ARTICLE IN PRESS

Deep-Sea Research Part II xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Deep-Sea Research Part II

journal homepage: www.elsevier.com/locate/dsr2

Heterogeneity of methane seep biomes in the Northeast Pacific

Sarah Seabrook[a,*], Fabio C. De Leo[b,c], Tamara Baumberger[d], Nicole Raineault[e], Andrew R. Thurber[a,f]

[a] College of Earth, Ocean, and Atmospheric Sciences, Oregon State University, Corvallis, OR 97331, United States
[b] Ocean Networks Canada, University of Victoria, PO Box 1700 STN CSC, Victoria, BC, Canada V8W 2Y2
[c] Department of Biology, University of Victoria, PO Box 3080, Victoria, BC, Canada V8W 2Y2
[d] National Oceanic and Atmospheric Administration, Pacific Marine and Environmental Laboratory, Newport, OR 97365, United States
[e] Ocean Exploration Trust, Narragansett, RI 02882, United States
[f] Department of Microbiology, College of Science, Oregon State University, Corvallis, OR 97331, United States

ABSTRACT

Methane seeps provide biogeochemical and microbial heterogeneity in deep-sea habitats. In the Northeast (NE) Pacific Ocean recent studies have found an abundance of seeps at varying spatial separations and within distinct biogeochemical environments ranging in oxygen, depth, and temperature. Here, we examine eight newly discovered seeps and two known seeps covering 800 km and varying across 2000 m water depth to identify: (1) novel megafaunal communities in this geographical region; (2) variations in the microbiome of seep habitats across the margin; (3) spatial and biogeochemical drivers of microbial diversity at seeps. In addition to authigenic carbonates, clam beds, microbial mats, and exposed hydrates - we also observed Siboglinidae tube worm bushes and an anomalous deep-sea barnacle adding to the overall habitats known from the NE Pacific. The microbial communities showed high variability in their spatial distribution and community structure. The seep communities formed distinct groups that included multiple groups of anaerobic methane oxidizing Archaea (ANME; 1, 2ab, 2c, and 3), often co-occurring within one site – however, there were also other sites with clearly dominant members (e.g. ANME-1s at Nehalem Bank). Sulfide oxidizers were dominated by the non-mat forming Campylobacterales and even though vertical gradients in redox potential typify seep sediments, in two cases there was not a significant change in community structure across the top five cm of sediment. We posit that these patterns were driven by 'bubble-turbation,' and bioirrigation by megafauna. A surprising latitudinal trend was observed in species diversity and richness with increasing richness significantly correlated to increasing latitude. Overall, our results demonstrate that heterogeneity is ubiquitous in the seep biome, spanning all faunal classes, and that the understanding of seeps and the drivers of the community structure can be improved by studying seeps at a range of spatial scales.

1. Introduction

Cold seep habitats are increasingly recognized for their ubiquity in the world's oceans (Brothers et al., 2013; Grupe et al., 2015; Johnson et al., 2015; Levin, 2005). Resulting from the upward advection of hydrocarbons through the sediment, seeps are important sources of energy and heterogeneity in many marine environments (Guilini et al., 2012; Levin and Sibuet, 2012). In these habitats, chemosynthetic microbial communities convert released hydrocarbons into energy that supports the surrounding ecosystem (summarized in Levin et al., 2016). Abiotic factors (i.e. fluid flux and composition) shape the microbial community (Knittel and Boetius, 2009; Sahling et al., 2002) which, in turn, structures the distribution of the associated macro- and megafauna (Cordes et al., 2010; Levin, 2005). A diversity of chemosynthetic production occurs within the sediment including: (1) the anaerobic oxidation of methane (AOM) carried out by Archaea belonging to the "ANME" group in consortia with sulfate-reducing bacteria (SRB; Knittel and Boetius, 2009; Orphan et al., 2001); (2) sulfide oxidation (thiotrophy) by mat forming microbes (i.e. *Beggiatoa* and *Thioploca)* and non-mat forming linages (i.e. Campylobacterales); and (3) aerobic methane oxidation performed by Gammaproteobacteria (Valentine, 2011). The distribution of these microbial taxa is thought to be driven by the rate of fluid flow from the subsurface (methane supply), which also impacts the metazoans present, including clam and Siboglinidae beds (Bernardino and Smith, 2010; Boetius and Suess, 2004). Recent studies have found methane to be deterministic in the

---

* Corresponding author.
E-mail address: seabroos@oregonstate.edu (S. Seabrook).

https://doi.org/10.1016/j.dsr2.2017.10.016

Please cite this article as: Seabrook, S., Deep-Sea Research Part II (2017), https://doi.org/10.1016/j.dsr2.2017.10.016

Table 1
Sampling sites off the Washington/Oregon/California Margin, 40–48°N, during E/V *Nautilus* cruise NA072 and off of British Columbia, Canada), 47–49°N, during TG Thompson (TN328), and E/V Nautilus (NA071) cruises.

Cruise Number	Date Sampled	Site	Latitude (N)	Longitude (W)	Depth (m)	Temperature (°C)	Oxygen (μmol /L)	Habitat description
NA072	June 2016	Juan de Fuca	48.1015	125.5544	150	6.67	90.48	Microbial mats[a]
NA072	June 2016	Nehalem Bank	45.8837	124.6434	190	6.75	66.61	Microbial mats (orange and white)[a]
NA072	June 2016	Astoria Canyon	46.2422	124.6494	850	3.76	4.78	Microbial mats[a], polychaete beds, exposed hydrates, pockmarks
NA072	June 2016	Astoria Canyon SW Wall SW wall	46.2224	124.6564	495	5.02	24.46	Clam beds[a],authigenic carbonates
NA072	June 2016	Heceta SW	43.9109	125.0756	1225	3.05	16.26	Siboglinidae assemblages[a], microbial mats[a], clam beds[a] polychaete beds, authigenic carbonates, exposed hydrates, pockmarks
NA072	June 2016	Coquille SW	42.7107	124.9011	615	4.61	7.43	Microbial mats[a], clam beds[a], authigenic carbonates
NA072	June 2016	Klamath Knoll	41.4274	124.8917	735	4.07	5.27	Clam beds[a], microbial mats (orange and white), authigenic carbonates
NA072	June 2016	Trinidad Canyon[b]	41.1385	124.9443	2149	1.98	56.90	Siboglindae assemblages, gooseneck barnacles, live and dead clam bed assemblages, patches of reduced sediment, authigenic carbonates
TN328, NA071	Sept 2015, May 2016	Clayoquot Slope	48.6707	126.8478	1250	2.85	13.4–22.3	Fine-grained sediments[a], methane gas, bubbles, extensive microbial mats, clam beds[a], methane gas hydrates
TN328, NA071	Sept 2015, May 2016	Barkley Canyon	48.3166	126.0508	985	3.61	6.7–13.4	Fine-grained sediments[a], microbial mats, methane gas hydrates, exposed hydrate mounds, clam beds
NA071	May 2016	Endeavour	47.9493	129.0983	2300	2.47	54.46	Mid-Ocean Ridge; Hydrothermal vent field rich with fissures, pillow lava deposits sulfide towers, rocky outcrops, fine grained sediment[a]
TN328	May 2015	Cascadia Basin	47.7627	127.7589	2660	1.78	72.56	Abyssal Plain; fine-grained sediment[a]

[a] Indicates habitat sediment core was taken from.
[b] Not sampled.

composition of the microbial community in comparison with non-seep habitats (Ruff et al., 2016).

Patterns in biogeography have traditionally been thought to be driven by either local environmental factors (e.g. biogeochemistry), historical geologic events (e.g. geographic isolation), or a combination of both. On large spatial scales (10–20 thousand km) the physical distance between microbial communities has been shown to drive the community structure, while at intermediate spatial scales (10–3000 km) both environmental conditions and physical distance structure the community, and at small spatial scales (0.1–0.3 km), the environmental conditions are the most deterministic (Martiny et al., 2006). Seep habitats exemplify the effect of local environmental variables with distinct vertical and horizontal gradients in the microbial community, which are governed primarily by the availability of electron acceptors (Knittel and Boetius, 2009; Lloyd et al., 2010; Ruff et al., 2015). The distinct biogeochemical processes present within seep habitats correlate to distinct indicator taxa (e.g. ANMEs and SRBs) that contrast with cosmopolitan species typically associated with non-seep marine sediments. The cosmopolitan species drive similarities among the microbial communities of marine sediments globally, particularly at the phylum level (Ruff et al., 2015). However, at the class level and lower these indicator taxa create distinctive microbiomes that are found across spatial scales, with suggestion that cold seeps are island-like habitats that do not necessarily fit traditional models of microbial biogeography (Ruff et al., 2015). Recent discoveries on the pervasiveness of seeps across continental margins provides the opportunity to further disentangle the role of spatial separation and fluid flow in structuring the seep microbiome.

The geologic dynamics of the Cascadia Margin are ideal for the formation of seep habitats. This margin is situated on the accretionary wedge that is associated with the Juan de Fuca subduction zone. The geologic setting of this region yields an environment suited for the migration of subsurface gases to the surface (e.g. Torres et al., 2004; Tréhu et al., 1999). Within the past few years, the known areas of seepage on the Cascadia margin have increased from a few to over five hundred (Bell et al., 2017; Johnson et al., 2015). This margin includes sites that have been studied extensively (e.g. Hydrate Ridge; Boetius and Suess, 2004) and have helped shape our current understanding of methane biogeochemistry (e.g. Hinrichs et al., 1999; Marlow et al., 2014; Orphan et al., 2001). Further, this is a region where productivity, seepage, and oxygen gradients are common and have been shown to impact the composition of fauna present in the region (De Leo et al., 2017; Guilini et al., 2012; Levin et al., 2010). More recently, advances made possible by the installation of Ocean Networks Canada's NEPTUNE cabled observatory, have been allowing continuous and long-term monitoring of cold seep environments in Barkley Canyon (Barkley Hydrates) and Clayoquot Slope, sites located ~500 km north from Hydrate Ridge. This, together with the exploration efforts along the rest of the margin, creates an opportunity for us to delve into the complexity of seep environments along the Cascadia Margin, including potential drivers of modulations in seepage and biogeography of seep fauna.

While we have learned much about the microbial fauna of the Cascadia Margin, most of this has been focused on a few known sites. Here we describe the habitat types, faunal associations, and the microbial communities present at 8 newly discovered seep sites along the margin, as well as at seep and non-seep sites that have being monitored for nearly 6 years in the context of the NEPTUNE cabled observatory. We use these data to ask:

1. What 'habitats' are present at the 8 recently discovered seep habitats?
2. What is the Cascadia Margin methane seep microbiome?
3. How variable is the microbial community among sites?
4. Are there any biogeographic patterns that suggest potential drivers of faunal distributions?

