

BACTERIAL COMMUNITY STRUCTURE IN THE AMUNDSEN SEA POLYNYA,  
ANTARCTICA

by

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(Under the Direction of Patricia Yager and Holly Bik)

ABSTRACT

In this study, we use 16 rRNA and taxonomic insights to understand that bacterial community in the Amundsen Sea Polynya, Antarctica. Polynyas are areas that are characterized by seasonal sea ice cover, massive algal blooms, and high productivity. One of the most productive polynyas is the Amundsen Sea Polynya (ASP) and Regional Ocean Models suggest the importance of an "iron conveyor belt," including iron-rich deep water being upwelled through cavities of ice shelves, though this has yet to be empirically assessed from a microbial perspective. Along with this, the microbial ecology of a recent discovery of a coastal current (CC) (0-200m) that flows westward has yet to be explored. We found that communities were distinct based on water mass, as well as certain locations within the Amundsen Sea Polynya, including along the CC. Heterotrophic bacteria tend to dominate in the surface waters, near the bloom, while bottom water communities tend to vary based on location within the polynya, indicating unique biogeochemical niches in the deep water.

INDEX WORDS: Environmental microbiology, Antarctica, coastal polynyas, microbial ecology, metagenomics

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BS, Georgia Southern University, 2021

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment  
of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2024

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December 2024

## DEDICATION

To my friends and family.

## ACKNOWLEDGEMENTS

I'd like to acknowledge the many members of the Bik lab for their help with the wet lab and statistics: Tiago for the major help with the wet lab and R code, Mirayana for the wet lab and organizational skills, and, Hunter and Alejandro for help with QIIME code.

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## INTRODUCTION

When the Southern Hemisphere becomes exposed to more sunlight in the austral summer, massive packs of sea ice from the previous winter begin to melt. In Antarctica, a known HNLC (High-Nutrient, Low Chlorophyll) zone is limited by both iron and light availability. The combination of open water and the immediate iron from the sea ice melt seeds phytoplankton blooms, primarily the haptophyte *Phaeocystis antarctica*. The Southern Ocean is responsible for deep-water formation and XX% of global carbon sequestration, storing and capturing atmospheric carbon dioxide. Most of this carbon processing comes from massive *P. antarctica* blooms and the bacteria accompanying them. Out of the entire Southern Ocean, coastal polynyas are responsible for XX% of primary productivity. A polynya is characterized as open water areas surrounded by sea ice, primarily caused by higher temperatures and catabatic winds, traveling from high to low pressure, pushing the sea ice out and away from the ice shelves. One of the most productive polynyas is the Amundsen Sea Polynya (ASP), clocking in at about XXX PP/year. *Phaeocystis* rapidly draws down carbon dioxide concentrations in these polynyas, supplying carbon and nutrients to the rest of the polynya and harboring a unique microbial heterotrophic community.

Previous studies in the ASP and other polynyas, like in the Ross Sea, found that these heterotrophic bacteria are essential to nutrient cycling and remineralization of the bloom. Many studies focus on the dynamics between bloom and bacterioplankton communities. However, few focus on other aspects that may influence the bacterial community and, in turn, nutrient overturning within the ASP. The surface communities are dominated by opportunistic

heterotrophic bacteria equipped with the ability to degrade algal-derived organic matter (Teeling et al., 2012). The bloom drives bacteria in the surface communities, but little is known about bottom-water communities and how they might change in response to detritus, sediments, or different water masses.

In the ASP, there are three distinct water masses: Antarctic Surface Water (AASW), Winter Water (WW), and Circumpolar Deep Water (CDW). AASW is warmer, with fresher water from summer solar heating and increased sea ice and glacial melt. AASW is typically characterized by a subsurface temperature minimum, separated by the colder, fresher water below (Park et al. 1998). The one below is WW, which is from winter sea ice production and convection, where strong winds cause evaporation and surface waters are overturned and sink while “warmer” waters rise to the surface (Kovalevsky et al., 2020). CDW, warmer and saltier water sourced from the Antarctic Circumpolar Current off the continental shelf, is at the very bottom. This water is a significant source of dissolved iron (dFe) to the polynya, accounting for 32% of the total dFe supply in the Amundsen Sea. The CDW is likely supplemented by mixing with high dFe shelf waters, where sediment dissolution and increased remineralization occur. CDW is upwelled toward the surface by bathymetric features and eddies, which allow microbial communities to access the highest dFe concentrations in the region (Smith et al. 2021). Another way CDW is supplied to the ASP is through the “meltwater pump.” A study by St-Laurent et al. (2017) highlighted the critical role of ice shelf melt-driven circulations (the “meltwater pump”) in transporting sediment-derived dissolved iron (dFe) to the upper 300 m of the water column. Following the bathymetry, the CDW eventually reaches underneath ice shelves, mainly the Getz Ice Shelf (GIS) and Dotson Ice Shelf (DIS) in the ASP, and begins to melt the underside of the shelf, which makes the water more buoyant and the outflow is provided back to the polynya.

Dinniman et al. found that sediment accounted for 39% of the total dFe supply, 75% of which is first advected into the ice-shelf cavity before delivery to the surface waters (2023). Glacial meltwater sources discharge nutrients, metals, particles, and freshwater microorganisms into the open water, and there are few to no studies looking at how this affects the microbial community at Dotson and in turn, nutrient cycling and the rest of the food web (Alcaman-Arias et al. 2021). Additionally, Regional Ocean Models suggest how bacteria may alter CDW before it travels into the cavities of the ice shelves is not well understood (St-Laurant et al. 2017).

## METHODS

### Sample collection

Samples were collected on the R/V NB Palmer expedition, ARTEMIS (January–February 2022) in the Amundsen Sea, Antarctica (Figure 1). Sampling was carried out with 12 L Niskin bottles mounted on an SBE 911; Sea-Bird Electronics) equipped with CTD (conductivity-temperature-depth) and oxygen sensors. Seawater samples were collected at varying depths, including surface and bottom waters. Intermediate samples vary, but most were taken at the mixed layer, temperature minimum (T-min), and an in-between sample. Individual depths varied per station and time according to the different depth thresholds, attempting to sample the three prevalent water masses (AASW, WW, CDW). Per sampling event, 8–12 L of seawater were filtered first onto a 2-3 $\mu$ m polycarbonate pre-filter, then equally diverged through replicate .2  $\mu$ m durapore filters (Millipore, Burlington, MA). Filters were stored in cryovials at -80C. Samples were extracted from each filter using the Zymo Research Quick-DNA Fecal/Soil Midiprep Kit according to the manufacturer's instructions (NA. cat 11-322MD). DOC contents were analyzed in Medeiros lab (University of Georgia). Dissolved iron data was provided by the Sherell lab (Rutgers University). Nutrient data provided by ARTEMIS was included in the analysis.

### Sample preparation

PCRs were set up in a dedicated laminar-flow hood that underwent daily bleach and UV light sterilization. The V3–V4 region of 16S rRNA genes were amplified using primers 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT). Sequencing was performed on a MiSeq platform (Illumina, San Diego, CA) using 2 × 300 bp paired-end

libraries according to the "16S Metagenomic Sequencing Library Preparation protocol" (Illumina). Both positive (ZymoBIOMICS™ Microbial Community Standard (Zymo Research, Irvine, CA)) and negative controls (molecular-grade water) were included in all PCRs. The following PCR profile was used for amplification of 16S rRNA gene fragments: 94°C for 3 min; 94°C for 45 s, 50°C for 60 s and 72°C for 90 s for 35 cycles; and 72°C for 10 min. PCR amplification success was evaluated with gel electrophoresis (agar 1%). Purification of PCR products was subsequently carried out using a magnetic bead purification protocol using Agencourt AMPure XP beads (Beckman Coulter, CA, USA) and following the manufacturer's protocol.

## Data analysis

Reads were processed into amplicon sequence variants (ASVs) using QIIME2 and the SILVA v138 database. Before analysis, the R package decontam was used to remove potential contaminants, using the prevalence and frequency method based on the controls. Communities were separated into “free-living” and “particle-associated”; The latter for bacteria on the 2-3  $\mu\text{m}$  pre-filter, and the former that was caught on the 0.2  $\mu\text{m}$  filters. based on what was caught on, >0.2  $\mu\text{m}$  Analysis and plotting were performed in RStudio, primarily using the phyloseq, vegan, ggplot2, qiime2R, ggplot2, indic species, ANCOMBC and ComplexHeatmap packages. Ordination figures were generated with the plot\_ordination function within phyloseq. The **distance** function was used to calculate beta-diversity (Bray-Curtis), the **adonis** function to perform an ANOVA, and the **TukeyHSD** for Tukey for the **multiplatt** function for indicator species analysis. The workflow figure has been created with Biorender.com. The ASV table and corresponding sequences were run through the PICRUSt2 pipeline for functional genomic

prediction, filtered based on desired KO (Kegg Orthology), and then imported into R. The bioinformatics workflow, including QIIME2 and PICRUSt2 pipeline, is available at <https://github.com/sierra-uga/ARTEMIS-MS>.

We used the R package oce for water mass categorization and can be found in Table X. We excluded the continental shelf station, STN 198, in many of the microbial community analyses within the ASP, due to the distinctly different community, altering the ordination and Bray-Curtis analyses. Based on velocity profiles provided by the ARTEMIS cruise, samples were denoted “Outflow” if the conservative velocity (m/s) was above ~0.1. Inflow stations were samples closest to the DIS, with the conservative velocity below ~0.1. (Table 1).

## RESULTS

### **Water mass**

We found that both free-living and particle associated communities were both statistically significant, both >200 and <200m, based on the location within the polynya ( $F = , p < 0.001$ ). When running a Tukey test, only the Dotson-OP category was significant in the upper 200m free-living communities (Table 1). The CCA of the communities show that the particle-associated is more related by the area within the polynya, while the surface communities are driven by bloom, as suggested in the typical communities found there. *Polaribacter* is a known algal-associated bacteria in high-latitude regions (pole-to-pole paper). Previous studies say that the bottom-water communities are heavily influenced by the surface communities, which are driven by the bloom. Our findings suggest that certain areas are in-fact driven by the bloom, like near the Dotson and Getz, but distinct communities in open polynya stations, especially in the free-living. The CDW communities are distinctly different based on the area in the polynya, suggesting that the presence of CDW is likely another factor driving the microbial community.

*Pseudoalteromonas*, of the *Pseudomonadaceae* family is only majorly present in STN2 and STN4, both stations that follow the Dotson Trough, were pure CDW has yet to be altered by meltwater and is likely not strictly influenced by bloom. Bloom markers near the Dotson are dominated by heterotrophic bacteria, mainly *Polaribacter spp.*, *Nitrocolacae*, STN89, an eastern notch station is distinctly different than bloom-marker communities, where the bloom was almost non-existent. The unique community underneath the bloom, found in Richert et al., mostly consists of opportunistic heterotrophic bacteria, like SUP05 clade, of the Thioglobacae family, or SAR 11 clade, known organoheterotrophs, capable of diverse genomic

abilities due to their small genomes and horizontal transfer. When comparing the communities together, Dotson-OP was significantly different in certain areas, particular stations, like STN 174, are different in each water mass, dominated by Saprinerceae, a known particle-associated taxa. The free-living and particle-associated communities don't appear to be influenced by the location in the polynya, more so the position in the water column in the mesopelagic, that includes both AASW and WW, where there are distinct nutrient and diversity shifts. The CDW, and only a few WW samples have *Pseudomonas*, a genetically diverse organism, a known pathogen or psychrophilic organism.

The community not only shifts because of the bloom, but also by DOC, potentially linked to the bloom, but also potentially by sloppy feeding, especially at stations like STN174, where the iron, cobalt, and magnesium are high at the bottom, and DOC is essentially depleted. In the surface waters, DOC follows the same gradient as temperature and oxygen, where sloppy feeding may play considerable role in the remineralization of organic matter, because of the microbial loop.

