

Supplementary Table S1: Biogeochemistry of samples obtained pre-, during, and post-discharge.

	Sample ID	CDOM	Methane (nM)	Ethane (nM)	Propane (nM)	i-Butane (nM)	Butane (nM)	Pentane (nM)	Methane Oxidation	
									Rate (nmol/L/d)	CH <sub>4</sub> turnover (days)
Pre-DC	1-PRE-MC118	nd	nd	nd	nd	nd	nd	nd	nd	nd
	2-PRE-MC118	nd	nd	nd	nd	nd	nd	nd	nd	nd
During DWH oil discharge	3-DUR-MC250	0.18	428	29	46	863	195	4	7.0	61.0
	4-DUR-MC250	0.25	210171	20338	15156	2965	3946	1238	2769.8	75.9
	5-DUR-MC250	0.18	160139	15592	10079	2472	2923	968	1643.2	97.5
	6-DUR-MC335	0.20	103654	9343	1688	162	39	bdl	1666.8	62.2
	7-DUR-MC295	0.37	160316	15546	10536	1943	3685	22	1030.9	155.5
	8-DUR-MC295	0.49	26	bdl	bdl	bdl	bdl	bdl	0.5	57.1
	9-DUR-MC252	0.81	104529	649	578	274	559	357	1984.0	52.7
	10-DUR-MC252	0.15	850	22136	15374	2956	6649	3813	12.9	66.0
	11-DUR-MC252	0.13	6	bdl	bdl	bdl	bdl	bdl	0.1	49.4
	12-DUR-MC252	0.38	122459	12055	8476	1692	3964	3497	1808.8	67.7
	13-DUR-MC205	0.08	42	bdl	1	22	8	8	0.4	104.0
Post-discharge	14-POST-MC422	nd	1	nd	nd	nd	nd	nd	0.1	13.5
	15-POST-MC298	nd	0	nd	nd	nd	nd	nd	0.0	19.5
	16-POST-MC297	nd	11	nd	nd	nd	nd	nd	0.0	nd
	17-POST-GC600	nd	758	nd	nd	nd	nd	nd	2.7	282.8
	18-POST-GC600	nd	824	nd	nd	nd	nd	nd	314.2	2.6
	19-POST-MC252	nd	2	nd	nd	nd	nd	nd	2.7	0.7
	20-POST-MC253	nd	0	nd	nd	nd	nd	nd	0	0

(table continued on next page)

Supplementary Table S1 (continued): Biogeochemistry of samples obtained pre-, during, and post-discharge.

	Sample ID	Cell Counts (cells/mL)	DOC (uM)	DIC (mM)	delta-DIC	NH <sub>4</sub> (uM)	NOx (uM)	PO <sub>4</sub> <sup>3-</sup> (uM)	DO (uM)	pH
Pre-DC	1-PRE-MC118	nd	nd	nd	nd	nd	nd	nd	168	nd
	2-PRE-MC118	nd	nd	nd	nd	nd	nd	nd	168	nd
During DWH oil discharge	3-DUR-MC250	1.57E+05	69	nd	nd	0.94	31.27	2.05	223	7.39
	4-DUR-MC250	6.92E+05	79	nd	nd	1.10	19.51	1.45	221	6.94
	5-DUR-MC250	3.49E+05	67	nd	nd	1.10	26.09	1.79	247	6.51
	6-DUR-MC335	1.59E+06	nd	2.32	-0.14	nd	20.61	1.38	nd	6.93
	7-DUR-MC295	1.54E+06	152	2.43	-0.01	0.85	18.02	1.25	200	7.72
	8-DUR-MC295	4.05E+05	139	2.32	0.21	0.72	14.46	1.00	198	7.82
	9-DUR-MC252	1.24E+06	79	2.35	0.10	0.82	21.67	1.65	196	nd
	10-DUR-MC252	2.88E+05	71	2.37	-0.06	0.75	13.75	1.13	230	nd
	11-DUR-MC252	1.46E+05	60	2.32	-0.05	0.53	30.61	2.37	164	7.91
	12-DUR-MC252	3.58E+05	65	2.26	0.15	1.04	23.07	1.62	231	7.78
	13-DUR-MC205	nd	83	2.30	0.08	0.78	28.37	1.68	219	7.92
Post-discharge	14-POST-MC422	nd	67	2.34	0.10	0.00	31.65	1.97	227	7.64
	15-POST-MC298	7.23E+04	45	2.33	1.12	0.00	30.00	1.95	199	7.78
	16-POST-MC297	3.55E+05	75	nd	nd	1.0	26.1	1.7	218	7.79
	17-POST-GC600	2.30E+05	42	1.89	nd	0.00	25.20	1.99	nd	7.99
	18-POST-GC600	1.38E+05	56	1.69	nd	0.44	27.35	1.61	207	7.93
	19-POST-MC252	1.25E+05	80	2.13	nd	0.69	26.95	1.54	210	7.9
	20-POST-MC253	1.55E+05	61	1.70	nd	0.31	24.05	1.55	240	7.95

**Supplementary Table S2: Statistics for high-quality pyrosequencing reads of the V3V5 and V6V4 regions of the 16S rRNA gene.**

Sample ID	Total reads		Reads assigned to Bacteria	
	V3V5	V6V4	V3V5	V6V4
1-PRE-MC118	54	26,632	54	23,473
2-PRE-MC118	18575	24,952	18,457	21,701
3-DUR-MC250	19,492	26,631	19,429	22,831
4-DUR-MC250	23,170	34,515	23,164	33,699
5-DUR-MC250	24,295	37,202	24,257	34,905
6-DUR-MC335	NA	9,081	NA	8,217
7-DUR-MC295	47,527	56,107	47,502	55,094
8-DUR-MC295	50,560	37,893	50,550	37,348
9-DUR-MC252	35,002	36,805	34,990	36,253
10-DUR-MC252	13,521	33,529	13,465	29,236
11-DUR-MC252	32,422	29,902	32,351	26,953
12-DUR-MC252	50,713	47,456	50,609	44,754
13-DUR-MC205	37,860	36,949	37,624	34,838
14-POST-MC422	23,978	39,248	23,901	37,265
15-POST-MC298	NA	33,641	NA	29,530
16-POST-MC297	NA	36,972	NA	32,386
17-POST-GC600	NA	106	NA	84
18-POST-GC600	17,958	52,604	17,907	51,024
19-POST-MC252	22,977	32,873	22,913	29,666
20-POST-MC253	28,602	35,226	28,474	30,700

Supplementary Table S3: Comparison of bacterial community composition\* based on relative read abundance (%) and OTU numbers (97% similarity threshold) of the V3V5 or V6V4 regions of 16S rRNA genes

Taxonomic identification	1-PRE-MC118				2-PRE-MC118				3-DUR-MC250				4-DUR-MC250				5-DUR-MC250			
	V3V5		V6V4		V3V5		V6V4		V3V5		V6V4		V3V5		V6V4		V3V5		V6V4	
	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)
<b>Total numbers</b>	100	38	100	811	100	1106	100	980	100	644	100	808	100	257	100	749	100	863	100	1428
<b>Acidobacteria</b>																				
Gp26	3.7	2	0.2	2	0.1	3	0.3	1	0.1	1	0.2	1	<0.1	1	<0.1	3	0.2	1	0.1	7
Acidobacteria_others	1.9	1	0.8	17	1.4	42	1.3	22	1.8	25	1.6	27	0.1	7	0.1	18	1.1	42	0.4	46
<b>Actinobacteria</b>	0	0	4.6	19	2.6	11	5.0	25	2.9	10	3.9	29	0.1	5	0.1	10	0.8	12	0.4	23
<b>Bacteroidetes</b>																				
Bacteroidia	0	0	0.1	8	1.0	6	0.6	9	<0.1	1	0	0	<0.1	1	<0.1	2	<0.1	2	<0.1	1
Flavobacteria	11.1	6	3.0	53	4.6	80	2.4	57	2.0	31	2.6	58	0.4	19	0.3	43	3.5	48	1.3	73
Sphingobacteria	0	0	0.9	11	1.3	30	1.0	18	0.7	16	1.3	33	0	3	0	6	0.2	14	0.2	20
Bacteroidetes_others	0	0	0	0	0	0	0	0	<0.1	0	<0.1	1	0	0	0	0	<0.1	0	0	0
<b>Chloroflexi</b>																				
SAR202	3.7	2	4.7	60	5.9	103	4.1	60	5.0	49	2.6	43	<0.1	3	0.1	11	0.8	45	0.3	42
Chloroflexi_others	0	0	0.2	7	0.1	4	0.2	13	0.2	8	0.2	9	0.0	0	<0.1	3	<0.1	8	<0.1	4
<b>Cyanobacteria</b>	0	0	<0.1	2	0.5	35	0	9	0	16	2	29	<0.1	2	<0.1	2	0.2	12	0.1	11
<b>Deferribacteres</b>	14.8	6	15.6	88	18.0	113	17.1	111	17.1	90	13.7	72	0.5	34	0.4	58	3.3	77	1.4	109
<b>Firmicutes</b>																				
Bacilli	0	0	0.9	11	0.4	11	0.4	7	0.1	3	0.1	4	0	0	0	0	<0.1	4	<0.1	1
Clostridia	0	0	0.3	6	0.4	14	0.5	16	0	0	0.1	1	0	0	<0.1	4	<0.1	3	<0.1	3
Erysipelotrichi	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Firmicutes_others	0	0	<0.1	1	0.5	2	0.2	1	0	0	0	0	0	0	0	0	0	0	0	0
<b>Gemmatimonadetes</b>	1.9	1	1.1	8	1.6	18	1.5	10	1.6	10	1.8	10	0.1	9	0.1	11	0.9	13	0.3	17
<b>Planctomycetes</b>																				
Phycisphaerae	1.9	1	0.3	4	1.0	22	1.0	19	1.8	17	0.6	8	0.2	11	0.1	12	1.6	21	0.3	28
Planctomycetacia	0	0	1.4	30	2.3	63	1.5	43	3.7	46	1.6	30	0.1	18	0.1	28	2.7	81	0.5	85
Planctomycetes_others	0	0	0.1	2	0	0	<0.1	1	<0.1	1	0.1	2	0	0	<0.1	1	<0.1	4	0	0
<b>Proteobacteria</b>																				
Alphaproteobacteria																				
SAR11	3.7	2	13.0	80	10.7	45	15.2	93	16.2	36	20.8	85	0.6	9	0.8	57	2.1	22	2.8	90
Alphaproteobacteria_others	11.1	5	6.0	94	5.2	109	6.3	114	3.5	54	5.7	86	0.2	18	0.3	45	2.7	91	1.3	120
Betaproteobacteria	0	0	0.4	8	<0.1	3	0.2	4	1.7	9	2.0	14	0.2	6	0.1	13	0.3	9	0.1	11
Deltaproteobacteria	31.5	7	14.1	88	24.0	164	14.1	119	14.6	82	12.0	64	0.6	21	0.5	49	3.3	105	1.3	105
Epsilonproteobacteria	0	0	0.5	7	0.4	8	0.2	6	<0.1	1	<0.1	2	0.1	5	<0.1	9	4.9	5	1.2	20
Gammaproteobacteria																				
Colwellia	0	0	0.4	6	0.0	1	0.7	9	0.4	5	1.5	15	8.7	9	16.6	82	13.1	14	27.7	125
Oceaniserpentilla	1.9	1	2.3	3	0.6	4	1.7	3	2.6	4	4.7	4	74.3	9	65.9	83	5.8	3	6.8	43
Cycloclasticus	0	0	0.1	1	0.6	5	1.5	2	1.0	5	3.1	6	12.6	8	13.2	50	42.1	13	46.8	138
Gammaproteobacteria_others	13.0	4	25.8	146	14.5	143	19.4	148	15.4	82	15.0	123	0.8	37	1.0	112	7.0	120	6.0	230
Proteobacteria_others	0	0	<0.1	1	0	0	0	0	0	0	<0.1	1	0	0	0	1	0	0	<0.1	1
<b>Verrucomicrobia</b>																				
Verrucomicrobiae	0	0	0.3	6	0.4	6	0.3	14	1.5	9	0.5	6	<0.1	5	<0.1	8	0.6	19	0.1	14
Verrucomicrobia_others	0	0	2.1	16	1.1	17	2.2	18	4.7	11	1.7	15	0.2	7	0.1	8	2.0	20	0.4	25
<b>Bacteria_others</b>	0	0	0.8	26	0.6	44	0.6	28	0.7	21	0.8	30	0.1	10	0.1	20	0.5	55	0.2	36

