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Unexpected scarcity of ANME archaea in hydrocarbon seeps within Monterey Bay

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Table S1: Monterey Bay sediment core information.

Site Name	Core Type	Dive Number	Core Number	Latitude; Longitude	Water Depth (m)	Sediment Horizons Sampled (cmbsf)	Substrate Type
Clam Field	Seep	DR1139	PC 75	36.7356; -122.0342	895	0-2.5, 2.5-5, 5-7.5, 7.5-10, 10-15, 15+	Patchy microbial mat
Clam Field	Seep-Edge	DR1139	PC 71	36.7356; -122.0342	895	0-2.5, 2.5-5, 5-7.5, 7.5-10, 10-15, 15+	Patchy microbial mat
Clam Field	Bkgd-5m	DR1139	PC 56	36.7356; -122.0342	895	0-2.5, 2.5-5, 5-7.5, 7.5-10, 10-15, 15+	Sand
Clam Field	Bkgd-100m	DR1139	PC 66	36.7365; -122.0342	908	0-2.5, 2.5-5, 5-7.5, 7.5-10, 10-15, 15+	Sand
Extrovert Cliff	Seep	DR1140	PC 64	36.7765; -122.0850	965	0-2.5, 2.5-5, 5-7.5, 7.5-10, 10-15	Thick microbial mat
Extrovert Cliff	Seep-Edge	DR1140	PC 44	36.7765; -122.0850	965	0-2.5, 2.5-5, 5-7.5, 7.5-10	Thick microbial mat
Extrovert Cliff	Bkgd-5m	DR1140	PC 79	36.7765; -122.0851	965	0-2.5, 2.5-5, 5-7.5, 7.5-10, 10-15, 15+	Sand
Extrovert Cliff	Bkgd-100m	DR1140	PC 63	36.7759; -122.0844	990	0-2.5, 2.5-5, 5-7.5, 7.5-10, 10-15, 15+	Sand

Table S2: Incubation scheme for Clam Field sediments. Five treatments with varying concentrations of methane (with a 20% 13C label) were performed in triplicate on sediment from each horizon.

	AMENDMENTS			
Desired Methane Partial Pressure (atm)	$^{13}CH_4$ Volume in Headspace (mL)	$^{12}CH_4$ Volume in Headspace (mL)	Argon Volume in Headspace (mL)	$^{15}NH_4^+$ Concentration in Slurry (μM)
0	0	0	80	100
0.25	2	8	70	100
0.5	4	16	60	100
1	8	32	40	100
2	16	64	0	100

Table S3: Gene targeting regions of primers used in this study.

Primer Name	Target Gene	Sequence (5' - 3')	Reference
515F-Y	16S rRNA	GTGYCAGCMGCCGCGGTAA	Parada et al. 2016
926R	16S rRNA	CCGYCAATTYMTTTRAGTTT	Parada et al. 2016
mcrA_F	*mcrA*	GGTGGTGTMGGATTCACACA R	shortened from Luton et al. 2002 as in Dekas et al. 2016
mcrA_R	*mcrA*	TTCATTGCRTAGTTWGGRTAG	shortened from Luton et al. 2002 as in Dekas et al. 2016

Table S4: Amplification of 16S rRNA and mcrA genes and transcripts in Monterey Bay samples with primer sets 515F-Y / 926R (Parada et al., 2016) and mcrA_F / mcrA_R (shortened from Luton et al. (2002) as in Dekas et al. (2016). Grey filled boxes indicate that amplification was observed in that extract with the given primer set.