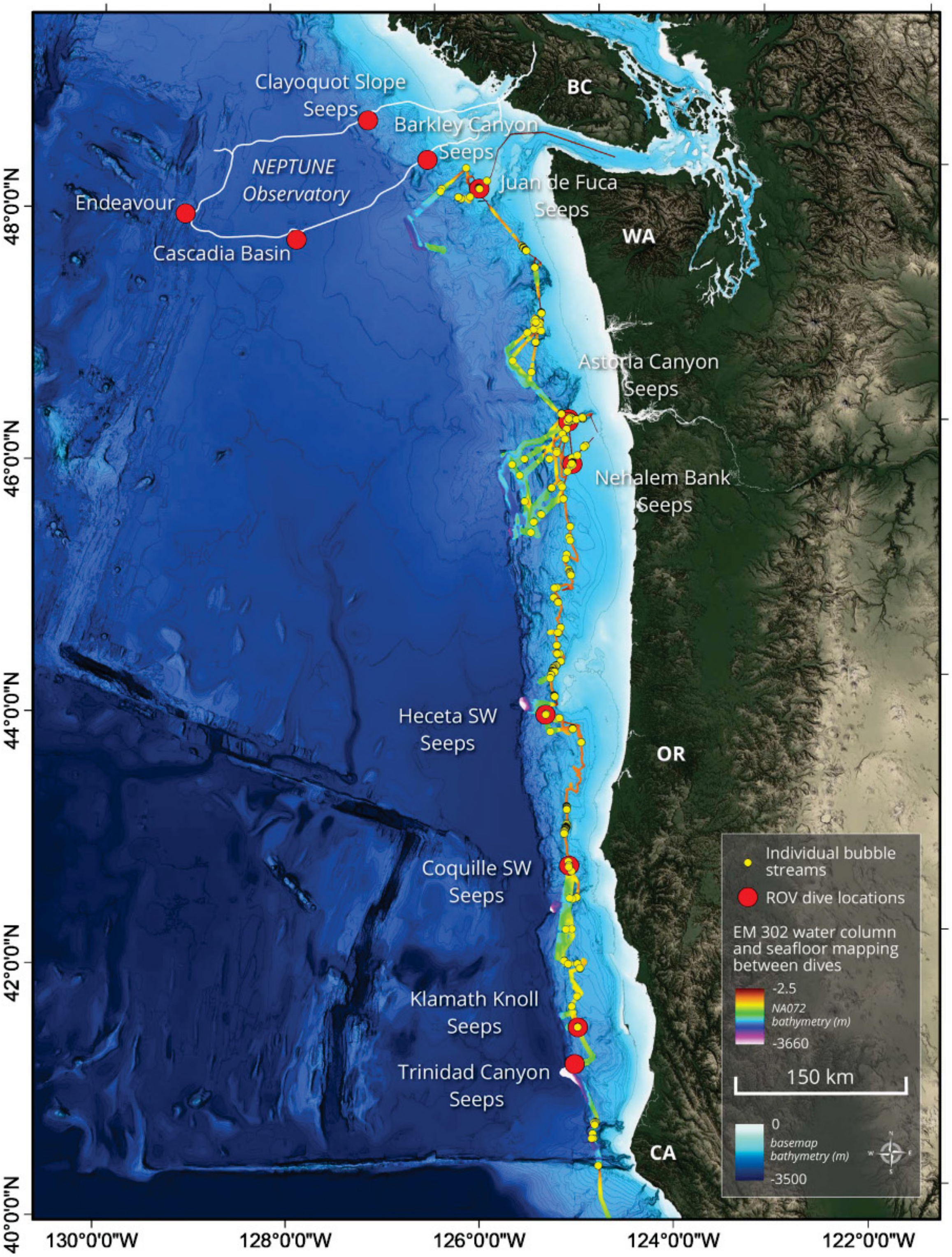

Fig. 1. Study sites included in this paper. Sites within the Neptune Observatory of Ocean Networks Canada are indicated as well as the sites sampled on the NA072 cruise aboard the E/V Nautilus with Ocean Exploration Trust. Yellow dots represent individual bubble plumes that were identified via multibeam, red dots represent sites explored with ROV. The NA072 sites and bathymetric data were compiled by S. Merle, NOAA PMEL and Oregon State University. The basemap was produced based on USGS Cascadia digital elevation model (report 99–369), and global seafloor topography data from Smith et al. (1997). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Methods

2.1. Study sites and sampling

Samples from the Oregon and Washington margins were collected during Cruise NA072 aboard the *E/V Nautilus* which focused on the Cascadia Margin, defined here as the region between 40–48°N off the west coast of North America. Push core samples (internal diameter 6.4 cm) were taken by the *ROV Hercules* from seep habitats at each of the sites (Table 1, Fig. 1). Upon retrieval, cores were extruded, sectioned at 1 cm intervals with the sides of the cores discarded to avoid smearing and frozen at −80 °C. Samples were also collected further north (47–49°N) along the Cascadia Margin off of British Columbia, Canada, and within the long-term seafloor monitoring sites of Ocean Networks Canada's (ONC) NEPTUNE cabled observatory (Barnes 2007). Push core samples of the same internal diameter were collected during two of ONC's yearly maintenance cruises, with *ROV Jason* aboard *R/V TG Thomson* (Cruise TN328, Sept 2015), and *ROV Hercules* aboard *E/V Nautilus* (Cruise NA071, May-June 2016; Table 1). These sediment cores were vertically sectioned at 0–1, 1–2, 2–3, and 3–5 cm intervals, and also preserved at − 80 °C.

2.2. DNA extraction and 16S rRNA gene amplification

DNA was extracted from 0.25 to 0.30 g of sediment from the vertically sectioned push core samples with the DNeasy PowerSoil Kit (Mobio/Qiagen) following manufacturer's instructions. The V4 region of the 16 S rRNA gene was PCR-amplified with 5PRIME HotMasterMix in triplicate with 515fb and 806rb primers that were bi-directionally barcoded to facilitate multiplexed sequencing following the Earth Microbiome Protocol (Caporaso et al., 2012; Apprill et al., 2015). Amplicons were pooled, quantified using a Qbit and cleaned using the Qiaquick PCR purification kit (Qiagen). Bi-directional sequencing was performed on the Illumina MiSeq platform using the V2 chemistry (2 × 250 bp) at the Center for Genome Research and Biocomputing (CGRB) at Oregon State University.

2.3. Microbial community analysis

Sequences were aligned, and quality filtered using mothur v39 (Schloss et al., 2011) and Usearch7 (64 bit; Edgar, 2010). Archaeal and bacterial sequences were clustered using QIIME v. 1.9.1 at 97% Operational Taxonomic Unit ($OTU_{0.03}$) and assigned by comparison to the Silva v123 database formatted for QIIME (https://www.arb-silva.de/download/archive/qiime/). For complete pipeline see Supplementary materials. The 16 S rRNA gene sequences are archived in the National Center for Biotechnology Information public database under accession SRP107137 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA386387).

To compare the community composition and structure across the sites we used both univariate and multivariate statistical analyses to visualize and quantify differences in the community structure. Aligned sequences were rarefied to the least abundant quality-filtered sequences per sample after omitting those with uncharacteristically low sequencing success (which was 16,801 sequences per sample for this project) after which they were summarized into discrete taxonomic levels on QIIME v. 1.9.1. Bray-Curtis similarity comparisons on log transformed rarified $OTU_{0.03}$s were used to generate nonmetric multidimensional scaling (nMDS) plots and cluster diagrams which were used to visualize patterns in the community structure. PERmutation Multivariate ANalysis Of VAriance (PERMANOVA, McArdle and Anderson, 2001) was used to identify significant differences among sites, regions, habitat types and sediment vertical fractions. The levels of similarity among samples and groups were measured using the SIMilarity PERrcentage Analysis (SIMPER, Clarke and Warwick, 2001), and species richness, diversity indexes and rarefaction curves were calculated and compared across sites. Multiple regression analysis was used to identify the main drivers diversity. The factors considered in these analyses were depth, bottom temperature, dissolved oxygen, and latitude. All multidimensional analyses were performed using the software package PRIMER v7 with the PERMANOVA+ add on (Clarke and Gorley, 2015; Anderson et al., 2008).

3. Results

3.1. Site descriptions

3.1.1. Juan de Fuca

This seep was found at 150 m depth and consisted of numerous patches of white thiotrophic mats (Table 1, Fig. 2a). No bubbling was observed in situ, although bubble plumes were observed by multibeam. In addition to the mat, there were patches of reduced (black) sediment (*sensu* Ritt et al., 2011) indicative of active methane release from the habitat. No seep-endemic megafauna nor authigenic (seep-derived) carbonates were observed at this site.

3.1.2. Astoria Canyon SW Wall

Two seeps were sampled from the Astoria Canyon region (Fig. 1), a shallow one (495 m) which was located on the wall of the canyon and a deeper one (see below). The shallow one (Astoria Canyon SW Wall) had dense vesocomyid clam and authigenic carbonates (Fig. 2b); the carbonates served as a substrate to macroinvertebrates including cold water corals. Bubble plumes were abundant at this site.

3.1.3. Astoria Canyon

The deeper site at Astoria Canyon, within the oxygen minimum zone (OMZ; 850 m, 4.78 µmol/L O_2), had a diversity of seep habitats including pockmarks, tube-dwelling polychaete beds (*sensu* Thurber et al., 2014), microbial mats, exposed hydrates and reduced sediments (Fig. 2c). At this site there were also gastropods with what appeared to be thiotrophic bacteria growing on their back. Bubbling was observed at various locations in the canyon, corresponding to multiple plumes in the multibeam data.

3.1.4. Nehalem Bank

The seep habitats discovered in Nehalem Bank (190 m) consisted of sparse microbial mats and episodic bubbling events were also observed. Both white and orange microbial mats (Fig. 2d) were found at this location and a persistent oil slick was found on the surface of the water overlying this site.

3.1.5. Heceta SW

The Heceta SW site (1225 m) had a mosaic of dense seep habitats (Fig. 2e,f). Habitats present included Siboglindae bushes (of the vestimentiferan type here and throughout) and vesocomyid clam beds, polychaete beds, sparse *Acharax* spp. individuals, as well as distributed authigenic carbonates and pockmarks. The varied seep habitats hosted a diversity of fauna including anemones, sponges, crustaceans, and gastropods. The site also had exposed hydrates and intense bubbling events.

3.1.6. Coquille SW

The Coquille SW site, within an OMZ (615 m, 7.43 µmol/L O_2), included authigenic carbonates, clam beds, and microbial mats (Fig. 2g). The clam shells had what appeared to be arborescent foraminifera covering them. Authigenic carbonates were distributed throughout the Coquille SW site and provided substrate for many sessile invertebrates, including cold water corals. Several bubbling events were observed during the exploration of this site.

3.1.7. Klamath Knoll

Occurring within an OMZ (735 m, 5.27 µmol/L O_2), this site consisted of extensive carbonate platforms with sediment channels interspersed and thin sediment layers on top of the authigenic carbonates. Microbial mats (orange and white variations) and clam beds were present, with the clam beds typically occurring within sediment channels. Many cold water coral species, sponges, and anemones were adhered to the abundant authigenic carbonates. A region of intense sporadic bubbling was found on the edge of a carbonate overhang (Fig. 2g).

3.1.8. Trinidad Canyon

Although not sampled, the few instances of seep endemic fauna at Trinidad Canyon (2149 m) were notable. At this site, we discovered evidence of what appeared to be a largely inactive seep habitats

