

ORIGINAL ARTICLE

Ocean currents shape the microbiome of Arctic marine sediments

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Prokaryote communities were investigated on the seasonally stratified Alaska Beaufort Shelf (ABS). Water and sediment directly underlying water with origin in the Arctic, Pacific or Atlantic oceans were analyzed by pyrosequencing and length heterogeneity-PCR in conjunction with physicochemical and geographic distance data to determine what features structure ABS microbiomes. Distinct bacterial communities were evident in all water masses. Alphaproteobacteria explained similarity in Arctic surface water and Pacific derived water. Deltaproteobacteria were abundant in Atlantic origin water and drove similarity among samples. Most archaeal sequences in water were related to unclassified marine Euryarchaeota. Sediment communities influenced by Pacific and Atlantic water were distinct from each other and pelagic communities. Firmicutes and Chloroflexi were abundant in sediment, although their distribution varied in Atlantic and Pacific influenced sites. Thermoprotei dominated archaea in Pacific influenced sediments and Methanomicrobia dominated in methane-containing Atlantic influenced sediments. Length heterogeneity-PCR data from this study were analyzed with data from methane-containing sediments in other regions. Pacific influenced ABS sediments clustered with Pacific sites from New Zealand and Chilean coastal margins. Atlantic influenced ABS sediments formed another distinct cluster. Density and salinity were significant structuring features on pelagic communities. Porosity co-varied with benthic community structure across sites and methane did not. This study indicates that the origin of water overlying sediments shapes benthic communities locally and globally and that hydrography exerts greater influence on microbial community structure than the availability of methane.

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Introduction

Many studies have characterized the composition and ecological role of microorganisms in methane-containing marine sediments around the world (Inagaki *et al.*, 2006; Heijs *et al.*, 2007; Parkes *et al.*, 2007; Pernthaler *et al.*, 2008; Wegener *et al.*, 2008; Harrison *et al.*, 2009; Hamdan *et al.*, 2012). These works assist in understanding the biological controls on oceanic methane and may contribute to establishing baselines to monitor change in marine sediments. This may be of particular importance in the Arctic Ocean due to its sensitivity to climate

change and abundance of methane (Kvenvolden *et al.*, 1993; McGuire *et al.*, 2009).

Methane biogeochemistry in marine sediment involves ancient enzymatic pathways (for example, sulfate reduction, anaerobic methanotrophy), and geographically cosmopolitan microorganisms (Battistuzzi *et al.*, 2004). Along with sulfate reducers, methanogens and methanotrophs, the Japan Sea candidates, Obsidian pool candidates and Chloroflexi, all of which have unclear biogeochemical roles, are routinely observed in high abundance in methane-containing sediments (Orphan *et al.*, 2001; Webster *et al.*, 2004; Inagaki *et al.*, 2006; Liao *et al.*, 2009; Hamdan *et al.*, 2011). These observations suggest that communities in methane-containing sediments in the global ocean bear similar characteristics (Harrison *et al.*, 2009; Knittel and Boetius, 2009). The phylogenetic and biogeochemical commonalities in these habitats imply that lineages have been conserved over time, possibly

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due to physical isolation and stable environmental conditions. An alternate explanation is recruitment of taxa from overlying waters. The latter agrees with the paradigm in microbial ecology that states 'Everything is everywhere; but the environment selects' (Baas-Becking, 1934). Due to their high densities, small size and absence of migration barriers, the probability of global dispersal of aquatic prokaryotes is high (Finlay, 2002; Fenchel, 2003; Martiny *et al.*, 2006; Caporaso *et al.*, 2012). Therefore, study is needed to ask the question if the apparent cosmopolitan nature of microbiomes in methane-containing marine sediments is the result of dispersal of cells in the hydrosphere or co-evolution in isolation. With this question in mind, the dispersal of sediment microbiomes was investigated on the Alaska Beaufort Shelf (ABS).

The ABS was selected for this study because it receives input from the Pacific and Atlantic Oceans and has a resident water mass derived from river drainage and sea ice melting (McLaughlin *et al.*, 2004). Pacific water enters the Arctic through the Bering Strait and flows east as a boundary layer along the ABS. Atlantic water enters the Arctic through the Fram Strait, and moves counter-clockwise along the ABS break. During summer, as ice melts and vertical mixing is reduced, water on the ABS is density stratified, resulting in a layer of Pacific origin water sandwiched between an Arctic surface layer, and a deep, cold and saline layer of Atlantic origin water (Carmack and Macdonald, 2002; McLaughlin *et al.*, 2004; Shimada *et al.*, 2005). The halocline (40–100 m) between surface and Pacific water and pycnocline (200 m) between Pacific and Atlantic water physically separates these masses (McLaughlin *et al.*, 2004; Pickart, 2004). During stratified periods, free-living microorganisms are resistant to sinking through boundary layers (Jonas, 1997; Hamdan and Jonas, 2006) and will become entrained in a water mass (Agogue *et al.*, 2011). Over an annual cycle, microorganisms from all layers may thermodynamically disperse and reach the sea floor. However, because planktonic prokaryotes are primarily free-living on the ABS, (Garneau *et al.*, 2009) it is reasonable to propose that the stratified period provides an opportunity to observe hydrographic influences on sediment microbiomes. Another factor making the ABS a candidate for study is the abundant methane in shelf and slope sediments (Kvenvolden *et al.*, 1993; McGuire *et al.*, 2009), which permits examination of methane-containing sediments in contact with water of Atlantic or Pacific origin but in close geographic proximity. Because of these features, the influence of water dispersal, the physical environment and local geochemistry on microbiome structure can be parsed.

The goals of this work were to study how water column stratification impacts microbiome composition, and to determine which environmental factors correlate with benthic and pelagic microbiome

structure. Data from this study and previous works on continental margins in the Pacific Ocean (Hamdan *et al.*, 2011, 2012) were used in a comparative assessment of microbial communities to address the role of the hydrosphere in shaping benthic microbiomes and distributing phylotypes to methane-containing sediments.

Materials and methods

Sample collection

Sediments were collected with a piston corer (PC) described in Hamdan *et al.* (2012). Cores were sampled at 20–70 cm intervals. For methane analysis, 2-cm holes were drilled into PC liners and 3-ml plugs were transferred to gas-tight 20 ml vials. Temperature was measured simultaneously. Cores were split horizontally, and sediment for genetic and gravimetric porosity analyses was taken from one half and stored at –80 and –20 °C, respectively. Pore-water was Rhizon extracted (Rhizosphere Research Products, Wageningen, NL, USA) from the other half.