	515F-Y / 926R		mcrA_F / mcrA_R	
Sample ID	**DNA**	**cDNA**	**DNA**	**cDNA**
CF_Seep_C75_0-2.5cm				
CF_Seep_C75_2.5-5cm				
CF_Seep_C75_5-7.5cm				
CF_Seep_C75_7.5-10cm				
CF_Seep_C75_10-15cm				
CF_Seep_C75_15+cm				
CF_Seep-Edge_C71_0-2.5cm				
CF_Seep-Edge_C71_2.5-5cm				
CF_Seep-Edge_C71_5-7.5cm				
CF_Seep-Edge_C71_7.5-10cm				
CF_Seep-Edge_C71_10-15cm				
CF_Seep-Edge_C71_15+cm				
CF_Bkgd-5m_C56_0-2.5cm				
CF_Bkgd-5m_C56_2.5-5cm				
CF_Bkgd-5m_C56_5-7.5cm				
CF_Bkgd-5m_C56_7.5-10cm				
CF_Bkgd-5m_C56_10-15cm				
CF_Bkgd-5m_C56_15+cm				
CF_Bkgd-100m_C66_0-2.5cm				
CF_Bkgd-100m_C66_2.5-5cm				
CF_Bkgd-100m_C66_5-7.5cm				
CF_Bkgd-100m_C66_7.5-10cm				

CF_Bkgd-100m_C66_10-15cm				
CF_Bkgd-100m_C66_15+cm				
EC_Seep_C64_0-2.5cm				
EC_Seep_C64_2.5-5cm				
EC_Seep_C64_5-7.5cm				
EC_Seep_C64_7.5-10cm				
EC_Seep_C64_10-15cm				
EC_Seep-Edge_C44_0-2.5cm				
EC_Seep-Edge_C44_2.5-5cm				
EC_Seep-Edge_C44_5-7.5cm				
EC_Seep-Edge_C44_7.5-10cm				
EC_Bkgd-5m_C79_0-2.5cm				
EC_Bkgd-5m_C79_2.5-5cm				
EC_Bkgd-5m_C79_5-7.5cm				
EC_Bkgd-5m_C79_7.5-10cm				
EC_Bkgd-5m_C79_10-15cm				
EC_Bkgd-5m_C79_15+cm				
EC_Bkgd-100m_C63_0-2.5cm				
EC_Bkgd-100m_C63_2.5-5cm				
EC_Bkgd-100m_C63_5-7.5cm				
EC_Bkgd-100m_C63_7.5-10cm				
EC_Bkgd-100m_C63_10-15cm				
EC_Bkgd-100m_C63_15+cm				

Table S5: Estimated ANME *mcrA* gene copy numbers, based on total *mcrA* gene copy numbers and ANME relative abundance in *mcrA* sequencing data.

Site Name	Core Type	Sediment Depth (cmbsf)	ANME *mcrA* Relative Abundance (%)	*mcrA* Copies (g^{-1} Dry Sediment)	ANME *mcrA* Copies (g^{-1} Dry Sediment)
Clam Field	Seep	0-2.5	0.54	4.15E+06	2.25E+04
Clam Field	Seep	2.5-5	0.56	3.46E+06	1.94E+04
Clam Field	Seep	5-7.5	0.20	2.22E+05	4.50E+02
Clam Field	Seep	7.5-10	0.31	2.53E+06	7.87E+03
Clam Field	Seep	10-15	2.63	4.07E+05	1.07E+04
Clam Field	Seep	15+	20.23	4.07E+05	8.23E+04
Extrovert Cliff	Seep	0-2.5	74.16	2.10E+07	1.56E+07
Extrovert Cliff	Seep	2.5-5	87.64	5.32E+07	4.67E+07
Extrovert Cliff	Seep	5-7.5	95.10	2.73E+07	2.60E+07
Extrovert Cliff	Seep	7.5-10	94.10	1.50E+07	1.41E+07
Extrovert Cliff	Seep	10-15	54.45	1.50E+07	8.17E+06
Clam Field	Bkgd-5m	0-2.5	22.90	7.30E+05	1.67E+05
Clam Field	Bkgd-5m	2.5-5	16.00	3.96E+05	6.33E+04
Clam Field	Bkgd-5m	5-7.5	11.69	5.88E+05	6.87E+04
Clam Field	Bkgd-5m	7.5-10	9.63	5.80E+05	5.59E+04
Clam Field	Bkgd-5m	10-15	41.75	1.09E+06	4.57E+05
Clam Field	Bkgd-5m	15+	51.30	1.34E+06	6.87E+05
Extrovert Cliff	Bkgd-5m	0-2.5	8.99	6.28E+05	5.65E+04
Extrovert Cliff	Bkgd-5m	2.5-5	9.23	8.45E+05	7.80E+04
Extrovert Cliff	Bkgd-5m	5-7.5	7.14	6.43E+05	4.59E+04
Extrovert Cliff	Bkgd-5m	7.5-10	2.60	3.19E+05	8.30E+03
Extrovert Cliff	Bkgd-5m	10-15	1.00	1.12E+06	1.11E+04
Extrovert Cliff	Bkgd-5m	15+	15.35	4.70E+05	7.21E+04

