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Supplementary Materials for

Macroecological drivers of archaea and bacteria in benthic deep-sea ecosystems

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The PDF file includes:

Supplementary Material and Methods

Sample collection and processing. Sediment samples were collected during seven oceanographic cruises conducted in the Arctic Ocean (July-August 2005), Atlantic Ocean (August 2005, June-July 2006 and September 2006) and Mediterranean Sea (August 2005, October 2005 and June 2006). In each sampling area, sediment samples were collected along a bathymetric transect at fixed depths using a hierarchical sampling strategy. At each sampling site, the top 15 cm of sediment was collected from independent multiple-corer deployments (n=3) for the analyses of microbial variables and biochemical composition of organic matter (in terms of chlorophyll-a and phaeopigment and protein, carbohydrate and lipid content). Sediment cores were sliced into horizons of 0-1 cm, 3-5 cm and 10-15 cm. Overall 228 samples were collected, including 174 samples from the 0-1 cm sediment horizon, 27 samples from the 3-5 cm sediment horizon and 27 samples from the 3-5 cm sediment horizon. Samples from the 3-5 cm and 10-15 cm sediment horizons were collected at three benthic deep-sea sites in the Arctic margin (located at 1279 m, 2545 m and 5570 m depth), two sites in the Atlantic margin (located at 1002 m and 2130 m depth) and four sites in the Mediterranean Sea (at 985 m and 2342 m depth in the NW Mediterranean and at 1263 m and 2325 m depth in the Central Mediterranean). For the determination of prokaryotic abundance, sediment samples were fixed in buffered formaldehyde (2%) and kept at 4°C until further laboratory analyses. For the analysis of the prokaryotic assemblage structure by catalysed reporter deposition–fluorescence in situ hybridisation (CARD-FISH), sediment samples were fixed with 2% paraformaldehyde (polymerised formaldehyde, dry chemical), washed with phosphate-buffered saline solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2mM KH_2PO_4, pH 7.6), and then stored in phosphate-buffered saline solution: 96% ethanol (1:1) at -20°C until further processing. The samples for qPCR analyses and the determination of the biochemical composition of organic matter were immediately frozen and stored at -20°C until further laboratory analyses.

table S1. Details of the station locations, temperature, and salinity of bottom waters, total phytopigment (CPE) and biopolymeric C (BPC) concentrations, and protein-to-carbohydrate ratio (PRT/CHO) in surface sediments (0 to 1 cm), net photosynthetic primary production (NPP), and organic C fluxes (OC fluxes). Standard deviations (±) are reported (n=3). na= not available.

