

BACTERIAL COMMUNITY STRUCTURE IN THE AMUNDSEN SEA POLYNYA,
ANTARCTICA

by

SIERRA BARTLETT

(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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MASTER OF SCIENCE

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DEDICATION

To Mary, who was my internship mom, good friend, and even better scientist.

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 (Thiele et al. 2022). In Antarctica, a known high-nutrient, low-chlorophyll (HNLC) zone is limited by both iron and light availability (Debeljak et al. 2023; Schine et al. 2021). The combination of open water and the immediate iron from the sea ice melt seeds phytoplankton blooms, primarily the haptophyte *Phaeocystis antarctica* (Ducklow et al. 2015; Richert et al. 2019; Wang et al. 2022). The Southern Ocean is responsible for deep-water formation and 18% of global carbon absorption, storing and capturing atmospheric carbon dioxide (Williams et al. 2015). Most of this carbon processing comes from massive *P. antarctica* blooms and the bacteria accompanying them. In the entire Southern Ocean, coastal polynyas are responsible for the majority for the primary productivity and carbon uptake. 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 (Richert et al. 2019; Choi et al. 2016; Dinniman et al. 2023). One of the most productive polynyas is the Amundsen Sea Polynya (ASP), at a maximum of about 2.5 gC/m per day (Yager 2021). *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 (Wang et al. 2022; Richert et al. 2019).

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; Richert et al. 2019; Delmont et al. 2015) It is also known that there are distinct free-living and particle-associated communities, with the later responsible for demineralizing algal material (Delmont et al. 2014, 2015). It is understood that 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 other bacteria.

In the ASP, there are three distinct water masses: Antarctic Surface Water (AASW), Winter Water (WW), and Circumpolar Deep Water (CDW) (Richert et al. 2019). 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, Charriaud, and Fieux 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, Bashmachnikov, and Alekseev 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 (Dinniman et al. 2023). The CDW is likely supplemented by mixing with high dFe shelf waters, where sediment dissolution and increased remineralization occur (Dinniman et al. 2023, 2020; St-Laurent et al. 2017). 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. Then, begins to melt the underside of the shelf, which makes the water more buoyant and the outflow is provided back to the polynya (Randall-Goodwin et al. 2015). 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 (Alcamán-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- Laurent et al. 2017).

The main questions of this study are:

1. How do bacteria communities (both free-living and particle-associated) change based on location within the ASP?
2. Do bacteria contribute to or come out of the Dotson outflow?
3. How do high and low iron communities differ? Are there any specific indicator taxa?
4. How do CDW communities change as they come onto the shelf and circulate in the rest of the polynya?

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 μ m polycarbonate pre-filter, then equally diverged through replicate .2 μ 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 μm pre-filter, and the former that was caught on the 0.2 μm filters. based on what was caught on, >0.2 μm 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

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 ($p < 0.001$). 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 vis 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

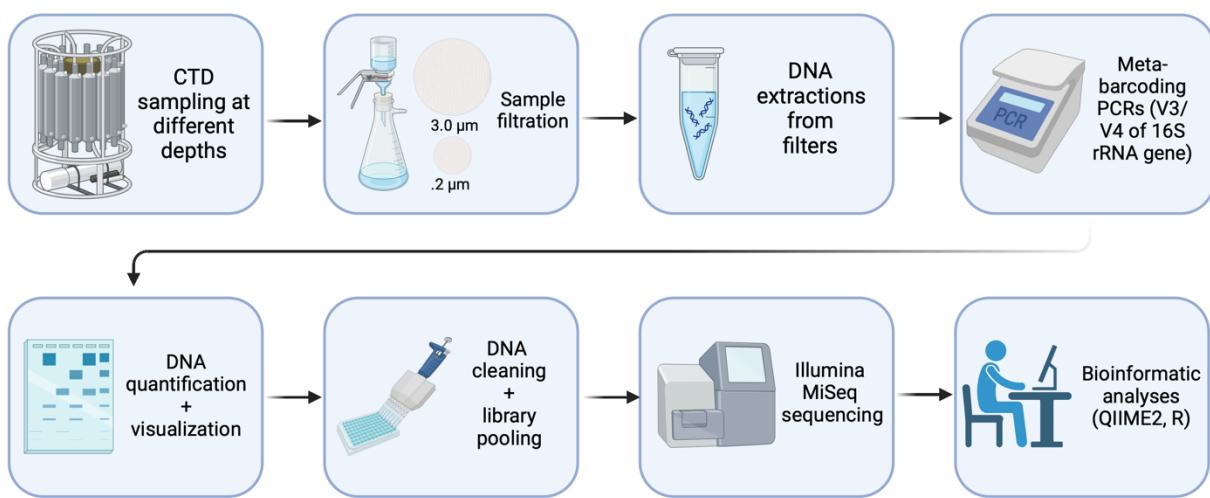


Figure 1. Pipeline of study.

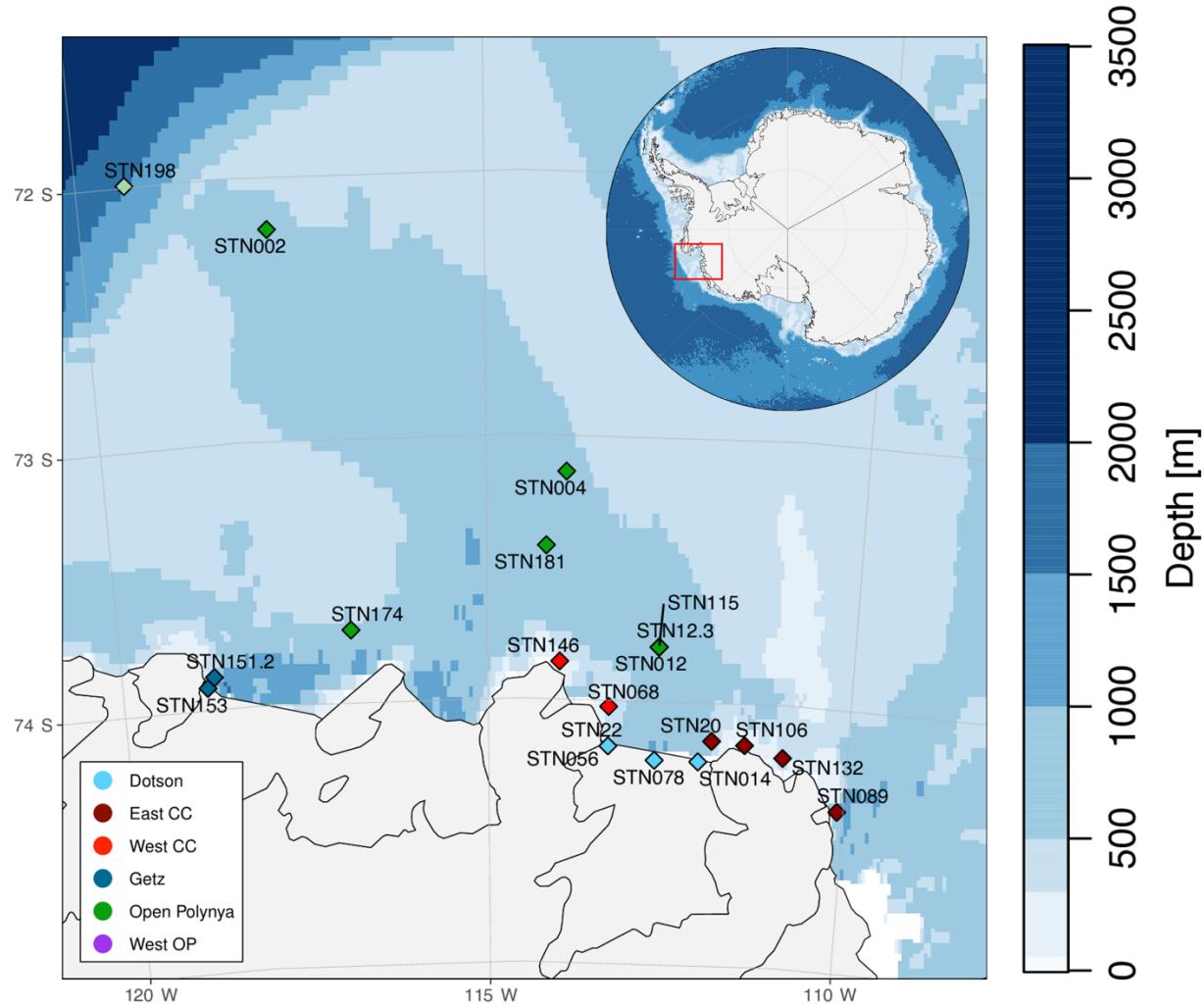


Figure 2. Station map with bathymetry. Colors are coordinated with location.