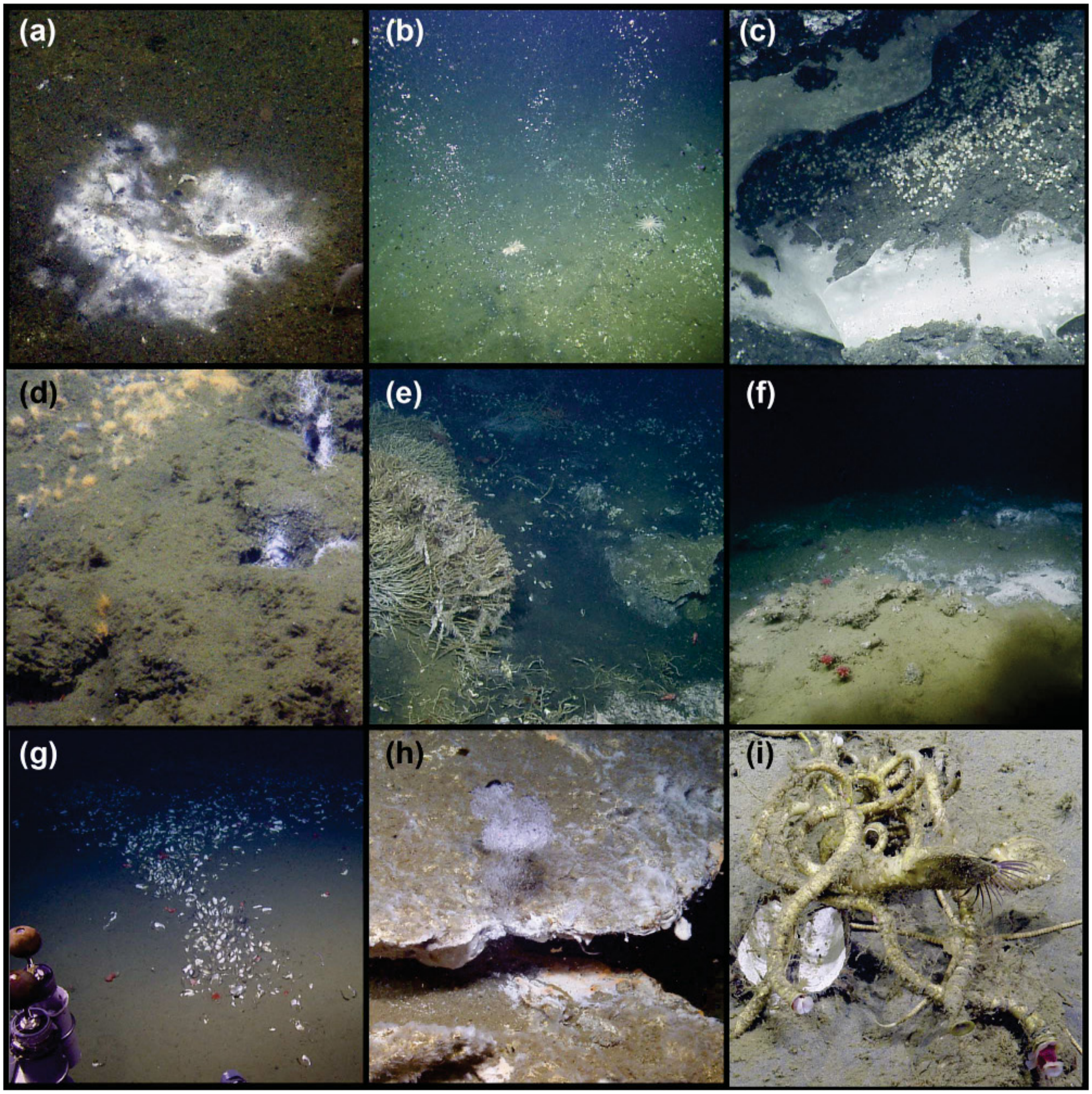

Fig. 2. Representative photos of sites sampled on the NA072 exploration of the Cascadia Margin: (a)microbial mat observed at Juan de Fuca seep, (b) authigenic carbonates, clam beds, and bubbles observed at Astoria Canyon SW Wall, (c) exposed hydrates and gastropods with possible thiotrophic bacteria on shells at Astoria Canyon, (d) orange and white microbial mats at Nehalem Bank, (e) Siboglinidae tube worm assemblages, clam beds and microbial mats at Heceta SW, (f) authigenic carbonates and microbial mats present at Heceta SW, (g) extensive clam bed at Coquille SW, (h) authigenic carbonate, microbial mats, and bubble 'fizz' at Klamath Knoll, (i) tube-worm assemblage and gooseneck barnacle at Trinidad Canyon. All photos copyright Ocean Exploration Trust, Inc.

including beds of clam shells and potentially inactive authigenic carbonates. However, we also discovered small beds of live clams, patches of reduced sediment, live Siboglindae bushes, and a live gooseneck barnacle assemblage (Fig. 2i). These live assemblages suggested that there was still some degree of methane flowing through the sediment, despite the other areas appearing to be dormant/extinct seeps.

3.1.9. *Barkley Canyon*

The two sampling sites in Barkley Canyon are located adjacent to the northern flank and at mid portion of the canyon (Fig. 1). Barkley Axis, at 987 m, intersects the core of the OMZ offshore Vancouver Island (ranging 6.7–13.4 µmol/L O_2), with the seafloor consisting of a mixture of sandy and muddy sediments and patches of microbial mats. The Barkley Hydrates site, at 870 m, is situated in a 1 km^2 plateau slightly less than a kilometer west from Barkley Axis. A mosaic of exposed hydrate mounds, bacterial mats and muddy sediments compose the seafloor at this location (Thomsen et al., 2012; Chatzievangelou et al., 2016).

3.1.10. *Clayoquot Slope*

This seep site at a depth of 1250 m (near the bottom of the OMZ with values ranging from 13.4 to 22.3 µmol/L O_2) is located 20 km landward of the toe of the Cascadia subduction zone, and presents localized and temporally variable methane bubble emissions (Römer et al., 2016). Clam beds (*Calyptogena* spp.), carbonate mounds with the presence of ampharetid polychaete thickets, and high densities of sea pens in conspicuous associations with the ophiuroid *Asteronix loveni* in the area.

3.1.11. Cascadia Basin

In this abyssal site, at 2660 m of depth and ~100 km offshore from the Cascadia subduction zone, the seafloor landscape is typical of abyssal settings with a nearly flat topography and fine grained sediments with very little spatial heterogeneity at 10's of km scales. Local benthic megafauna is mostly constituted of echinoderms (holothurians and seastars), and macrourid fishes.

3.1.12. Endeavour

Located at the northern segment of the medium rate (6 cm/yr) spreading center known as the Juan de Fuca Ridge, the Endeavour vent field is perhaps one of the best-studied vent sites worldwide (Kelley et al., 2012). At 2300 m of depth, the sampling site at Endeavour is comprised of a highly three-dimensionally structured habitat with very tall sulfide towers and black smokers, pillow lava and other basaltic rock formations. The core sampled at Endeavour for this project was retrieved from sediment that did not overlay the vents and is considered to be reference sediment.

3.2. Overall trends in the microbial communities

A total of 1,663,299 sequences across the 104 samples were used to explore the microbial community structure. This resulted in the identification of 30,652 Bacterial and Archaeal OTU's recovered, with 19.6% belonging to Archaea and the remaining 24,617 OTUs classified as bacteria. The microbial communities identified in this study were most similar (indicated by SIMPER similarity percentages) within regional groupings: Oregon/California Margin seeps (40.6%), British Columbia Seeps (40.8%), and British Columbia reference sites (43.8%) (Fig. 3). Although there was only one sample from the Washington Margin, it was most similar to the British Columbia seeps (33.7%); the Washington Margin seep was 30% similar to the Oregon/California Margin seeps. There were significant differences among these groupings (PERMANOVA pseudo-F = 12.708, $p \leq 0.001$, significance of the pairwise comparisons based on this model are indicated below). The Oregon/California Margin seeps were significantly different from the British Columbia reference sites ($p \leq 0.001$) and the British Columbia seep sites ($p \leq 0.001$) and not significantly different from the Washington Margin seep ($p = 0.060$). The British Columbia seep sites were significantly different from the British Columbia reference sites ($p < 0.001$) but not significantly different from the Washington Margin sample ($p = 0.260$), while the British Columbia reference sites were significantly different from the Washington Margin sample ($p = 0.040$).

Within the aforementioned regional groupings, the microbial community structure was significantly different between the different habitat types (clam bed vs. microbial mat) of the Oregon/California and Washington Margin seep sites (PERMANOVA, pseudo-F = 11.41, $p \leq 0.001$, Fig. S1). We were unable to test the role of habitat in structuring the community at other locations, due to limited samples for comparison of both habitats at a given site. There were also significant differences between the vertical fractions of sediment sampled (pseudo-F = 2.00, $p < 0.001$, Table S1). The pairwise comparison based on this model revealed no differences among vertical fractions that were next to each other (i.e. 1–2 cm and 2–3 cm; $p > 0.050$) however there was a significant difference between those samples that were most separated vertically (i.e. 0–1 cm and 4–5 cm, and 0–1 cm and 3–5 cm; $p < 0.003$).

Surprisingly, there was an apparent decrease in species richness from north to south (Fig. 4). To quantify what factors may be driving this pattern, we utilized a multiple regression analysis including depth, oxygen saturation, temperature and latitude as explanatory variables. This identified that latitude best explained this pattern of OTU richness, solely explaining 29% of the variance ($r^2 = 0.29$, $p < 0.001$). All of the other factors except for oxygen correlated with each other and latitude so, due to concerns of co-variance, were excluded from further analysis. This covariance should be kept in mind when considering latitude as a driver of variance. Oxygen did not significantly increase the fit of the model. Exploring trends in the microbial community further with a multiple regression analysis based on the Shannon Diversity indexes for the samples and including the aforementioned variables revealed both latitude and oxygen concentration as the drivers of variance in OTU diversity. Notably, latitude ($p < 0.001$) was again the most significant driver, explaining 22% of the variance, while latitude and oxygen saturation ($p = 0.006$) together explained 30% of the variance.

3.3. Microbial community composition

The abundances of the top 19 bacterial Orders varied among sites with the most obvious differences between the groupings of Oregon Margin seep sites, Washington Margin seep site, British Columbia seep sites, and British Columbia reference sites (two-way crossed ANOSIM; $r_{spearman} = 0.87$, $p_{spearman} \leq 0.001$; Fig. 5). Within the Oregon and Washington Margin seep sites, the order Desulfobacterales was the most dominant, making up 20 ± 1.5% (± SE here and throughout) of the

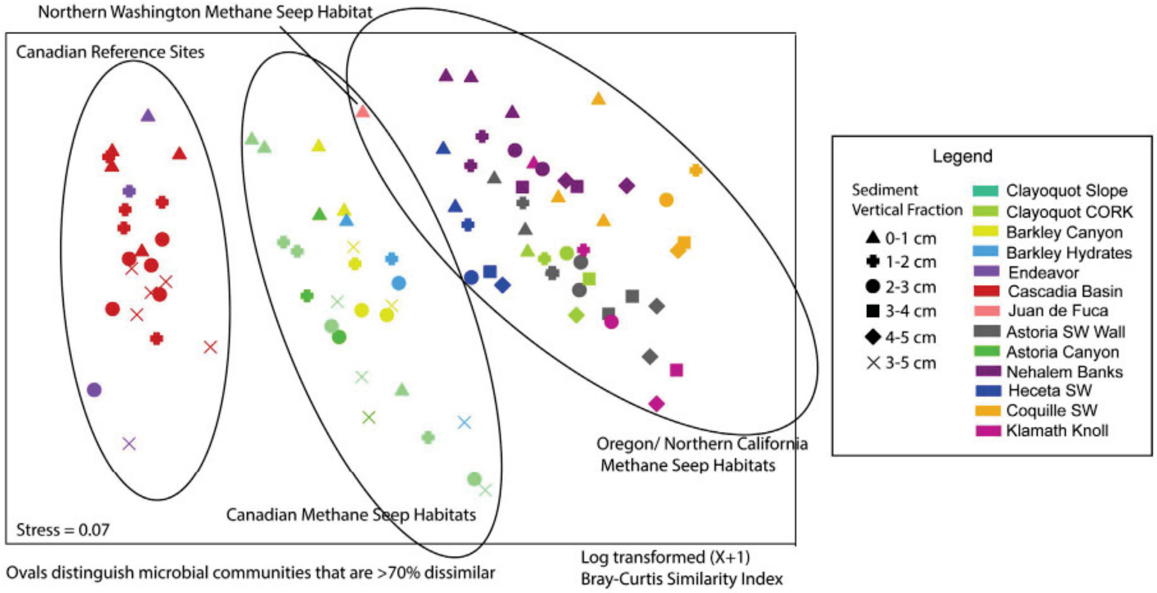

Fig. 3. nMDS plot of the ordination of the microbial community present at all sites sampled. Data was log-transformed (X + 1) and a resemblance matrix was plotted with the Bray-Curtis similarity index. Stress value indicated (accepted if stress value is < 0.17).