Area	Latitude (°N)	Longitude (°E)	Depth (m)	Temperatura (°C)	Salinity	CPE (μg g^{-1})	BPC (mg C g^{-1})	PRT/CHO	NPP (mg C m^{-2} d^{-1})	OC fluxes (mg C m^{-2} d^{-1})
Arctic Ocean	79.1334	6.0928	1279	-0.74	34.89	32.3 ± 9.6	8.0 ± 0.9	1.4	2429	64.6
	79.1081	4.6004	1914	-0.83	34.91	13.8 ± 2.0	3.3 ± 0.3	0.7	1935	40.0
	78.6098	5.0700	2338	-0.90	34.91	11.9 ± 3.4	4.0 ± 0.6	0.4	882	16.2
	79.0651	4.1801	2462	-0.90	34.90	10.5 ± 2.6	2.8 ± 0.2	0.7	1061	18.8
	79.1336	2.8424	2545	-0.84	34.92	16.0 ± 2.5	4.2 ± 0.5	0.7	615	10.7
	79.0634	3.6580	3158	-0.90	34.90	3.1 ± 0.8	2.8 ± 0.5	1.1	1053	16.1
	79.0600	3.5815	3485	-0.72	34.49	3.4 ± 1.5	1.1 ± 0.2	1.4	1054	15.2
	79.0650	4.1796	3997	-0.67	34.93	10.1 ± 5.6	2.7 ± 0.5	1.6	1166	15.5
	79.0643	3.3360	5108	-0.55	34.92	4.5 ± 1.1	1.6 ± 0.3	2.9	895	10.3
	79.1336	2.8424	5571	-0.51	34.82	22.3 ± 7.3	5.9 ± 0.8	1.4	689	7.6
Atlantic Ocean	55.650	-15.9333	469	10.00	35.46	2.9 ± 0.6	na	na	1258	64.8
	55.452	-15.7661	1091	6.77	35.16	3.3 ± 0.7	0.6 ± 0.1	0.2	1271	37.4
	55.373	-15.6531	1488	6.00	34.96	1.5 ± 0.3	0.3 ± 0.02	0.1	1328	32.1
	55.208	-15.7793	1958	3.50	34.92	8.7 ± 1.5	0.8 ± 0.1	0.3	1299	26.5
	55.082	-15.7722	2459	2.00	34.94	39.0 ± 15.9	2.4 ± 0.3	0.6	1609	28.6
	40.1667	-9.6671	416	11.35	35.55	3.7 ± 0.1	0.34 ± 0.1	0.7	1144	64.0
	40.1667	-9.8333	959	10.69	36.09	3.8 ± 1.0	0.7 ± 0.1	1.1	947	30.3
	40.1668	-9.9333	1463	8.59	35.88	4.9 ± 0.8	0.9 ± 0.2	0.7	907	22.2
	40.1667	-9.9998	3475	2.57	34.84	6.5 ± 2.6	1.3 ± 0.2	1.2	838	12.1
	40.1665	-10.1669	3981	2.52	34.82	9.5 ± 1.1	2.2 ± 0.5	0.6	714	9.5
	40.6014	-10.3681	3416	2.61	34.92	3.9 ± 0.2	2.3 ± 0.4	0.5	640	9.4
	40.0778	-10.3756	4277	2.48	34.90	7.5 ± 0.5	2.5 ± 0.3	0.5	731	9.4
	40.1668	-10.9999	4902	2.47	34.86	3.4 ± 1.2	2.0 ± 0.2	0.5	508	6.0
	39.6135	-9.1904	458	na	na	46.9 ± 2.9	2.6 ± 0.7	1.3	2087	109.3
	39.5803	-9.4253	740	na	na	34.6 ± 0.2	na	na	1297	49.2
	39.5968	-9.4038	897	na	na	42.1 ± 2.2	2.7 ± 0.5	1.1	1253	41.9
	39.5040	-9.8434	3231	2.63	34.87	21.7 ± 2.3	2.1 ± 0.3	0.9	854	12.9
	39.5000	-9.9403	3530	2.54	34.92	47.9 ± 1.6	na	na	674	9.7
	39.5942	-10.3211	4340	2.48	34.14	23.7 ± 2.7	na	na	677	8.6
	39.5934	-10.3334	4363	2.49	34.80	20.0 ± 7.4	2.1 ± 0.5	0.8	716	9.1

	38.4935	-9.4788	445	11.85	35.74	36.2 ± 6.9	3.4 ± 0.3	0.9	1511	80.7
	38.4652	-9.4749	1021	12.05	36.28	54.8 ± 13.4	2.7 ± 0.4	1.5	1501	46.1
	38.3631	-9.5096	2100	4.77	35.20	31.8 ± 3.6	2.3 ± 0.4	1.3	1689	33.0
	38.3115	-9.7025	2975	2.85	34.95	13.4 ± 3.1	1.7 ± 0.1	1.3	1211	19.2
	38.3333	-9.8584	3914	2.48	34.91	8.7 ± 0.8	1.6 ± 0.2	0.5	987	13.3
	38.3558	-9.9789	4513	2.50	34.09	16.2 ± 3.5	3.5 ± 0.5	0.7	699	8.7
	38.4168	-10.0833	4689	2.51	34.90	3.8 ± 2.5	0.9 ± 0.2	0.5	529	6.4
	37.83283	-8.48533	1002	11.6	36.29	10.5 ± 6.0	1.7 ± 0.3	0.5	541	16.8
	37.83333	-8.25000	2130	5.45	35.34	7.8 ± 3.5	1.3 ± 0.2	1.1	538	10.4
	37.8333	-9.91667	2908	2.75	34.95	9.0 ± 0.9	1.6 ± 0.3	0.7	1398	22.5
	37.8336	-10.5001	3908	2.43	34.88	2.8 ± 0.6	1.3 ± 0.3	0.7	451	6.1
	37.8334	-11.0002	4987	2.55	34.90	6.9 ± 2.2	1.1 ± 0.2	1.1	401	4.7
Mediterranean Sea	42.1475	3.5843	398	13.34	38.49	12.4 ± 0.7	2.9 ± 0.5	1.2	661	38.1
	42.1287	3.7772	985	13.02	38.45	2.9 ± 0.6	0.9 ± 0.1	0.3	584	18.4
	42.1175	4.0457	1887	13.07	38.44	1.5 ± 0.2	1.3 ± 0.2	0.4	533	11.1
	42.0797	4.6817	2342	13.10	38.43	9.5 ± 2.0	2.4 ± 0.5	0.8	544	10.0
	36.9001	15.2733	607.4	na	na	22.1 ± 3.1	2.6 ± 0.3	3.9	339	14.7
	36.8714	15.3386	1263	13.60	38.57	9.4 ± 2.0	1.7 ± 0.3	1.6	318	8.5
	36.8491	15.3411	2062	13.65	38.53	9.0 ± 5.6	1.8 ± 0.2	5.4	318	6.3
	36.8177	15.3625	2325	13.10	38.50	16.8 ± 2.6	2.8 ± 0.4	0.9	333	6.1
	34.9835	23.8095	520	14.17	38.83	na	na	na	218	10.5
	35.0113	23.6971	1081	13.70	38.75	12.2 ± 1.2	1.3 ± 0.3	0.1	228	6.8
	35.0281	23.6143	1903	13.70	38.70	19.1 ± 3.2	1.0 ± 0.3	0.2	228	4.7
	35.1475	23.7142	2420	13.50	38.76	12.9 ± 2.6	0.9 ± 0.1	0.2	218	3.9
	35.0847	23.5536	3553	13.60	38.75	23.5 ± 4.7	1.2 ± 0.2	0.2	228	3.3
	35.0524	23.4921	3600	13.60	38.65	24.2 ± 5.1	1.5 ± 0.4	0.3	228	3.2
	34.7244	24.1280	507	13.90	38.85	24.4 ± 15.2	0.8 ± 0.1	0.4	206	10.1
	34.6031	24.1426	3617	na	na	38.3 ± 0.9	0.9 ± 0.2	0.02	175	2.5