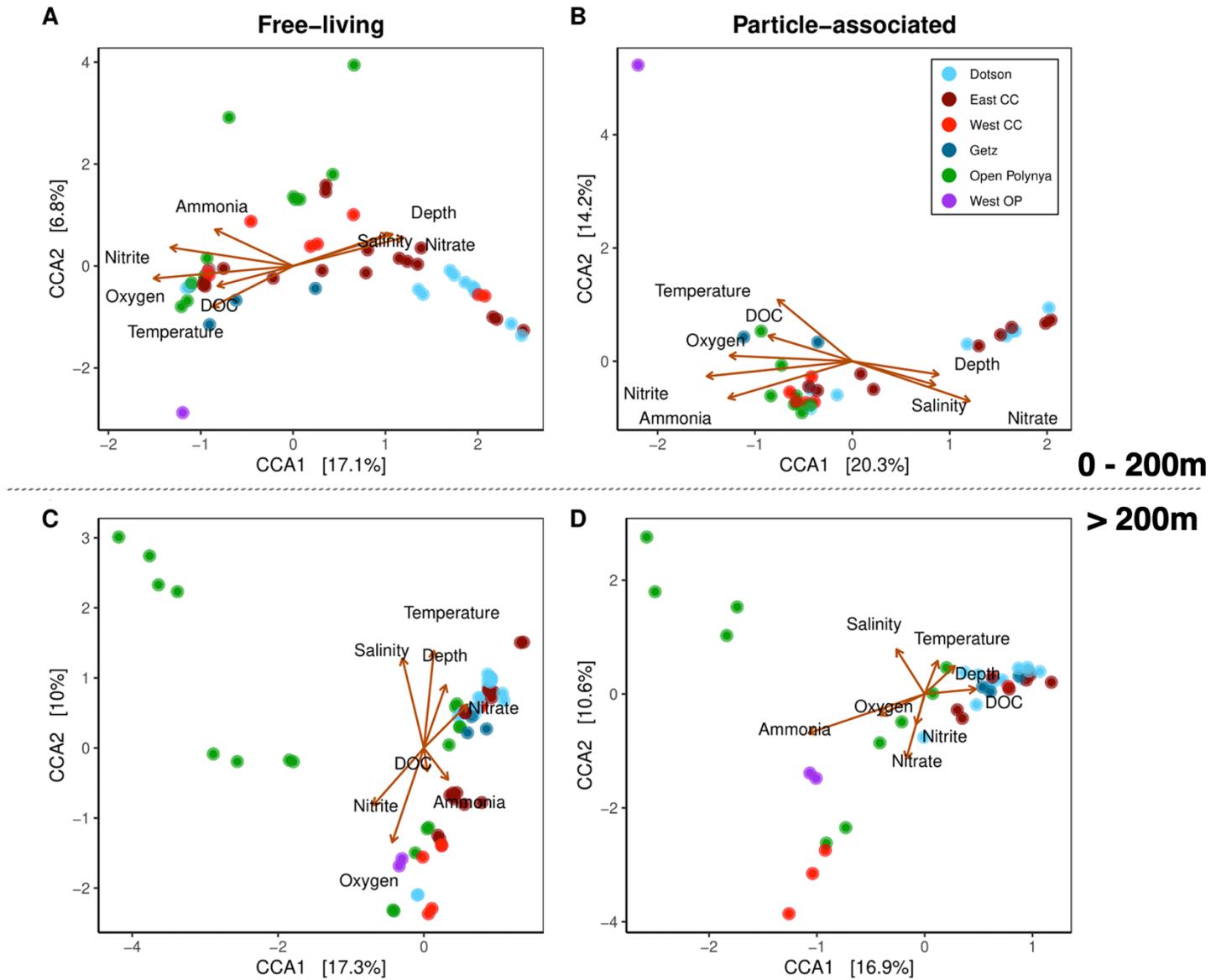


Figure 3. Canonical Correspondence Analysis (CCA) of free-living (**A, C**) and particle-associated (**B, D**) bacterial communities based on **location** within the ASP. First row CCAs are surface-200m and bottom row CCAs are 200m-bottom.