Water samples and hydrodynamic profiles of temperature, salinity, density and dissolved oxygen (DO) were obtained with a Seabird 911 CTD rosette fitted with 10 l Niskin bottles. For methane analysis, water was placed in 20 ml vials and a 4-ml headspace of nitrogen was used for analysis. A minimum of 2 l of water for phylogenetic analysis was filtered through Sterivex filters (Millipore, Billerica, MA, USA). Remaining water was removed and filters were sealed, and frozen at –80 °C.

Multitag pyrosequencing and phylogenetic analysis

Genomic DNA was extracted from sediment and filters using the FastDNA SPIN kit for soil (MP Biomeicals Inc., Santa Ana, CA, USA). Filter capsules were cracked and the filter was removed aseptically and placed in the Lysing tubes. DNA was visualized on a 1% agarose gel with ethidium bromide and diluted with diethylpyrocarbonate (DEPC) treated water. Approximately 10 ng of DNA was used as PCR template. Prior to multitag pyrosequencing (MTPS), samples were analyzed by length heterogeneity-PCR (LH-PCR) as a quality control for linear amplification and to normalize PCR yield before pooling. Amplification of V1 and V2 of the small subunit rRNA gene was performed using the primers 6-FAM-27F and 355R for bacteria and 1HK and 589R for archaea (Hamdan *et al.*, 2011, 2012). Universal archaeal primers UA571F and UA1204R were evaluated; however, limited product was obtained. Positive (*Escherichia coli* and *Sulfolobus solfataricus*) and negative controls (DEPC water) accompanied reactions. The PCR mixture and run conditions are described in Hamdan *et al.* (2012). Product was visualized on an agarose gel with ethidium bromide and quantitated. Products were diluted, mixed with ILS-600 (Promega, Madison, WI, USA) and HiDi formamide, and analyzed on an ABI3130.

For MTPS, 60 forward bacterial primers (27F) tagged on the 5' end with a 8-base barcode along with the emulsion PCR-A titanium adapter were used with a reverse fusion primer (355R) and emulsion PCR-B titanium adapter (Hamdan *et al.*, 2011, 2012). Thirty forward archaeal barcoded primers (1HKF) and a reverse fusion primer (589R) with titanium adapters were also synthesized. Samples were amplified for 30 cycles with a 10-min final extension step (Hamdan *et al.*, 2012) using barcoded primers. Amplified product was visualized and quantitated, diluted for normalization and pooled. Pooled product was quantitated by Quant-iT PicoGreen assay (Invitrogen, Grand Island, NY, USA) on a Beckman Coulter DTX 880 and diluted for use in 454 emulsion PCR. Sequencing was performed on the Roche (Branford, CT, USA) GS-FLX Jr. Data were analyzed by the GS run browser with signal processing for shotgun or paired end and processed in the QIIME pipeline (qiime.sourceforge.net). Sequences <200 bp long, with quality scores <25, with multiple Ns and chimeras (identified with ChimeraSlayer) were excluded. Operational taxonomic units (OTUs) were clustered with CD-HIT at 97% similarity. Sequences were aligned with PyNAST against the Greengenes core set and phylogeny was assigned with RDP. BLAST searches against GenBank were also performed to provide information on the numerous OTUs that were not classified by RDP. The abundance of OTUs was annotated with the number of reads and relative abundance was calculated using the total reads from each barcode.

Geochemical analysis

Methane was determined with a Shimadzu 14-A gas chromatograph and flame ionization detector (Hamdan *et al.*, 2011). Equilibration concentrations for water were calculated as described in Wiesenburg and Guinasso (1979). Chloride concentration measured with a Dionex DX-120 ion chromatograph was used to calculate salinity in sediments. Phosphate, nitrate and nitrite were determined according to Murphy and Riley (1962) and Grasshoff *et al.* (1983).

Statistical analysis

UniFrac was used to quantify the relatedness of phylogenies (Lozupone *et al.*, 2006). The weighted UniFrac metric was computed, and values were Bonferroni corrected. PCoA was conducted on UniFrac distances to display results. MDS (multi-dimensional scaling analysis) and hierarchical cluster analysis were conducted on Bray-Curtis similarity data for LH-PCR results from this study and previous works (Hamdan *et al.*, 2011, 2012) to sample display assemblages. MTPS and LH-PCR similarity data were applied to the Biota-Environmental matching procedure (Bio-Env, Primer-E), to determine the abiotic factors that co-varied with

community structure. A geographic distance matrix was also used in Bio-Env. Bio-Env provides a rank correlation coefficient (ρ , high=1, low=0) to attribute covariance to specific variables. The similarity percentage (SIMPER, Primer-E) analysis was used to determine the sequences that contributed most to similarity within, and dissimilarity between groups of samples identified by water mass and sample type. SourceTracker (Knights *et al.*, 2011) was used to identify sources and sinks of OTUs in samples. The analysis was conducted at a rarefaction depth of 1000. Results are reported as proportion estimates.

Results

Study area

Samples were collected on board the USCGC *Polar Sea* along three transects located north and east of Barrow Alaska (Figure 1; Table 1). This area receives freshwater input primarily from the Coleville River (Figure 1), and secondarily from the Kuparuk and Meade Rivers. Seismic images identified areas with gas in the sediment, and the bottom simulating reflector, the seismic signature of gas below the hydrate stability zone. Sediments were collected from water depths ranging from 136 to 490 m (Table 1). Sediment samples discussed are from the upper 1 m of the sediment column from PC3, PC8 and PC13 so that hydrographic influences could be constrained. The age of sediments from PC13 and a core collected 1 km from PC8 was determined by radiocarbon analysis of bacterial FAMES (fatty acid methyl esters) (Dougherty, 2012). FAMES in the upper 1 m of the sediment column at these locations averaged 2518 and 3613 radiocarbon years, respectively, and the fraction modern was 0.63 and 0.73. Radiocarbon age increased and the fraction modern decreased significantly below 1 m depth at both sites. The observed fractions modern indicates recent organic matter deposition (Griffith *et al.*, 2010). Radiocarbon ages are consistent with another study in the same region, which reported that sedimentation is controlled by current flow across the ABS (Darby *et al.*, 2009).

