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MICROBIAL DIVERSITY AND COMMUNITY COMPOSITION IN THE OPEN OCEAN AND MARINE SEDIMENT

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BY

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ABSTRACT

Over the last fifteen years, there has been a large increase in the literature on microbial community composition in marine sediment (Inagaki et al., 2006, 2015; Biddle et al., 2012; Briggs et al., 2012; Breucker et al., 2013; Lloyd, 2014; Teske et al., 2014; Nunoura et al., 2016; Walsh et al., 2016; Petro et al., 2017; Harrison et al., 2018; Hoshino et al., 2020), and seawater (Quaiser et al., 2011; Hamdan et al., 2013; Walsh et al., 2016; Medina-Silva et al., 2018; Mestre et al., 2018; Quero et al., 2019). As molecular study of these biomes progresses, and the tools available for detailed analyses expand, it has become important to evaluate those tools for their effectiveness and limitations. By combining environmental microbial investigations with evaluation of some of the most common genetic protocols, I have characterized microbial diversity and community composition in (i) Pacific, Atlantic, and Arctic seawater and (ii) Pacific and Atlantic sediment, and I have identified the common results obtainable using (i) two different 16S ribosomal RNA gene (rRNA) hypervariable regions of interest, and (ii) the two amplicon analysis pipelines most commonly used to determine microbial diversity and community composition.

My first manuscript, “Influence of 16S rRNA Hypervariable Region on Estimates of Bacterial Diversity and Community Composition in Seawater and Marine Sediment”, looks at the bacterial diversity and community composition of deep-ocean sediment and overlying seawater from one site in the Central North Atlantic and one site in the Equatorial Pacific. In each case, we amplified both the V4 and V6 hypervariable regions of the 16S rRNA gene of each sample and clustered the sequences into

operational taxonomic units (OTUs) of 97% similarity. In doing so, we determined that while OTU-level diversity metrics and community composition are quite different between the two tags, (i) vertical patterns of relative diversity are broadly the same, (ii) community composition is very similar for both tags at the class level, and (iii) while the open ocean communities are very similar between the Pacific and Atlantic oceans, the sediment communities of each ocean differ greatly.

My second manuscript, "Patterns of Relative Bacterial Richness and Community Composition in Seawater and Marine Sediment are Robust for both Operational Taxonomic Units and Amplicon Sequence Variants", examines how the choice of bioinformatic analysis pipeline affects characterization of taxonomic richness and community composition in seawater (from 12 sites in the North Atlantic Ocean and Canadian Arctic) and sediment (from two sites in the North Atlantic). For all samples, we amplified the V4-V5 hypervariable region of the 16S rRNA gene and analyzed each sample in two different ways: (i) by clustering its reads into 97%-similar OTUs, and (ii) by assigning sequences to unique amplicon sequence variants (ASVs). By comparing the results obtained with each method, we determined that (i) for both OTUs and ASVs, estimates of taxonomic richness depend on the number of sequences analyzed, (ii) bacterial community composition between the two methods is broadly similar in all samples at the taxonomic levels of phyla to families, and (iii) broad-scale patterns of relative richness and community composition are similar with both methods.

My third manuscript, “Microbial Community Composition of Canadian Arctic Seawater”, leverages the results of the first two manuscripts to characterize microbial community composition in the Northwest Passage of the Arctic Ocean and illustrate its connection to community composition in other major oceans. Here, we analyzed from three to six water-column depths at eleven locations throughout the Canadian Arctic and Davis Strait, as well as one site in the North Atlantic by amplifying the V4-V5 hypervariable region of the 16S rRNA gene and clustering the sequences into 97%-similar OTUs. While we report specifically on the OTU-based results, we also analyzed each sample with the ASV methods described in manuscript two to test our conclusions. Our results show that (i) vertical profiles of OTU-level richness (and ASV-level richness) in Arctic seawater are similar to those in other oceans, and (ii) the predominant primary producers in Northwest Passage communities appear to be eukaryotes, not bacteria. In the latter respect, the Northwest Passage communities resemble coastal communities of other ocean regions, rather than open-ocean communities of the North Atlantic and North Pacific.

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DEDICATION

To my father, Michael Kerrigan

For encouraging me in the life-long pursuit of knowledge,

and inspiring me to always be the best possible version of myself

PREFACE

This dissertation is written in manuscript format and consists of the following three manuscripts: The first manuscript, "Influence of 16S rRNA Hypervariable Region on Estimates of Bacterial Diversity and Community Composition in Seawater and Marine Sediment", characterizes the bacterial diversity and community composition of the open ocean and seafloor sediment with respect to the 16S rRNA hypervariable region of analysis. This manuscript was published in *Frontiers in Microbiology* in July of 2019. The second manuscript, "Patterns of Relative Bacterial Richness and Community Composition in Seawater and Marine Sediment are Robust for both Operational Taxonomic Units and Amplicon Sequence Variants", details the complimentary results from both a 97%-similar, OTU-clustered approach, and a unique, ASV approach. This manuscript is being prepared for submission to *Frontiers in Microbiology* for the summer of 2021. The third manuscript, "Microbial Community Composition of Canadian Arctic Seawater", leverages the findings in the first two manuscripts to characterize the microbial diversity and community composition of the seawater in the Canadian Arctic, and how that community relates to other oceanic communities around the world. This manuscript is being prepared for submission in the summer of 2021 to a yet undetermined journal.

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MANUSCRIPT I

INFLUENCE OF 16S RRNA HYPERVARIABLE REGION ON ESTIMATES OF BACTERIAL DIVERSITY AND COMMUNITY COMPOSITION IN SEAWATER AND MARIND SEDIMENT

by

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Abstract

To assess the influence of 16S rRNA tag choice on estimates of microbial diversity and/or community composition in seawater and marine sediment, we examined bacterial diversity and community composition from a site in the Central North Atlantic and a site in the Equatorial Pacific. For each site, we analyzed samples from four zones in the water column, a seafloor sediment sample, and two subseafloor sediment horizons (with stratigraphic ages of 1.5 Ma and 5.5 Ma). We amplified both the V4 and V6 hypervariable regions of the 16S ribosomal RNA gene (rRNA) and clustered the sequences into operational taxonomic units (OTUs) of 97% similarity to analyze for diversity and community composition. OTU richness is much higher with the V6 tag than with the V4 tag, and subsequently OTU-level community composition is quite different between the two tags. Vertical patterns of relative diversity are broadly the same for both tags, with maximum taxonomic richness in seafloor sediment and lowest richness in subseafloor sediment at both geographic locations. Genetic dissimilarity between sample locations is also broadly the same for both tags. Community composition is very similar for both tags at the class level, but very different at the level of 97%-similar OTUs. Class-level diversity and community composition of water-column samples are very similar at each water depth between the Atlantic and Pacific. However, sediment communities differ greatly from the Atlantic site to the Pacific site. Finally, for relative patterns of diversity and class-level community composition, deep sequencing and shallow sequencing provide similar results.

Introduction

Over the last ten years, there has been a large increase in literature on microbial community composition in marine sediment (Inagaki et al., 2006, 2015; Blazejak and Schippers, 2010; Biddle et al., 2012; Briggs et al., 2012; Durbin and Teske, 2012; Mills et al., 2012; Breucker et al., 2013; Colwell and D'Hondt, 2013; Orsi et al., 2013, 2017; Lloyd, 2014; Teske et al., 2014; Bienhold et al., 2016; Brandt and House, 2016; Jones et al., 2016; Nunoura et al., 2016; Labonté et al., 2017; Petro et al., 2017; Harrison et al., 2018; Orsi, 2018), and how it compares to community composition in the overlying water (Quaiser et al., 2011; Hamdan et al., 2013; Walsh et al., 2016a). Seawater and marine sediment contain roughly equal numbers of microbial cells (Kallmeyer et al., 2012), but bacterial taxonomic richness is generally much higher in the ocean than in the sediment (Walsh et al., 2016a). The ability to characterize these communities in detail is due to the advent of high-throughput sequencing (HTS) technology, which allows sequencing of either a large number of samples on a single sequencing run, or deep sequencing of a small number of samples (hundreds of thousands to millions of amplicons per sample). Consequently, HTS is now the dominant method for analyzing environmental microbiology because it is able to capture a large portion of the community in each sample. However, HTS is limited by its capacity to sequence relatively short regions of DNA, and therefore relies on analysis of only one or two of the hypervariable regions of the 16S rRNA gene to identify taxonomic diversity and community composition. Most recent studies of microbial diversity in seawater and marine sediment typically analyzed a single 16S hypervariable region (e.g. Inagaki et al., 2006; Sogin et al., 2006; Huber et al.; 2007,

Mills et al., 2012; Gibbons et al., 2013; Beinhold et al., 2016; Brandt and House, 2016; Jones et al., 2016; Nunoura et al., 2016; Walsh et al., 2016a; Labonté et al., 2017; Orsi et al., 2017; Starnawski et al., 2017; Harrison et al., 2018).

While HTS has become a valuable tool in microbial ecology, previous studies have shown that estimates of taxonomic diversity and community composition depend on which hypervariable region is analyzed (Liu et al., 2008; Youssef et al., 2009; Mysara et al., 2017). This dependence is problematic because different studies have analyzed different regions (V3 from Mills et al., 2012; V4 from Starnawski et al., 2017 and Orsi et al., 2017; V6 from Huber et al., 2007 and Bienhold et al., 2016; V1-V3 from Harrison et al., 2018; V2-V5 from Inagaki et al., 2006; V4-V6 from Walsh et al., 2016a; V5-V6 from Jones et al., 2016; V6-V9 from Brandt and House, 2016). Reliance of different studies on different regions hinder synthesis of these studies to draw broad conclusions about distributions of microbial richness and community composition in the open ocean and marine sediment.

To determine what information from a 16S study is consistent across hypervariable regions and help provide a stable foundation for synthesizing results from studies of different hypervariable regions, we separately amplified both the V4 and V6 hypervariable region of the bacterial 16S rRNA gene from multiple horizons in the ocean and marine sediment of the Atlantic and Pacific Oceans. Sampled horizons range from the surface, sunlit water to 5.5-Ma subseafloor sediment in individual sites from both oceans. Our goals are to (*i*) assess the extent to which interpretations of

marine microbial diversity and community composition depend on the hypervariable region chosen, and (*ii*) identify the results that can be used to make conclusions about these communities regardless of hypervariable region.

Materials and methods

Sample collection and DNA extraction

We collected samples from water-column filters and sediment cores from Site 8 of R/V Knorr cruise 195-3 (00°00.36'N 147°47.50'W, water depth 4360m) in the Equatorial Pacific and Site 15 of R/V Knorr cruise 223 (33°29.01'N 054°09.98'W, water depth 5510m) in the North Atlantic. At each site, we collected 7 samples; 4 water-column samples at depths corresponding to the Chlorophyll-a maximum (*Chl-a*), Oxygen Minimum Zone (OMZ), bulk deep water, and bottom water, and 3 sediment samples at depths from the sediment-water interface, sediment dated approximately 1.5 million years old (Ma), and sediment dated approximately 5.5 Ma. We extracted DNA from both the water filters and sediment with the MOBIO PowerSoil DNA Isolation Kit (Mo Bio Laboratories, A Qiagen Company, Carlsbad, CA), following the manufacturer's protocol. For the water-column samples, we filtered 10L of seawater through 0.22 µm Sterivex filters (Millipore Sigma, Billerica, MA), then shredded each filter and placed the pieces in two of the MOBIO PowerSoil bead tubes for extraction using the standard PowerSoil protocol. For all but two sediment samples, we used duplicate subsamples of 0.25g of sediment for DNA extraction. For the remaining two sediment samples (taken from 7 meters below sea floor (mbsf) (1.5 Ma) and 26mbsf (5.5 Ma) at Site 8), we used 12 subsamples of 0.25g

of sediment due to the extremely low biomass of the material. In addition to the sediment samples, the entire PowerSoil extraction protocol was completed on an empty sample tube to analyze for lab and kit contamination during post-sequencing processing. Sediment age was approximated for each depth by dividing basement age (Müller et al., 2008) by sediment thickness (Divins, 2003), and assuming a constant sediment deposition rate.

PCR amplicon construction and sequencing

From each extract, we amplified the V4 and V6 hypervariable regions of the 16S rRNA gene using forward and reverse primers from Caporaso et al., 2011, and The Visualization and Analysis of Microbial Population Structures (VAMPS) center (https://vamps.mbl.edu/resources/primers.php), respectively. The V4 primers are 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The V6 primers are a combination of 4 forward primers, 967F1 (5'-CTAACCGANGAACCTYACC-3'), 967F2 (5'-CNACGCGAAGAACCTTANC-3'), 967F3 (5'-CAACGCGMARAACCTTACC-3'), 967F4 (5'-ATACGCGARGAACCTTACC-3'), and one reverse primer, 1064R (5'-CGACRRCCATGCANCACCT-3'). We performed a 20-µl PCR reaction in triplicate for each sample, in which each reaction contained a mixture of 0.1 µl Platinum HF Taq Polymerase (Life Technologies, Carlsbad, CA), 2 µl Platinum HF Buffer (10x), 0.8 µl $MgSO_4$ (50mM), 0.16 µl dNTPs (25Mm mix), 0.1 µl of each primer (50µM), 0.1µl Bovine Serum Albumin (Fermentas Life Sciences, Carlsbad, CA), and between 1 and 10 µl of the extracted DNA in addition to a reaction containing 10µl of water

and no extract to analyze for PCR reagent contamination during post-sequencing processing. The thermocycler program for both the V4 and V6 regions was set to an initial denaturation temperature of 94°C for 2 minutes; 30 cycles (37 cycles for sediment depths 7mbsf and 26mbsf at Site 8) of 94°C for 15 seconds, 60°C for 30 seconds, and 68°C for 60 seconds; and then a final extension of 68°C for 5 minutes. We then pooled and cleaned the amplicons for each sample using the Agencourt AMPure PCR Purification kit (Beckman Coulter Life Sciences, Indianapolis, IN), and then sequenced the amplicons on the Illumina MiSeq at the University of Rhode Island Genomics and Sequencing Center. Sequencing of the V4 and V6 datasets was conducted using Illumina V2 chemistry with 2 x 250 cycles and V3 chemistry with 2 x 75 cycles, respectively.

Sequence analysis

To process and analyze the sequences, we used mothur v.1.38.1 (Schloss et al., 2009), and followed the mothur MiSeq SOP (Kozich et al., 2013) (December 2016). In addition to the steps in the SOP, we removed from all samples any reads that were sequenced from the PowerSoil extraction kit blank and PCR blank (described above) to account for possible lab and reagent contamination. Prior to clustering, we randomly subsampled all samples to 230,097 sequences (lowest number of reads in any sample) to make direct comparisons when conducting community analyses. Unless otherwise stated, we performed and average-neighbor clustering analysis at 97% sequence similarity for both the V4 and V6 hypervariable regions. Additionally, we removed singletons and doubletons of OTUs from each sample to mitigate the

likelihood of including OTUs based solely on sequencing error (NCBI BioProject PRJNA423041, Accession numbers SAMN08204781 through SAMN08204808).

Results

Taxonomic richness

In total, 12,316 OTUs were identified from all samples by clustering the V4 tags. 58,087 OTUs were identified by clustering the V6 tags. Despite the large difference in total number of OTUs from the two tags, the V4 and V6 tag profiles exhibit similar relative vertical patterns of taxonomic richness at both the Atlantic and Pacific sites (Fig. 1). In each case, bacterial richness is highest in seafloor sediment 0-1 cm below the sediment-water interface, and then diminishes with increasing sediment age, consistent with established trends in the deep subseafloor (Walsh et al., 2016a, b). In the water column, richness is highest in the Oxygen Minimum Zone (OMZ). These vertical distributions of relative richness in the water column broadly match distributions previously reported for the Southern Ocean and other Pacific sites, based on analysis of the entire V5-V8 and V4-V6 hypervariable region (Signori et al., 2014; Walsh et al., 2016a, respectively). To further illustrate the similarity in patterns of relative abundance, we directly compared the absolute numbers of bacterial richness between the V4 and V6 tags (Fig. 2), and found a very strong correlation ($R^2 = 0.885$).

Taxonomic evenness

For each sample, we calculated the Shannon's Equitability Index ($E_H = {}^{H}/_{\ln S}$), where H is the Shannon Index and S is the taxonomic richness (total number of OTUs)

for each sample. This results in a measure of OTU evenness on a scale from 0 to 1, where 1 is a value of total evenness (each OTU in the sample contains the same number of sequences). Although evenness is consistently higher with the V6 tag than with the V4 tag, both E_H profiles exhibit broadly similar vertical patterns, with highest evenness at the sediment-water interface (Fig. 3). For all of the water column samples and the two seafloor sediment samples, the V6 E_H values are between 7% and 27% larger than the V4 values, indicating a more even distribution of OTUs. However, in the remaining subseafloor sediment of both the Pacific and Atlantic, the V6 E_H values are between 94% and 127% larger than the V4 values. This indicates the subseafloor sediment communities are dominated by one or two V4-defined OTUs, but contain relatively even distributions of V6-defined OTUs.

Community composition

Similarity/dissimilarity of community compositions from different samples

To compare the genetic similarity/dissimilarity between different samples, we completed a two-dimensional, Principle Coordinate Analysis (PCoA). We calculated both Jaccard and Bray-Curtis distance matrices for use in the PCoA for comparison. As shown by the Jaccard results in Figure 4, PCoA of the V4-based OTUs and PCoA of the V6-based OTUs yield very similar sample groupings. For the Jaccard-based PCoAs (Fig. 4), the two axes plotted explain 73% and 85% of the total variation for the V4-based and V6-based OTUs, respectively. The Bray-Curtis analysis (not shown) groups the samples similarly to the Jaccard analysis with a total variation of 54% and

57% explained by the first two axes for the V4-based and V6-based OTUs, respectively.