Though the inflow and outflow aren't inherently different, the outflow might influence the communities by supplying more iron to the bloom, via the cavity at about ~400m at the ice shelf face. A Tukey test showed that both free-living and particle-associated communities were not different ( $F=$  ,  $p < 0.5$ ), and this could be because there isn't enough time within the cavity for significant community changes, and instead the outflow more influences the communities that are following the coastal current in the surface, shown by the CCA, where western CC is distinctly different from the Dotson stations, including the outflow. Western CC and Dotson bottom-water stations do not include pure CDW, as it was likely categorized as WW-CDW, with the fresh meltwater addition, making it mCDW.

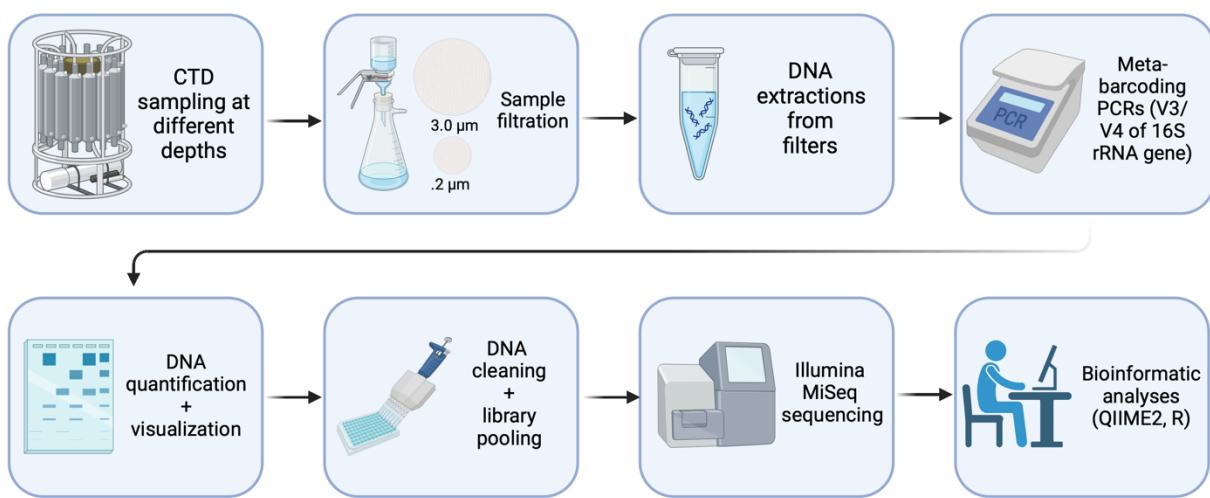
As previous studies, like Richert et al. suggest, the surface waters are dominated by opportunistic heterotrophic taxa, and leads to a less diverse community in the surface waters. Many of the previous bacterial studies in the ASP do not incorporate the inflow and outflow, and how the deep CDW travels into the ASP via bathymetry.

## DISCUSSION

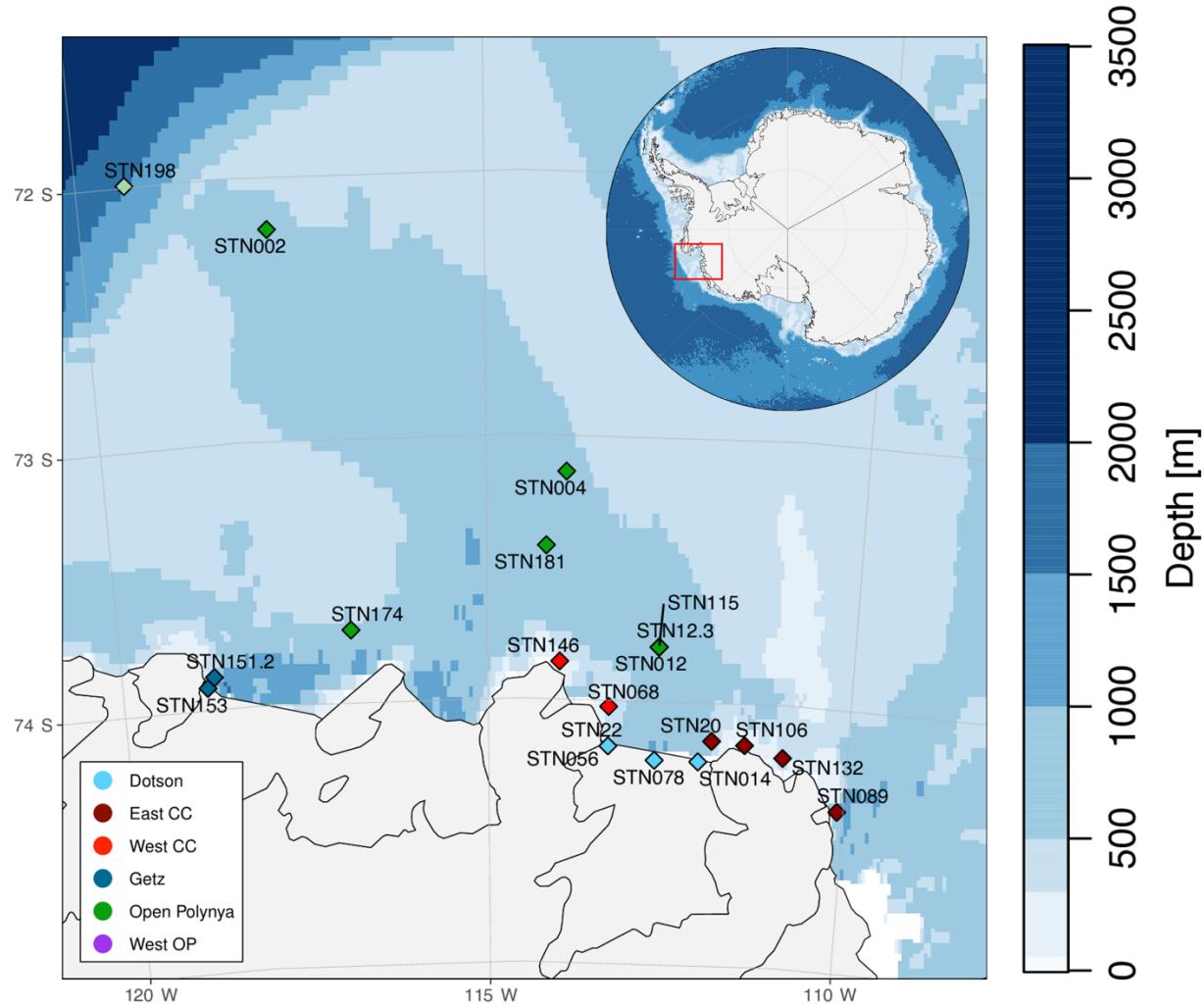
Along the ice shelf, a previously overlooked dynamic delivers nutrients to the polynya. Due to the easterly winds, a coastal current runs along the ice shelves in the upper 200 meters, approximately the thickness of the ice shelf itself. This coastal current comes from the Pine Island Glacier in the east, near Twaite, the fastest melting glacier in Antarctica, and travels along the DIS toward GIS in the west. The coastal current not only brings iron (from the abundance of sea ice in the eastern notch) but also could aid in bringing the outflow from the DIS (iron-rich water) to the polynya.

Several iron-related taxa (*Alteromonadales*, *Oceanospirillales*, and *Rhodobacterales*) are present in surface and bottom water samples. The main bacterial contributors are different, indicating a unique biogeochemical role. Bottom water from both free-living and particle-associated samples exhibits a more diverse bacterial community. Though our results do not show a significant difference in surface and bottom water, this could be due to myriad factors, including the location within the polynya, pre-bloom dynamics (no detritus to breakdown), insufficient samples, etc. Our hypothesis is partially confirmed by the Tukey tests. The next step will be to do the same analysis for archaea because they are known to be extremophiles and are also incredibly important to the biogeochemistry of marine ecosystems. Previous studies have shown they have an untapped importance to the Southern Ocean and often get overlooked in microbial community analysis. Overall, this project shows a clear distinction between open polynya and ice shelf, indicating an unexplored importance for the proposed coastal current and the biogeochemical implications.