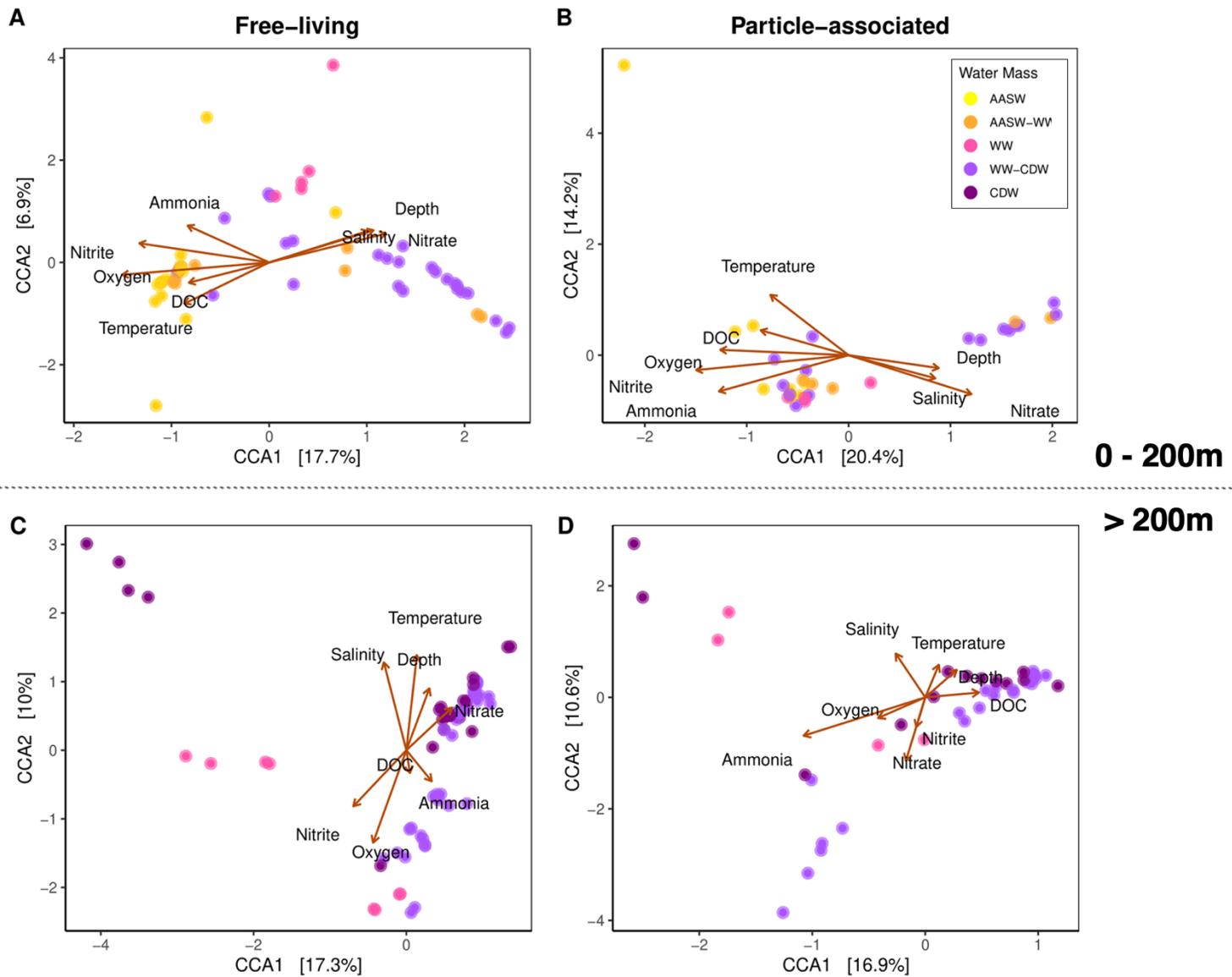


Figure 4. Canonical Correspondence Analysis (CCA) of free-living (**A, C**) and particle-associated (**B, D**) bacterial communities based on defined **water mass**. First row CCAs are surface-200m and bottom row CCAs are 200m-bottom.

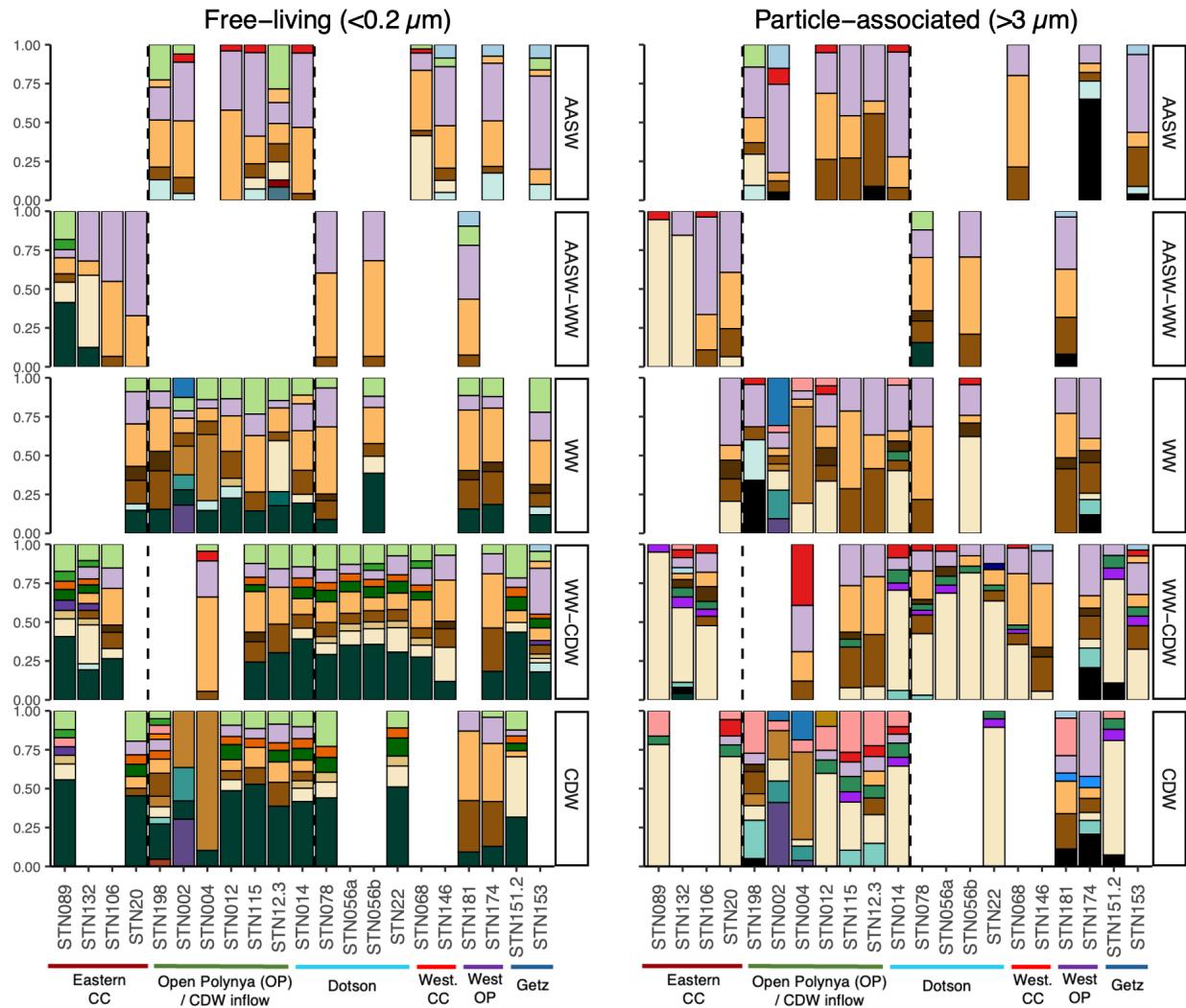


Figure 5a. Relative abundance for free-living (left) and particle-associated (right) communities at the family level. Each row represents a water mass, labeled on the right.

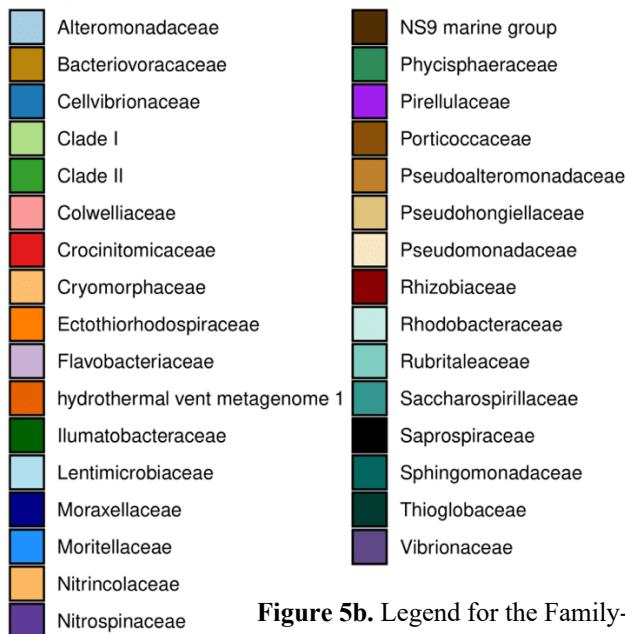


Figure 5b. Legend for the Family-level taxa that corresponds with Figure 5a.