For both the V4-based and V6-based analysis, the water-column samples are more similar at each water depth regardless of geographic location (Fig. 4). The subseafloor samples resemble each other closely within each geographic location, but differ greatly from one location to the other (Fig. 4). These results are consistent with other studies conducted in the water column using the V6 rRNA tag (Zinger et al., 2011) and subseafloor using a universal tag similar to V4 (Nunoura et al., 2016).

Comparison of abundant taxa. To examine the taxonomic composition of the samples, we chose the top 20 most abundant OTUs from each sample (similar to Inagaki et al. [2006], Agogué et al. [2011], and Petro et al. [2017]). These taxa encompass all of the individual OTUs that are responsible for approximately 1% or more of the total sequences in each sample, which is a typical cutoff for considering OTUs to be abundant (Campbell et al., 2015; Inagaki et al., 2015; Kirkpatrick et al., 2019; Tseng et al., 2015). Jing et al. (2013) showed that using more than the 10 most abundant OTUs is sufficient to visualize changes in OTU community composition between samples. Our top OTUs account for 13% to 100% of the total sequences in each sample, with a total of 163 OTUs, and 170 OTUs for the V4 and V6 tag datasets, respectively.

For a broad look at bacterial community composition, we separately grouped these 163 V4 tage and 170 V6 tags by taxonomic class (Fig. 5). Although the total numbers of OTUs from each hypervariable region are similar (163 OTUs for V4 and 170 OTUs for V6), the number of class-level taxa returned from the V4 tags is almost double that of V6 (32 and 18 taxa, respectively). Most of this difference in richness is attributable to V4-based taxa that occur in low abundance and are from the Atlantic seafloor sediment sample, in particular. Except for this V4-based Atlantic community, the communities in all other samples are dominated by relatively few classes of bacteria and show a high degree of similarity between the V4 and V6 hypervariable region.

At both the Atlantic and Pacific sites, the water-column communities are broadly similar; they are dominated by *Cyanobacteria* and *Alphaproteobacteria* near the surface, sunlit water, and mostly *Gammaproteobacteria* in deeper water, similar to other 16S rRNA studies of water-column composition (DeLong et al., 2006; Brown et al., 2009; Jing et al., 2013; Tseng et al., 2015; Medina-Silva et al., 2018). In contrast, community composition in the sediment is quite different between the Atlantic and Pacific. Samples of older sediment in the Atlantic (dated approximately 1.5 Ma and 5.5 Ma) are dominated by *Dehalococcoidia* and an unclassified *Atribacteria*, similar to other deep-sediment community composition studies (Inagaki et al. 2006; Blazejak and Schippers, 2010; Breuker et al., 2013; Teske et al., 2014; Inagaki et al., 2015; Brandt and House, 2016; Nunoura et al., 2016; Labonté et al., 2017; Petro et al., 2017; Orsi, 2018). In contrast, the communities in Pacific samples of the same age are primarily dominated by *Alphaproteobacteria*, an unclassified *Aerophobetes*, and some

Dehalococcoidia. Inter-basin differences between the seafloor sediment samples are even more striking. The community of the Pacific seafloor sample is dominated by *Gammaproteobacteria* and *Alphaproteobacteria*, bearing a strong resemblance to the community composition in the overlying water column, as well as seafloor sediment from the South Atlantic (Schauer et al., 2010; Mills et al., 2012; Orsi et al., 2013; Bienhold et al., 2016; Jones et al., 2016) with additional, relatively rare taxa that give this sample its high taxonomic richness. As discussed above, the community in the Atlantic seafloor sample is quite diverse and other than one or two relatively abundant taxa, V4-based and V6-based community compositions are not in strong agreement. The V4-based Atlantic seafloor community contains a fairly even distribution of fourteen taxa slightly dominated by *Phycisphaerae*, *Anaerolineae*, *Epsilonproteobacteria*, an unclassified *Aminicenantes*, and an unclassified *Atribacteria*. The V6-based community is not quite as evenly spread over 8 taxa and dominated by *Deltaproteobacteria* and *Phycisphaerae*.

To examine community composition at a finer taxonomic level, we plotted the vertical distributions of the 163 abundant V4-based OTUs and the 170 V6-based OTUs (Fig. 6). The 163 V4-based OTUs exhibit a similar depth pattern of community composition in both the Atlantic and Pacific (as seen with the V4 class-level analysis), with between one and four dominant OTUs in each sample (Fig. 6a). The water-column communities in both the Atlantic and Pacific are dominated by three V4-based OTUs of the genera *Halomonas*, *Idiomarina* and, *Erythrobacter* in the three mid-water samples, and two OTUs associated with photosynthetic metabolism of the genus

Prochlorococcus in the upper, sunlit region, consistent with previous studies of the water column (DeLong et al.; 2006, Brown et al., 2009; Campbell et al., 2015; Tseng et al., 2015). In the sediment samples, the Atlantic and Pacific communities are distinct. In the Atlantic, the subseafloor sediment (1.5 Ma and 5.5 Ma) is dominated by one V4-based OTU associated with an unclassified genus of *Atribacteria*, whereas subseafloor sediment of the same age in the Pacific is dominated by two OTUs associated with the genus *Methlyobacterium* and an unclassified genus of *Aerophobetes*. Consistent with the E_H values, the near-seafloor sedimentary communities exhibit very little bias towards any single V4-based OTU.

In contrast to the 163 V4-based OTUs (Fig. 6a), and in contrast to the class-level V6-based analysis (Fig. 5), the 170 V6-based OTUs do not exhibit clean depth-related patterns of taxonomic dominance (Fig. 6b). Instead, many V6-based OTUs constituted a few percent of each sample, consistent with the calculated E_H values (Fig. 3).

Sampling Bias

Because the top 20 OTUs in each sample incorporates a greater percentage of total sequence reads for the V4-based analysis than for the V6-based analysis, it is possible that some of these disparities between the V4-based and V6-based results can be attributed to under-sampling of the V6-based communities by restricting the comparison to the top 20 OTUs in each sample. In order to test this possibility, we analyzed the top 200 V6 OTUs and compared them to the top 20 V4 OTUs in each of the three Atlantic sediment samples. We chose the three Atlantic sediment samples

because they exhibit a consistent pattern in V4-based community composition, and might reasonably be expected to exhibit a similarly consistent pattern with V6 tags if under-sampling was the cause of the differences. We chose the top 200 V6 OTUs because it provides similar percentage coverage to the top 20 V4 tags for the same samples.

At the class level, this analysis led the number of *Phycisphaerae* reads in the V6 dataset to more closely match the number in the V4 dataset. However, it also added fifteen classes to the V6 community not found in the V4 community, and it still produced very different compositions for the V4 and V6 seafloor communities (Fig. S1). The class-level V4-based and V6-based communities of the 1.5 Ma and 5.5 Ma sediment samples in the Atlantic resemble each other more closely than do the class-level V4- and V6-based seafloor communities; this similarity of the subseafloor V4- and V6-based communities is mainly due to the dominance of *Dehalococcoidia* and unclassified *Atribacteria*, which were also similar with only the top 20 OTUs in each sample.

Despite the higher percentage of OTU coverage provided by the 200 most abundant V6 tags in each sediment sample, the top 200 V6-based OTUs still do not exhibit a clear pattern of taxonomic dominance (Fig. S2). This result agrees with the evenness metrics calculated for the V6 dataset, in which all the values are very high ($E_H > 0.69$ for all samples) and all OTUs, not just the 20 or 200 most abundant, are taken into

consideration. This result suggests that the apparent differences in V4-based versus V6-based communities are not due to an under-sampling of the V6 dataset.

Sub-sampled datasets

To compare the patterns of taxonomic richness and community composition from our deep sequencing run to those that would result from a shallow sequencing run, we randomly pulled 10,000 sequences from each sample and performed the same clustering and analysis as with the full dataset. While the absolute numbers of 97%-similar OTUs were proportionally reduced for each sample, the patterns of relative OTU abundance remained the same as in the full dataset across both hypervariable regions and sample sites. Community composition was also not strongly affected, with the community composition pattern of the top 20 OTUs nearly identical to those for the full data set. This result is consistent with previous studies that found no significant difference in diversity analysis between shallow and deep sequencing results (Kuczynski et al., 2010; Caporaso et al., 2012).

Discussion

Taxonomic richness patterns

All four of our vertical profiles of OTU richness (2 sites x 2 hypervariable regions) are similar in nature (Fig. 1), indicating that the fundamental pattern in each of them broadly represents the relative richness of bacterial OTUs in the open ocean and marine sediment. In each case, water-column bacterial richness peaks in the OMZ and sedimentary richness peaks at the seafloor, in agreement with previous studies of the

water column (Signori et al., 2014; Walsh et al., 2016a) and the sediment (Walsh et al., 2016a; Petro et al., 2017). For both hypervariable regions at both sites, taxonomic richness declines with sediment age, also in agreement with previous studies (Walsh et al., 2016b; Kirkpatrick et al., 2019). However, richness declines much more strongly in the Pacific V4 dataset than in the Atlantic V4 dataset, or either V6 dataset. This result suggests that sedimentary properties (e.g. geographic location, sediment composition, and sediment age), affects measures of taxonomic richness differently using different hypervariable regions. It also shows that in certain geographic locations, sediment up to 5.5-Ma in age may contain a large fraction of the taxonomic richness in the overlying water column.

Taxonomic richness depends on hypervariable region

Although the V4 and V6 datasets are generally similar in their vertical profiles of taxonomic richness, the large difference between V4-based OTU numbers and V6-based OTU numbers at each sampling horizon illustrates that choice of one hypervariable region over another can significantly impact the number of clustered OTUs (Lebret et al., 2016). To further illustrate this inference, the V4-based richness values are strongly correlated to the V6-based richness values for each sample (R^2 = 0.885) (Fig. 2). However, the y-intercept of approximately 4500 on the V6 axis implies that V6 OTU richness is highly inflated. Previous studies have shown that V4-based taxonomic richness closely matches the OTU richness based on the entire 16S rRNA gene (Tremblay et al., 2015), whereas V6-based richness is consistently higher (Youssef et al., 2009). This dependence of OTU richness on hypervariable region

complicates comparison studies that rely on different hypervariable regions. Such comparison is further complicated by a recent study which shows that, regardless of hypervariable region, clustering algorithms consistently group together sequences into single OTUs that would define separate OTUs using the entire 16S gene at the same similarity level (97%) (Mysara et al., 2017). Even when clustered at a more restrictive level (98% or 99%), the returned number of OTUs for different hypervariable regions is between 30% and 87% (this includes differences between hypervariable regions) of the OTU number for the entire 16S gene (Mysara et al., 2017). Their percentage of "overmerged" OTUs also varies between different bacterial families, and OTUs within each family are either more conserved, or less conserved, and depended on which hypervariable tag is analyzed (Mysara et al., 2017). Over- and under-merging species into OTUs depending on family and hypervariable region complicates discussion of absolute richness.

Community evenness

E_H values indicate that where the V4 tags define a community skewed toward one or two OTUs, these dominant V4-based OTUs may be broken down into several strains using the V6 tags. This result is particularly noticeable in the subseafloor sediment samples (from approximately 1.5 Ma and 5.5 Ma), in which many V6-based OTUs merge into only a few V4-based OTUs. Additionally, the V6-based communities exhibit much greater evenness than the V4-based communities. Although 16S V6 clustering produces much higher numbers of 97%-similar OTUs than V4 clustering or whole 16S clustering, V6 tag sequences still measure real differences in diversity (Liu

et al., 2008). Consequently, a V6-based analysis will identify higher subseafloor diversity (and higher potential diversification) than a V4-based analysis (e.g. Starnawski et al., 2017).

Community composition

The PCoA analysis clearly shows that community composition using 97%-similar OTUs is similar for each environment in the water column and the sediment, regardless of the hypervariable region (V4 or V6) (Fig. 4). This result indicates that even at the 97%-similar OTU level, patterns of similarity or dissimilarity of community compositions obtained using a single hypervariable region are robust. The PCoA results also strengthen the finding that while V6 tag sequences return a higher number of 97%similar OTUs, the much higher evenness values of the V6-based community in the subseafloor sediment samples (Fig. 3) is due to the much higher diversity of genetically-similar reads in the V6 dataset relative to the V4 dataset, rather than hypervariable-region bias.

The V4-based communities and V6-based communities are broadly similar to each other at the class level (Fig. 5). Regardless of the total number of 97%-similar OTUs, each sample is dominated by the same class (or classes) of bacteria in both the V4-based and V6-based analyses. This similarity indicates that apparent community composition analysis of the most abundant taxa in a sample is not strongly affected by the hypervariable region chosen (Lebret et al., 2016). In our data, there are two distinct exceptions to this general pattern. First, in both the Atlantic and Pacific Chl-a

maximum samples, the V4-based community is dominated by relatively similar numbers of *Gammaproteobacteria*, *Alphaproteobacteria*, and *Cyanobacteria*. However, the V6-based community is mostly dominated by *Alphaproteobacteria*, which is similar to other studies using only the V6 tag (Agogué et al., 2011; Zinger et al., 2011). Second, in the subseafloor sediment of both the Atlantic and Pacific, the V6-based community contains a high number of *Chloroflexi*, consistent with other subseafloor studies (Inagaki et al., 2006; Petro et al., 2017), whereas the V4-based community contains very few *Chloroflexi* reads, but includes a large percentage of *Firmicutes* that are completely absent in the V6-based community.

Despite the similarity of the V4 and V6 PCoA results and the visibly-similar patterns of class-level community compositions using the V4 and V6 datasets, strip charts of V4-based community compositions differ greatly from strip charts of V6-based community compositions at the level of 97%-similar OTUs (Fig. 6). The V4-based OTU communities exhibit a vertical pattern of composition similar to that at the class level, with dominance of each water-column or sediment sample by one or two OTUs in both the Atlantic and Pacific. The V4-based samples tend to be more visibly similar between separate samples taken from the same site and the same general environment (e.g. deep water or subseafloor sediment). For the V6-based communities, this visual similarity is obscured by high taxonomic richness and evenness. For example, the V4-based OTU community of the 1.5 Ma Atlantic subseafloor generally resembles the V4-based 5.5 Ma Atlantic community (Fig. 6a), but the V6-based communities from the same samples appear to resemble each other much less closely (Fig. 6b). In short,

the community relatedness indicated by the PCoA analyses of the 97%-similar OTUs is not readily visible in strip charts of V6-based community compositions. This result may be due to the much higher diversity of genetically-similar reads in the V6 dataset relative to the V4 dataset.

This point can be further illustrated by examining a single, V4-based OTU in the oldest Atlantic sediment sample (5.5 Ma), identified as an unclassified *Atribacterium.* At the 97% similarity level, it is identical to the same V4-based OTU in the other two Atlantic sediment samples (seafloor and 1.5 Ma). However, examination of the same sample using the V6 tag reveals this V4-based *Atribacterium* OTU to contain 14 distinguishable V6-based OTUs, 13 of which were observed only in the two subseafloor sediment samples and seven of which were observed only in either the 1.5 Ma sample or the 5.5 Ma sample. These sample-to-sample differences in presence or absence of V6 OTUs may be due to (i) under-sampling of the populations resident in the samples, (ii) variation in the V6 hypervariable region of 16S rRNA in the founding seafloor populations over the past 5.5 Mys, or (iii) diversification within the subseafloor community over the past 5.5 Mys which is detectible in the V6 region but not the V4 region. Identification of 6 of the 13 V6-based OTUs in both the 1.5-Ma sample and the 5.5-Ma sample indicates that the greater diversity in the V6 dataset is largely real, and not an artifact of sequencing error.

Conclusions

Our comparison of paired V4 and V6 16S tags in natural samples of seawater and marine sediment confirms that estimates of bacterial OTU diversity and evenness depend on the 16S tag used. However, both tags yield similar patterns of taxonomic richness and evenness in the seawater and sediment at both the Atlantic site and Pacific site. And, when directly comparing the genetic distances of one sample from another (PCoA), the grouping of OTUs based on sample location is the same regardless of the tag used. Based on taxonomic assignment at the class level, community composition is broadly similar for both tags. However, at the 97%-similar OTU level, the V4 and V6 tags yield different community compositions. This is perhaps because many marine bacteria are unclassified due to lack of a reference database. For both tags, water-column community composition is similar across geographic locations, but sediment community composition differs substantially from the Atlantic site to the Pacific site. Finally, deep sequencing provides no differences in patterns of relative diversity or in community composition of the most abundant taxa.

Acknowledgments

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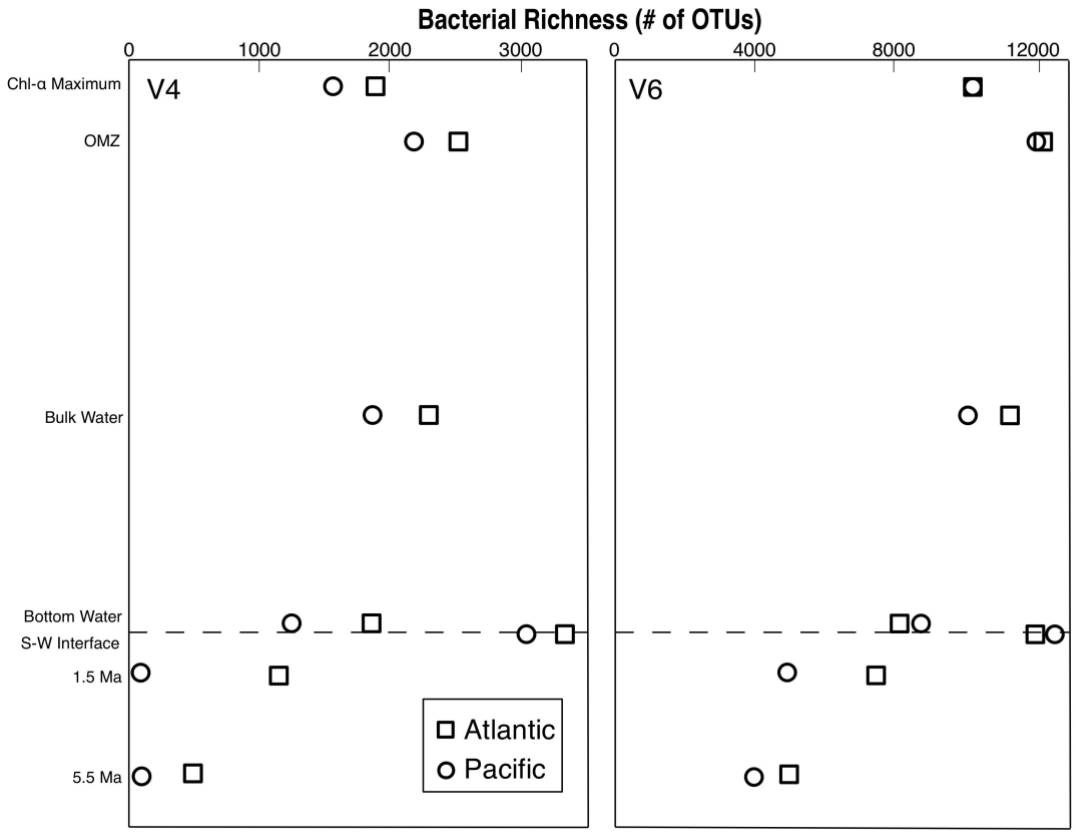

Figure 1. Bacterial richness profiles from the Pacific and Atlantic analyzed with both the V4 and V6 hypervariable region of 16S rRNA.