(table continued on next page)

Supplementary Table S3 (continued): Comparison of bacterial community composition based on relative read abundance (%) and OTU numbers (97% similarity threshold) of the V3V5 or V6V4 regions of 16S rRNA genes

Taxonomic identification	6-DUR-MC335				7-DUR-MC295				8-DUR-MC295				9-DUR-MC252				10-DUR-MC252			
	V3V5		V6V4		V3V5		V6V4		V3V5		V6V4		V3V5		V6V4		V3V5		V6V4	
	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)
<b>Total numbers</b>	0	0	100	199	100	287	100	347	100	377	100	329	100	338	100	548	100	693	100	1090
<b>Acidobacteria</b>																				
Gp26	-	-	<0.1	1	<0.1	3	<0.1	1	<0.1	2	0.1	1	<0.1	1	<0.1	2	0.2	1	0.1	1
Acidobacteria_others	-	-	0.1	5	0.1	18	0.1	12	0.3	23	0.1	16	0.1	15	0.1	12	2.2	30	1.0	28
<b>Actinobacteria</b>	-	-	0.2	6	0.1	9	0.1	10	0.1	10	0.1	7	0.0	7	0.1	12	1.3	11	1.2	31
<b>Bacteroidetes</b>																				
Bacteroidia	-	-	0	0	<0.1	4	<0.1	1	<0.1	2	0	0	0	0	0	0	0.1	4	<0.1	2
Flavobacteria	-	-	2.7	19	0.3	19	0.3	29	0.6	23	0.3	22	0.3	27	0.1	23	5.4	36	3.3	59
Sphingobacteria	-	-	0.2	5	0.1	5	0	6	0.1	4	0.1	4	0.1	6	0	6	0.3	12	0.8	39
Bacteroidetes_others	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	0	<0.1	0
<b>Chloroflexi</b>																				
SAR202	-	-	0	0	0	0	<0.1	4	<0.1	6	<0.1	5	0.1	12	<0.1	8	5.0	52	2.7	64
Chloroflexi_others	-	-	0	0	0	0	0	0	<0.1	1	<0.1	3	0	0	0	0	0.1	7	0.1	13
<b>Cyanobacteria</b>	-	-	<0.1	2	<0.1	2	<0.1	2	0.1	5	<0.1	5	<0.1	6	<0.1	3	0	13	0	27
<b>Deferribacteres</b>	-	-	0.3	5	0.2	16	0.1	12	0.3	28	0.2	20	0.3	29	0.2	37	13.3	84	12.1	96
<b>Firmicutes</b>																				
Bacilli	-	-	0.2	3	<0.1	2	0	0	<0.1	1	<0.1	1	0.0	0	<0.1	1	0.2	3	<0.1	2
Clostridia	-	-	0.1	4	0	0	0	0	0	0	0	0	<0.1	1	<0.1	2	<0.1	0	0.2	8
Erysipelotrichi	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Firmicutes_others	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Gemmatimonadetes</b>	-	-	<0.1	1	0.3	10	0.1	8	0.3	13	0.1	7	0.1	10	0.1	8	1.9	13	1.3	9
<b>Planctomycetes</b>																				
Phycisphaerae	-	-	0.1	4	0.2	8	<0.1	5	0.2	12	0.1	5	0.2	11	0.1	11	2.5	15	1.1	12
Planctomycetacia	-	-	0.3	10	0.3	24	0.1	16	0.4	41	0.1	21	0.1	28	0.1	16	4.2	47	1.7	54
Planctomycetes_others	-	-	0	0	0	0	0	0	0	0	0	0	<0.1	1	<0.1	1	<0.1	2	0	1
<b>Proteobacteria</b>																				
Alphaproteobacteria																				
SAR11	-	-	3.8	22	0.5	16	0.6	36	1.0	24	0.8	37	0.5	16	0.7	45	7.6	29	17.1	101
Alphaproteobacteria_others	-	-	1.0	15	0.1	19	0.2	36	0.3	33	0.3	43	0.2	28	0.2	38	4.5	63	6.5	123
Betaproteobacteria	-	-	0.5	3	0.3	11	0.5	10	0.3	12	0.3	10	<0.1	5	<0.1	6	0.7	6	1.1	17
Deltaproteobacteria	-	-	0.2	7	0.6	20	0.4	20	1.4	36	0.6	13	0.4	35	0.2	36	15.7	87	11.5	113
Epsilonproteobacteria	-	-	0.4	6	0.1	5	<0.1	6	<0.1	1	<0.1	2	<0.1	2	<0.1	2	0.1	5	<0.1	4
Gammaproteobacteria																				
Colwellia	-	-	35.3	20	0.5	9	3.6	23	0.2	6	1.2	14	2.0	8	3.7	41	0.9	4	3.3	20
Oceaniserpentilla	-	-	1.9	1	92.3	13	88.6	33	92.7	16	93.7	26	79.6	16	77.3	72	5.8	3	9.6	9
Cycloclasticus	-	-	46.5	9	3.0	5	4.1	8	0.5	5	0.6	4	15.3	7	16.3	47	1.4	6	3.3	6
Gammaproteobacteria_others	-	-	5.8	42	0.6	46	0.7	50	0.7	52	0.9	53	0.6	44	0.7	93	17.5	98	17.6	171
Proteobacteria_others	-	-	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	0	0	0	0	0
<b>Verrucomicrobia</b>																				
Verrucomicrobiae	-	-	0.1	1	0.1	5	<0.1	5	<0.1	4	0.1	2	<0.1	6	<0.1	7	1.5	11	0.5	14
Verrucomicrobia_others	-	-	<0.1	1	0.1	4	0.0	4	0.2	2	0.1	4	0.1	5	0.1	9	6.1	17	2.2	19
<b>Bacteria_others</b>	-	-	0.2	7	0.1	14	0.1	10	0.1	15	<0.1	4	0.1	12	<0.1	10	1.1	34	0.9	47

(table continued on next page)

Supplementary Table S3 (continued): Comparison of bacterial community composition based on relative read abundance (%) and OTU numbers (97% similarity threshold) of the V3V5 or V6V4 regions of 16S rRNA genes