With the exception of PC13, sediment samples were obtained in proximity to water samples (Figure 1a). Although samples were not obtained from the water column directly above PC13, CTD data demonstrate that hydrodynamic properties in the upper 600 m of the water column at CTD31 were equivalent to that observed above PC13.

Determination of hydrographic water masses

Water masses were determined from depth profiles of temperature, salinity, density and DO according to McLaughlin *et al.* (2004) (Table 1; Figure 1). Arctic surface water (<40 m) at all sites had water temperatures <0.7 °C, salinity ranging from 26 to 29

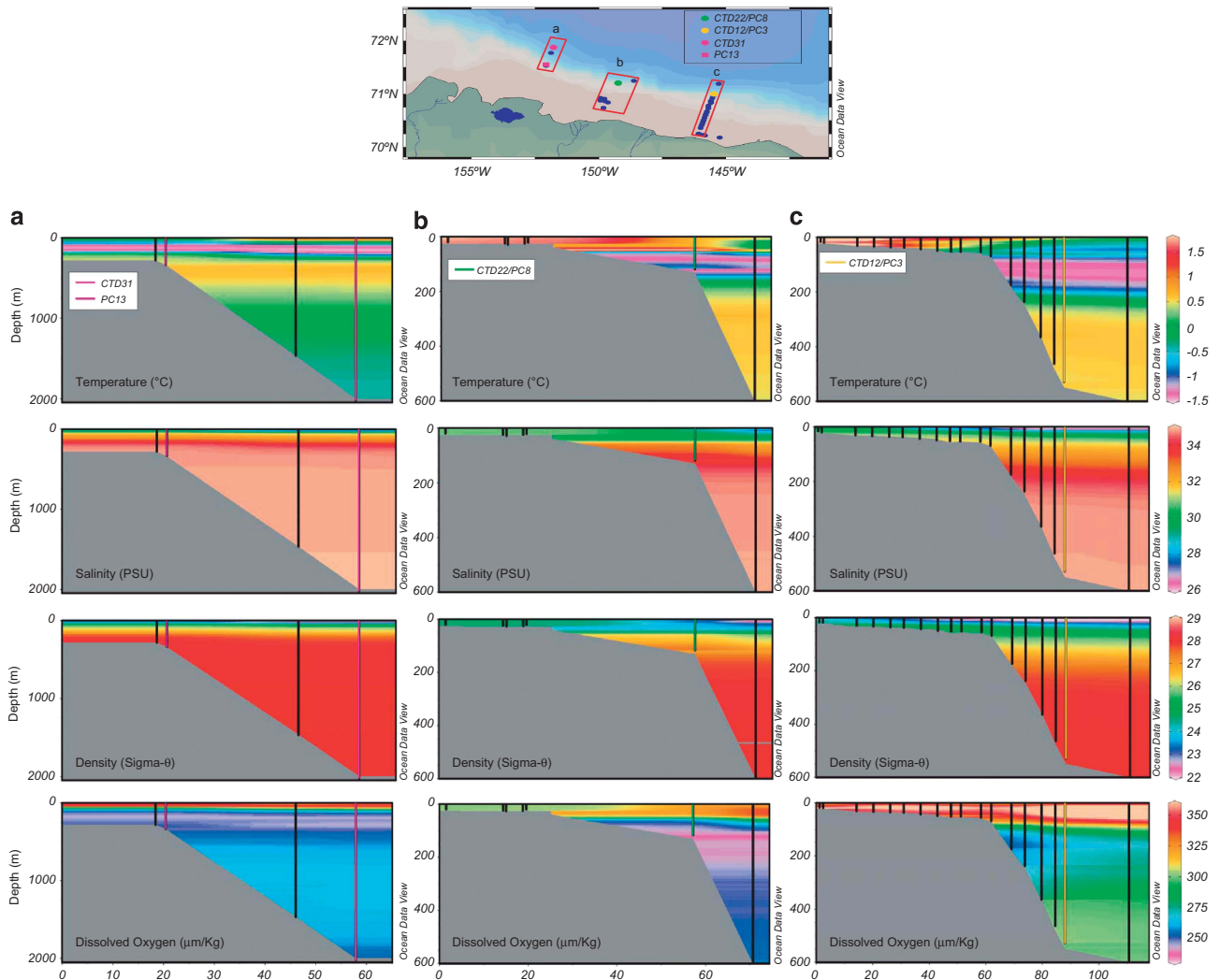


Figure 1 Hydrographic properties of the ABS. The upper map depicts the three regions sampled (a–c). Lower panels are contour plots of temperature, salinity, density and DO in areas (a–c) from the map. Water column locations used to construct contour plots but not used for discrete sample analysis are depicted as blue dots on the map and black vertical lines in a–c. Piston core (PC) and water column (CTD) discrete sampling locations are identified as colored dots on the map, and vertical lines in panels a–c. Contour plots and map were generated in Ocean Data View.

PSU, and density $< 24 \text{ kg m}^{-3}$ (Figure 1). A sharp increase in salinity was observed below $\sim 20 \text{ m}$ at all stations concomitant with an increase in temperature and DO. The temperature maximum was between 20 and 50 m. Between 40 and 60 m strong gradients in temperature, DO and density were evident marking the upper boundaries of the Pacific layer. Between 50–115 m at CTD22, and 100–180 m at CTD12 and CTD31, the temperature minimum was observed in association with a secondary halocline and pycnocline denoting the depths of the Pacific water mass. Below these depths, at CTD12 and CTD31, salinity gradually increased to 34.8 PSU. A tertiary thermocline was observed between ~ 200 and 300 m at CTD12 and CTD31 along with the DO minimum, marking the transition between Atlantic and Pacific water. Below 400 m at CTD12 and CTD31 temperature declined and salinity, DO and density moderately increased

towards the sea floor. With these data, samples were placed in three categories indicative of their oceanic origin (Table 1) and sediments were coded according to their immediately overlying water mass.