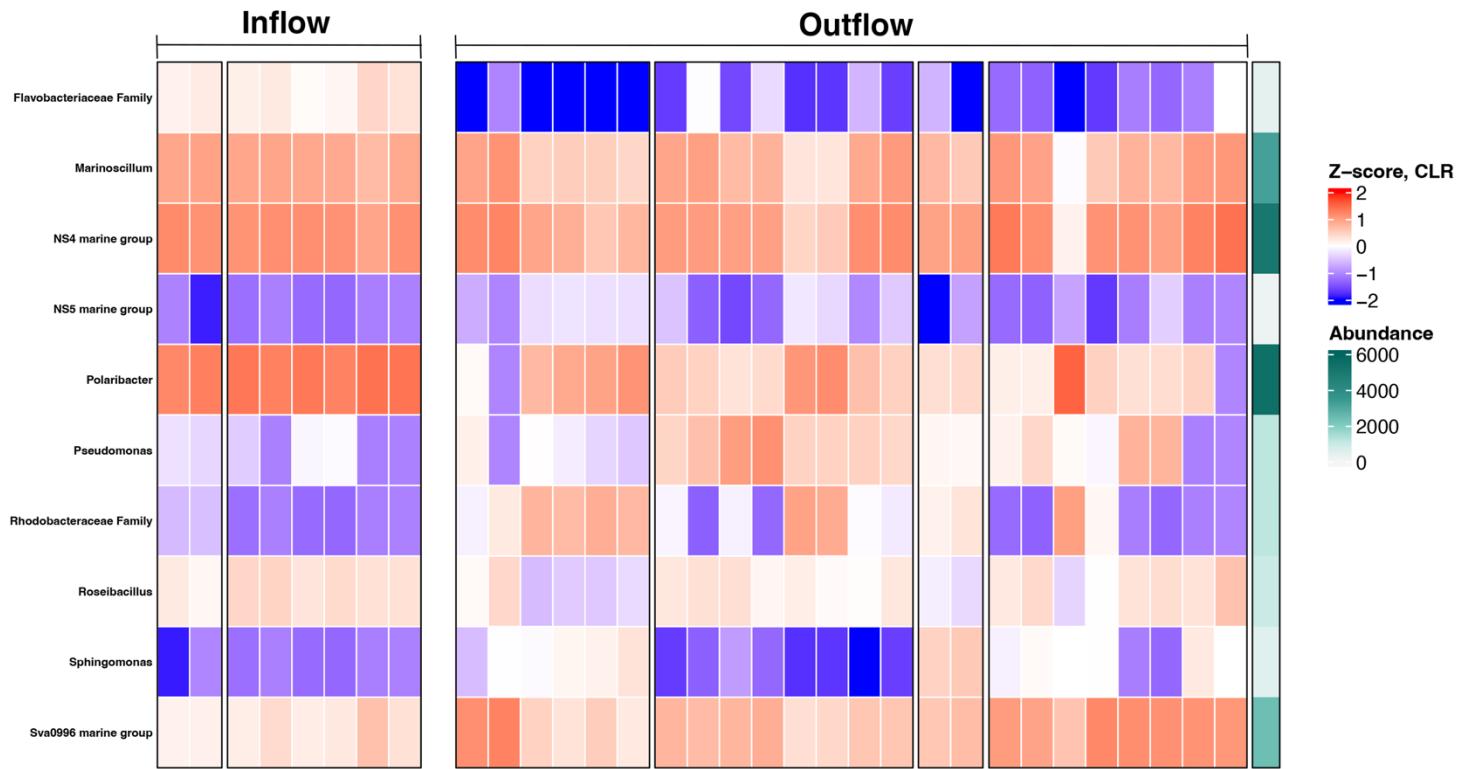


Figure 6. Significant free-living taxa from ANCOM-BC analysis for inflow vs. outflow samples. Z-score derived from log-transformed data. No significant change in particle-associated taxa from ANCOM-BC analysis.

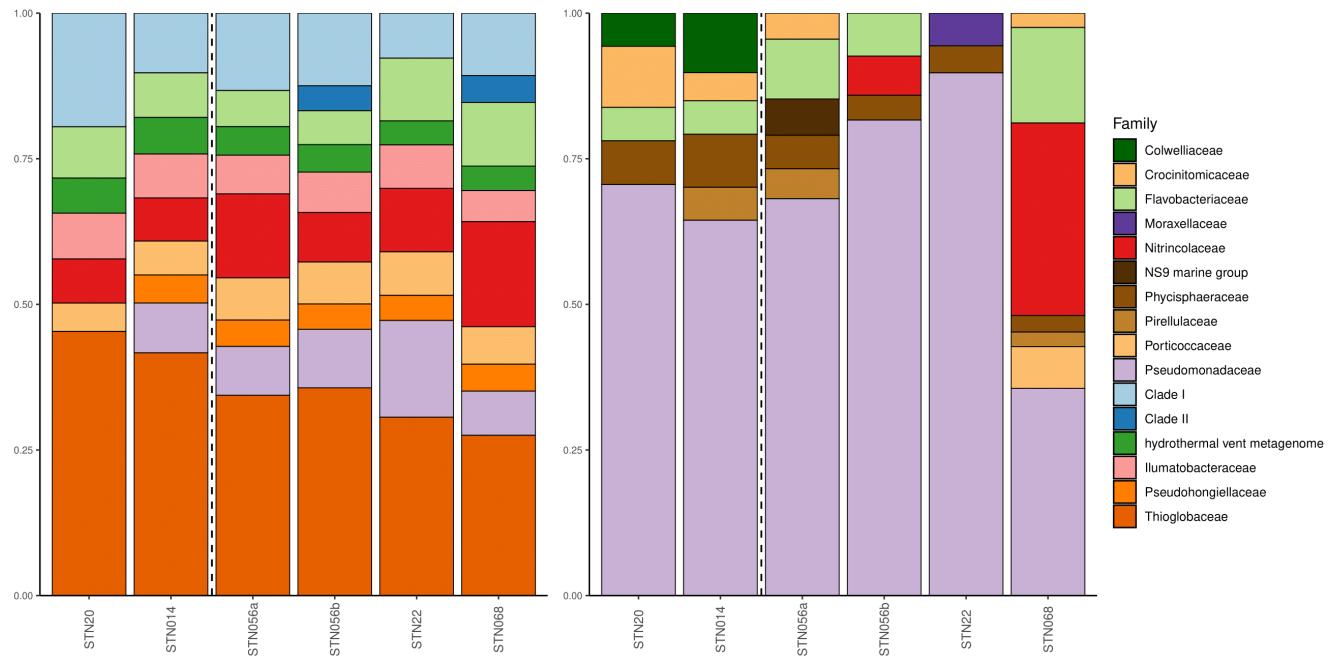


Figure 7. Relative abundance for free-living (left) and particle-associated (right) communities at the family level. In each community, inflow stations are before the black dashed line, while outflow are after.

