

# MARINE MICROBIAL COMMUNITIES IN A RAPIDLY CHANGING ARCTIC

By

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## Abstract

Bacteria are essential to marine ecosystems as they remineralize organic matter, cycle carbon, nitrogen, and other key elements, and support higher trophic levels through food webs. The Bering and Chukchi Seas off the coast of Alaska are known as some of the most productive regions in the world, with intense seasonal algal blooms. However, little is known about bacterial communities in this region compared with other marine organisms. In addition, excessive heat transport from the Pacific Ocean leads to raised temperatures, sea ice retreat, and harmful algal bloom formation. Since the Bering Strait is the Pacific gateway to the Arctic Ocean, investigating how marine bacterial communities in this region are shaped by algal blooms and environmental change will lead to a better understanding of the potential for future regime shifts on the Arctic microbial ecosystem.

In this study, 259 samples were collected at 68 stations across the Bering and Chukchi Seas during the Arctic Shelf Growth, Advection, Respiration and Deposition Rate Experiments (ASGARD) cruise in June 2017, when sea surface temperatures in the region were anomalously warm. Each sample was collected from different depths (surface, middle, and bottom) and with two different filtration methods: direct filtration on 0.2  $\mu\text{m}$  filters at “survey” stations (Chapter 2) and sequential filtration on 20  $\mu\text{m}$ , 3  $\mu\text{m}$ , and 0.2  $\mu\text{m}$  filters at the “process” stations (Chapter 3). Amplicon sequencing of the bacterial and archaeal 16S small subunit ribosomal RNA gene was conducted, producing 3076 amplicon sequencing variants (ASVs) which were used as proxies for microbial species.

Hierarchical clustering of ASV relative abundance was used to explore microbial community structure in the two datasets: 11 clusters were investigated in the survey samples and 4 clusters in the process samples. All clusters were dominated by three major bacterial classes (Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia). Community structure was related to water mass, salinity, and bloom stage, but not to depth. The 11 survey clusters ranged from Gammaproteobacteria-dominant and Bacteroidia-poor assemblages in warm/cold Shelf Water (Division A clusters) to Bacteroidia-rich assemblages spanning pre- to post-bloom conditions (Divisions B and C clusters). In the four process clusters, communities separated into a free-living Anadyr Water cluster (FL1) and particle-associated clusters (PA2–4), several of which were enriched in Bacteroidia. Multivariate analysis with dbRDA identified temperature, salinity, DO,  $\text{NO}_3$  and fluorescence as environmental factors that differentiated bacterial communities in the survey samples, while salinity was the sole significant environmental factor distinguishing communities in the process samples.

The results are consistent with the microbial clusters being shaped by a combination of water-mass origin, physicochemical conditions, and bloom stage. These findings suggest that

changes in temperature and salinity, as well as shifts in the magnitude, timing and spanning of blooms, could substantially affect the bacterial communities of this region in the future.

Finally, since archaea are also important members of marine microbial communities, the ecology of Marine Group II (MGII) archaea in the Arctic Ocean was reviewed (Chapter 4). Understanding the distribution and ecological role of this representative marine archaeon complements the analyses of bacteria and will contribute to comprehensively understand the microbial ecosystem of the Arctic Ocean, including the Bering and Chukchi Seas.

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# Chapter 1. General Introduction and Method

## 1. Introduction

### 1.1 Importance of Microbes in the Marine Ecosystems

Oceans harbor a wide diversity of life, the majority of which are microorganisms. Microbial communities are essential to marine ecosystems as contributors to primary production and organic matter decomposition, and as drivers of biogeochemical cycles (Azam & Malfatti, 2007; Falkowski et al., 2008). Microbes channel carbon and energy to higher trophic levels through the “microbial loop” (Legendre & Le Fèvre, 1995) by remineralizing organic matter and rescuing it from the large, dissolved pool, in turn driving the cycling of key elements such as carbon, nitrogen, and sulfur (Jiao et al., 2010; Jørgensen et al., 2019; Nixon, 1981; Zehr & Ward, 2002). Through the microbial carbon pump, microbes transform labile organic matter into recalcitrant dissolved organic carbon that resists degradation and can be stored in the ocean over long timescales (Jiao et al., 2024).

### 1.2 General Description of the Arctic Ocean

Microbes have an important role in sustaining ecosystems in the Arctic Ocean, a geographically unique marine environment. The Arctic Ocean is seasonally covered with sea ice and though it accounts for only 3 % of the world’s oceans by area, it contains about 20 % of the world’s continental shelf ( $5.1 \times 10^6 \text{ km}^2$ ; Tomczak & Godfrey, 1994). The Arctic Ocean is mostly enclosed by land, and about half of its area is over continental shelves (Serreze et al., 2007a; Tomczak & Godfrey, 1994); thus, it is defined as a mediterranean sea, which has only limited connections with other global oceans and where ocean circulation is primarily driven by thermohaline circulation. Moreover, although the Arctic Ocean holds about only 1 % of the world's ocean volume, it is the only ocean whose watershed exceeds its own surface area, and it accounts for roughly 11 % of global riverine input (Carmack, 2007; McClelland et al., 2011). Along with river input, rapid coastal erosion driven by permafrost thaw is a major source of terrigenous inputs (Fritz et al., 2017). However, the Arctic Ocean is generally known as nitrogen-limited, which influences primary production in this region (Arrigo et al., 2024; Simpson et al., 2008). Primary production is mainly driven by eukaryotic algae, and this differentiates the Arctic Ocean from other oceans; cyanobacteria such as *Synechococcus* are rare in the Arctic (Huang et al., 2012; Jungblut et al., 2010; Pedrós-Alió et al., 2015).

### 1.3 Impact of Environmental Change on Microbial Communities in the Arctic Ocean

There is increasing global concern that the Arctic Ocean is changing because of human influences including global warming, contaminant deposition, and marine transportation. Sea-ice extent and thickness have decreased substantially in recent years (Rothrock et al., 2003; Stroeve et al., 2005; Stroeve et al., 2007) in a feedback cycle known as “Arctic amplification” caused by increased absorption of solar radiation in formerly ice-covered waters; the Arctic Ocean has warmed four times faster than other oceans (Rantanen et al., 2022; Serreze & Barry, 2011). The temperature rise is also driven by the advection of heat from the Pacific and Atlantic Oceans. On the Atlantic side, enhanced Atlantic water inflow has accelerated “Atlantification”, warming Arctic waters and causing a shift toward Atlantic-like hydrographic properties (Polyakov et al., 2020). On the Pacific side, heat is transported through Bering Strait and the amount of transported heat from the Pacific Ocean to Barrow Canyon has increased 50 % since 2000 (Itoh et al., 2026; Watanabe & Hasumi, 2009). In addition, increased carbon absorption causes Arctic Ocean acidification (Tehrar et al., 2020). Sea ice loss also causes freshening (McPhee et al., 1998) and alters water column stratification (Peralta-Ferriz & Woodgate, 2015; Polyakov et al., 2020), preventing vertical mixing (McLaughlin & Carmack, 2010; McPhee et al., 2009), inhibiting upward transport of nutrients into the euphotic zone and influencing the composition and primary productivity of pelagic microbial communities (Tremblay & Gagnon, 2009).

These environmental changes also influence microbial communities in the Arctic Ocean. Microbial communities (including bacteria, archaea, eukaryotes, and viruses) are susceptible to climate change, as reported after the extensive loss of multiyear ice (MYI) in 2007 and 2012 (Comeau et al., 2011; Kraemer et al., 2024). Moreover, net primary production increased by 57 % from 1998 to 2018 as primary producers accessed more sunlight due to sea ice retreat (Arrigo & van Dijken, 2015; Lewis et al., 2020). Increased primary production may seem beneficial at first glance, but it also has the risk of promoting harmful algal blooms (HABs). For instance, in summer 2022 a bloom of the toxic dinoflagellate *Alexandrium catenella* occurred in the Chukchi Sea that was exceptional in its spatial extent, intensity, and toxicity relative to previously recorded blooms (Fachon et al., 2025). These observations highlight microbial communities as first responders to rapid transformation of the Arctic Ocean, and thus, monitoring microbial communities and their responses is necessary to understand the ongoing environmental change happening there.

### 1.4 Importance of Studying Microbial Community in the Bering and Chukchi Seas

In this study, microbial communities in the Bering and Chukchi Seas were targeted as

this region is the gateway to the Arctic Ocean on the Pacific side and is known as “the Green Belt” because it is one of the most productive regions in the world (Grebmeier, 2012; Grebmeier et al., 2006; Springer et al., 1996). The continental shelf spanning the Bering and Chukchi Seas is shallow (about 50 m) and heat and nutrients are transported from Pacific inflows (Woodgate et al., 2005) and the upwelling and mixing induces large seasonal phytoplankton blooms.

The Bering Sea is located between the North Pacific Ocean and the Arctic Ocean and is generally ice-free from late June to late October, with sea ice extent typically greatest in March (Francis & Hunter, 2007; Mysak & Manak, 1989). However, annual variability is large and is linked to wind patterns (Niebauer, 1998; Sasaki & Minobe, 2005; Walsh & Sater, 1981). Changes in ice extent also affects the timing of the spring bloom. If ice remains after mid-March, the bloom forms early, next to the ice; if the ice is gone, the bloom usually forms in May, once the water stratifies (Hunt et al., 2002; Stabeno et al., 2001). In winter and early spring (November to April), the water column is generally well mixed due to convection and mixing due to winds and tides. On the other hand, the water is stratified due to winds and tidal mixing and surface heat fluxes (Kinder & Schumacher, 1981; Overland et al., 1999). As the mixed layer becomes shallower, phytoplankton can receive more light and lose less energy through respiration, triggering the spring bloom (Eslinger & Iverson, 2001; Sambrotto et al., 1986). In summer, phytoplankton run short of nutrients as the bloom depletes the nutrients near the surface. In late summer, a strong pycnocline restricts wind mixing in the water column, though occasional summer mixing brings up new nutrients and briefly boosts production (Sambrotto et al., 1986; Whitley et al., 1986).

The Chukchi Sea is a marginal sea between the Pacific and the Arctic Ocean and is known for its broadness ( $\approx 62,000 \text{ km}^2$ ) and shallowness ( $\approx 50 \text{ m}$ ). The Chukchi shelf is ice covered during winter; sea ice retreat begins in spring, and the shelf is largely ice-free by September (Ladd et al., 2016). In late summer, the water column is stratified by the formation of a pycnocline, resulting in a well-mixed surface layer overlying a bottom layer. Surface cooling, storms, and brine rejection can all weaken this stratification and cause vertical mixing during winter (Ladd et al., 2016). Heat is supplied by upwelling events from Atlantic waters, induced by easterly winds and shelf waves (Aagaard & Roach, 1990; Pickart et al., 2009, 2011). It can significantly affect the local surface heat budget (Aagaard & Roach, 1990; Hirano et al., 2016). While Atlantic water is typically observed near Barrow Canyon (Aagaard & Roach, 1990; Garrison & Paquette, 1982; Itoh et al., 2013; Mountain et al., 1976), rare events have been reported in which it penetrates more than 200 km onto the Chukchi shelf (Bourke & Paquette, 1976). Primary production in the Chukchi Sea is strongly controlled by light and nutrient availability, and nutrients are largely transported from the Pacific Ocean through Bering Strait. Nutrient-rich, cold and saline Pacific waters play an important role in sustaining under-ice

primary production during early spring (Arrigo et al., 2014; Lowry et al., 2014), and a spring phytoplankton bloom develops shortly after sea ice retreats. Net primary production in the Chukchi Sea has recently increased substantially, nearly doubling between 1998 and 2018. This increase has largely been attributed to enhanced inflow of Pacific water, which supplies additional nutrients to the region (Lewis et al., 2020).

Various water masses have been described in the Bering and Chukchi regions. Coachman et al. (1975) initially defined three different kinds of water currents and differentiated water masses into Alaskan Coastal Water (ACW, eastern Bering Strait), Bering Shelf Water (BSW, central Bering Strait) and Anadyr Water (AnW, western Bering Strait), using salinity boundaries that vary from year to year, and this definition became the standard nomenclature (Coachman et al., 1975; Springer et al., 1996; Walsh et al., 1989). Within the Chukchi Sea, AnW and BSW merge into a water mass named Bering Sea Water, which originated in AnW, found in the western Chukchi and is far richer in nutrients than the ACW in the eastern Chukchi (Walsh et al., 1989). Various water-mass classifications have been proposed (Danielson et al., 2017; Gong and Pickart, 2016; Lin et al., 2019; Pickart et al., 2019; Pisareva et al., 2015). Recently, Danielson et al. (2020) developed a detailed scheme that distinguishes warm and cool variants of coastal and shelf waters across seasons. They introduced a simplified, all-season water-mass classification for both shelves that splits coastal (wCW/cCW) and shelf (wSW/cSW) waters into warm/cool variants, instead of ACW/BSW categories (Coachman et al., 1975), which cannot capture the strong seasonal variability or the full distribution of water masses across these large shelf regions (at the cost of finer sub-class resolution).

### 1.5 Previous Analyses of the Arctic Marine Microbial Community

Previous studies have analyzed the distribution of phytoplankton (Arrigo et al., 2014; Hansell et al., 1989; Sigler et al., 2014) and phytoplankton-associated zooplankton (Brodeur et al., 1999; Hopcroft et al., 2010; Matsuno et al., 2016; Spear et al., 2019) in the Bering-Chukchi Seas, which hosts extensive spring phytoplankton blooms (Arrigo et al., 2012; Sigler et al., 2014). Bacterial communities in western Arctic seas have been investigated with clone libraries and fluorescence in situ hybridization (FISH) (Chen et al., 2008; Malmstrom et al., 2007; Zeng et al., 2012; Zeng et al., 2013a), amplicon sequencing (Han et al., 2014; Kirchman et al., 2010; Lee et al., 2019; McFarlin et al., 2017; Walker et al., 2023; Zeng et al., 2013b), denaturing gradient gel electrophoresis (DGGE) (Zeng et al., 2012), and flow cytometry (Connel et al., 2018). These studies illustrated that the bacterial communities in the Bering and Chukchi Seas are less diverse than those in warmer and temperate waters and are mainly dominated by Alphaproteobacteria, Gammaproteobacteria and Bacteroidia, which are known as responders to algal blooms, degrading complex algal substrates (Buchan et al., 2014; Bunse et al., 2017;

Needham & Fuhrman, 2016; Teeling et al., 2012; Teeling et al., 2016). Also, microbial community composition in this region is strongly structured by spatial and hydrographic context, varying with water mass, depth, and latitude. Most notably, these communities respond to sea-ice melt and to associated gradients in salinity, temperature, and nutrient concentrations.

However, those studies included relatively few samples, and mostly from surface waters. Despite its importance, bacterial communities in this region remain poorly understood compared to other marine organisms. Consequently, microbial distributional patterns, environmental and biological factors that determine community composition, and functional responses to algal blooms are still largely unknown. As the Bering and Chukchi Seas are the Pacific gateway to the Arctic Ocean, any environmental change happening in this region would propagate to and shape the future state of the Arctic Ocean. Microbial communities can be the first responder of environmental change as they are highly sensitive to such change and thus, understanding microbial community composition in this region is important to predict the impact of future environmental change on Arctic marine ecosystems.

## 1.6 Objectives of This Study

Despite its ecological importance and rapid environmental change, the bacterial community structure of the Bering and Chukchi Seas, and the factors that shape it, remain incompletely understood. Using 16S rRNA gene amplicon sequencing of 259 samples collected from different depths (surface, middle, and bottom) at 68 stations during the Arctic Shelf Growth, Advection, Respiration and Deposition Rate Experiments (ASGARD) 2017 cruise, this thesis aimed to clarify how bacterial communities in this region are structured, how they respond to a diatom bloom and to the physicochemical properties of different water masses, and how they may be affected by ongoing environmental change. Marine archaea were also considered, because they are an important part of Arctic marine microbial ecosystems. The thesis is based on two complementary sample sets and one literature review, and each chapter addresses a distinct objective.

Chapter 2 used the survey samples, collected with 0.2  $\mu\text{m}$  filters (the free-living fraction) across the full set of stations. This chapter aimed to (i) characterize the composition and spatial structure of bacterial communities in the Bering and Chukchi Seas, (ii) examine how the diatom bloom influenced community composition, (iii) identify relationships between the communities and physicochemical parameters of water masses from which they derived, and (iv) assess how physicochemical changes associated with ongoing warming may affect these communities.

Chapter 3 used the process samples, collected by sequential filtration (three size fractions: 20, 3, and 0.2  $\mu\text{m}$ ). In addition to the four aims of Chapter 2, applied here to the size-

fractionated samples, this chapter aimed to (v) compare free-living and particle-associated communities to determine whether the environmental drivers of change differ by fraction.

Chapter 4 is a review of Marine Group II archaea, one of the most representative marine archaea. This chapter aimed to summarize their ecology, distribution, and lifestyle in temperate and Arctic regions, and, on that basis, to discuss future directions for research on Marine Group II archaea in the Arctic Ocean and to show how this research could help us comprehensively understand the Arctic marine microbial ecosystem.

By linking bacterial community structures to bloom dynamics and to physicochemical properties of water masses, this study provides a baseline for understanding how the microbial communities in this region can respond to environmental change and highlight their potential as sentinels of change.

## 2. Materials and Methods

Sample processing and analysis methods that overlap between the two studies in Chapters 2 and 3 are provided here, with study-specific methods found in their respective chapters.

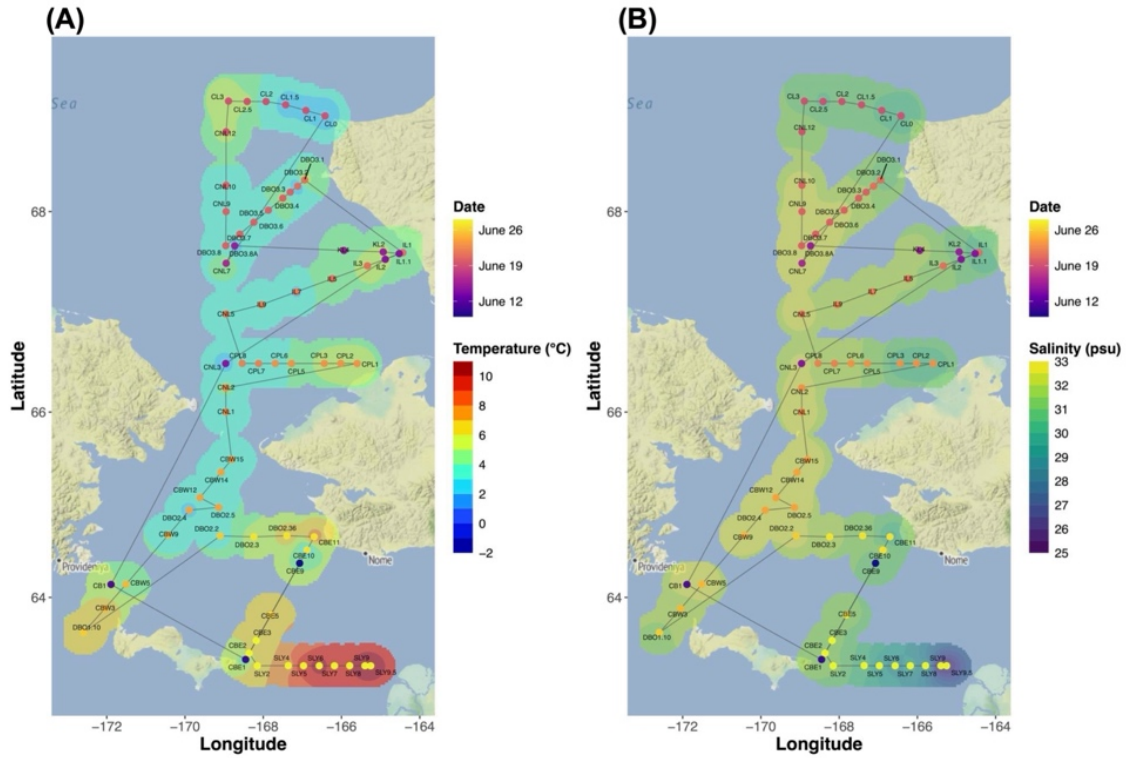
### 2.1 Sample collection

Seawater sampling was conducted during the ASGARD project from June 9–28, 2017 aboard *R/V Sikuliaq*. The ASGARD 2017 cruise visited 68 unique stations, many of which were associated with the Distributed Biological Observatory (DBO), including the DBO2 line in the northern Bering Sea and the DBO3 line in the southern Chukchi Sea (Figure 1). Samples were collected from surface, middle (pycnocline) and bottom depths between 2.5 m (surface) and 55 m (bottom), with modal depths per station at 5 m, 20 m, and 40 m. Seawater was sampled from Niskin bottles on a rosette with an attached Sea-Bird CTD (Sea-Bird Electronics Inc., Bellevue, WA, USA).

Samples were collected in two phases, here labeled “survey” and “process” stations. The survey stations (62 total) were relatively short stops where only the CTD rosette and zooplankton nets were deployed; process stations (12 total) were full-day stops, including four CTD casts conducted for sensor profiling, nutrient analysis, zooplankton incubations, and more. Some stations were visited during both phases. Figure 1 shows the sampling stations of ASGARD 2017 and the near-surface hydrography during the cruise obtained by the flow-through thermosalinograph. The average of near-surface temperature was 4.9 °C (range 1.1–10.9 °C), and salinity 31.6 (range 25.0–32.9). Six stations (CBE1, CBW5, CL1, CL3, DBO3.3, and IL) were sampled in both the survey and process phases. All geographic maps were produced in R with the ggmap package (Kahle & Wickham, 2013). Basemap data are © Stadia Maps, © Stamen Design, © OpenMapTiles and © OpenStreetMap contributors.