Geochemistry and porosity

Sediment methane concentration was highest in PC13 where concentrations exceeded $100 \mu\text{M}$ (Table 1). In PC3, methane was $< 1 \mu\text{M}$ and up to $4 \mu\text{M}$ was observed in PC8. At all sites, water column methane was significantly lower than sediment concentrations. Water column methane was highest in CTD22 ($0.05 \mu\text{M}$). Nitrate, nitrite and phosphate data were available for CTD12 and CTD22. Phosphate and nitrite (< 0.6 and $0.0 \mu\text{M}$) were depleted in surface water at both sites (Table 1), and elevated (1.8 and $0.3 \mu\text{M}$) at 150 m in CTD12. Nitrate was also depleted at the surface ($0.0 \mu\text{M}$), although it exceeded

Table 1 Description of samples collected from the Alaska Beaufort Shelf during September 2009

Station ID	Date	Time (UTC) (hours)	Sample ID	Water mass	Water column depth (m)	Sediment depth (mbsf)	Max water depth (m)	Latitude (N)	Longitude (W)	Temp (°C)	Salinity (PSU)	Density (Sigma-θ)	Methane (µM)	PO ₄ (µM)	NO ₂ (µM)	NO ₃ (µM)	DO (µMol kg ⁻¹)	
Water column CTD12	20-Sep-09	1959	CTD12.05 ^a	Surface	10	—	550	70.99	145.47	-0.49	27.2	21.8	0.01	0.6	0.0	0.0	407.0	
			CTD12.06	Pacific	150	—	550	70.99	145.47	145.47	-1.40	33.0	26.6	0.01	1.8	0.3	12.0	287.5
			CTD12.07	Atlantic	316	—	550	70.99	145.47	145.47	0.56	34.7	27.8	0.02	1.0	0.0	13.6	291.9
			CTD12.08	Atlantic	526	—	550	70.99	145.47	145.47	0.53	34.8	27.9	0.00	0.9	0.0	13.2	301.3
			CTD22.09	Surface	3	—	130	71.20	149.22	149.22	0.57	29.2	23.4	0.01	0.3	0.0	0.0	323.4
CTD22	23-Sep-09	0341	CTD22.10	Pacific	60	—	130	71.20	149.22	-1.13	32.8	26.3	0.02	ND	ND	ND	247.0	
			CTD22.11	Pacific	115	—	130	71.20	149.22	149.22	-0.57	34.2	27.5	0.05	ND	ND	ND	240.2
			CTD31.12	Surface	5	—	2017	71.87	151.76	151.76	0.72	26.4	21.1	0.01	ND	ND	ND	327.3
CTD31	24-Sep-09	1622	CTD31.13	Atlantic	1,000	—	2017	71.87	151.76	-0.01	34.8	28.1	0.01	ND	ND	ND	264.3	
			CTD31.14	Atlantic	2,017	—	2017	71.87	151.76	151.76	-0.50	34.9	28.0	0.00	ND	ND	ND	253.1
Sediment PC3	20-Sep-09	2319	3.59	Atlantic influenced	—	0.2	490	70.97	145.49	-0.70	32.2 ^b	25.9	0.29	ND	ND	ND	ND	
			3.58	Atlantic influenced	—	0.5	490	70.97	145.49	145.49	-0.40	32.5 ^b	26.1	0.44	ND	ND	ND	ND
			3.57	Atlantic influenced	—	1.1	490	70.97	145.49	145.49	-1.20	31.6 ^b	25.4	0.41	ND	ND	ND	ND
PC8	22-Sep-09	0320	8.110	Pacific influenced	—	0.1	136	71.20	149.22	1.20	30.9 ^b	24.7	0.64	ND	ND	ND	ND	
			8.109	Pacific influenced	—	0.3	136	71.20	149.22	149.22	1.00	31.8 ^b	25.5	1.14	ND	ND	ND	ND
			8.108	Pacific influenced	—	0.5	136	71.20	149.22	149.22	0.80	31.8 ^b	25.5	2.23	ND	ND	ND	ND
			8.107	Pacific influenced	—	0.7	136	71.20	149.22	149.22	0.70	31.7 ^b	25.4	3.73	ND	ND	ND	ND
PC13	24-Sep-09	0628	13.212	Atlantic influenced	—	0.2	342	71.55	152.06	0.90	32.5 ^b	26.0	2.94	ND	ND	ND	ND	
			13.211	Atlantic influenced	—	0.5	342	71.55	152.06	152.06	1.10	32.5 ^b	26.0	11.09	ND	ND	ND	ND
			13.210	Atlantic influenced	—	0.7	342	71.55	152.06	152.06	0.50	32.3 ^b	25.9	27.15	ND	ND	ND	ND
13.209	Atlantic influenced	—	0.9	342	71.55	152.06	152.06	0.20	32.0 ^b	25.7	127.53	ND	ND	ND	ND			

Abbreviations: DO, dissolved oxygen; LH, length heterogeneity; mbsf, meters below sea floor; nd, no data; PC, piston core.

^aLH-PCR analysis only.

^bDerived from chlorinity.

12 μm deeper in CTD12. Sediment porosity ranged from 0.5% to 0.8% (Supplementary Table S1) and was lowest in samples from PC8.

Microbial diversity

On average, 4000 sequences were obtained per sample (Table 2). The number of OTUs and diversity was generally higher for bacteria than archaea. Sequence coverage varied substantially between bacteria and archaea, especially in sediment samples. The Chao 1 statistic was used to estimate the depth of coverage provided by MTPS with the understanding that the statistic scales with sequencing effort. According to the Chao 1 estimate, bacteria were under sequenced by 21–55%. Archaeal OTUs were near or exceeded the number predicted by the Chao1 estimate. The number of archaeal sequences per sample varied considerably (37–6526) and in general, more sequences were

obtained from water than sediment. Bacterial sequences were more evenly distributed (~ 1472 per sample) across sample types. Archaeal sequence abundance was low in surface water samples (< 10 m) (Table 2), and template concentration in CTD12.05 was too low for MTPS. Diversity estimates in general were not significantly different between water and sediment. Sample 8.110 had the highest diversity (Table 2) and surface water samples had the lowest diversity.