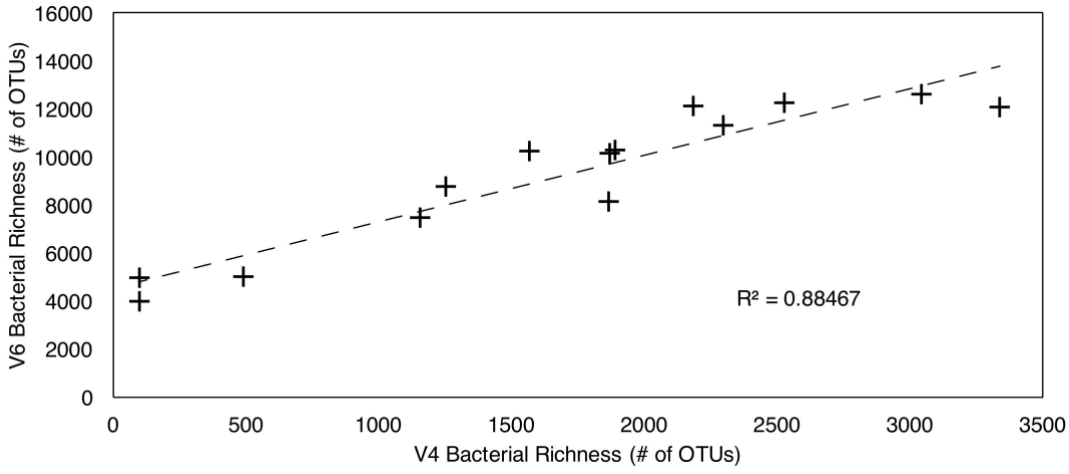

Figure 2. Linear regression of V4 to V6 hypervariable region richness values. $R^2 = 0.88467$.

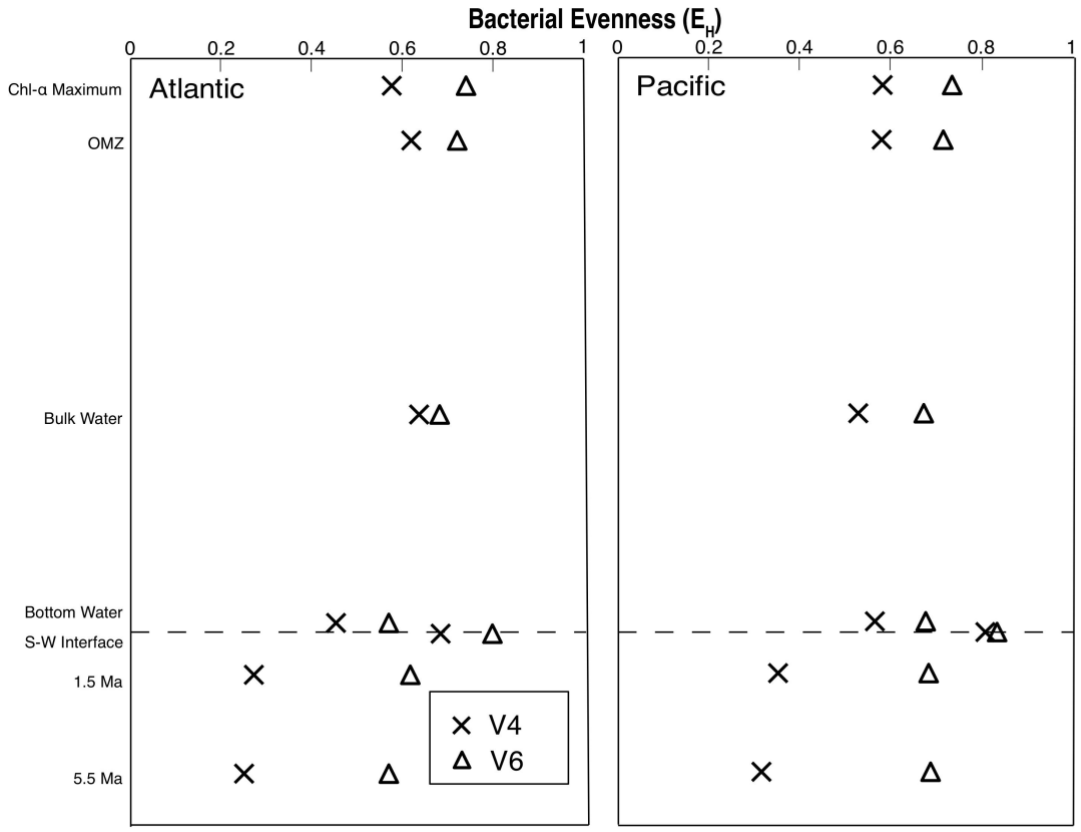

Figure 3. Bacterial evenness (E_H) profiles from the Pacific and Atlantic analyzed with both the V4 and V6 hypervariable region of 16S rRNA.

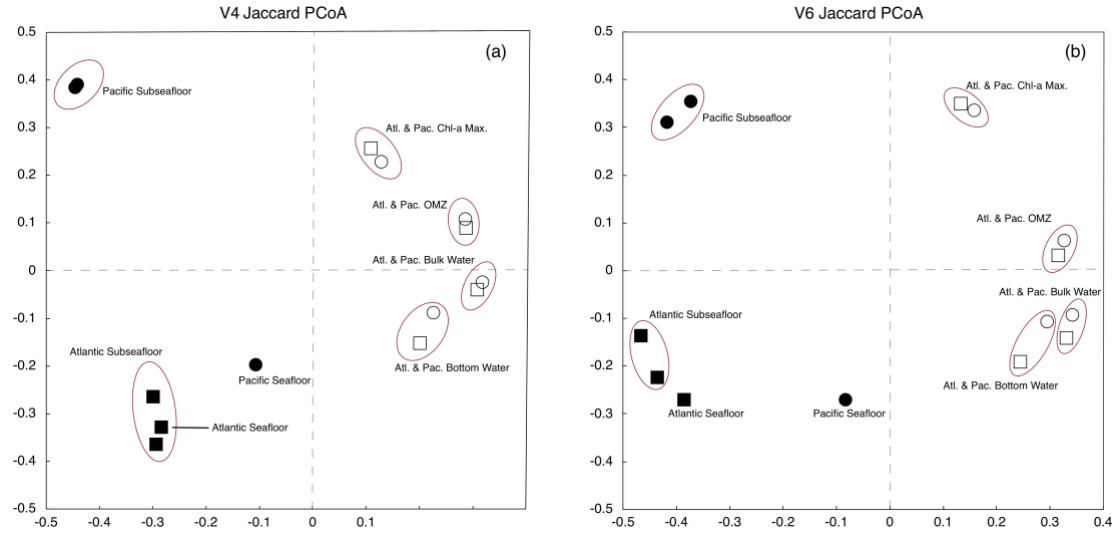

Figure 4. Jaccard Principle Coordinate Analysis (PCoA) of V4-based (a) and V6-based (b) tag sequences. Atlantic samples are squares and Pacific samples are circles. The sediment is depicted with darkened markers, and the water column is depicted with open markers.

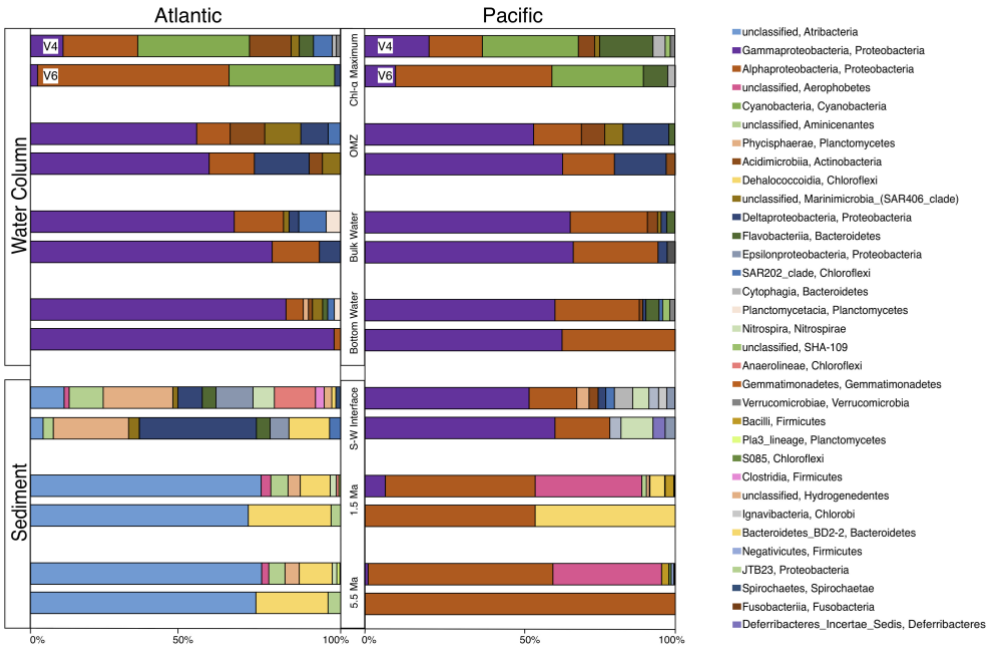

Figure 5. Class level community composition for the Atlantic and Pacific. At each depth horizon, the top bar displays community composition using the V4 tags, and the bottom bar displays community composition using the V6 tags.

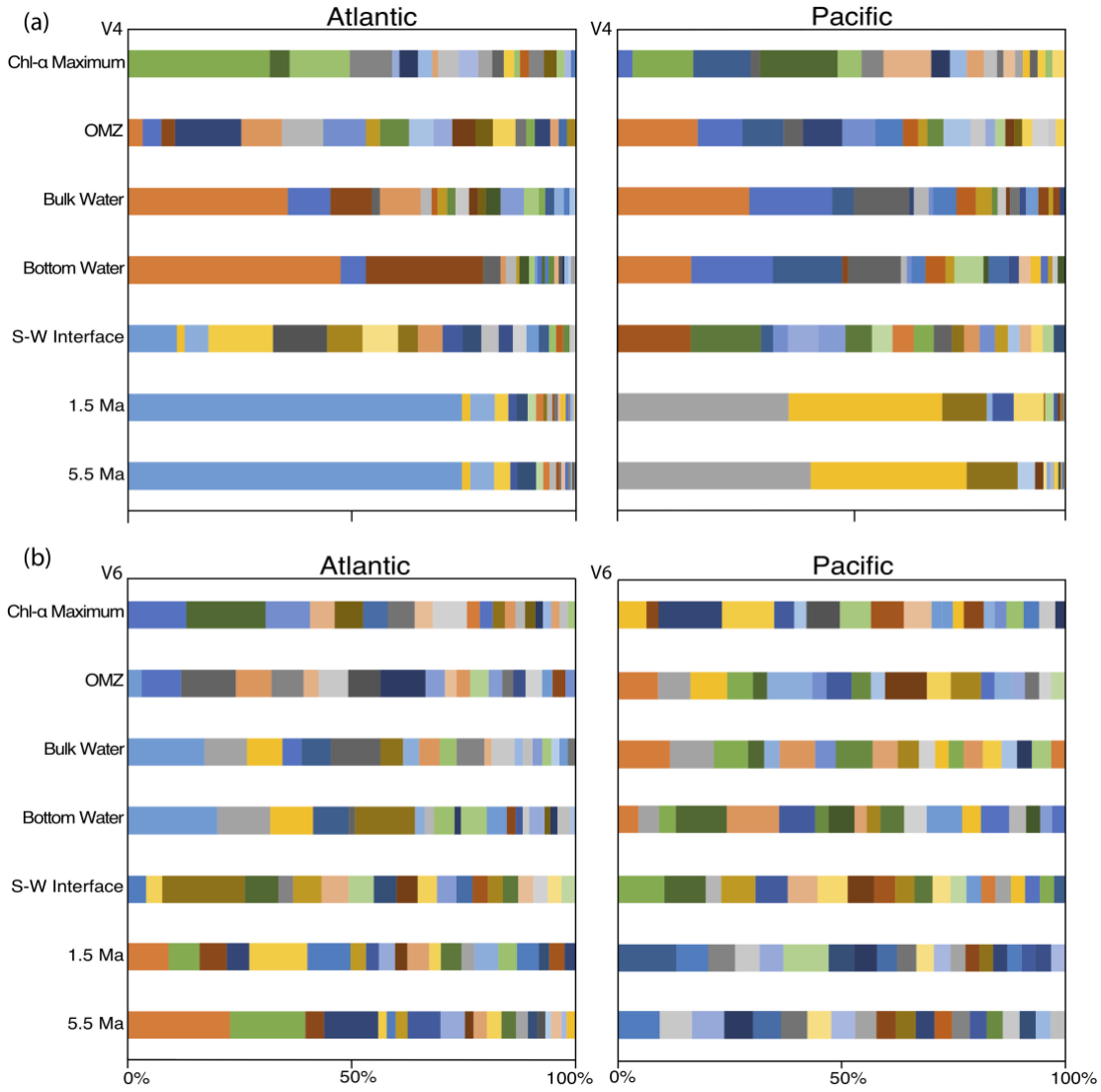

Figure 6. OTU level (97% similarity) community composition of the top 20 OTUs from the V4 hypervariable region (panel a) and the top 20 OTUs from the V6 hypervariable region (panel b). Colors correspond to identical OTUs between the Atlantic and Pacific within hypervariable region, but not across regions.

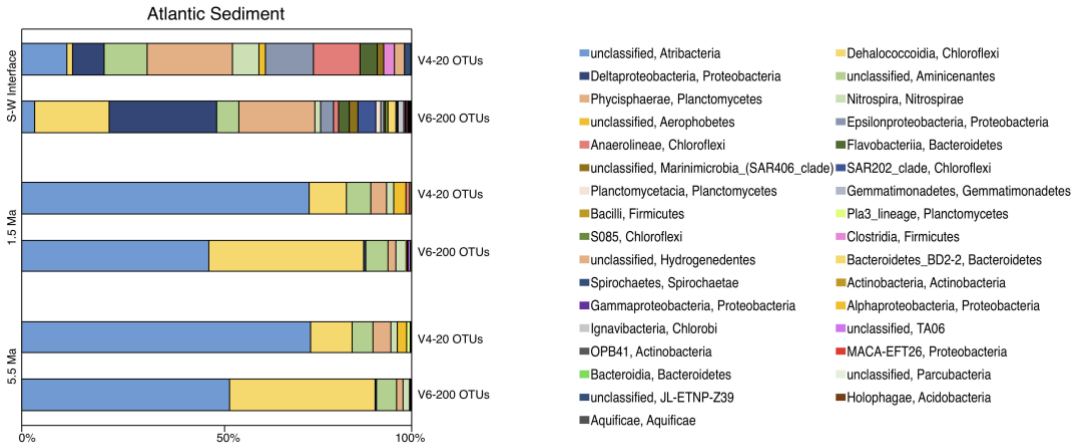

Figure S1. OTU level (97% similarity) community composition of the top 20 V4 OTUs compared to the top 200 V6 OTUs.

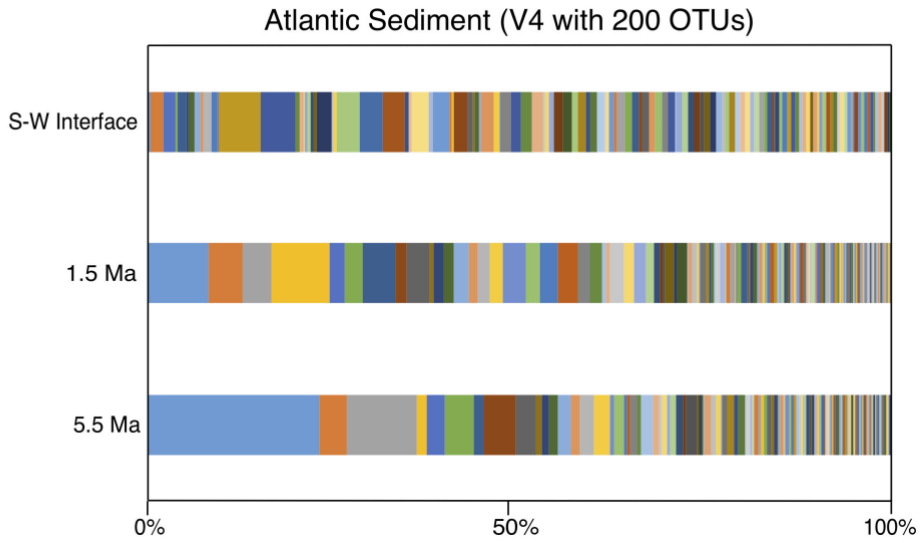

Figure S2. OTU level (97% similarity) community composition analysis of the top 200 OTUs based on V6 tag sequences for the Atlantic sediment.