Taxonomic identification	11-DUR-MC252				12-DUR-MC252				13-DUR-MC205				14-POST-MC422				15-POST-MC298			
	V3V5		V6V4		V3V5		V6V4		V3V5		V6V4		V3V5		V6V4		V3V5		V6V4	
	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)
<b>Total numbers</b>	100	911	100	986	100	1273	100	1405	100	1283	100	1391	100	635	100	973	0	0	100	777
<b>Acidobacteria</b>																				
Gp26	0.1	3	0.2	1	<0.1	4	0.1	1	0.2	3	0.2	3	<0.1	1	<0.1	1	-	-	0.1	1
Acidobacteria_others	0.7	21	0.5	21	0.5	29	0.4	26	1.2	44	0.9	31	0.3	21	0.1	19	-	-	0.4	19
<b>Actinobacteria</b>	2.5	19	4.3	31	0.9	15	1.3	27	2.1	16	2.8	22	0.9	12	0.7	17	-	-	1.2	20
<b>Bacteroidetes</b>																				
Bacteroidia	0.1	5	<0.1	9	26.4	28	12.1	41	<0.1	4	<0.1	0	<0.1	1	<0.1	2	-	-	<0.1	1
Flavobacteria	1.7	41	2.1	68	1.8	59	1.3	62	3.0	79	2.3	82	53.9	58	22.7	74	-	-	3.5	53
Sphingobacteria	0.6	15	0.6	17	0.5	26	0.4	25	0.5	22	0.5	28	0.7	13	0.3	16	-	-	0.5	9
Bacteroidetes_others	0	0	0	0	<0.1	0	<0.1	0	<0.1	0	<0.1	0	0	0	0.0	0	-	-	0	0
<b>Chloroflexi</b>																				
SAR202	4.4	90	2.1	62	2.0	99	1.3	58	3.9	112	2.7	90	0.8	21	0.4	28	-	-	1.2	38
Chloroflexi_others	0.1	8	0.1	8	0.1	16	0.1	13	0.1	10	0.1	12	<0.1	2	<0.1	8	-	-	0.2	5
<b>Cyanobacteria</b>	0	17	0	14	0	31	0.1	20	0	25	0	26	0.9	11	0.5	9	-	-	0.2	11
<b>Deferribacteres</b>	18.3	119	14.3	110	7.3	113	7.0	115	15.0	121	11.7	136	4.3	58	3.1	85	-	-	14.2	99
<b>Firmicutes</b>																				
Bacilli	0.6	6	0.5	16	0.1	7	0.1	7	0.1	7	<0.1	2	0.1	4	<0.1	5	-	-	0.2	5
Clostridia	0.2	11	0.3	21	18.6	96	12.6	132	<0.1	3	0.1	6	<0.1	1	<0.1	3	-	-	0.1	3
Erysipelotrichi	0	0	0	0	2.0	10	1.4	11	<0.1	1	0	0	0	0	0.0	0	-	-	<0.1	2
Firmicutes_others	0	0	0	0	0	0	0	0	0	0	0	0	<0.1	1	<0.1	1	-	-	0	0
<b>Gemmatimonadetes</b>	1.0	12	0.6	8	0.7	10	0.5	9	1.6	18	0.9	17	0.6	7	0.2	8	-	-	0.2	5
<b>Planctomycetes</b>																				
Phycisphaerae	0.8	18	0.3	9	1.0	27	0.4	18	1.4	24	0.8	22	0.9	16	0.3	19	-	-	0.4	11
Planctomycetacia	1.9	37	0.9	37	1.7	75	0.6	52	2.1	87	1.4	68	2.0	54	0.7	50	-	-	1.1	33
Planctomycetes_others	<0.1	3	<0.1	1	<0.1	3	<0.1	1	<0.1	2	<0.1	1	<0.1	2	<0.1	1	-	-	0	0
<b>Proteobacteria</b>																				
Alphaproteobacteria																				
SAR11	16.2	53	14.5	97	2.9	32	5.7	90	13.0	60	12.4	97	4.6	24	7.4	95	-	-	41.5	98
Alphaproteobacteria_others	3.0	85	5.3	124	2.5	128	3.5	164	3.5	121	5.4	162	3.3	84	3.1	134	-	-	7.3	111
Betaproteobacteria	0.8	23	1.0	18	0.5	27	0.8	17	0.5	21	0.9	13	0.1	6	2.0	9	-	-	0.4	9
Deltaproteobacteria	14.1	157	8.7	106	7.4	177	5.2	151	13.9	201	10.4	181	3.3	61	1.5	69	-	-	7.6	87
Epsilonproteobacteria	0.0	0	0.1	4	0.1	5	<0.1	10	0.1	4	<0.1	2	0.1	2	0.1	4	-	-	0.1	6
Gammaproteobacteria																				
Colwellia	0.2	8	2.5	15	0.1	7	2.0	27	0.3	7	2.6	24	<0.1	1	0.3	19	-	-	<0.1	1
Oceaniserpentilla	13.1	3	26.1	9	11.7	7	31.4	40	12.3	6	17.5	21	<0.1	4	0.1	2	-	-	0	0
Cycloclasticus	1.1	5	2.7	5	0.5	6	1.9	13	6.9	11	10.3	22	2.8	6	3.4	7	-	-	0.1	1
Gammaproteobacteria_others	13.9	91	10.2	121	6.3	143	8.0	196	14.0	173	12.9	218	19.8	117	52.1	220	-	-	17.4	112
Proteobacteria_others	0	0	0	1	<0.1	0	0	0	<0.1	0	0	0	0	0	0	0	-	-	0	0
<b>Verrucomicrobia</b>																				
Verrucomicrobiae	0.9	9	0.2	10	1.8	18	0.4	10	0.6	17	0.3	17	0.1	8	0.1	8	-	-	0.2	3
Verrucomicrobia_others	3.1	21	1.4	16	2.2	31	1.1	21	3.0	31	1.9	24	0.3	15	0.6	19	-	-	1.4	17
<b>Bacteria_others</b>	0.4	31	0.4	27	0.4	44	0.3	48	0.5	53	0.7	64	0.2	24	0.2	41	-	-	0.4	17

(table continued on next page)

Supplementary Table S3 (continued): Comparison of bacterial community composition based on relative read abundance (%) and OTU numbers (97% similarity threshold) of the V3V5 or V6V4 regions of 16S rRNA genes

Taxonomic identification	16-POST-MC297				17-POST-GC600				18-POST-GC600				19-POST-MC252				20-POST-MC253			
	V3V5		V6V4		V3V5		V6V4		V3V5		V6V4		V3V5		V6V4		V3V5		V6V4	
	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)	Reads (%)	OTUs (#)
<b>Total numbers</b>	0	0	100	917	0	0	100	42	100	1287	100	1640	100	1899	100	2997	100	4644	100	6835
<b><i>Acidobacteria</i></b>																				
Gp26	-	-	0.2	1	-	-	0	0	0.1	3	0.1	1	0.1	2	0.2	4	0.2	8	0.3	15
<i>Acidobacteria</i> _others	-	-	1.6	29	-	-	0.0	0	1.4	50	0.5	38	1.2	61	0.9	63	3.6	248	2.8	290
<b><i>Actinobacteria</i></b>	-	-	2.4	17	-	-	4.9	2	1.2	16	1.1	26	0.5	14	0.6	28	1.3	49	1.8	108
<b><i>Bacteroidetes</i></b>																				
<i>Bacteroidia</i>	-	-	0	0	-	-	0	0	< 0.1	6	< 0.1	2	0.2	24	0.2	22	< 0.1	3	< 0.1	12
<i>Flavobacteria</i>	-	-	2.5	44	-	-	1.2	2	4.7	61	1.8	91	2.9	102	2.3	127	2.8	139	2.5	172
<i>Sphingobacteria</i>	-	-	0.5	16	-	-	1.2	1	0.9	37	0.3	40	1.5	87	1.4	92	2.4	186	2.1	243
<i>Bacteroidetes</i> _others	-	-	0	0	-	-	0	0	< 0.1	0	< 0.1	3	0.5	0	0.3	9	< 0.1	0	< 0.1	2
<b><i>Chloroflexi</i></b>																				
SAR202	-	-	2.9	54	-	-	0	0	3.6	80	1.5	78	4.6	138	3.0	138	5.2	165	3.8	218
<i>Chloroflexi</i> _others	-	-	0.1	11	-	-	0	0	0.4	40	0.2	50	0.2	24	0.2	35	0.8	108	1.1	153
<b><i>Cyanobacteria</i></b>	-	-	1	11	-	-	1	1	0	24	0	28	0	48	0	36	0	68	0	46
<b><i>Deferribacteres</i></b>	-	-	14.8	105	-	-	3.7	3	12.5	96	5.9	118	8.5	115	10.6	262	10.7	151	9.1	411
<b><i>Firmicutes</i></b>																				
<i>Bacilli</i>	-	-	0.1	2	-	-	1.2	1	0.1	1	< 0.1	7	< 0.1	3	< 0.1	3	< 0.1	9	< 0.1	6
<i>Clostridia</i>	-	-	0.1	3	-	-	0	0	< 0.1	0	0.1	9	0.3	28	0.3	39	0.1	16	0.3	48
<i>Erysipelotrichi</i>	-	-	0	0	-	-	0	0	0	0	< 0.1	1	< 0.1	4	0.1	6	< 0.1	2	< 0.1	1
<i>Firmicutes</i> _others	-	-	0	0	-	-	0	0	0	0	< 0.1	1	0.3	8	0.3	9	< 0.1	3	< 0.1	1
<b><i>Gemmatimonadetes</i></b>	-	-	1.6	8	-	-	0	0	1.9	16	0.4	9	1.2	18	0.8	30	2.3	79	1.4	96
<b><i>Planctomycetes</i></b>																				
<i>Phycisphaerae</i>	-	-	0.8	17	-	-	0	0	1.3	26	0.3	28	0.7	28	0.7	51	2.2	184	2.4	297
<i>Planctomycetacia</i>	-	-	2.8	60	-	-	0	0	3.5	120	1.2	81	1.0	73	1.6	123	5.9	524	4.7	530
<i>Planctomycetes</i> _others	-	-	0.1	2	-	-	0	0	0.1	7	0	3	< 0.1	1	0.1	10	0.3	24	0.7	54
<b><i>Proteobacteria</i></b>																				
<b><i>Alphaproteobacteria</i></b>																				
SAR11	-	-	25.1	99	-	-	48.1	10	9.8	35	9.0	90	5.2	29	15.1	129	5.1	35	12.6	166
<i>Alphaproteobacteria</i> _others	-	-	7.4	115	-	-	9.9	6	4.0	110	2.9	154	3.1	139	4.6	226	5.1	282	6.6	531
<i>Betaproteobacteria</i>	-	-	0.3	6	-	-	1.2	1	0.1	4	0.1	10	0.1	5	0.1	4	0.2	9	0.6	16
<i>Deltaproteobacteria</i>	-	-	13.9	114	-	-	3.7	3	18.8	195	6.9	247	17.8	315	13.9	419	18.2	762	14.6	1032
<i>Epsilonproteobacteria</i>	-	-	< 0.1	1	-	-	0	0	0.9	23	0.3	32	30.1	68	15.1	175	0.9	37	0.6	60
<b><i>Gammaproteobacteria</i></b>																				
<i>Colwellia</i>	-	-	0.1	3	-	-	0	0	< 0.1	4	0.1	9	0.1	4	0.5	15	< 0.1	10	0.2	11
<i>Oceaniserpentilla</i>	-	-	< 0.1	1	-	-	0	0	0	0	< 0.1	1	0.5	2	1.0	12	< 0.1	2	< 0.1	2
<i>Cycloclasticus</i>	-	-	< 0.1	2	-	-	0	0	0.1	4	< 0.1	1	0.9	6	1.1	10	0.1	3	0.3	12
<i>Gammaproteobacteria</i> _others	-	-	18.7	138	-	-	22.2	10	32.0	202	65.5	326	13.1	249	18.3	484	25.0	709	22.9	1175
<i>Proteobacteria</i> _others	-	-	0	0	-	-	0	0	0	0	0	1	< 0.1	2	< 0.1	2	< 0.1	3	< 0.1	8
<b><i>Verrucomicrobia</i></b>																				
<i>Verrucomicrobiae</i>	-	-	0.7	9	-	-	0	0	0.4	16	0.2	11	0.1	8	0.3	18	0.7	72	0.7	70
<i>Verrucomicrobia</i> _others	-	-	2.1	16	-	-	1.2	1	0.8	25	1.0	28	0.3	21	1.4	40	0.6	42	1.5	74
<b><i>Bacteria</i>_others</b>	-	-	0.6	33	-	-	0.0	1	0.8	86	0.4	116	4.5	273	4.5	376	5.7	712	6.1	975