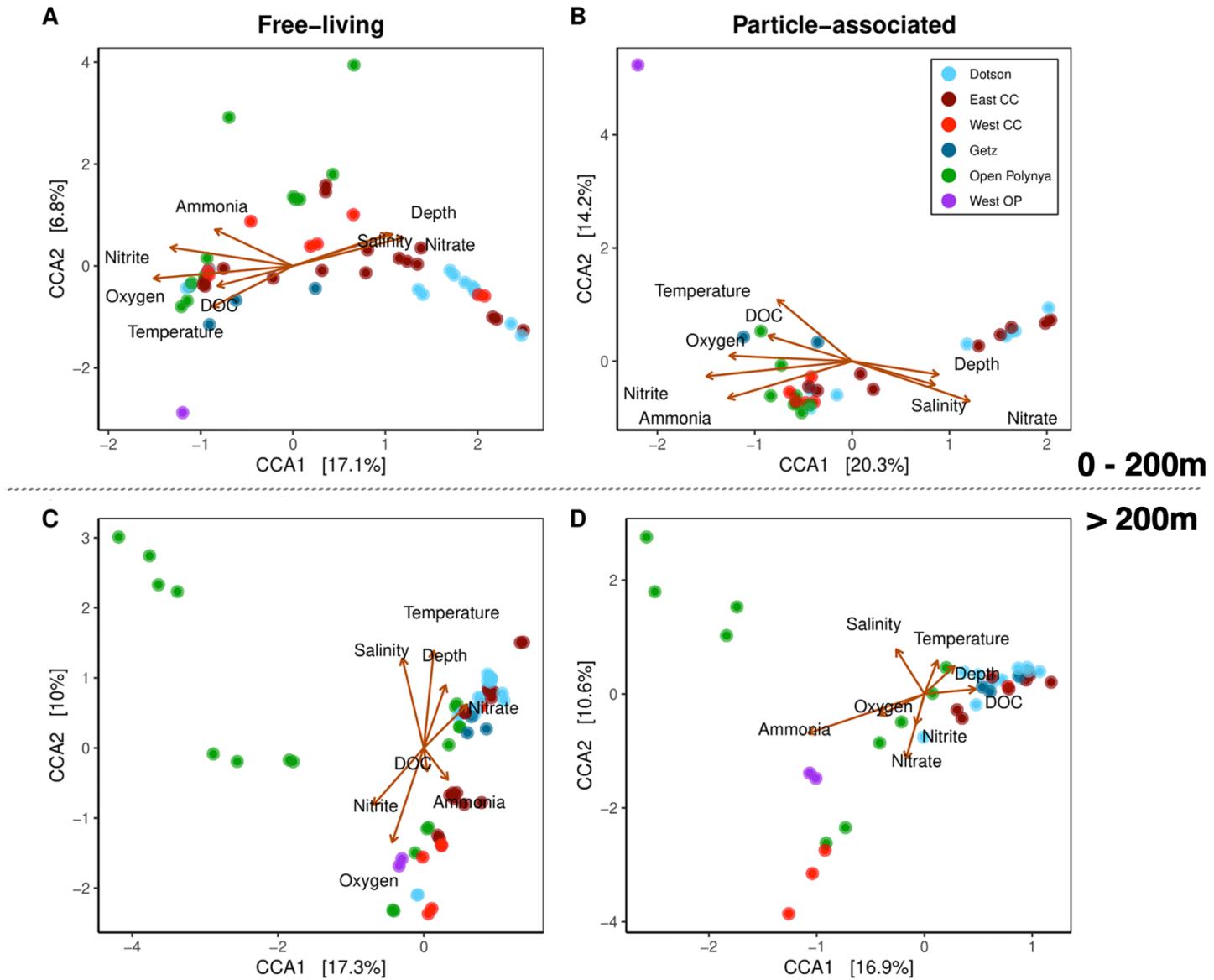
## FIGURES



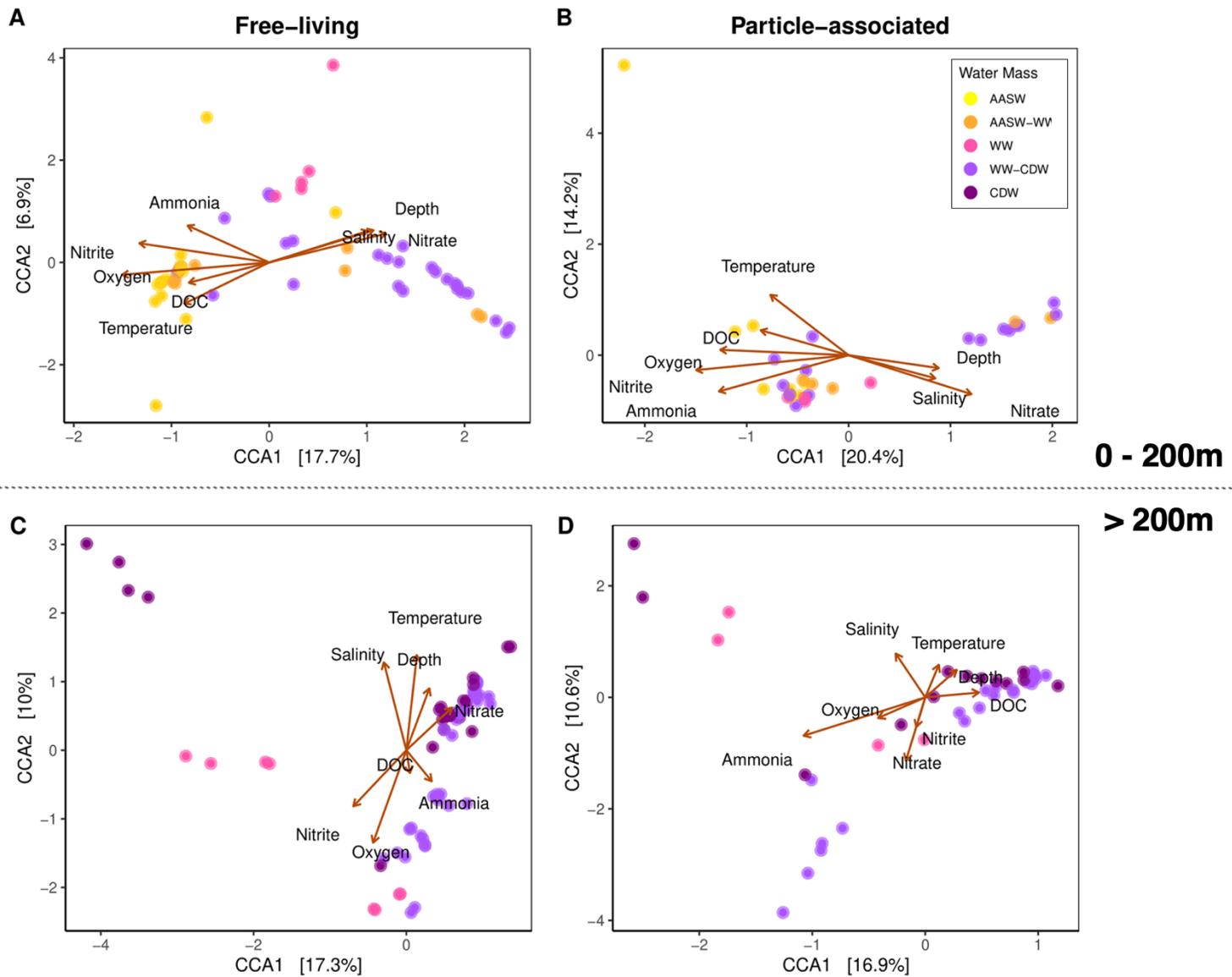
**Figure 1.** Pipeline of study.



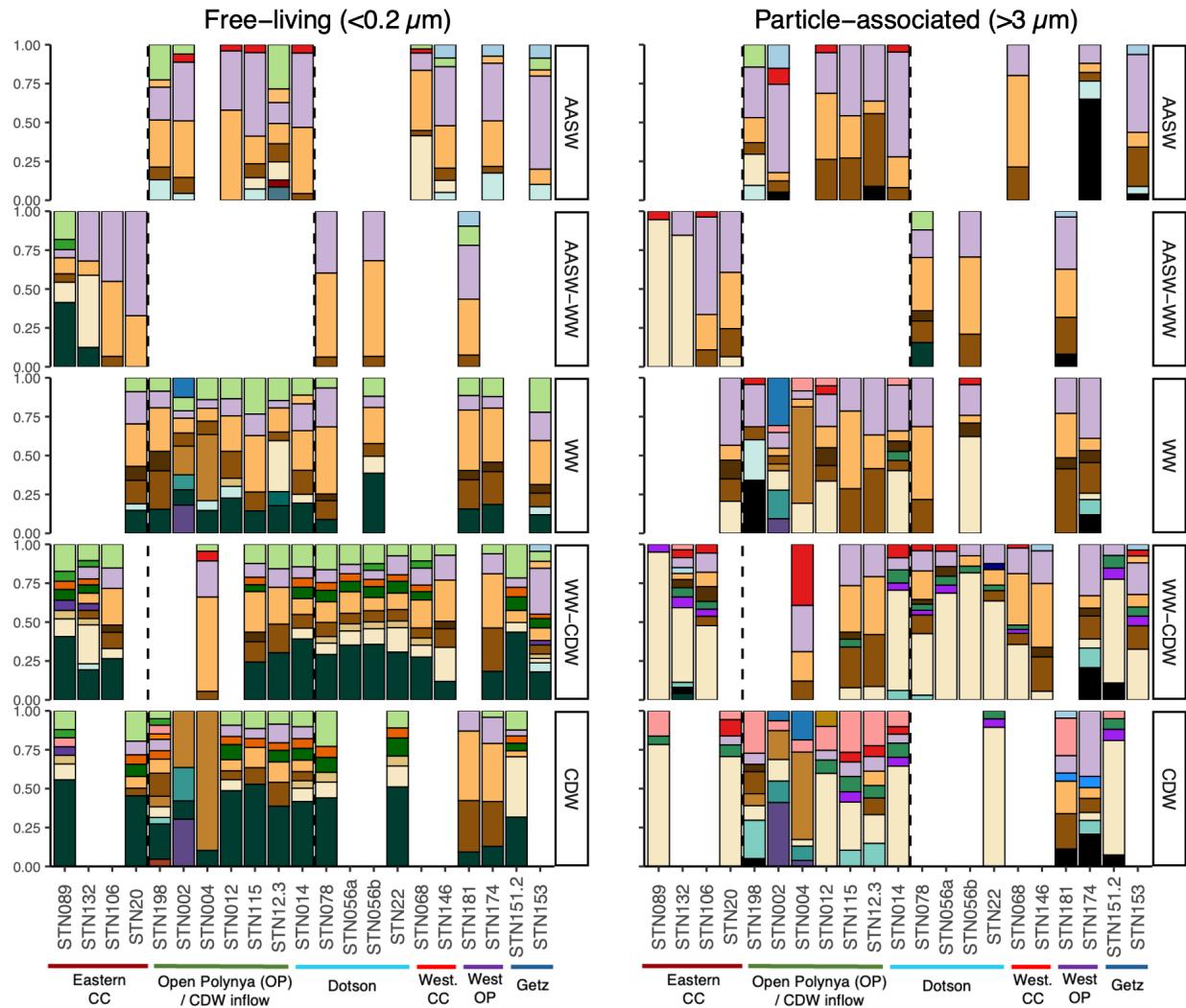
**Figure 2.** Station map with bathymetry. Colors are coordinated with location.



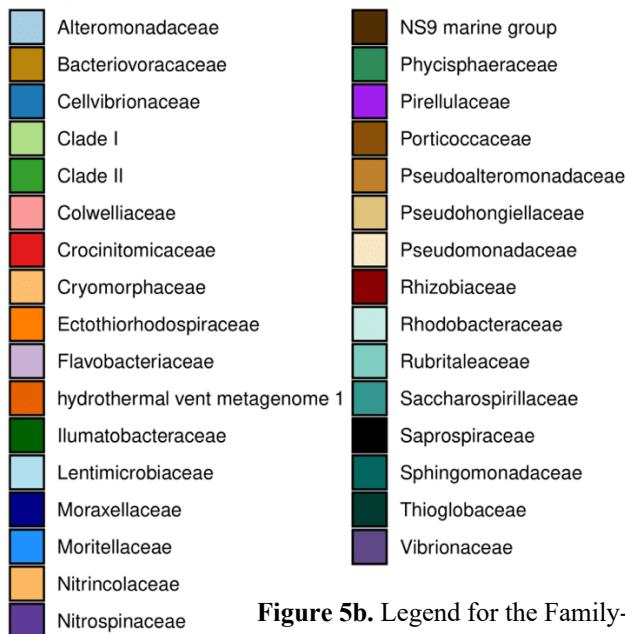
**Figure 3.** Canonical Correspondence Analysis (CCA) of free-living (**A, C**) and particle-associated (**B, D**) bacterial communities based on **location** within the ASP. First row CCAs are surface-200m and bottom row CCAs are 200m-bottom.



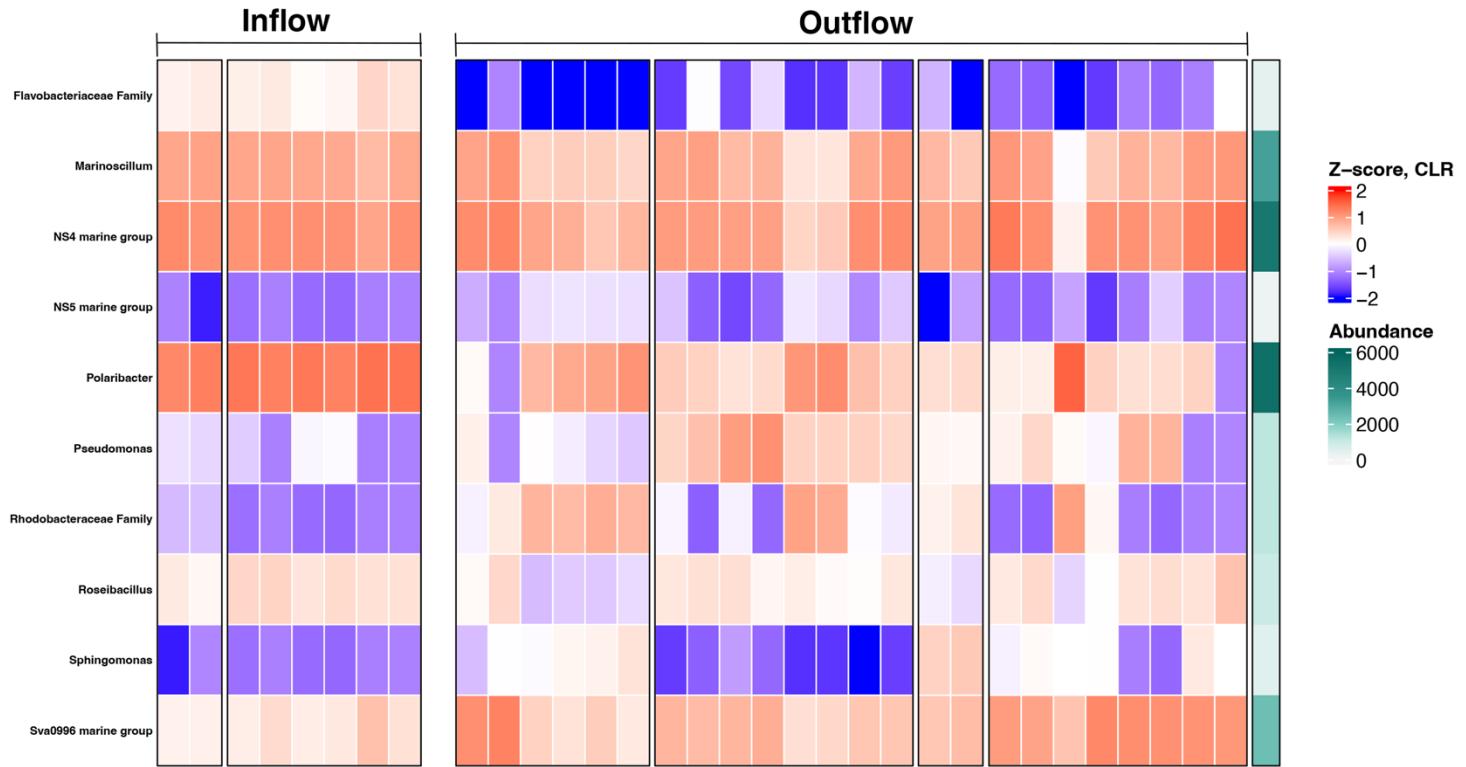
**Figure 4.** Canonical Correspondence Analysis (CCA) of free-living (**A, C**) and particle-associated (**B, D**) bacterial communities based on defined **water mass**. First row CCAs are surface-200m and bottom row CCAs are 200m-bottom.