MANUSCRIPT II

PATTERNS OF RELATIVE BACTERIAL RICHNESS AND COMMUNITY COMPOSITION IN SEAWATER AND MARINE SEDIMENT ARE ROBUST FOR BOTH OPERATIONAL TAXONOMIC UNITS AND AMPLICON SEQUENCE VARIANTS

by

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Abstract

To understand the relative influences of operational taxonomic units (OTUs) and amplicon sequence variants (ASVs) on patterns of marine microbial diversity and community composition, we examined bacterial diversity and community composition of seawater from 12 sites in the North Atlantic Ocean and Canadian Arctic and sediment from two sites in the North Atlantic. For the seawater analyses, we included samples from three to six zones in the water column of each site. For the sediment analyses, we included over 20 sediment horizons at each of two sites. For all samples, we amplified the V4-V5 hypervariable region of the 16S ribosomal RNA (rRNA) gene. We analyzed each sample in two different ways: (i) by clustering its reads into 97%-similar (OTUs), and (ii) by assigning sequences to unique, (ASVs). OTU richness is much higher than ASV richness for every sample, but both OTUs and ASVs exhibit similar vertical patterns of relative diversity in both the water column and the sediment. Bacterial richness is highest just below the photic zone in the water column, and at the seafloor in the sediment. For both OTUs and ASVs, estimates of taxonomic richness depend on the number of sequences analyzed. Community composition between the two methods is broadly similar in all samples at the taxonomic levels of phyla to families. While the two methods yield different richness values, broad-scale patterns of relative richness and community composition are similar with both methods.

Introduction

In recent years, high-throughput sequencing (HTS) has dominated studies of microbial diversity and community composition in marine environments (Inagaki et al., 2006; Bienhold et al., 2016; Kirkpatrick et al., 2019) and other environments (He et al., 2015; Tremblay et al., 2015; Berry et al., 2017; Glassman and Martiny, 2018). The ability to sequence either a large number of samples on a single sequencing run, or deep sequencing of a small number of samples (millions to tens of millions of amplicons per sample) generates terabytes of data for a single study. For this purpose, some studies have relied on operational taxonomic units (OTUs) (Huse et al., 2010; Jing et al., 2013; Bienhold et al., 2016; Kirkpatrick et al., 2019), whereas others have relied on amplicon sequence variants (ASVs) (Callahan et al., 2017; Hoshino et al., 2020; Fadeev et al., 2021). Studies that rely on OTUs typically cluster sequences at an operationally defined level of similarity (usually 97%) (Ward et al., 1998; Roesch et al., 2007; Huse et al., 2010; He et al., 2015; Mysara et al., 2017). This clustering is intended to reduce the impact on diversity estimates of errors that accumulate during PCR amplification and genetic sequencing (Huse et al., 2010; Kunin et al., 2010). Alternatively, studies that rely on ASVs use algorithms intended to remove errors associated with sequencing and return individual, unique sequences that represent individual taxa (Rosen et al., 2012; Eren et al., 2013; Callahan et al., 2016; Edgar, 2016). These sequences (ASVs or ESVs – exact sequence variants) differ from each other by as little as a single nucleotide change (Rosen et al., 2012; Callahan et al., 2016; Edgar, 2016).

Reliance of previous marine studies solely on OTUs or on ASVs has hindered synthesis of OTU-based studies with ASV-based studies to draw broad conclusions about distributions of microbial richness and community composition in the open ocean and marine sediment. Fortunately, patterns have begun to emerge from recent studies that compared the two methods using mock communities (Nearing et al., 2018; Prodon et al., 2020) and/or communities from diverse non-marine environments (soil [Nearing et al., 2018; Joos et al., 2020], plant-associated communities [Joos et al., 2020], fecal matter [Nearing et al., 2018; Prodon et al., 2020], and dairy products [Xue et al., 2018]). These previous studies have mostly focused on the differences between the OTU and ASV (we include ESVs, zero-radius OTUs, and other exact sequence nomenclature) methods, and the shortcomings of each. Here, we present an investigation that highlights the similarities and dissimilarities of OTU-based results and ASV-based results for the same microbial communities from seawater and marine sediment.

Our goals are to (i) assess the extent to which interpretations of marine microbial diversity and community composition depend on the analytical approach chosen (OTU or ASV), and (ii) identify how their respective results can be used to make conclusions regardless of each pipeline. For this purpose, we have characterized the bacterial diversity and community composition of seawater and marine sediment by clustering genetic sequences into 97% similar OTUs with the mothur pipeline (Schloss et al., 2009), and identifying ASVs from the same samples with the DADA2 pipeline (Callahan et al., 2016).

Materials and Methods

Sample collection and DNA extraction

We collected the seawater samples from twelve geographic locations in the western North Atlantic (*RV Falkor* expedition FK003b, and *RV Knorr* expedition KN223) and Canadian Arctic (*Icebreaker Oden* expedition NWP2019) (Fig. 1). We collected the water and measured oceanographic properties using a 24-Niskin bottle CTD rosette (SeaBird SBE 9). Once the CTD/Niskin system was on deck, we used a 50 in, D Bar clamp to seal the top and bottom of each 12 L Niskin bottle. The vent plugs of each Niskin bottle were replaced with t-fittings and a chain of plastic tubing was attached to each t-fitting to connect all the bottles to a common manifold which was connected to an air compressor. A cylindrical, 0.22 µm Sterivex membrane filter (Millipore Sigma, Billerica, MA) was connected to the petcock valve of each Niskin bottle, and the pressure manifold was pressurized between 8 and 10 psi to create a filtration rate between 66 and 80 mL/min to filter the entire contents of each bottle. Once each Niskin bottle was empty, we removed and sealed the filters and stored them in the shipboard -80 °C freezers until they were transported to the University of Rhode Island (URI), Graduate School of Oceanography (GSO) for post-expedition DNA extraction using the DNeasy PowerWater Sterivex Kit (Qiagen, Germantown, MD) following the manufacturer's protocol. We targeted a combination of standard depths and oceanographic horizons (sea surface, O_2-minimum, chlorophyll-maximum, thermocline, and deep-water) and analyzed two to six sampling depths at each station between 3 and 5500 m water depth depending on location (Table S1).

We collected the sediment samples from two locations in the North Atlantic (*RV Knorr* expedition KN223, Sites 15 and 16) (Fig. 1). We collected sediment samples from multiple sediment depths in cores that collectively span the interval from the seafloor to as much as 40 meters below seafloor (mbsf) (Table S2). Sediment coring was conducted using a multi-coring sampler (0 to 30 cm below seafloor (cmbsf)), a single, gravity-driven coring device (up to 4 mbsf), and a longer, piston-coring device (up to 40 mbsf). We subsampled the sediment using sterile 60 cc syringes that had been modified by removing the tapered tip and immediately froze the subsamples at -80°C for post-expedition DNA extraction at URI, GSO. For each sediment sample, we extracted DNA in duplicate, 0.25-g subsamples using the DNeasy PowerSoil Pro Kit (Qiagen, Germantown, MD) following the manufacturer's protocol, and pooled the duplicate extracts for amplification. Sediment age was approximated for each depth by dividing basement age (Müeller er al., 2008) by sediment thickness (Divins, 2003), and assuming a constant sediment deposition rate. Both sites exhibit similar dissolved chemistry profiles, with oxygen undetectable below 2 to 3 cmbsf (Murray et al., 2014).

PCR amplicon construction and sequencing

From each extract, we amplified the V4-V5 hypervariable region of the 16S rRNA gene using forward and reverse primers from Parada et al., 2016. We performed a 20-µl PCR reaction for each sample which contained a mixture of 0.4 µl Platinum SuperFi II DNA Polymerase (Invitrogen, Carlsbad, CA), 4 µl SuperFi II Buffer (5x), 0.2 mM dNTPs, 0.5 µM of each primer, and 0.1 µl Bovine Serum Albumin (Thermo

Scientific, Carlsbad, CA). We amplified each water sample in a single PCR reaction with 5 µl of DNA template and each sediment sample in triplicate with 12 µl of DNA template. To account for possible PCR reagent contamination during PCR amplification, we amplified three samples (one for each batch of polymerase used) containing only laboratory water and no extract. Additionally, we completed the full DNeasy PowerWater Sterivex protocol using a sterile Sterivex filter for each kit and the full DNeasy PowerSoil Pro protocol with no sediment for each kit to account for possible kit contamination. The thermal cycler program for all reactions began with an initial denaturation temperature of 98°C for 30 seconds followed by 35 cycles of 98°C for 10 seconds, 60°C for 10 seconds, and 72°C for 15 seconds, followed by a final extension of 72°C for 5 minutes. The triplicate sediment samples were then pooled, and we cleaned all of the samples using the Agencourt AMPure PCR Purification Kit (Beckman Coulter Life Sciences, Indianapolis, IN). Finally, all samples were sent to the University of Rhode Island Genomics and Sequencing Center and sequenced on an Illumina MiSeq using the Illumina MiSeq V3 chemistry at 2 x 300 cycles (NCBI BioProject PRJNA718862).

Sequence analyses

To process and analyze the sequences as 97%-similar OTUs, we used mothur v.1.42.3 (Schloss et al., 2009) and followed the mothur MiSeq SOP (Kozich et al., 2013, revision 6/24/19). To process and analyze the same sequences as ASVs, we used DADA2 v.1.16.0 (Callahan et al., 2016) and followed the DADA2 Pipeline Tutorial (1.16). In both cases, we removed from all samples any reads that were sequenced

from the PowerWater and PowerSoil extraction kits, as well as any reads from the PCR negative controls (described above), to account for possible lab and reagent contamination. Due to the low number of sequences in each of these negative controls, there was no impact to the results of each pipeline.

For our OTU analysis, each sample contained between 11 and 98,030 sequences prior to clustering. We randomly subsampled all samples to 10,000 sequences prior to clustering to make direct comparisons when conducting community analysis (Weiss et al., 2017). Although the 10k cutoff removed two of our smallest samples, we chose this value based on our previous study which showed that samples with 10,000 sequences provide the same, broad-scale patterns of relative taxonomic abundance as the same samples with much greater numbers of sequences (Kerrigan et al., 2019). Unless otherwise stated, we performed an opti-clust (Westcott and Schloss, 2017) clustering analysis at 97% sequence similarity. Additionally, we removed singletons and doubletons of OTUs from each sample to mitigate the likelihood of including OTUs based solely on sequencing error. To illustrate the effect of sequence number on estimates of taxonomic richness using OTUs, we compared OTU richness or representative samples based on total reads to OTU richness of the same samples based on 10,000 randomly subsampled sequences.

For our standard ASV analysis, we completed the DADA2 pipeline with all sequences. Unless otherwise stated, the ASV results presented are based on the non-rarefied samples. By default, the DADA2 algorithm removes singletons as part of its

process. To illustrate the effect of sequence number on estimates of taxonomic richness using ASVs, we compared ASV richness of representative samples based on total reads to ASV richness of the same samples based on 10,000 randomly subsampled sequences.

Ecological metrics and statistical analyses

We used R v 4.0.4 (R Core Team, 2021) to perform all diversity, community composition, and statistical analyses, primarily employing packages *phyloseq* (McMurdie and Holmes, 2013), *ggplot2* (Wickham, 2013), *dbplyr* (Wickham et al., 2021), and *vegan* (Oksanen et al., 2020). Our diversity analyses included calculations of OTU richness, as well as the Shannon and Simpson diversity metrics. We also calculated the Shannon's Equitability Index ($E_H = {}^{H}/_{ln\ S}$) (Pielou, 1966), where H is the Shannon Index and S is the taxonomic richness for each sample. For each diversity metric, we directly compared the values between the OTU and ASV analysis and plotted the linear regression with resulting Pearson's r and p-values. We also calculated the mean of the OTU and ASV values for all samples, and the associated standard error (SE). Finally, we calculated and plotted abundance curves for each sample with both the maximum number of sequences in each sample and the 10k-sequence subsampled data for both OTUs and ASVs.

Our community composition analyses included both taxonomic assignment of OTUs and ASVs from phylum to family, and dissimilarity calculations at the OTU/ASV level. For taxonomic classification, we assigned phylogenies to each OTU/ASV based

on the Silva reference database (v138) and then constructed relative taxonomic composition profiles for each sample by calculating the contribution of each taxon as a percentage of the whole sample. To compare the OTU/ASV-level community composition between samples, we calculated two-dimensional Bray-Curtis distance matrices, and plotted the results using Nonmetric Multidimensional Scaling (NMDS) ordination (Fig. 4). We also calculated an environmental fit ordination and overlaid the resulting parameter vectors (salinity, temperature, fluorescence, and oxygen) on the NMDS figures to help explain and visualize underlying trends in sample groupings.

Results

Taxonomic diversity

In total, 30,651 bacterial OTUs were generated from all samples by clustering a total of 564,364 sequences with the mothur pipeline. 18,696 bacterial ASVs were generated from all samples from a total of 2,402,375 sequences with the DADA2 pipeline. The large difference in total initial sequences for the two pipelines was due to rarefication of samples prior to clustering in the mothur pipeline, and not in the DADA2 pipeline. Although more than four times as many sequences were used to determine total ASVs over OTUs, nearly twice as many OTUs were generated as ASVs (mean = 1,102 ± 36 (SE) and 744 ± 38 (SE) for OTUs and ASVs, respectively). Despite this difference in their total numbers, OTU and ASV profiles exhibit similar vertical patterns of relative taxonomic richness (total number of OTUs or ASVs in each sample) in both the water column and the sediment (Fig. 2). In the water column, bacterial richness is highest at

or just below the Chlorophyll-a maximum. In the sediment, bacterial richness is highest at the sediment-water interface, and diminishes with increasing sediment depth and age, consistent with previously established trends (Walsh et al., 2016; Kerrigan et al., 2019; Kirkpatrick et al., 2019). The relative richness of bacterial OTUs in each sample is linearly correlated to the relative richness of bacterial ASVs (r=0.82, *p-value* < 0.00001), with average ASV richness generally lower than OTU richness (*slope* = 0.76) (Fig. 3).

The Shannon and Simpson diversity indices for OTUs and ASVs are similarly correlated (r = 0.87 (*p-value* < 0.00001) and r = 0.80 (*p-value* < 0.00001), respectively). Shannon diversity values are relatively high for both OTUs and ASVs (4.16 to 6.90) with ASV-based values generally higher than OTU-based values (slope = 0.80). The average ASV-based Simpson diversity values are also high and span a narrower range (0.96 to 0.99) than the OTU-based values (slope = 0.24) (Fig. 3).

Community evenness, based on the Shannon's Equitability Index (E_H), was also similar between OTU and ASV data. This index is a measure of community evenness on a scale from 0 to 1, where 1 is a value of total evenness (in which each OTU or ASV in the sample contains the same number of sequences). As with taxonomic richness and the diversity indices, OTU-based E_H values are linearly correlated to ASV-based E_H values (r = 0.88, *p-value* < 0.00001) (Fig. 3). Also, as with the diversity indices, ASV-based E_H values are generally higher and span a narrower range that OTU-based E_H values (Fig. 3).

To illustrate the effect of sequence numbers on estimates of taxonomic richness for both OTUs and ASVs, we analyzed all of the samples at both their entirety of amplicon sequences and randomly subsampled to 10,000 sequences per sample, as described above. We use two of the North Atlantic deep-sea sediment samples to illustrate the consequences and implications of the random subsampling (Fig. 4). Before subsampling and processing with either pipeline these samples (1 and 2) contained approximately 67k and 100k sequences, respectively. After completing the mothur OTU clustering of the full data set, sample 1 contained 56,630 sequences with 5,975 OTUs and sample 2 contained 79,914 sequences with 7,981 OTUs. At the end of the DADA2 ASV pipeline with the full data set, sample 1 contained 30,924 sequences with 391 ASVs, and sample 2 contained 49,501 sequences with 343 ASVs. The OTUs and ASVs yield very different rarefaction curves. For both samples, the OTU curve continues with a positive slope, whereas the ASV curve approaches an asymptote very quickly. Reducing the total number of reads by random subsampling significantly changes the rarefaction curves for both OTUs and ASVs. After randomly subsampling both samples to 10,000 sequences, the OTU pipeline retained 7,426 sequences with 598 OTUs in sample 1 and 7,478 sequences with 758 OTUs in sample 2. The ASV pipeline retained 6,631 sequences with 278 ASVs and 6,350 sequences with 200 ASVs for the same samples. For both OTUs and ASVs, the subsampled OTU and ASV numbers yield rarefaction curves with much lower rates of increase than the curves defined by the full numbers of sequences in each sample (Fig. 4). The ASV-

based curves for the subsamples approach much lower asymptotes than the asymptotes for the ASV-based curves for the total sequences in each sample.

Community composition

To compare the OTU-based community composition to the ASV-based community composition for all samples, we calculated Bray-Curtis distance matrices, and plotted the results using Nonmetric Multidimensional Scaling (NMDS) ordination (Fig. 5). To help explain and visualize underlying trends in sample groupings, we added environmental factors to the NMDS figures (salinity, temperature, fluorescence, and oxygen for the water column, and sediment depth and sediment age for the sediment). For both the water-column samples and the sediment samples, the OTU-based results and the ASV-based results grouped the samples similarly, with each grouping explained by environmental factors. For the water column, both the OTU-based analysis and the ASV-based analysis grouped the shallow-water and Arctic-water samples to the bottom right, which corresponded to higher oxygen and fluorescence values, and grouped the deeper and more southerly samples to the left, which corresponded to higher salinity and temperature values (Zinger et al., 2011). For the sediment, the OTU-based results and the ASV-based results both grouped the deeper (older) samples to the right along the x axis (Fig. 5).