\*Phyla breakdown to class if read abundance of class ≤1%; Groups selected for oligotyping (*Colwellia*, *Oceaniserpentilla*, *Cycloclasticus*, and SAR11) are presented individually.

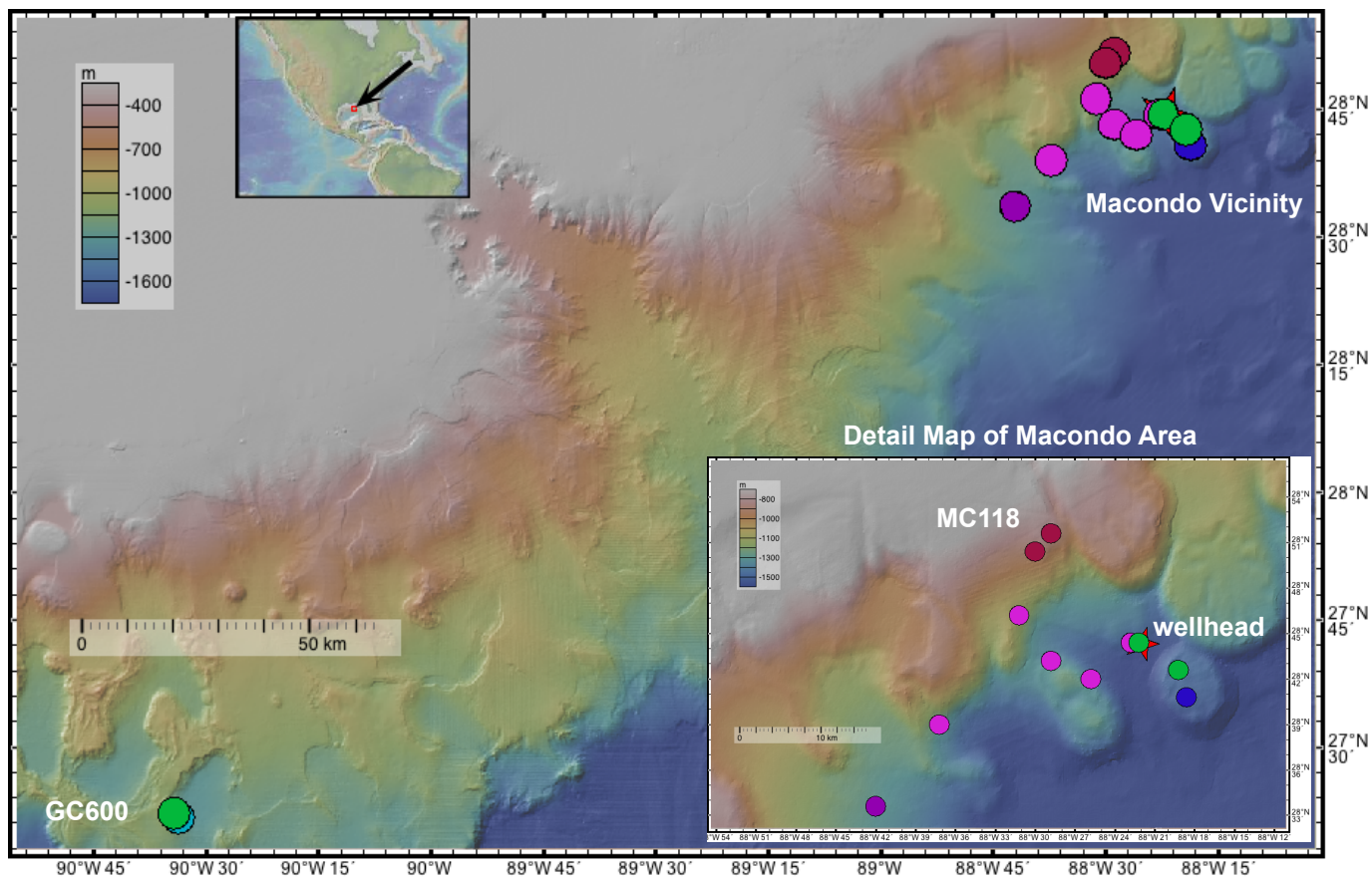
Supplementary Table S4: Diversity indexes of non-rarefied, rarefied and mean rarefied (average of 100 calculations) OTU tables.

Sample ID	Non-rarefied							Rarefied							Rarefied (mean of 100 calculations)						
	Single-tons (S)	Individuals (N)	Good's coverage (C)	Shannon's Index (H)	Richness (R)	Evenness (E)	Inverse Simpson (1/D)	Single-tons (S)	Individuals (N)	Good's coverage (C)	Shannon's Index (H)	Richness (R)	Evenness (E)	Inverse Simpson (1/D)	Single-tons (S)	Individuals (N)	Good's coverage (C)	Shannon's Index (H)	Richness (R)	Evenness (E)	Inverse Simpson (1/D)
1-PRE-MC118	117	23473	1.00	5.07	811	0.76	52.81	119	21701	0.99	5.07	802	0.76	52.79	117	21701	0.99	5.07	811	0.76	52.79
2-PRE-MC118	226	21701	0.99	5.01	980	0.73	45.97	226	21701	0.99	5.01	980	0.73	45.97	226	21701	0.99	5.01	980	0.73	45.97
3-DUR-MC250	106	22831	1.00	4.86	808	0.73	40.94	112	21701	0.99	4.86	807	0.73	40.89	106	21701	1.00	4.86	808	0.73	40.93
4-DUR-MC250	492	33699	0.99	1.73	749	0.26	2.32	371	21701	0.98	1.71	551	0.27	2.31	492	21701	0.98	1.72	749	0.26	2.32
5-DUR-MC250	872	34905	0.98	2.88	1428	0.40	5.39	674	21701	0.97	2.87	1058	0.41	5.42	872	21701	0.96	2.88	1428	0.40	5.39
6-DUR-MC335	75	8217	0.99	2.07	777	0.39	21.40	75	21701	0.99	2.07	199	0.39	3.47	148	21701	0.99	4.46	777	0.67	21.37
7-DUR-MC295	115	55094	1.00	0.78	347	0.13	1.30	116	21701	0.99	0.75	244	0.14	1.29	176	21701	0.99	0.78	347	0.13	1.30
8-DUR-MC295	123	37348	1.00	0.59	329	0.10	1.17	115	21701	0.99	0.58	268	0.10	1.16	124	21701	0.99	0.59	329	0.10	1.16
9-DUR-MC252	357	36253	0.99	1.10	548	0.17	1.67	270	21701	0.99	1.09	386	0.18	1.67	358	21701	0.98	1.10	548	0.17	1.67
10-DUR-MC252	215	29236	0.99	4.77	1090	0.68	34.29	232	21701	0.99	4.77	1012	0.69	34.36	215	21701	0.99	4.77	1090	0.68	34.30
11-DUR-MC252	240	26953	0.99	4.24	986	0.62	12.77	263	21701	0.99	4.24	943	0.62	12.75	240	21701	0.99	4.24	986	0.62	12.77
12-DUR-MC252	501	44754	0.99	4.23	1405	0.58	9.99	448	21701	0.98	4.21	1065	0.60	9.96	640	21701	0.97	4.23	1405	0.58	9.98
13-DUR-MC205	531	34838	0.98	4.43	1391	0.61	20.15	492	21701	0.98	4.42	1158	0.63	19.91	531	21701	0.98	4.43	1391	0.61	20.16
14-POST-MC422	423	37265	0.99	3.71	973	0.54	12.05	363	21701	0.98	3.70	764	0.56	11.97	426	21701	0.98	3.71	973	0.54	12.04
15-POST-MC298	148	29530	0.99	4.46	199	0.67	3.47	160	8217	0.99	4.45	731	0.68	21.24	75	8217	0.99	2.07	199	0.39	3.47
16-POST-MC297	137	32386	1.00	4.80	917	0.70	33.12	172	21701	0.99	4.79	858	0.71	32.93	137	21701	0.99	4.80	917	0.70	33.18
17-POST-GC600	29	84	0.65	3.29	42	0.88	16.18	29	84	0.65	3.29	42	0.88	16.18	29	84	0.65	3.29	42	0.88	16.18
18-POST-GC600	763	51024	0.99	3.60	1640	0.49	6.87	581	21701	0.97	3.57	1092	0.51	6.83	1044	21701	0.95	3.60	1640	0.49	6.86
19-POST-MC252	1759	29666	0.94	5.60	2997	0.70	58.89	1506	21701	0.93	5.57	2511	0.71	59.03	1759	21701	0.92	5.60	2997	0.70	58.94
20-POST-MC253	4600	30700	0.85	6.74	6835	0.76	93.88	3685	21701	0.83	6.66	5375	0.77	92.45	4600	21701	0.79	6.74	6835	0.76	93.94