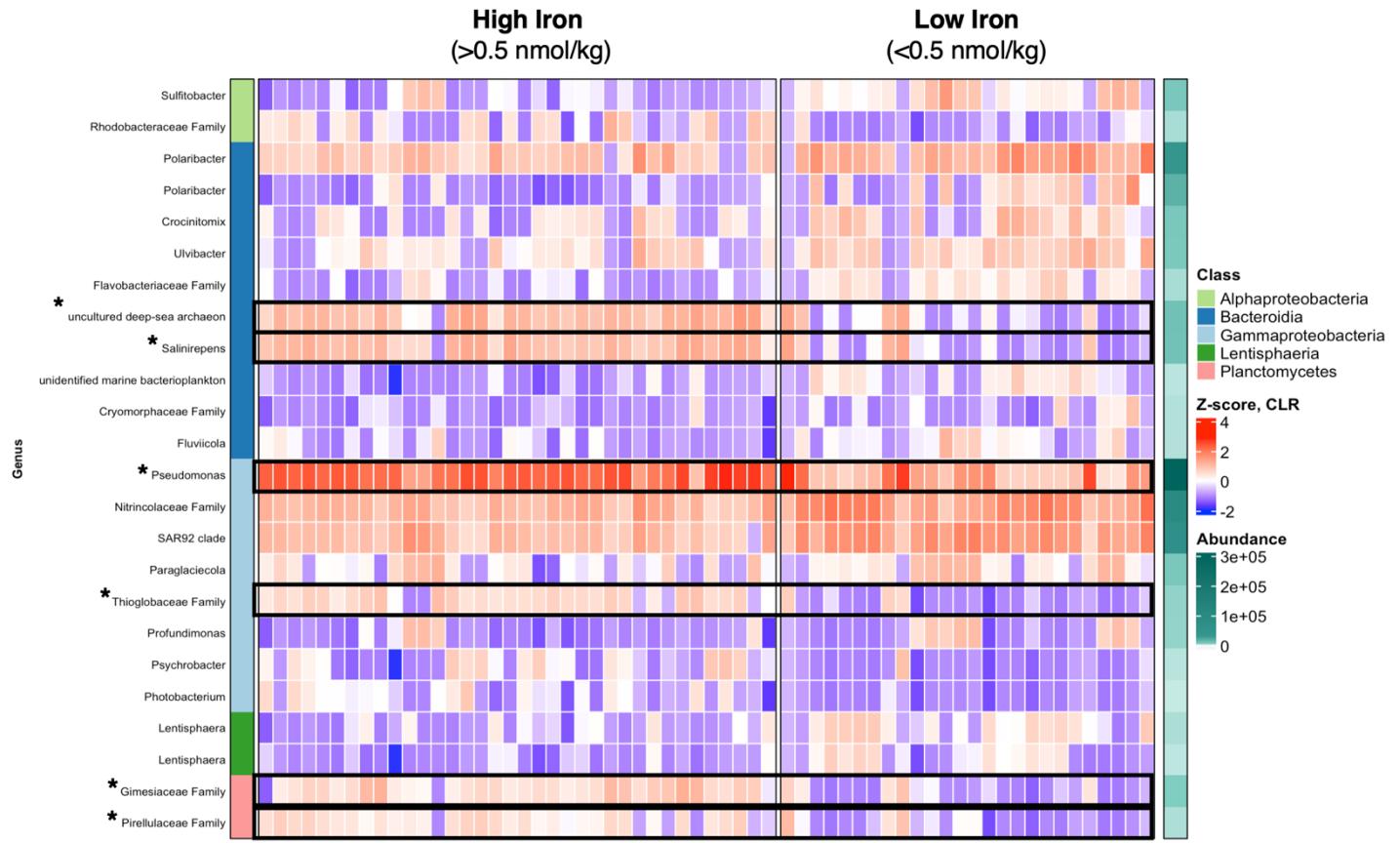


Figure 8. Significant particle-associated taxa from ANCOM-BC analysis for high (>0.5 nmol/kg) or low (<0.5 nmol/kg) dFe concentrations. Z-score derived from log-transformed data. See Supplementary Information for significant free-living taxa.

TABLES

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

Water Mass	Temperature Range	Salinity Range
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.

Inflow	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
Outflow	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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SUPPLEMENTARY FIGURES

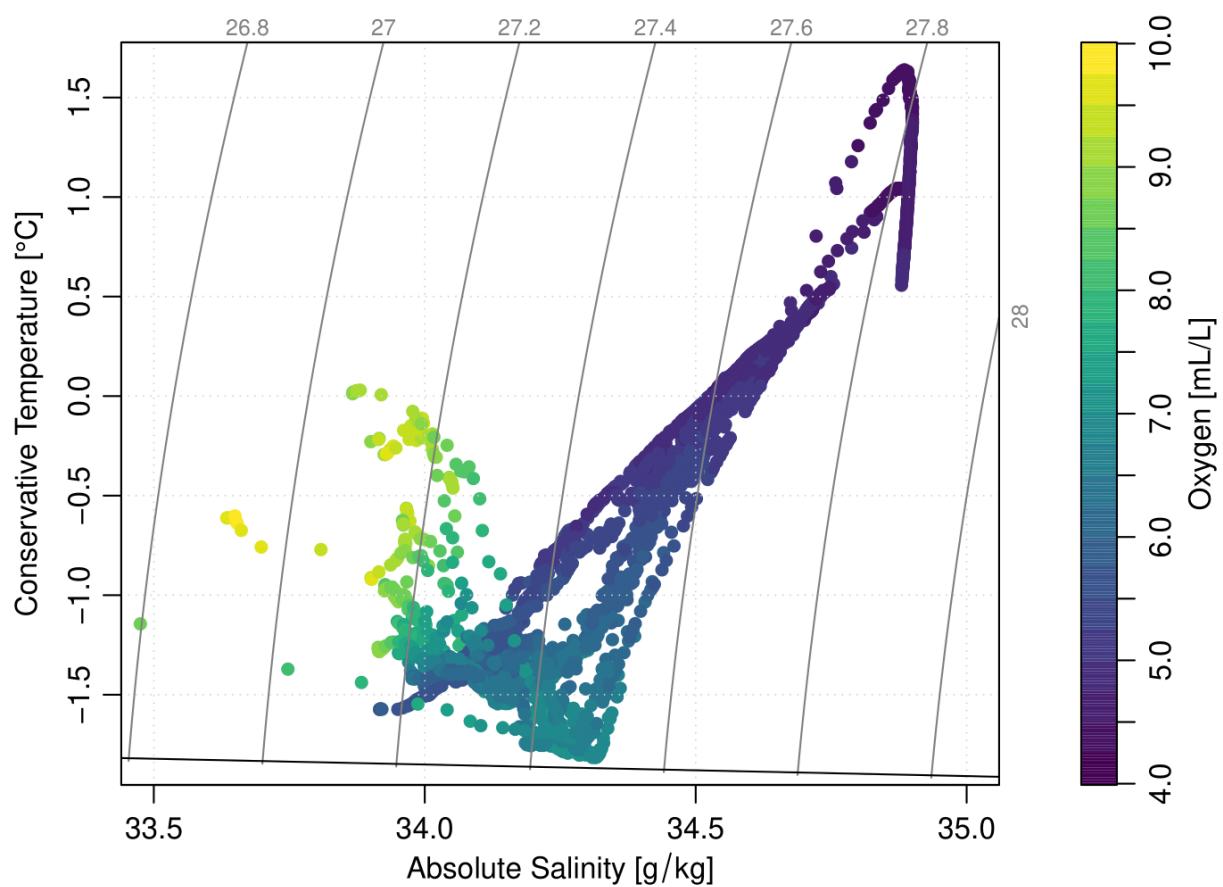


Figure S1. Temperature-Salinity plot for all ARTEMIS stations with DNA samples.

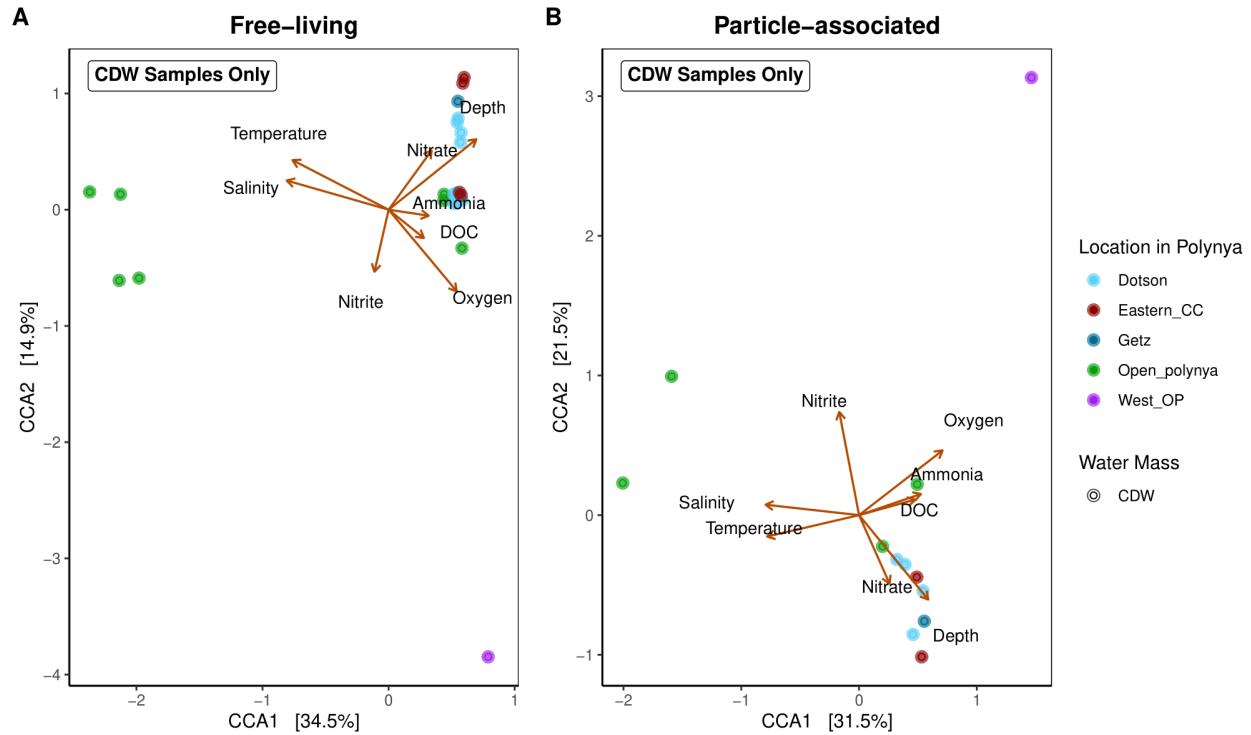


Figure S2. Canonical Correspondence Analysis (CCA) of free-living (**A**) and particle-associated (**B**) of samples that are characterized in CDW water mass, which are bottom water samples.