In addition to OTU- and ASV-level community similarity, we mapped each OTU and ASV in our study to the Silva reference database (v138). We then calculated the relative abundance of each OTU and each ASV in each sample and compared the

bacterial results of each sample at the phylum, class, order, and family level. We chose five of the most diverse samples from one water-column and one sediment sample for illustration and compared the OTU- and ASV-based results of the most prevalent taxa at each phylogenetic level (Fig. 6). These examples show that the OTU-based results and the ASV-based results yield very similar patterns of taxonomic abundance. With either OTUs or ASVs, the water-column populations are dominated by *Proteobacteria* at the phylum level, *Alphaproteobacteria* at the class level, and *SAR 11* at the order level. OTUs and ASVs also yield similar abundance patterns for the sediment samples, which are dominated by the phylum *Planctomycetota*, the class *Phycisphaerae*, and the order *MSBL9*.

Discussion

Taxonomic richness

The vertical profiles of relative bacterial richness in both the water column and sediment were similar in nature between the OTU and ASV analysis (Fig. 2). This result indicates that the variation in relative taxonomic richness from one marine environment to another is captured by both OTUs and ASVs. In agreement with previous OTU-based studies, bacterial richness peaks in the water column at or just below the photic zone and decreases with increasing ocean depth (Signori et al., 2014; Walsh et al., 2016). Also, in agreement with previous OTU-based studies, bacterial richness in the sediment is highest at the seafloor and decreases with increasing depth (Walsh et al., 2016; Petro et al., 2017; Kirkpatrick et al., 2019).

While the OTU and ASV pipelines produced similar vertical patterns of relative richness, the large difference between OTU richness and ASV richness for each sample reinforces previous recognition that the choice of OTUs or ASVs significantly impacts estimates of total bacterial richness (Glassman and Martiny, 2018; Nearing et al., 2018; Xue et al., 2018; Prodon et al., 2020; Joos et al., 2020). In this study, the mothur-based OTU richness of each sample averages 1.77 times DADA2-based ASV richness of that sample. This could be caused either by the OTU pipeline accumulating and clustering sequencing errors, or by the ASV pipeline discarding real sequences during filtering and error modeling. However, the ratio of OTUs to ASVs can vary considerably by altering the user-defined parameters in each pipeline or by analysis with other pipelines (Prodon et al., 2020). In some cases, the ratio is even reversed; in comparing the UPARSE OTU clustering and ASV algorithms, Glassman and Martiny (2018) found bacterial OTU richness of leaf-litter communities to be, on average, half the ASV richness. However, in this study of marine communities, and in the previous studies of non-marine communities, patterns of relative variation in taxonomic richness between samples remain robust regardless of the method used. This result, of robust between-sample variation in relative richness within studies with large differences in richness between studies, does not solely depend on the choice of bioinformatic pipeline. It also depends on the specific 16S rRNA region amplified (Liu et al., 2008; Youssef et al., 2009; Mysara et al., 2017; Kerrigan et al., 2019).

The absence of an asymptote in the OTU-based rarefaction curves for the full datasets in Figure 4 shows that these OTUs cannot be used to identify an absolute number of

taxa in these samples. This issue is further demonstrated by the difference between the 10,000-read OTU-based rarefaction curves and the full-dataset OTU-based curves, which shows that the slope and rate of change in slope of these curves depends on the number of sequences used to define the curves. This issue is also known from studies of soil and plant-associated bacterial communities (e.g., Roesch et al., 2007; Joos et al., 2020). It has been ascribed to OTU instability (He et al., 2015) and to accumulation of sequencing errors with increasing numbers of reads (Joos et al., 2020). Unlike the OTU-based curves in Figure 4, the ASV-based curves for the same sample approach asymptotes quickly. However, as with the OTU-based curves, reducing the total number of reads to 10,000 per sample greatly changes the ASV-based rarefaction curves, leading to a much lower asymptote for both samples (Fig. 4). As with the OTU-based rarefaction curves, this issue has been previously observed for ASV-based rarefaction curves for bacterial communities associated with soil and plants (Joos et al., 2020). For ASV-based curves, the dependence of slope and asymptote on the total number of reads used to define the curve may be at least partly due to removal of singletons by the standard ASV pipeline. Although both the OTU approach and the ASV approach appear to capture similar patterns of variation in relative richness from one sample to another, this dependence of rarefaction curves on total read numbers indicates that the outputs of the standard pipelines for OTUs and ASVs are not good predictors of absolute richness.

Community composition

Our NMDS analyses reveal similar ordination patterns for both OTU-based community composition and ASV-based composition in both the water column and the sediment (Fig. 5). In the water column, samples associated with the Chlorophyll-a maximum are grouped together across samples, and distinct from those from deeper depths (Fig. 5). Each ordination also distinguishes the much deeper, and more southerly water samples taken from the North Atlantic from the other samples (Fig. 5). Inclusion of the environmental factors specific to each sample as an ordination overlay to the NMDS distribution shows that OTU-based ordination differences between OTUs and ASVs exhibit similar relationships to the environmental properties that control community composition. In short, as similarly seen in a recent study of leaf-litter bacteria and fungi (Glassman and Martiny, 2018), the OTU and ASV pipelines produce similar cross-sample diversity patterns for bacterial communities in seawater and marine sediment.

Direct comparison shows that OTU-based taxonomic assignments broadly match ASV-based assignments for the communities in both the water column and the sediment (Fig. 6). This similarity between the OTU- and ASV-based results is illustrated in Figure 6 by the water-column communities from our site located off the Southeast coast of Greenland, where the five sampled horizons, representing five distinct oceanographic zones, were dominated by *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria* in similar proportions using both OTUs and ASVs. At each taxonomic level from phylum to family, we show the finer scale composition of the most

abundant, or most common taxon (Fig. 6). Although there are some small differences, such as some of the ASV reads mapping to the *SAR 11 Clade II* while OTU reads remain *unclassified* in the deepest water sample, the results are generally very similar. Both pipelines returned results that broadly agree with previous OTU-based investigations of water-column community composition in different regions of the open ocean (DeLong et al., 2006; Brown et al., 2009; Jing et al., 2013; Tseng et al., 2015; Medina-Silva et al., 2018).

To compare OTU-based composition to ASV-based composition for the marine sedimentary communities, Figure 6 shows results for five of the most diverse deep-sea sediment samples from the North Atlantic. Both the OTU and ASV pipelines reveal similar communities in each sample, from the seafloor to 26 meters below seafloor (mbsf) (Fig. 6). These samples range in sediment age from approximately 0 to approximately 7 million years (Ma) (Fig. 6). As with our example seawater communities, the major taxonomic contributors are the same for both the OTU-based results and the ASV-based results and agree with the broad community composition of previous studies (Inagaki et al., 2006; Nunoura et al., 2016; Petro et al., 2017; Kerrigan et al., 2019).

In short, the OTU pipeline and the ASV pipeline yield very similar patterns of relative richness and community composition for both seawater and marine sediment. In combination with recent comparisons of OTU-based results and ASV-based results for bacterial and fungal communities in leaf litter (Glassman and Martiny, 2018) and for

bacterial and fungal communities associated with soil and plants (Joos et al., 2020), this result indicates that the OTU approach and the ASV approach yield similar ecological conclusions for diverse marine and terrestrial environments. This consistent similarity points to the potential of using both approaches simultaneously to check for consistency and gain confidence in the ecological conclusions from environmental genetic investigations.

Conclusions

Our comparison of OTU-based results and ASV-based results demonstrates that both methods yield similar patterns of relative taxonomic richness and evenness in seawater and marine sediment. NMDS groupings of the sampled communities and their relationships to environmental factors are also similar for both methods. For individual samples, from the phylum level to the family level, community composition is broadly similar for both methods.

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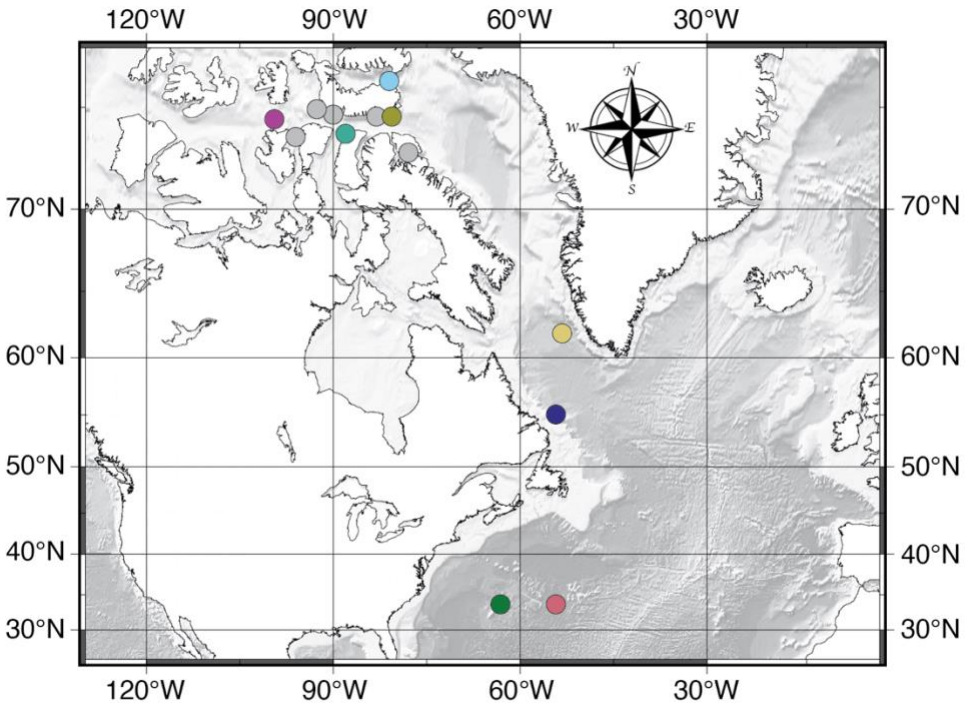

Figure 1. Sampling locations for this study. Data presented in this manuscript includes all sample locations except for the data in Figures 2 and 5, which only include the colored circles. The colors correspond to the same colors in Figures 2 and 5.

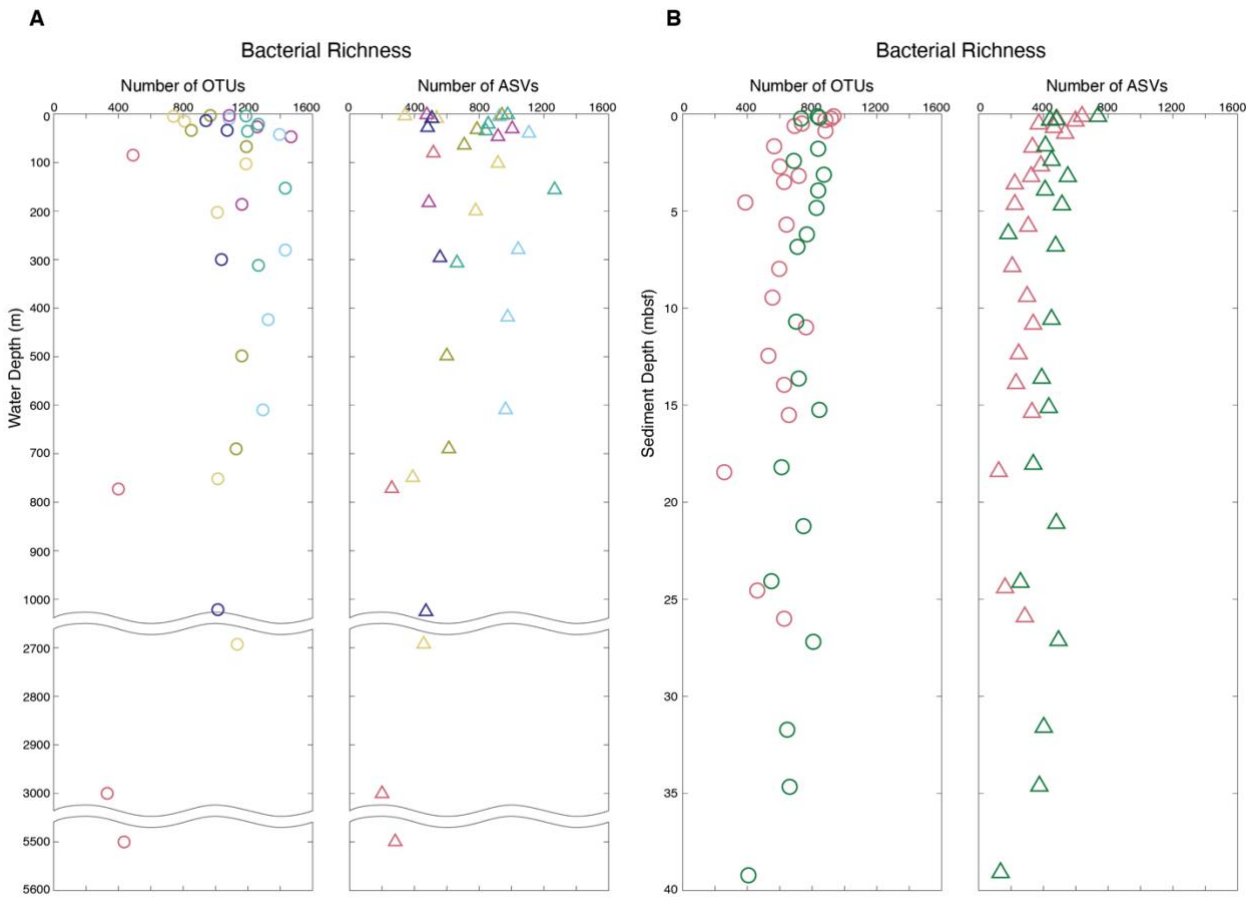

Figure 2. Bacterial richness profiles of the water column and marine sediment analyzed with both OTU- and ASV-based methods. All Arctic sample sites exhibit similar profiles and only four of the nine Arctic sites are presented here for ease of visualization. Sample colors correspond to sample sites plotted in Figure 1. (a) Bacterial richness of water-column OTUs (right) and ASVs (left). (b) Bacterial richness of marine-sediment OTUs (right) and ASVs (left)

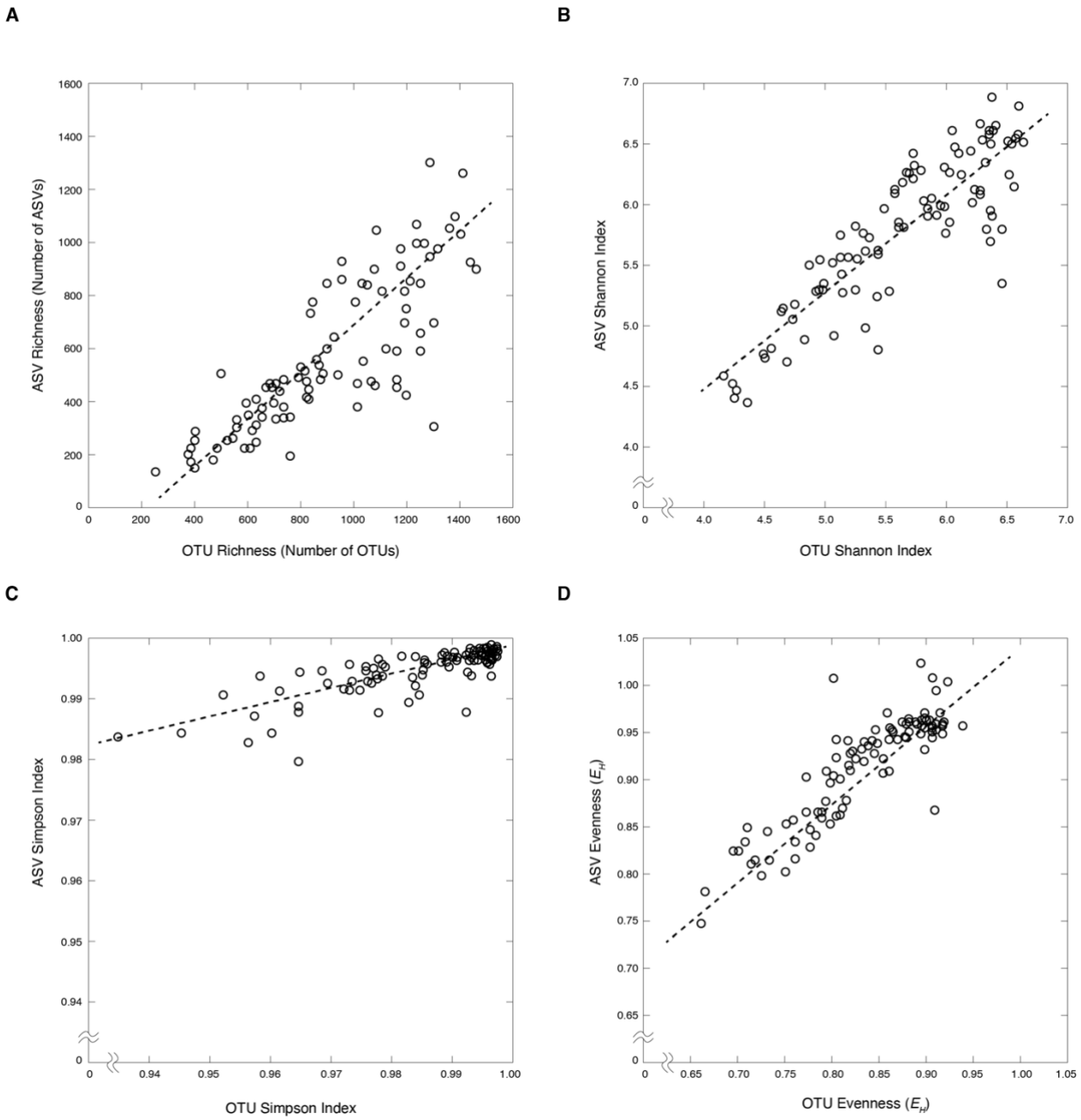

Figure 3. Bacterial diversity metrics of OTUs vs. ASVs for the water column (blue) and marine sediment (red). (a) Bacterial richness (r = 0.82, slope = 0.76). (b) Shannon index (r = 0.87, slope = 0.80). (c) Simpson index (r = 0.80, slope = 0.24). (d) Bacterial evenness (r = 0.88, slope = 0.76)