**Supplementary Table S5. Significant ( $\alpha < 0.05$ ) and Storey's FDR-controlled ( $q < 0.05$ ) Spearman rank correlations between untransformed environmental parameters and oligotype abundances.**

Oligotype	Methane	Methane Oxidation rate	Cell counts	DOC	NOx	CDOM
Oceaniserpentilla_17	0.74	-	0.77	-	-	-
Oceaniserpentilla_20	-	-	-	0.73	-	-
Cycloclasticus_01	-	0.71	-	-	-	-
Cycloclasticus_04	0.73	-	-	-	-	-
Cycloclasticus_06	0.72	0.74	-	-	-	-
Cycloclasticus_30	0.78	0.76	0.79	-	-	-
Colwellia_01	0.74	0.71	-	-	-	-
Colwellia_02	0.71	-	-	-	-	-
Colwellia_03	0.73	-	0.74	-	-	-
Colwellia_04	0.78	0.75	-	-	-	-
Colwellia_06	0.77	-	-	-	-	-
Colwellia_08	-	-	0.76	-	-	-
Colwellia_09	0.81	0.75	-	-	-	-
SAR11_04	-	-	-0.75	-	-	-
SAR11_06	-	-	-	-	-	-0.85
SAR11_16	-	-	-	-	-	-0.86
SAR11_30	-	-	-0.75	-	0.73	-
SAR11_37	-	-0.70	-	-	-	-
SAR11_42	-0.75	-0.71	-0.81	-	-	-
SAR11_43	-	-0.71	-	-	-	-
SAR11_48	-	-	-0.76	-	0.71	-
SAR11_51	-	-	-	-	0.72	-
SAR11_53	-	-	-0.79	-	0.79	-
SAR11_59	-	-	-0.75	-	-	-
SAR11_66	-0.72	-0.72	-0.78	-	0.76	-
SAR11_67	-	-	-	-	-	-0.92
SAR11_68	-	-	-	-	0.84	-
SAR11_69	-	-	-	-	0.75	-
SAR11_73	-	-	-	-	0.77	-
SAR11_83	-	-	-0.75	-	-	-
SAR11_88	-	-	-0.75	-	-	-
SAR11_90	-0.71	-0.71	-	-	-	-
SAR11_95	-	-	-0.78	-	-	-
SAR11_96	-	-	-	-	0.71	-



Kleindienst et al. Supplementary Figure S1

**A** *Oceaniserpentilla*

Samples	Pre-DC		During discharge																Post-discharge			
	1-PRE-MC118	2-PRE-MC118	3-DUR-MC250	4-DUR-MC250	5-DUR-MC250	6-DUR-MC335	7-DUR-MC295	8-DUR-MC295	9-DUR-MC252	10-DUR-MC252	11-DUR-MC252	12-DUR-MC252	13-DUR-MC205	14-POST-MC422	15-POST-MC298	16-POST-MC297	17-POST-GC600	18-POST-GC600	19-POST-MC252	20-POST-MC253		
Pre-DC	1-PRE-MC118	0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
	2-PRE-MC118	0.0	0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	NA	NA	NA	1.0	1.0	1.0	
During discharge	3-DUR-MC250	1.0	1.0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	NA	NA	NA	1.0	1.0	0.0
	4-DUR-MC250	1.0	1.0	0.0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	NA	NA	NA	0.2	1.0	0.0
	5-DUR-MC250	1.0	1.0	0.0	0	0	0	0	0	0	0	0	0	0	0	0.1	NA	NA	NA	0.2	1.0	0.0
	6-DUR-MC335	1.0	1.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0	0.2	NA	NA	NA	0.2	1.0	0.0
	7-DUR-MC295	1.0	1.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0	0.2	NA	NA	NA	0.2	1.0	0.0	
	8-DUR-MC295	1.0	1.0	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0.2	NA	NA	NA	0.2	1.0	0.0	
	9-DUR-MC252	1.0	1.0	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0.2	NA	NA	NA	0.2	1.0	0.0	
	10-DUR-MC252	1.0	1.0	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0.1	NA	NA	NA	0.2	1.0	0.0	
	11-DUR-MC252	1.0	1.0	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0.2	NA	NA	NA	0.2	1.0	0.0	
	12-DUR-MC252	1.0	1.0	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0.2	NA	NA	NA	0.2	1.0	0.0	
	13-DUR-MC205	1.0	1.0	0.0	0.0	0.0	0.0	0	0	0	0	0	0	0	0.2	NA	NA	NA	0.2	1.0	0.0	
Post-discharge	14-POST-MC422	1.0	1.0	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0	NA	NA	NA	0.1	0.5	0.2	
	15-POST-MC298	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	NA	
	16-POST-MC297	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	NA	
	17-POST-GC600	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	NA	
	18-POST-GC600	1.0	1.0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	NA	NA	NA	0	0.6	0.3	
	19-POST-MC252	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.5	NA	NA	NA	NA	0.6	0	1.0	
	20-POST-MC253	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	NA	NA	NA	0.3	1.0	0	0	

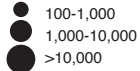
**B** *Cycloclasticus*

Samples	Pre-DC		During discharge																Post-discharge			
	1-PRE-MC118	2-PRE-MC118	3-DUR-MC250	4-DUR-MC250	5-DUR-MC250	6-DUR-MC335	7-DUR-MC295	8-DUR-MC295	9-DUR-MC252	10-DUR-MC252	11-DUR-MC252	12-DUR-MC252	13-DUR-MC205	14-POST-MC422	15-POST-MC298	16-POST-MC297	17-POST-GC600	18-POST-GC600	19-POST-MC252	20-POST-MC253		
Pre-DC	1-PRE-MC118	0	0.0	0.0	0.0	0.5	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.1	1.0	1.0	1.0	NA	0.9	0.5	1.0	
	2-PRE-MC118	0.0	0	0	0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.9	0.9	NA	1.0	0.4	1.0	
During discharge	3-DUR-MC250	0.0	0.0	0	0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	NA	1.0	0.4	1.0	
	4-DUR-MC250	0.0	0.0	0	0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	NA	1.0	0.4	1.0	
	5-DUR-MC250	0.5	0.4	0.4	0.4	0	0.4	0.4	0.3	0.4	0.3	0.4	0.3	0.5	0.6	0.6	NA	1.0	0.5	1.0	0	
	6-DUR-MC335	0.0	0.0	0.0	0.4	0	0	0	0	0	0	0	0	0	1.0	1.0	1.0	NA	1.0	0.3	1.0	
	7-DUR-MC295	0.0	0.0	0.0	0.0	0.4	0	0	0	0	0	0	0	0	1.0	1.0	1.0	NA	1.0	0.3	1.0	
	8-DUR-MC295	0.1	0.0	0.0	0.0	0.3	0.0	0.0	0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	NA	1.0	0.3	1.0	0	
	9-DUR-MC252	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	NA	1.0	0.4	1.0	0	
	10-DUR-MC252	0.1	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0	0.0	0.0	0.0	1.0	1.0	1.0	NA	1.0	0.3	1.0	0	
	11-DUR-MC252	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0	0.0	0.0	0	1.0	1.0	1.0	NA	1.0	0.4	1.0	0	
	12-DUR-MC252	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0	0	0	1.0	1.0	1.0	NA	1.0	0.4	1.0	0	
	13-DUR-MC205	0.1	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0	0	1.0	1.0	1.0	NA	1.0	0.3	1.0	0	
Post-discharge	14-POST-MC422	1.0	0.9	1.0	1.0	0.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0	0	0.2	NA	1.0	1.0	1.0	0	
	15-POST-MC298	1.0	0.9	1.0	1.0	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0	0.3	NA	1.0	1.0	1.0	1.0	0	
	16-POST-MC297	1.0	0.9	1.0	1.0	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.2	0.3	0	NA	1.0	1.0	1.0	0	
	17-POST-GC600	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	
	18-POST-GC600	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	NA	0	1.0	1.0	0	
	19-POST-MC252	0.5	0.4	0.4	0.4	0.5	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	NA	1.0	1.0	1.0	0	
	20-POST-MC253	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	NA	1.0	1.0	1.0	0	