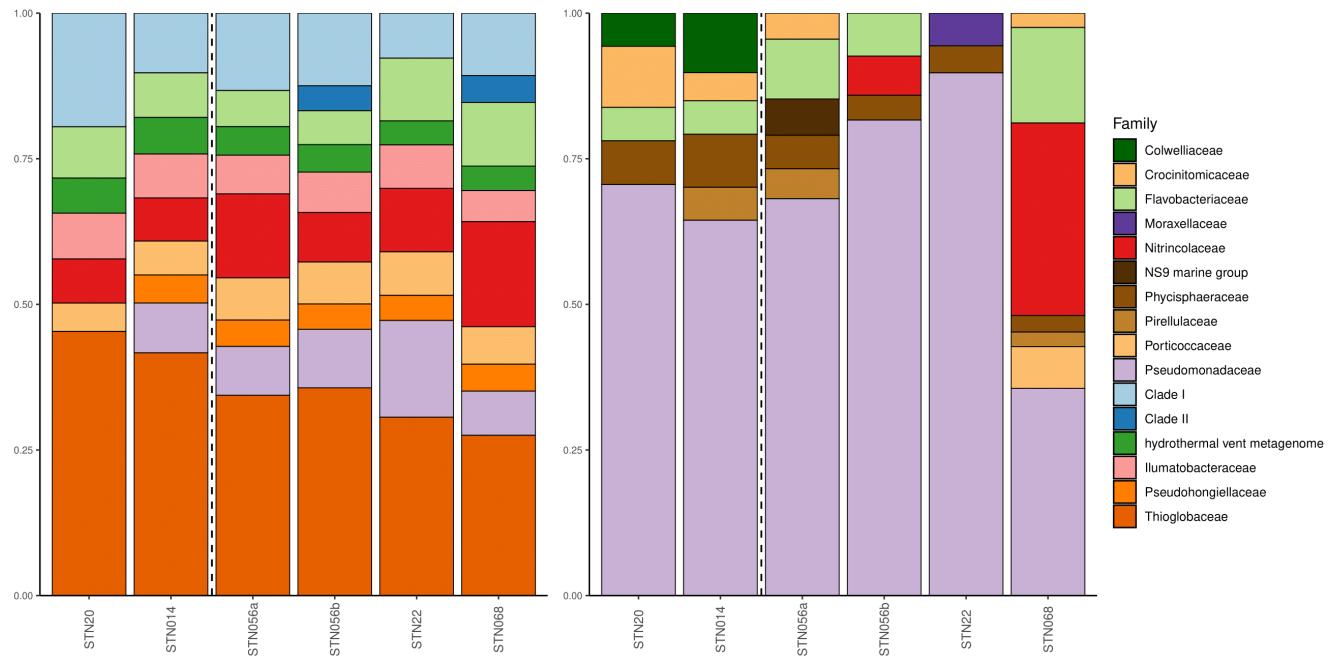
**Figure 5a.** Relative abundance for free-living (left) and particle-associated (right) communities at the family level. Each row represents a water mass, labeled on the right.



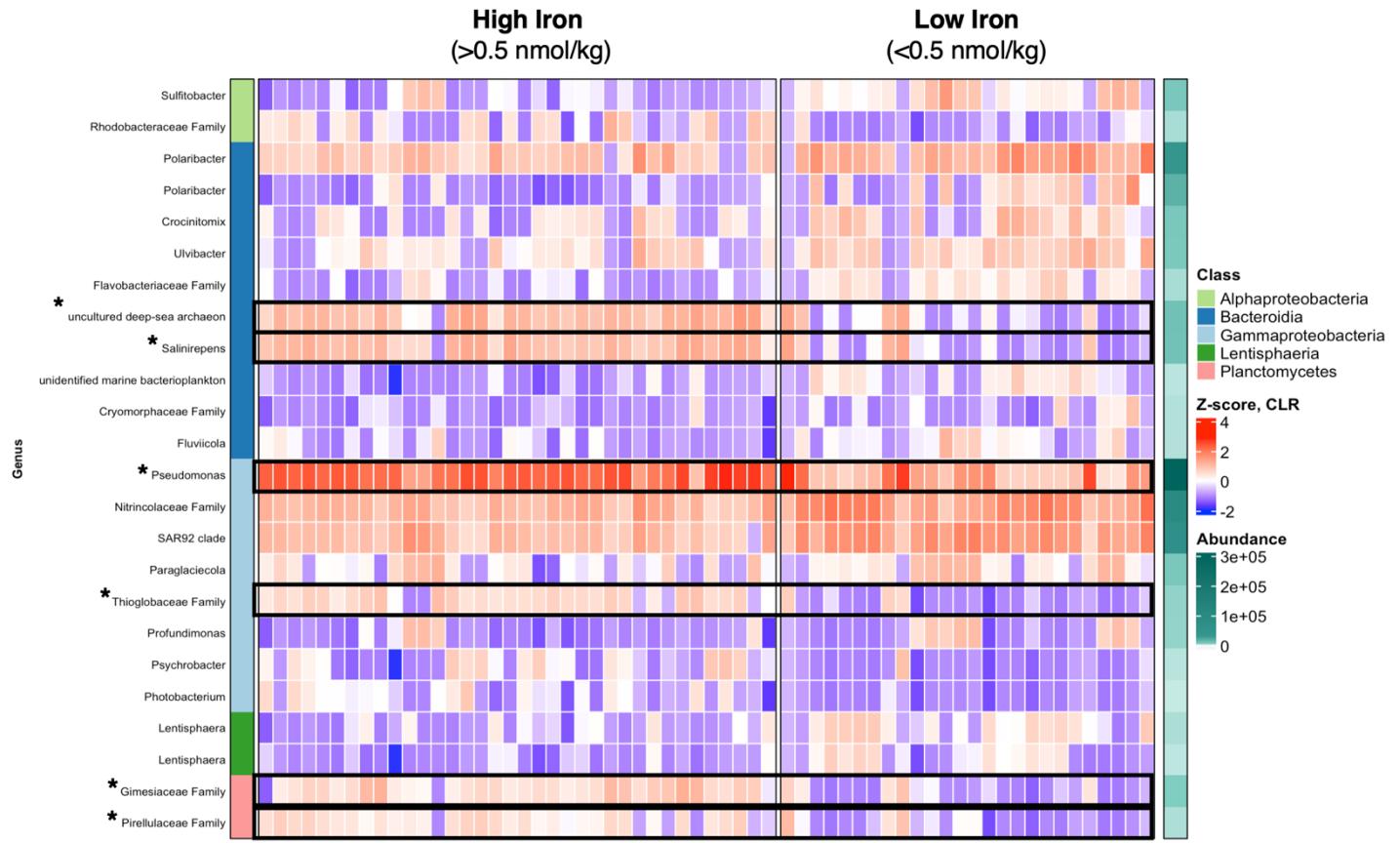
**Figure 5b.** Legend for the Family-level taxa that corresponds with Figure 5a.



**Figure 6.** Significant free-living taxa from ANCOM-BC analysis for inflow vs. outflow samples. Z-score derived from log-transformed data. No significant change in particle-associated taxa from ANCOM-BC analysis.



**Figure 7.** Relative abundance for free-living (left) and particle-associated (right) communities at the family level. In each community, inflow stations are before the black dashed line, while outflow are after.



**Figure 8.** Significant particle-associated taxa from ANCOM-BC analysis for high ( $>0.5$  nmol/kg) or low ( $<0.5$  nmol/kg) dFe concentrations. Z-score derived from log-transformed data. See Supplementary Information for significant free-living taxa.

## TABLES

**Table 1.** Water mass characterization with temperature and salinity ranges.

| <b>Water Mass</b> | <b>Temperature Range</b> | <b>Salinity Range</b> |
|-------------------|--------------------------|-----------------------|
| AASW              | -1.15 – 0.5              | 32.75 – 33.87         |
| AASW-WW           | -2 – -1.15               | 33.5 – 33.87          |
| WW                | -2 – -1.4                | 33.88 – 34.5          |
| WW-CDW            | -1.4 – 0.1               | 33.88 – 34.5          |
| CDW               | 0.1 – 2                  | 33.88 – 35            |

**Table 2.** Inflow and outflow stations and depth.

| <b>Inflow</b>  | STN014  | -74.23    | -112.08    |
|----------------|---------|-----------|------------|
|                | STN014  | -74.23    | -112.08    |
|                | STN014  | -74.23    | -112.08    |
|                | STN20   | -74.15    | -111.9     |
|                | STN20   | -74.15    | -111.9     |
| <b>Outflow</b> | STN22   | -74.1753  | -113.34488 |
|                | STN056a | -74.18    | -113.34    |
|                | STN056a | -74.18    | -113.34    |
|                | STN056a | -74.18    | -113.34    |
|                | STN056b | -74.18    | -113.34    |
|                | STN068  | -74.02906 | -113.34464 |
|                | STN068  | -74.02906 | -113.34464 |
|                | STN068  | -74.02906 | -113.34464 |

**Table 3.** Indicator species analysis of significant taxa in **free-living** outflow community. No significant indicator taxa appeared for particle-associated community.

| ASV # | index | Inflow | Outflow | .value.b | Order              | Family                                 | Genus   |
|-------|-------|--------|---------|----------|--------------------|--|---|
| Seq58 | 2     | 0      | 1       | 0.027    | Verrucomicrobiales | Rubritaleaceae                         | Roseibacillus                                 |
| Seq71 | 2     | 0      | 1       | 0.015    | Flavobacteriales   | Cryomorphaceae                         | Vicingus                                      |
| Seq78 | 2     | 0      | 1       | 0.009    | Cellvibrionales    | Cellvibrionaceae                       | uncultured 5                                  |
| Seq91 | 2     | 0      | 1       | 0.005    | Alteromonadales    | Colwelliaceae                          | Colwellia                                     |
| Seq93 | 2     | 0      | 1       | 0.009    | SAR86 clade        | uncultured Oceanospirillales bacterium | uncultured Oceanospirillales bacterium Family |
| Seq94 | 2     | 0      | 1       | 0.023    | Phycisphaerales    | Phycisphaeraceae                       | JL-ETNP-F27                                   |
| Seq97 | 2     | 0      | 1       | 0.044    | Burkholderiales    | Methylophilaceae                       | OM43 clade                                    |