table S2. Output of the in silico analysis dealing with the coverage of probes targeting 16*S* rRNA used in the present study. For each probe, it is shown the comparison between the percentages of coverage with 0 and 1 mismatch and the relative sequences (in brackets) obtained using the updated SILVA database at June 2015.

Probe	Sequence 5'-3'	Target	Coverage (no mismatch)	Coverage (one mismatch)
EUB338-I	GCTGCCTCCCGTAGGAGT	*Bacteria*	76% (343904)	80% (362065)
EUB338-II	GCAGCCACCCGTAGGTGT	*Planctomycetales*	72% (2981)	74% (3061)
EUB338-III	GCTGCCACCCGTAGGTGT	*Verrucomicrobiales*	84% (938)	86% (959)
NONEUB	ACTCCTACGGGAGGCAGC	Negative control	-	-
ARCH915	GTGCTCCCCCGCCAATTCCT	*Archaea*	72% (13408)	79% (14779)
CRN537	TGACCACTT AGGTGCTG	*Marine Group I*	68% (1545)	74% (1663)
EURY806	CACAGCGTTTACACCTAG	*Marine Group II*	71% (248)	73%(357)

table S3. Statistical analysis testing for differences in the distribution of the different microbial components. Output of the PERMANOVA analysis testing for differences in the abundances of bacteria (**A**), archaea (**B**), *MG-I Thaumarchaeota* (**C**) and *MG-II Euryarchaeota* (**D**) in the top 1 cm of deep-sea sediments of the different oceanic regions and sampling areas investigated. *** P<0.001, **P<0.01, *P<0.05, ns=not significant. The output of post-hoc tests is also reported.

A) Bacteria					
Factor	**df**	**MS**	**Pseudo-F**	**P**	**Post-hoc**
Oceanic region	2	1.19	4.69	**	Arctic > Atlantic, Mediterranean
Area	3	0.30	1.21	ns	
Residual	51	0.25			
Total	56				
A) Archaea					
Factor	**df**	**MS**	**Pseudo-F**	**P**	**Post-hoc**
Oceanic region	2	11.44	17.44	***	Arctic > Atlantic, Mediterranean
Area	3	1.03	1.57	ns	
Total	51	0.66			
Oceanic region	56				
C) *MG-I Thaumarchaeota*					
Factor	**df**	**MS**	**Pseudo-F**	**P**	**Post-hoc**
Oceanic region	2	13.86	15.36	***	Arctic > Atlantic, Mediterranean
Area	3	0.71	0.79	ns	
Residual	51	0.90			
Total	56				
D) *MG-II Euryarchaeota*					
Factor	**df**	**MS**	**Pseudo-F**	**P**	**Post-hoc**
Oceanic region	2	7.29	9.34	***	Arctic > Atlantic, Mediterranean
Area	3	1.78	2.28	ns	
Residual	51	0.78			
Total	56				

fig. S1. Depth-related patterns of total prokaryotic abundances (obtained using SYBR Green I) in the top 1 cm of sediments collected in the different oceanic regions. Means and standard deviations (n = 3) are reported.

