



Limited carbon cycling due to high-pressure effects on the deep-sea microbiome

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Supplementary information

Supplementary Table 1. Station details on prokaryotic activity measurements performed in this study. Information is provided for the on-deck atmospheric pressure samples collected by the sampling rosette with the conductivity-temperature-depth (CTD) system.

Cruise or sampling site	Area	St	Date	Latitude (°)	Longitude (°)	Depth (m)	Temp (°C)	Salinity	DO (μmol kg ⁻¹)
MODUPLAN	North Atlantic Ocean	8	5-Aug-2014	43.0000 N	9.7165 W	445	11.39	35.67	189
MODUPLAN	North Atlantic Ocean	14	8-Aug-2014	42.9998 N	10.6667 W	1140	10.60	36.12	171
MODUPLAN	North Atlantic Ocean	108	10-Aug-2014	43.0000 N	12.6667 W	1778	4.35	35.05	233
MODUPLAN	North Atlantic Ocean	59	19-Aug-2014	43.9000 N	3.7833 W	1974	3.91	35.03	229
MODUPLAN	North Atlantic Ocean	60	20-Aug-2014	43.8000 N	3.7833 W	990	9.72	35.78	175
MODUPLAN	North Atlantic Ocean	57	21-Aug-2014	44.2917 N	3.7832 W	1975	4.10	35.04	228
Rovinj*	Adriatic Sea	N/A	25-Oct-2014	45.0856 N	13.6394 E	20	19.50	N/D	N/D
RadProf15	North Atlantic Ocean	11	1-Aug-2015	43.0253 N	10.0173 W	2465	3.14	34.96	220
RadProf15	North Atlantic Ocean	111	5-Aug-2015	43.0010 N	14.0328 W	3152	2.79	34.94	213
SO248	Pacific Ocean	16†	26-May-2016	49.9999 N	179.5501 E	999	2.73	34.36	26
SO248*	Pacific Ocean	17	29-May-2016	54.0013 N	179.5813 E	501	3.63	34.08	26
M139*	Atlantic Ocean	A1†	12-Jul-2017	15.8860 N	68.9148 W	2002	4.12	34.98	197
M139	Atlantic Ocean	A3†	22-Jul-2017	23.5538 N	48.0838 W	3998	2.29	34.89	227
M139	Atlantic Ocean	A3†	22-Jul-2017	23.5538 N	48.0838 W	2000	3.68	35.00	223
M139	Atlantic Ocean	A5_6†	31-Jul-2017	10.3388 N	36.9597 W	3999	2.33	34.89	221
M139	Atlantic Ocean	A5_6†	31-Jul-2017	10.3388 N	36.9597 W	3001	2.75	34.93	225
M139	Atlantic Ocean	A5_6†	31-Jul-2017	10.3392 N	36.9617 W	474	8.56	34.90	69
MOBYDICK	Southern Ocean	M2_1†	26-Feb-2018	50.6160 S	72.0010 E	448	2.25	34.38	194
MOBYDICK	Southern Ocean	M3_1†	3-Mar-2018	50.6835 S	68.0622 E	1499	2.00	34.76	190
MOBYDICK	Southern Ocean	M2_2†	6-Mar-2018	50.6252 S	72.0134 E	400	2.16	34.30	211
MOBYDICK*	Southern Ocean	M1	8-Mar-2018	49.8502 S	74.9017 E	2499	1.01	34.72	197
MOBYDICK	Southern Ocean	M2_3	16-Mar-2018	50.6159 S	72.0013 E	175	1.72	33.99	302
MOBYDICK	Southern Ocean	M3_3	18-Mar-2018	50.6877 S	68.0664 E	1498	2.07	34.76	188
RadProf18	North Atlantic Ocean	111	24-Aug-2018	42.9998 N	14.0350 W	3443	2.63	34.93	224
RadCan18	North Atlantic Ocean	C3	27-Aug-2018	43.7668 N	6.1672 W	743	10.23	35.67	182
POSEIDON	Atlantic Ocean	5	7-Mar-2019	36.1575 S	44.3407 W	2565	2.41	34.82	204
POSEIDON	Atlantic Ocean	9	12-Mar-2019	25.4064 S	35.0020 W	3156	2.66	34.91	237
POSEIDON	Atlantic Ocean	13	16-Mar-2019	14.4742 S	30.6714 W	2962	2.69	34.90	229
POSEIDON	Atlantic Ocean	19	23-Mar-2019	5.9091 N	29.7460 W	2375	3.16	34.95	229
POSEIDON	Atlantic Ocean	22	26-Mar-2019	13.5957 N	29.7139 W	3942	2.37	34.89	218
POSEIDON	Atlantic Ocean	27	31-Mar-2019	23.7935 N	23.9188 W	4002	2.40	34.90	219

*Sample used for instrumental test; †Metaproteomics stations¹; N/A: not applicable; N/D: not determined; DO-dissolved oxygen measured with an oxygen sensor (Sea-Bird SBE 43 or Aanderaa 4831F).