Phylogenetic composition

The most abundant pelagic bacterial group was the Alphaproteobacteria (Figure 2), with the majority associated with the SAR11 clade. These were most abundant in surface water and least abundant in Atlantic water (Figure 2; Supplementary Figure S1). Alphaproteobacteria were $< 10\%$ of sediment OTUs. Gammaproteobacteria and Bacteroidetes were well

Table 2 Descriptive statistics and diversity estimates for bacterial and archaeal sequences

Summary statistics		Count									
Raw input sequences		223 119									
Length outside bounds of 200 and 1000		20 212									
Ambiguous bases		4296									
Missing quality score		0									
Qual score below minimum of 25		6545									
Max homopolymer run exceeds limit of 6		2167									
Sequences written to selected samples		87 008									
Raw length min/max/avg		40/957/371									
Wrote length min/max/avg		200/479/344									
Sample statistics											
Sample ID	Water mass	Sequence count		OTUs/sample		Chao1		Simpsons		Shannon	
		Bacteria	Archaea	Bacteria	Archaea	Bacteria	Archaea	Bacteria	Archaea	Bacteria	Archaea
<i>Water</i>											
CTD12.06	Pacific	3107	6526	477	226	758	181	0.04	0.12	4.58	3.23
CTD12.07	Atlantic	1995	2424	364	167	666	192	0.02	0.05	4.68	3.72
CTD12.08	Atlantic	2408	5625	488	258	778	189	0.02	0.05	4.99	3.82
CTD22.09	Surface	2316	935	255	35	386	54	0.07	0.49	3.99	1.26
CTD22.10	Pacific	2172	3641	408	176	697	169	0.05	0.06	4.48	3.56
CTD22.11	Pacific	2212	8059	475	304	859	214	0.03	0.07	4.83	3.74
CTD31.12	Surface	4578	63	331	20	419	17	0.07	0.14	4.04	2.43
CTD31.13	Atlantic	3074	4059	551	210	836	201	0.02	0.05	5.04	3.79
CTD31.14	Atlantic	2135	4109	573	157	1039	131	0.01	0.06	5.41	3.48
<i>Sediment</i>											
3.59	Atlantic	2160	139	857	32	1471	41	0.00	0.10	6.19	2.77
3.58	Atlantic	3102	37	1006	17	1452	11	0.01	0.08	6.20	2.55
3.57	Atlantic	2011	515	726	52	1154	57	0.01	0.08	5.90	3.18
8.110	Pacific	2749	1092	1097	113	1600	47	0.01	0.02	6.30	4.13
8.109	Pacific	1271	178	354	28	595	32	0.02	0.08	4.96	2.83
8.108	Pacific	2648	941	459	35	734	38	0.03	0.11	4.65	2.65
8.107	Pacific	2899	128	601	12	822	13	0.02	0.21	5.26	1.85
13.212	Atlantic	1019	872	426	115	926	179	0.02	0.04	5.18	3.89
13.211	Atlantic	1033	370	475	70	1037	91	0.01	0.04	5.60	3.62
13.210	Atlantic	1339	1323	463	133	879	61	0.03	0.11	4.97	3.25
13.209	Atlantic	1472	272	458	39	914	51	0.02	0.08	5.20	2.92
Average		2285	2065	542	110	901	98	0.02	0.10	5.12	3.13
Total		45 700	41 308	6204	901						

Abbreviation: OTU, operational taxonomic unit.

represented in the water column (Figure 2). Bacteroidetes abundance decreased with depth and Gammaproteobacteria increased with depth (Figure 2; Supplementary Figure S1). Gammaproteobacteria were related to 11 orders and the Bacteroidetes were associated with four orders.

The Chloroflexi were 20% and 14% of OTUs in Pacific and Atlantic influenced sediments, respectively (Figure 2). The Firmicutes were more abundant in Pacific than Atlantic influenced sediments and averaged 5% in Pacific and Atlantic waters. Deltaproteobacteria were concentrated in Atlantic influenced sediments (~20%), and were at least 10% of sequences from all sample groups except surface water. Epsilonproteobacteria were most abundant in Atlantic influenced sediments, and OTUs affiliated with the *Sulfurovum* and *Sulfurimonas* accounted for up to 13% of sequences in PC13 (Supplementary Figure S1).

The Euryarchaeota dominated archaea in terms of OTU abundance (Figure 3) and diversity. Over half of water sequences fell into a category that could not be annotated beyond Euryarchaeota with either RDP or GenBank. Over 80% of surface waters sequences and over 400 OTUs fell in this category. Sequences related to the methanogenic Methanomicrobia were most abundant in Atlantic influenced sediments. In Pacific influenced sediments, Crenarchaeota related to the Thermoprotei were abundant, accounting for 43% of sequences (Figure 3). Crenarchaeota were ~10% of Atlantic influenced sediment OTUs, and minimally abundant in pelagic samples (Supplementary Figure S2).

Community structure

The weighted UniFrac metric was computed for bacteria and archaea separately. Bacterial samples distributed into five groups (Figure 4a). Surface samples from CTD12 and CTD31 were co-located. Pacific and Atlantic water samples grouped near each other, but formed separate groups. PC3 and PC13, from areas with immediately overlying Atlantic water clustered together. PC8 collected within the Pacific water mass formed a distinct cluster. There was no correlation between sediment and pelagic communities. A similar clustering pattern for archaea in sediment was observed (Figure 4b). The majority of pelagic archaeal samples were in a single group, however, Pacific samples CTD12.06 and CTD22.11 collected from similar depths (150 and 115 m, respectively; Table 1) grouped apart.

SIMPER analysis revealed that differences in bacterial and archaeal communities were explained by one or a few OTUs (Supplementary Tables S2 and S2). Dissimilarity was generally lower between groups of the same sample type (water or sediment) (Supplementary Table S3), and highest between sediment and surface water. Bacteria in Atlantic and Pacific sediment had the lowest dissimilarity relative to other comparisons (Supplementary Table S3) because both groups were influenced by two shared OTUs, a Chloroflexi and a Firmicutes (Supplementary Table S2). The former accounted for 18% of OTUs in Pacific sediments and controlled dissimilarity between this and other groups. The Firmicutes OTU was also important to similarity in Atlantic water (6–12% of sequences),