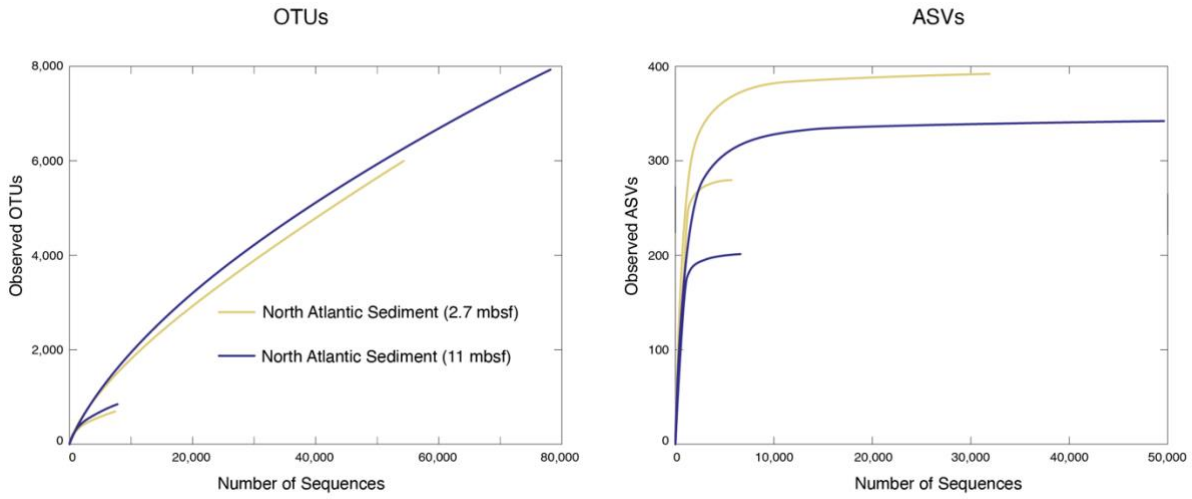

Figure 4. Bacterial taxonomic abundance curves of OTUs (left) and ASVs (right) for two marine-sediment samples at both their full number of sequences, and a subsampled, 10,000-sequence dataset

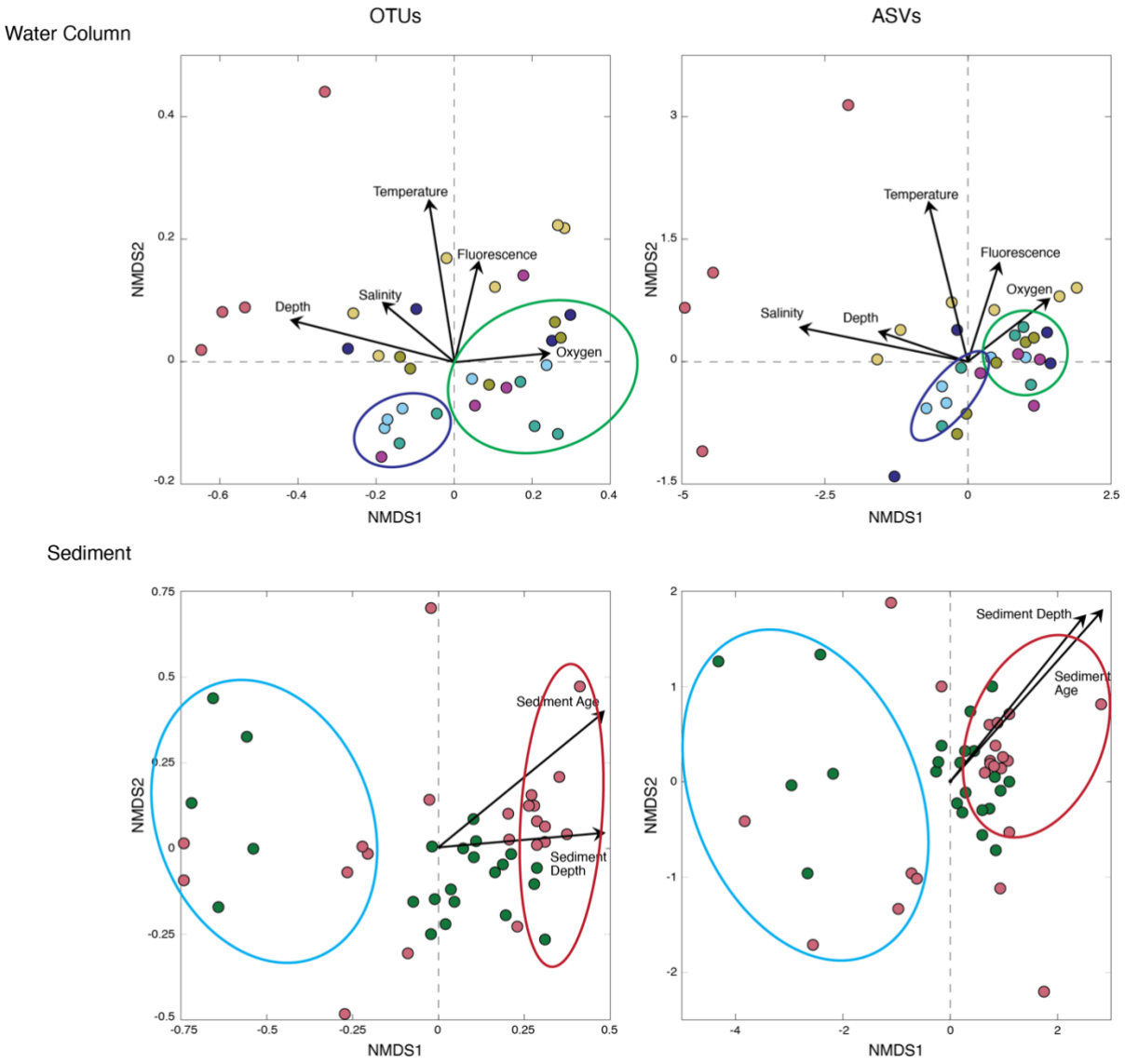

Figure 5. Bray-Curtis Nonmetric Multidimensional Scaling (NMDS) of the water column (top) and marine sediment (bottom). All Arctic samples exhibit similar groupings, and only four of the nine Arctic sites are presented here for ease of visualization. Sample colors correspond to sample sites plotted in Figure 1. OTUs (left) and ASVs (right) are overlayed with environmental factors for both the water column (salinity, temperature, fluorescence, and oxygen) and sediment (sediment depth and sediment age)

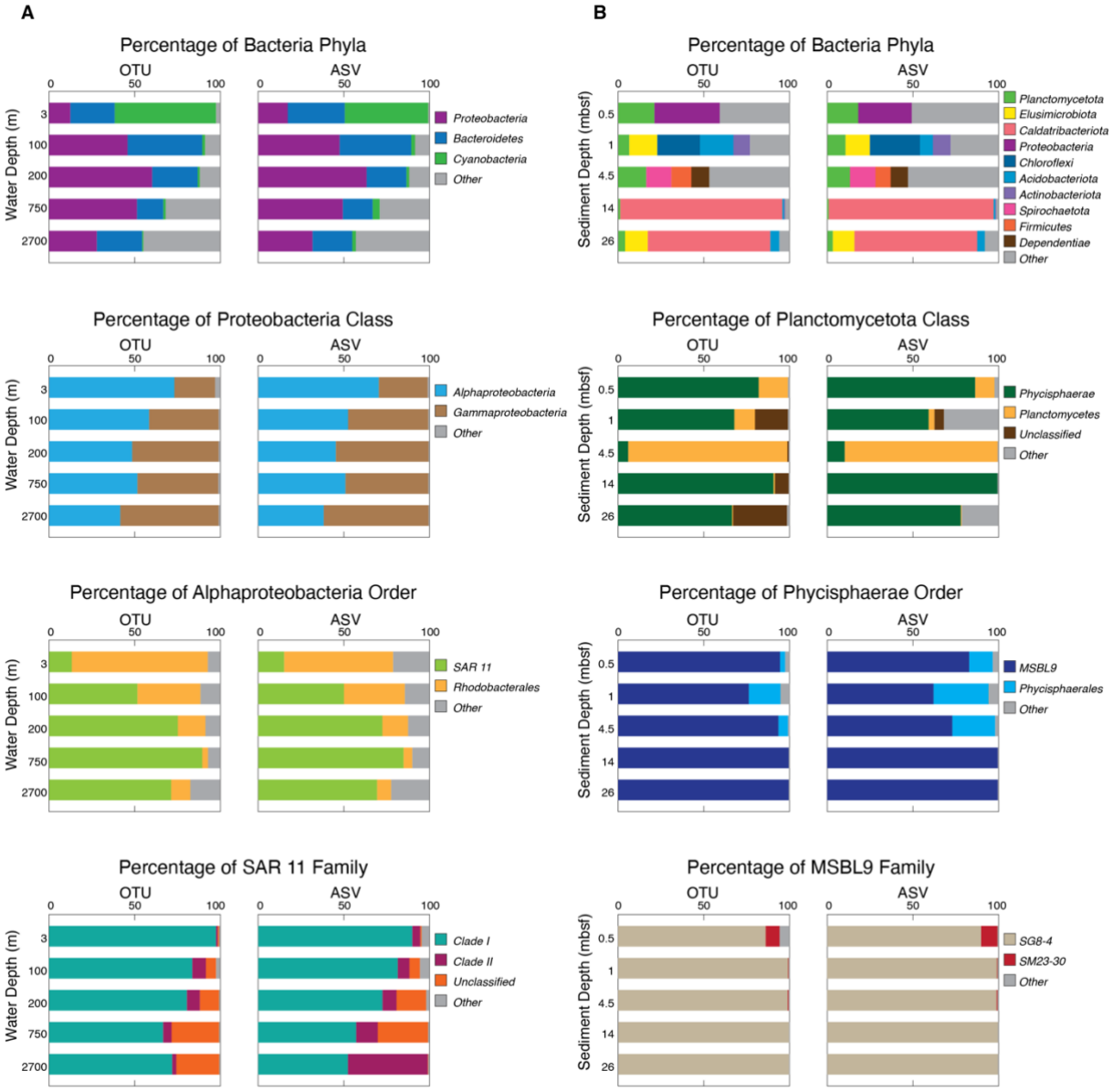

Figure 6. Bacterial community composition as analyzed with both OTUs and ASVs in the water column and marine sediment. (a) Water-column community composition of the Bacteria domain, Proteobacteria phylum, Alphaproteobateria class, and SAR 11 order with OTUs (left) and ASVs (right). (b) Marine-sediment community composition of the Bacteria domain, Planctomycetota phylum, Phycisphaerae class, and MSBL9 order with OTUs (left) and ASVs (right)

Table S1. Water-column samples

Cruise	*Site*	*Lat/Long*	*Water Depth (m)*	*Sample Depth (m)*
Northwest Passage Project July - August 2019	3	75°57'N 080°50'W	630	7 40 280 420 609
	5	72°47'N 078°15'W	780	20 60 380 600 770
	16	74°26'N 081°34'W	712	1 30 65 500 690
	21	74°40'N 093°07'W	146	10 25 136
	23	74°26'N 099°17'W	191	1.5 26 45 182
	24	73°45'N 096°49'W	220	1 30 100 210
	30	74°28'N 090°31'W	263	27 251
	40	73°29'N 088°55'W	330	1.5 20 35 150 308
	51	74°29'N 083°11'W	450	1.8 30 225 425
R/V Falkor Cruise 003b July 2012	8	61°53'N 053°16'W	2890	3 10.4 101 201 751 2690
	10	55°09'N 054°16'W	1032	10.3 29 299 1046
R/V Knorr Cruise 223 October - December 2014	15	33°29'N 054°10'W	5510	81 771 3000 5505

Table S2. Sediment samples

Cruise	*Site*	*Lat/Long*	*Water Depth (m)*	*Sample Depth (mbsf)*	*Sediment Age (Ma)*
R/V Knorr Cruise 223	15	33°29'N	5510	0.13	0.03
October - December 2014		054°10'W		0.26	0.07
				0.52	0.14
				0.65	0.17
				0.2	0.05
				0.9	0.24
				1.7	0.45
				3.2	0.85
				4.62	1.23
				2.7	0.72
				3.5	0.93
				5.7	1.52
				8	2.13
				9.5	2.53
				11	2.9
				12.5	3.33
				14	3.72
				15.5	4.12
				18.5	4.92
				21.5	5.72
				24.5	6.52
				26	6.92
	16	33°41'N	4545	0.13	0.01
		057°37'W		0.26	0.03
				0.39	0.04
				0.2	0.02
				1.7	0.17
				2.4	0.24
				3.9	0.39
				4.77	0.48
				0.2	0.02
				3.2	0.32
				4.7	0.47
				6.2	0.63
				6.9	0.7
				10.7	1.08
				13.7	1.38
				15.2	1.54
				18.2	1.84
				21.2	2.14
				24.2	2.44
				27.2	2.75
				31.7	3.2
				34.7	3.5
				39.2	3.96

MANUSCRIPT III

MICROBIAL COMMUNITY COMPOSITION OF CANADIAN ARCTIC SEAWATER

by

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Abstract

The Arctic Ocean connects the Pacific and Atlantic oceans, making it a key pathway for potentially linking marine microbial communities throughout the world. To characterize the microbial community composition of the Arctic Ocean, we examined bacterial and archaeal diversity and community composition from eleven sites in the Canadian Arctic Ocean (Northwest Passage), and three sites in the North Atlantic Ocean. For each site, we analyzed samples from three to six water depths. We amplified the V4-V5 hypervariable region of the 16S ribosomal RNA gene (rRNA) and analyzed each sample by clustering into operational taxonomic units (OTUs) of 97% similarity. Vertical profiles of taxonomic richness in the Arctic seawater are similar to those in other oceans, with highest relative richness at depths just below the chlorophyll maximum. At the 97%-similar OTU level, the Northwest Passage communities are distinct from communities in the Davis Strait, the North Atlantic, and the Central Pacific. At the phylogenetic class level, the Northwest Passage communities are broadly similar to those of the Atlantic and Pacific oceans. However, unlike open-ocean Atlantic and Pacific communities, *Cyanobacteria* are almost totally absent from the Northwest Passage communities. In this regard, the Northwest Passage communities resemble coastal communities in other parts of the world ocean, where primary production is dominated by eukaryotes (e.g. diatoms).

Introduction

Nowhere is understanding marine microbiology more important than in the Arctic region, due to both the rapid climatic changes particular to this region, and the understudied nature of this environment (Vincent, 2010). The rate of Arctic temperature increase is almost twice that of other regions (Screen and Simmons, 2010; Dai et al., 2019). Fully understanding the future effect of this drastic climate change on Arctic marine communities requires immediate knowledge of present-day Arctic communities.

Largely because the Arctic is a challenging environment, few studies have focused on the microbial communities in its waters (Galand et al., 2009; Kirchman et al., 2010; Comeau et al., 2011; Hamdan et al., 2013; Quero et al., 2019). Some studies have already analyzed both the bacteria and archaea that make up the microbial communities (Galand et al., 2009; Comeau et al., 2011; Hamdan et al., 2013). One of these studies showed that bacterial community composition is different in Pacific-sourced Arctic water and in the underlying sediment than in Atlantic-sourced water and in the sediment beneath it (Hamdan et al., 2013). However, vertical successions of microbial communities in the Arctic Ocean, and their relationships to microbial communities in other oceans are not fully characterized. Additionally, while these studies have locally characterized the microbial communities, and others have hinted at the importance of Arctic microbes to the marine food web (Jungblut, et al., 2010; Harding et al., 2018), the relationship of Arctic-ocean microbes to the rest of the world

remains poorly understood. Understanding these relationships could provide valuable insight into the global changes to come (Vincent, 2010).

To advance knowledge of microbial communities in the Arctic Ocean, and their relationship to communities in the North Atlantic and Pacific oceans, we completed a high-throughput sequencing (HTS) study of 16S rRNA from locations throughout the Northwest Passage (NWP) in the Canadian Arctic, the Davis Strait, and the North Atlantic open ocean. At each location, sampled horizons range from the sea surface to just above the seafloor, and include oceanographic horizons that occur in all three regions. We compare these results to those of other studies in the Arctic and Pacific Oceans to strengthen the global picture of oceanic microbial diversity and community composition. Our goals are to (*i*) build robust vertical profiles of microbial diversity and community composition in the NWP of the Arctic Ocean, (*ii*) identify similarities and differences between these communities and communities in the open Atlantic and Pacific Oceans, and (*iii*) examine the connectivity of microbial communities in all three oceans.

Materials and Methods

Sample collection and DNA extraction

We collected water-column samples at eleven sites from the Northwest Passage (NWP) in the Canadian Arctic aboard the *Icebreaker Oden* in the summer of 2019 (expedition NWP2019). For comparison, we also analyzed water-column samples from two sites in the Davis Strait (*RV Falkor* expedition FK003b), and one site in the

North Atlantic (*RV Knorr* expedition KN223) (Fig. 1). We collected the seawater and measured oceanographic properties using a 24-Niskin bottle CTD rosette (SeaBird SBE 9). Once the CTD/Niskin system was on deck, we used a 50 in, D Bar clamp to seal the top and bottom of each 12 L Niskin bottle. The vent plugs of each Niskin bottle were replaced with t-fittings and a chain of plastic tubing was attached to each t-fitting to connect all the bottles to a common manifold which was connected to an air compressor. A cylindrical, 0.22 µm Sterivex membrane filter (Millipore Sigma, Billerica, MA) was connected to the petcock valve of each Niskin bottle, and the pressure manifold was pressurized between 8 and 10 psi to create a filtration rate between 66 and 80 mL/min to filter the entire contents of each bottle. Once each Niskin bottle was empty, we removed and sealed the filters and stored them in the shipboard -80 °C freezers until they were transported to the University of Rhode Island (URI), Graduate School of Oceanography (GSO) for post-expedition DNA extraction using the DNeasy PowerWater Sterivex Kit (Qiagen, Germantown, MD) following the manufacturer's protocol. We targeted a combination of standard depths and oceanographic horizons (sea surface, O_2-minimum, chlorophyll-maximum, thermocline, and deep-water) and analyzed two to six sampling depths at each station between 3 and 5500 m water depth depending on location (Table S1).