\* sig taxa in only outflow, none for particle associated

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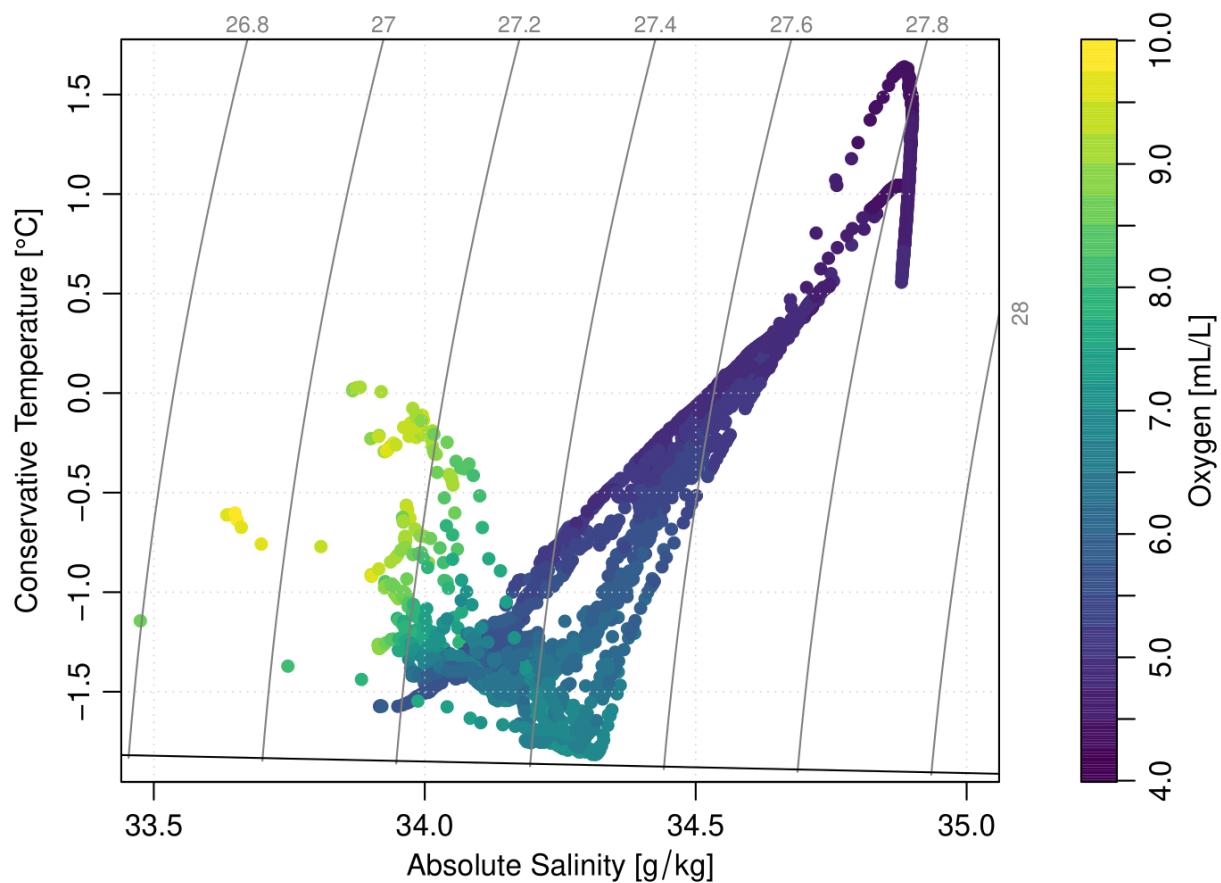
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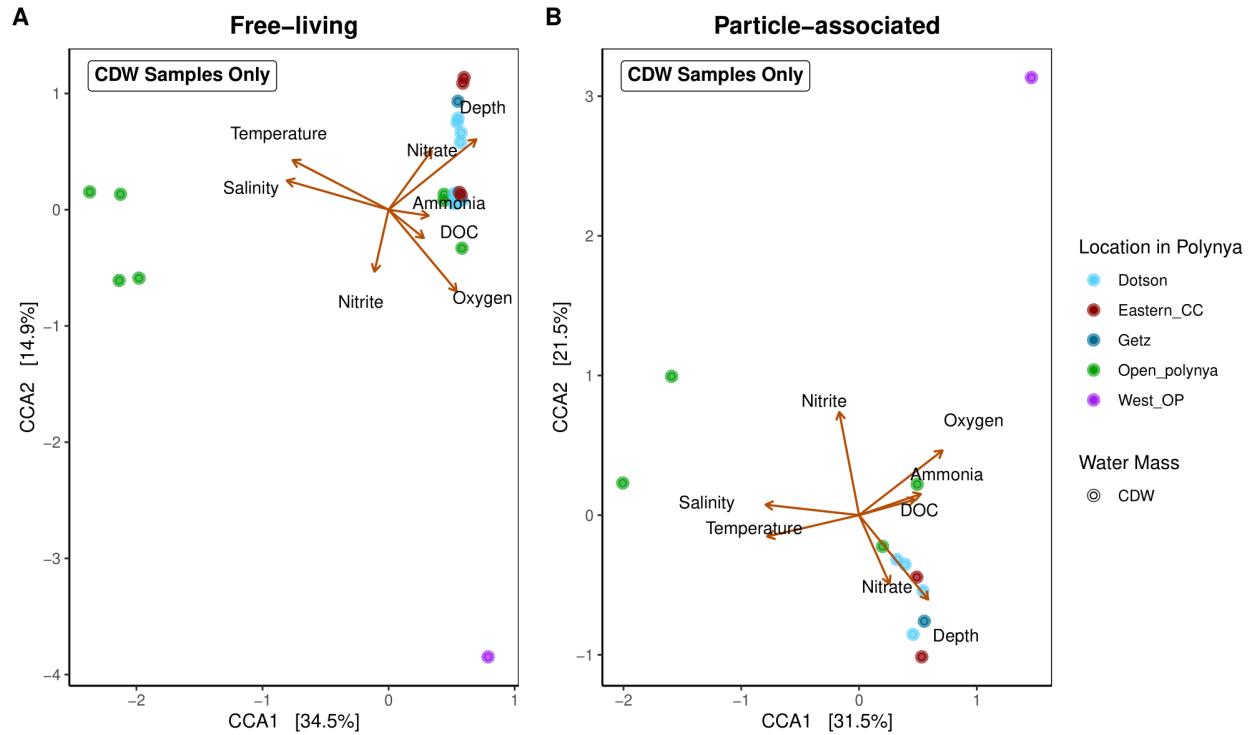
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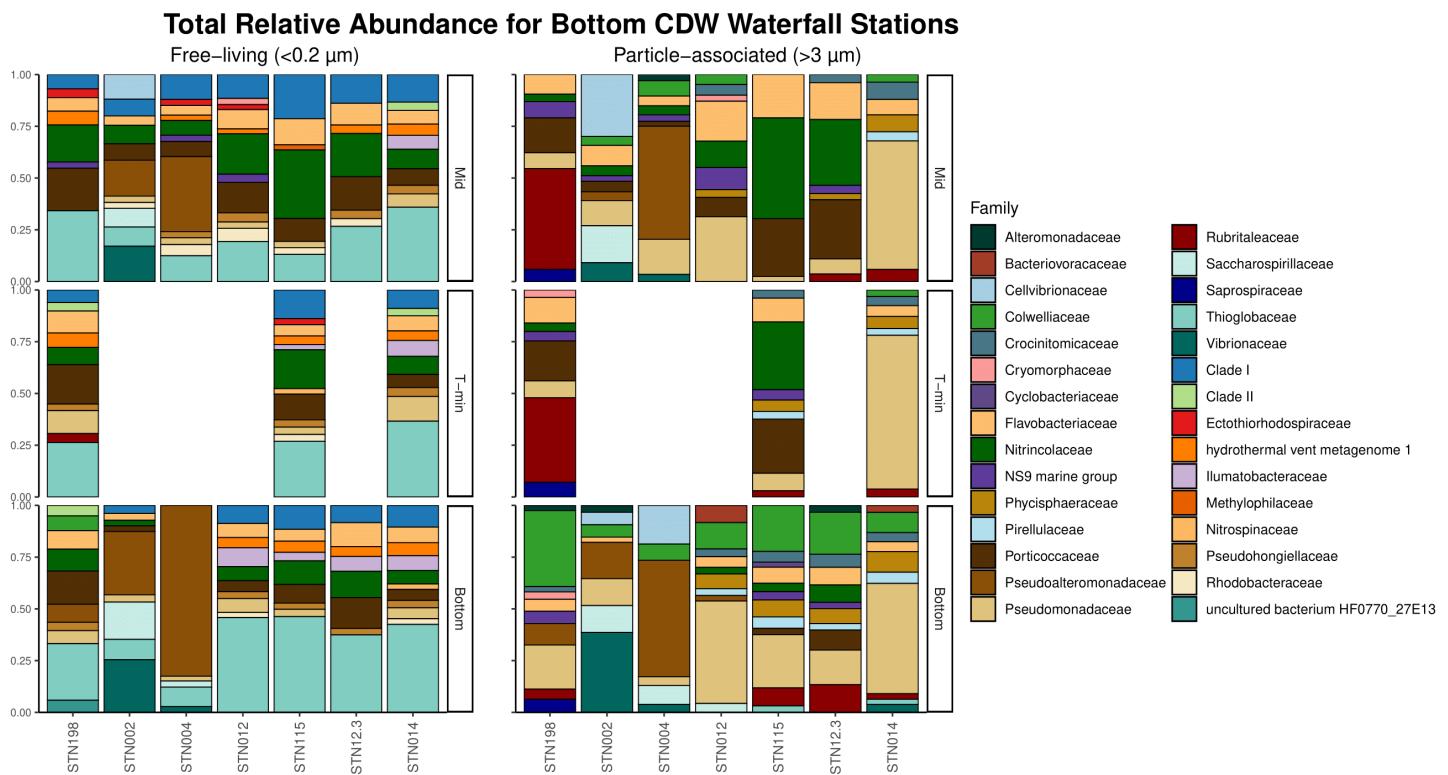
## SUPPLEMENTARY FIGURES