Table S6: Methane diffusive flux calculations.

Site Name	Core Type	Average Sediment Porosity	Ds (m^2/yr)	Methane Conc. Gradient (μM / cm)	Methane Conc. Gradient ($mmol\ m^{-3}\ m^{-1}$)	Flux ($mmol\ m^{-4}\ yr^{-1}$)
Clam Field	Seep	0.771	2.62E-02	8.79	8.79E+02	-17.7
Clam Field	Seep-Edge	0.774	2.63E-02	9.09	9.09E+02	-18.5

Table S7: Taxonomic affiliations of the 20 most abundant 16S rRNA ASVs (cDNA) in Clam Field seep sediment (> 2.5 cmbsf) according to SILVA release 138 (Quast et al., 2013), and the average potential relative activity across samples (%).

	ASV	Phylum	Specific Taxonomic Group	Clam Field Seep Average Relative Potential Relative Activity (%)
1	ASV.7	Desulfobacterota	Desulfatiglans	13.76
2	ASV.33	Bacteroidota	Bacteroidetes BD2-2	9.51
3	ASV.396	Chloroflexi	SCGC-AB-539-J10	8.29
4	ASV.16	Bacteroidota	Draconibacterium	7.84
5	ASV.39	Verrucomicrobiota	R76-B128	7.49
6	ASV.215	Desulfobacterota	Desulfatiglans	5.96
7	ASV.379	Acetothermia	Acetothermiia	5.39
8	ASV.50	Chloroflexi	Thermomarinilinea	5.12
9	ASV.146	Bacteroidota	Labilibacter	4.95
10	ASV.281	TA06	TA06	4.50
11	ASV.70	Thermoplasmatota	Marine Benthic Group D and DHVEG-1	4.29
12	ASV.60	Verrucomicrobiota	R76-B128	4.27
13	ASV.478	Desulfobacterota	Desulfobacterales	4.18
14	ASV.31	Bacteroidota	Bacteroidetes BD2-2	3.65
15	ASV.3	Desulfobacterota	Desulfobacteraceae	3.61
16	ASV.2	Campylobacterota	Sulfurovum	3.57
17	ASV.5	Desulfobacterota	SEEP-SRB4	3.45
18	ASV.489	Chloroflexi	AB-539-J10	3.40
19	ASV.20	Proteobacteria	endosymbionts	3.34
20	ASV.120	Verrucomicrobiota	MSBL3	3.32