**Figure 1**

*Sampling Stations and Near-surface Hydrography During the ASGARD 2017 Cruise*



*Note.* Sampling stations and near-surface hydrography during the ASGARD 2017 cruise. All 68 unique stations visited across the Bering and Chukchi Seas are shown. Where a station was occupied more than once, the shallowest sample is plotted. Each station is colored by its sampling timing, is connected with the grey line in chronological order to indicate the cruise track, and is labeled by its station name based on pre-determined lines: SLY (St. Lawrence Island to Yukon River transect), CBE (Chirikov Basin East transect), CB (Chirikov Basin Transect), CBW (Chirikov Basin West transect), CPL (Cowpatch Line transect), DBO (Distributed Biological Observatory), CNL (Convention Line transect), IL (Isilivik Lagoon transect), KL (Kivalina Line transect), and CL (Cape Lisburne transect). The filled background shows the near-surface (A) temperature and (B) salinity, interpolated from the station values by inverse-distance weighting and masked to within 30 km of a station. Six common stations and one incubation were excluded from this map.

## 2.2 Sample filtration

Seawater samples were transferred from Niskin bottles to 20 L cubitainers and stored at 4 °C until filtration within six hours. Using a peristaltic pump, 1–5 L of seawater per depth

was filtered. The survey samples were directly filtered onto 0.2 µm-pore size Sterivex cartridge filters to collect microbes (Millipore Sigma, Burlington, MA, USA). Process samples were sequentially filtered through a 47 mm-diameter 20 µm-pore size nylon net filter, then a 47 mm-diameter 3 µm-pore size membrane filter, and finally through a 0.2 µm-pore size Sterivex cartridge filter (all filters from Millipore Sigma, Burlington, MA, USA). Filters were stored at -20 °C until processing. Samples for nutrient analysis were drawn from the CTD rosette immediately after recovery using 60 mL syringes, filtered through 0.45 µm Nuclepore filters, and stored frozen at -20 °C or colder until analysis on shore. Nutrients were measured colorimetrically on a segmented-flow autoanalyser following World Ocean Circulation Experiment protocols (Gordon et al., 1993), the chemistry of which is described in detail by Mordy et al. (2025). Nitrate was reduced to nitrite on a cadmium column and quantified as the resulting azo dye at 520 nm, with nitrate obtained by subtracting the nitrite measured on an unreduced sample stream. Nitrate (NO<sub>3</sub>), ammonium (NH<sub>4</sub>), phosphate (PO<sub>4</sub>), and silicate concentrations were provided by S. Danielson (University of Alaska Fairbanks, unpublished data).

### 2.3 DNA extraction, amplification, and sequencing

DNA was extracted from filters using the DNeasy PowerWater kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. PCR-amplification of 16S rRNA genes was used for analysis of prokaryotic communities. The KAPA HiFi HotStart PCR Kit (Kapa Biosystems, Wilmington, MA, USA) was used for PCR-amplification of 16S rRNA genes using the following conditions: one cycle at 98 °C for 1-min, 26 cycles at 98 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and one cycle at 72 °C for five minutes. Earth Microbiome Project primers (Thompson et al., 2017) were used to target the V4 hypervariable region of 16S SSU rRNA: forward primer 515F-revised (Parada et al., 2016) (5'-GTGYCAGCMGCCGCGGTAA-3') and reverse primer 806RB-revised (Apprill et al., 2015) (5'-GGACTACNVGGGTWTCTAAT-3'). Amplified DNA was dual-indexed using unique adapters (Glenn et al., 2016) before TruSeq library preparation and sequencing on an Illumina MiSeq in the DNA Core Lab in University of Alaska, Fairbanks (Fairbanks, USA).

### 2.4 Analysis of 16S rRNA gene sequencing

Sequences were demultiplexed and primers were removed with cutadapt v2.8 (Martin, 2014). The amplicon sequencing data was analyzed for amplicon sequence variants (ASVs) using DADA2 in R (Divisive Amplicon Denoising Algorithm 2; Callahan et al., 2016), performing quality control, error correction, merging, chimera checks and taxonomic

classification. The SILVA database v138.2 (Quast et al., 2012) was used to classify ASVs. The analyses described in the following sections were performed in R (version 4.5.2). All R scripts and outputs are publicly available at [https://github.com/ReiSHIBUE/ASGARD2017\\_analysis](https://github.com/ReiSHIBUE/ASGARD2017_analysis).

## 2.5 Hierarchical Clustering

For beta diversity analysis, Bray-Curtis dissimilarities were calculated using the *vegan* package in R (Oksanen et al., 2024). Samples were hierarchically clustered using Ward's minimum variance method and plotted with the R packages *vegan*, *fastcluster* (Müllner, 2013), and *gplots* (Warnes et al., 2009). Hierarchical clustering was conducted independently for each dataset. ASVs were normalized by the fourth-root-transformation. Bray-Curtis dissimilarities among samples were computed from the fourth-root-transformed ASV relative-abundance. Sample clusters were defined by cutting the dendrogram using `stats::cutree`, and labels were assigned hierarchically following the dendrogram topology.

Bubble plots were generated to visualize relative abundances of ASVs belonging to three major bacterial classes (Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia). For each sample, ASV relative abundances were summed to the genus level and averaged across the samples within each cluster, giving a mean relative abundance (%) per genus per cluster.

## 2.6 Water Mass Characterization

A temperature–salinity (T-S) diagram was constructed to characterize the water masses sampled and to visualize their relationship with the microbial community clusters. Potential density isopycnals were overlaid for reference, and water mass boundaries followed the classification of Danielson et al. (2020), reproduced in Table 1. All plots were produced with *ggplot2* in R (Wickham, 2016). Seawater density isolines were computed from the EOS-80 equation of state. The correlation between each cluster and water mass was assessed by Fisher's exact test (9,999 Monte Carlo replicates), while the significance of correlations between each cluster and depth was tested with a Kruskal-Wallis test.

Samples were assigned to the Bering or Chukchi by latitude (south or north of the Bering Strait at 65.7 °N, respectively), and potential density was calculated from temperature and salinity using the EOS-80 equation of state at surface pressure.

**Table 1**

*Water Mass Definitions in the Northern Bering and Chukchi Seas, Modified From Danielson et al. (2020)*

| Water mass                          | Abbreviation | Temperature range (°C) | Salinity range    |
|-------------------------------------|--------------|------------------------|-------------------|
| Anadyr Water                        | AnW          | $0 < T < 3$            | $32.5 < S < 33.8$ |
| Ice Melt Water & cool Coastal Water | IMW, cCW     | $-2 < T < 3$           | $22 < S < 30.8$   |
| cool Shelf Water                    | cSW          | $0 < T < 3$            | $30.8 < S < 32.5$ |
| warm Coastal Water                  | wCW          | $3 < T < 14$           | $18 < S < 30.8$   |
| Warm Shelf Water                    | wSW          | $3 < T < 14$           | $30.8 < S < 33.4$ |
| Modified Winter Water               | MWW          | $-1 < T < 0$           | $30.8 < S < 33.8$ |
| Winter Water                        | WW           | $-2 < T < -1$          | $30.8 < S < 35$   |

*Note.* Atlantic Water and Bering Basin Water (AtlW and BBW) are not included in this table because no samples in this study fell within their temperature–salinity ranges. Temperature is in °C and salinity is unitless (PSU).

## 2.7 Environmental Characterization of Sample Clusters

Boxplots were generated for each measured environmental variable (temperature, salinity, dissolved oxygen, nitrate, phosphate, ammonium, chlorophyll fluorescence, chlorophyll a, sampling depth, and latitude) to explore how environmental conditions varied among microbial community clusters. Each cluster was visualized with median, interquartile range, and overlaid individual sample points using ggplot2 in R.

To classify each microbial community cluster by its bloom stage, principal component analysis (PCA) was performed on nine standardized environmental variables (with  $\log(1+x)$ -transformation of right-skewed nutrient and fluorescence variables). PC1 was observed to capture the dominant gradient between pre- and post-bloom conditions, and was rescaled to the [0, 1] interval and interpreted as a “bloom index” (0 = pre-bloom, 1 = post-bloom). The index is derived from environmental data alone and uses no microbial information, so the assignment of bloom stages to clusters is independent of the clustering itself. The distribution of the bloom index across clusters was then used to assign a representative bloom stage to each cluster.

Parameter selection was conducted by calculating variance inflation factors (VIF) between each environmental parameter using the R package car (Fox & Weisberg, 2019) and GGally (Schloerke et al., 2024). Temperature (°C), salinity, depth (m), DO ( $\mu\text{mol/kg}$ ),  $\text{NO}_3$  ( $\mu\text{M}$ ) and FIECO-AFL (chlorophyll fluorescence,  $\text{mg m}^{-3}$ ) were selected for further analysis. A distance-based redundancy analysis (dbRDA) was performed, and ordinations were used to conduct ANOVA between environmental parameters and microbial ASVs using the vegan

package. dbRDA plots were generated using ggplot2. Bray–Curtis distances were calculated from the fourth-root-transformed ASV relative abundance matrices. Five parameters were used for this analysis (temperature, salinity, DO, NO<sub>3</sub>, and FIECO-AFL) and only samples with complete environmental data were analyzed. The significance of the overall model, the marginal contribution of each variable, and each constrained axis were tested by permutational ANOVA with 999 permutations and the constrained variance was reported as the adjusted R<sup>2</sup>. Ordinations were visualized with ggplot2, with the environmental variables displayed using stats::biplot.

ASV richness, defined as the number of ASVs present (relative abundance > 0) in the filtered clustering set, was compared across each depth using Kruskal–Wallis rank-sum tests. Tests were performed overall and separately within each community grouping, followed by Bonferroni correction for the number of within-group tests.

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## **Chapter 2. Prokaryotic Community Structures in the Bering and Chukchi Seas**

### **4. Introduction**

Microbes are the most abundant organisms in the global oceans, are essential in marine ecosystems, and include bacteria, archaea, algae, protists, fungi, and viruses. Marine organisms at higher trophic levels graze microbes and consume organic matter that is produced by microbes, and microbes contribute to marine nutrient cycling through the remineralization of organic matter into inorganic nutrients like nitrate and phosphate, which are then taken up by other organisms.

The Arctic Ocean is a habitat for many forms of charismatic fauna, but the fact that microbes are supporting their lives is often overlooked. To understand the role of microbes in the Arctic Ocean, studying their diversity, distribution, and function is necessary. The Arctic Ocean harbors unique marine ecosystems, and the loss of microbial diversity due to climate change is likely to cause shifts in microbial community structure, leading to the loss of unique microbial species and their influence on biogeochemical cycles and marine food webs in Arctic marine ecosystems (Comeau et al., 2011; Kraemer et al., 2024). Shifts in microbial community composition may also accompany the increased occurrence of harmful algal blooms in the region. In fact, massive harmful algal blooms (HABs) were recently observed in the Bering Strait (Fachon et al., 2025). HABs may also have disproportionate impacts on people in Indigenous communities who utilize the waters for subsistence livelihoods (Nolan, 2022). Therefore, studying microbial biodiversity is important because a loss of diversity could have unforeseen consequences, not only for the marine ecosystem itself but also for the communities that depend on it.

Microbial biogeography is the study of the distributions of microbes in time and space. Knowing the baseline microbial distributions at specific locations allows us to assess the effects of environmental change on microbial communities over time. For example, psychrophiles (cold-loving microbes) are abundant in the Arctic Ocean and can only survive in cold environments. If sea surface temperatures rise, the abundance of psychrophiles will likely decrease; monitoring their abundance will help us understand the effect of climate change on the Arctic microbial community. Also, since some psychrophiles in the Arctic Ocean are endemic to that region, their loss may act as a bioindicator of climate change and potentially provide forewarning of larger scale changes in ecosystem function. Microbial function includes the role of each microbe in biogeochemical cycling and marine food webs, including primary production, secondary production, and remineralization of inorganic species of carbon, nitrogen, phosphorous, and silicon. As mentioned above, microbes support marine organisms at higher trophic levels, so microbial compositional changes may influence the efficiency of food web functionality and lead

to changes in the entire ecology, for example by changes in the phenology (timing) of algal blooms. In addition, studying microbial function helps us understand the mechanisms by which microbes evolved and adapted their metabolism and physiology to the constraints present in the cold, dark (or light), Arctic Ocean.

Finally, studying microbial function will benefit researchers in other fields as well, for example biogeochemical modelers observe that microbes are essential drivers of Arctic biogeochemical cycling, and that microbial compositional change can impact those cycles, which are ultimately linked to climate change. By studying microbial functionality in the Arctic Ocean, we will help determine the relative importance of microbes in the Arctic marine ecosystem, and whether there are unique aspects of Arctic microbial communities there compared to other oceans. Due to global warming, the Arctic region is warming four times faster than other regions (Serreze & Barry, 2011). Also, Comeau et al. (2011) and Kraemar et al. (2024) reported that microbial community composition in the Arctic Ocean changed dramatically after the huge loss of multiyear ice (MYI) in 2007 and 2012. Along with global warming, seasonal sea ice melt can also change microbial compositions in the Arctic Ocean. Freshwater input from sea ice melt during spring can influence microbial communities in the Arctic Ocean (Campbell et al., 2018) and microbial food webs (Kellogg et al., 2019) in Arctic estuaries. Those results imply that climate change may change the component of microbial community which leads to the loss of microbial diversity, unique microbes, and their unique metabolism.

Studying microbes in the Arctic Ocean will help us understand the Arctic marine ecosystem and assess of the impact of climate change on the ecosystem. With metagenomic techniques, biogeography, metabolism, and diversity of Arctic marine microbes have been analyzed in the Arctic Ocean. However, compared with other oceans, we only have limited information about the Arctic marine microbial communities due to their relative inaccessibility. Therefore, in this study, we analyze the microbial community in the Bering and Chukchi Seas, which are at the entrance to the Arctic Ocean on the Pacific side and are known as areas with high productivity (Grebmeier, 2012; Grebmeier et al., 2006; Springer et al., 1996).

Here, using 16S rRNA gene amplicon sequencing of 181 samples collected at three depths (surface, middle, and bottom) across 62 stations during the Arctic Shelf Growth, Advection, Respiration and Deposition Rate Experiments (ASGARD) 2017 cruise, we aim to (i) characterize the composition and spatial structure of bacterial communities in the Bering and Chukchi Seas, (ii) examine how the diatom bloom influenced community composition, (iii) identify relationships between the communities and physicochemical parameters of water masses from which they derived, and (iv) assess how physicochemical changes associated with ongoing warming may affect these communities.

By linking community structure to bloom dynamics and to the physicochemical

properties of water masses, this study provides a baseline for understanding how microbial communities in this region respond to environmental change and evaluates their potential as sentinels of that change.

## 5. Methods

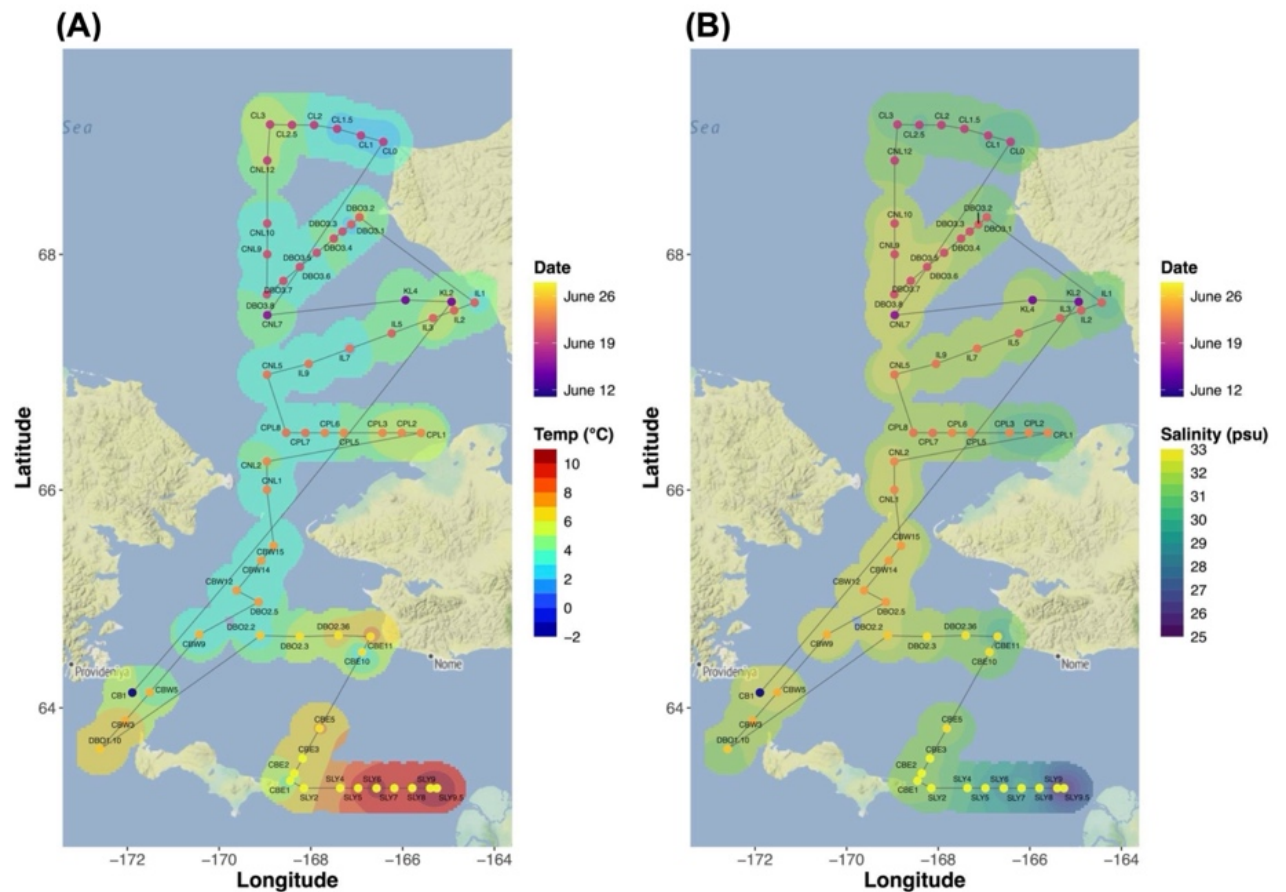
See section 2 for additional Materials and Methods.

### 5.1 Sample collection

Seawater sampling was conducted during the ASGARD project from June 9–28, 2017 aboard R/V Sikuliaq. The ASGARD 2017 cruise visited 62 “survey” stations (Figure 2), which were brief stops to collect water for nutrients, microbes, and conduct zooplankton tows.

**Figure 2**

*Survey Stations and Near-surface Hydrography During the ASGARD 2017 Cruise*



### 5.2 Sample filtration

Samples from the survey stations were directly filtered onto 0.2  $\mu$ m-pore size Sterivex cartridge filters to collect microbes (Millipore Sigma, Burlington, MA, USA).

### 5.3 Analysis of 16S rRNA gene sequencing

In total, 3076 prokaryotic amplicon sequence variants (ASVs) were obtained from 16S rRNA gene and samples with fewer than 1000 reads were excluded. Of the 3076 ASVs, those detected with  $\geq 2$  reads in each dataset numbered 442 in the survey. Read counts were converted to relative abundances within each sample and ASVs were retained only if their maximum relative abundance across samples exceeded 0.001 (0.1 %) and they occurred in two or more samples. This filtering yielded 258 ASVs from the 181 survey samples.

### 5.4 Hierarchical Clustering

For beta diversity analysis, Bray-Curtis dissimilarities were calculated from the fourth-root-transformed relative abundance matrix (258 ASVs in 181 survey samples) and hierarchically clustered using Ward's minimum variance method. Clusters were defined by cutting the dendrogram, and labels were assigned hierarchically following the dendrogram topology: each top-level branch was given a division-level name, and within each division the subsequent splits defined nested sub-clusters (e.g., division-level  $\rightarrow$  first split  $\rightarrow$  second split). For the survey dataset, the resulting dendrogram was cut at  $k = 10$  and the groups were separated into three divisions (A, B, C), yielding a final set of 11 clusters (A1, A2, B1, B2a, B2b, C1a, C1b1, C1b2, C2a, C2b1, and C2b2) following manual separation of C2b into C2b1 and C2b2.

For the further separation in Division C, a top-down tree walk was conducted and the dendrogram was interpreted against an environmental ordination. The nine environmental variables (temperature, salinity, DO, NO<sub>3</sub>, PO<sub>4</sub>, silicate, NH<sub>4</sub>, fluorescence and depth) were standardized and ordinated by principal component analysis (PCA), and the first nine components (PC1–PC9) were retained and interpreted from their loadings. Division C clusters were subdivided, and at each split of the dendrogram, the two descendant subclusters were compared along each PC separately: for every PC, a one-dimensional Euclidean distance was computed from the sample PC scores and tested by pairwise permutational multivariate analysis of variance (PERMANOVA) with 999 permutations, with permutational analysis of multivariate dispersions (PERMDISP) used to check homogeneity of dispersion. Significance of PERDISP was assessed by Tukey's honestly significant difference (HSD) test. Within each node, the p-values across the nine PCs were BH-adjusted, and the principal component(s) showing the most significant separation were regarded as the environmental axis driving that split.