Supplementary Table 2. Leucine incorporation rates and incubation conditions under *in situ* and atmospheric pressure conditions.

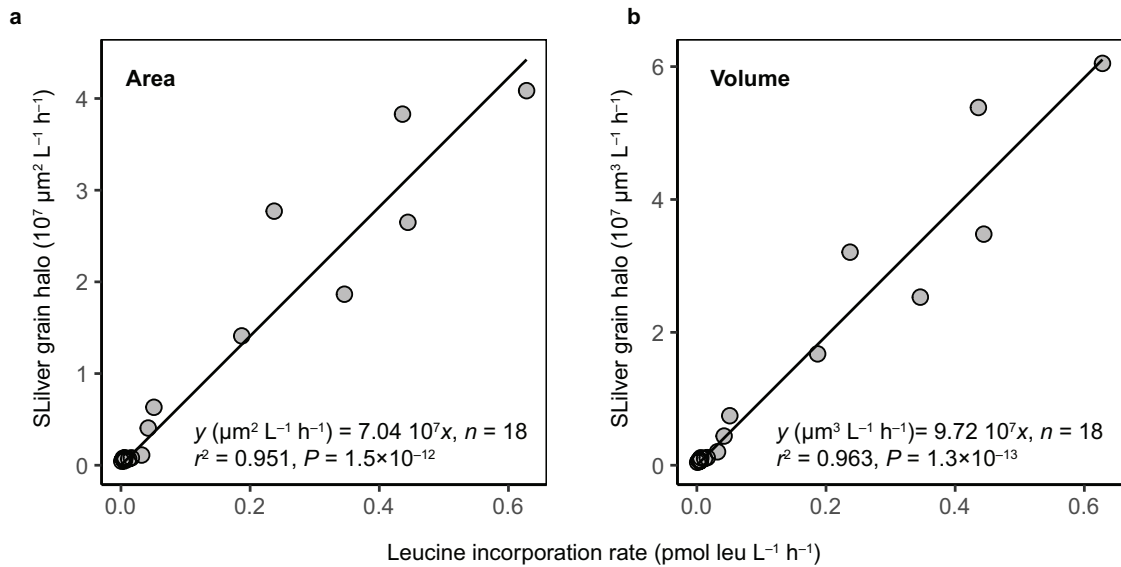
Cruise/ sampling site	St.	Atmospheric pressure condition					<i>In situ</i> condition						
		Depth (m)	Inc. temp. (°C)	Duration (h)	Leu incorp.		Depth (m)	Inc. temp. (°C)	Duration (h)	Leu incorp.			
					Mean n	SD or mean – repl.				Mean n	SD or mean – repl.		
MODUPLAN	8	445	10	6.2	0.400	1	N/A	500	11.4	6.0	0.289	2	0.035
	14	1140	10	8.0	0.088	2	0.000	1150	10.6	8.0	0.046	2	0.003
	108	1778	4	10.4	0.028	1	N/A	1800	4.4	9.5	0.013	2	0.006
	59	1974	4	8.1	0.077	1	N/A	2000	3.9	8.1	0.009	2	0.004
	60	990	10	8.2	0.074	2	0.032	1000	9.7	8.0	0.053	2	0.002
	57	1975	4	8.1	0.033	2	0.006	2000	4.1	7.7	0.008	2	0.002
RadProf15	11	2465	0–3	12.2	0.047	3	0.017	2500	3.1	11.8	0.011	2	0.004
	111	3152	2.5	12.1	0.014	3	0.010	3200	2.8	12.0	0.007	2	0.000
SO248	16	999	4	8.1	0.088	3	0.003	1000	2.7	7.5	0.058	2	0.018
M139	A3	3998	2.5	12.0	0.011	3	0.000	3983	2.3	12.0	0.003	3	0.000
	A3	2000	2.5	10.2	0.016	3	0.000	1995	3.7	10.0	0.004	3	0.001
	A5_6	3999	2.5	11.0	0.033	3	0.002	4013	2.3	10.6	0.004	2	0.000
	A5_6	3001	2.5	10.7	0.013	3	0.001	3002	2.8	11.0	0.004	2	0.000
	A5_6	474	7	6.0	0.336	3	0.022	453	8.6	6.0	0.205	2	0.018
MOBYDICK	M2_1	448	2.2	7.5	1.228	3	0.023	450	2.2	2.8	1.089	2	0.030
	M3_1	1499	1.9	7.0	0.070	3	0.002	1470	2.0	5.8	0.047	2	0.002
	M2_2	400	1.4	7.3	0.651	3	0.023	400	2.2	7.2	0.484	2	0.048
	M2_3	175	1.4	4.1	1.400	3	0.050	175	1.7	4.2	1.118	2	0.038
	M3_3	1498	1.7	7.2	0.060	3	0.008	1500	2.1	7.6	0.044	2	0.002
RadProf18	111	3443	2.6	8.3	0.007	3	0.000	3501	2.6	8.0	0.003	2	0.000
RadCan18	C3	743	10.2	3.3	0.440	3	0.004	751	10.2	3.3	0.243	2	0.006
POSEIDON	5	2565	2.4	7.6	0.023	2	0.002	2560	2.4	7.0	0.013	2	0.001
	9	3156	2.7	6.9	0.004	2	0.001	3155	2.7	6.8	0.003	2	0.000
	13	2962	2.7	6.8	0.006	2	0.001	2961	2.7	6.6	0.002	2	0.000
	19	2375	3.2	7.5	0.012	2	0.000	2375	3.2	7.2	0.004	2	0.001
	22	3942	2.4	8.2	0.005	2	0.000	3941	2.4	8.3	0.002	2	0.000
	27	4002	2.4	8.6	0.005	2	0.000	4000	2.4	8.4	0.001	2	0.000