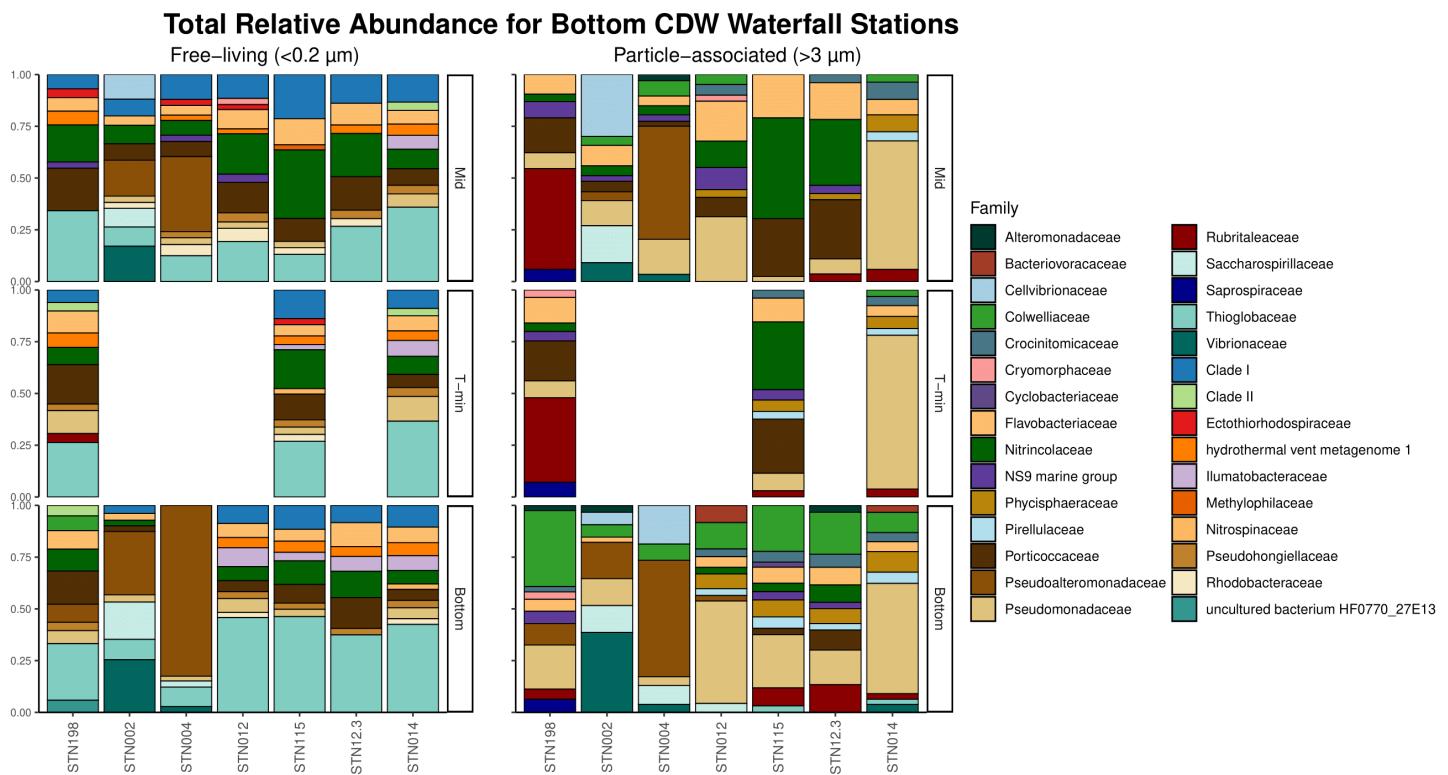


Figure S3. Relative abundance of stations coming from off the continental shelf at the family level.

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

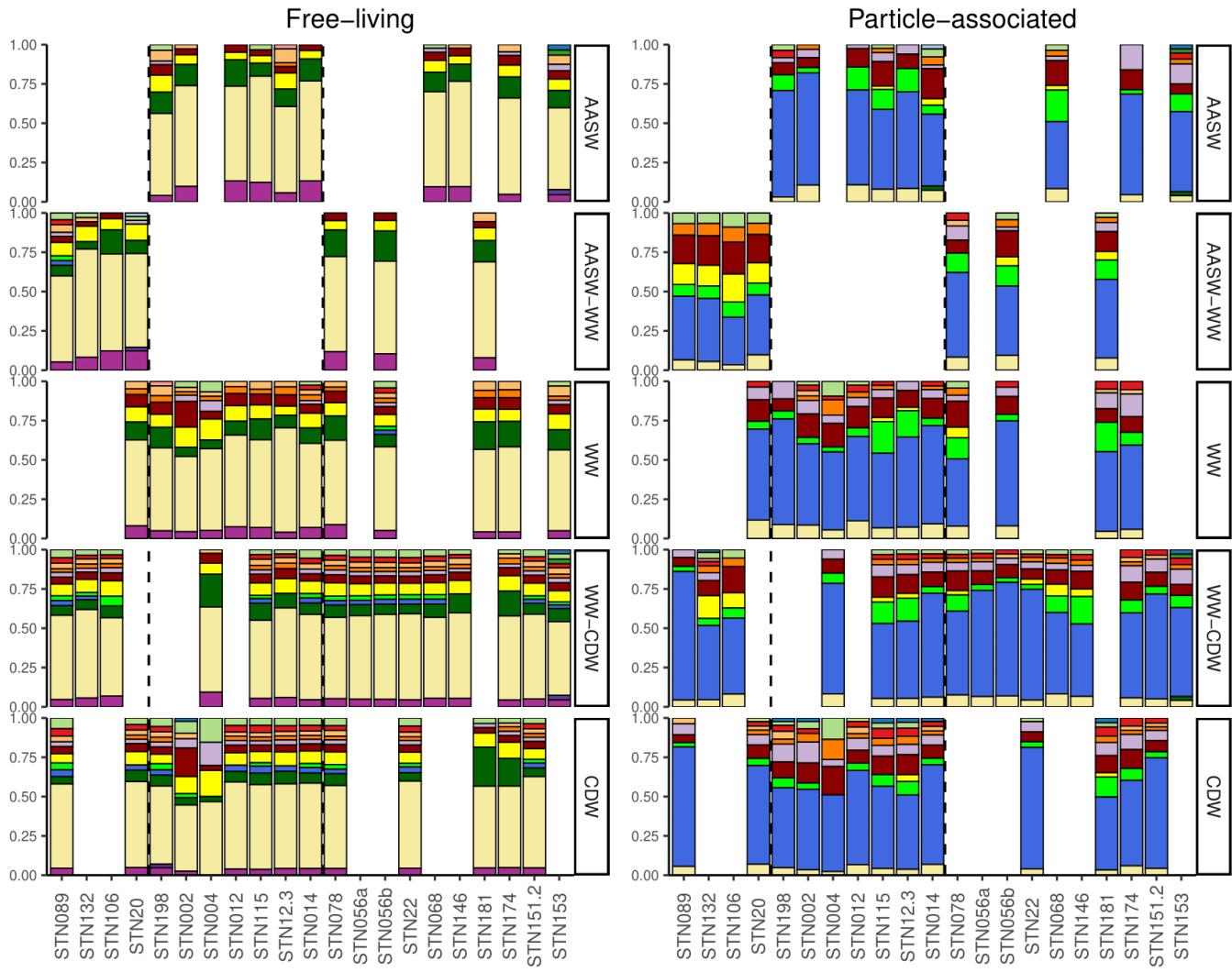


Figure S4a. Relative abundance of PICRUSt2 KO numbers by water mass and station. Free-living on left and particle-associated on the right.