PCR amplicon construction and sequencing

From each extract, we amplified the V4-V5 hypervariable region of the 16S rRNA gene using forward and reverse primers from Parada et al., 2016. We performed a 20-µl PCR reaction for each sample. The reaction medium contained a mixture of 0.4 µl

Platinum SuperFi II DNA Polymerase (Invitrogen, Carlsbad, CA), 4 µl SuperFi II Buffer (5x), 0.2 mM dNTPs, 0.5 µM of each primer, 0.1 µl Bovine Serum Albumin (Thermo Scientific, Carlsbad, CA), and 5 µl of DNA template. To account for possible PCR reagent contamination during PCR amplification, we amplified three samples (one for each batch of polymerase used) containing only laboratory water and no extract. Additionally, we completed the full DNeasy PowerWater Sterivex protocol using a sterile Sterivex filter for each kit to account for possible kit contamination. The thermal cycler program for each sample began with an initial denaturation temperature of 98°C for 30 seconds, followed by 35 three-step cycles (98°C for 10 seconds, 60°C for 10 seconds, 72°C for 15 seconds), and a final extension of 72°C for 5 minutes. We then cleaned all of the samples using the Agencourt AMPure PCR Purification Kit (Beckman Coulter Life Sciences, Indianapolis, IN). Finally, all samples were sent to the University of Rhode Island Genomics and Sequencing Center for sequencing on an Illumina MiSeq using the Illumina MiSeq V3 chemistry at 2 x 300 cycles (NCBI BioProject PRJNA718862).

OTU construction

To process and analyze the sequences as 97%-similar OTUs, we used mothur v.1.42.3 (Schloss et al., 2009), following the mothur MiSeq SOP (Kozich et al., 2013, revision 6/24/19). From each sample, we removed any reads that were sequenced from the PowerWater extraction kits, as well as the PCR negative controls (described above) to account for possible lab and reagent contamination. Due to the low number of sequences in each of these negative controls, there was no significant impact to the

results in any sample. Prior to clustering, each sample contained between 11 and 98,030 sequences. We randomly subsampled all samples to 10,000 sequences prior to clustering to make direct comparisons when conducting community analysis (Weiss et al., 2017). Although the 10k cutoff removed two of our smallest samples, we chose this value based on our previous study which showed that samples with 10,000 sequences provide the same, broad-scale results as those at deeper sequencing depths (Kerrigan et al., 2019). Unless otherwise stated, we performed an opti-clust (Westcott and Schloss, 2017) clustering analysis at 97% sequence similarity. We removed singletons and doubletons of OTUs from each sample to mitigate the likelihood of including OTUs based solely on sequencing error. We also removed any OTUs associated with mitochondria and chloroplasts.

Additionally, we used DADA2 v.1.16.0 (Callahan et al., 2016) and followed the DADA2 Pipeline Tutorial (1.16) to process and analyze the sequences as amplicon sequence variants (ASVs). We compared the 97%-similar OTU results to the ASV results for consistency across all diversity and community composition metrics presented in this manuscript. While all results in this manuscript were based on the 97%-similar OTU analyses, the ASV-based results were in complete agreement.

Ecological analyses

We used R v 4.0.4 (R Core Team, 2021) to perform all diversity, community composition, and statistical analyses, primarily employing packages *phyloseq* (McMurdie and Holmes, 2013), *ggplot2* (Wickham, 2013), *dbplyr* (Wickham et al.,

2021), and *vegan* (Oksanen et al., 2020). Our diversity analyses included calculations of OTU richness, as well as the Shannon and Simpson diversity metrics. We also calculated the Shannon's Equitability Index ($E_H = {}^{H}/_{ln\ S}$) (Pielou, 1966), where H is the Shannon Index and S is the taxonomic richness for each sample. In addition to the raw values of each of the above diversity indices, we combined each of the NWP samples into one of four separate bins based on environmental data and calculated the mean and standard deviation for each of the bins to show the statistically significant variation in the vertical patterns of each index. Bin one includes all samples from the sea surface to approximately 30 m below the surface and is characterized by peak chlorophyll levels, minimum salinity and temperature values, and maximum oxygen levels (Fig. 3). Bin two includes all samples from 30 to 200 m below sea surface and is characterized by the rapid drop in oxygen levels, very low chlorophyll levels, and near constant temperature and salinity (Fig. 3). Bin four includes all samples from 200 to 400m and is characterized with a distinct thermocline and halocline, and a gradual decrease in oxygen levels (Fig. 3). Bin four includes all samples from 400 m to the sea floor and is characterized by stable values of all environmental parameters (Fig. 3).

Our community composition analyses included both taxonomic assignment of OTUs at the phylum and class levels, and dissimilarity calculations at the OTU level. For taxonomic classification, we assigned phylogenies to each OTU based on the Silva reference database (v138) and then constructed a relative taxonomic composition profile for each sample by calculating the contribution of each taxa as a percentage of the whole sample. To compare the OTU-level community composition between

samples, we calculated two-dimensional Bray-Curtis distance matrices, and plotted the results using Nonmetric Multidimensional Scaling (NMDS) ordination (Fig. 4). We also calculated an environmental fit ordination and overlayed the resulting parameter vectors (salinity, temperature, fluorescence, and oxygen) over the NMDS figures to help explain and visualize underlying trends in sample groupings.

Results

Taxonomic diversity

In total, 20,231 OTUs were identified from 306,915 sequences by clustering into 97%-similar OTUs which included 18,046 bacterial OTUs and 2,185 archaeal OTUs. In general, bacterial richness is lowest in the surface waters, peaks at or just below the Chlorophyll-a maximum, and then decreases or remains fairly constant with depth (Fig. 2). Archaeal richness at all water depths is lower than bacterial richness (Comeau et al., 2011; Hamdan et al., 2013; Tseng et al., 2015). It is lowest in the surface water and increases substantially with depth (Fig. 2) (Brown et al., 2009). The Shannon and Simpson diversity indices for bacteria exhibit similar vertical patterns, with the lowest values in surface waters, increasing with depth to just below the Chlorophyll-a maximum, and then remaining relatively constant with increasing depth (Fig. 3). The Shannon and Simpson diversity indices for archaea exhibit vertical patterns similar to the bacterial patterns, although the very low archaeal richness in surface waters likely skews the diversity indices and, in general, archaeal diversity is relatively constant with depth (Fig. 3).

The Shannon's Equitability Index (E_H) provides a measure of taxonomic evenness on a scale from 0 to 1, where 1 is a value of total evenness (each OTU in the sample contains the same number of sequences). For bacteria, the vertical pattern is similar to the richness patterns, where evenness values are lowest in surface waters, increase just below the Chlorophyll-a maximum, and remain relatively constant with increasing depth (Brown et al, 2009; Agogue et al., 2011). Archaeal evenness is also similar across geographic locations, but again affected by low OTU richness in surface water, and communities at all depths are generally relatively even (Fig. 3).

Community Composition

To compare the OTU-level community composition between samples, we calculated two-dimensional Bray-Curtis distance matrices, and plotted the results using Nonmetric Multidimensional Scaling (NMDS) ordination (Fig. 4). To help explain and visualize underlying trends in sample groupings, we added environmental factors to the NMDS figures (salinity, temperature, fluorescence, and oxygen). For bacteria, the shallow-water samples from the Davis Strait and NWP group together to the bottom right, corresponding to higher oxygen and fluorescence values, whereas the deeper samples from the NWP and Davis Strait group to the left with all samples from the North Atlantic, corresponding to higher salinity and temperature values. (Fig. 4a). For archaea, although the shallow-water samples from the Davis Strait and NWP contain relatively few OTUs, they still group to the right, corresponding to higher oxygen and fluorescence values, and the deeper samples from the NWP and Davis Strait group to

the left with all samples from the North Atlantic, corresponding to higher salinity and temperature values (Fig. 4b).

In addition to examining OTU-level community similarity, we mapped each OTU to the Silva reference database (v138). We then calculated the relative abundance of each bacterial and archaeal OTU in each sample at the phylum and class level (Fig. S1). The bacterial populations are dominated by the *Bacteroidetes* and *Proteobacteria* phyla, and the *Bacteroidia*, *Alphaproteobacteria*, and *Gammaproteobacteria* classes with greater proportions of *Bacteroidia* in the shallow-water samples from each location. The archaeal populations are heavily dominated by the *Chrenarchaeota* phylum, and the *Nitrososphaeria* class at all water depths.

Discussion

Vertical patterns of microbial richness in the Northwest Passage are similar to those in the Pacific and Atlantic Oceans. As in those regions (Signori et al., 2014; Tseng et al., 2015; Walsh et al., 2016; Medina-Silva et al., 2018; Kerrigan et al., 2019), bacterial richness is highest just below the Chlorophyll-a maximum and slightly lower at greater depths (Fig. 2a). This result is further highlighted by the Shannon and Simpson diversity indices (Fig. 3). While previous studies have reported highest bacterial richness in the oxygen-minimum zone (e.g., Walsh et al., 2016), we cannot test this association in the Northwest Passage, where water depth is relatively shallow (146 to 780 meters below sea level(mbsl)) (Table S1), and there is not a clear oxygen-minimum zone (Fig. 2d). The vertical pattern of archaeal richness in the NWP is also

similar to that in other locations, with very few taxa in shallow water and increasing taxonomic richness with depth (Fig. 2b) (Brown et al., 2009).

While the vertical pattern of taxonomic richness is similar to that in other parts of the world, community composition is different than in open-ocean regions. At the OTU level, our ordination analysis distinguishes the bacterial communities of the NWP from those of the Davis Strait and the North Atlantic (Fig. 4a). In general, samples taken from the NWP group together in the bottom right of the NMDS ordination, in contrast to the North Atlantic samples, which plot to the far left and top of the ordination. Between these groupings are the samples from the Davis Strait, as well as samples from two of the NWP sites that are closest to Baffin Bay. In each case, the groupings are tied more closely to the environmental factors of the water masses, rather than the geographic location, similar to previous findings (Galand et al., 2009; Zinger et al., 2011; Hamdan et al., 2013; Jing et al., 2013). The NMDS ordination of archaeal community composition is not as visually distinct as that of bacteria, but similar regarding the grouping of samples (Fig. 4b). Because the ordination is based on a Bray-Curtis calculation that compares the OTUs of one sample to another, fewer total OTUs per sample can result in ordination values that are more different from one sample to another, and perhaps create an NMDS with samples that are more spread out. Both figures illustrate that although all of these sites are physically connected by ocean water, environmental parameters (temperature, salinity, etc.) isolate each geographically distinct community (Agogue et al., 2011; Zinger et al., 2011; Hamdan et al., 2013; Sunagawa et al, 2015; Bienhold et al., 2016), and although the class-level

community composition may be very similar (especially with regard to archaeal communities) (Fig. S1), there are OTU-level differences that contribute to this distinction.

Comparison of the bacterial classes abundant in communities from different regions clarifies the similarities and differences between the Arctic (Northwest Passage) communities and communities elsewhere (Fig. 5). For this comparison, we included surface and deep-water communities from three other studies; two in the Arctic (Kirchman et al., 2010; Comeau et al., 2011), and one in the Pacific Ocean (Kerrigan et al., 2019), and we grouped the NWP sites as two locations; one for the bulk of the sites, and one for the three sites bordering Baffin Bay. Figure 5 also shows the major ocean currents in the greater Arctic region, to show the potential for interaction or isolation between sites (Fig. 5). We assigned all of the communities to one of two broad water-depth categories: near-surface (less than approximately 100 mbsl) or deep (greater than 100 m). Regardless of ocean region, the near-surface communities are characterized by varying relative abundances of *Bacteroidetes*, *Cyanobacteria*, *Gammaproteobacteria*, and *Alphaproteobacteria* (Fig. S1). However, nearly all of the OTUs associated with *Cyanobacteria* in the near-surface NWP and Davis Strait samples are classified as *Chloroplasts* while a large proportion of the North Atlantic surface water is associated with true *Cyanobacteria*, either *Prochlorococcus* or *Synechococcus*. Once the chloroplast-associated OTUs were removed from all samples (*see Methods*), the NWP communities are distinctly different from communities in open-ocean regions. In the Pacific and Atlantic Oceans, the surface

water contains a large proportion of *Cyanobacteria* and these are the predominant primary producers of the low-nutrient, open ocean (DeLong et al., 2006; Campbell et al., 2015; Kerrigan et al., 2019). In contrast, our NWP and Davis Strait samples contain very few *Cyanobacteria*, suggesting that the majority of primary production is due to eukaryotes, most likely diatoms (Jing et al., 2013). This absence of *Cyanobacteria* differs from previous Arctic studies, which reported *Cyanobacteria* as prevalent in the high Arctic (Jungblut, et al., 2010) and a key component for fixation of biologically available nitrogen (Harding, et al., 2018). However, a lack of *Cyanobacteria* is characteristic of coastal communities in other oceanic regions (Campbell et al., 2015). Consequently, their absence from the NWP and Davis Strait communities may result from coastal influence, rather than a signature of Arctic open-ocean communities. Although most of the Arctic deep-water samples are devoid of *Cyanobacteria*, the sample taken from a depth of approximately 750 m just southwest of Greenland in the Davis Strait contains a high proportion of true *Cyanobacteria*, similar to the surface water of the North Atlantic (Fig. 5). This site is located at one of the major deep-water formation areas of the North Atlantic, and directly fed by the Irminger Current bifurcation of the North Atlantic Drift.

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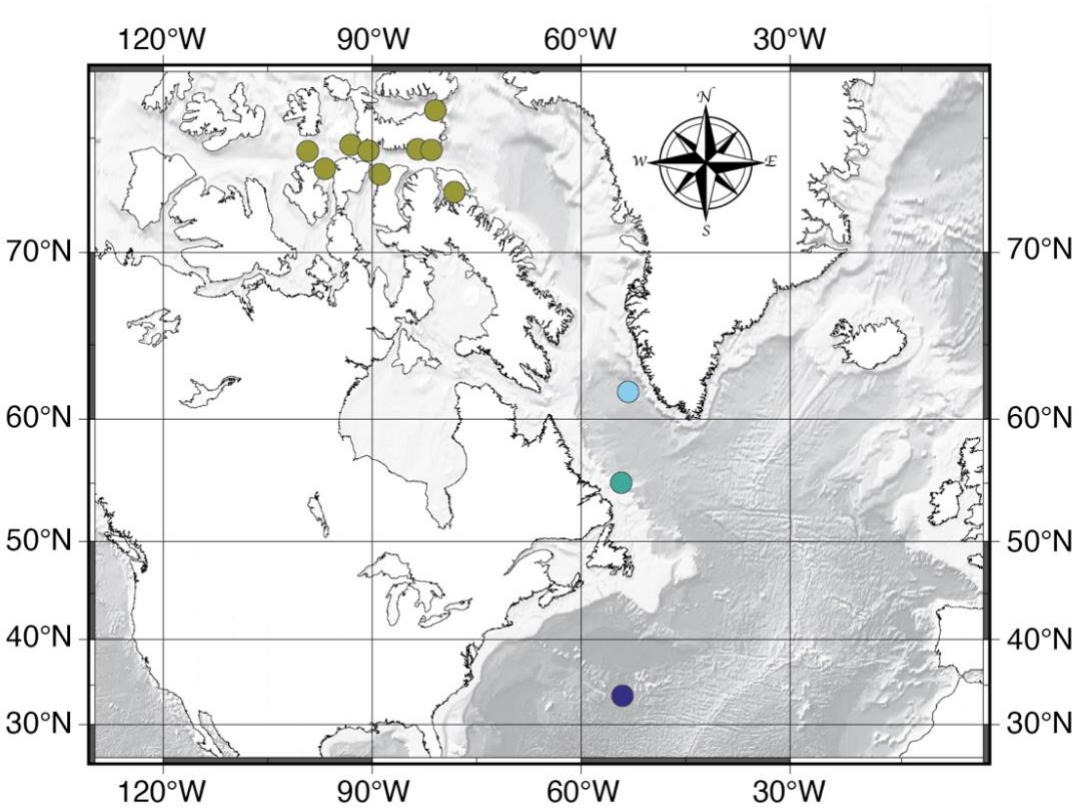

Figure 1. Map of sample sites. NWP sites are denoted by the olive-colored circles. Davis Strait sites from the *R/V Falk*or are cyan and teal, and the North Atlantic site from the *R/V Knorr* is indigo. Site colors correspond to the color of data presented in Figure 2 and 4.