Inc. temp.: incubation temperature; Duration: incubation time; Leu incorp.: leucine incorporation rate in pmol leu L⁻¹ h⁻¹; mean – repl.: |mean-replicate| of duplicate bottle incubations; standard deviation (SD) was used when triplicates were available; N/A: not applicable.

Supplementary Table 3. Oligonucleotide probes applied in MICRO-CARD-FISH analyses.

Target organisms	Probe	Sequence (5' to 3')	FA (%)
Bacteria	EUB338 ²	GCTGCCTCCCGTAGGAGT	55
	EUB338-II ³	GCAGCCACCCGTAGGTGT	
	EUB338-III ³	GCTGCCACCCGTAGGTGT	
SAR11 clade	SAR11-152R ⁴	ATTAGCACAAGTTTCCYCGTGT	45
	SAR11-441R ⁴	TACAGTCATTTTCTTCCCCGAC	
	SAR11-542R ⁴	TCCGAACTACGCTAGGTC	
	SAR11-732R ⁴	GTCAGTAATGATCCAGAAAGYTG	
SAR202 clade	SAR202-312 ⁵	TGTCTCAGTCCCCCTCTG	55
SAR406 clade	SAR406-97 ⁶	CACCCGTTCCGCCAGTTTA	60
Thaumarchaeota Marine Group I	Cren554 ⁷	TTAGGCCCAATAATCMTCTCT	20
	Cren537 ⁸	TGACCACTTGAGGTGCTG	
<i>Alteromonas/Colwellia</i>	Alt1413 ⁹	TTTGCATCCCCTCCCAT	55
Bacteroidetes	CF319a ¹⁰	TGGTCCGTGTCTCAGTAC	55
Euryarchaeota Marine Group II	Eury806 ⁸	CACAGCGTTTACACCTAG	20
Negative control Bacteria	NON338 ¹¹	ACTCCTACGGGAGGCAGC	55

FA: Formamide concentration of the hybridization buffer



Supplementary Fig. 1. Single-cell activity measurements. Relationship of bulk leucine incorporation rates and silver grain halo around cells expressed as **a**, area and **b**, volume. All the MICRO-CARD-FISH samples under *in situ* and atmospheric pressure conditions at 9 stations (see Methods) were used to obtain the regressions. Plots are shown as mean values of 8 technical replicates which correspond to the number of probes used. A slightly better correlation between bulk leucine incorporation and silver grain halo volume than with halo area was found. Consequently, single-cell leucine uptake was calculated using the regression with halo volume in this study.

Supplementary references:

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