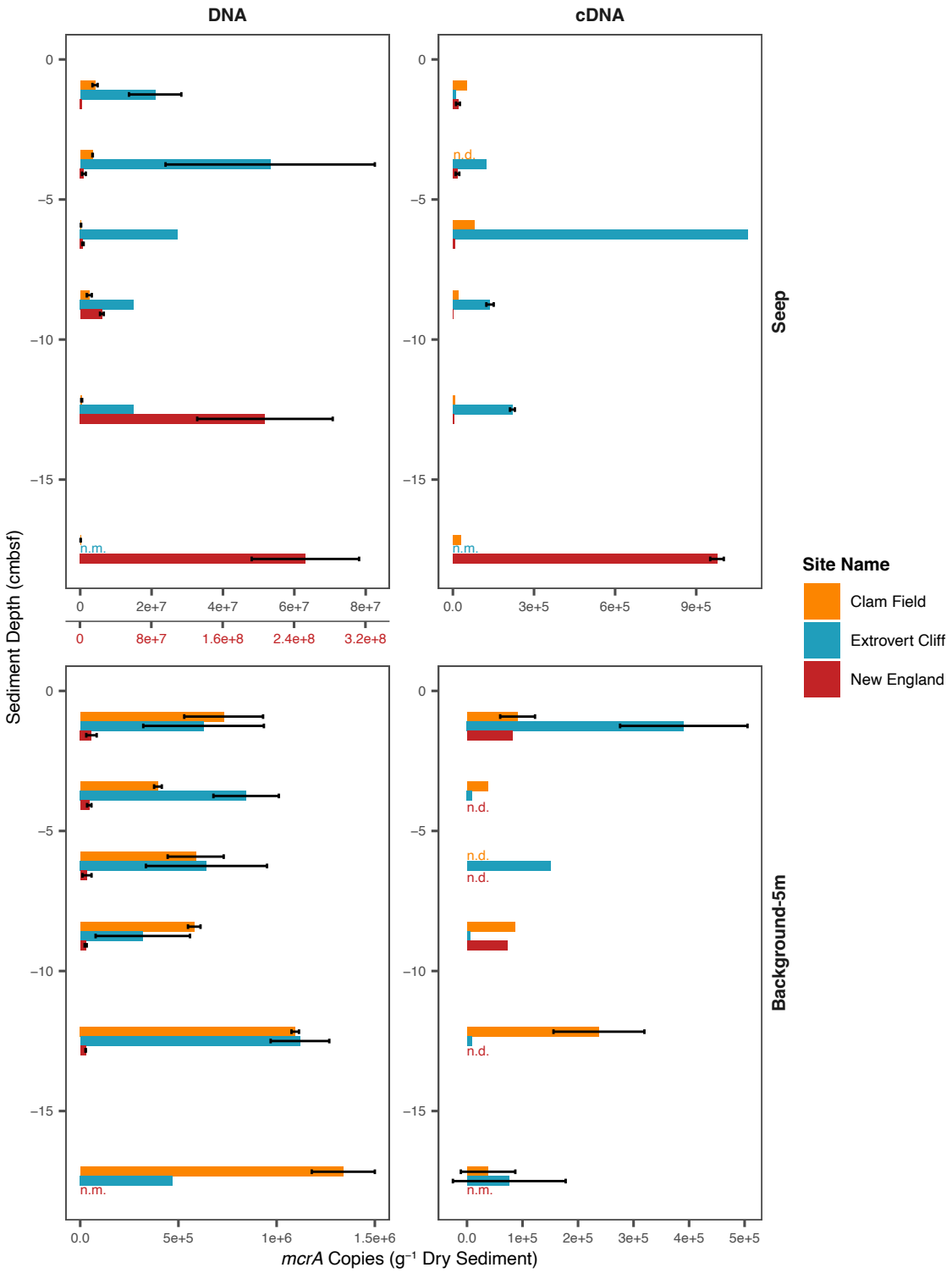

Fig. S1: Comparison of *mcrA* gene (DNA; left) and transcript (cDNA; right) abundance with sediment depth in Monterey Bay (Clam Field and Extrovert Cliff sites) and the US Atlantic Margin (New England seep). Data are from within seep (top) and background sediment (5 meters outside seep; bottom). Values with error bars are the means of two

technical replicates; error bars represent the standard deviation. (Note that *mcrA* gene concentrations in New England seep sediments are an order of magnitude higher than they appear and are measured on an alternative x-axis in red.)

n.d. – *mcrA* ddPCR assay conducted, but *mcrA* not detected
n.m. – not measured (assay not conducted)