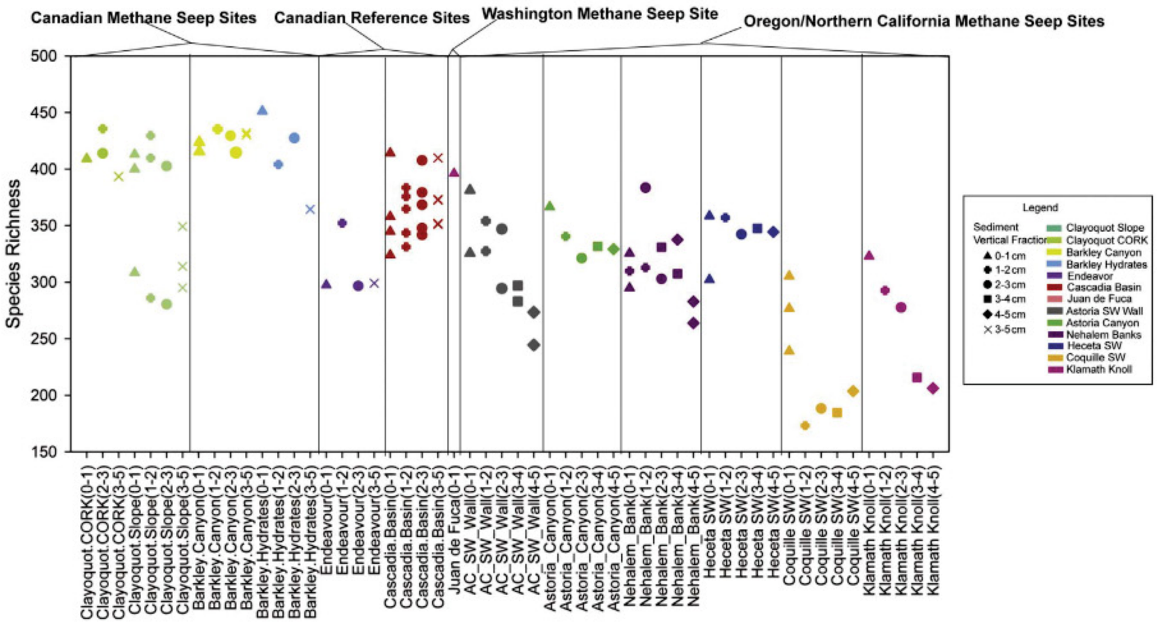

Fig. 4. Species richness at all sites sampled, with major groupings identified. Samples are plotted from North to South.

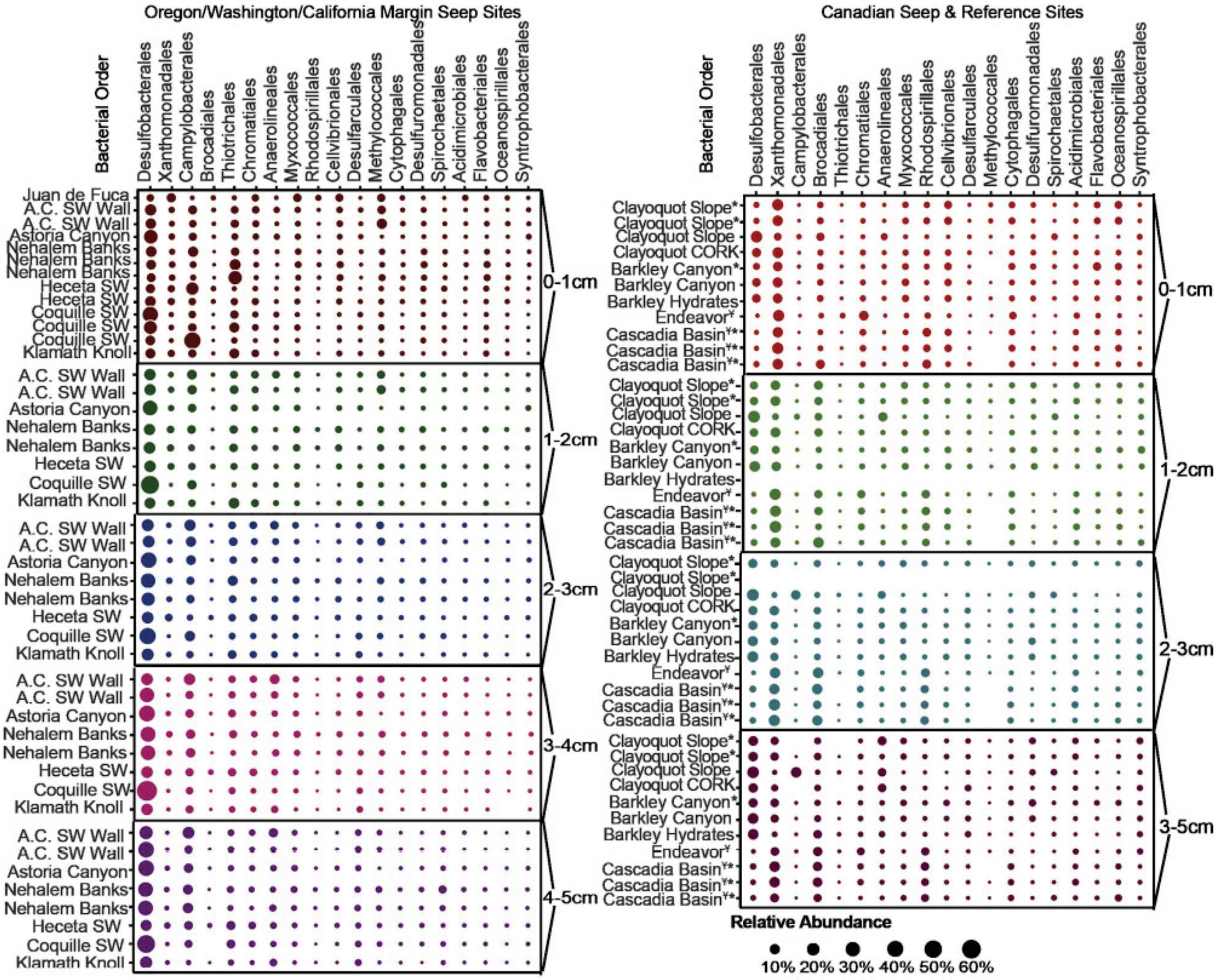

Fig. 5. Relative abundance of the most dominant Bacterial Orders at all sites sampled with the size of the circle representing the percent of the overall microbial community.

community composition. SRB's *Desulfobulbus*, *SEEP-SRB1*, and *SEEP-SRB2* were the main taxa within the Desulfobacterales at the Oregon margin seep sites comprising 6 ± 1.0%, 4 ± 0.5%, 1 ± 0.5% of the relative abundance, respectively. Camplyobacterales (*Sulfurovum* (4 ± 0.4%) and *Sulfurimonas* (2 ± 0.4%)), Thiotrichales (4.9 ± 0.5%), Chromatiales (2.5 ± 0.2%), Anaerolineales (2.3 ± 0.3%), and Methylococcales (2.3 ± 0.3%) were also abundant. In the sample from the Washington margin, Xanthomonadales was the most abundant bacteria at 8% along with the Deltaproteobacteria Sh765B-TzT-29 (6.7%), Myxococcales (6.6%), Methylococcales (5.9%), Cellvibrionales (5.6%), and Desulfobacterales (4.4%).

At the British Columbia reference sites, Xanthomonadales was the most abundant bacteria observed making up 11.7 ± 0.4% of the community. The Deltaproteobacteria Sh765B-TzT-29 was the next most abundant at the non-seep sites (6.5 ± 3% of the community). Brocadiales (associated with Anaerobic Ammonia Oxidation [ANNAMOX]; 6.4 ± 0.7%), Rhodospirillales (5.5 ± 0.2%), and Chromatiales (2.8 ± 0.3%) were also abundant.

In the British Columbia seep samples, Deulfobacterales was the most abundant at 8.5 ± 0.7% followed by Xanthomonadales (6.8 ± 0.6%), the Deltaproteobacteria Sh765B-TzT-29(6.2 ± 0.5%), and Brocadiales (3.6 ± 0.2%). There is variation with in this overall trend, however, particularly with the core that was taken from an area that appeared to be experiencing the greatest seep input (i.e. black sediment indicative of a reduced environment) that was sampled at Clayoquot Slope, where Campylobacterales increases in abundance throughout the core to 11.5% of the relative abundance at 3–5 cm, despite averaging 0.9 ± 0.5% for the British Columbia seep samples overall.

The most dominant Archaea were those normally associated with methane seep and benthic habitats with the orders ANME, Woesearchaea, Marine Group I, Thermoplasmata, and Marine Benthic Group B dominating (Fig. 6). The phylum Woesearchaea was the most abundant in the Washington Margin seep site, British Columbia seep sites, and British Columbia reference samples ranging from 4.0% of the microbial community at Cascadia Basin to 0.5% at the Juan de Fuca seep. The seep sites off of Oregon/California had ANME-1 as the most abundant Archaeal taxa, for which they composed 3.7 ± 1.3% of the community composition. Some of these sites had co-occurrences of all ANME types whereas others had just one ANME group dominating.

3.4. Vertical patterns in the abundance of key players in the seep microbiome of the Oregon Margin

3.4.1. Anaerobic methane oxidizers (ANME)

The distribution of ANME varied among sites in its composition and vertical distribution (Fig. 7). ANME-1b dominated, composing 5 ± 3.5% of the microbial community at 5 cm sediment depth, in both cores from Astoria Canyon SW Wall (Cores A and B). However, in Astoria Canyon SW Wall-B, ANME-2ab (2.7%) and ANME-1 (2.2%) were also present below 2–3 cm. Although ANME-1's did not dominate between 0 and 5 cm in the Nehalem Bank cores (Cores A and B), ANME-1a was present at 3–4 cm with a peak of 4.2% in Nehalem Bank-B. Only Nehalem Bank-A was sequenced below 5 cm and it showed an impressive dominance of ANME-1 peaking at 52% of the microbial community at 8–9 cm depth. Klamath Knoll was largely dominated by ANME-1a, with a sharp peak of 12.3% at 3–4 which coincided with a small peak of 2% in ANME-1.

While ANME-1 appeared to dominate the ANME community in certain cores, others had abundant ANME-2 lineages that showed differential depth distributions. For example, the ANME community between 0 and 5 cm at Nehalem Bank (Cores A and B) was dominated by ANME-2ab with a peak at the 4–5 cm depth (4.4 ± 3.4%). In addition, Astoria Canyon and Coquille SW samples had both ANME-2ab and ANME-2c increasing throughout the top 5 cm of sediment with ANME-2ab the most dominant (0.7% and 0.8%, respectively). Astoria Canyon also had a peak (0.2%) in the relative abundance of ANME-3 at 3–4 cm, but it was not dominant. The core sampled at Heceta SW had low overall relative abundance of ANME, however the ANME community that was present was dominated by ANME-3 with a peak at 3–4 cm depth, albiet at a low relative abundance (0.025%) of the microbial community; this contrasted with the other sites where ANME-2 and ANME-1 lineages dominated.

3.4.2. Sulfate reducing bacteria (SRB)

Deltaproteobacteria associated with sulfate reduction, which are in many cases known to be the syntrophic partners of ANME, followed similar trends of clear changes in vertical distributions and among site variance in composition (Fig. 8). Desulfobulbus, comprising an average of 6.8 ± 1.0% of the relative abundance of the microbial community, was the most dominant SRB in the 0–3 cm range at all sites, continuing to be the most dominant down to 5 cm at Astoria Canyon (peak at 3–4 cm of 15%), Heceta SW (peak at 2–3 cm of 7.8%), and Coquille SW (peak at 3–4 cm of 4.5%). After 3–4 cm in Astoria Canyon SW Wall (Cores A and B), SEEP-SRB1 becomes the most dominant SRB at 10 ± 1.0% relative abundance. This is coupled with an increase in both Desulfococcus and SEEP-SRB2 after 2–3 cm to 2.8% and 3.7%, respectively, in Astoria Canyon SW Wall-B. Similarly, in Nehalem Bank (Cores A and B), SEEP-SRB1 increases in relative abundance after 3–4 cm to 5.8 ± 2.6% at 4–5 cm concurrent with an increase in Nehalem Bank-B of Desulfococcus (1.8%) and SEEP-SRB2 (1.5%) to 4–5 cm. Desulfococcus also increases in Nehalem Bank-A to 7% of the relative abundance at 5 cm, and SEEP-SRB2 - although making up only 0.6% of the microbial community at 4–5 cm, increases to 26% of the relative abundance at 5–6 cm. Seep-SRB1 dominates in the Klamath Knoll core, reaching a maximum relative abundance of 11% at 4–5 cm. Desulfobulbus decreases throughout the 0–5 cm from 2% to 0.5% of the relative abundance in the Klamath Knoll core and SEEP-SRB2 increases from 0.01% to 2.6%, respectively.