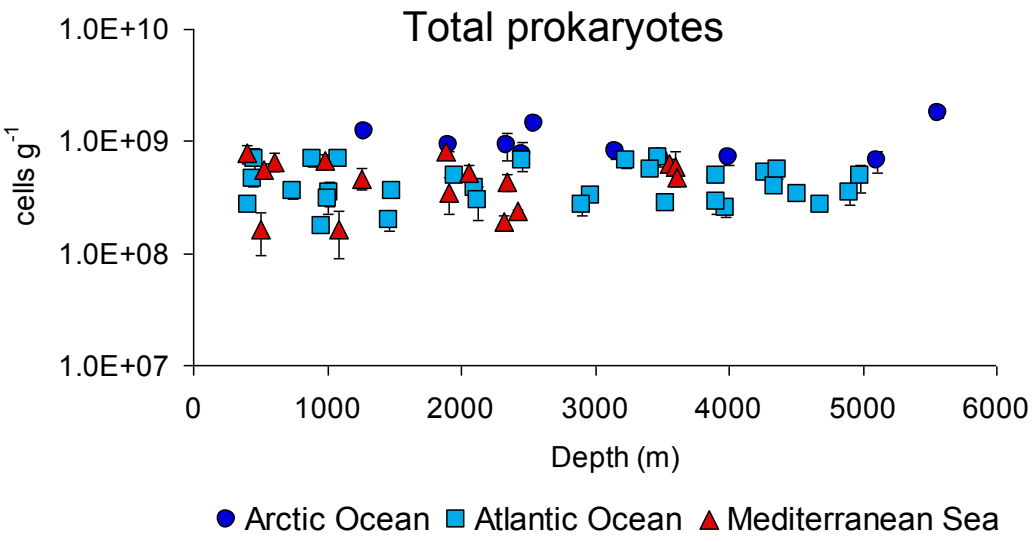

fig. S2. Comparison of the abundances of bacteria and archaea obtained by CARD-FISH, with the number of 16*S* rDNA copies of bacteria and archaea obtained in surface sediments of different oceanic regions. (**A**) Comparison between bacterial abundances and the number of bacterial 16*S* rDNA copies; (**B**) Comparison between archaeal abundances and the number of archaeal 16*S* rDNA copies. Means and standard deviations (n = 3) are reported.