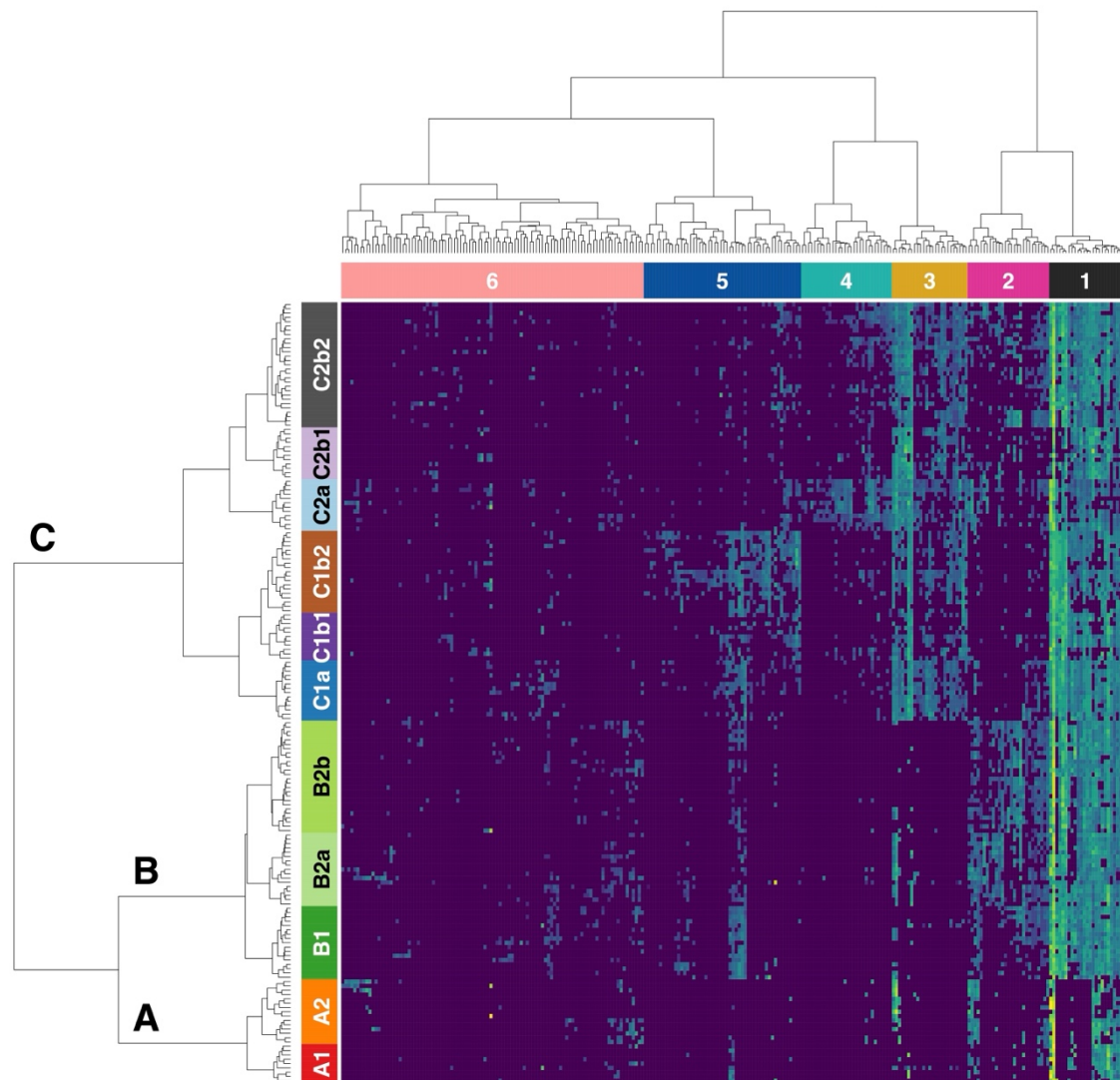
## 6. Results

### 6.1 Hierarchical Clustering

In total, 181 samples were assigned to three divisions (A, B, and C) and those were further categorized into 11 clusters based on the dendrogram (Figure 3): Cluster A1 (red; n = 9), Cluster A2 (orange; n = 15), Cluster B1 (dark green; n = 17), Cluster B2a (light green; n = 17), Cluster B2b (yellow-green; n = 26), Cluster C1a (dark blue; n = 14), Cluster C1b1 (purple; n = 11), Cluster C1b2 (brown; n = 19), Cluster C2a (light blue; n = 12), Cluster C2b1 (lavender; n = 12), and Cluster C2b2 (grey; n = 29). In addition, 258 filtered-ASVs were grouped into six Assemblages: Assemblage 1 (black; # of ASVs = 24), Assemblage 2 (pink; # of ASVs = 27), Assemblage 3 (yellow; # of ASVs = 25), Assemblage 4 (light blue; # of ASVs = 30), Assemblage 5 (dark blue; # of ASVs = 52), and Assemblage 6 (light red; # of ASVs = 100).

### Figure 3

*Heatmap of ASVs in the Survey Dataset*



*Note.* Hierarchical clustering was conducted based on relative abundance of 258 ASVs (columns), present in at least two of 181 samples (rows) with maximum relative abundances greater than or equal to 0.1 %. Samples were assigned to three primary divisions (A, B, and C) in 11 clusters based on the dendrogram (Cluster A1 to C2b2). ASVs were assigned to one of six assemblages (columns) based on the dendrogram.

Likewise, ASVs were assigned to one of six assemblages based on clustering (Figure 3). Mean relative abundance of each ASV was calculated, revealing that uncultured Nitrincolaceae, *Sulfitobacter*, SAR11 clade Ia, *Polaribacter*, *Ulvibacter*, and OM182 clade were highly abundant throughout all sample clusters and were mostly assigned to Assemblage 1. Therefore, we categorized them as the cosmopolitan ASVs.

Clusters A1 and A2 were distinguished by their low diversity and lack of numerous

ASVs from Assemblage 1 assigned to the Bacteroidetes, including *Polaribacter*, *Ulvibacter* (two ASVs), uncultured Cryomorphaceae, uncultured Flavobacteriaceae, *Tenacibaculum*, NS11-12 marine group and *Fluviicola*. A2 and B2a were differentiated from A1 and B2b respectively by showing high abundance of two SAR86 clade ASVs.

Division B clusters (B1, B2a, and B2b) were defined by ASVs in Assemblage 2, which were mostly dominated by Bacteroidetes such as *Polaribacter* (three ASVs), NS4 marine group (two ASVs), *Lutibacter* (two ASVs), *Lutimonas*, and uncultured Cryomorphaceae (two ASVs), representing particle-attached copiotrophs associated with phytoplankton blooms and Gammaproteobacteria categorized as OMG (Oligotrophic Marine Group) such as SAR92 (four ASVs), OM182 clade (two ASVs), and OM60/NOR5 clade.

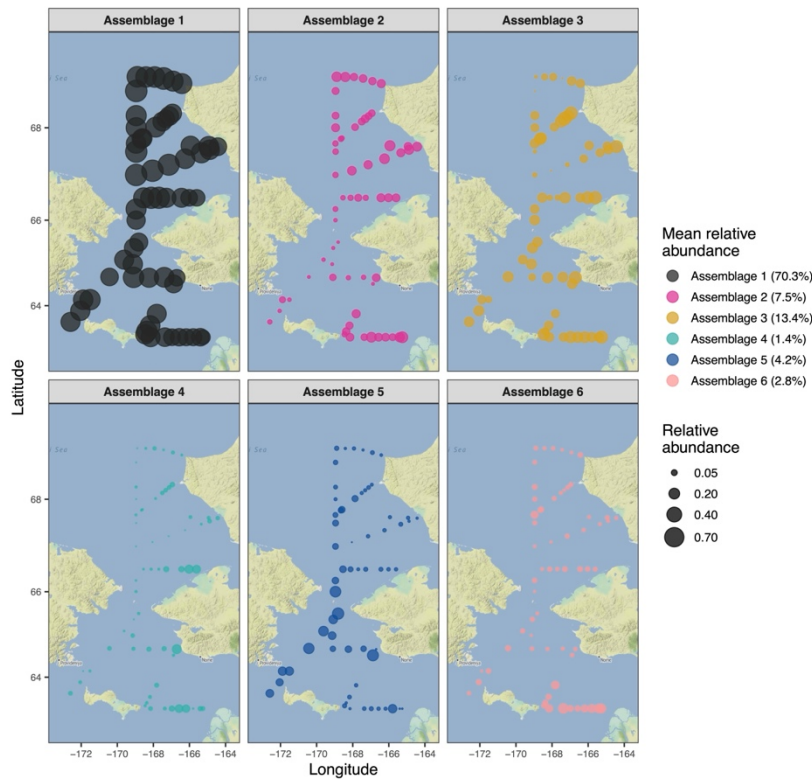
Division C clusters (C1a, C1b1, C1b2, C2a, C2b1 and C2b2) were characterized by ASVs in Assemblage 3, which included numerous Bacteroidetes ASVs (predominantly NS marine groups ASVs), OMG group (SAR86 clade and OM60/NOR5), methylotrophs (OM43 clade, *Methylothermus*, and Methylophagaceae) and other heterotrophic marine bacteria. C1 groups (C1a, C1b1, and C1b2) were distinct from C2 groups (C2a, C2b1, and C2b2) by taxonomically diverse ASVs in Assemblage 5, including *Colwellia*, Marine Group II archaea, and other ASVs with low bootstrap values, suggesting unreliability of taxonomic annotations in this column cluster. ASVs in Assemblage 5 also divided other clusters in Division C. For instance, Division B and C1 group shared some ASVs including sulfur-oxidizing bacteria ASVs (two Thiotrichales ASVs) and NS4 and NS9 marine groups ASVs. Also, C1b1 and C1b2 were separated by ASVs, comprised of various bacteria including Flavobacteriales ASVs (NS marine groups, uncultured Cryomorphaceae, and *Fluviicola*), chemolithoautotrophic bacteria (SUP05 cluster and SAR324), and oligotrophic clades (SAR86). C2b1 and C2b2 were also divided by ASVs such as sediment associated bacteria (*Illumatobacter* and *Urania-1B-19* group), *Colwellia*, SAR11 clade II, NS7 marine group, and *Alteromonas*. C2a and C2b1/C2b2 were divided by taxonomically distinct ASVs in Assemblage 4, including Flavobacteriales (NS5/7 marine groups and Cryomorphaceae), oligotrophs (SAR11 clade II/III/IV and AEGEAN-169), methyltroph (OM43 clade), chemolithotrophs (Nitrosomonadaceae, Thiotrichaceae, and Chromatiales), and Cyanobacteria (*Aterocyanobacterium* (UCYN-A)).

ASVs in each assemblage showed different biogeographic patterns (Figure 4) and community structures. ASVs in Assemblage 1 were highly abundant throughout the Bering and Chukchi Seas and its mean relative abundance was 70.3 %. Those ASVs exhibited a cosmopolitan-like distributional pattern and globally dominant taxa such as *Nitrospiraceae* and SAR11 clade Ia (Giovannoni, 2017; Thiele et al., 2022; Wietz et al., 2021) were highly dominant in this Assemblage. This constant distributional pattern in both seas may be attributed to the fact that both seas share the same Pacific-origin water. One study reported that microbial

communities above 44°N showed similar community structures (Milke et al., 2022). ASVs in Assemblage 2 and 5 showed different patterns. For example, Assemblage 5 ASVs showed positive correlation with NO<sub>3</sub> on distance-based redundancy analysis (dbRDA,  $F = 21.4$ ,  $p = 0.001$ , permutations = 999, adjusted  $R^2 = 0.37$ ) while Assemblage 2 ASVs showed negative correlations with it ( $F = 19.6$ ,  $p = 0.001$ , permutations = 999, adjusted  $R^2 = 0.35$ ). This difference can be attributed to whether abundant ASVs were found in the area where Anadyr Current flows. The origin of AnW is nutrient-rich water from deep (Coachman et al., 1975) and ASVs in Assemblage 5 were more abundant in that area. Also, Nitrosopumilus that is well-known ammonia oxidizer in deep water (Könneke et al., 2005), were relatively more abundant in this Assemblage. On the other hand, ASVs in Assemblage 2 were more abundant in the east which is influenced by Alaskan Coastal Current and Bering Shelf Current. Therefore, both assemblages showed different correlation with NO<sub>3</sub>. ASVs in Assemblage 4 were more abundant around the Alaskan coast. Those ASVs were abundant in Cluster C2a, that showed lower salinity than other clusters (Figure 8). Also, SAR11 clade III was relatively more abundant in this Assemblage and one of their subclades is known as freshwater bacteria (Laghdass et al., 2005). Those results suggest that ASV in this Assemblage may prefer the freshwater-influenced community.

#### **Figure 4**

*Sampling Sites of ASVs in Six Assemblages in the Survey Dataset*

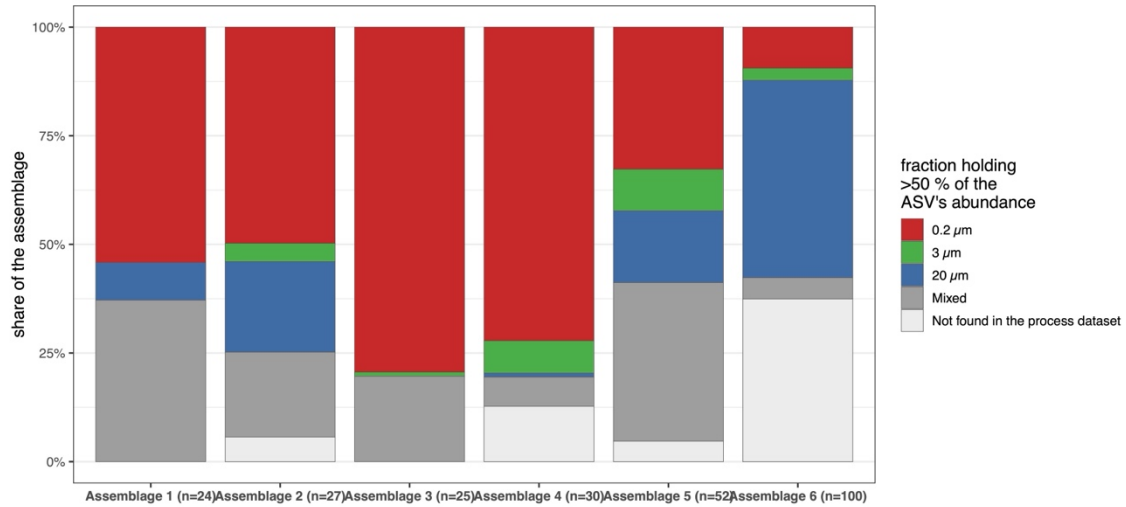


*Note.* Each Assemblage is assigned to different colors. The dots correspond to mean relative abundance of ASVs in six assemblages. Mean relative abundance of ASVs in each Assemblage is shown as legend.

ASVs in Assemblage 3 showed cosmopolitan-like distribution and showed positive correlations with salinity on dbRDA ( $p = 0.004$ ) but did not show specific pattern like assemblage 2 and 5. However, most of them were collected with 0.2  $\mu\text{m}$  filter, while ASVs in Assemblage 6 were mainly collected with 20  $\mu\text{m}$  filter (Figure 5). This result suggested that Assemblage 3 is largely composed of free-living microbes, while Assemblage 6 is mostly composed of particle-attached microbes. ASVs in Assemblage 3 separated Division C clusters and were mainly composed of Bacteroidia, OMG group, and methylotrophs. On the other hand, 100 ASVs were assigned to Assemblage 6, but they did not show specific abundance pattern on heatmap (Figure 3). This result implies that Assemblage 6 is a patchy group of ASVs from the rare biosphere and cannot be represented by any single dominant ASV.

## Figure 5

*Relative Abundance of ASVs in Six Assemblages With Each Filter in the Survey Dataset*



*Note.* ASVs whose relative abundance were greater than 50 % were assigned to each filter-sizes. Each filter is represented as different colors (red: 0.2  $\mu\text{m}$ , green: 3  $\mu\text{m}$ , blue: 20  $\mu\text{m}$ ). Gray color means that ASVs whose abundance is spread across the size fractions, with no single filter holding more than 50 % of the ASV's abundance. White color means that ASVs are absent from the process dataset.

Overall PERMANOVA indicated significant differences in community composition among the 11 clusters ( $R^2 = 0.587$ ,  $F = 24.13$ ,  $p < 0.001$ ), while PERMDISP also revealed significant heterogeneity in within-cluster dispersion ( $F = 7.65$ ,  $p < 0.001$ ). Since PERMANOVA is sensitive to both differences in group centroids and within-group dispersion, the co-significance of PERMDISP suggests that the PERMANOVA result may be partly influenced by unequal dispersions, particularly for pairs involving Cluster A2.

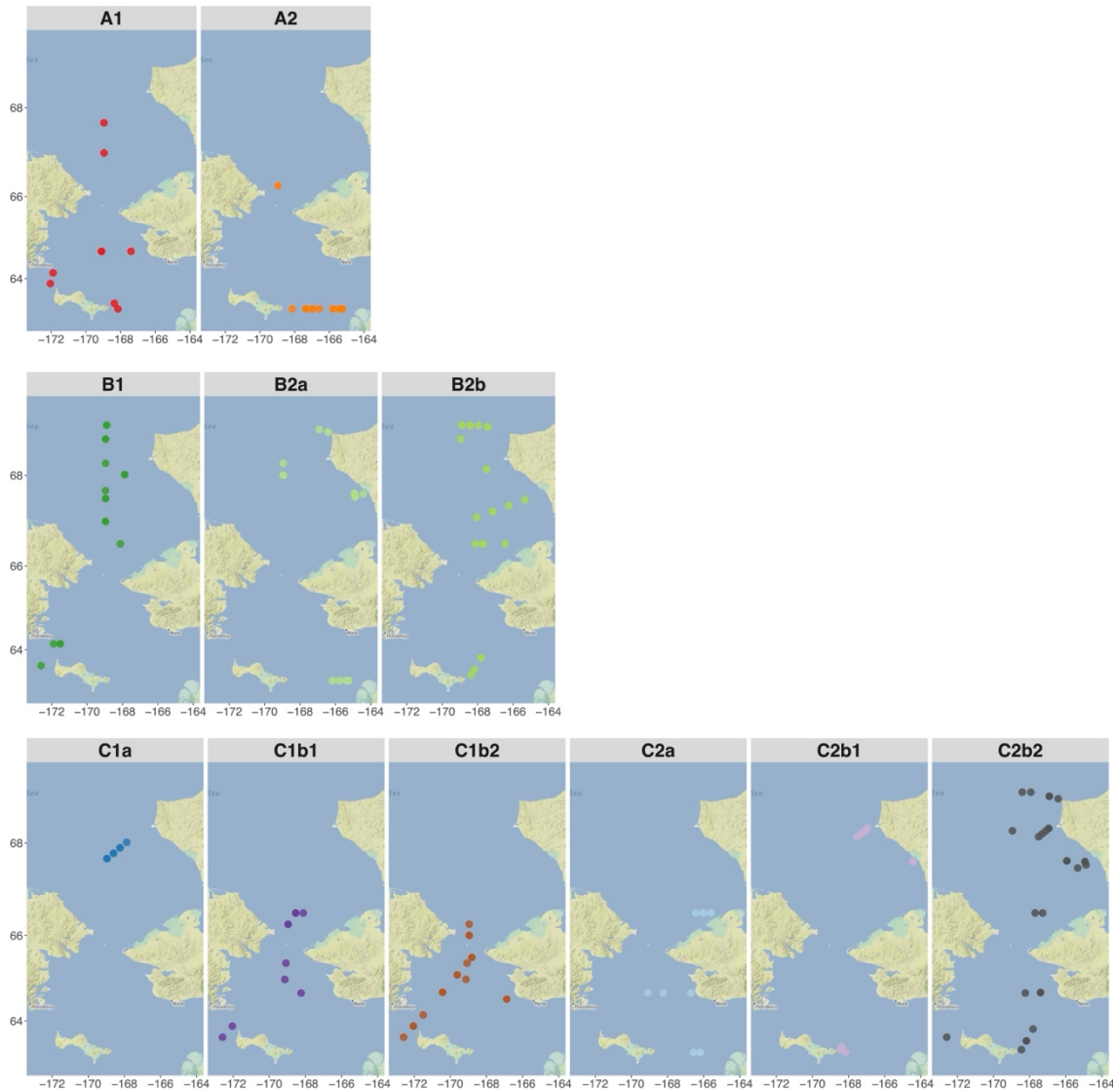
Therefore, pairwise PERMANOVA and PERMDISP were conducted to determine which specific cluster pairs differed in composition and which clusters were responsible for the dispersion heterogeneity. Pairwise PERMANOVA revealed that all cluster combinations had significantly distinct microbial communities (average  $R^2 = 0.4182$ , BH-adjusted  $p < 0.001$ ). On the other hand, pairwise PERMDISP was non-significant in the 55 pairwise comparisons not involving A2, indicating that PERMANOVA significance for these pairs can be attributed to true differences in community composition rather than dispersion. Cluster A2 was the sole source of this heterogeneity, exhibiting the highest mean distance to centroid (0.336) and significantly greater dispersion than other clusters except for Cluster A1 (Tukey's HSD,  $p < 0.05$ ), suggesting that A2 encompasses a broader range of microbial community compositions compared to other clusters. However, it should also be noted that A2 had the lowest median sequencing depth (5,470 reads) among the 11 clusters, which may have inflated Bray-Curtis dissimilarities and contributed to the elevated dispersion.

## 6.2 Geographic and Hydrographic Distribution of Sample Clusters

The geographic distribution of the 11 clusters revealed distinct spatial patterns (Figure 6). Division A clusters (A1 and A2) were largely confined to the southern portion of the study area. Especially, Cluster A2 was restricted to the stations in St. Lawrence to Yukon River Transect (SLY). Division B clusters were broadly distributed across the Bering and Chukchi regions and Cluster B2b spanned the widest geographic range from Cape Lisburne Transect (CL) to Chirikov Basin East Transect (CBE) (16 stations). Within Division C, Cluster C1a was the most geographically restricted cluster, occurring exclusively at DBO3 stations (four stations) near the northern Chukchi Sea. In contrast, Cluster C2b2 had the broadest distribution (21 stations), which like Cluster B2b spanned the entire study area from south to north. Clusters C1b1 and C1b2 were mainly concentrated in Chirikov Basin West (CBW) and DBO1–2 stations, while Cluster C2a and C2b1 were mostly found in CBE and Cowpatch Line Transect (CPL). No significant association was detected between microbial communities in each cluster and depth types (Fisher's exact test,  $p = 0.94$ ), indicating that the 11 clusters were not driven by sampling depth.