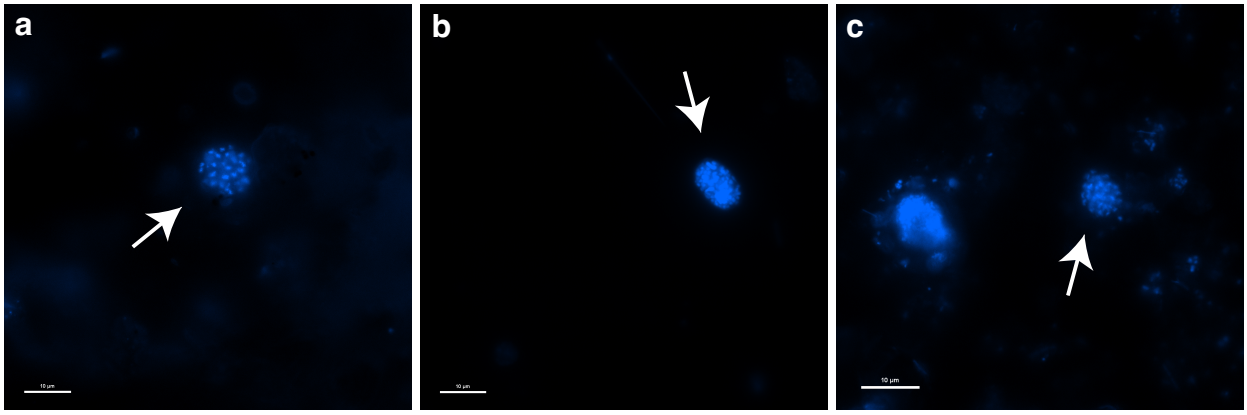

Fig. S2: Aggregates of putative ANME archaea from seeps at Extrovert Cliff (a), as well as from Shallop Canyon East (b) and New England Seep (c) (examined as positive controls), visualized with a DAPI stain. No aggregates were recovered from Clam Field. Scale bar is 10 μm.