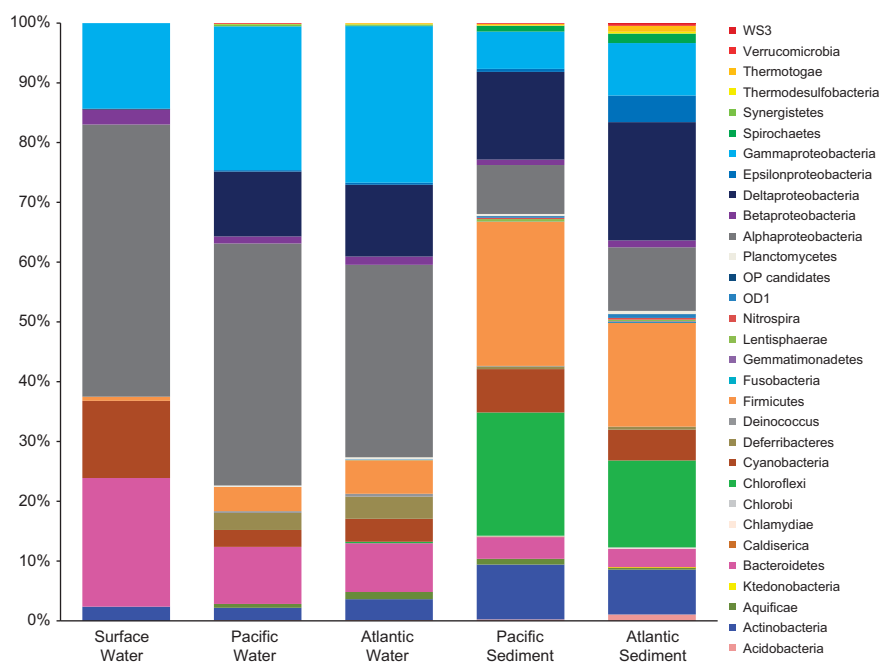


Figure 2 Bacterial community composition in sample groups from the ABS identified by water mass (Atlantic, Pacific, surface) and sample type (sediment, water).

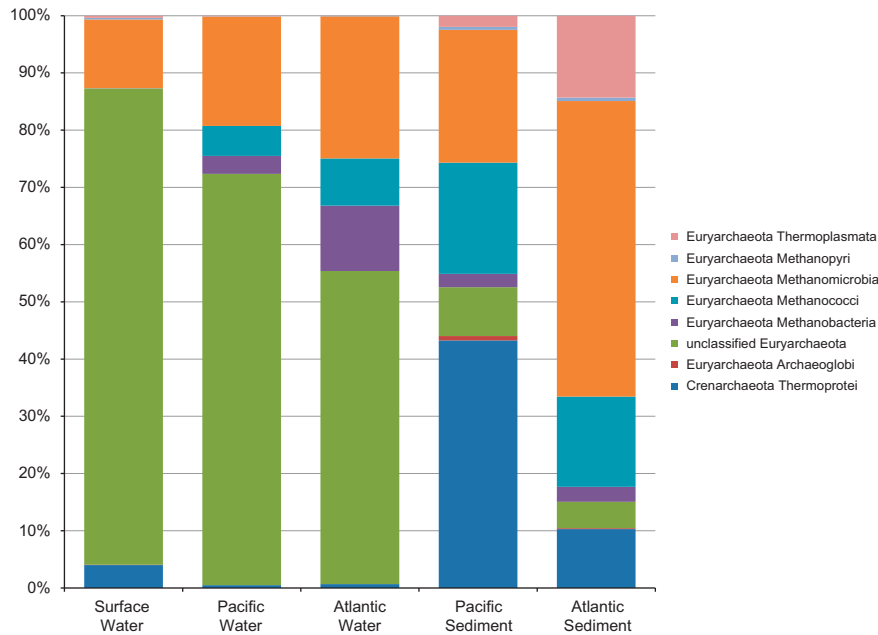


Figure 3 Archaeal community composition in sample groups from the ABS identified by water mass (Atlantic, Pacific, surface) and sample type (sediment, water).

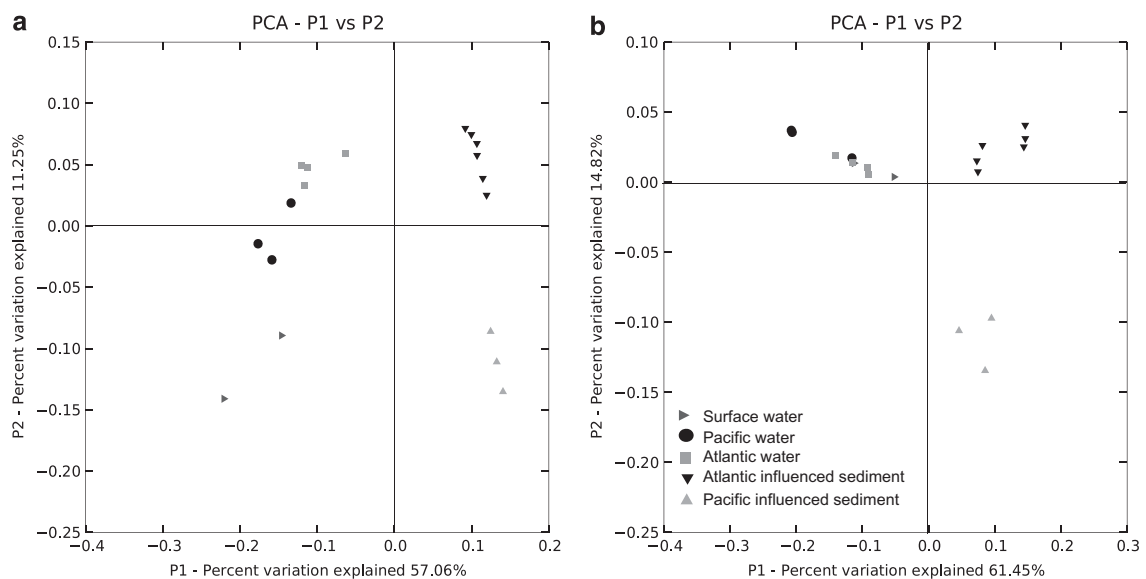


Figure 4 Weighted UniFrac distances for sediment and water column Multitag Pyrosequenced data for bacteria (a) and archaea (b) from the ABS.

and was influential in all dissimilarity comparisons with Atlantic water or sediment (Supplementary Table S3). However, similarity in Atlantic water was driven by an unclassified Deltaproteobacteria (Supplementary Table S2), which was the main discriminator between Atlantic sediment and water (Supplementary Table S3). A SAR11 affiliated OTU contributed most to bacterial similarity in Pacific and surface water samples. While the SAR11 OTU may explain the lower dissimilarity between Pacific and surface water (Supplementary Table S2), it drove dissimilarity in all other surface water pairings.