### **Figure 6**

*Maps of Sampling Stations for Each of the 11 Sample Clusters*



*Note.* Sampling stations of the eleven clusters are listed. Cluster A1 (red;  $n = 9$ ), Cluster A2 (orange;  $n = 15$ ), Cluster B1 (dark green;  $n = 17$ ), Cluster B2a (light green;  $n = 17$ ), Cluster B2b (yellow-green;  $n = 26$ ), Cluster C1a (dark blue;  $n = 14$ ), Cluster C1b1 (purple;  $n = 11$ ), Cluster C1b2 (brown;  $n = 19$ ), Cluster C2a (light blue;  $n = 12$ ), Cluster C2b1 (lavender;  $n = 12$ ), and Cluster C2b2 (grey;  $n = 29$ ).

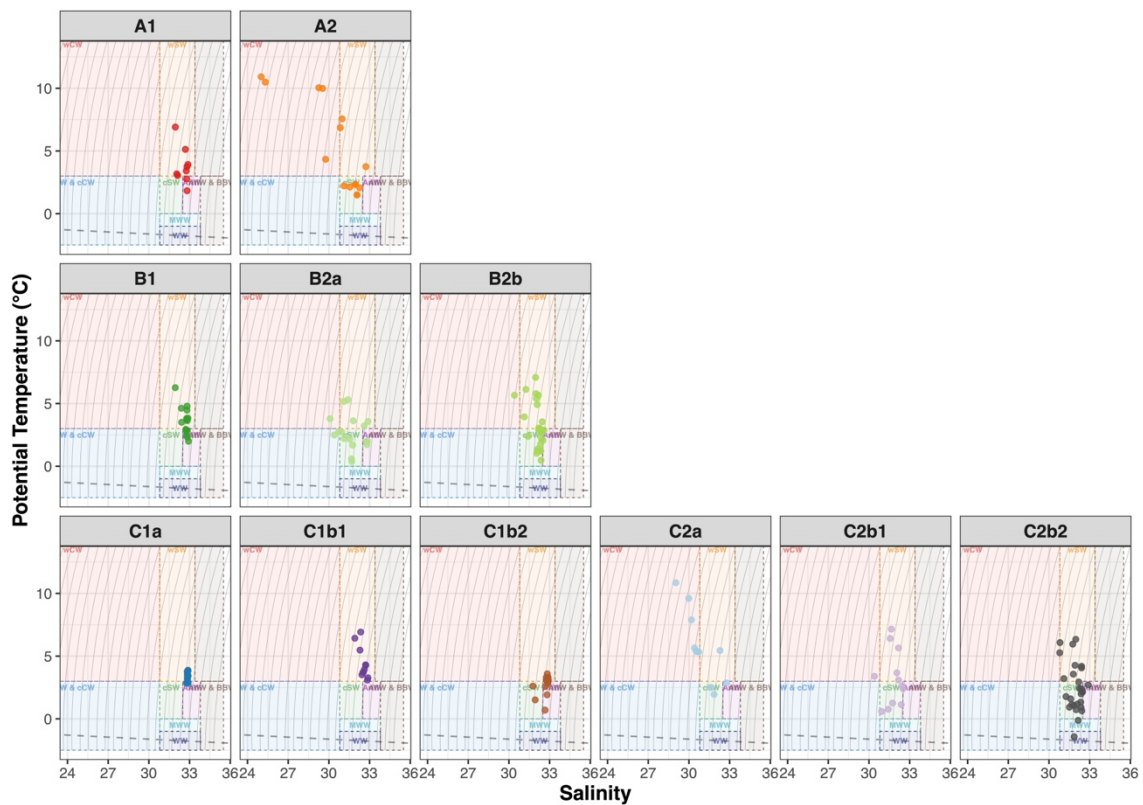
To examine whether the spatial distribution of the 11 clusters corresponded to distinct water masses, a T-S diagram of all survey samples was plotted (Figure 7), and each sample cluster overlaid on the water mass definitions from Danielson et al. (2020). The proportion of samples classified into each water mass was calculated for each cluster from the contingency table. Table 2 shows the water masses from which samples in each cluster were primarily and secondarily collected. The statistical significance of the association between clusters and water

masses was highly significant (Fisher's exact tests with simulated p-values, 9,999 Monte Carlo replicates,  $p < 0.001$ ).

Most samples in each cluster were found from warm Shelf Water (wSW,  $n = 81$ , 44.8 %), cool Shelf Water (cSW,  $n = 53$ , 29.3 %), and Anadyr Water (AnW,  $n = 26$ , 14.4 %). Cluster A1, C1a, and C1b2 were primarily found from wSW and secondarily from AnW. Cluster B1 was mainly sampled from the same water masses, but samples were collected in roughly equal proportions from wSW and AnW. Many samples in Cluster A2, B2a, B2b, C2b1, and C2b2 were found from shelf waters (cSW and wSW), while Cluster C2a was the only cluster which was mainly sampled from warm Coastal Water (wCW). Cluster A2 occupied the broadest T-S range (salinity 25.0 – 32.7, temperature 1.5 – 10.9 °C), spanning wCW and wSW, which may explain why the pairs involving this cluster did not show the statistical significance in pairwise PERMDISP (Tukey HSD  $p < 0.05$ ).

**Figure 7**

*Water Mass Characteristics of the 11 Sample Clusters*



*Note.* Water mass definitions were adapted from Danielson et al. (2020). T-S diagram with water mass boundaries was separated by the 11 clusters. Dashed rectangles indicate the T-S ranges of each water mass: warm Coastal Water (wCW), cool Coastal Water (cCW), Ice Melt Water (IMW), warm Shelf Water (wSW), cool Shelf Water (cSW), Anadyr Water (AnW), Modified Winter Water

(MWW), Winter Water (WW), Atlantic Water (AtlW), Bering Basin Water (BBW). Gray contours represent isopycnals (lines of equal density,  $\sigma_\theta$ , at 0.5 kg/m<sup>3</sup> intervals). The dashed black line indicates the freezing point.

**Table 2**

*The Proportion of Samples in the 11 Clusters, Classified Into Each Water Mass*

| Cluster                           | Primary water mass | Secondary water mass |
|-----------------------------------|--------------------|----------------------|
| A1                                | wSW (78 %)         | AnW (22 %)           |
| A2                                | cSW (47 %)         | wCW (33 %)           |
| B1                                | wSW (53 %)         | AnW (47 %)           |
| B2a                               | cSW (35 %)         | wSW (29 %)           |
| B2b                               | cSW (54 %)         | wSW (35 %)           |
| C1a                               | wSW (79 %)         | AnW (21 %)           |
| C1b1                              | wSW (100 %)        | —                    |
| C1b2                              | wSW (63 %)         | AnW (26 %)           |
| C2a                               | wCW (67 %)         | cSW (17 %)           |
| C2b1                              | cSW / wSW (42 %)   | —                    |
| C2b2                              | cSW (59 %)         | wSW (28 %)           |
| Fisher's exact test (Monte Carlo) |                    |                      |
| Replicates                        | <i>p</i>           | Significance         |
| B = 9,999                         | 0.0001             | ***                  |

*Note.* The primary water mass was defined as the water mass with the highest proportion of samples, and the secondary water mass as the one with the second highest proportion. Water-mass abbreviations are defined in Table 1. An em dash (—) indicates that no second water mass was present. \*\*\*  $p < 0.001$ .

### 6.3 Physicochemical Characterization of Sample Clusters

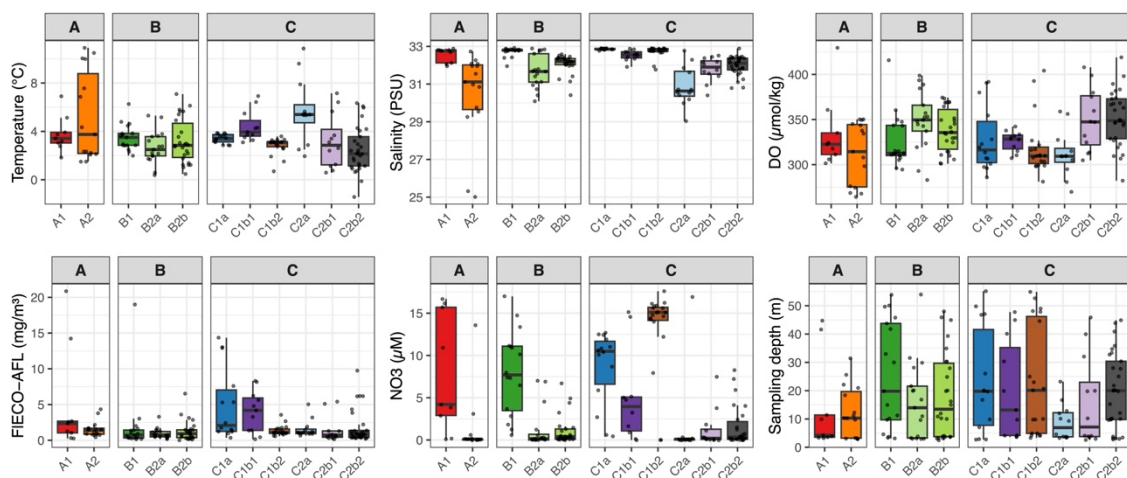
Physicochemical characteristics of the 11 clusters were shown in Figure 8. Clusters within Division A were sampled from relatively shallow depths (median: A1, 4.2 m; A2, 10.3 m), and A2 showed relatively low salinity (median: 31.1), reflecting the influence of wCW. Nutrient concentrations in Division A were generally low, with A2 showing near-complete

depletion of  $\text{NO}_3$  (median:  $0.1 \mu\text{M}$ ). Division B clusters exhibited contrasting nutrient conditions. Cluster B1 had high concentrations of  $\text{NO}_3$  (median:  $7.7 \mu\text{M}$ ), suggesting active organic matter remineralization by heterotrophic bacteria. In contrast, B2a and B2b were nutrient-depleted ( $\text{NO}_3$  median:  $0.15$  and  $0.4 \mu\text{M}$ , respectively), indicating post-bloom conditions.

Division C displayed the greatest environmental heterogeneity. Clusters C1b2 and C1a had the highest nutrient concentrations ( $\text{NO}_3$  median:  $15.1$  and  $10.5 \mu\text{M}$ , respectively), consistent with pre-bloom conditions where nutrients had not yet been consumed by phytoplankton. In contrast, Cluster C1b1 exhibited the highest FIECO-AFL (median:  $4.2 \text{ mg/m}^3$ ) values among all clusters, suggesting active phytoplankton bloom conditions. C2 subclusters (C2a, C2b1, and C2b2) were uniformly nutrient-depleted ( $\text{NO}_3$  median:  $0.1 - 0.4 \mu\text{M}$ ), which is the characteristic of post-bloom environments. Among these, C2a had the highest temperature (median:  $5.4^\circ\text{C}$ ) and the lowest salinity (median:  $30.6$ ), reflecting wCW influence (Table 2). On the other hand, C2b2 had the lowest temperature (median:  $2.1^\circ\text{C}$ ), consistent with its association with cSW and MWW (Table 2). Each cluster contains samples from all three depths. Therefore, some of the within-cluster variability shown here comes from vertical differences, not from differences between communities. This applies most to salinity and  $\text{NO}_3$ , which both change with depth. The clusters did not differ in how their samples were distributed among depths (Fisher's exact test,  $p = 0.94$ ), so comparisons between clusters should not be affected. Even so, showing each variable by cluster and by depth together would give a more complete picture, and we leave this for future work.

**Figure 8**

*Boxplots of Environmental Variables for the 11 Sample Clusters, Grouped by Primary Divisions (A, B, and C)*



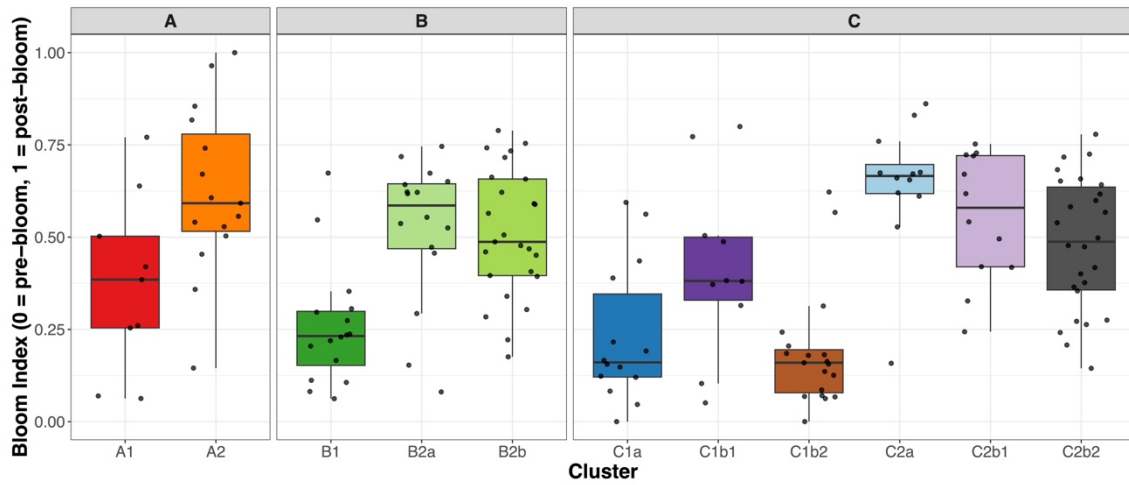
*Note* Each panel shows one of six variables: temperature (°C), salinity (PSU), DO (μmol/kg), FIECO-AFL (chlorophyll fluorescence, mg/m<sup>3</sup>), NO<sub>3</sub> (μM), and sampling depth (m). Individual samples were overlaid as jittered points. Boxes represent the interquartile range (IQR), with the horizontal line indicating the median. Whiskers extend to the most extreme values within 1.5 × IQR.

Nine environmental variables with near-complete data coverage ( $\leq 3.9$  % missing values; 174 out of 181 samples) were used for PCA: temperature, salinity, DO, NO<sub>3</sub>, PO<sub>4</sub>, Sil, NH<sub>4</sub>, FIECO-AFL, and sampling depth. Variables with higher proportions of missing data (e.g., chlorophyll a, 10.5 %; POC/PON, 35.4 %) were not included to maximize the number of samples retained in the analysis. PC1 (48.1 % of total variance explained) was interpreted as a “bloom index”, as it was primarily driven by positive loadings of nutrient concentrations (NO<sub>3</sub>, PO<sub>4</sub>, NH<sub>4</sub>, and Sil), salinity, and depth, reflecting the progression of phytoplankton bloom. High PC1 values indicated pre-bloom (nutrient-repleted) conditions, while low PC1 values indicated post-bloom (nutrient-depleted) conditions.

The bloom index, which is derived from environmental variables only, revealed distinct bloom stages within each division (Figure 9). Cluster A1 (median: 0.39) represented pre-bloom conditions since NO<sub>3</sub> concentration was higher than Cluster A2 (median: 0.59), which showed post-bloom conditions (Figure 8). Cluster B1 (median: 0.23) was the most pre-bloom cluster, with high residual nutrient concentrations, while Cluster B2a (median: 0.59) and Cluster B2b (median: 0.49) were in post-bloom stages. Division C exhibited the widest range of bloom stages: Cluster C1b2 (median: 0.16) and Cluster C1a (median: 0.16) were the most pre-bloom clusters among all 11 clusters, while Cluster C2a (median: 0.67), Cluster C2b1 (median: 0.66), and Cluster C2b2 (median: 0.49) were in post-bloom stages. Cluster C1b1 (median: 0.38) was categorized into the pre-bloom cluster, it can also be represented as during-bloom cluster since it showed highest median of chlorophyll a concentration (8.03 μg/l) and higher median of NO<sub>3</sub> (3.95 μM) than other Division C post-bloom clusters (C2a, C2b1, and C2b2).

## **Figure 9**

*Bloom Index (Rescaled PC1) for the 11 Sample Clusters, Grouped by Primary Divisions (A, B, and C)*



*Note.* The bloom index was derived from PC1 of a PCA on nine environmental variables, rescaled from 0 (pre-bloom, nutrient-replete) to 1 (post-bloom, nutrient-depleted). Boxes represent the interquartile range with the median shown as a horizontal line. Individual samples are overlaid as jittered points.

Since Division C ( $n = 97$ ) was the most environmentally heterogeneous division, encompassing the full range of bloom stages from pre-bloom (C1a, C1b1, C1b2), and post-bloom (C2a, C2b1, and C2b2) and spanning six water masses (wSW, cSW, AnW, wCW, MWW, and WW), pairwise PERMANOVA was conducted on each PC axis separately, with p-values corrected for multiple testing within each node (Benjamini-Hochberg correction across 9 PC axes; Figure 10). The primary split between C1 and C2 was best explained by PC1 ( $R^2 = 0.34$ ,  $p = 0.009$ ), representing the nutrient gradient (the bloom index). The separation between C1a and C1b was mainly associated with PC6 ( $R^2 = 0.24$ ,  $p = 0.027$ ), reflecting the  $\text{NH}_4$ –salinity–depth gradient, although PC6 explained only 3.2 % of the total environmental variance. The differentiation of C1b1 from C1b2 was most strongly associated with PC5 ( $R^2 = 0.70$ ,  $p = 0.023$ ), which represents a silicate–salinity–temperature gradient, though PC5 accounted for only 5.5 % of the total variance. Although the splits of C1a vs C1b and C1b1 vs C1b2 were explained by PC axes with relatively low explained variance (PC6, 3.2 %; PC5, 5.5 %), it suggested that the finer-scale subdivisions within Division C were driven by relatively minor environmental gradients not captured by the dominant PC axes. The split of C2a from C2b was driven by PC2 ( $R^2 = 0.32$ ,  $p = 0.014$ ), the DO–temperature gradient. No PC axis significantly differentiated C2b1 from C2b2 (all BH-adjusted  $p > 0.88$ ), suggesting that this split was driven by differences in microbial community composition, which was not captured by the measured environmental variables.

**Figure 10**

*Results of pairwise PERMANOVA on Individual Primary Component (PC) Axes for Each Hierarchical Split Within Division C*

| Dendrogram node | PC1 (48.1%)<br>Nutrients+, Sal+, Depth+ | PC2 (28.7%)<br>DO+ vs Temp- | PC3 (12.2%)<br>FIECO- | PC4 (7.2%)<br>Depth- vs Sal+ | PC5 (5.5%)<br>Sil+ vs Sal- | PC6 (3.2%)<br>NH4+ vs Sal- | PC7 (2.0%)<br>DO-, Temp- | PC8 (0.7%)<br>PO4+ vs Sil- | PC9 (0.5%)<br>NO3+ vs PO4- |
|-----------------|-----------------------------------------|-----------------------------|-----------------------|------------------------------|----------------------------|----------------------------|--------------------------|----------------------------|----------------------------|
| C1 vs C2        | R <sup>2</sup> =0.34                    | R <sup>2</sup> =0.07        | R <sup>2</sup> =0.06  | ns                           | ns                         | ns                         | R <sup>2</sup> =0.06     | ns                         | ns                         |
| C1a vs C1b      | ns                                      | ns                          | ns                    | ns                           | R <sup>2</sup> =0.19       | R <sup>2</sup> =0.24       | R <sup>2</sup> =0.17     | ns                         | ns                         |
| C1b1 vs C1b2    | ns                                      | ns                          | ns                    | ns                           | R <sup>2</sup> =0.70       | ns                         | ns                       | ns                         | ns                         |
| C2a vs C2b      | ns                                      | R <sup>2</sup> =0.32        | ns                    | ns                           | ns                         | ns                         | ns                       | ns                         | R <sup>2</sup> =0.29       |
| C2b1 vs C2b2    | ns                                      | ns                          | ns                    | ns                           | ns                         | ns                         | ns                       | ns                         | ns                         |

ns Significant Top PC

*Note.* Pairwise PERMANOVA was conducted with 999 permutations. P-values were adjusted within each node using the Benjamini-Hochberg method. Each cell represents the PERMANOVA result for a given dendrogram node (rows) and PC axis (columns). The dominant variable loadings for each PC axis are shown alongside, with (+) and (−) indicating the direction of each variable's contribution. Dark blue cells indicate the top PC axis for each node, defined as the axis with the highest R<sup>2</sup> among those with significant PERMANOVA (BH-adjusted  $p < 0.05$ ) and non-significant PERMDISP ( $p > 0.05$ ). Light blue cells indicate other significant PC axes. Gray cells indicate non-significant results (ns). R<sup>2</sup> values and significance levels are shown within each significant cell (Significance levels: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).

A dbRDA was conducted to examine the correlations between environmental variables and microbial community composition across the 11 sample clusters (Figure 11). Variance inflation factors (VIF) revealed strong multicollinearity among nutrients (NO<sub>3</sub>, VIF = 31.3; PO<sub>4</sub>, VIF = 22.5; Sil, VIF = 15.7). Variables PO<sub>4</sub>, Sil, NH<sub>4</sub>, and sampling depth were sequentially removed to reduce multicollinearity, resulting in a final set of five variables (temperature, salinity, DO, NO<sub>3</sub>, and FIECO-AFL; all VIF < 3). These variables were scaled to zero mean and unit variance prior to analysis. Seven samples with missing environmental data were excluded, resulting in 174 samples used in this model.

