity

13.1-14.5% salinity

24.1-36.0% salinity

Figure S3. Heat maps of microbial amplicons similarity matrix of MdS ponds. The heat maps (A: *Bacteria*; B: *Archaea*) were obtained by inferring the Bray-Curtis distances on ASV counts and clustering the samples using the UPGMA algorithm. The letters A, B and C accompanying the names of the ponds refer to the values of the experimental replicas. On the right of the heat maps, corresponding salinity and pH values are shown (coloured cycles define pond salinity ranges).