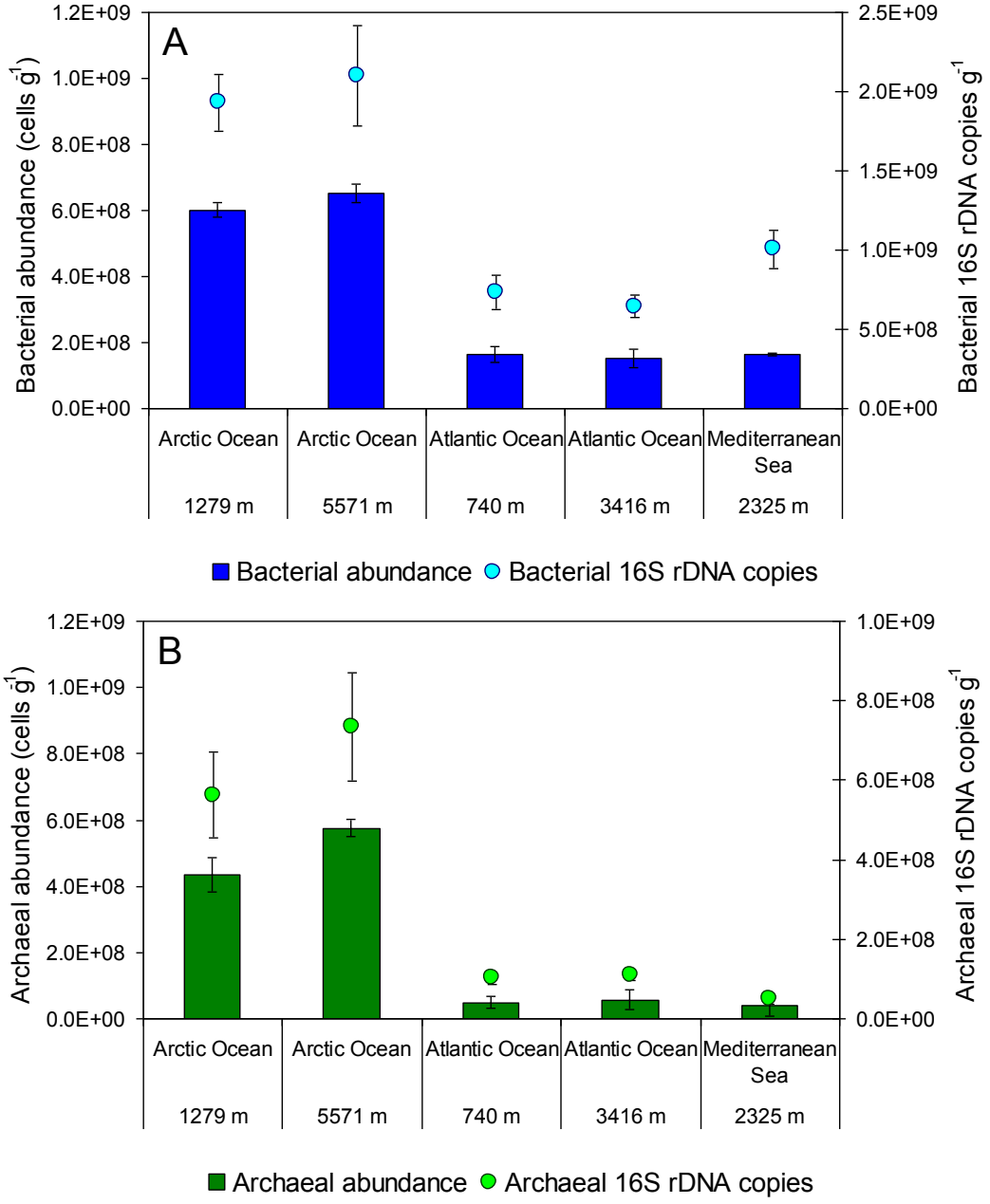

fig. S3. Output of the regression tree analysis carried out to identify environmental factors explaining the distribution of MG-I Thaumarchaeota in the top 1 cm of sediments collected in the different oceanic regions. The significance level and the percentage of the explained variance of the predictor variable is reported along with the number of sampling sites for each oceanic region at the terminal nodes.

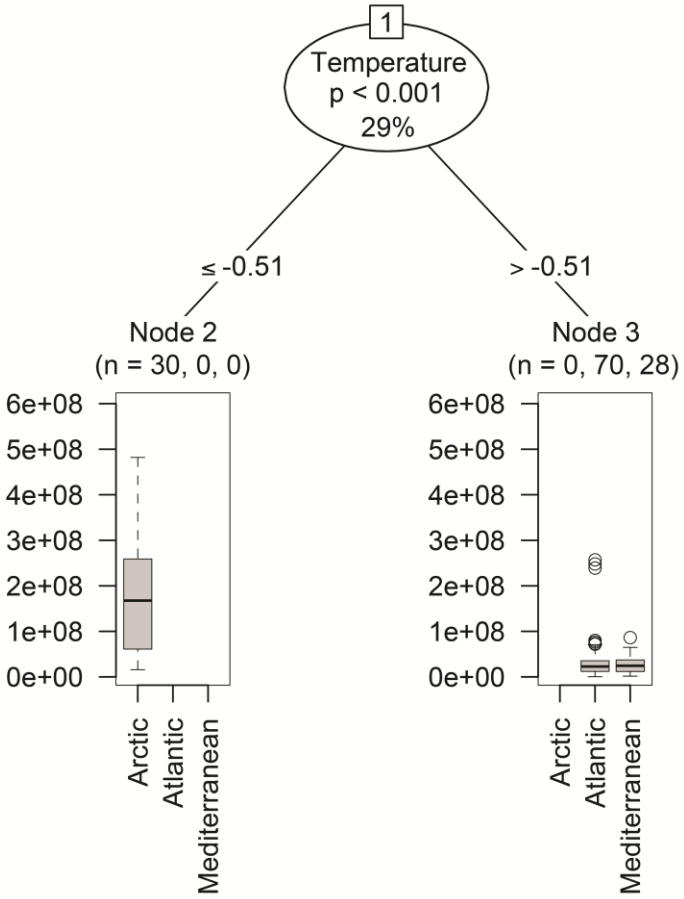

fig. S4. Output of the regression tree analysis carried out to identify environmental factors explaining the distribution of MG-II Euryarchaeota in the top 1 cm of sediments collected in the different oceanic regions. The significance level and the percentage of the explained variance of each predictor variable is reported along with the number of sampling sites for each oceanic region at the terminal nodes. OC flux = organic C that reaches the seafloor through particle sinking (expressed as mg C m^{-2} d^{-1}); BPC= Biopolymeric C concentrations in the sediment (expressed as mg C g^{-1}); PRT:CHO= protein to carbohydrate ratio in the sediment (adimensional).