**Figure S1.** Temperature-Salinity plot for all ARTEMIS stations with DNA samples.

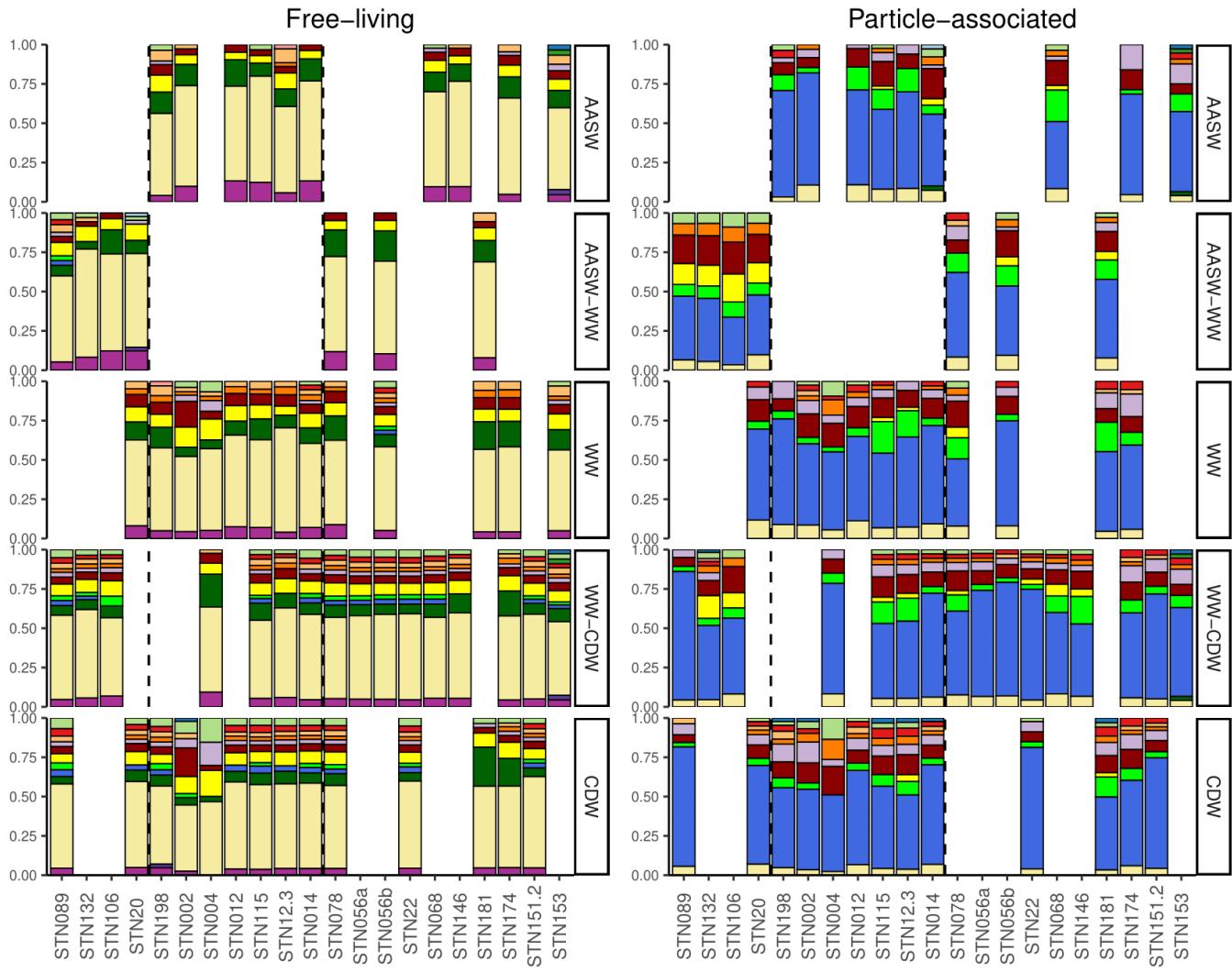


**Figure S2.** Canonical Correspondence Analysis (CCA) of free-living (**A**) and particle-associated (**B**) of samples that are characterized in CDW water mass, which are bottom water samples.

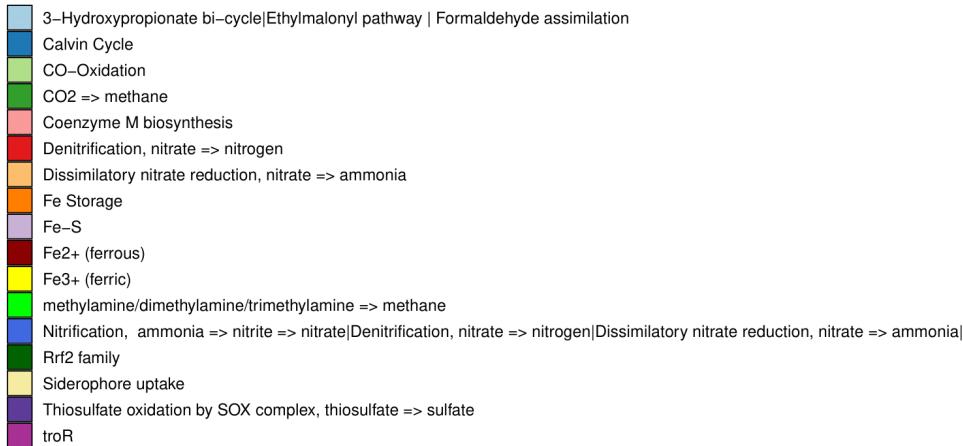


**Figure S3.** Relative abundance of stations coming from off the continental shelf at the family level.

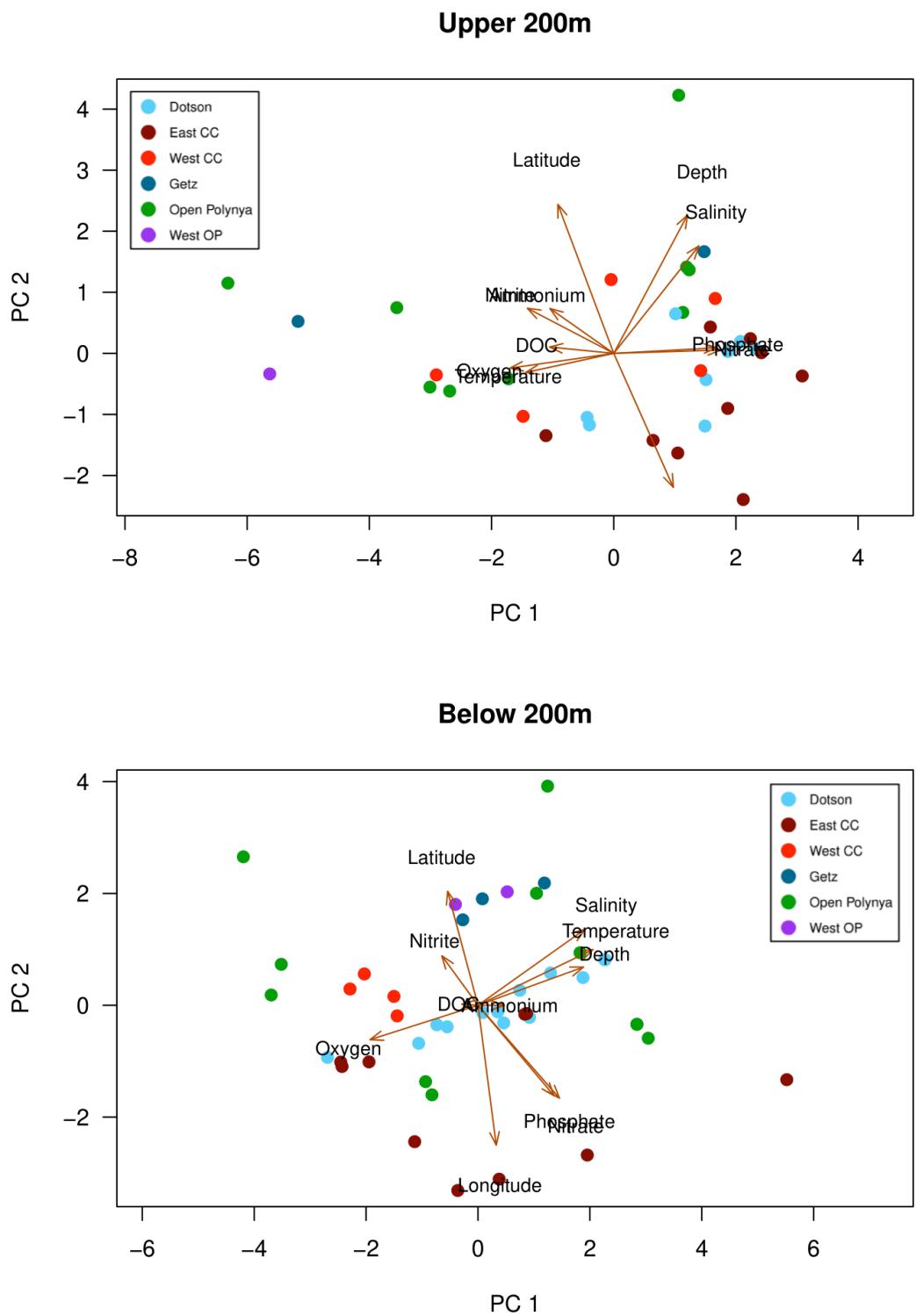
Free-living on left and particle-associated on the right.



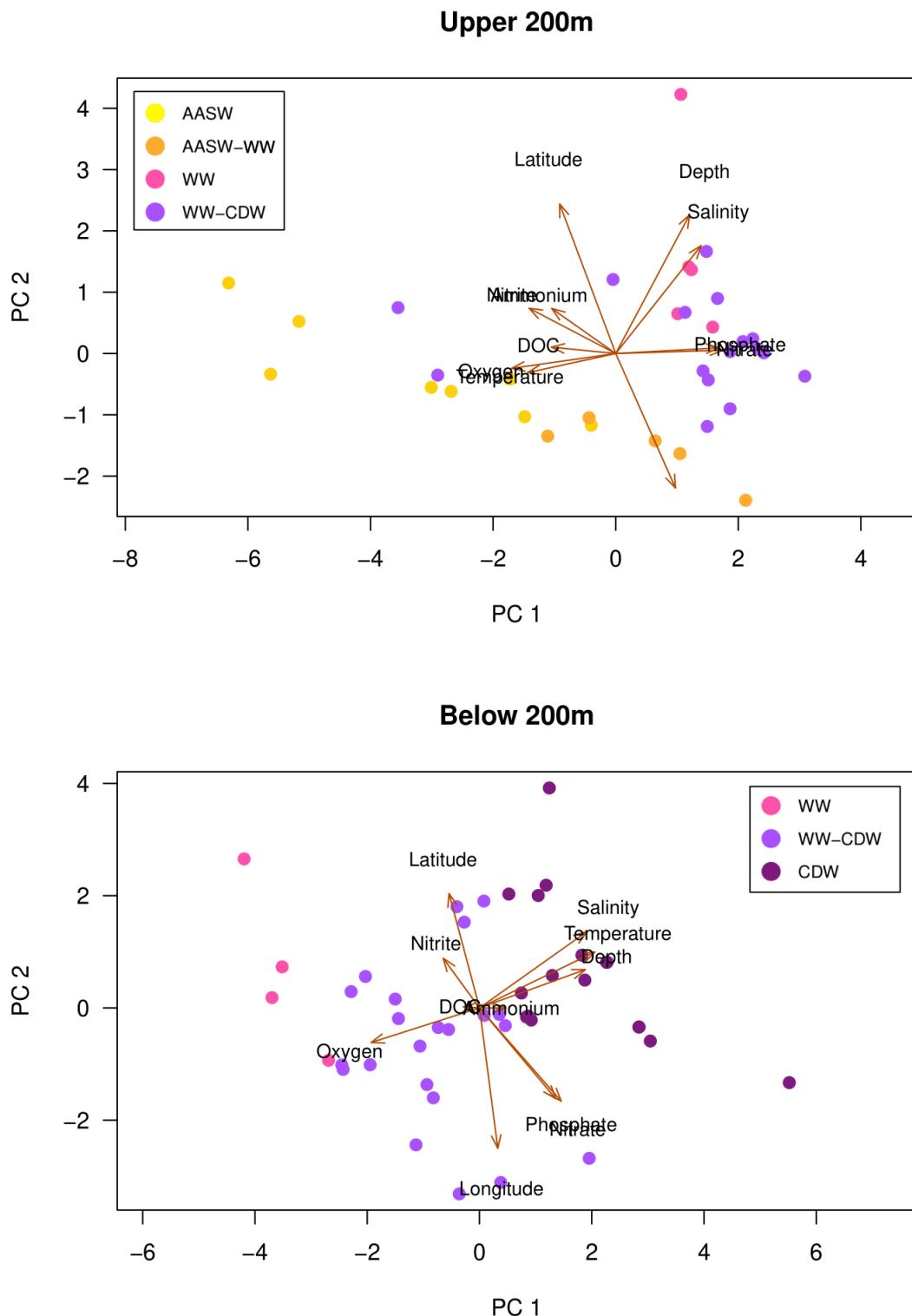
**Figure S4a.** Relative abundance of PICRUSt2 KO numbers by water mass and station. Free-living on left and particle-associated on the right.