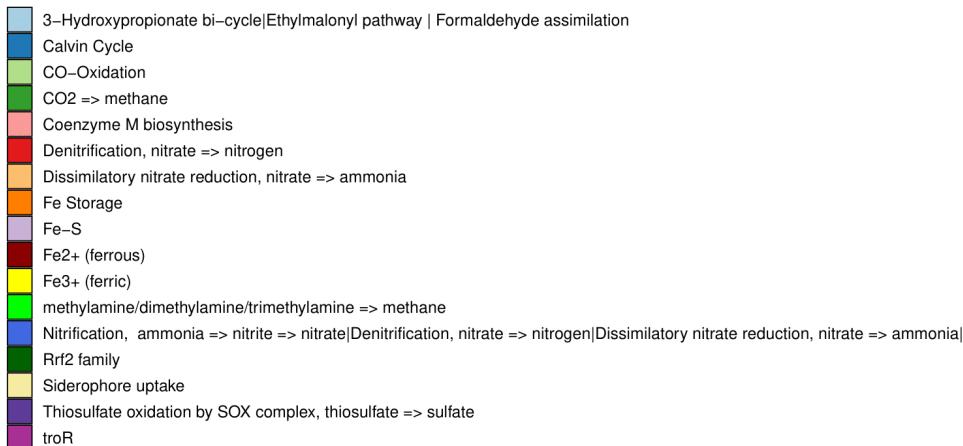


Figure S4b. Legend that corresponds to **Figure S4a**.

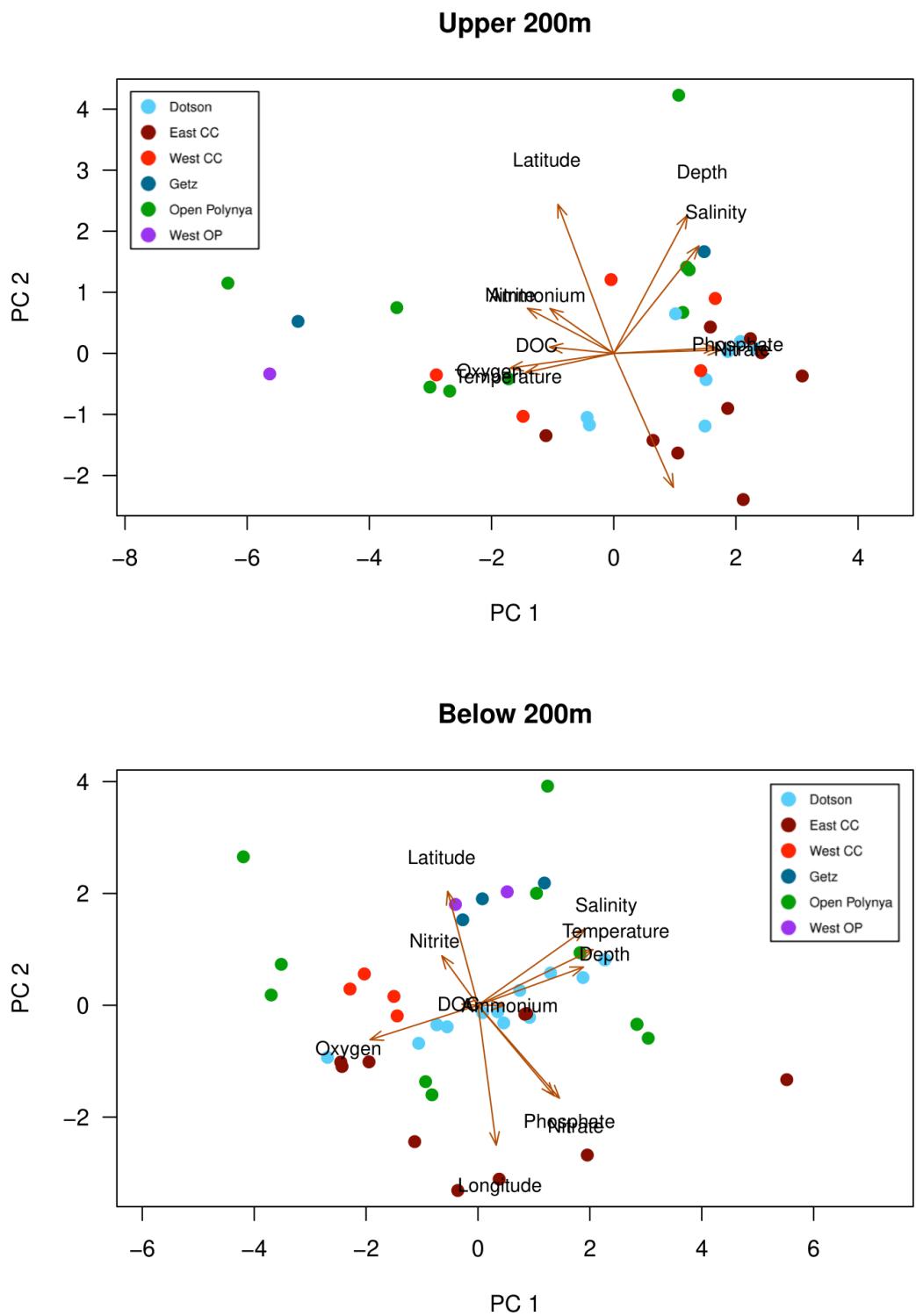


Figure S5. PCA of sample metadata by location in the ASP. Top PCA is surface-200m and bottom PCA is 200-bottom.