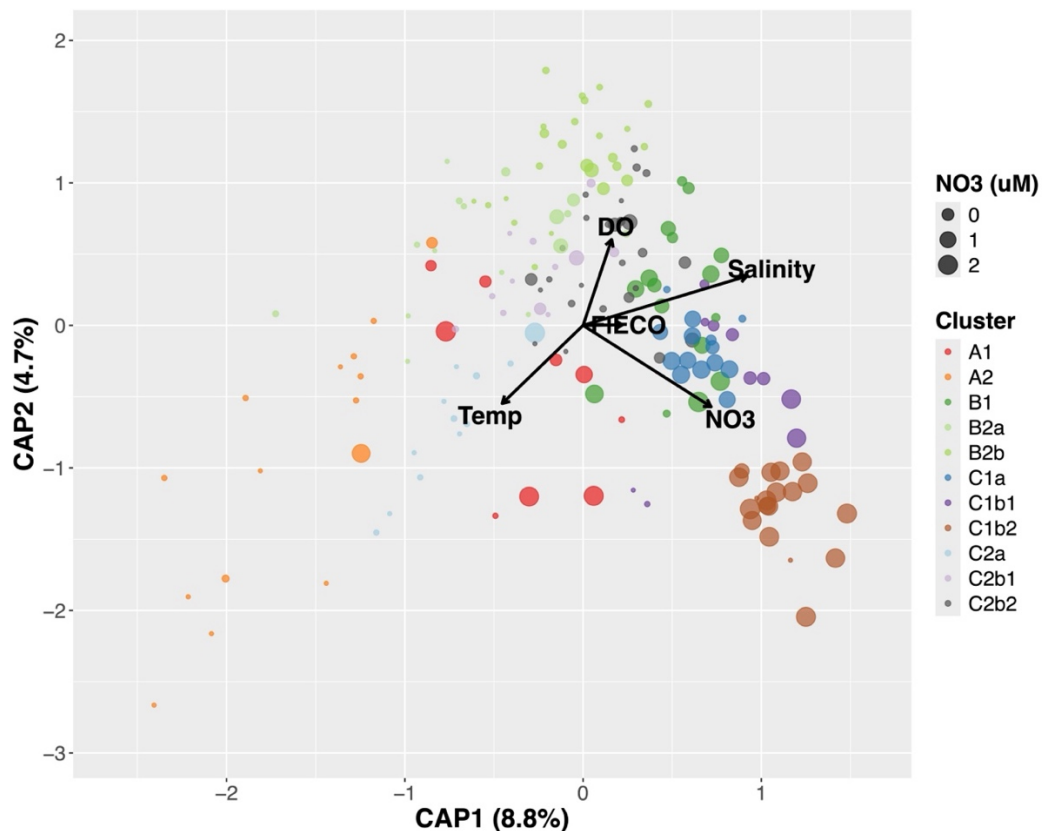
The dbRDA model was statistically significant ( $F = 7.00$ ,  $p = 0.001$ , permutations = 999), with the five environmental variables explaining 14.8 % of the total variation in community composition (adjusted R<sup>2</sup> = 0.148). All five variables significantly contributed to the model and salinity ( $F = 8.44$ ,  $p = 0.001$ ) and NO<sub>3</sub> (−0.57) and temperature (−0.55).

On the CAP ordination, pre-bloom clusters (C1b2, C1a, and B1) were positioned toward high CAP1 values, driven by high salinity and NO<sub>3</sub>, while wCW-influenced post-bloom clusters (A2 and C2a) were positioned at low CAP1 and low CAP2, driven by high temperature and low salinity. Post-bloom clusters in cSW (B2b, C2b2, B2a, and C2b1) occupied high CAP2

positions, associated with high DO. Division A clusters were mostly differentiated by temperature and salinity, while Division B clusters were by DO and salinity. Within Division C, C1 clusters were associated with NO<sub>3</sub> and fluorescence, while C2 clusters were differentiated by DO and temperature.

**Figure 11**

*Distance-Based Redundancy Analysis (dbRDA) of the 11 Clusters Constrained by Five Environmental Variables*



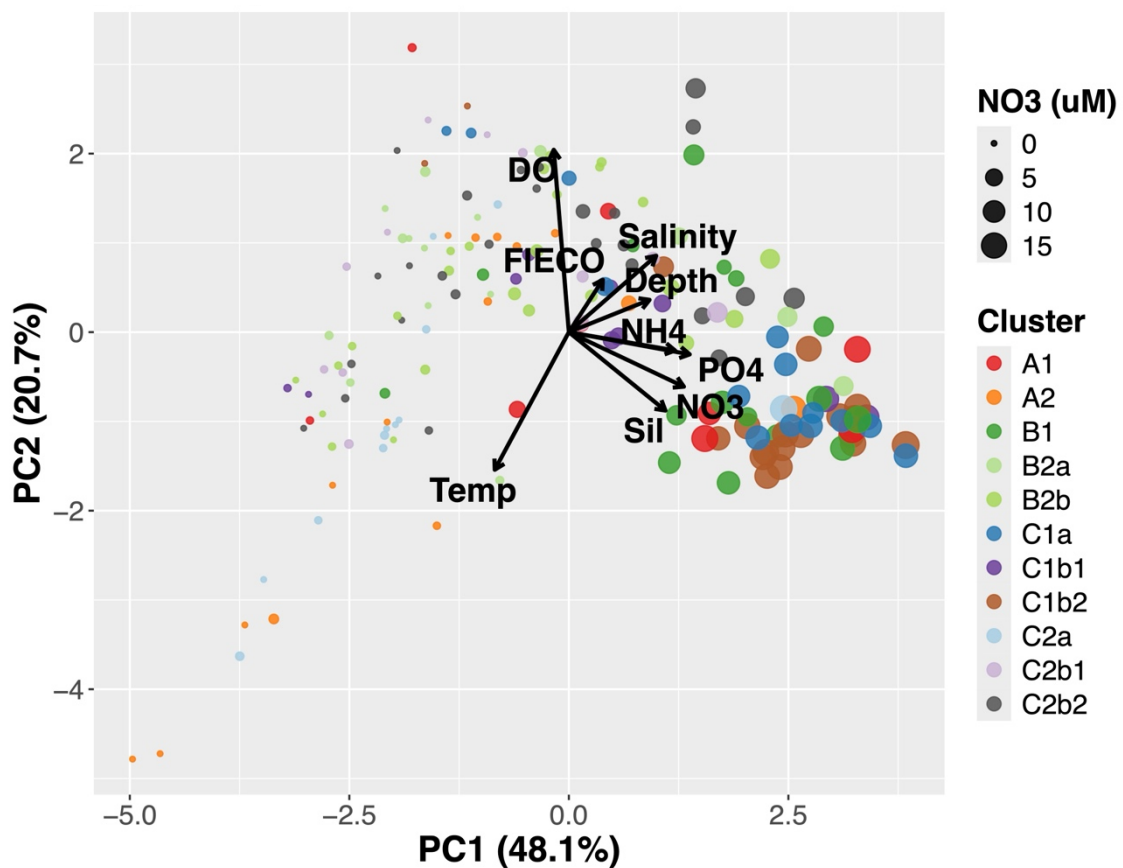
*Note.* Temperature, salinity, DO, NO<sub>3</sub>, and FIECO-AFL were used in the dbRDA. Samples are plotted on the first two canonical axes (CAP1, 8.8 %; CAP2, 4.7 %), colored by the 11 sample clusters, with dot size scaled to NO<sub>3</sub> concentration (μM). Arrows indicate the direction and relative strength of each environmental variable's contribution to the constrained ordination.

A principal component analysis of the nine standardised environmental variables ( $n = 174$  samples) resolved a single dominant axis of variation (Figure 12). PC1 explained 48.1 % and PC2 20.7 % of the total variance (68.8 % cumulative). PC1 was defined by a contrast between the inorganic nutrients, salinity and depth on its positive side (PO<sub>4</sub>, loading 0.46; NO<sub>3</sub>, 0.44; NH<sub>4</sub>, 0.40; silicate, 0.37; salinity, 0.33; depth, 0.31) and temperature on its negative side (−0.28),

describing a gradient from warm, fresher, nutrient-poor surface/coastal waters to cold, saline, nutrient-rich, deeper waters. PC2 was mainly driven by dissolved oxygen (0.68) opposed to temperature ( $-0.51$ ). Samples were distributed as a continuous curved shape along PC1 rather than as discrete groups. The samples curve into an arch rather than falling into separate groups. This arch is a well-known effect that appears when the data are shaped by one strong gradient, so PC2 mostly reflects a curve of that same gradient rather than a truly separate axis. The 11 clusters line up in order along PC1. Division A and B clusters sit at the low-to-middle end (warmer, fresher, lower-nutrient water) and Division C clusters at the high end (cold, salty, high-nutrient, deep water). In other words, the clusters are not sharply separate communities but different points along one continuous environmental gradient.

**Figure 12**

*PCA Biplots of Nine Environmental Variables in the Survey Dataset*



*Note.* Principal component analysis (PCA) of the environmental data in the survey dataset is shown. Nine variables (temperature, salinity, dissolved oxygen, NO<sub>3</sub>, PO<sub>4</sub>, silicate, NH<sub>4</sub>, fluorescence and depth) were used, with nutrients and fluorescence log-transformed and all variables standardized prior to PCA. Each point is a sample, positioned by its PC1 and PC2,

colored by sample clusters, and sized by nitrate concentration ( $\mu\text{M}$ ). Black arrows show the loadings of the environmental variables. Axes are labelled with the percentage of variance explained (PC1 48.1 %, PC2 20.7 %,  $n = 174$ ).

## 7. Discussion

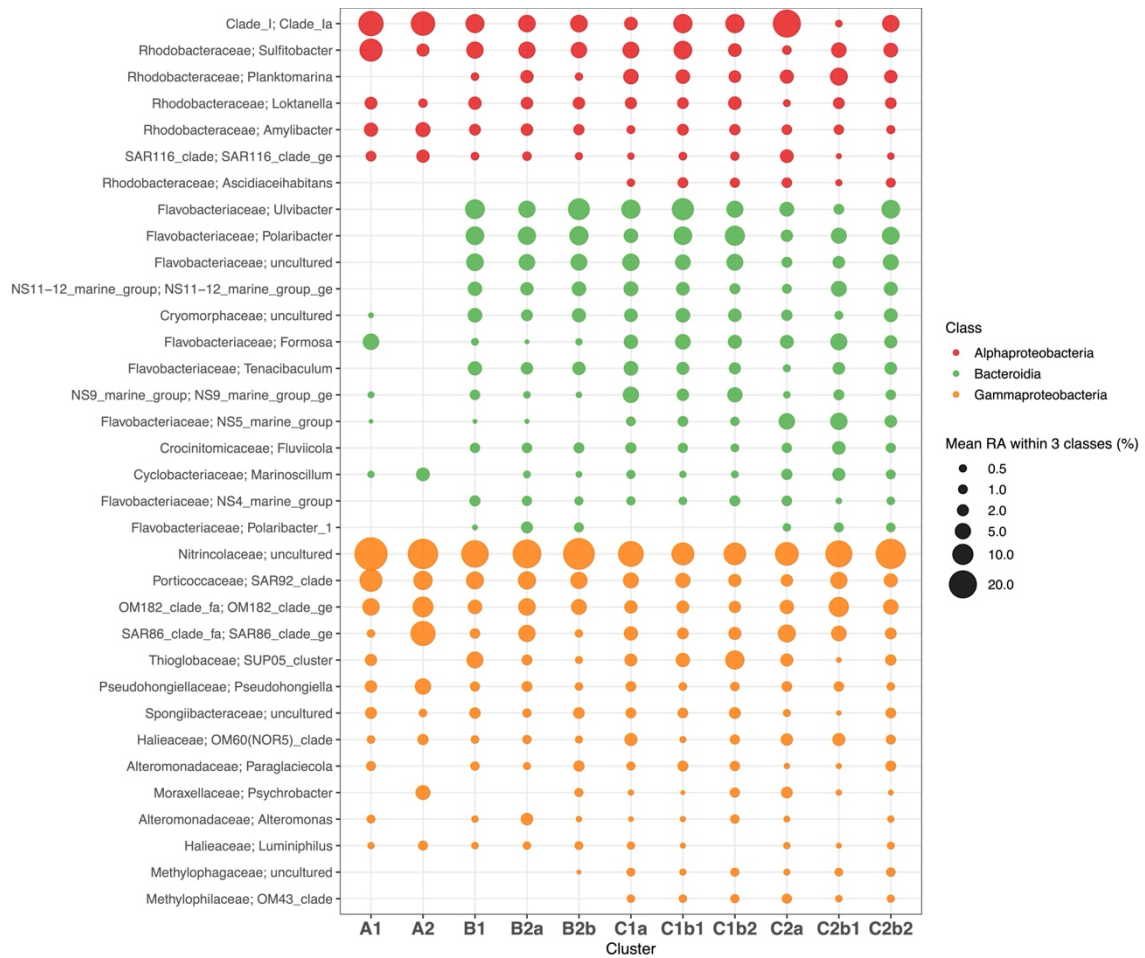
### 7.1 Ecological Characterization of the 11 Clusters and Comparison With Prior Studies

Hierarchical clustering revealed that the surveyed samples harbored distinct microbial communities. Most of ASVs were classified to three major classes: Bacteroidia, Alphaproteobacteria or Gammaproteobacteria (Figure 13); among each are cosmopolitan free-living taxa and representatives known to respond to algal blooms and contribute to the degradation of algal substrates (Buchan et al., 2014; Bunse et al., 2017; Needham & Fuhrman, 2016; Teeling et al., 2012; Teeling et al., 2016).

Within the Alphaproteobacteria, ASVs classified as SAR11 clade Ia and SAR116 clade, and Rhodobacteriaceae ASVs such as *Sulfitobacter*, *Amylibacter*, *Planktomarina*, and *Loktanella* were found; these taxa are known to respond to phytoplankton blooms and decomposing organic matter (Bergen et al., 2016; Buchan et al., 2014; Hahnke et al., 2015; Nelson et al., 2012; Sidhu et al., 2023; Takebe et al., 2024; Teeling et al., 2012). Among Gammaproteobacteria, ASVs classified as uncultured Nitrospiraceae, SAR92, OM182, SAR86, SUP05 cluster, and OM60 (NOR5) were abundant, which were also known as degraders of organic matters and association with algal blooms (Cho & Giovannoni, 2004; Dupont et al., 2012; Francis et al., 2021; Morris & Spietz, 2022; Redondo-Río et al., 2024; Ruff et al., 2014; Sakami et al., 2016; Sidhu et al., 2023; Thiele et al., 2022; Wietz et al., 2021; Xue et al., 2021; Yang et al., 2015). For Bacteroidetes, ASVs classified as *Polaribacter*, *Ulvibacter*, uncultured Cryomorphaceae, uncultured Flavobacteriaceae, *Formosa*, *Fluviicola*, *Lutibacter*, *Lutimonas*, and NS marine groups (NS4, 5, 9, 10, and 11-12) were highly abundant; they are representative particle-attached and algae-associated bacteria, contributing to the degradation of algal substrates (Avcı et al., 2020; Bowman, 2020; Choi & Cho, 2006; Ko et al., 2025; Liu et al., 2019; Mann et al., 2013; Meziti et al., 2015; Ngugi & Stingl., 2018; O'Sullivan et al., 2005; Priest et al., 2022; Priest et al., 2023; Thiele et al., 2022; Wietz et al., 2021).

### Figure 13

*Bubble Plot of Major Genera Within Three Bloom-Responsive Bacterial Classes (Bacteroidia, Gammaproteobacteria, Alphaproteobacteria) Across the 11 Clusters*



*Note.* Rows correspond to genera, columns to sample clusters. Bubble size represents the mean relative abundance (%) of the genus in each cluster among all taxa in the plot, and color represents class. Displayed genera satisfy two objective criteria: occurrence in > 10 % of samples ( $n = 181$ ) and cumulative within-class relative abundance  $\leq 95$  % ( $n = 34$  genera: 7 Alphaproteobacteria, 13 Bacteroidia, 14 Gammaproteobacteria).

Division A clusters lacked some Bacteroidia ASVs including the eight ASVs missing in Assemblage 1 (Figure 3); two *Ulvibacter* ASVs and one *Polaribacter*, *Fluviicola*, uncultured Cryomorphaceae, Tenacibaculum, NS11-12 marine group, and uncultured Flavobacteriaceae. Those ASVs mostly belonged to Bacteroidetes and were highly abundant across samples, and are particularly abundant in sea ice, suggesting recently melted ice may have played a role in structuring communities in the other Divisions. Also, from the boxplots (Figure 8), unique physicochemical characteristics were not found in Cluster 1 except for the high variance of temperature. However, this might be the reason for the lack of those ASVs as temperature can be the main factor for bacterial abundance (Korvelic et al., 2022), but further investigation is required to answer this question.

In the survey data, total relative abundance of ASVs which belong to Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia accounted for 94.7 %, which is consistent with previous studies conducted in this region (Chen et al., 2008; Lee et al., 2019; Zeng et al., 2012; Zeng et al., 2013a). Remarkably, relative abundance of Gammaproteobacteria ASVs was higher (42.7 %) than Alphaproteobacteria (20.4 %) and Bacteroidia (31.6 %). Bacteroidetes are known for their associations with algal blooms and their contribution to decomposing high molecular weight compounds produced by phytoplankton (Needham & Fuhrman, 2016; Needham et al., 2018). It is also known that the succession of Proteobacteria and Bacteroidetes is often triggered by algal blooms (Bunse & Pinhassi, 2017), and thus, our results were consistent with previous findings, implying the evidence of its association with algal blooms.

Remarkably, Cluster A1 and A2 showed low relative abundance of Bacteroidia (5.5 and 2.9 %, respectively), and this can be ascribed to the factors that caused the eight microorganisms to be missing from these clusters shown in hierarchical analysis (Figure 3). Cluster A1 and C2a showed high abundance of Alphaproteobacteria (34 and 33.1 %, respectively), implying that warm temperature might trigger the abundance of Alphaproteobacteria, as both clusters were largely influenced by wCW and wSW respectively.

Cluster C2a showed high abundance of SAR11 clade Ia, which is known as one of the most abundant bacteria in oligotrophic marine environment (Giovannoni, 2017), and this can match with the fact that Cluster C2a was the only cluster influenced by nutrient-scarce wCW. Cluster A1 showed high concentration of  $\text{NO}_3$  (Figure 8), so this is not consistent with the possible reason of the abundance of Alphaproteobacteria in Cluster C2a. However, since the mean relative abundance of Bacteroidia ASVs were substantially low in Cluster A1, the proportion of Proteobacteria became extremely high, and thus, we need to interpret this result carefully.

Some pre-bloom clusters such as Cluster C1a and C1b1 showed high abundance of Bacteroidia (40.3 and 40.2 %, respectively), and those clusters were mainly influenced by wSW. However, C1a also showed relatively high abundance of Gammaproteobacteria along with other pre-bloom clusters such as B1 and C1b2. Those three clusters showed balanced proportion of Gammaproteobacteria to Bacteroidia (the difference between the proportions of the two was within 3 %). On the other hand, some post-bloom clusters such as Cluster B2a, B2b, C2b1, and C2b2 showed high abundance of Gammaproteobacteria (46.6, 42.9, 43.3, and 41.7 %, respectively). Clusters B2b and C2b2 were influenced by cSW, while Clusters B2a and C2b1 were in the mixed water mass conditions but were more relatively closely associated with the cSW (Table 2).

Previous studies have been surveyed microbial community composition in the Arctic

Ocean. For example, a pyrosequencing study conducted in the Chukchi Sea, the Beaufort Sea, and Franklin Bay, reported that Gammaproteobacteria and Alphaproteobacteria shared 22-30 % of all sequences, and the two most abundant taxa (at 97 % similarity) were SAR11 and an uncultured Bacteroidetes (Kirchman et al., 2010). Ghiglione et al (2012) conducted amplicon sequencing in both Arctic and Antarctic waters, including the Chukchi Sea and found that most taxa in both polar oceans belonged to Gammaproteobacteria, Alphaproteobacteria, and Bacteroidia and several taxa such as SAR11, SAR324, known for their ubiquity and having versatile metabolisms (Malfertheiner et al., 2022). Also, *Polaribacter*, one of the representative bloom-associated bacteria, was the Arctic-specific taxa, underscoring the importance of investigating the link between phytoplankton dynamics and Bacteroidetes succession in the Arctic Ocean. Pedrós-Alió et al. (2015) also reported that microbial communities in temperate regions were dominated by Alphaproteobacteria (approximately 50 %), while microbial communities in the Arctic Ocean contained nearly equal proportions of Alphaproteobacteria and Gammaproteobacteria (approximately 20 % each). Also, the proportion of Bacteroidetes in the Arctic Ocean is higher than the global average (18 % vs. 4 %) (Pedrós-Alió et al., 2015).

Metagenomic analyses also reported the dominance of Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes in the Arctic Ocean. For example, Cao et al. (2020) analyzed metagenomes from the poles to temperate region and revealed that 90 taxa in phylum level, which was classified from 24,504 taxa, mostly belonged to Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes showing their high relative abundance in surface and deep-water samples from the Arctic Ocean. Royo-Llonch et al. (2021) analyzed 530 MAGs, which were reconstructed from the Arctic Ocean, and found that more than half of MAGS belonged to Gammaproteobacteria (22.8 %), Bacteroidota (20.9 %), Alphaproteobacteria (11.5 %), and the most abundant 12 MAGs were from unknown species in those three class/phylum and other phyla such as Actinobacteria and SAR324.