**Figure S4b.** Legend that corresponds to **Figure S4a.**



**Figure S5.** PCA of sample metadata by location in the ASP. Top PCA is surface-200m and bottom PCA is 200-bottom.



**Figure S6.** PCA of sample metadata by water mass in the ASP. Top PCA is surface-200m and bottom PCA is 200-bottom.

## SUPPLEMENTARY TABLES

**Table S1.** Nutrient ranges for all stations combined.

| Nutrient    | Range        |
|-------------|--------------|
| Salinity    | 33.49 – 34.7 |
| Temperature | -1.8 – 1.11  |
| Depth       | 2 – 1271     |
| Nitrate     | 9.22 – 38.6  |
| Phosphate   | 0.87 – 2.74  |
| Nitrite     | 0 – 0.08     |
| Ammonia     | 0.03 – 0.65  |
| Oxygen      | 4.31 – 9.35  |
| DOC         | 34.6 – 77.6  |

**Table S2.** Nutrient ranges per station.

| Station | Location     | Date    | Latitude | Longitude | Salinity      | Temperature  | Depth     | Nitrite     | Nitrate       | Ammonia     | Phosphate   | Oxygen      | D    |
|---------|--------------|---------|----------|-----------|---------------|--------------|-----------|-------------|---------------|-------------|-------------|-------------|------|
| STN014  | Dolson       | 1/20/22 | -74.23   | -112.08   | 33.84 – 34.53 | -1.42 – 0.48 | 40 – 860  | 0 – 0.06    | 26.53 – 34.86 | 0.03 – 0.16 | 1.87 – 2.39 | 4.61 – 7.52 | 43.8 |
| STN22   | Dolson       | 1/23/22 | -74.18   | -113.34   | 33.88 – 34.38 | -1.27 – 0.1  | 2 – 610   | 0 – 0.02    | 32.44 – 33.39 | 0.05 – 0.12 | 2.25 – 2.3  | 4.73 – 5.49 | 37.8 |
| STN056a | Dolson       | 1/26/22 | -74.18   | -113.34   | 33.88 – 34.15 | -1.37 – 0.56 | 100 – 310 | 0.01 – 0.02 | 32.56 – 33.29 | 0.09 – 0.18 | 2.18 – 2.24 | 4.96 – 5.65 | 42.4 |
| STN078  | Dolson       | 1/31/22 | -74.23   | -112.69   | 33.83 – 34.54 | -1.5 – 0.48  | 20 – 1040 | 0.01 – 0.06 | 27.72 – 32.92 | 0.24 – 0.46 | 1.97 – 2.28 | 4.58 – 7.03 | 39.6 |
| STN20   | Eastern CC   | 1/22/22 | -74.15   | -111.90   | 33.81 – 34.44 | -1.52 – 0.13 | 25 – 499  | 0.01 – 0.06 | 26.6 – 33.69  | 0.08 – 0.23 | 1.96 – 2.32 | 4.8 – 7.55  | 38.6 |
| STN089  | Eastern CC   | 2/3/22  | -74.39   | -110.09   | 33.77 – 34.7  | -1.53 – 1.11 | 2 – 1271  | 0.01 – 0.02 | 36.66 – 38.6  | 0.1 – 0.48  | 2.58 – 2.7  | 4.31 – 5.65 | 41.4 |
| STN106  | Eastern CC   | 2/5/22  | -74.16   | -111.44   | 33.82 – 34.04 | -1.35 – 1.3  | 20 – 318  | 0.03 – 0.05 | 32.51 – 36.53 | 0.16 – 0.35 | 2.09 – 2.56 | 5.82 – 6.88 | 39.6 |
| STN132  | Eastern CC   | 2/11/22 | -74.20   | -110.90   | 33.82 – 34.09 | -1.43 – 1.24 | 30 – 505  | 0.01 – 0.02 | 31.56 – 32.23 | 0.09 – 0.24 | 2.12 – 2.19 | 5.61 – 6.23 | 41.8 |
| STN153  | Geltz        | 2/15/22 | -73.92   | -118.84   | 33.76 – 34.39 | -1.37 – 0    | 2 – 548   | 0.02 – 0.08 | 10.97 – 33.15 | 0.05 – 0.5  | 1.13 – 2.18 | 4.63 – 8.78 | 34.6 |
| STN002  | Open Polynya | 1/15/22 | -72.20   | -117.69   | 33.49 – 34.66 | -1.73 – 0.93 | 20 – 517  | 0.02 – 0.08 | 9.22 – 33.62  | 0.08 – 0.41 | 0.87 – 2.26 | 4.52 – 9.35 | 43.4 |
| STN004  | Open Polynya | 1/16/22 | -73.14   | -113.95   | 33.88 – 34.58 | -1.8 – 0.59  | 30 – 555  | 0.03 – 0.07 | 16.96 – 33.86 | 0.03 – 0.65 | 1.42 – 2.27 | 4.56 – 8.63 | 42.  |
| STN012  | Open Polynya | 1/18/22 | -73.80   | -112.67   | 33.81 – 34.57 | -1.79 – 0.57 | 30 – 730  | 0.02 – 0.07 | 18.3 – 34.11  | 0.11 – 0.43 | 1.5 – 2.3   | 4.55 – 8.62 | 40.2 |
| STN115  | Open Polynya | 2/7/22  | -73.80   | -112.67   | 33.81 – 34.58 | -1.43 – 0.58 | 35 – 730  | 0.02 – 0.07 | 22.98 – 37.39 | 0.19 – 0.59 | 1.92 – 2.74 | 4.58 – 8.27 | 42.9 |
| STN174  | West OP      | 2/17/22 | -74.39   | -111.26   | 33.79 – 34.34 | -1.36 – 0.21 | 10 – 727  | 0.01 – 0.08 | 20.71 – 33.71 | 0.1 – 0.51  | 1.45 – 2.3  | 5.34 – 9.11 | 40.2 |
| STN088  | Western CC   | 1/29/22 | -74.03   | -113.34   | 33.79 – 34.11 | -1.34 – 0.71 | 30 – 257  | 0.02 – 0.05 | 20.94 – 33.16 | 0.09 – 0.27 | 1.62 – 2.23 | 5.09 – 8.57 | 44.1 |
| STN146  | Western CC   | 2/13/22 | -73.86   | -114.02   | 33.77 – 34.1  | -1.17 – 0.85 | 30 – 268  | 0.03 – 0.07 | 18.47 – 32.84 | 0.17 – 0.63 | 1.54 – 2.25 | 5.29 – 8.55 | 43.1 |

**Table S3.** Indicator species for **free-living** communities based on location, separated into surface-200m (upper 200m) and 200-bottom (below 200m).

| FREE-LIVING |       |    |         |        |            |            |       |                         |  |       |  |
|-------------|-------|----|---------|--------|------------|------------|-------|-------------------------|--|-------|--|
| Upper 200m  |       |    |         |        |            | Lower 200m |       |                         |  |       |  |
| ASV #       | Index | OP | West CC | Dotsen | Eastern CC | Getz       | Order | Family                  | Genus  | Genus |  |
| Seq37       | 3     | 0  | 0       | 0      | 0          | 1          | 0.042 | Flavobacteriales        | Flavobacteriaceae Family                     |       |  |
| Seq955      | 1     | 0  | 0       | 1      | 0          | 0          | 0.031 | Arcto9FB-4 marine group | HF050_16O23 Family                           |       |  |
| Seq235      | 2     | 0  | 0       | 0      | 0          | 1          | 0.022 | Flaonomyctetes          | Gemmisicetaceae                              |       |  |
| Seq147      | 2     | 0  | 0       | 0      | 0          | 0          | 0.031 | Optitales               | Punciceococcaceae                            |       |  |
| Seq1485     | 2     | 0  | 0       | 0      | 0          | 0          | 0.028 | uncultured              | Pelagicoccus                                 |       |  |
| Seq9768     | 2     | 0  | 0       | 0      | 0          | 0          | 0.022 | Pedobacteriales         | uncultured marine bacterium                  |       |  |
| Seq9661     | 3     | 0  | 0       | 0      | 0          | 0          | 0.022 | Aclotonanoidales        | Pedobacteriaceae Family                      |       |  |
| Seq9628     | 3     | 0  | 0       | 0      | 0          | 0          | 0.022 | Aclotonanoidales        | Candidatus Aclotonanna                       |       |  |
| Seq153      | 4     | 0  | 1       | 0      | 0          | 0          | 0.016 | Victamicrobacteriales   | uncultured bacterium AD246-D7-1A             |       |  |
| Seq171      | 5     | 0  | 1       | 0      | 0          | 0          | 0.031 | Oleospirales            | Oleospira                                    |       |  |
| Seq234      | 5     | 0  | 1       | 0      | 0          | 0          | 0.009 | Lentisphaerales         | Lentisphaera                                 |       |  |
| Seq125      | 5     | 0  | 1       | 0      | 0          | 0          | 0.022 | OM182 clade             | marine gamma proteobacterium HTCC2178 Family |       |  |
| Seq134      | 6     | 0  | 0       | 1      | 0          | 0          | 0.016 | Calvinbionales          | uncultured marine bacterium                  |       |  |
| Seq155      | 6     | 0  | 0       | 0      | 1          | 0          | 0.014 | Thiomicrosporales       | Theiobaccae Family                           |       |  |
| Seq255      | 6     | 0  | 0       | 1      | 0          | 0          | 0.022 | Planctomyctetes         | uncultured bacterium HF050_40221             |       |  |
| Seq529      | 6     | 0  | 0       | 1      | 0          | 0          | 0.025 | Optitales               | MB100 marine group                           |       |  |
| Seq110      | 7     | 0  | 0       | 1      | 0          | 0          | 0.022 | Deltavircoctales        | uncultured Parvibaculum sp.                  |       |  |
| Seq386      | 7     | 0  | 0       | 1      | 0          | 0          | 0.031 | Flavobacteriales        | NS4 marine group                             |       |  |
| Seq161      | 10    | 0  | 0       | 0      | 1          | 1          | 0.016 | Nitospinaces            | uncultured marine microorganism              |       |  |
| Seq192      | 10    | 0  | 0       | 0      | 0          | 1          | 0.022 | Spiringtonomataces      | LS-NOB                                       |       |  |
| Seq444      | 10    | 0  | 0       | 0      | 0          | 1          | 0.034 | Victamicrobacteriales   | Sphingomonadaceae                            |       |  |
| Seq255      | 15    | 1  | 0       | 0      | 0          | 0          | 0.032 | Cellvibrionales         | uncultured                                   |       |  |
| Seq123      | 16    | 0  | 0       | 1      | 0          | 1          | 0.026 | SAR11 clade             | SAF92 clade                                  |       |  |
| Seq128      | 16    | 0  | 0       | 1      | 0          | 1          | 0.034 | Nitospinaces            | Clade II                                     |       |  |
| Seq132      | 16    | 0  | 0       | 1      | 1          | 1          | 0.012 | Steroidobacteriales     | Nitrospinaceae                               |       |  |
| Seq144      | 16    | 0  | 0       | 0      | 1          | 1          | 0.026 | Cytophagales            | Weisfeliaeae                                 |       |  |
| Seq148      | 16    | 0  | 0       | 0      | 1          | 1          | 0.031 | Rhodospirillales        | Cycloabacteriaceae                           |       |  |
| Seq156      | 16    | 0  | 0       | 1      | 1          | 1          | 0.025 | Deltavircoctales        | AEGEAN-169 marine group                      |       |  |
| Seq185      | 16    | 0  | 0       | 1      | 1          | 1          | 0.031 | Micronichiales          | uncultured bacterium KM3-47-46               |       |  |
| Seq19       | 16    | 0  | 0       | 1      | 1          | 1          | 0.044 | Microthrixaceae         | Sva096 marine group                          |       |  |
| Seq195      | 16    | 0  | 0       | 1      | 1          | 1          | 0.031 | Rhodospirillales        | Illumobacteriaceae Family                    |       |  |
| Seq199      | 16    | 0  | 0       | 1      | 1          | 1          | 0.022 | SAR202 clade            | uncultured                                   |       |  |
| Seq260      | 16    | 0  | 0       | 0      | 1          | 1          | 0.023 | SAR86 clade             | hydrothermal vent metagenome                 |       |  |
| Seq34       | 16    | 0  | 0       | 0      | 1          | 1          | 0.031 | SAR86 clade             | hydrothermal vent metagenome                 |       |  |
| Seq38       | 16    | 0  | 0       | 1      | 1          | 1          | 0.009 | SAR11 clade             | Clade I                                      |       |  |
| Seq387      | 16    | 0  | 0       | 1      | 1          | 1          | 0.022 | Opitales                | Punciceococcaceae                            |       |  |
| Seq389      | 16    | 0  | 0       | 1      | 1          | 1          | 0.034 | Thiotrichales           | Thiotrichaceae                               |       |  |
| Seq563      | 16    | 0  | 0       | 1      | 1          | 1          | 0.022 | SAR202 clade            | uncultured bacterium HF050_03M05             |       |  |
| Seq616      | 16    | 0  | 0       | 1      | 1          | 1          | 0.022 | Kilonellales            | Kilonellaceae                                |       |  |
| Seq167      | 26    | 1  | 0       | 0      | 1          | 1          | 0.039 | Flavobacteriales        | Flavobacteriaceae                            |       |  |
|             |       |    |         |        |            |            |       |                         | NSS marine group                             |       |  |