3.4.3. Sulfide oxidizing bacteria

The sulfide-oxidizing Thiotrichales (mat-forming) order and the *Sulfurovum* (non-mat forming) genera also showed differential trends in their vertical distribution in the Oregon/California Margin seep samples (Fig. 5). At Astoria Canyon SW wall (Cores A and B), Thiotrichales decreased in relative abundance from 5.7 ± 0.5% of the microbial community at 0–1 cm depth to 2.8 ± 0.01% at 4–5 cm depth. Similarly at Nehalem Bank (Cores A and B) and Astoria Canyon, Thiotrichales decreased from 18.8 ± 0.6% and 3.4% at 0–1 cm to 2.7 ± 0.8% and 2.0% at 4–5 cm, respectively. Divergent from this, at Heceta SW and Coquille SW, Thiotrichales increased in relative abundance from 4.3% and 2.6% of the microbial community at 0–1 cm to 7.4% and 6.7% at 4–5 cm. There were no clear trends in the relative abundance of Thiotrichales at Klamath Knoll. At Nehalem Bank (Cores A and B) and Astoria Canyon, *Sulfurovum* increased in relative abundance from 0.6 ± 0.2% and 1.5% at 0–1 cm to 5.9 ± 1% and 3.4% at 4–5 cm, respectively. Similarly, *Sulfurovum* increased in relative abundance as well at Heceta SW from 2.7% at 0–1 cm to 4.1% at 4–5 cm. However, at Coquille SW and Klamath Knoll, *Sulfurovum* decreased in relative abundance from 13.7% and 3.0% of the relative abundance at 0–1 cm to 2.9% and 0.8% at 4–5 cm, respectively.

4. Discussion

4.1. Overall patterns in distribution and abundance

A range of seep habitats were observed throughout the explorations of the Cascadia Margin. This included: microbial mats (both orange and white variations), clam and tube-dwelling polychaete beds, siboglinid assemblages, and sparse instances of *Archarax* spp. and gooseneck barnacles. The heterogeneity within and among seep biomes is highlighted by this variation in the observed megafaunal assemblages. Notable is the discovery of vestimentiferan siboglinids at Heceta SW

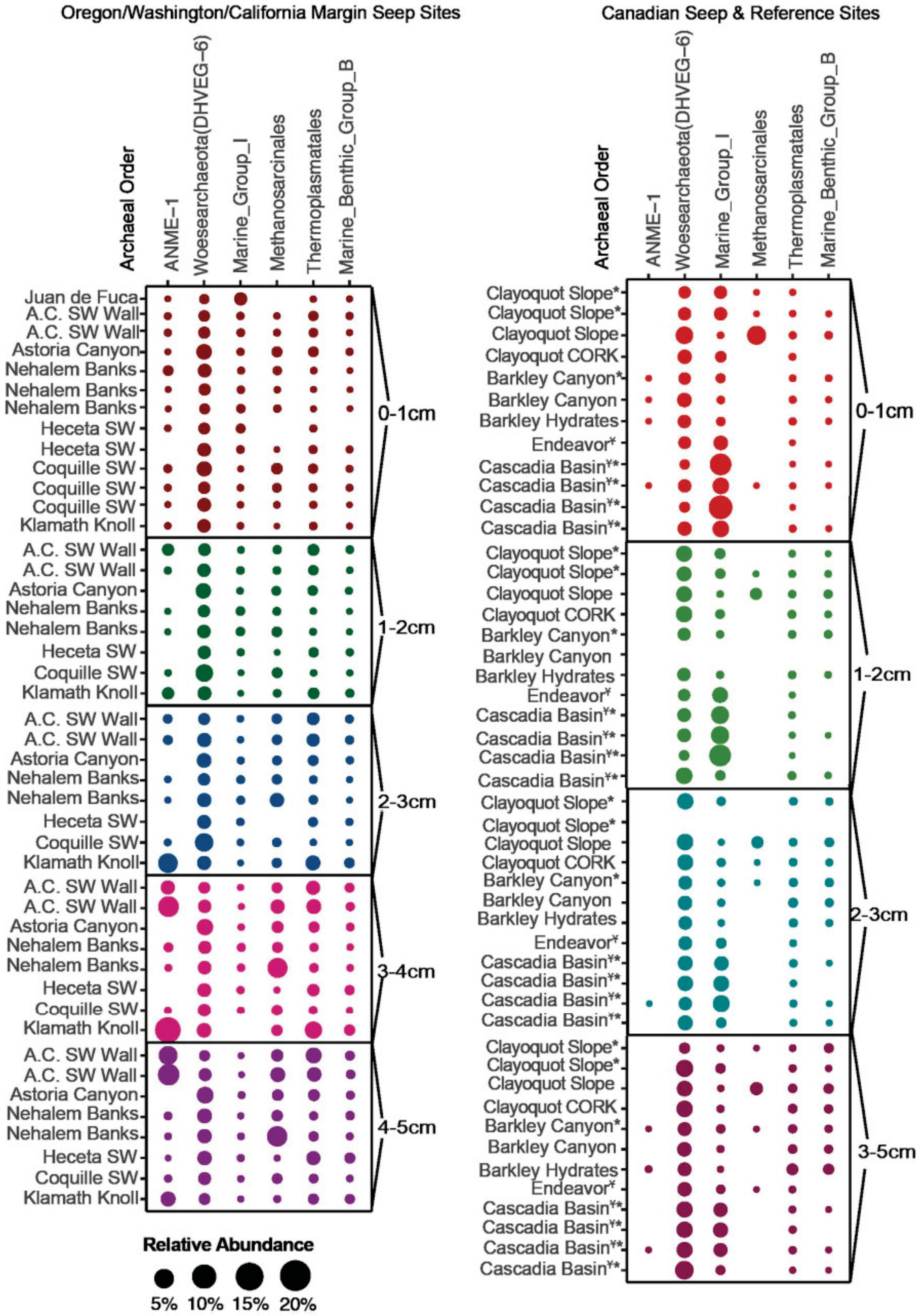

Fig. 6. Relative abundance of the most dominant Archaeal Orders at all sites sampled with the size of the circle representing the percent of the overall microbial community.

and Trinidad Canyon, adding to the limited reports of this group within seep habitats in the NE Pacific (see Bernardino and Smith, 2010; Grupe et al., 2015; Kulm et al., 1986). The discovery of vestimentiferan siboglinids at Heceta SW was particularly interesting because of the similarity of this habitat to Hydrate Ridge and Eel River Basin, which occur in areas that also experience lower oxygen concentrations associated with the OMZ and have been extensively explored but conspicuously lack vestimentiferan siboglinids (Levin et al., 2010). One

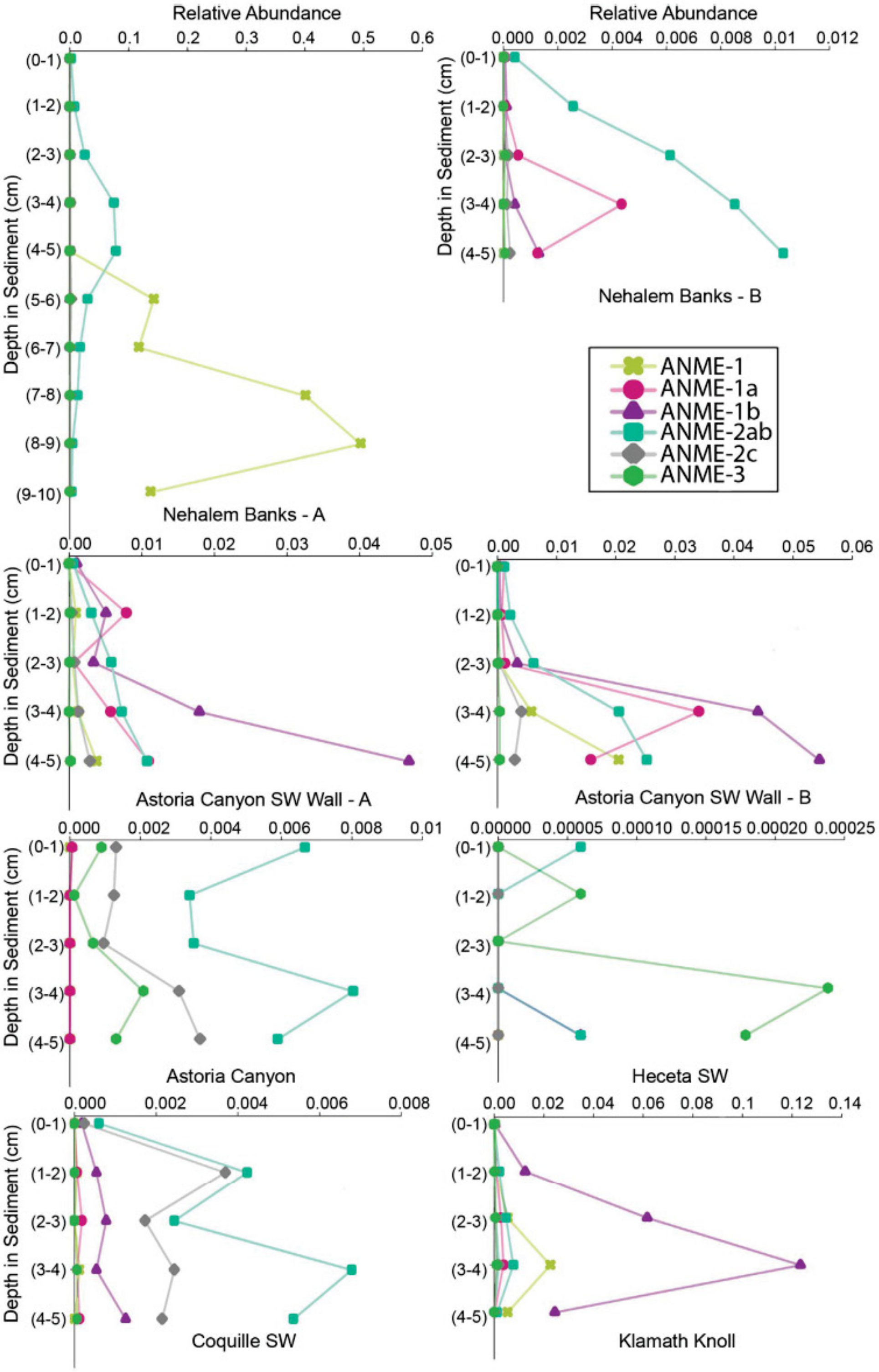

Fig. 7. Vertical distribution of the relative abundance of anaerobic methane oxidizers (ANME) groups within sediment cores from Oregon and California margin seep sites.

guiding hypothesis on why these taxa do not occur at Eel River Basin and Hydrate Ridge has been that they both occur within or near the upper bounds of the OMZ and low oxygen has been suggested to exclude vestimentiferan siboglinids (and bathymodiolin mussels) from these locations. While this may be the case for why the vestimentiferan siboglinids are present at Trinidad Canyon, a site that is well below the permanent OMZ (56.90 μmol/L O_2), Heceta SW lies just below the most intense core of the OMZ and has hypoxic conditions (16.26 μmol/L O_2). Eel River Basin methane seep occurs slightly shallower than Heceta SW and in even more normoxic water, making the occurrence of vestimentiferan siboglinids at Heceta SW surprising and challenging the notion that oxygen concentration alone drives the lack of vestimentiferan siboglinids. The discovery of gooseneck barnacles at Trinidad Canyon was also notable as the gooseneck barnacles include groups that are vent-endemic with interesting biogeographical and evolutionary distributions (Herrera et al., 2015) but are not common members of methane seeps (but see Yamaguchi et al., 2004). Large gooseneck barnacles do include non-reducing habitat groups as well. While it is unclear whether the one we observed is a previously unknown seep endemic, or just a deep-sea species taking advantage of the