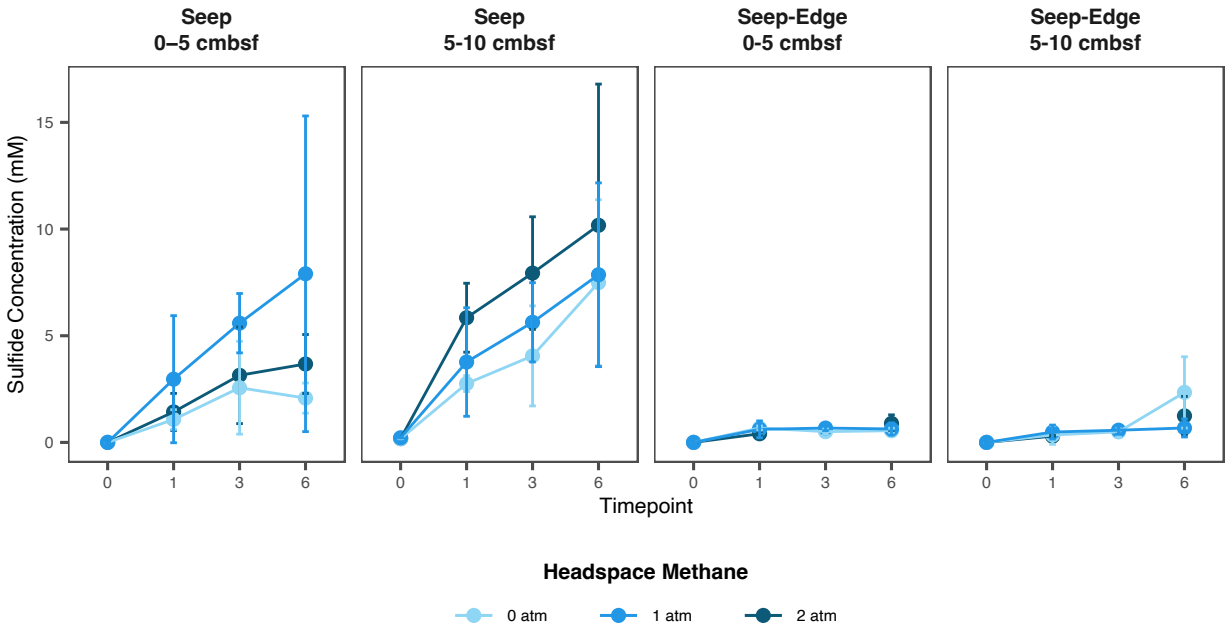

Fig. S3: Sulfide concentrations with time in incubations of Clam Field seep sediment. Sediment from four separate sediment horizons (sediment source delineated by each box) was incubated under three methane headspace treatments – ranging from 0 to 2 atm methane – and sampled at 0-, 1-, 3-, and 6-month timepoints. Error bars represent the standard deviation of the mean of three biological replicates.

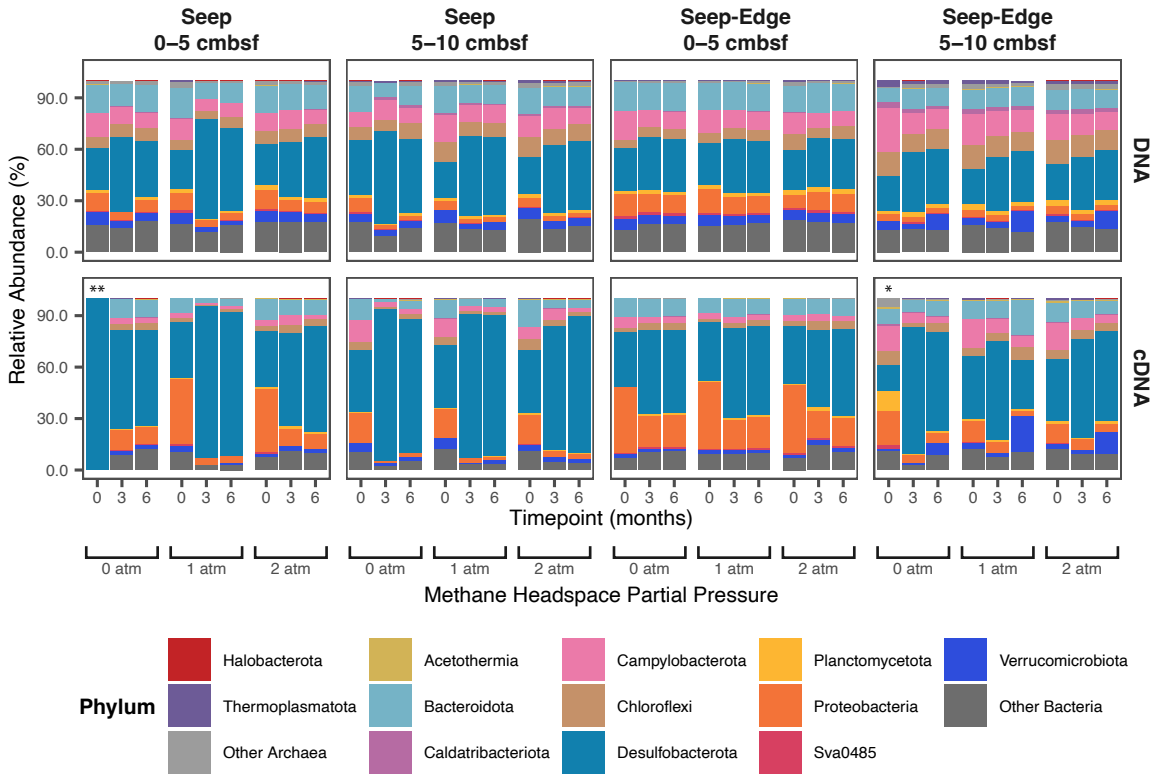

Fig. S4: Relative abundance (%) of Archaea and Bacteria phyla with time in Clam Field seep incubations, as inferred by 16S rRNA gene (DNA) and 16S rRNA (cDNA) sequencing. Sediment from four separate sediment horizons (sediment source delineated by each box) was incubated under three methane headspace treatments – ranging from 0 to 2 atm methane. Each of the twelve incubations was sampled at 0-, 3-, and 6-month timepoints.