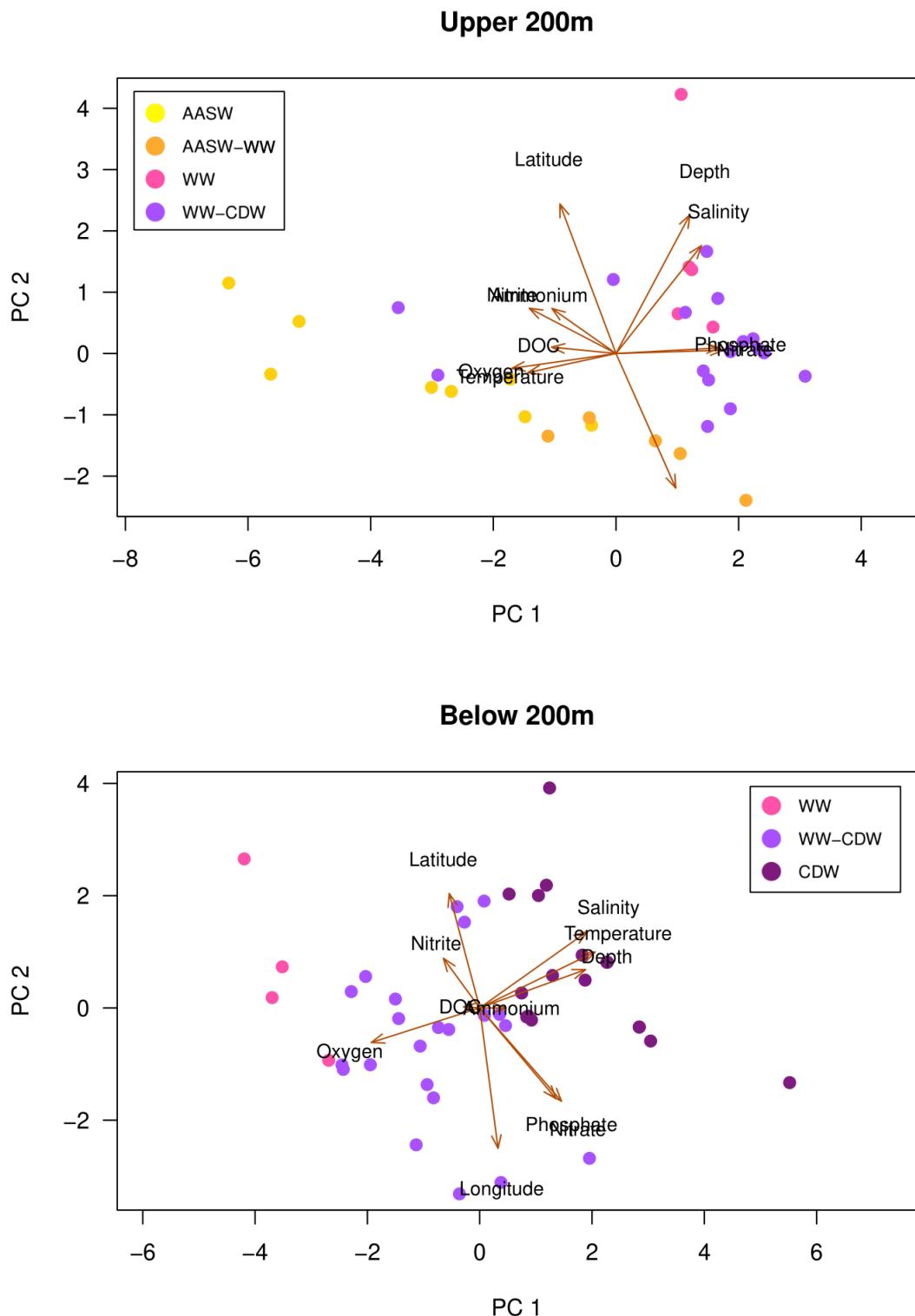


Figure S6. PCA of sample metadata by water mass in the ASP. Top PCA is surface-200m and bottom PCA is 200-bottom.

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	Family	Genus
Seq37	3	0	0	0	0	1	0.042	Flavobacteriales			Flavobacteriaceae Family
Seq955	1	0	0	0	0	0	0.031	Arcto-9FB-4 marine group	HF050_16O23		
Seq235	2	0	0	0	0	0	0.022	Planctomyctetes	HF050_16O23 Family		
Seq147	2	0	0	0	0	0	0.031	Optitales	uncultured 8	Pelagicoccus	
Seq485	2	0	0	0	0	0	0.028	uncultured	uncultured marine bacterium	Pedobacteriaceae Family	3
Seq768	2	0	0	0	0	0	0.022	Pedobacteriales	Candidatus Acilonanana	Pedobacteriaceae Family	
Seq961	3	0	0	0	0	0	0.022	Aeolimoniales	uncultured bacterium AD246-D7-1A	Aeolimonaceae	
Seq928	3	0	0	0	0	0	0.016	Victamicrobales	Oleospira	Victamicrobales	
Seq153	4	0	1	0	0	0	0.031	Oceanospirillales	Lentisphaeraea	Saccharospirillaceae	
Seq171	5	0	1	0	0	0	0.009	Lentisphaerales	HTCC2178	marine gamma proteobacterium HTCC2178 Family	
Seq234	5	0	1	0	0	0	0.022	OM182 clade	uncultured marine bacterium	uncultured marine bacterium	
Seq125	5	0	1	0	0	0	0.016	Calvibacteriales	Theobacaea	Theobacaea Family	
Seq134	6	0	0	1	0	0	0.014	Thiomicrobiales	uncultured bacterium HF50_4021	Thiomicrobiales	
Seq155	6	0	0	0	0	0	0.022	Planctomyctetes	MB100 marine group	uncultured	
Seq255	6	0	0	1	0	0	0.025	Optitales	MB100 marine group	Puniceococcaceae	
Seq529	6	0	0	1	0	0	0.022	Deltaproteobacteria	uncultured	uncultured	
Seq110	7	0	0	1	0	0	0.022	Flavobacteriales	Flavobacteriaceae	uncultured marine microorganism	
Seq386	7	0	0	1	0	0	0.031	SAR202 clade	Nitrospinae	uncultured marine microorganism Family 1	
Seq161	10	0	0	0	0	1	0.016	Nitrosopinales	Nitrospinae	Nitrospinae Family	
Seq192	10	0	0	0	0	1	0.022	Spiringtonomatales	Spiringtonomataceae	Spiringtonomataceae	
Seq444	10	0	0	0	0	1	0.034	Victamicrobales	uncultured	Victamicrobales	
Seq255	15	1	0	0	0	0	0.032	Cellvibrionales	Porticocaceae	uncultured family 1	
Seq123	16	0	0	1	0	1	0.026	SAR11 clade	Clade II	SAR11 clade	
Seq128	16	0	0	1	0	1	0.034	Nitrosopinales	Nitrosopinae	Nitrosopinae Family	
Seq132	16	0	0	1	0	1	0.012	Steroidobacteriales	Weissellaceae	Weissellaceae	
Seq144	16	0	0	0	0	1	0.026	Cytophagales	Cytophagaceae	Cytophagaceae	
Seq148	16	0	0	0	0	1	0.031	Rhodospirillales	AEGEAN-169 marine group	AEGEAN-169 marine group	
Seq156	16	0	0	1	0	1	0.025	Deltaproteobacteria	uncultured	Deltaproteobacteria	
Seq185	16	0	0	1	0	1	0.031	Micronichiales	Microthrixaceae	Microthrixaceae	
Seq19	16	0	0	1	0	1	0.044	Rhodospirillales	Illuminobacteriaceae	Illuminobacteriaceae Family	
Seq195	16	0	0	1	0	1	0.031	Magnipediales	Magnipediaceae	Magnipediaceae	
Seq199	16	0	0	1	0	1	0.022	SAR202 clade	hydrothermal vent metagenome	hydrothermal vent metagenome Family 3	
Seq260	16	0	0	0	0	1	0.023	SAR202 clade	uncultured Chloroflexi bacterium HF-4000_28F02	uncultured Chloroflexi bacterium HF-4000_28F02	
Seq34	16	0	0	0	0	1	0.031	SAR86 clade	hydrothermal vent metagenome	hydrothermal vent metagenome Family 1	
Seq38	16	0	0	0	0	1	0.009	SAR11 clade	Clade I	SAR11 clade	
Seq387	16	0	0	0	0	1	0.022	Opitales	Puniceococcaceae	Puniceococcaceae	
Seq389	16	0	0	0	0	1	0.034	Thiotrichales	Thiotrichaceae	Thiotrichaceae	
Seq563	16	0	0	0	0	1	0.022	SAR202 clade	Kilonellales	uncultured bacterium HF050_03M05	
Seq616	16	0	0	0	0	1	0.022	Kilonellales	Kilonellaceae	Kilonellaceae	
Seq167	26	1	0	0	0	1	0.039	Flavobacteriales	Flavobacteriaceae	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.

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	Pseudomonadaceae	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	Lentisphaeraceae	Lentisphaeraea
Seq188	5	0	1	0	0	0	0.045	Oceanospirillales	Profoundimonas
Seq215	5	0	1	0	0	0	0.033	Alteromonadaceae	Pseudalteromonadaceae
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	OM43 clade

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