**C** *Colwellia*

Samples	Pre-DC		During discharge																Post-discharge			
	1-PRE-MC118	2-PRE-MC118	3-DUR-MC250	4-DUR-MC250	5-DUR-MC250	6-DUR-MC335	7-DUR-MC295	8-DUR-MC295	9-DUR-MC252	10-DUR-MC252	11-DUR-MC252	12-DUR-MC252	13-DUR-MC205	14-POST-MC422	15-POST-MC298	16-POST-MC297	17-POST-GC600	18-POST-GC600	19-POST-MC252	20-POST-MC253		
Pre-DC	1-PRE-MC118	0	0.0	0.8	0.9	0.6	0.9	0.8	1.0	0.7	0.8	1.0	0.9	0.9	0.6	1.0	1.0	NA	0.6	1.0	0.7	
	2-PRE-MC118	0.0	0	0.8	0.9	0.6	0.9	0.8	1.0	0.7	0.8	1.0	0.9	0.9	0.6	1.0	1.0	NA	0.6	1.0	0.7	
During discharge	3-DUR-MC250	0.8	0.8	0	0.3	0.2	0.1	0.2	0.4	0.4	0.3	0.4	0.3	0.1	0.2	0.9	NA	0.6	0.5	0.3	0	
	4-DUR-MC250	0.9	0.9	0.3	0	0.6	0.4	0.1	0.3	0.5	0.1	0.0	0.1	0.3	0.3	1.0	NA	0.7	0.2	0.3	0	
	5-DUR-MC250	0.6	0.6	0.2	0.6	0	0.2	0.4	0.6	0.3	0.6	0.8	0.6	0.2	0.2	1.0	NA	0.5	0.8	0.4	0	
	6-DUR-MC335	0.9	0.9	0.1	0.4	0.2	0	0.3	0.6	0.5	0.4	0.5	0.4	0.2	0.3	1.0	NA	0.6	0.5	0.4	0	
	7-DUR-MC295	0.8	0.8	0.2	0.1	0.4	0.3	0	0.2	0.2	0.2	0.1	0.1	0.1	1.0	1.0	NA	0.6	0.4	0.2	0	
	8-DUR-MC295	1.0	1.0	0.4	0.3	0.6	0.6	0.2	0	0.1	0.3	0.3	0.2	0.2	0.4	1.0	NA	0.8	0.5	0.5	0	
	9-DUR-MC252	0.7	0.7	0.4	0.5	0.3	0.5	0.2	0.1	0	0.5	0.6	0.4	0.1	0.3	1.0	NA	0.7	0.8	0.5	0	
	10-DUR-MC252	0.8	0.8	0.3	0.1	0.6	0.4	0.2	0.3	0.5	0	0.1	0.0	0.3	0.2	1.0	NA	0.6	0.1	0.1	0	
	11-DUR-MC252	1.0	1.0	0.4	0.0	0.8	0.5	0.2	0.3	0.6	0.1	0	0.1	0.4	0.4	1.0	NA	0.7	0.1	0.3	0	
	12-DUR-MC252	0.9	0.9	0.3	0.1	0.6	0.4	0.1	0.2	0.4	0.0	0.1	0	0.3	0.2	1.0	NA	0.6	0.1	0.2	0	
	13-DUR-MC205	0.9	0.9	0.1	0.3	0.2	0.2	0.1	0.2	0.1	0.3	0.4	0.3	0	0.2	1.0	NA	0.6	0.6	0.4	0	
Post-discharge	14-POST-MC422	0.6	0.6	0.2	0.3	0.2	0.3	0.1	0.4	0.3	0.2	0.4	0.2	0.2	0	1.0	NA	0.4	0.4	0.1	0	
	15-POST-MC298	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.3	NA	0	NA	0.7	1.0	1.0	0	
	16-POST-MC297	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.3	0	NA	0.7	1.0	1.0	0	0	
	17-POST-GC600	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	
	18-POST-GC600	0.6	0.6	0.6	0.7	0.5	0.6	0.6	0.8	0.7	0.6	0.7	0.6	0.6	0.4	0.7	NA	0	0.7			