Archaeal within group similarity was lower than observed for bacteria (Supplementary Table S2). In all groups, unclassified marine isolates were most influential on SIMPER comparisons. The most abundant archaeal OTUs generally did not provide the greatest contribution to similarity, and unlike the bacteria, the most influential OTUs generally were not shared across groups. This was most evident in sediment groups, and explains their high dissimilarity to each other (Supplementary Table S3). An unclassified Euryarchaeote accounted for >10% of within group similarity in all pelagic groups

(Supplementary Table S3). The abundance of this OTU in Pacific and Atlantic water explains their low dissimilarity to each other (Supplementary Table S3). Conversely, it drove dissimilarity between all pelagic and sediment pairings.

Community structuring features

Bio-Env analysis indicates that salinity ($\rho > 0.66$) and density ($\rho > 0.62$) were the principal structuring elements on bacterial and archaeal pelagic microbiomes. However, temperature and methane had minimal influence on pelagic community structure ($\rho < 0.01$). A suite of sediment geochemical variables was used to determine the features that structured sediment communities (Supplementary Table S1). Bio-Env indicates that porosity, wt% carbon and % nitrogen, all provided ρ -values > 0.40 for bacteria and archaea.

The impact of vertical and horizontal geographic distance on microbiome structure was addressed with Bio-Env. The distance in km from CTD31 was calculated for all sites. Geographic distance, the maximum water depths for sediment samples and the depth of collection for water samples were considered in the analysis. The test indicated that distance was not an important structuring element on pelagic microbiomes ($\rho = 0.007$); however, water depth was ($\rho = 0.60$, bacteria; $\rho = 0.50$, archaea). Distance moderately co-varied with sediment archaea ($\rho = 0.30$) and to a lesser extent, sediment bacteria ($\rho = 0.18$). The maximum water depth co-varied consistently with sediment bacteria and archaea ($\rho > 0.37$).

SourceTracker was used to address the influence of water masses on sediment microbiomes. CTD samples were source terms and sediment samples were sink terms, and the proportion of OTUs from Atlantic, Pacific and Surface water, and unknown sources were calculated. The source of OTUs to ABS sediments is largely unknown (Supplementary Figure S3). However, the influence of Atlantic water was evident on all Atlantic influenced sediments. Up to 9% of bacterial OTUs at PC3 and PC13 were attributed to Atlantic water. However, the Pacific influence was not evident in PC8 bacterial samples. In only one archaeal sample from PC8 a proportion of OTUs were related to Pacific water. The proportion of OTUs shared between water masses was also estimated (Supplementary Figure S4). Bacterial OTUs in surface and bottom water samples were dominated by their respective source. This was also the case for Archaeal OTUs with the exception of surface sample CTD31.12 in which 35% of OTUs were attributed to the underlying Pacific water mass. Most samples from intermediate depths had a mix of sources from adjacent water masses.

Discussion

Community composition

Few previous works have explored microbial diversity in Arctic pelagic communities (Bano *et al.*,

2004; Galand *et al.*, 2009, 2010; Kirchman *et al.*, 2010) and none has examined sediment communities. Others have observed Proteobacteria including the SAR11 and Bacteroidetes to be prominent in Arctic surface waters (Galand *et al.*, 2010; Kirchman *et al.*, 2010) and abundant Delta-proteobacteria in deeper waters (Galand *et al.*, 2010). The dominance of unclassified Euryarchaeota in Beaufort Shelf waters and minimal abundance of Crenarchaeota has also been observed (Galand *et al.*, 2006, 2009). Despite the distinct water masses on the ABS, the pelagic archaeal community was relatively homogeneous. Recent works highlight inconsistent patterns in pelagic archaea around the world (Martin-Cuadrado *et al.*, 2008) and the general lack of knowledge regarding archaeal population dynamics in the ocean. Although archaeal composition in stratified waters on the ABS requires further study, due to the consistency between this and previous studies of prokaryotes in the Arctic, these results are a robust characterization of ABS communities, which permits examination of the features that shape them.

Hydrographic influences on ABS communities

Prokaryotic community structure in open water is dictated by physical constraints on dispersal and environmental variability (Martiny *et al.*, 2006; Follows *et al.*, 2007). A previous study observed that water masses are a factor explaining the distribution of pelagic microbiomes in the Arctic ocean (Galand *et al.*, 2010). Our data agree with that previous work where bacteria are concerned; however, no consistent separation by water mass was observed for archaea. The SourceTracker analysis suggests that there is a greater degree of mixing of archaeal OTUs in different water masses than for bacteria (Supplementary Figure S4). However, this may be a function of the relative homogeneity of abundant archaeal OTUs in pelagic samples.

Salinity is a significant structuring element on microbial composition in coastal environments (Crump *et al.*, 2004; Auguet *et al.*, 2010) and density gradients limit the physical dispersion of plankton (Galand *et al.*, 2009). Salinity and density are related in open water, and while both impact physical dispersal, salinity also exerts physiological control on the expression of phylotypes. Bio-Env indicates that salinity and density most strongly co-varied with pelagic microbiome structure. As in previous works (Auguet *et al.*, 2010), temperature, a physiological constraint on enzyme activity, was not an important structuring element on pelagic communities. Despite the presence of methane in the water column (Table 1) and methanogen-related OTUs (Figure 3), methane had no observable influence on community structure. Overall, these results suggest that physical isolation of water masses not physiological constraints is responsible for pelagic microbiome structure on the ABS and agree with the findings of Galand *et al.* (2010).

The sampling of nearby (<100 km) sediments influenced by different water masses provided a unique opportunity to determine the influence of hydrography on benthic microbiomes. SourceTracker provided some evidence for water deposition of OTUs in sediments, particularly at PC3 and PC13, and Bio-Env indicated the covariance between microbiome structure and water depth. A separate cluster of samples was evident for PC8 (Figure 4). Because PC8 was in physical contact with Pacific origin water, it may be assumed that hydrography was primarily responsible for this cluster. However, the influence of the *in situ* environment must be considered. SourceTracker reported that a lower proportion of OTUs in PC8 were derived from the water column relative to PC3 and PC13 (Supplementary Figure S3). The lower porosity in PC8 (Supplementary Table S1) compared with PC3 and PC13 and the strong covariance between sediment communities and porosity as indicated by Bio-Env suggests that porosity may physically influence OTUs deposition in sediment. However, because of the covariance between nitrogen and carbon with sediment community structure, a combined influence of permissible physical and geochemical conditions for OTU deposition and recruitment is most likely.