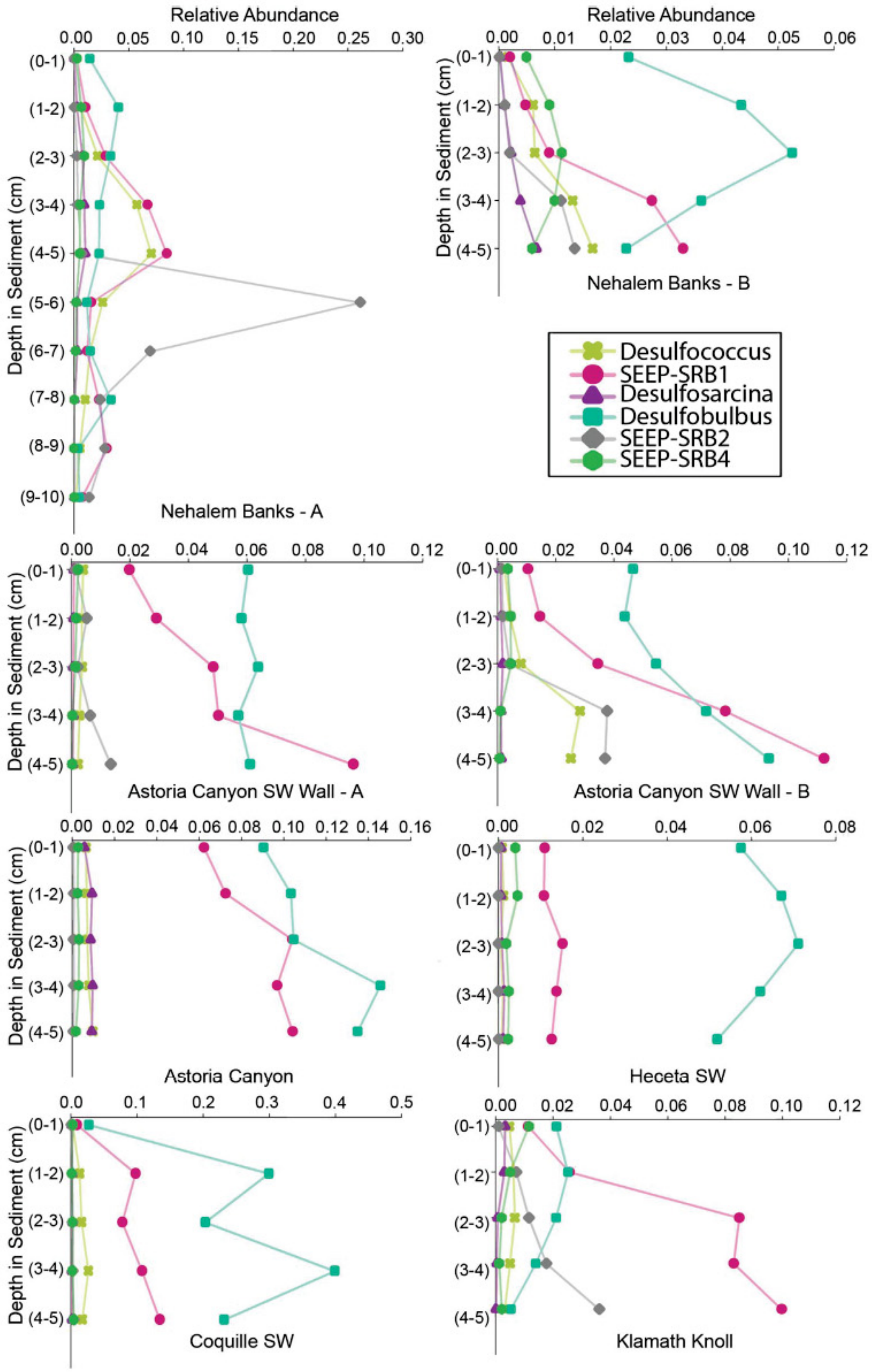

Fig. 8. Vertical distribution of the relative abundance of the main sulfate reducing Deltaproteobacteria (SRB) within sediment cores from Oregon and California margin seep sites.

hard substrate provided by the Siboglinidae tubes, this increases the fauna known to inhabit seeps in this region.

Seep communities are a result of both the current and historic dynamics of the region with multiple successional patterns proposed. Consequentially, fluid flow rate and duration of seepage can influence the fauna present. At many seep systems the rate of fluid flow is thought to drive the patterns of fauna (e.g. Bernardino et al., 2010; Sahling et al., 2002). Microbial mats occur in areas of higher methane and sulfide flux whereas clam beds have lower or oscillating flow regimes (Tryon et al., 2002) and Siboglinidae's are intermediate in their flux regime (summarized in Bernardino et al., 2010). Taxonomic distribution may also be driven by successional stages of a seep in addition to seepage rate. It has been posited that upon the onset of seepage, microbial mats appear which are then replaced by clam beds and/or siboglinid assemblages as the seep matures. As the seepage continues, authigenic carbonates form as a byproduct of the anaerobic oxidation of methane that in turn provide substrate for background communities (Bowden et al., 2013; Cordes et al., 2009). Thus, the extent and rate of seepage can both lead to similar patterns of fauna. While this presents a challenge for interpreting certain faunal groups in seep habitats others are more clear. For example, habitats that had a diversity of seep habitats (defined by the fauna present) and that had extensive carbonate features (i.e. Heceta SW, Astoria Canyon sites, Coquille SW, and Klamath Knoll) are likely seeps that have been persistent for long time periods and/or experience sustained and higher flow than others. In contrast sites with mostly microbial mats (i.e. Juan de Fuca and Nehalem Bank) may either be relatively recent features or experience lower flux that does not lead to the development of more expansive or diverse habitats.

Another surprising trend was that many of the taxa that are often found as dominant in seeps co-occurred to a greater extent than previously expected and a few taxa were more dominant than expected. Particularly, the different ANME lineages often co-occur but one dominates (Knittel and Boetius, 2009). Here we observed some of the sites with extreme dominance (e.g. Nehalem Bank and ANME-1) and other sites where there was a more even distribution (Astoria Canyon SW Wall). Additionally, ANME-2 lineages have been shown to be dominant at other known seeps in the region (i.e. Hydrate Ridge; Knittel and Boetius, 2009) and although we observed this at some sites, in many instances ANME-1 lineages comprised the majority of the ANME present. Further ANME- 3, often found in mud volcanoes, was present at some of our sites, including being the dominant ANME type at Heceta SW (albeit at a low total percentage of the microbial community). The spatial scale and survey approach taken here can help us understand the distribution of microbial taxa and both further refine and augment distribution patters (such as those proposed by Ruff et al., 2015).

4.2. Small scale variability

Methane seeps are an area with large gradients in the biogeochemical composition over small vertical spatial scales within the sediment. The sediment has often become anoxic within the first mm of the sediment and sulfate concentrations can approach zero within the top few cm of sediment in areas of high seepage. These steep chemoclines lead to niche specification within the microbial community (Boetius and Suess, 2004; Cordes et al., 2010; Macalady et al., 2008). The sulfide oxidizing groups of bacteria are thought to be influenced by these steep chemoclines (Pjevac, 2014). Microbial mats, most often dominated by Thiotrichales of the generas *Beggiatoa* and *Thioploca,* have been found to be more prominent in diffusive controlled niches with high sulfide/oxygen levels and are often most abundant at the sediment surface (Macalady et al., 2008). This contrasts with *Sulfurovum* and other Camplyobacerterales types that are thought to dominate in areas of increased sulfide flux and lower concentrations of oxygen, sometimes even using nitrate rather than oxygen as the electron acceptor (Grünke et al., 2011; Nguyen, 2016; Pjevac, 2014). Within the microbial mats at the Cascadia Margin seeps, we found Thiotrichales to be more abundant in the surface sediment with the Camplyobacterales (i.e. *Sulfurovum* another sulfide oxidizing group), more dominant in deeper sediment layers. Thus, the distribution of Thiotrichales and Camplyobacterales observed at out sites fit the distribution patterns found elsewhere, with Thiotrichales dominating the surface and better oxygenated regions with the Camplyobacterales found deeper in the sediment.

Clam beds, which often have a sulfide peak at or below 4 cm depth in the sediment, were present at Astoria Canyon SW Wall, Heceta SW, Coquille SW and Klamath Knoll (Sahling et al., 2002; Valentine et al., 2005). At these sites *Sulfurovum* was found to increase with depth down to 5 cm, except from at Coquille SW and Klamath Knoll where *Sulfurovum* decreased from 0 to 1 cm to 4–5 cm. The core from Klamath Knoll was taken from a clam bed within a sediment filled channel, a physically different environment than the other clam beds sampled. This potentially contributed to its divergence in community structure, and in the abundance of *Sulfurovum,* due to the hydrodynamics caused by the adjacent carbonate outcrops. Another core that had a unique vertical distribution of the microbial community compared to the other cores was a core that was collected from a clam bed in Coquille SW. The uniqueness of this core could have been partially driven by what appeared to be aborescent foramnifera growing on clam shells and tufts of microbial mat distributed throughout the bed. The presence of protists such as aborescent foramnifera in seep environments has been shown to increase habitat heterogeneity and contribute to carbon cycling (Pasulka et al., 2017). The marked difference in the microbial community between this core, collected from a clam bed, and the others sampled in clam beds was indicated with a greater similarity of this core to other cores collected from microbial mats at 0–1 cm (Fig. S1). In this particular case it is difficult to disentangle if the abundance of the protists is driving this or if the protists are a result of the biogeochemistry of the environment.

Both the Astoria Canyon core and the Heceta SW core showed little vertical trend in abundance in microbial taxa. The Astoria Canyon core, taken from a dense microbial mat, was from an area of significant bubbling and surrounded by exposed hydrates. Likely, in this core, the lack of a clear vertical trend with the microbial community was due to 'bubble-turbation' of the sediment. This is similar to what occurs at mud volcanoes such as the Håkon Mosby Mud Volcano (HMMV), where sediment mixing alters the microbial community in the surface sediment (Lösekann et al., 2007). Unlike HMMV, aerobic methanotrophs did not comprise > 50% of the microbial community in this site (maximum of 11.5% of total microbial community), which may reflect the lower availability of oxygen within overlying water (Table 1). The high abundance of SRBs and ANMEs within the top few cms of sediment also suggest that oxygen penetration was limited within the Astoria Canyon microbial mat sampled. The other location that showed a similar trend was from a clam bed at Heceta SW with large clams present; there were also a few siboglinids within the sediment. Clams both bioirrigate and bioturbate the sediment (Wallmann et al., 1997), potentially smearing biogeochemical gradients, and often occur in areas where there is fluid flow both into and out of the sediment (Orphan et al., 2004; Tryon et al., 2002, 1999). So unlike the Astoria canyon core that we posit is bubble-turbated, the faunal turbation at this site likely led to the absence of a clear vertical trend with the microbial community within the top 5 cms. Further, sulfate leakage from the roots of siboglinids has been shown to occur, a process which stimulates the anaerobic oxidation of methane around the root area (Cordes et al., 2005, 2003). Thus the presence of siboglinids could have been removing vertical gradients and creating horizontal gradients, as a result of both the sulfate leakage from the roots, leading to horizontal rather than vertical chemoclines and the more 'smeared' structure of the microbial community at Heceta SW.

4.3. Biogeographic patterns

A surprising trend was the latitudinal gradient in seep microbial community. While we were unable to heavily replicate sampling from within any one site, our approach did allow us to collect a snap shot of the microbial community across significant spatial scales and environments. The sites ranged in depth from 190 m to 2149 m (Table 1), spanned 800 km of latitude, and varied from sparse microbial mats to massive hydrate outcrops with extensive bubble emission. While on a fundamental level, this highlighted the diversity of seep habitats and dynamics on the margin, it also allowed a holistic view of the seepage beyond one particular site, complimenting those studies that provide high resolution at a site-level spatial scale. With this approach we found that there were significant shifts in the community composition going from the sites off Vancouver Island to the more southerly sites.

The composition of seep communities studied here were dominated by SRBs, methanotrophs, and sulfate oxidizing bacteria while the non-seep sites were dominated by cosmopolitan bacteria associated with deep-sea sediments. While the role of seepage rates, successional patterns, and background fauna have been used to explain overall patterns of diversity in seep sediments in the past, the results here add to this by suggesting that large scale latitudinal trends may have an overarching influence on these previously observed patterns. Previous studies have described increased diversity and species richness of macrofauna in non-seep sites, with this observation attributed often to higher niche specification of seep communities (Grupe et al., 2015). This is in contrast to our findings, as we observed increased species richness of microbial taxa at more northerly seep sites compared to our reference sites.