**Table S4.** Indicator species for particle-associated communities based on location, separated into surface-200m (upper 200m) and 200-bottom (below 200m). No significant taxa for upper 200m.

| PARTICLE-ASSOCIATED            |       |    |         |        |            |      |             |                   |                                      |  |  |  |  |  |
|--------------------------------|-------|----|---------|--------|------------|------|-------------|-------------------|--------------------------------------|--|--|--|--|--|
| Upper 200m                     |       |    |         |        | Lower 200m |      |             |                   |                                      |  |  |  |  |  |
| No significant indicator taxa. |       |    |         |        |            |      |             |                   |                                      |  |  |  |  |  |
|                                |       |    |         |        |            |      |             |                   |                                      |  |  |  |  |  |
| ASV #                          | Index | OP | West CC | Dotson | Eastern CC | Getz | p-value, bh | Order             | Family                               |  |  |  |  |  |
| Seq235                         | 2     | 0  | 0       | 0      | 1          | 0    | 0.047       | Planctomycetales  | Gemmatae                             |  |  |  |  |  |
| Seq801                         | 2     | 0  | 0       | 0      | 0          | 0    | 0.042       | Vibrionales       | Arctic95B-14                         |  |  |  |  |  |
| Seq1526                        | 3     | 0  | 0       | 0      | 0          | 0    | 0.027       | Methylomonadaceae | uncultured Verrucomicrobia bacterium |  |  |  |  |  |
| Seq156                         | 3     | 0  | 0       | 0      | 0          | 0    | 0.047       | Deltaproteobact.  | Marine Methyiotrophic Group 2        |  |  |  |  |  |
| Seq272                         | 3     | 0  | 0       | 0      | 0          | 0    | 0.033       | Phytophtheraceae  | uncultured bacterium KN3-47-46       |  |  |  |  |  |
| Seq380                         | 3     | 0  | 0       | 0      | 0          | 0    | 0.009       | Pirellulales      | CL500-3                              |  |  |  |  |  |
| Seq407                         | 3     | 0  | 0       | 0      | 0          | 0    | 0.009       | Nanocystostacae   | Pirellulaceae Family                 |  |  |  |  |  |
| Seq452                         | 3     | 0  | 0       | 0      | 0          | 0    | 0.022       | Rubrobacteraceae  | Nannocystaceae                       |  |  |  |  |  |
| Seq482                         | 3     | 0  | 0       | 0      | 0          | 0    | 0.009       | Planctomycetales  | uncultured marine bacterium Family 2 |  |  |  |  |  |
| Seq653                         | 3     | 0  | 0       | 0      | 0          | 0    | 0.027       | Oligofiales       | uncultured sediment bacterium        |  |  |  |  |  |
| Seq171                         | 5     | 0  | 1       | 0      | 0          | 0    | 0.047       | Lentisphaeraceae  | Lentisphaera                         |  |  |  |  |  |
| Seq183                         | 5     | 0  | 1       | 0      | 0          | 0    | 0.027       | Oceanospirillales | uncultured 1                         |  |  |  |  |  |
| Seq420                         | 7     | 0  | 0       | 1      | 0          | 0    | 0.042       | Pirellulales      | Blastopirellula                      |  |  |  |  |  |
| Seq95                          | 10    | 0  | 0       | 1      | 1          | 1    | 0.028       | Pirellulales      | Phodopirellula                       |  |  |  |  |  |
| Seq6                           | 15    | 1  | 1       | 0      | 0          | 0    | 0.047       | Celvibrionales    | SAR92 clade                          |  |  |  |  |  |
| Seq1                           | 16    | 0  | 0       | 1      | 1          | 1    | 0.009       | Pseudomonadaceae  | Pseudomonas                          |  |  |  |  |  |
| Seq155                         | 16    | 0  | 0       | 1      | 1          | 1    | 0.042       | Planctomycetales  | uncultured bacterium HF0500-40D21    |  |  |  |  |  |
| Seq308                         | 16    | 0  | 0       | 1      | 1          | 1    | 0.047       | Pirellulales      | uncultured                           |  |  |  |  |  |

**Table S5.** Indicator species for free-living communities based on location for CDW samples ONLY.

| FREE-LIVING |       |      |         |    |        |         |            |                     |   |
|-------------|-------|------|---------|----|--------|---------|------------|---------------------|---|
| ASV #       | index | Gelz | West OP | OP | Dotson | East CC | p.value_bh | Order               | Family  |
| Seq260      | 1     | 0    | 0       | 0  | 1      | 0       | 0.014      | SAR202 clade        | uncultured Chloroflexi bacterium HF4000_28F-02 Family |
| Seq134      | 2     | 0    | 0       | 0  | 0      | 1       | 0.031      | Thiobacillaceae     | Thiobacillaceae Family                                |
| Seq1405     | 3     | 1    | 0       | 0  | 0      | 0       | 0.045      | Anaerolineales      | uncultured 24   |
| Seq1671     | 3     | 1    | 0       | 0  | 0      | 0       | 0.045      | Coxiellales         | Coxiella  |
| Seq210      | 3     | 1    | 0       | 0  | 0      | 0       | 0.037      | Burkholderiales     | Nitrosomonadaceae                                     |
| Seq228      | 3     | 1    | 0       | 0  | 0      | 0       | 0.046      | Cellvibrionales     | Haleaceae Family                                      |
| Seq326      | 3     | 1    | 0       | 0  | 0      | 0       | 0.045      | Pseudomonadales     | Pseudomonadaceae Family                               |
| Seq447      | 3     | 1    | 0       | 0  | 0      | 0       | 0.024      | Opitutales          | Pelagicoccus  |
| Seq661      | 3     | 1    | 0       | 0  | 0      | 0       | 0.024      | Actinomarinaires    | Candidatus Actinomarinira                             |
| Seq117      | 5     | 0    | 1       | 0  | 0      | 0       | 0.014      | Flavobacteriales    | uncultured 2  |
| Seq171      | 5     | 0    | 1       | 0  | 0      | 0       | 0.031      | Lentisphaerae       | Lentisphaeraceae                                      |
| Seq188      | 5     | 0    | 1       | 0  | 0      | 0       | 0.045      | Oceanospirillales   | Profoundimonas  |
| Seq215      | 5     | 0    | 1       | 0  | 0      | 0       | 0.033      | Alteromonadaceae    | Pseudodalteromonadaceae                               |
| Seq3        | 5     | 0    | 1       | 0  | 0      | 0       | 0.029      | Oceanospirillales   | Nitriliclavaceae                                      |
| Seq359      | 5     | 0    | 1       | 0  | 0      | 0       | 0.037      | Fibrobacterales     | Fibrobacteraceae                                      |
| Seq43       | 5     | 0    | 1       | 0  | 0      | 0       | 0.031      | Alteromonadaceae    | Alteromonadaceae                                      |
| Seq95       | 5     | 0    | 1       | 0  | 0      | 0       | 0.024      | Flavobacteriales    | Paraglaciecola  |
| Seq6        | 5     | 0    | 1       | 0  | 0      | 0       | 0.033      | Cellvibrionales     | Planibacter   |
| Seq775      | 5     | 0    | 1       | 0  | 0      | 0       | 0.045      | Campylobacteriales  | SAR92 clade   |
| Seq91       | 5     | 0    | 1       | 0  | 0      | 0       | 0.045      | Chitinophagales     | uncultured 13   |
| Seq834      | 5     | 0    | 1       | 0  | 0      | 0       | 0.045      | Saprospiraceae      | uncultured 18   |
| Seq117      | 6     | 0    | 0       | 0  | 1      | 1       | 0.029      | Arcothiobacteriales | Saprospiraceae Family                                 |
| Seq155      | 6     | 0    | 0       | 0  | 0      | 1       | 0.014      | Chitinophagales     | Halarcobacter   |
| Seq195      | 6     | 0    | 0       | 0  | 0      | 1       | 0.029      | Springbacteriales   | uncultured deep-sea archaeon                          |
| Seq58       | 6     | 0    | 0       | 0  | 0      | 1       | 0.011      | Rubritaleaceae      | uncultured bacterium HF0500_40021                     |
| Seq563      | 7     | 1    | 0       | 0  | 0      | 1       | 0          | SAR202 clade        | uncultured bacterium HF0500_03M05                     |
| Seq157      | 10    | 1    | 0       | 0  | 0      | 1       | 0.014      | SAR11 clade         | uncultured marine bacterium 440                       |
| Seq161      | 10    | 1    | 0       | 0  | 0      | 1       | 0.011      | Nitrospinales       | Clade II  |
| Seq265      | 10    | 1    | 0       | 0  | 0      | 1       | 0.024      | Rhodospirillales    | LS-NOB  |
| Seq110      | 16    | 1    | 0       | 0  | 0      | 1       | 0.031      | Flavobacteriales    | unidentified marine bacterioplankton 2                |
| Seq132      | 16    | 1    | 0       | 0  | 0      | 1       | 0.011      | Steroidobacterales  | NS4 marine group                                      |
| Seq199      | 16    | 1    | 0       | 0  | 0      | 1       | 0.013      | SAR202 clade        | Woeseia   |
| Seq220      | 16    | 1    | 0       | 0  | 0      | 1       | 0.011      | Phycisphaeraeales   | hydrothermal vent metagenome Family 3                 |
| Seq98       | 16    | 1    | 0       | 0  | 0      | 1       | 0.013      | Phycisphaeraeales   | Phycisphaeraeace                                      |
| Seq85       | 16    | 1    | 0       | 0  | 0      | 1       | 0.031      | SAR11 clade         | Clade I   |
| Seq97       | 16    | 1    | 0       | 0  | 0      | 1       | 0.024      | Pirellulales        | Rhodopirellula  |
|             |       |      |         |    |        |         |            | Burkholderiales     | Methylphilaceae                                       |

\* no sig indicator taxa for particle-associated  
\* no western CC has pure CDW