Microbiome structure in methane-containing marine sediments

Because this study indicates that hydrography influences nearby sediment microbiomes on the

ABS, an attempt was made to explore this in locations at greater geographic distance. Microbiomes in methane-containing sediment from the mid-Chilean margin (Hamdan *et al.*, 2012) and the Hikurangi margin (Hamdan *et al.*, 2011) were studied previously. LH-PCR data from these previous works and the ABS were used in MDS and Bio-Env analysis to evaluate which features (that is, physical constraints, dispersal, *in situ* environment) are responsible for community composition across a larger geographic area. LH-PCR data were used because previous works employed older MTPS platforms and thus, data were not comparable. Samples from <1 m sediment depth from the Chilean and Hikurangi margins and ABS were considered.

The MDS analysis revealed that samples from each individual area generally coordinated (Figure 5). Bio-Env suggests that small geographic distance between samples from individual areas co-varied well with community composition ($\rho=0.47$). As observed for ABS sediments, porosity and water depth were also important physical structuring features ($\rho=0.36$ and $\rho=0.31$, respectively). Carbon and nitrogen content could not be evaluated across study areas, but carbon co-varied with bacteria from the Hikurangi margin (Hamdan *et al.*, 2011). Although samples from the Chilean margin, Hikurangi margin and PC13 contained methane, it did not co-vary with community composition ($\rho=0.03$).

All ABS water samples clustered apart from sediment samples (Figure 5). Atlantic influenced sediments formed a distinct cluster and three of four

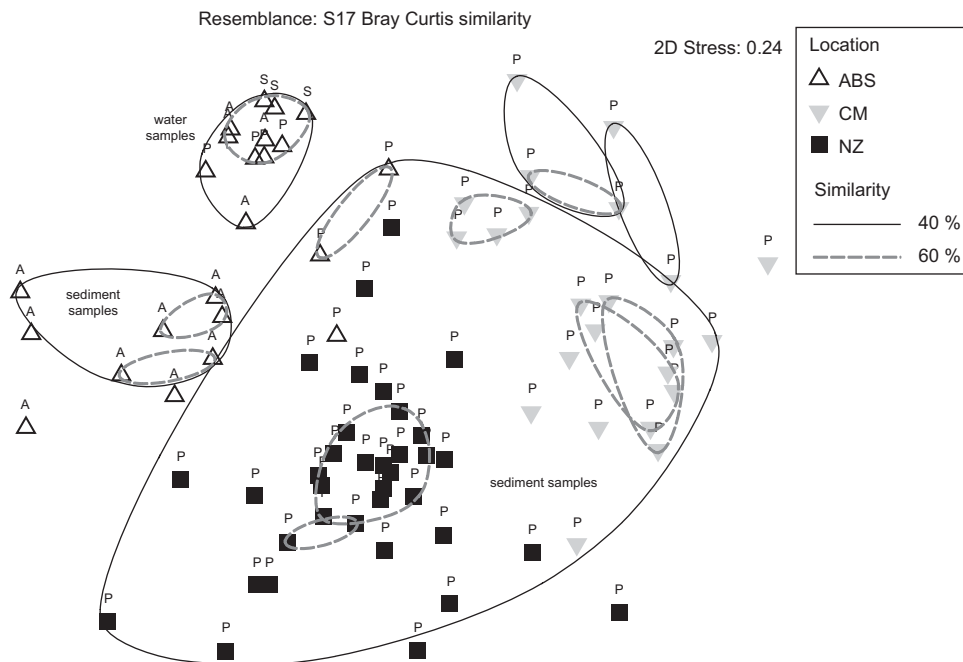


Figure 5 MDS of length heterogeneity-PCR data for samples collected during this study on the ABS and from the Chilean margin (CM) and the Hikurangi margin of New Zealand (NZ) during previous studies. Contours were generated by a hierarchical cluster analysis on Bray-Curtis similarity data. Samples are coded according to the water mass they were collected in (A—Atlantic, P—Pacific and S—Arctic surface).

Pacific ABS sediment samples coordinated within the cluster containing most Pacific samples from the Chilean and Hikurangi margins despite the fact that ABS sites were >12 000 km from the other Pacific sites. Sample 8.110 from the surface of PC8 (Table 2) grouped with water samples from the ABS. This is intriguing as SourceTracker suggested that this sample had greater interaction with OTUs from the overlying water column than others from PC8.

This comparative analysis provides evidence that historical events and the contemporary environment both contribute to marine sediment microbiomes. Specifically, Bio-Env indicates that near proximity is the principal structuring feature on sediment microbiomes. However, SourceTracker results for the ABS suggest that marine sediments are a sink for OTUs in the water transiting them. The grouping of Pacific ABS samples with other Pacific samples (Figure 5) indicates that phylogenetic integrity of pelagic microbiomes is retained during dispersal across long distance. This agrees with studies that demonstrate high bacterial dispersal rates within water masses (Schauer *et al.*, 2010). Bio-Env results for depth and porosity also indicate that local physical conditions influence microbiomes in methane-containing sediments to a greater extent than local geochemistry, possibly by regulating the rate of pelagic OTU deposition.

In keeping with ‘Everything is everywhere; but the environment selects’ (Baas-Becking, 1934), this study indicates that OTUs are transported in oceanic masses over long distances, and local sediment features control recruitment from the water column. Because of the limited influence of local geochemistry, in particular methane concentration, this work indicates that co-evolution in isolation does not explain similarity in microbiome composition in methane-containing sediments. Instead, it suggests that dispersal and the physicochemical environment shape benthic microbiomes on a continual basis. This work is a first attempt at describing hydrographic influences on microbiomes in methane-containing sediments. In order to firmly establish the theories posited here, a comprehensive study and samples from a range of geographic distances are needed. This work demonstrates the importance of establishing ecological baselines for communities in marine sediments so that environmental change and any resulting effects that modify the structure of communities involved in methane regulation in the ocean can be monitored and assessed.

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