Here, variance of both species richness and diversity with latitude suggest that latitude may be a driver in the composition and richness of the methane seep microbiome. The additional variance of diversity with oxygen saturation highlights the potential impact that variations in oxygen saturation, observed in areas such as OMZ, could have upon microbial communities. Together, this supports previous work indicating impacts on the heterogeneity of marine environments from variations in oxygen saturation (such as in OMZs; Guilini et al., 2012; Pasulka et al., 2016), while also revealing a potential new driver of community composition in seep microbiomes. We would like to point out that in some cases the areas sampled as seep habitats within the ONC array system off Vancouver Island did not appear to experience the same extent of seepage as those sampled more southerly, even though very active seep sites do occur in within the ONC sites. They were, however, very clearly seep habitats as ANME were present, and importantly, if one omits all of the British Columbia samples from the analysis there is still a latitudinal trend (Fig. 4). Additional focused surveying efforts along the NE Pacific margin would help to further elucidate these possible trends in seep biogeography.

Intriguing trends in biogeographic patterns of reducing habitat communities are beginning to identify large scale patterns in species composition and diversity in microbial (Ruff et al., 2015) and animal communities (Bachraty et al., 2009; Van Dover et al., 2002). Biogeography of the deep sea is thought-provoking due to the dispersal mechanisms of fauna that must coexist with slow water movements, cold but stable temperatures, and low food supply. Reducing habitats in this context serve as additional disparate islands that aid in the survival of fauna leading to patterns where distance and chemical similarity have resulted in interesting constructs of the mechanisms that communities use to disperse. The patterns and explanations of biogeography in deep-sea macro- and megafauna communities are interesting (Baco et al., 1999; Herrera et al., 2015; Hilariio et al., 2015) and microbial communities have also been identified as having clear biogeographic and seasonal patterns in their distribution and composition (Ladau et al., 2013; Ruff et al., 2015). To understand the factors that drive these patterns of observed species distributions, sampling needs to occur on nested scales where similarity at different distances and the factors that drive those (or add to them) are quantified. Here, we have added to the overall understanding of large scale faunal distributions, adding to limited reports of siboglinids in the NE Pacific and the discovery of the gooseneck barnacle that may or may not be a seep endemic. Further, we have quantified both regional and local variability of the microbial community in seep habitats, contributing to important global comparisons that exist (i.e. Ruff et al., 2015).

5. Conclusions

Select seep habitats in the NE Pacific have been heavily studied (Hydrate Ridge, Eel River Basin), but we are just beginning to appreciate the extent and variability of seepage in the NE Pacific. Seeps are important to global carbon cycling, energy flow, and overall functioning of the earth system (Brooks et al., 2017; Sweetman et al., 2017; Thurber et al., 2014; Levin et al., 2016). In just two years, directed study of the continental margin off of Washington, Oregon, and Northern California resulted in > 500 individual bubble plumes (Bell et al., 2017). The seep habitats discovered in this study covered a range of depths (150–2149 m), latitudes, and oxygen concentrations. In addition to the microbial mats, authigenic carbonates and vesicomyid clam beds that are known in the NE Pacific, we also observed siboglinid tubeworm assemblages in a habitat that intersects the top of the OMZ and is similar to well-studied seep habitats (Eel River Basin and Hydrate Ridge) in which they have not been found. We found significant differences in the microbial community composition between regional groupings, habitat type (microbial mat vs. clam bed), and vertical fraction within the sediment. Trends in species richness were driven by changes in latitude while observed variance in diversity were driven by both latitude and oxygen concentration. This compliments previous studies where the main drivers of the seep microbial community were seafloor temperature and electron acceptor availability (Ruff et al., 2015). This study highlights the variability and complexity that exists within seep communities of the NE Pacific. This study also presents a framework for further studies of seep habitats by providing an overview of how variances in community structure can be used to more comprehensively constrain how geographic location and chemical, physical and geological dynamics contribute to the composition and functioning of seep ecosystems, beyond what we have learned from individual sites.

Acknowledgements

We would like to thank R. Embley, S. Merle, and the Captains and crew of the NA072 cruise aboard the E/V *Nautilus*. Cruise NA072 was operated by the Ocean Exploration Trust Inc. and funded by the NOAA Office of Exploration and Research. We also thank the Canada Foundation for Innovation for ship and ROV time devoted to the collection of push core samples during two of Ocean Networks Canada-NEPTUNE observatory maintenance cruises (TN328, NA071). We are indebted to the generosity of ONC in providing samples to facilitate this study and we particularly thank K. Douglas for assembling the final layout for the basemap in Fig. 1. A portion of S. Seabrook's time in the preparation of this manuscript was supported by the U.S. National Science Foundation, Office of Polar Programs 1642570 award to A.R. Thurber. A portion of this project was supported by the International network for scientific investigation of deep-sea ecosystems (INDEEP) supported by Foundation Total. This work is under PMEL contribution number 4673.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dsr2.2017.10.016.

References

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK.

Apprill, A., McNally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat. Microb. Ecol. 75, 125–137.

Bachraty, C., Legendre, P., Desbruyères, D., 2009. Biogeographic relationships among deep-sea hydrothermal vent faunas at global scale. Deep. Res. Part I Oceanogr. Res. Pap. 56, 1371–1378. http://dx.doi.org/10.1016/j.dsr.2009.01.009.

Baco, A.R., Smith, C.R., Peek, A.S., Roderick, G.K., Vrijenhoek, R.C., 1999. The phylogenetic relationships of whale-fall vesicomyid clams based on mitochondrial COI DNA sequences. Mar. Ecol. Prog. Ser. 182, 137–147. http://dx.doi.org/10.3354/meps182137.

Barnes, C. R. (2007). Building the world's first regional cabled ocean observatory (NEPTUNE): Realities, challenges and opportunities. In Oceans Conference Record (IEEE). ⟨https://doi.org/10.1109/OCEANS.2007.4449319 ⟩.

Bell, K.L.C., J., Flanders, A., Bowman, N.A.R., 2017. New frontiers in ocean exploration: the E/V Nautilus, NOAA Ship Okeanos Explorer, and R/V Falkor 2016 field season. Oceanography 30, 94. http://dx.doi.org/10.5670/oceanog.2016.supplement.01.

Bernardino, A.F., Smith, C.R., 2010. Community structure of infaunal macrobenthos around vestimentiferan thickets at the San Clemente cold seep, NE Pacific. Mar. Ecol. 31, 608–621. http://dx.doi.org/10.1111/j.1439-0485.2010.00389.x.

Boetius, A., Suess, E., 2004. Hydrate Ridge: a natural laboratory for the study of microbial life fueled by methane from near-surface gas hydrate. Chem. Geol. 205, 291–310. http://dx.doi.org/10.1016/j.chemgeo.2003.12.034.

Bowden, D.A., Rowden, A.A., Thurber, A.R., Baco, A.R., Levin, L.A., Smith, C.R., 2013. Cold Seep Epifaunal communities on the Hikurangi Margin, New Zealand: composition, succession, and vulnerability to human activities. PLoS One 8. http://dx.doi.org/10.1371/journal.pone.0076869.

Brooks, J.M., Kennicutt, M.C., Fisher, C.R., Macko, S.A., Cole, K., Bidigare, R.R., Vetter, R. D., 2017. Deep-Sea Hydrocarbon Seep Communities: Evidence for Energy and Nutritional Carbon Sources Published by: American Association for the Advancement of Science Stable. ⟨http://www.jstor.org/stable/1700859⟩ References Linked references are available on JS 238, pp. 1138–1142.

Brothers, L.L., Dover, C.L. Van, German, C.R., Kaiser, C.L., Yoerger, D.R., Ruppel, C.D., Lobecker, E., Skarke, A.D., 2013. Evidence for extensive methane venting on the southeastern U . S . Atlantic margin 807–810. ⟨http://dx.doi.org/10.1130/G34217.1⟩.

Caporaso, J.G., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. http://dx.doi.org/10.1038/ismej.2012.8.

Chatzievangelou, D., Doya, C., Thomsen, L., Purser, A., Aguzzi, J., 2016. High-frequency patterns in the abundance of benthic species near a cold-seep – an internet operated vehcicle application. Plos One. http://dx.doi.org/10.1371/journal.pone.0163808.

Clarke, K.R., Gorley, R.N., 2015. PRIMER v7: User Manual/Tutorial. PRIMER-E Ltd, Plymouth, 296pp.

Clarke, K.R., Warwick, R.M., 2001. Change in marine communities: an approach to statistical analysis and interpretation. PRIMER-E.

Cordes, E.E., Bergquist, D.C., Fisher, C.R., 2009. Macro-ecology of Gulf of Mexico cold seeps. Ann. Rev. Mar. Sci. 1, 143–168. http://dx.doi.org/10.1146/annurev.marine.010908.163912.

Cordes, E.E., Bergquist, D.C., Shea, K., Fisher, C.R., 2003. Hydrogen sulphide demand of long-lived vestimentiferan tube worm aggregations modifies the chemical environment at deep-sea hydrocarbon seeps. Ecol. Lett. 6, 212–219. http://dx.doi.org/10.1046/j.1461-0248.2003.00415.x.

Cordes, E.E., Cunha, M.R., Galéron, J., Mora, C., Olu-Le Roy, K., Sibuet, M., Van Gaever, S., Vanreusel, A., Levin, L.A., 2010. The influence of geological, geochemical, and biogenic habitat heterogeneity on seep biodiversity. Mar. Ecol. 31, 51–65. http://dx.doi.org/10.1111/j.1439-0485.2009.00334.x.

Cordes, E.E., Hourdez, S., Predmore, B.L., Redding, M.L., Fisher, C.R., 2005. Succession of hydrocarbon seep communities associated with the long-lived foundation species Lamellibrachia luymesi. Mar. Ecol. Prog. Ser. 305, 17–29. http://dx.doi.org/10.3354/meps305017.

De Leo, F.C., Gauthier, M., Nephin, J., Mihály, S., Juniper, S.K., 2017. Bottom trawling and oxygen minimum zone influences on continental slope benthic community structure off Vancouver Island (NE Pacific). Deep-Sea Research II 137, 404–419.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. http://dx.doi.org/10.1093/bioinformatics/btq461.

Grünke, S., Felden, J., Lichtschlag, A., Girnth, A., De Beer, D., Wenzhöfer, F., Boetius, A., 2011. Niche differentiation among mat-forming, sulfide-oxidizing bacteria at cold seeps of the Nile Deep Sea Fan (Eastern Mediterranean Sea). Geobiology 9, 330–348. http://dx.doi.org/10.1111/j.1472-4669.2011.00281.x.

Grupe, B.M., Krach, M.L., Pasulka, A.L., Maloney, J.M., Levin, L.A., Frieder, C.A., 2015. Methane seep ecosystem functions and services from a recently discovered southern California seep. Mar. Ecol. 36, 91–108. http://dx.doi.org/10.1111/maec.12243.

Guilini, K., Levin, L.A., Vanreusel, A., 2012. Progress in Oceanography Cold seep and oxygen minimum zone associated sources of margin heterogeneity affect benthic assemblages, diversity and nutrition at the Cascadian margin (NE Pacific Ocean). Prog. Oceanogr. 96, 77–92. http://dx.doi.org/10.1016/j.pocean.2011.10.003.

Herrera, S., Watanabe, H., Shank, T.M., 2015. Evolutionary and biogeographical patterns of barnacles from deep-sea hydrothermal vents. Mol. Ecol. 24, 673–689. http://dx.doi.org/10.1111/mec.13054.

Hilariio, A., Metaxas, A., Gaudron, S.M., Howell, K.L., Mercier, A., Mestre, N.C., Ross, R.E., Thurnherr, A.M., Young, C., 2015. Estimating dispersal distance in the deep sea: challenges and applications to marine reserves. Front. Mar. Sci. 2, 1–14. http://dx.doi.org/10.3389/fmars.2015.00006.

Hinrichs, K., Hayes, J.M., Sylva, S.P., 1999. Methane-consuming archaebacteria in marine sediments, 398, 802–805.

Johnson, H.P., Miller, U.K., Salmi, M.S., E.A.S, 2015. Analysis of bubble plume distributions to evaluate methane hydrate decomposition on the continental slope. Geochem. Geophys. Geosyst. 3825–3839. http://dx.doi.org/10.1002/2015GC005955.Received.