*Sample contains fewer than 1,000 or **25 reads.

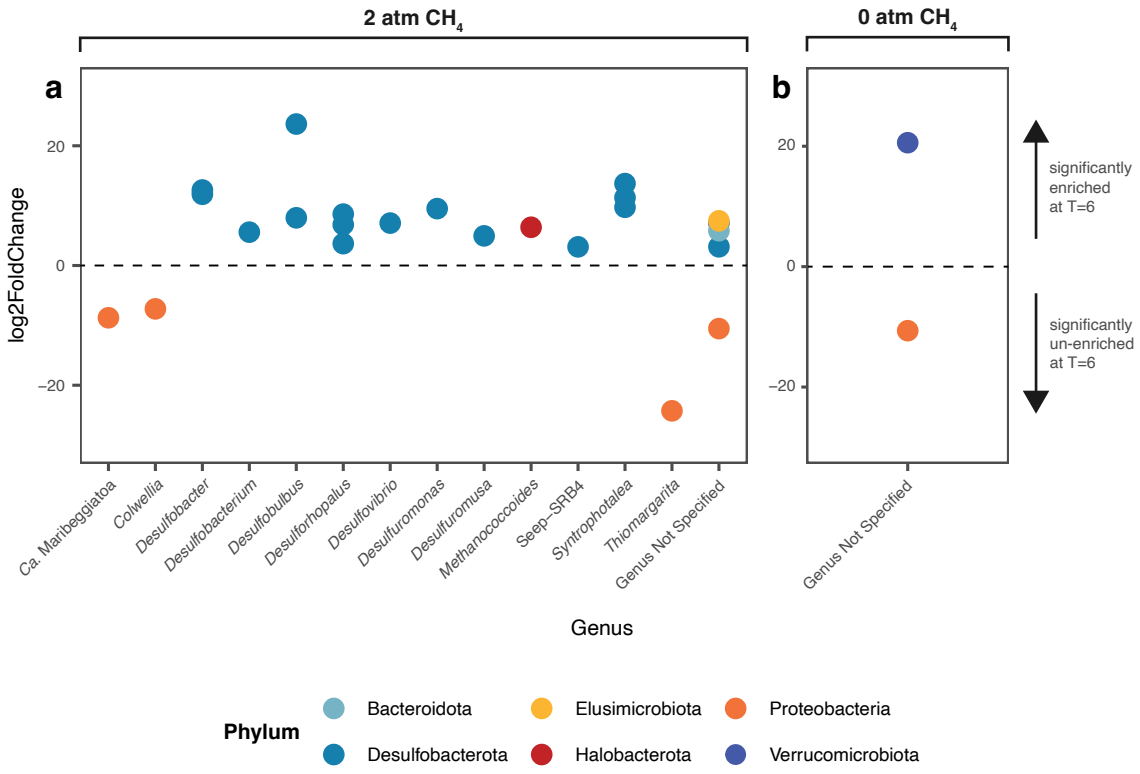

Fig. S5: ASVs which were significantly over-enriched or under-enriched in incubations at the 6-month timepoint vs 0-month under a methane headspace of 2 atm (A) or 0 atm (B). Taxa with a positive log2fold change were significantly over-enriched at 6 months, while taxa with a negative change were significantly under-enriched.