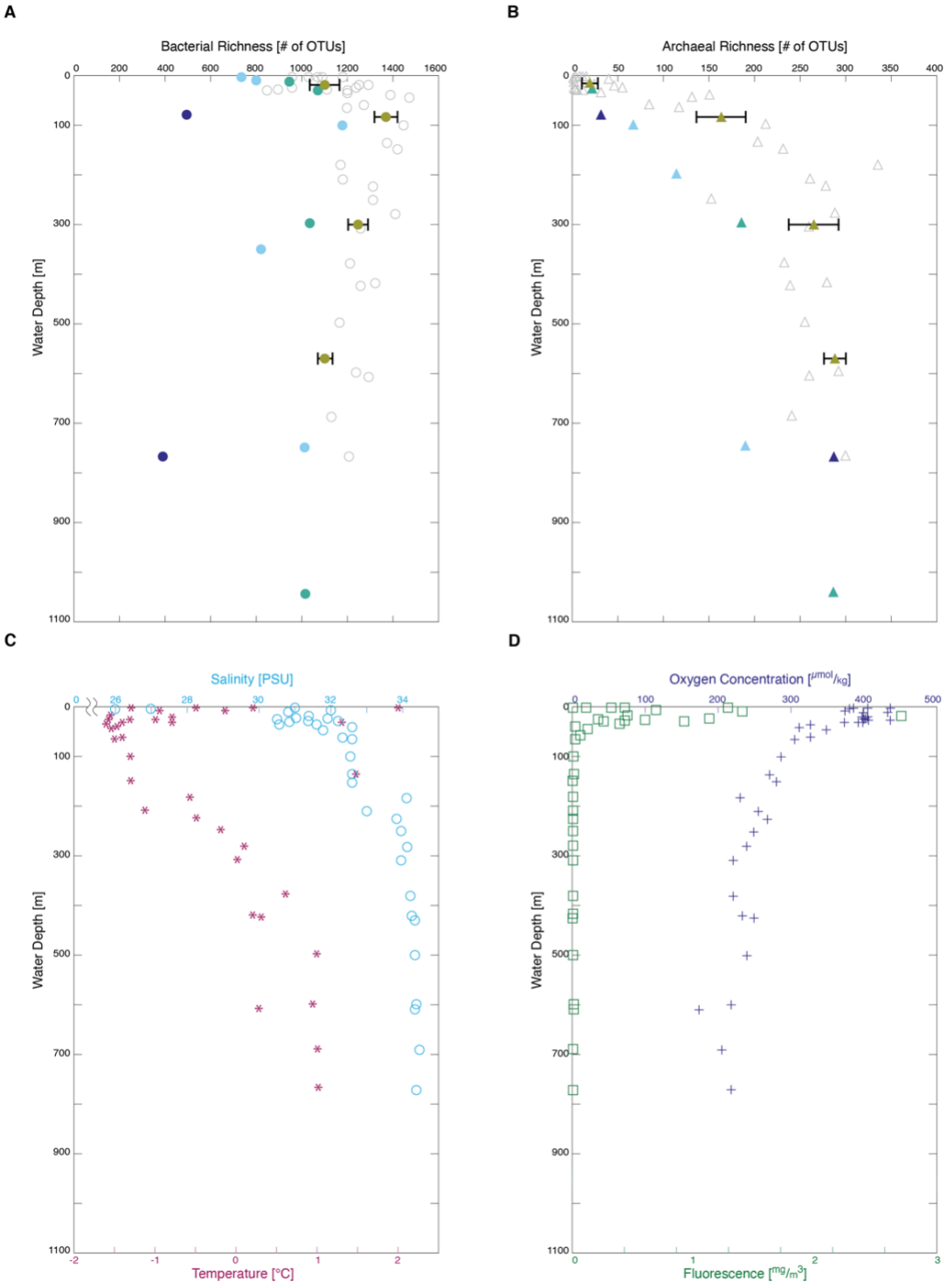

Figure 2. Taxonomic richness profiles of bacteria (circles)(a) and archaea (triangles)(b) in the water column, and relationship to water-mass parameters. All samples from the Arctic are plotted as grey outlines in panels A and B with average values and associated standard deviation error bars (*see Methods*) are overlayed in olive green. Comparison values from the Davis Strait and North Atlantic are plotted with colors corresponding to Figure 1. (c) Arctic salinity and temperature values with sampling depth. (d) Arctic oxygen concentration and fluorescence values with sampling depth.

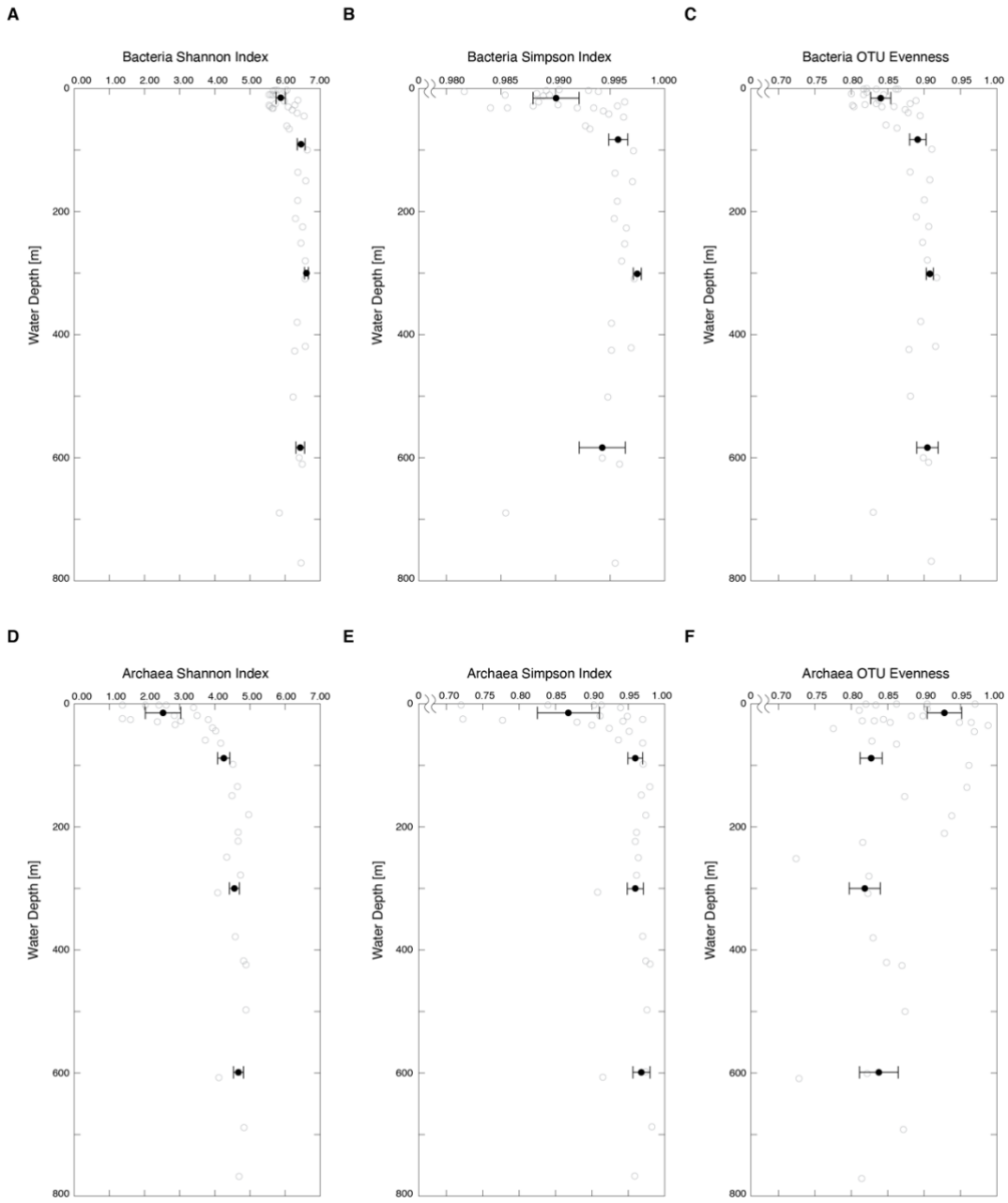

Figure 3. Diversity metrics of OTUs for bacteria and archaea from the Arctic sample sites (cyan circles from Figure 1). (a) Bacterial Shannon index. (b) Bacterial Simpson index. (c) Bacterial evenness. (d) Archaeal Shannon index. (e) Archaeal Simpson index. (f) Archaeal evenness. All sample values are plotted as open grey circles in each panel with closed black circles and associate error bars corresponding to the average and standard deviation values for each depth bin (*see Methods*).

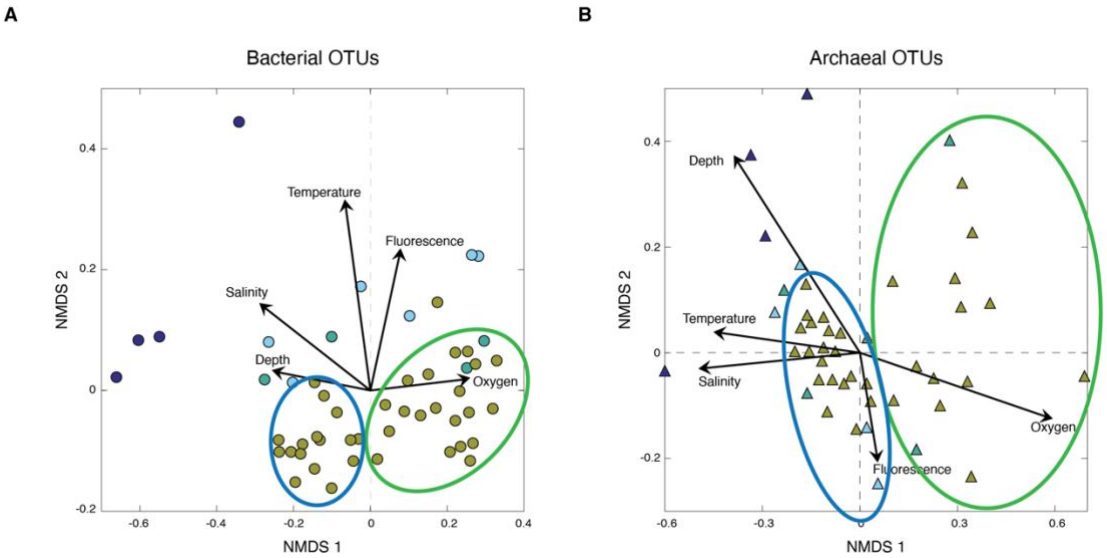

Figure 4. Bray-Curtis Nonmetric Multidimensional Scaling (NMDS) of the bacteria (left) and archaea (right). Colors are indicative of similar water-mass properties or geographic location (green: shallow-water samples from the NWP and Davis Strait, indigo: deeper-water samples from the NWP, red: deeper-water samples from the more southerly site in the Davis Strait, orange: deeper-water samples from the more northerly site in the Davis Strait, olive: all samples from the site in the North Atlantic). Ordinations are overlayed with environmental factors for both the bacteria and archaea (salinity, temperature, fluorescence, and oxygen).

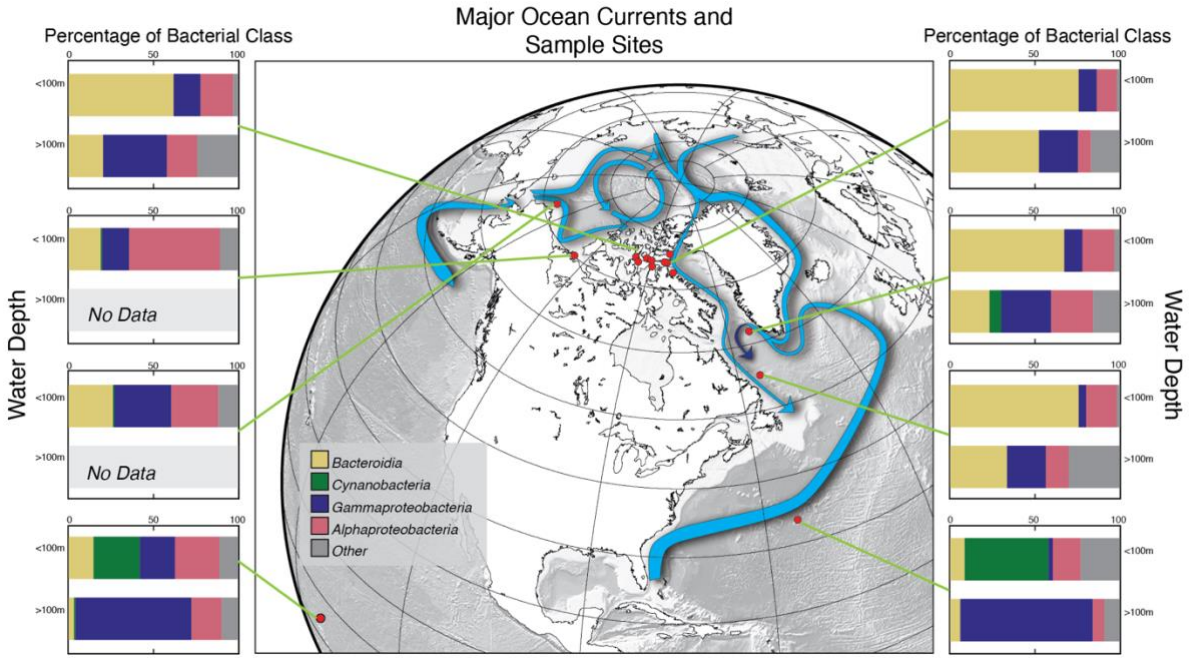

Figure 5. Relative abundance of bacterial class in surface (0 to 100 m) and deep (> 100 m) water. Included are data from the Equatorial Pacific (Kerrigan et al., 2019) and two sites in the western Canadian Arctic (Kirchman et al., 2010; Comeau et al., 2011).

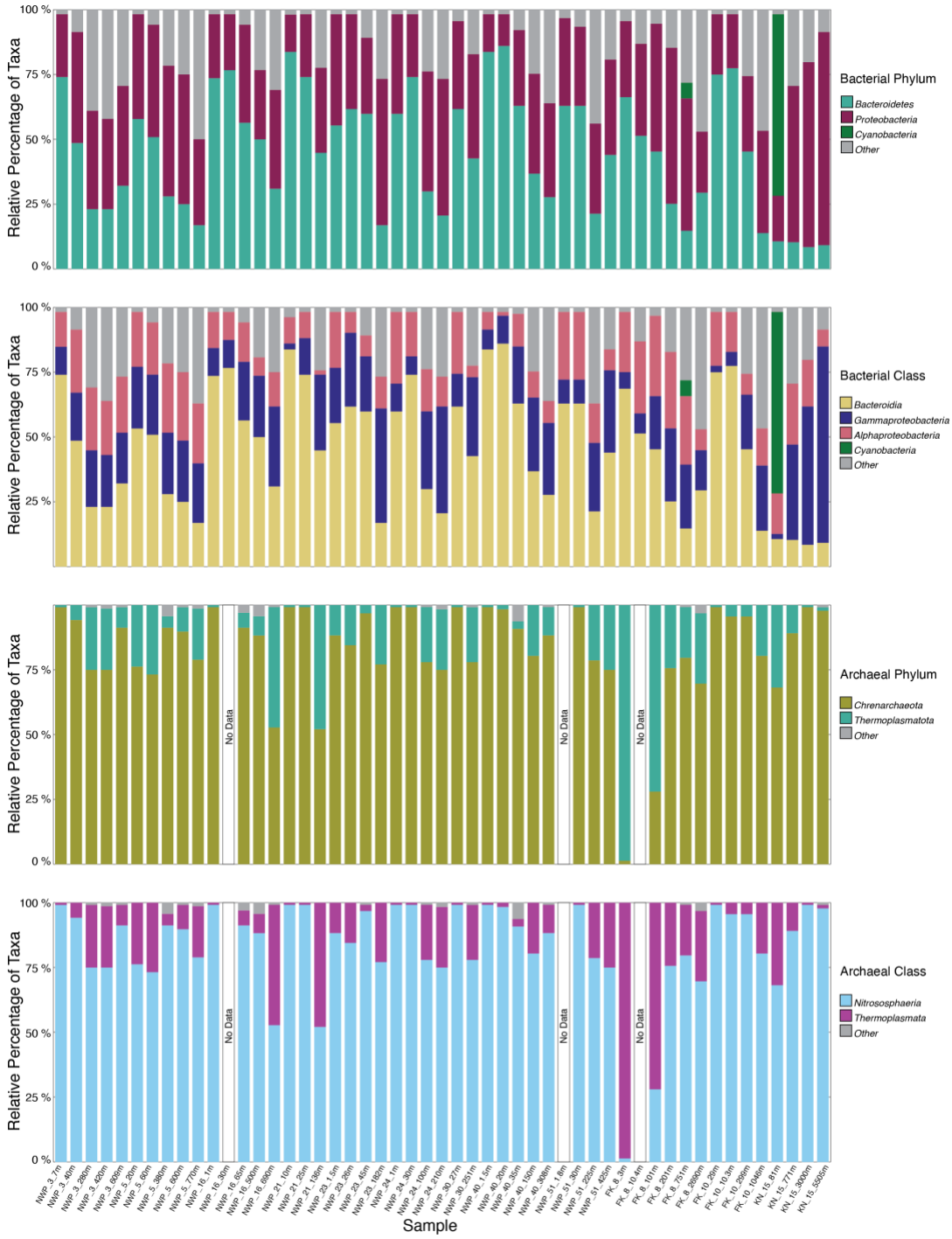

Figure S1. Relative abundance of bacterial and archaeal phylum and class. Samples are labeled by expedition_sample site_water depth (Table S1).

Table S1. Sample Data

Cruise	*Site*	*Lat/Long*	*Water Depth (m)*	*Sample Depth (m)*
Northwest Passage Project July - August 2019	3	75°57'N 080°50'W	630	7 40 280 420 609
	5	72°47'N 078°15'W	780	20 60 380 600 770
	16	74°26'N 081°34'W	712	1 30 65 500 690
	21	74°40'N 093°07'W	146	10 25 136
	23	74°26'N 099°17'W	191	1.5 26 45 182
	24	73°45'N 096°49'W	220	1 30 100 210
	30	74°28'N 090°31'W	263	27 251
	40	73°29'N 088°55'W	330	1.5 20 35 150 308
	51	74°29'N 083°11'W	450	1.8 30 225 425
R/V Falkor Cruise 003b July 2012	8	61°53'N 053°16'W	2890	3 10.4 101 201 751 2690
	10	55°09'N 054°16'W	1032	10.3 29 299 1046
R/V Knorr Cruise 223 October - December 2014	15	33°29'N 054°10'W	5510	81 771 3000 5505

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