The association between bacteria and algal blooms has been studied from temperate regions to the Arctic Ocean. In the North Sea, SAR11 clade taxa were abundant in pre-algal bloom communities, Bacteroidetes such as *Formosa*, *Polaribacter*, and *Ulvibacter* were abundant in during-algal bloom communities, and Gammaproteobacteria such as SAR92 clade dominated post-algal bloom communities (Liu et al., 2013; Sintès et al., 2013; Teeling et al., 2012; Teeling et al., 2016). Those studies were conducted in temperate regions and not in the Arctic Ocean, but our result is consistent with their findings. The study from Franklin Bay found that Bacteroidetes and Alphaproteobacteria taxa such as *Roseobacter* (one of the most abundant marine bacteria and an aerobic anoxygenic photosynthesizer; Brinkhoff et al., 2008), showed the highest abundance in the chlorophyll-a maximum, while Gammaproteobacteria became abundant below the chlorophyll-a maximum (Alonso-Sáez et al., 2008). Microbial

community analysis through the northern Barents Sea into the east of Svalbard found that during spring and summer, when sea ice algal bloom occurs, Gammaproteobacteria ASVs such as SAR92, OM60, and Nitrospiraceae and Bacteroidetes ASVs such as *Formosa*, *Polaribacter*, and NS9 marine group became abundant, while SAR11 and some nitrifiers dominated winter samples (Thiele et al., 2023). The metagenomic time-series study, which was conducted in Dease Strait, revealed that 80 % of reads were classified into Alphaproteobacteria (33.2 %), Gammaproteobacteria (28.7 %), and Bacteroidia (21.6 %) (Redondo-Río et al., 2024). Remarkably, Alphaproteobacteria was dominant in the pre-bloom community, while Bacteroidetes increased their abundance in the during-bloom community. Gammaproteobacteria also increased alongside the bloom, though they started to decrease in relative abundance as Bacteroidetes exponentially increased.

In summary, we found that Bacteroidia was relatively more abundant in wSW-influenced pre-bloom clusters, while Gammaproteobacteria was more dominant in cSW-influenced post-bloom clusters. For Alphaproteobacteria, we can assume that they were more abundant in oligotrophic environment, excluding the atypical Cluster A2. Previous studies also illustrated the dynamics of Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes in the Arctic marine bacterial community responding to phytoplankton blooms and are consistent with the results of our study, suggesting that blooms can be a key driver shaping the seasonal succession of specific bacterial taxa in the Arctic marine ecosystem.

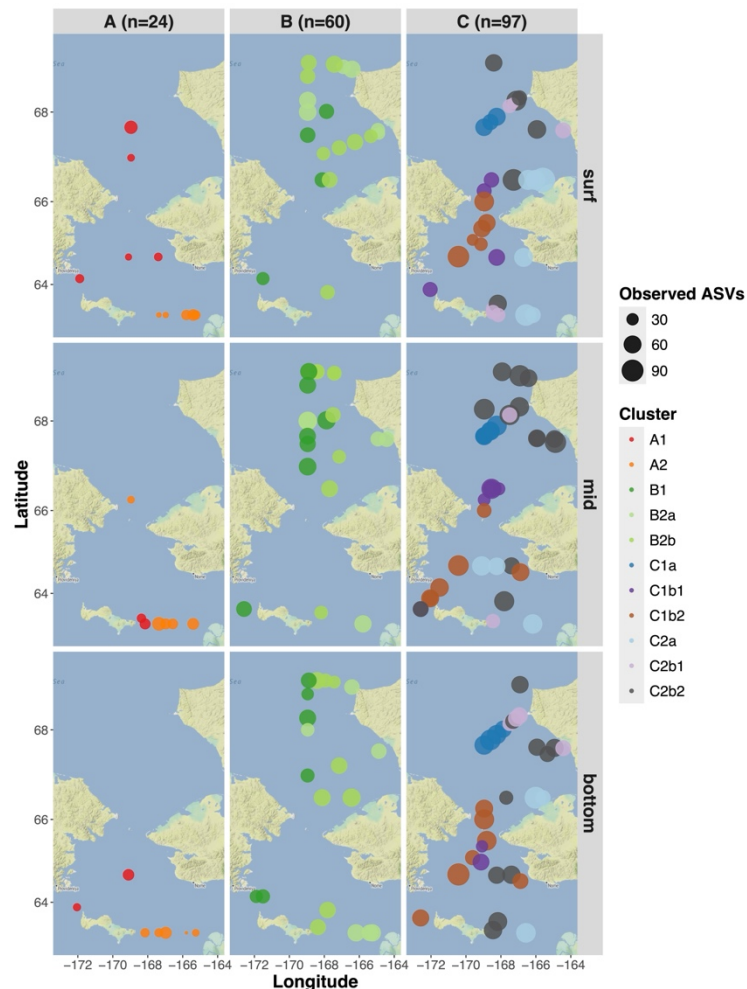
## 7.2 Microbial Community Difference in Depth

In this study, 181 samples were collected from Bering Sea ( $n = 72$ ) and Chukchi Sea ( $n = 109$ ) from different depths: surface ( $n = 24$ ), middle ( $n = 22$ ), and bottom ( $n = 26$ ) in the Bering Sea; surface ( $n = 36$ ), middle ( $n = 42$ ), and bottom ( $n = 31$ ) in the Chukchi Sea. This differentiated our study from previous studies which collected less samples from the same depth in this region (Han et al., 2014; Kirchman et al., 2010; Lee et al., 2019; Zeng et al., 2013a; Zeng et al., 2013b). However, the observed ASV richness within each division was largely independent of sampling depth (Figure 14). In Division A, the median richness was 19.0, 25.0, and 18.5 observed ASVs (bottom, middle, surface); in Division B, 47.5, 50.0, and 44.0; and in Division C, 56.0, 59.5, and 57.0. Kruskal–Wallis tests revealed no significant depth effect for any division after Bonferroni correction ( $\alpha = 0.017$ ): Division A ( $\chi^2 = 3.00$ ,  $df = 2$ ,  $p = 0.223$ ), Division B ( $\chi^2 = 2.26$ ,  $df = 2$ ,  $p = 0.324$ ), and Division C ( $\chi^2 = 1.19$ ,  $df = 2$ ,  $p = 0.551$ ). Consistently, richness did not differ significantly across depths when all samples were pooled ( $\chi^2 = 4.43$ ,  $df = 2$ ,  $p = 0.109$ ). This result indicates that the depth at which a sample was collected did not, by itself, predict ASV richness in this region, and that richness was similar across the three depths during the sampling period. This does not mean that vertical gradients

were not important. Salinity and nutrient concentrations still varied with depth, but the depth label added no information beyond those variables.

**Figure 14**

*ASV Richness Across Survey Stations, Shown by Depth (Surface, Middle, and Bottom) and Division (A, B, and C)*



*Note.* Dot size indicates observed ASV richness (ASVs present within the filtered 258 ASVs). Each dot corresponds to 11 sample clusters.

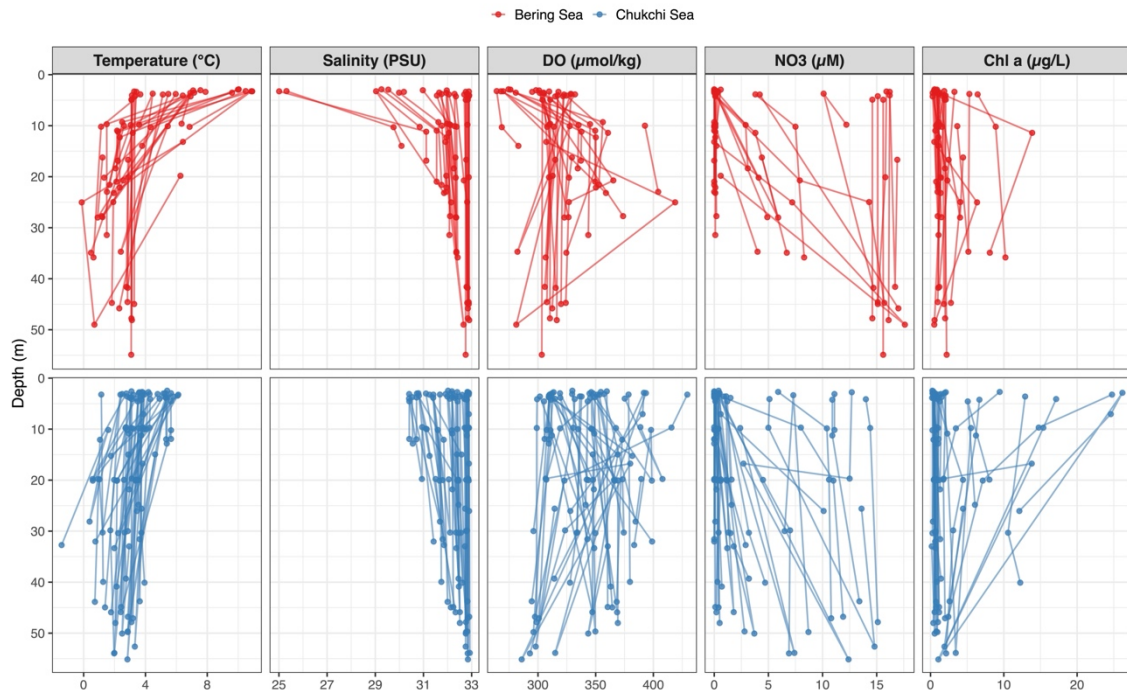
In both the Bering and Chukchi Seas, very weak thermocline and halocline were observed, whereas no clear clines were evident for the other measured parameters (DO, NO<sub>3</sub>, and chlorophyll-a) (Figure 15). Density anomaly ( $\Delta\sigma_t$ ) was also calculated to see the strength of stratification in the Bering and Chukchi Seas during June 2017. The Bering Sea exhibited relatively greater vertical stratification than the Chukchi Sea (mean  $\Delta\sigma_t = 1.26$  vs.  $0.43 \text{ kg m}^{-3}$ , respectively). However, stratification within the Bering Sea was spatially heterogeneous, with

40 % of stations classified as stratified ( $\Delta\sigma_t > 1$ ) and another 40 % as well-mixed ( $\Delta\sigma_t \leq 0.5$ ). In contrast, the Chukchi Sea was predominantly well-mixed, with 64 % of stations classified as well-mixed and only 18 % as strongly stratified (mean  $\Delta\sigma_t = 0.43 \text{ kg m}^{-3}$ ). The water column in the Bering Sea is stratified during spring when wind weakens and insolation increases (Hunt et al., 2002). However, it is also known that occasional summer mixing happens in this sea (Sambrotto et al., 1986; Whitledge et al., 1986). The Chukchi Sea was single-layered in this study.

Another possible factor explaining the lack of differences in microbial communities between the surface and deep layers is the transport of microbes to deeper waters with sinking particles. Mestre et al. (2018) examined seawater samples and reported that the dominant prokaryotes on sinking particles in the deep ocean overlapped with those living in surface waters. Also, Boeuf et al. (2019) found that Alphaproteobacteria and Flavobacteria MAGs, which were most prevalent in surface waters, were scarce in deep-sea POM DNA but were relatively abundant in the RNA pool especially in summer, suggesting that these surface-derived bacteria can be exported from the surface to the deep ocean in active-state by attaching to sinking particles, although the authors concluded that sediment trap samples showed distinct surface and deep prokaryotic communities. Those studies compared samples from surface to 4,000 m deep but given that the Bering and Chukchi Seas are shallow ( $\approx 50 \text{ m}$ ), particle-associated bacteria might be transported quickly from surface waters to bottom waters.

## Figure 15

*Vertical Profiles of Five Environmental Variables in the Bering and Chukchi Seas*



*Note.* It includes all samples for which the respective variable was measured. Sample sizes differed among variables and between seas. For temperature, salinity, and DO,  $n = 72$  (Bering) and 109 (Chukchi); for  $\text{NO}_3$ ,  $n = 71$  (Bering) and 103 (Chukchi); and for chlorophyll a,  $n = 66$  (Bering) and 96 (Chukchi). Samples were assigned to the Bering Sea ( $\text{lat} < 65.7^\circ\text{N}$ ) or the Chukchi Sea ( $\text{lat} \geq 65.7^\circ\text{N}$ ). Each point represents a single sample plotted at its sampling depth (Y-axis, reversed).

### 7.3 Association of Dominant Bacterial Class with Diatom Bloom

Clustering of the survey samples may have been influenced by biological factors as well as environmental conditions. During the ASGARD 2017 cruise, the high abundance of some diatoms and zooplanktons species, or groups were reported.

For example, Krause et al. (2021) examined diatom growth and productivity in the northern Bering and Chukchi Seas in June 2017 and reported that diatom biomass ( $\text{bSiO}_2$ ,  $\mu\text{mol Si l}^{-1}$ ) was among the highest at station DBO3.8. Consistent with this, Nielsen et al. (2023) found that diatoms dominated the phytoplankton community in the same region in June 2017, as indicated by elevated diatom-associated fatty acid biomarkers (16:1n-7, 16:4n-1, and EPA) and their close association with the June 2017 samples in the Non-Metric Multidimensional Scaling (NMDS) ordination. Lalande et al. (2021) investigated spatial and temporal variation in zooplankton and phytoplankton composition. At DBO3 station in the southern Chukchi Sea, they observed elevated chlorophyll-a fluxes ( $\approx 5 \text{ mg m}^{-2} \text{ d}^{-1}$ ) together with a high abundance of the centric diatoms *Chaetoceros* and *Thalassiosira*, both of which are widespread bloom-

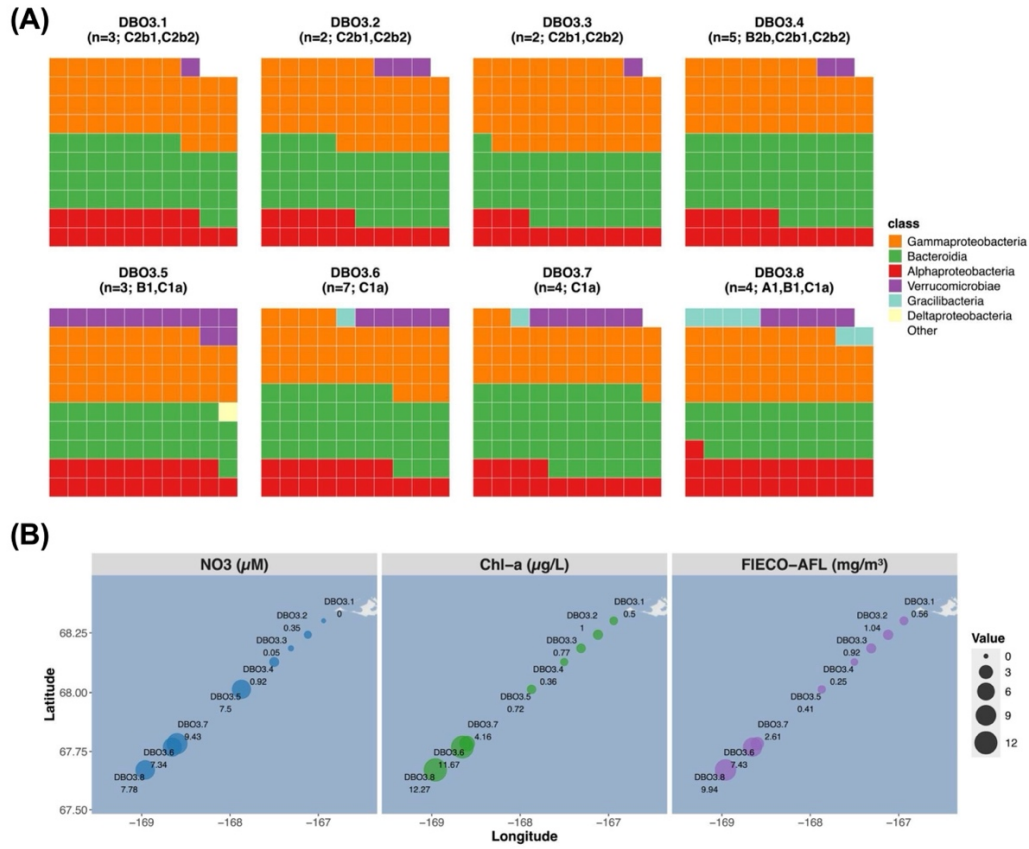
forming taxa (De Luca et al., 2019; Park et al., 2016). Because these observations spanned late June to July and coincided with the ASGARD 2017 cruise, they suggest that a pelagic bloom developed around DBO3 during this period. Lekanoff (2020) also conducted clustering analysis of diatom communities in the Bering and Chukchi region. She reported that samples from the ASGARD 2017 cruise were dominated by *Chaetoceros* and *Thalassiosira*, while samples around DBO3 lines were mostly dominated by *Thalassiosira*-rich cluster, which is consistent to the result from Lalande et al. (2021). The association between *Polaribacter* and *Chaetoceros* was reported by microcosm experiments (Takebe et al., 2024), so the results from those studies indicate an association between Bacteroidia and some specific diatom taxa.

Along with diatoms, high abundance of some zooplankton was also reported around DBO stations during the ASGARD 2017 cruise. For instance, *Neocalanus*, a dominant copepod in the northern Pacific Ocean (Conover, 1988; Gonzalez et al., 2025), and polychaete larvae, a globally abundant meroplankton stage (Pamungkas et al., 2019), became highly abundant at DBO2 (the northern Bering Sea) and DBO3 stations during ASGARD2017 due to the increased inflow of Pacific waters (Lalande et al., 2021).

Based on our results, six clusters contained samples from DBO3 stations: 1 station in Cluster A1 and B2b, 2 stations in Cluster B1, 4 stations in Cluster C1a, C2b1, and C2b2. Especially, all samples in Cluster C1a were collected from DBO3 stations (DBO 3.5–3.8 stations,  $n = 14$ ). Waffle plot for microbial class composition at eight DBO3 stations (Figure 16A) showed that Gammaproteobacteria, Bacteroidia, and Alphaproteobacteria were highly abundant in each DBO3 station (33–47 % for Gammaproteobacteria, 29–45 % for Bacteroidia, and 17–21 % for Alphaproteobacteria). In addition, chlorophyll-a, FIECO-AFL, and  $\text{NO}_3$  concentration were relatively high around DBO3 stations, especially offshore stations (DBO3.6, 3.7, and 3.8) (Figure 16B), which was consistent with the results from the previous study (Gonzalez et al., 2025) and suggesting that this can be attributed to an active diatom bloom, observed during June 2017 (Krause et al., 2021; Lalande et al., 2021; Lekanoff, 2020; Nielsen et al., 2023).

## Figure 16

*Waffle Plots Showing Class-Level Taxonomic Composition and Map of Nitrate, Chlorophyll-a, and FIECO-AFL Concentrations at DBO3 Stations*



**Note.** Samples in six clusters (A1, B1, B2b, C1a, C2b1, and C2b2) were collected in eight stations (DBO3.1-3.8). (A) n in each panel label indicates the number of samples collected at each DBO3 station. Each waffle panel comprises 100 grid cells (10 × 10), with one cell representing 1 % of the within-assemblage relative abundance. Colors indicate taxonomic class; the top 5 classes are shown individually, and the rest are grouped as "Other"; (B) Each point represents each DBO3 station. Point size is proportional to the concentration of each parameter. This map shows the depth-averaged concentration of each parameter.

Compared to other transects, DBO3 showed relatively high abundance of Bacteroidia (Table 3). DBO3 and other four transects (KL, CL, and IL in the Chukchi Sea and CBW in the Bering Sea) showed higher abundance of Gammaproteobacteria and Bacteroidia. Also, the ratio of Alphaproteobacteria: Gammaproteobacteria: Bacteroidia at five transects were approximately 2: 4: 4, suggesting that microbial communities were mainly led by Gammaproteobacteria or Bacteroidia. This pattern further implies that diatom blooms occurred not only along DBO3 but also at the other stations during the ASgard 2017. In this paper, I focused on DBO3 stations because the pelagic bloom of *Chaetoceros* and *Thalassiosira* was observed around this transect during the ASgard 2017 cruise (Lalande et al., 2021; Lekanoff, 2020). The bacterial phylum,

Bacteroidetes, is known as the association of bloom and the contribution of decomposing complex organic matter such as polysaccharide (Fernández-Gómez et al., 2013; Krüger et al., 2019). *Thalassiosira* is known for its association with Alphaproteobacteria (Mönnich et al., 2020; Pavlovska et al., 2026; Pontiller et al., 2022; Tisserand et al., 2020), Bacteroidetes (Gärdes et al., 2011; Kamalanathan et al., 2019; Mönnich et al., 2020) and Gammaproteobacteria (Pavlovska et al., 2026; Tran et al., 2023). Also, zooplankton such as *Neocalanus* and polychaetes are known to have associations with marine bacteria (De Corte et al., 2014; Kunihiro et al., 2011; Shoemaker et al., 2015; Tan et al., 2015). Taken together, the high abundance of Bacteroidia and Gammaproteobacteria at DBO3 stations and the transects nearby (KL, IL, and CNL) is possibly attributed to the diatom bloom observed in June 2017 and bloom-associated microbial activities.

**Table 3**

*Composition of Three Bacterial Classes (Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia) at the 12 Transects in the Survey Data*

| Transect | <i>n</i> | Alphaproteobacteria<br>(%) | Gammaproteobacteria<br>(%) | Bacteroidia (%) |
|----------|----------|----------------------------|----------------------------|-----------------|
| KL       | 8        | 12.3                       | 43.5                       | 40.9            |
| DBO3     | 30       | 16.9                       | 38.0                       | 40.2            |
| CBW      | 16       | 21.6                       | 33.7                       | 38.3            |
| CL       | 20       | 14.0                       | 49.2                       | 37.6            |
| IL       | 13       | 14.9                       | 39.6                       | 36.6            |
| DBO1     | 4        | 21.2                       | 37.2                       | 34.3            |
| CPL      | 16       | 22.4                       | 35.6                       | 33.6            |
| DBO2     | 11       | 21.5                       | 44.6                       | 31.7            |
| CBE      | 15       | 20.5                       | 42.9                       | 31.3            |
| CNL      | 22       | 23.2                       | 38.6                       | 30.4            |
| CB       | 2        | 14.7                       | 43.5                       | 27.1            |
| SLY      | 24       | 26.4                       | 58.4                       | 5.3             |

*Note.* The number of samples is shown in the *n* column. Each proportion is the median relative abundance of the class.