**Oligotype sequence #**

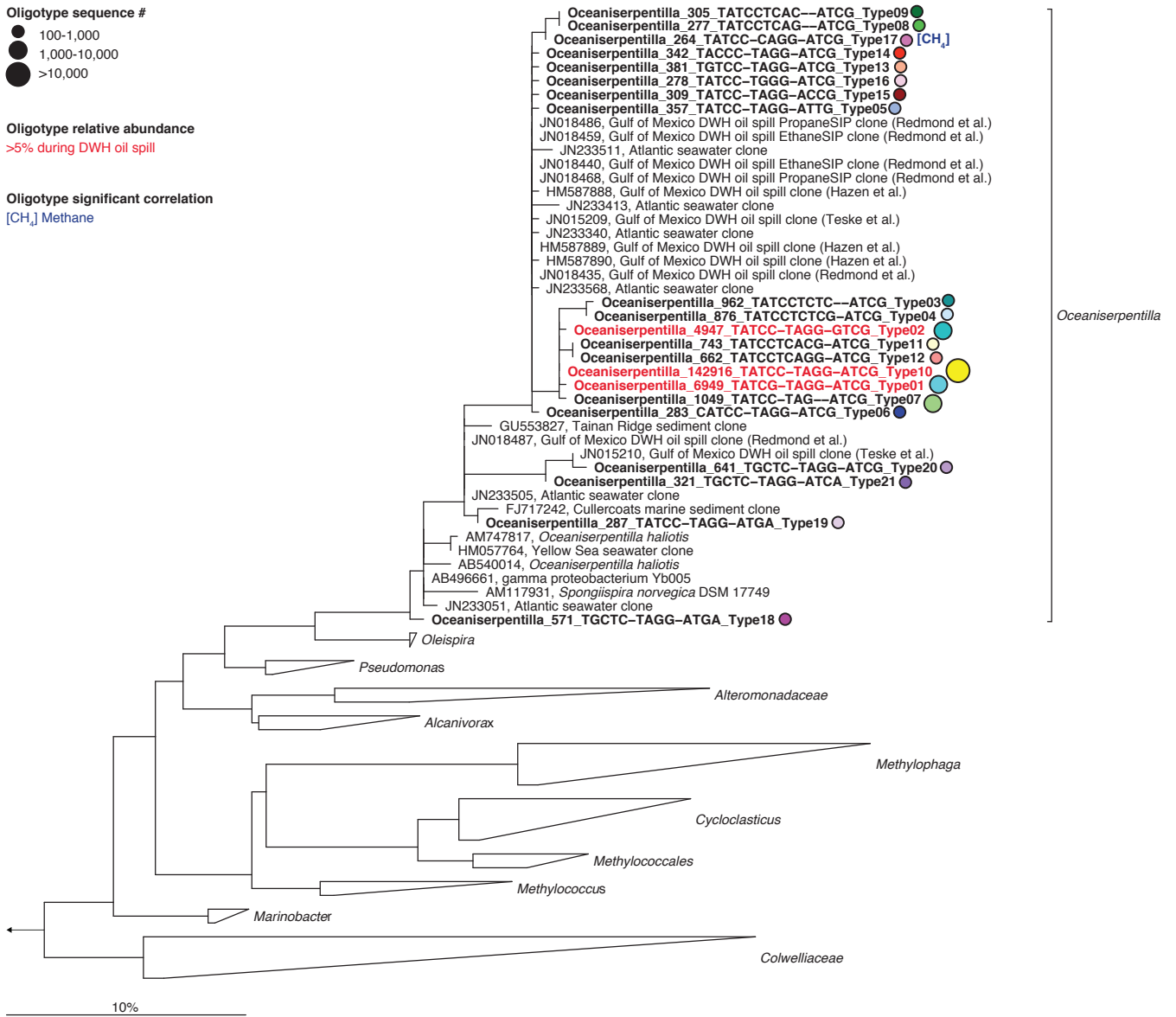


**Oligotype relative abundance**

>5% during DWH oil spill

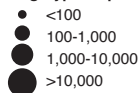
**Oligotype significant correlation**

[CH<sub>4</sub>] Methane



**Kleindienst et al. Supplementary Figure S3**

Oligotype sequence #



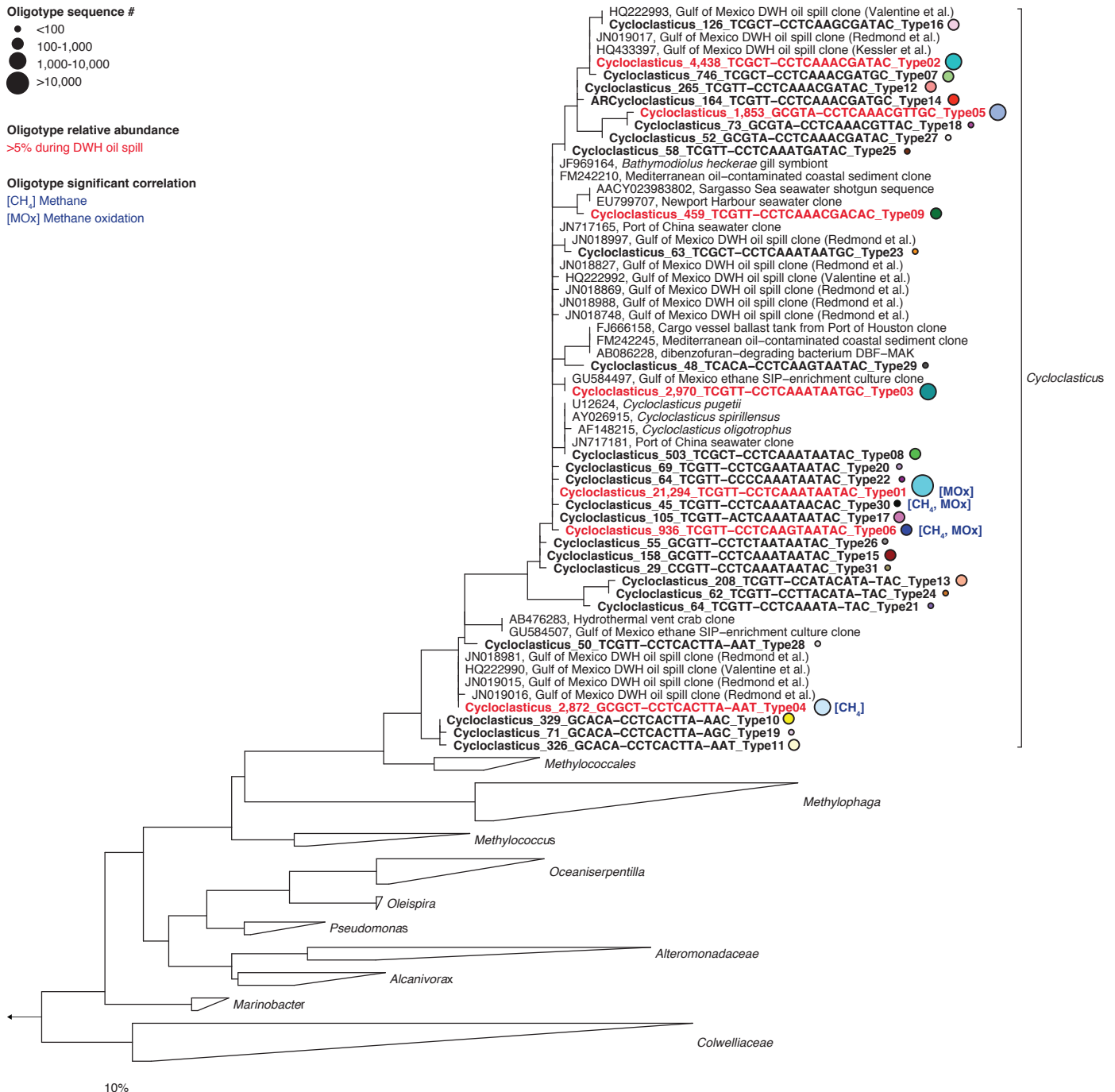
Oligotype relative abundance

>5% during DWH oil spill

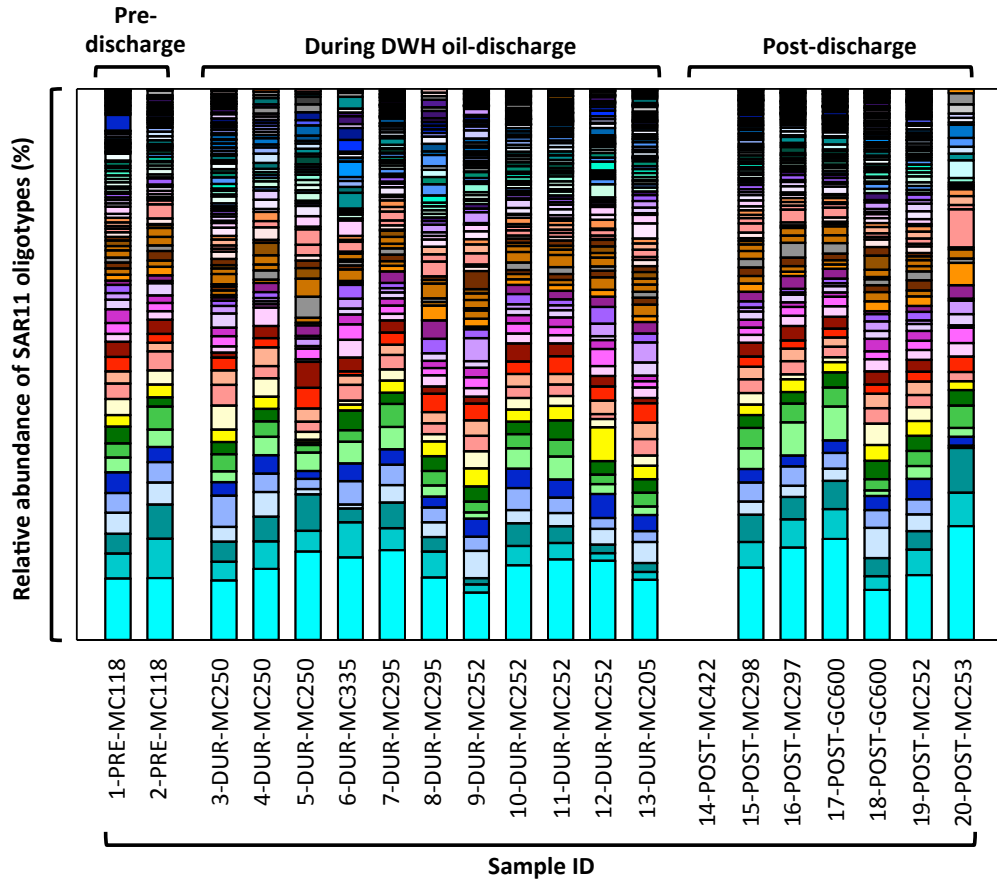
Oligotype significant correlation

[CH<sub>4</sub>] Methane

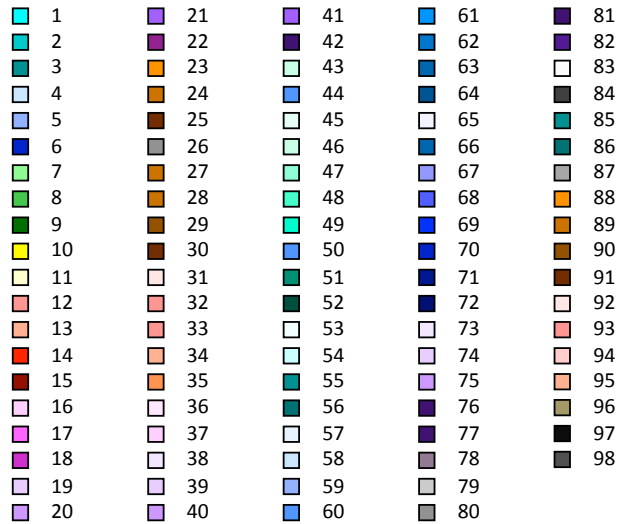
[MOx] Methane oxidation



Kleindienst et al. Supplementary Figure S4



**SAR11 oligotype #**



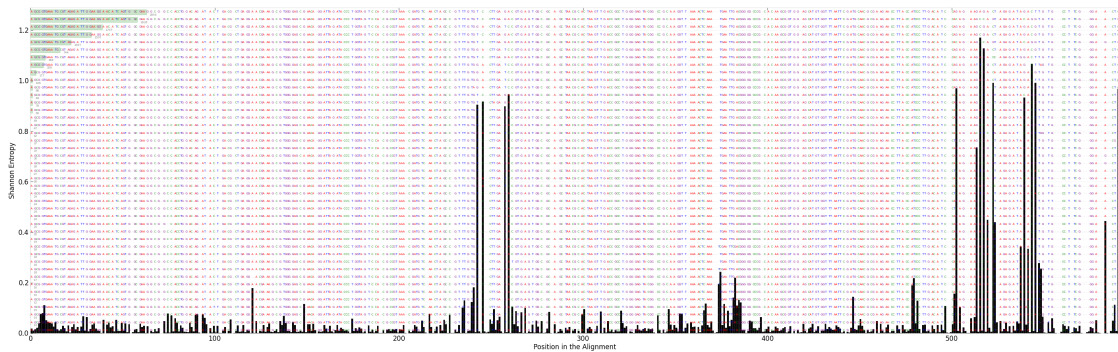
## **Supplementary Material - Example oligotyping procedure of *Colwellia* sp. sequences.**

Oligotyping (1) uses information entropy in aligned nucleotide sequences in order to partition closely-related (>97% similarity) sequences into more meaningful biological and/or ecological groups, or oligotypes. Here we demonstrate the oligotyping procedure on sequences that had been assigned to the genus *Colwellia* (Gammaproteobacteria). The full pipeline and python scripts for oligotyping are available for download at <http://oligotyping.org>.

**Step 1. Sequence pre-processing.** We used 454 pyrosequencing of the bacterial 16S rRNA V4-V6 regions (2, 3) to profile our samples. At the time of the experiment, the GS FLX Titanium chemistry involved unidirectional sequencing from the end of the V6 region into the V4 region. As sequencing progresses along each molecule, the per-base quality decays, and noise and sequencing error rate increases. In order to minimize sequencing error artifacts in oligotyping, we opted to trim the end of the read (in the V4 region) to a known nucleotide sequence before substantial quality decay, in a process known as anchor trimming. This procedure significantly reduces artificially introduced noise. We anchor trimmed all sequences from our datasets that underwent the oligotyping procedure to minimize such sequencing errors.

**Step 2. Sequence alignment and entropy decomposition.** The next steps in the procedure are alignment and entropy decomposition. We extracted sequences that classified as *Colwellia* from the Greengenes database, which had been aligned along the full 16S rRNA region. These fifty-two sequences were used as a template, against which we aligned the 26,217 anchor-trimmed *Colwellia* sequences from our datasets. After

alignment, we trimmed off stray ends so that all sequences under consideration started and ended at the same position. Next, we used the oligotyping script entropy-analysis to calculate per-base Shannon entropy values of the aligned sequences. The script generates a bar chart of entropy along the sequences, as well as a text file of entropy values per base (Fig. 1).