Kelley, D.S., Carbotte, S.M., Caress, D.W., Clague, D.A, Delaney, J.R., Gill, J.B, Hadaway, H., Holden, J.F., Hooft, E.E.E., Kellogg, J.P., LilLey, M.D., Stoermer, M., Toomey, D., Weekly, R., Wilcock, W.S.D., 2012. Endeavour segment of the Juan de Fuca Ridge: One of the most remarkable places on Earth. Oceanography 25 (1), 44–61.

Knittel, K., Boetius, A., 2009. Anaerobic oxidation of methane: progress with an unknown process. Annu. Rev. Microbiol. 63, 311–334. http://dx.doi.org/10.1146/annurev.micro.61.080706.093130.

Kulm, L.D., Suess, E., Moore, J.C., Carson, B., Lewis, B.T., Ritger, S.D., Kadko, D.C., Thornbug, T.M., Embley, R.W., Rugh, W.D., Massoth, M.G., Langseth, Cochrane, G.R., Scamman, R.L., 1986. Oregon Subduction Zone: venting, Fauna, and Carbonates. Science (80-.) 231, 561–566.

Ladau, J., Sharpton, T.J., Finucane, M.M., Jospin, G., Kembel, S.W., O'Dwyer, J., Koeppel, A.F., Green, J.L., Pollard, K.S., 2013. Global marine bacterial diversity peaks at high latitudes in winter. ISME J. 7, 1669–1677. http://dx.doi.org/10.1038/ismej.2013.37.

Levin, L.A., 2005. Ecology of Cold Seep Sediments: Interactions of Fauna with Flow, Chemistry and Microbes, 1–46.

Levin, L.A., Baco, A.R., Bowden, D., Colaço, A., Cordes, E., Cunha, M.R., Demopoulos, A., Gobin, J., Grupe, B., Le, J., Metaxas, A., Netburn, A., Rouse, G.W., Thurber, A.R., Tunnicliffe, V., Van Dover, C., Vanreusel, A., Watling, L., 2016. Hydrothermal vents and methane seeps: rethinking the sphere of influence. Front. Mar. Sci. 3, 72. http://dx.doi.org/10.3389/fmars.2016.00072.

Levin, L.A., Mendoza, G.F., Gonzalez, J.P., Thurber, A.R., Cordes, E.E., 2010. Diversity of bathyal macrofauna on the northeastern Pacific margin: the influence of methane seeps and oxygen minimum zones. Mar. Ecol. 31, 94–110. http://dx.doi.org/10.1111/j.1439-0485.2009.00335.x.

Levin, L.A., Sibuet, M., 2012. Understanding continental margin biodiversity: a new imperative. Ann. Rev. Mar. Sci. 4, 79–112. http://dx.doi.org/10.1146/annurev-marine-120709-142714.

Lloyd, K.G., Albert, D.B., Biddle, J.F., Chanton, J.P., Pizarro, O., Teske, A., 2010. Spatial structure and activity of sedimentary microbial communities underlying a Beggiatoa spp. mat in a Gulf of Mexico hydrocarbon seep. PLoS One 5. http://dx.doi.org/10.1371/journal.pone.0008738.

Lösekann, T., Knittel, K., Nadalig, T., Fuchs, B., Niemann, H., Boetius, A., Amann, R., 2007. Diversity and abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby Mud Volcano, Barents Sea. Appl. Environ. Microbiol. 73, 3348–3362. http://dx.doi.org/10.1128/AEM.00016-07.

Macalady, J.L., Dattagupta, S., Schaperdoth, I., Jones, D.S., Druschel, G.K., Eastman, D., 2008. Niche differentiation among sulfur-oxidizing bacterial populations in cave waters. ISME J. 2, 590–601. http://dx.doi.org/10.1038/ismej.2008.25.

Marlow, J.J., Steele, J.A., Ziebis, W., Thurber, A.R., Levin, L. a., Orphan, V.J., 2014. Carbonate-hosted methanotrophy represents an unrecognized methane sink in the deep sea. Nat. Commun. 5, 5094. http://dx.doi.org/10.1038/ncomms6094.

Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Øvreås, L., Reysenbach, A., Smith, V.H., 2006. Microbial biogeography : putting microorganisms on the map. Nat. Rev. Microbiol. 4, 102–113. http://dx.doi.org/10.1038/nrmicro1341.

Nguyen, P.M., 2016. Microbial sulfur transformations in novel laboratory-scale constructed wetlands treating artificial wastewater. Helmholtz Cent. Environ. Res.

Orphan, V.J., Sylva, S.P., Hayes, J.M., Delong, E.F., 2001. Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. Appl. Environ. Microbiol. 67, 1922–1934. http://dx.doi.org/10.1128/AEM.67.4.1922.

Orphan, V.J., Ussler, W., Naehr, T.H., House, C.H., Hinrichs, K.U., Paull, C.K., 2004. Geological, geochemical, and microbiological heterogeneity of the seafloor around methane vents in the Eel River Basin, offshore California. Chem. Geol. 205, 265–289. http://dx.doi.org/10.1016/j.chemgeo.2003.12.035.

Pasulka, A.L., Goffredi, S.K., Tavormina, P.L., Dawson, K.S., Levin, L.A., Rouse, G.W., Orphan, V.J., 2017. Colonial tube-dwelling ciliates influence methane cycling and microbial diversity within methane seep ecosystems. Front. Mar. Sci. 3, 1–17. http://dx.doi.org/10.3389/fmars.2016.00276.

Pasulka, A.L., Levin, L.A., Steele, J.A., Case, D.H., Landry, M.R., Orphan, V.J., 2016. Microbial eukaryotic distributions and diversity patterns in a deep-sea methane seep ecosystem. Environ. Microbiol. 18, 3022–3043. http://dx.doi.org/10.1111/1462-2920.13185.

Pjevac, P., 2014. Co-existence and Niche Differentiation of Sulfur Oxidizing Bacteria in Marine Environments.

Ritt, B., Pierre, C., Gauthier, O., Wenzhöfer, F., Boetius, A., Sarrazin, J., 2011. Diversity and distribution of cold-seep fauna associated with different geological and environmental settings at mud volcanoes and pockmarks of the Nile Deep-Sea Fan. Mar. Biol. 158, 1187. http://dx.doi.org/10.1007/s00227-011-1679-6.

Römer, M., Riedel, M., Scherwath, M., Heesemann, M., Spence, G.D., 2016. Tidally controlled gas bubble emissions: a comprehensive study using long-term monitoring data from the NEPTUNE cabled observatory offshore Vancouver Island. Geochemistry, Geophysics, Geosystems 17, 3797–3814.

Ruff, S.E., Biddle, J.F., Teske, A.P., Knittel, K., Boetius, A., Ramette, A., 2015. Global dispersion and local diversification of the methane seep microbiome. Proc. Natl. Acad. Sci. U.S.A. 112, 4015–4020. http://dx.doi.org/10.1073/pnas.1421865112.

Ruff, S.E., Kuhfuss, H., Wegener, G., Lott, C., Ramette, A., Wiedling, J., Knittel, K., Weber, M., 2016. Methane seep in shallow-water permeable sediment harbors high diversity of anaerobic methanotrophic communities, Elba, Italy. Front. Microbiol. 7, 1–20. http://dx.doi.org/10.3389/fmicb.2016.00374.

Sahling, H., Rickert, D., Lee, R.W., Linke, P., Suess, E., 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. Mar. Ecol. Prog. Ser. 231, 121–138. http://dx.doi.org/10.3354/meps231121.

Schloss, P.D., Gevers, D., Westcott, S.L., 2011. Reducing the effects of PCR amplification and sequencing Artifacts on 16s rRNA-based studies. PLoS One 6. http://dx.doi.org/10.1371/journal.pone.0027310.

Smith, W.F., Sandwell, D.T., 1997. Global sea floor topography from satellite altimetry and ship depth soundings. Science 277 (5334), 1956–1962.

Sweetman, A.K., Thurber, A.R., Smith, C.R., Levin, L.A., Mora, C., Wei, C.-L., Gooday, A.J., Jones, D.O.B., Rex, M., Yasuhara, M., Ingels, J., Ruhl, H.A., Frieder, C.A., Danovaro, R., Würzberg, L., Baco, A., Grupe, B.M., Pasulka, A., Meyer, K.S., Dunlop, K.M., Henry, L.-A., Roberts, J.M., 2017. Major impacts of climate change on deep-sea benthic ecosystems. Elem. Sci. Anth 5, 4. http://dx.doi.org/10.1525/elementa.203.

Thurber, A.R., Sweetman, A.K., Narayanaswamy, B.E., Jones, D.O.B., Ingels, J., Hansman, R.L., 2014. Ecosystem function and services provided by the deep sea. Biogeosciences 11, 3941–3963. http://dx.doi.org/10.5194/bg-11-3941-2014.

Thomsen, L., Barnes, C., Best, M., Chapman, R., Pirenne, B., Thomson, R., Vogt, J., 2012. Ocean circulation promotes methane release from gas hydrate outcrops at the NEPTUNE Canada Barkley Canyon node. Geophys. Res. Lett. 39, n/a–n/a. http://dx.doi.org/10.1029/2012GL052462.

Torres, M.E., Teichert, B.M.A., Tréhu, A.M., Borowski, W., Tomaru, H., 2004. Relationship of pore water freshening to accretionary processes in the Cascadia margin: fluid sources and gas hydrate abundance. Geophys. Res. Lett. 31, 1–4. http://dx.doi.org/10.1029/2004GL021219.

Tréhu, A.M., Torres, M.E., Moore, G.F., Suess, E., Bohrmann, G., 1999. Temporal and spatial evolution of a gas hydrate-bearing accretionary ridge on the Oregon continental margin. Geology 27, 939–942. http://dx.doi.org/10.1130/0091-7613(1999)027<0939:TASEOA>2.3.CO;2.

Tryon, M.D., Brown, K.M., Torres, M.E., 2002. Fluid and chemical flux in and out of sediments hosting methane hydrate deposits on Hydrate Ridge, OR, II: hydrological processes. Earth Planet. Sci. Lett. 201, 541–557. http://dx.doi.org/10.1016/S0012-821X(02)00732-X.

Tryon, M.D., Brown, K.M., Torres, M.E., Tréhu, A.M., McManus, J., Collier, R.W., 1999. Measurements of transience and downward fluid flow near episodic methane gas vents, Hydrate Ridge, Cascadia. Geology 27, 1075–1078. http://dx.doi.org/10.1130/0091-7613(1999)027<1075:MOTADF>2.3.CO;2.

Valentine, D.L., 2011. Emerging topics in marine methane biogeochemistry. Ann. Rev. Mar. Sci. 3, 147–171. http://dx.doi.org/10.1146/annurev-marine-120709-142734.

Valentine, D.L., Kastner, M., Wardlaw, G.D., Wang, X., Purdy, A., Bartlett, D.H., 2005. Biogeochemical investigations of marine methane seeps, Hydrate Ridge, Oregon. J. Geophys. Res. 110, G02005. http://dx.doi.org/10.1029/2005JG000025.

Van Dover, C.L., German, C.R., Speer, K.G., Parson, L.M., Vrijenhoek, R.C., 2002. Evolution and biogeography of deep-sea vent and seep invertebrates. Science (80-) 295, 1253–1257. http://dx.doi.org/10.1126/science.1067361.

Wallmann, K., Linke, P., Suess, E., Bohrmann, G., Sahling, H., Schlüter, M., Dählmann, A., Lammers, S., Greinert, J., von Mirbach, N., 1997. Quantifying fluid flow, solute mixing, and biogeochemical turnover at cold vents of the eastern Aleutian subduction zone. Geochim. Cosmochim. Acta 61, 5209–5219. http://dx.doi.org/10.1016/S0016-7037(97)00306-2.

Yamaguchi, T., Newman, W.A., Hashimoto, J., 2004. A cold seep barnacle (Cirripedia: neolepadinae) from Japan and the age of the vent/seep fauna. J. Mar. Biol. Assoc. U. K. 84, 111–120. http://dx.doi.org/10.1017/S0025315404008975h.