## 7.4 Influence of Water Mass on Microbial Communities

The objective of this study is investigating the influence of water mass on microbial communities in the Bering and Chukchi Seas. As Table 2 showed, the 11 sample clusters were influenced by different water masses. Clusters B1, C1a, and C1b2, which were wSW-influenced and pre-bloom clusters, showed correlations with  $\text{NO}_3$  and salinity on dbRDA (Figure 11). Also, those three clusters showed higher  $\text{NO}_3$  concentration than other clusters (median: 15.1, 10.5, and 7.7; mean  $\pm$  SD:  $13.17 \pm 5.05$ ,  $8.62 \pm 4.39$ , and  $7.97 \pm 5$  in Cluster C1b2, C1a, and B1, respectively, Figure 8) and contained balanced proportions of relative abundance of major bacterial classes (approximately 2: 4: 4, Alphaproteobacteria: Gammaproteobacteria: Bacteroidia). However, samples in those clusters were collected from different geographical locations (B1 in the Chukchi Sea including DBO3 stations, C1a in DBO3 stations, and C2b2 in the Bering Sea), across a latitudinal range spanning more than 300 km. This result suggests that these communities were transported with Pacific water flowing northward through the Bering Strait or transported by water that formed preferable environmental conditions for those clusters.

In addition, the six assemblages differed in where they were most abundant, each reaching its highest relative abundance in a different part of the study area (Figure 4). This shows that the communities are spatially structured. However, location and water mass are not independent here. Water flows northward across the shelf, so where a sample was collected also reflects how long its water has been modified by biological activity. Therefore, the spatial pattern reflects both water-mass origin and bloom progression, and the two cannot be separated with these data.

Post-bloom clusters showed the influence of water mass. For example, A2 and C2a, both wCW-influenced clusters (Table 2), showed low loadings on CAP1 and CAP2 but showed correlations with temperature on dbRDA (Figure 11). C2a had highest temperature (median: 5.4; mean  $\pm$  SD:  $5.68 \pm 2.69$ , Figure 8), while A2 showed the third highest median and the second highest mean  $\pm$  SD in temperature (median: 3.75; mean  $\pm$  SD:  $5.2 \pm 3.68$ , Figure 8). In addition, compared to other clusters, the relative abundance of Alphaproteobacteria was higher in these clusters (33.1 % in C2a and 25.3 % in A2) though most clusters showed less than 20 % of their abundance. Geographically, sampling locations of Cluster A2 and C2a are different as samples in A2 and C2a were mostly collected from SLY Transect and Alaskan coasts respectively. wCW is nutrient-scarce water, and both A2 and C2a showed the lowest median (0.1) of mean  $\pm$  SD ( $1.19 \pm 3.52$  and  $1.48 \pm 4.86$ , respectively) of  $\text{NO}_3$  concentration. Also, the most dominant Alphaproteobacteria ASV in Cluster A2 and C2a was SAR11 clade Ia, the representative marine bacteria in oligotrophic environment (Giovannoni, 2017), suggesting that oligotrophic condition

in wCW favored the microbial communities in those post-bloom clusters, which were led by Alphaproteobacteria.

Other four post-bloom clusters also represented distinct characteristics. For instance, Cluster B2a and C2b1, which were influenced by both cSW and wSW (Table 2), showed high DO concentration (median: 349.58 and 347.39; mean  $\pm$  SD:  $349.14 \pm 32.5$  and  $350.32 \pm 34.51$ , respectively, Figure 8) and had correlations with DO concentration (Figure 11). Cluster B2b and C2b2, which were cSW-influenced clusters (Table 2), also showed similar pattern with DO (Figure 8 and Figure 11) and lower nutrient concentrations (median:  $< 0.5 \mu\text{M}$  in those four clusters, Figure 8). Also, microbial communities in these four clusters were led by Gammaproteobacteria (46.6 % in B2a, 43.3 % in C2b1, 42.9 % in B2b, and 41.7 % in C2b2, Figure 16A). Mostly, Gammaproteobacteria is known as increasing their abundance in post-bloom conditions (Liu et al., 2013; Sintes et al., 2013; Teeling et al., 2012; Teeling et al., 2016), and dominant ASVs in these four clusters (Figure 13) were uncultured Nitrospiraceae, SAR92, OM182, and SAR86, which were known as bloom-responding carbon degraders (Sidhu et al., 2023; Thiele et al., 2022; Wietz et al., 2021; Yang et al., 2015). Sampling locations of Cluster B2b, C2b1, and C2b2 were mainly from the Chukchi Sea (B2b from CL and IL, C2b1 from DBO3, and C2b2 from KL, IL, and DBO3). On the other hand, most samples in Cluster B2a were collected from both the northern Chukchi Sea (CNL, 29.4 %) and the southern Bering Sea (SLY, 23.5 %). Although samples in Cluster C2B1 were largely collected from DBO3 stations (50 %), it also contained samples from the southern Bering Sea (SLY, 8.3 %; CBE, 25.0 %). This can be the reason why Cluster B2a and C2b1 showed similarities in water mass conditions and microbial community composition.

Those results support our interpretation that microbial community composition across the Bering and Chukchi Seas is structured by water mass origin and bloom-stage progression along with geographic distance. However, we must remember the fact that sea water temperature in this region anomalously increased and experienced early-ice retreat due to the heat wave during June 2017 (Baker et al., 2020; Danielson et al., 2020; Walsh et al., 2018). Mooring study also reported that heat transport from Pacific Ocean to Canada Basin increased 1.5 times over 20 years. (Ito et al., 2026). Danielson et al. (2020) analyzed water mass distribution in the Bering and Chukchi Seas and showed that before 2014, cSW was prevalent in upper water column through this region due to seasonal warming and sea ice melt. In this paper, we investigated the proportion of influential water mass on each sample cluster (Table 2) and found that primary water masses of most sample clusters were either wSW or cSW. Given the impact of the heat wave, sample clusters classified as having wSW as the primary water mass might have been cSW-influenced clusters in fact.

The Bering and Chukchi regions are known for their high productivity, but it is also known that they are vulnerable to sea ice loss. Ballinger & Overland (2022) reported that dramatic loss of sea ice in the Bering and Chukchi regions and shifted atmospheric patterns led the Alaskan Arctic to enter a new climate regime in 2017-2021, and it may cause the unprecedented extreme events such as delaying spring blooms and disrupting marine food webs. Furthermore, microbial communities in the Arctic Ocean are influenced by sea ice loss. For example, Comeau et al (2011) analyzed post-bloom communities in the subsurface chlorophyll maximum layer in Beaufort Sea before and after the loss of MYI in 2007 and found that the abundance of Bacteroidetes dramatically decreased after 2007, and more than half of this reduction was attributed to *Polaribacter* and unidentified Flavobacteriaceae. On the other hand, the relative abundance of Alphaproteobacteria was consistently high, and Gammaproteobacteria did not change their abundance much. Kraemer et al. (2024) also conducted time-series analyses of microbial community composition in the Canada Basin and reported that the abundance and diversity of Bacteroidetes dramatically decreased after sea ice loss, and the majority of decreasing ASVs were members of the Bacteroidetes and Proteobacteria. These studies collectively demonstrated the significant impact of sea ice dynamics on the composition and succession of Bacteroidetes in Arctic marine microbial communities. In this paper, we found that wSW-influenced clusters and cSW-influenced clusters had different microbial community compositions (Figure 3; Table 2; Figure 13), implying that future environmental shift probably alters the microbial compositional change in this region, and thus, we need to monitor microbial communities as a sentinel of environmental change.

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## Chapter 3. Particle-Associated Prokaryotic Community

### Structures in the Bering and Chukchi Seas

#### 9. Introduction

Microbial communities are central to marine ecosystems, contributing to primary production and organic-matter decomposition and driving biogeochemical cycles (Azam & Malfatti, 2007; Falkowski et al., 2008). As primary producers they channel carbon and energy to higher trophic levels, and by remineralizing organic matter they cycle key elements such as carbon, nitrogen, and sulfur; through the microbial carbon pump they further convert labile organic matter into long-lived recalcitrant dissolved organic carbon (Jiao et al., 2024; Legendre & Le Fèvre, 1995).

The Arctic Ocean is a geographically unique setting for these processes. Although it represents only about 3 % of the global ocean by area and roughly 1 % by volume, it holds about 20 % of the world's continental shelf and receives close to 11 % of global riverine input (Carmack, 2007; Tomczak & Godfrey, 1994). It is largely enclosed by land, seasonally ice-covered, and generally nitrogen-limited, with primary production dominated by picoeukaryotic algae rather than cyanobacteria such as *Synechococcus* (Pedrós-Alió et al., 2015; Simpson et al., 2008).

The region is changing rapidly. Sea-ice extent and thickness are declining, and Arctic amplification has warmed the region roughly four times faster than the global ocean (Rantanen et al., 2022; Serreze & Barry, 2011). Warming is reinforced by heat advected from the Atlantic (Atlantification) and the Pacific, alongside acidification, freshening, and stronger stratification that suppress the vertical supply of nutrients (Polyakov et al., 2020; Tremblay & Gagnon, 2009). These changes reach the microbial communities as well. For instance, their composition shifted after the major losses of MYI in 2007 and 2012 (Comeau et al., 2011). Net primary production rose by about 57 % from 1998 to 2018 (Lewis et al., 2020). New harmful algal blooms have also appeared, including the toxic *Alexandrium catenella* bloom in the Chukchi Sea in 2022 (Fachon et al., 2025). Because they respond to drastic environmental change quickly, microbial communities serve as sensitive early indicators of Arctic change.

This study focuses on the Bering and Chukchi Seas, the Pacific gateway to the Arctic Ocean and among the most productive regions on Earth (Grebmeier, 2012; Springer et al., 1996). The shallow (~50 m) Bering–Chukchi shelf receives heat and nutrients from Pacific inflow that fuel seasonal phytoplankton blooms whose timing depends strongly on sea-ice cover and water-column stratification (Hunt et al., 2002; Woodgate et al., 2005). Nutrient-rich Pacific Winter Water sustains under-ice production, and net primary production in the Chukchi Sea has nearly

doubled since the late 1990s (Arrigo et al., 2014; Lewis et al., 2020). The region's waters are classically divided into Alaskan Coastal, Bering Shelf, and Anadyr waters (Coachman et al., 1975), recently refined into an all-season scheme of warm and cool coastal and shelf water masses (Danielson et al., 2020).

Marine bacterial communities can be broadly divided into two microbial lifestyles: free-living (FL) cells suspended in the water column and particle-associated (PA) cells attached to suspended or sinking particles. The FL and PA fractions of marine bacterial communities have been compared from estuaries and coastal waters to the open sea (Crespo et al., 2013; Hollibaugh et al., 2000; LaMontagne & Holden, 2003; Rieck et al., 2015; Roth Rosenberg et al., 2021; Selje & Simon, 2003; Zhang et al., 2007). These studies have generally shown that FL and PA communities are taxonomically distinct, with many taxa restricted to a single fraction. For example, only about a quarter of the operational taxonomical units (OTUs; proxies for bacterial species) were shared between fractions in the northwestern Mediterranean (Crespo et al., 2013), and more than half were PA-exclusive in the Baltic Sea (Rieck et al., 2015). A recurrent pattern has emerged in which FL communities are dominated by Alphaproteobacteria, whereas PA communities are enriched in Gammaproteobacteria and Bacteroidetes (Crespo et al., 2013; Zhang et al., 2007). These temperate studies establish the FL–PA distinction as an ecologically meaningful but environmentally influenced feature of marine bacterial communities.

In the Arctic, FL and PA fractions of bacterial communities have been examined mainly in fjords, on productive shelves, and in river-influenced seas (Jain & Krishnan, 2017; Jain et al., 2019, 2021; Kellogg & Deming, 2009; Ortega-Retuerta et al., 2013; Sperling et al., 2013). As in temperate systems, FL and PA communities are generally distinct, especially in open, offshore waters, and tend to show the same taxonomic contrast. Bacteroidetes and Gammaproteobacteria are enriched in the PA fraction, whereas Alphaproteobacteria dominate the FL fraction (Ortega-Retuerta et al., 2013). PA fractions also tend to harbour higher diversity (Jain et al., 2019). However, the strength of this separation is not uniform and is closely tied to the distinctive hydrography of the Arctic. Glacial meltwater, terrestrial runoff, and water mass gradients can act on the two fractions differently, so that FL communities often vary more among locations while PA communities remain comparatively similar (Jain & Krishnan, 2017; Ortega-Retuerta et al., 2013). More specifically, the biochemical quality of particles can structure PA communities and favour polymer-degrading taxa such as Bacteroidetes and Verrucomicrobia (Jain et al., 2019). This is especially true of their carbohydrate content. The two lifestyles are increasingly regarded as spatially connected assemblages shaped by both dispersal and local environmental selection rather than as fixed habitats (Jain et al., 2021). Together, these studies show that the FL–PA distinction persists in cold, seasonally productive, and freshwater-influenced polar waters, but that its expression is strongly governed by water masses and particle supply.

FL and PA communities in the Bering and Chukchi region have also been studied. For example, Qin et al. (2025) compared suspended and sinking particles at ten western-Arctic stations including the Bering and Chukchi regions. They found that eukaryotic communities differed between particle types, while prokaryotic communities differed only slightly, at the level of individual taxa, and carbon sinking flux increased with chlorophyll a and diatom-associated taxa. In the same region, the study extends the FL/PA perspective to sinking particles, suggesting a pathway by which diatom blooms may drive carbon export. However, despite such study, how bacterial communities in this region are structured in response to algal blooms remains only partly understood. Given the intense algal blooms and high primary productivity that characterize these seas, further work is needed to understand how bloom dynamics shape the structure of local bacterial communities.

Here, using 16S rRNA gene amplicon sequencing of 78 samples collected at three depths (surface, middle, and bottom) across 12 stations during the Arctic Shelf Growth, Advection, Respiration and Deposition Rate Experiments (ASGARD) 2017 cruise, we aim to (i) characterize the composition and spatial structure of bacterial communities in the Bering and Chukchi Seas, (ii) examine how the diatom bloom influenced community composition, (iii) identify relationships between the communities and physicochemical parameters of water masses from which they derived, and (iv) assess how physicochemical changes associated with ongoing warming may affect these communities, and (v) compare free-living and particle-associated communities to determine whether the environmental drivers of change differ by fraction.

This study links community structure to bloom dynamics and to physicochemical properties of water masses. Through this approach, it provides a baseline for understanding how microbial communities in this region respond to environmental change, and how different lifestyles (free-living vs. particle-associated) shape microbial communities.

## 10. Methods

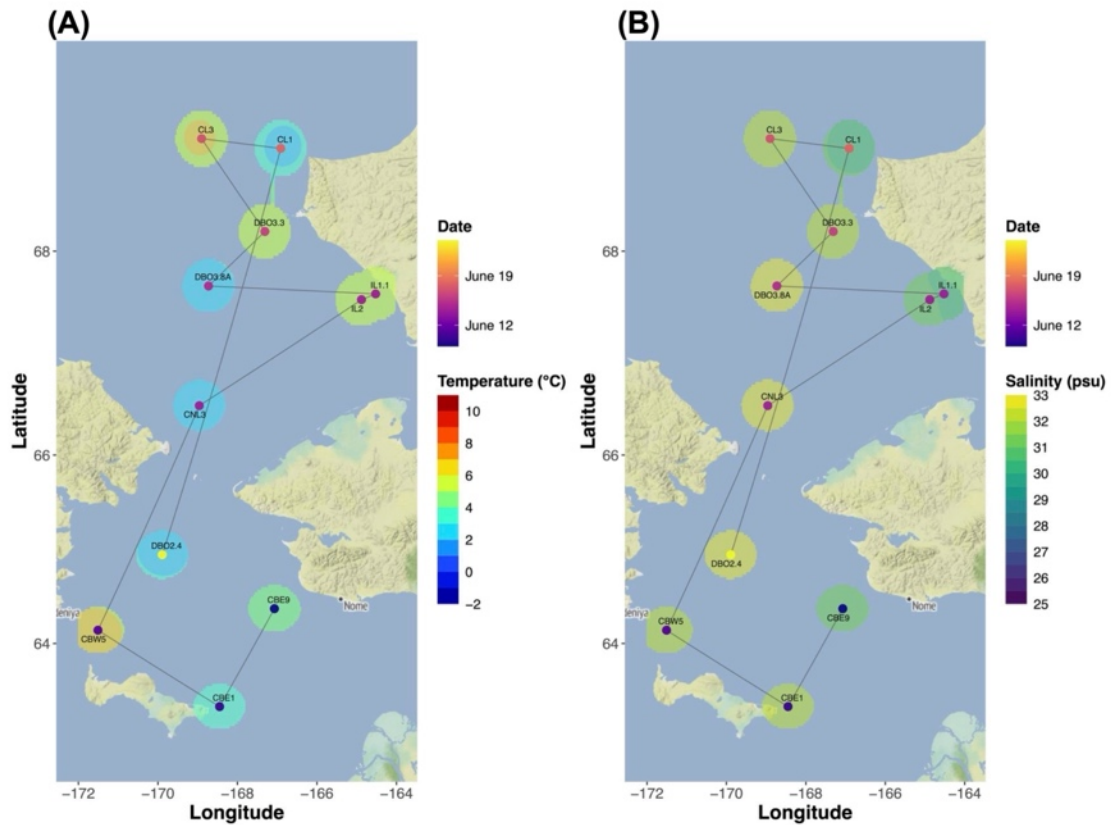
See Section 2 for detailed Materials and Methods.

### 10.1 Sample collection

Seawater was sampled during the ASgard project between 9 and 28 June 2017 aboard the R/V Sikuliaq. Over the ASgard 2017 cruise, 12 process stations were occupied, and four casts were carried out for sensor profiling, nutrient analysis, and zooplankton incubation at those stations.

**Figure 17**

*Process Stations and Near-surface Hydrography During the ASgard 2017 Cruise*



### 10.2 Sample filtration

Samples were collected with sequential filtration through a 47 mm-diameter 20  $\mu$ m-pore size nylon net filter, then a 47 mm-diameter 3  $\mu$ m-pore size membrane filter, and finally through a 0.2  $\mu$ m-pore size Sterivex cartridge filter (all filters from Millipore Sigma, Burlington, MA, USA).

### 10.3 Analysis of 16S rRNA gene sequencing

Altogether, the 16S rRNA gene comprised of 3,076 prokaryotic ASVs in total. Of these 3,076 ASVs, 374 were detected ( $> 2$  reads) in each dataset in the 78 processing samples that retained  $\geq 5,000$  reads. Read counts were converted to relative abundances within each sample, and an ASV was retained only when its maximum relative abundance across samples exceeded 0.001 (0.1 %) and it was present in more than two samples. This filtering step left 221 ASVs from the 78 process samples.

### 10.4 Hierarchical Clustering

For the beta-diversity analysis, Bray–Curtis dissimilarities were derived from the fourth-root-transformed relative-abundance matrix with the *vegan* package in R (Oksanen et al., 2024). Samples were then clustered by Ward’s minimum-variance method and displayed as a heatmap built with the R packages *vegan*, *fastcluster* (Müllner, 2013), and *gplots* (Warnes et al., 2009). For the process dataset, Bray–Curtis dissimilarities among samples were computed from the fourth-root-transformed ASV relative-abundance matrix (221 ASVs across 78 process samples) and the samples were grouped using Ward’s method. Cutting the dendrogram at  $k = 4$  yielded four clusters. The clustering was based on ASV composition alone; filter pore size was not used as an input. The first split of the dendrogram nevertheless separated samples collected on 0.2  $\mu\text{m}$  filters from those collected on 3 and 20  $\mu\text{m}$  filters. On this basis the four clusters were labelled as one free-living cluster (FL1) and three particle-associated clusters (PA1–PA3). These labels are operational, describing the size fraction in which each community was recovered rather than a directly observed lifestyle. This division was also examined through a top-down tree walk, carried out differently from the hierarchical clustering used for the survey dataset. Here, environmental variables were log-transformed (nutrients), scaled, and reduced to principal components explaining  $\geq 90$  % of cumulative variance; at each internal node with  $\geq 5$  samples per child, PC scores were compared with Wilcoxon rank-sum tests and adjusted across PCs with the Benjamini–Hochberg procedure (significance at BH-adjusted  $p < 0.05$ ).

ASV richness, defined as the number of ASVs present (relative abundance  $> 0$ ) in the filtered clustering set (221 ASVs), was compared among depths using Kruskal–Wallis rank-sum tests. The tests were run overall and separately within each community grouping (one free-living cluster and three particle-associated clusters), and p-values were Bonferroni-corrected for the number of within-group tests.