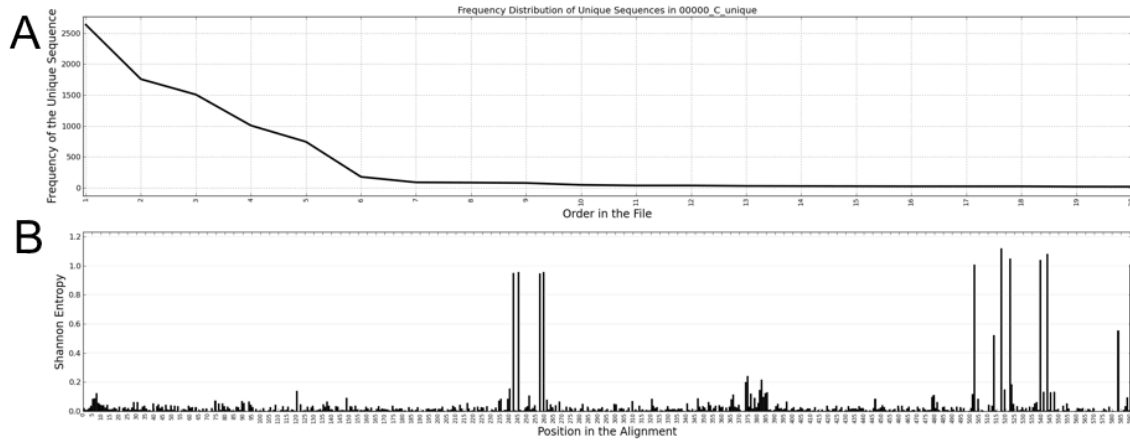


**Fig. 1.** Entropy values of each base of the aligned, anchor-trimmed sequences. From left to right, the reads proceed into the end of the V6 region.

**Step 3. Oligotype calculation.** At this stage, the script oligotype takes the entropy values, the aligned sequences, and either the highest entropy position(s) (by default, the first highest position) or user-provided entropy positions (as many as provided), along with other optional variables, and disaggregates the sequences into oligotypes based on the provided position(s). For *Colwellia*, the highest entropy position at 515 separated sequences into five oligotypes, naming them by their nucleotide or gap position. These four oligotypes were “C”, “A”, “T”, “G”, and “-”. Sequences within each oligotype were uniqued, and entropy values were calculated for these sequences to determine if this step of oligotyping had sufficiently reduced entropy within oligotypes, or if oligotyping with additional positions would improve entropy reduction. If the majority of sequences within

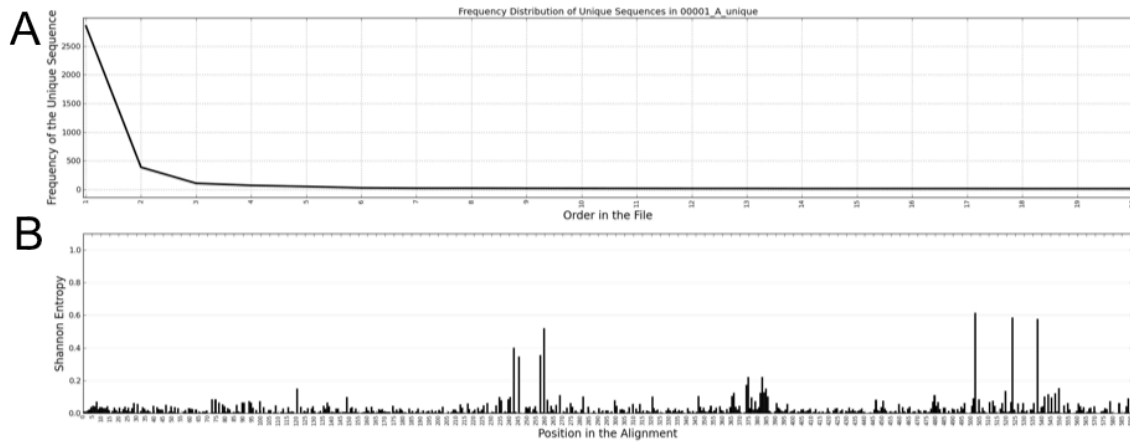


each oligotype do not fall into the most abundant unique bin, then additional oligotyping is recommended using selected positions that describe where sequences disagree (Fig. 2).



**Fig. 2.** Abundance distribution of sequences in the “C” oligotype of *Colwellia* sequences (A) and entropy values at each position (B). In A, unique sequences are ranked in abundance within each oligotype, and their ranks are plotted on the horizontal axis. The frequencies of each unique sequence are plotted on the vertical axis. In B, the entropy values for the sequences in the “C” oligotype are shown.

As seen in Fig. 2, the most abundant unique sequence in the “C” oligotype had over 2500 representatives, and the next most abundant unique sequence had over 1500 representatives. Careful evaluation of these two unique bins suggested that including other positions in the oligotyping step would separate these bins into different oligotypes. Looking at the other oligotypes and their unique sequences, we observed that their entropy decomposition was more saturated (Fig. 3). Unlike for “C”, including additional entropy positions from these oligotypes would not refine these unique sequence bins.



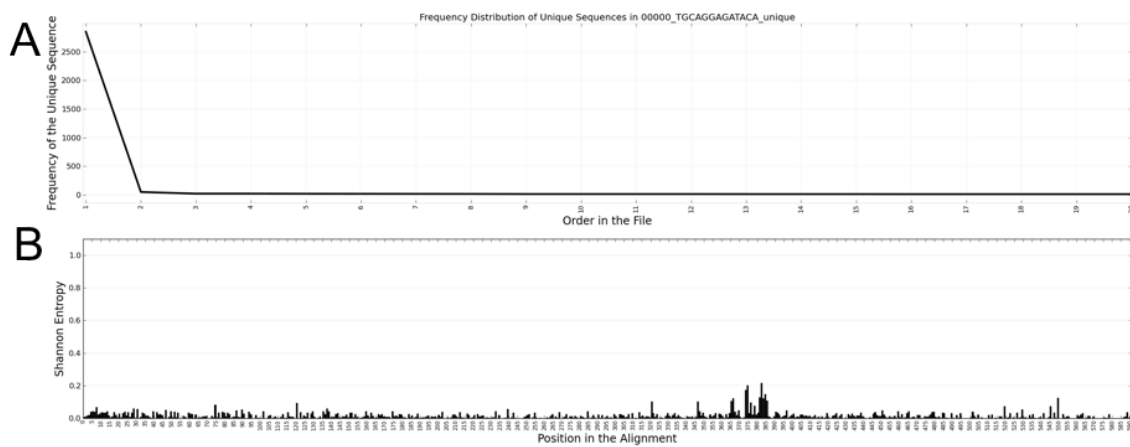
**Fig. 3.** Abundance distribution of sequences in the “A” oligotype of *Colwellia* sequences (A) and entropy values at each position (B). Profiles are similar for “G”, “T”, and “-“ oligotypes.

Oligotyping with the first entropy position 515 and an additional position determined from examining the “C” oligotype bin, 543, generated 19 oligotypes. Much like with the first round of oligotyping, further scrutiny of unique sequences and entropy within each oligotype indicated the selection of additional positions for oligotyping. Additionally, the distribution of oligotypes had a long tail of low-abundance oligotypes. Optional parameters that can be used in oligotyping include the elimination of: oligotypes that are not present across a set number of samples; oligotypes that fall below a minimum abundance proportion of all sequences; and oligotypes with unique sequence bins below a minimum abundance threshold. To refine the oligotyping procedure, we opted to retain an oligotype that: appeared in at least 10% of the samples analyzed; was at least 0.1% of all *Colwellia* sequences in at least one sample; and in which the most abundant unique sequence was at least 0.05% of all *Colwellia* sequences. There were 19 samples in which 26,217 *Colwellia* sequences were observed, and we determined 13 nucleotide positions in total that yielded informative oligotypes (Table 1).

**Table 1.** *Colwellia* oligotypes after procedure using 13 high-entropy positions.

Abundance	Oligotype name	Closest BLAST hit	% similarity (100 unless otherwise stated)
5,568	TGCAGGAGATACA	JX559223	
5,521	TGCAGGAGCTAGA	JN019019	
3,590	TGACGGAGCTACA	JN018895	
3,389	TGCAGGAGCTACA	JQ580281	
2,045	TGCAGGAGCTAGG	JN019001	
1,477	TGACGGAACTACA	JN019018	99.7
737	TGACGGATTTACA	JN860307	
716	TGCAGGAGACGCA	JN018476	
362	TGACGGAGCAACA	JN018895	99.7
253	TGACGGAGATACA	NR_074565	
208	TAACGGAGCTACA	JN018463	
125	TGCGGGAGACGCA	JN018476	99.7
105	TGACGGACTG-GA	JN018894	98.0
85	TGCAGGAGTTACA	JN233061	99.4
82	TGCAGGAGCTACG	GQ348314	99.7
62	TGACGGAGACGCA	JQ800146	
56	CGACGGAGCTAGA	JN018439	
43	TGCAGGAACTACA	HQ203927	
39	TGCAGGAGATATA	JX879002	
38	GCGCGGACTTATA	JQ862019	96.3
32	TTCAGGAGCATGA	HQ326394	
28	TGCAGGGGTTACA	JN233061	99.2
27	TGCAGGAATTATA	<u>AY172255</u>	99.2
24	TGACGGAGTTACA	EU491928	
20	TGCAGGATCTAGA	JN019019	98.9
19	TGCGGGAGCTAGA	JN019019	99.7

Entropy graphs and abundance curves within each oligotype suggested entropy had been sufficiently explained, and that the oligotyping procedure was saturated (Fig. 4). These oligotypes all have the same taxonomic assignment of *Colwellia* and their nucleotide sequences are more than 97% similar. However, they have unique closest matches in the NCBI nr database, or are at most 96.3% similar to existing sequences in the database.



**Fig. 4.** Abundance distribution of sequences in the “TGCAGGAGATACA” oligotype of *Colwellia* sequences (A) and entropy values at each position (B). Profiles are similar for the other oligotypes.

## References

1. Eren AM, *et al.* (2013) Oligotyping: differentiating between closely related microbial taxa using 16S rRNA gene data. *Methods Ecol. Evol.* 4(12):1111–1119.
2. Sogin ML, *et al.* (2006) Microbial diversity in the deep sea and the underexplored “rare biosphere”. *Proc. Natl. Acad. Sci. USA* 103(32):12115-12120.
3. Huber JA, *et al.* (2007) Microbial Population Structures in the Deep Marine Biosphere. pp 97-100.