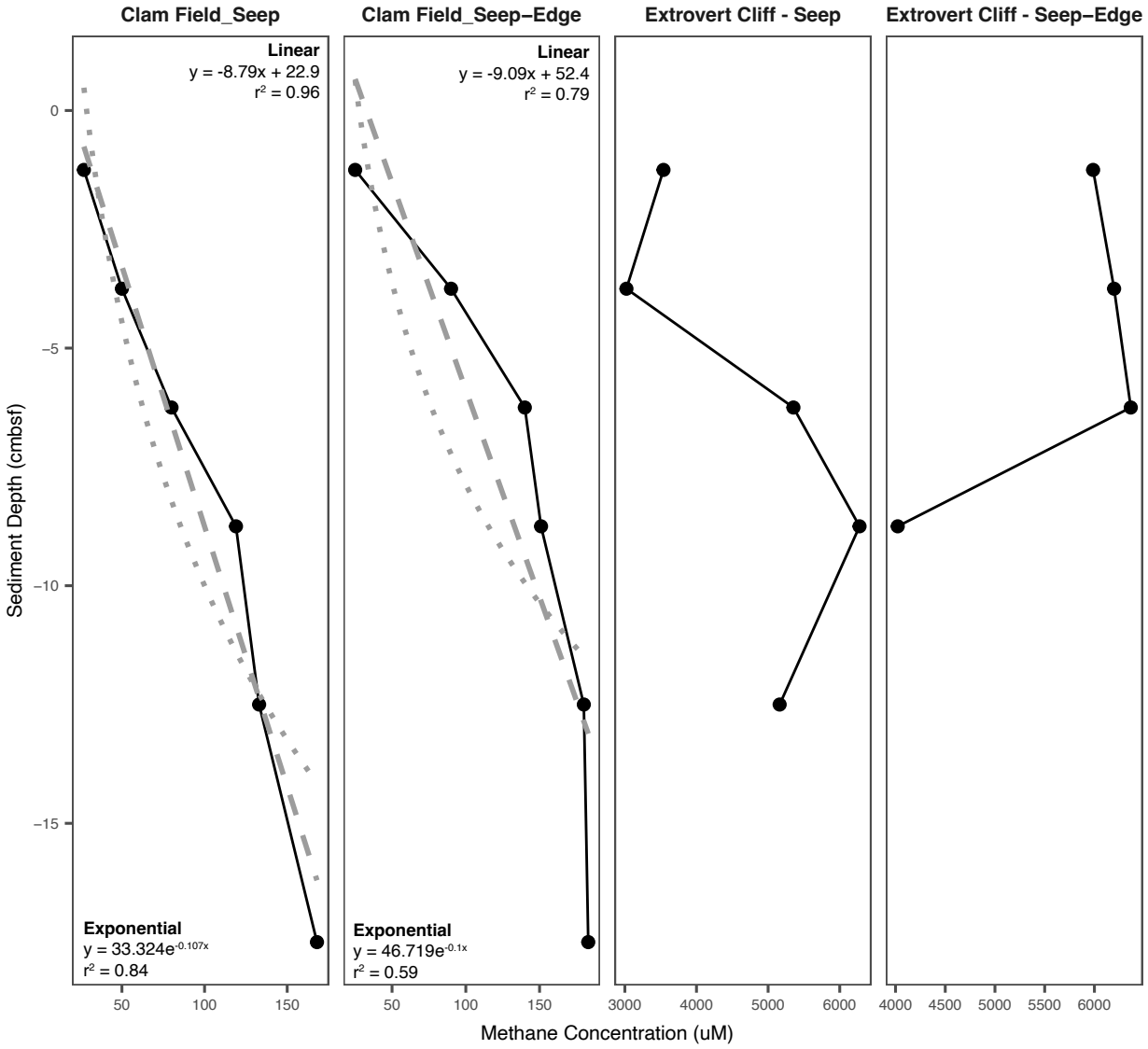

Fig. S6: Methane concentrations with depth in seep cores from Clam Field and Extrovert Cliff. The fit of linear (dashed line) and exponential (dotted line) models are evaluated at Clam Field; linear model slopes (quantifying methane concentration with sediment depth) were used to calculate diffusive methane flux.