Ternary plots were produced in R using the *Ternary* package; normalized mean relative abundances of each ASV in each filter fraction were used as input (0.2  $\mu\text{m}$ :  $n = 23$  samples, 3  $\mu\text{m}$ :

$n = 27, 20 \mu\text{m}: n = 28).$

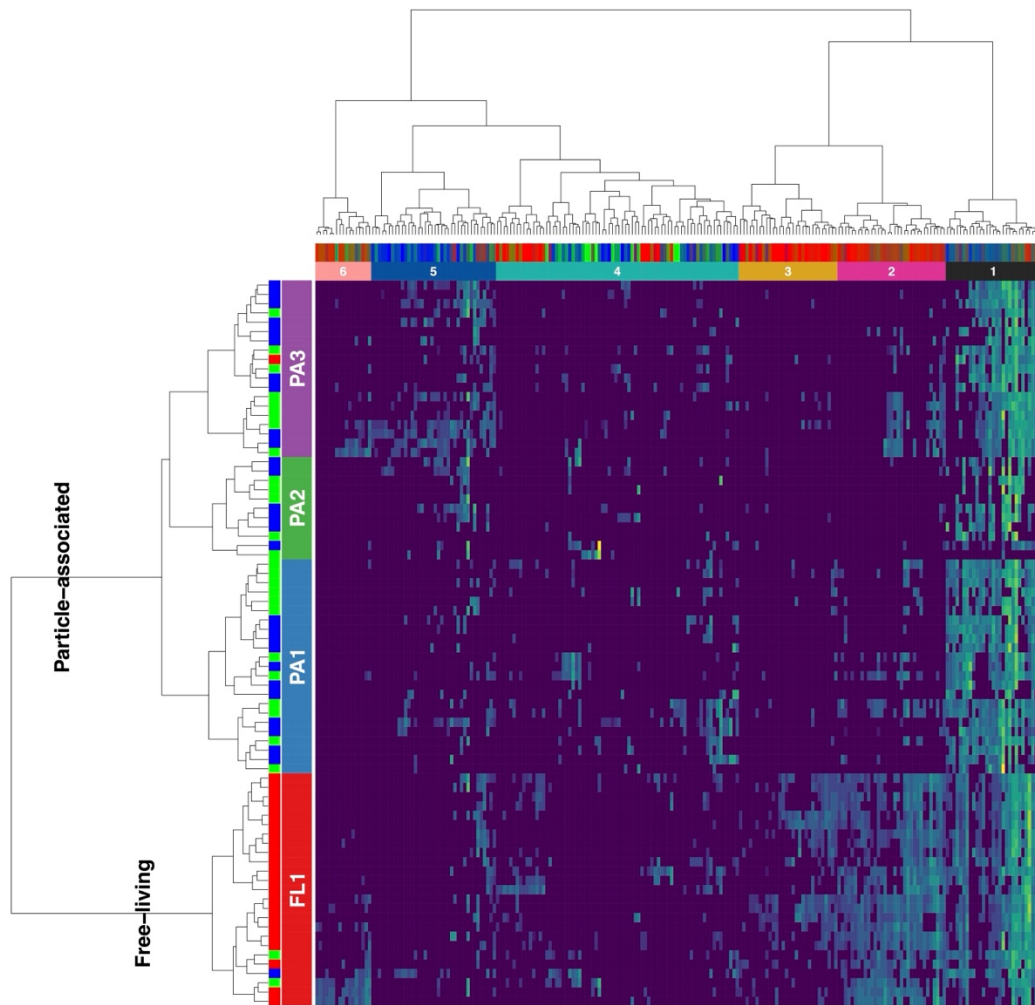
## 11. Results

### 11.1 Hierarchical Clustering

The 78 samples were assigned to four sample clusters for this analysis (Figure 18). Out of 78 samples, 75 contained data for all nine parameters (temperature, salinity, DO, NO<sub>3</sub>, PO<sub>4</sub>, Sil, NH<sub>4</sub>, FIECO-AFL, and depth). PC1–PC4 captured 92.1 % of the variance. A top-down tree walk was performed to test for environmental differences among subclusters, but no internal node remained significant after Benjamini–Hochberg correction. We retained four clusters for further investigation defined by Bray–Curtis distance and Ward’s method clustering ( $k = 4$ ), with Cluster 1 corresponding to the 0.2  $\mu\text{m}$  fraction and Clusters 2–4 to large-sized fractions (3 and 20  $\mu\text{m}$ ). In addition, 221 filtered-ASVs were grouped into six assemblages: Assemblage 1 (black; # of ASVs = 29), Assemblage 2 (pink; # of ASVs = 33), Assemblage 3 (yellow; # of ASVs = 30), Assemblage 4 (light blue; # of ASVs = 74), Assemblage 5 (dark blue; # of ASVs = 38), and Assemblage 6 (light red; # of ASVs = 17).

#### **Figure 18**

*Heatmap of ASVs in the Process dataset*



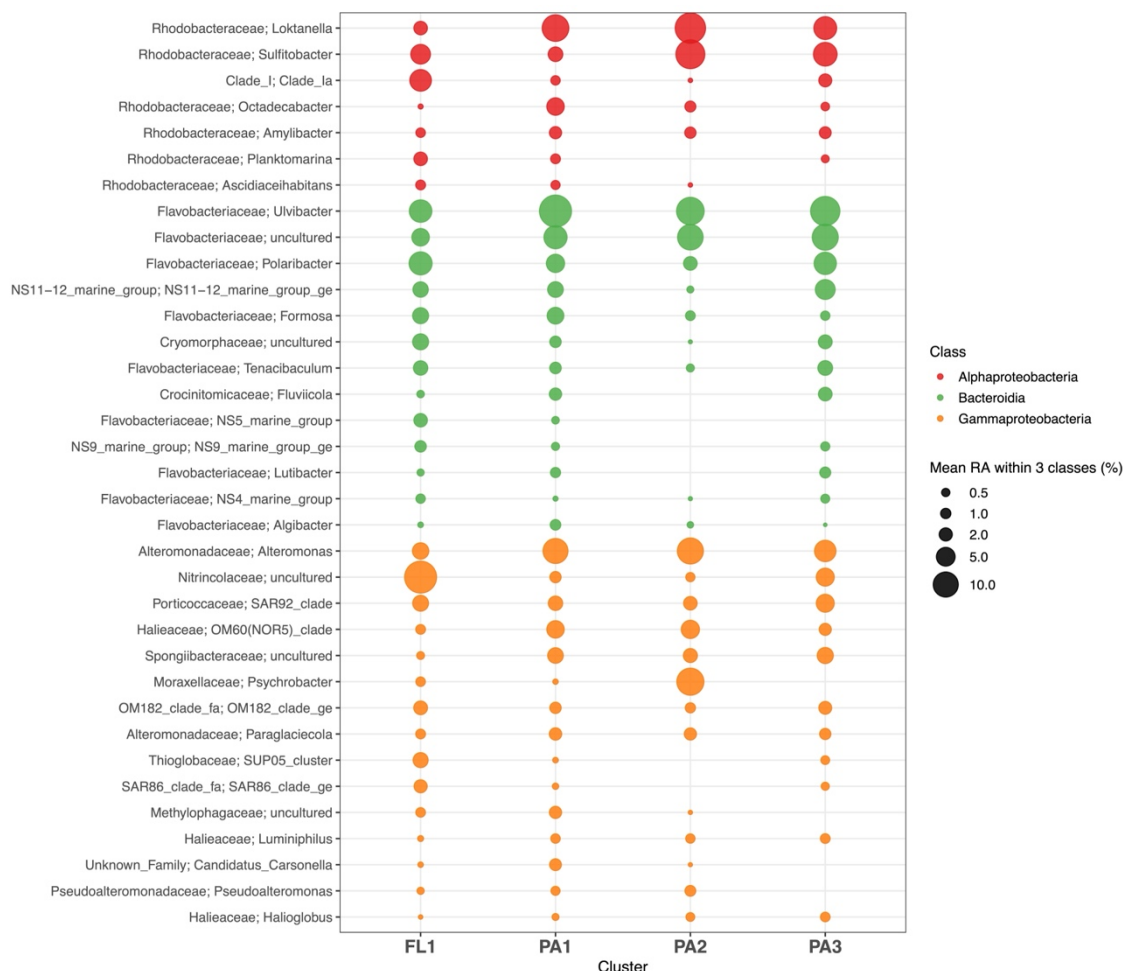
*Note.* Hierarchical clustering was conducted based on relative abundance of 221 ASVs (columns), present in at least 5,000 quality-filtered reads across 78 seawater samples (rows), based on fourth-root-transformed relative abundances (viridis colour scale; dark = low, yellow = high). Samples and ASVs were both clustered by Bray–Curtis dissimilarity with Ward's method (ward.D). The dendrograms are shown on the left (samples) and top (ASVs). Samples were separated into four clusters: one free-living cluster (FL1) and three particle-associated clusters (PA1–PA3), indicated by the colored bar immediately left of the heatmap and by the labels within it. The first branch of the dendrogram ( $k = 2$ ) separated the FL sample from the PA samples, as annotated on the tree. The second, thin row bar encodes the filter size of each sample ( $0.2\ \mu\text{m}$  = red,  $3\ \mu\text{m}$  = green,  $20\ \mu\text{m}$  = blue). ASVs are colored by an RGB blend of their distribution across the three size fractions (red =  $0.2\ \mu\text{m}$ , green =  $3\ \mu\text{m}$ , blue =  $20\ \mu\text{m}$ ) and are further grouped into six assemblages (numbered 1–6).

The relative abundances of the most abundant ASVs revealed differentiation in

community composition across the four clusters (Figure 19), though ASVs from the three major bacterial class were abundant throughout each of the four clusters. Alphaproteobacteria ASVs classified as SAR11 clade Ia are known to be free-living and were most abundant in Cluster FL1. Bacteroidetes ASVs classified as *Ulvibacter* are known to be common in algal phycospheres and were most abundant in particle-associated Clusters PA1-3. Gammaproteobacteria ASVs classified as uncultured Nitrincolaceae, SUP05, and SAR86 are known to be free-living and were most abundant in Cluster FL1. ASVs classified as *Psychrobacter* are common in sea ice and were unusually abundant in Cluster PA2.

**Figure 19**

*Bubble Plot of Major Genera Within Three Bloom-Responsive Bacterial Classes (Bacteroidia, Gammaproteobacteria, Alphaproteobacteria) Across the Four Clusters*



*Note.* Rows correspond to genera, columns to sample clusters. Bubble size represents the mean relative abundance (%) of the genus in each cluster in each class, and color represents class. Displayed genera satisfy two criteria: occurrence in > 10 % of samples (n = 78) and cumulative

within-class relative abundance  $\leq 95\%$  ( $n = 35$  genera: 7 Alphaproteobacteria, 13 Bacteroidia, 14 Gammaproteobacteria).

Three PA Clusters shared a high relative abundance of ASVs belonging to three major Bacteria classes. Cluster PA1 was dominated by *Ulvibacter*, *Loktanella*, and *Alteromonas*, with Bacteroidia being the most abundant class (42.4 %). Cluster PA2 showed the most evenly elevated abundances and contained ASVs not prominent elsewhere: *Psychrobacter*, *Sulfitobacter*, and *Alteromonas*. The abundance of *Psychrobacter* can be attributed to AnW and MWW which influenced this cluster. Also, this cluster lacked some Bacteroidia ASVs such as *Fluviicola*, NS5 and 9 marine groups, and *Lutibacter*, which were abundant in other clusters along with other Bacteroidia ASVs. Instead, given the higher abundance of Gammaproteobacteria (36.1 %) and the highest median of FIECO-AFL, the bloom stage of this cluster might be shifted toward post-bloom conditions. Cluster PA3 was characterized by Bacteroidia ASVs including *Ulvibacter* and displayed the most diverse, including *Polaribacter*, uncultured Flavobacteriaceae, and the NS11-12 marine group. This cluster was AnW/cSW-influenced and comprised pre-bloom communities from the deepest samples, and Bacteroidia was the most abundant class (47.7 %), suggesting that it harbored typical of particle-attached degraders of sinking diatom aggregates.

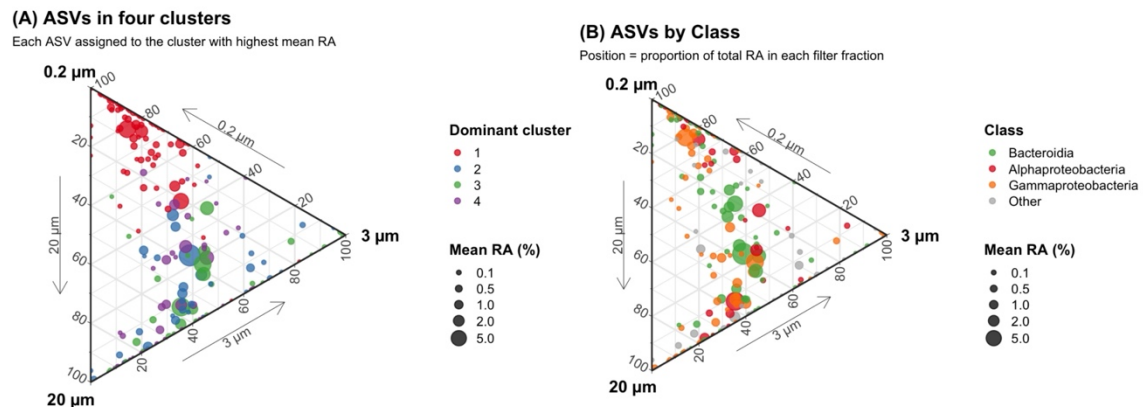
In Chapter 2, the 11 survey clusters were assigned to different bloom stages. They were also significantly associated with water mass (Fisher's exact test,  $p < 0.001$ ), but the association was not one-to-one, as five clusters shared wSW as their primary water mass. Pre-bloom clusters tended to occur in wSW and post-bloom clusters in cSW, with one coastal cluster as an exception. On the other hand, in the process samples, there was no clear one-to-one correspondence between each water mass and bloom stage of the four clusters, as clusters primarily associated with the same water mass (AnW). However, each cluster differed in their bloom stages and the dominant bacterial class, driven by differences in size fractions.

The first split in the clustering dendrogram (Figure 18) discriminated free-living (FL; Cluster FL1; 0.2  $\mu\text{m}$  filter fraction) and particle-associated (PA; Clusters PA1-3; 3  $\mu\text{m}$  and 20  $\mu\text{m}$  fractions). We did not use pore size to build the clusters. The samples separated this way based on their ASV composition alone. The partition of ASVs across the 0.2  $\mu\text{m}$ , 3  $\mu\text{m}$ , and 20  $\mu\text{m}$  filter fractions (Figure 20) demonstrated size-selectivity of some ASVs. ASVs in Cluster FL1 were particularly highly concentrated in the 0.2  $\mu\text{m}$  fraction (most had  $> 60\%$  normalized mean relative abundance; while ASVs in Clusters PA1-3 overlapped substantially with each other and typically had  $< 40\%$  normalized mean relative abundance in the 0.2  $\mu\text{m}$  fraction. This supports the result of hierarchical analysis (Figure 18) that separated process samples into FL (Cluster FL1) and PA (Clusters PA1-3) fractions and shows that the three PA clusters share

particle-associated microbial community structures.

**Figure 20**

*Ternary Diagrams of ASVs Across the 0.2, 3, and 20  $\mu$ m Filter Fractions in the Process Dataset*



*Note.* Each point represents one of 221 ASVs that passed the abundance filter; its position on the ternary indicates the normalized mean relative abundance distributed among the 0.2  $\mu$ m (free-living, top vertex), 3  $\mu$ m (lower-right vertex), and 20  $\mu$ m (bottom vertex) fractions, while point size encodes the mean within-sample relative abundance of the ASV across the 78 samples. (A) ASVs colored by the four sample clusters, defined as the cluster in which the ASV reached its highest mean relative abundance; (B) ASVs colored by taxonomic class: Bacteroidia (green), Alphaproteobacteria (red), and Gammaproteobacteria (orange); all other classes are pooled as "Other" (grey).

The three major bacteria classes were present in both FL and PA fractions (Figure 20; Table 4). However, the most abundant ASVs were rarely found to be specific to one size fraction and were typically intermediate between 2 or 3 size fractions. Gammaproteobacteria had higher mean abundance (40.4 %) in the 0.2  $\mu$ m fraction than other fractions (29.1 % in 3  $\mu$ m and 30.5 % in 20  $\mu$ m), making them the most free-living-biased class of the three. Bacteroidia showed balanced portions of their abundance in each filter fractions, but the portion was relatively higher in 3  $\mu$ m (36.0 %) than in other filter fractions (32.0 % in 0.2  $\mu$ m and 20  $\mu$ m). Alphaproteobacteria also showed balanced portions in each filter, but their abundance was somewhat higher in large-sized filter fractions than in 0.2  $\mu$ m filter fraction (35.1 % in 20  $\mu$ m, 34.3 % in 3  $\mu$ m, and 30.6 % in 0.2  $\mu$ m), driven by abundant Rhodobacteraceae members such as *Sulfitobacter* and *Loktanella*, known as bloom-responsive Alphaproteobacteria (Bergen et al., 2016; Sidhu et al., 2023). All three classes were present in every fraction, but each leaned toward a different one. Niche partitioning at the class level thus appears to come from abundance shifts, not from being absent from any fractions.

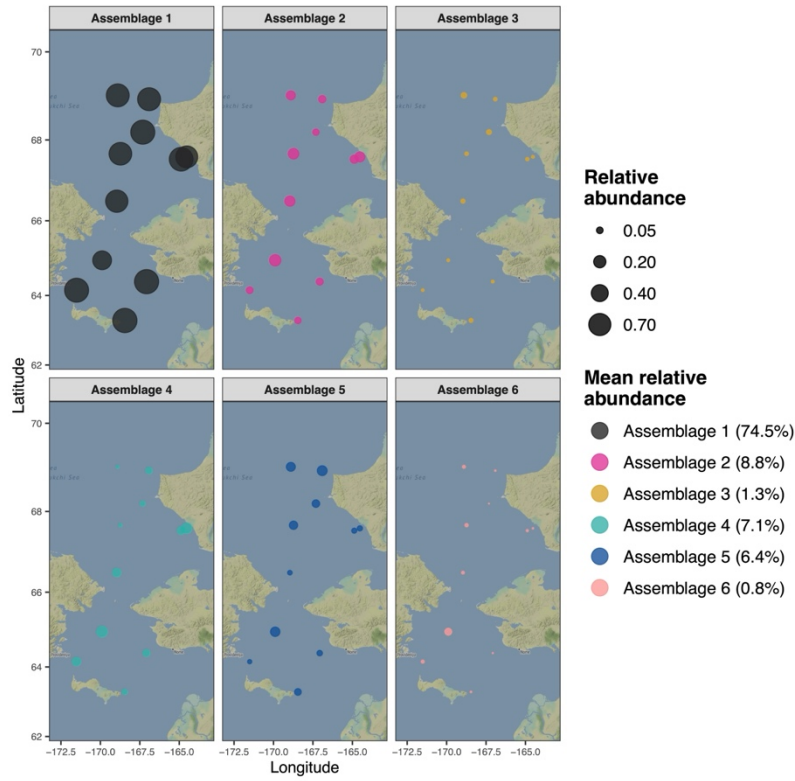
**Table 4***The Ratio of Three Bacterial Classes in Each Size Fraction*

| Filter size (µm) | <i>n</i> | Alphaproteobacteria<br>(%) | Gammaproteobacteria<br>(%) | Bacteroidia (%) |
|------------------|----------|----------------------------|----------------------------|-----------------|
| 0.2              | 23       | 30.6                       | 40.4                       | 32.0            |
| 3                | 27       | 34.3                       | 29.1                       | 36.0            |
| 20               | 28       | 35.1                       | 30.5                       | 32.0            |

*Note.* The within-sample relative abundance of all ASVs for each class was summed within each filter fraction (0.2 µm: *n* = 23; 3 µm: *n* = 27; 20 µm: *n* = 28). The per-fraction mean was then calculated across samples, and the three filter totals were normalized to sum to 1, yielding the proportion of each class's overall relative abundance contributed by each fraction.

ASVs in six assemblages were also investigated. Likewise, ASVs in the survey dataset, Assemblage 1 ASVs showed cosmopolitan-like distribution, and their mean relative abundance was 74.5 % (Figure 21). However, compared to the survey dataset, six assemblages in the process dataset showed weaker biogeographical patterns as the number of samples are smaller than the survey dataset and the four sample clusters were separated based on FL and PA lifestyles, not the water mass differences.

**Figure 21***Sampling Sites of ASVs in Six Assemblages in the Process dataset*

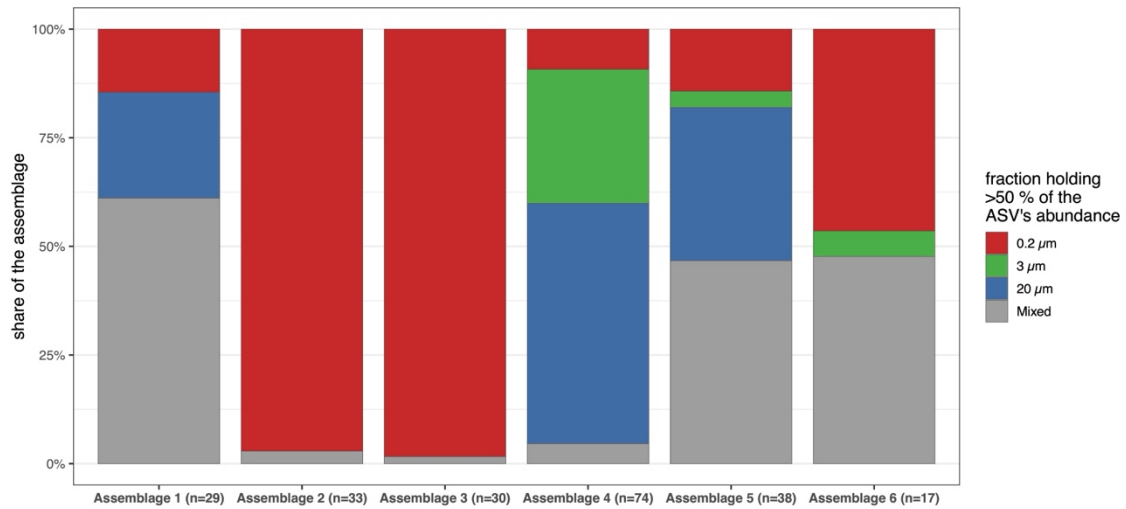


*Note.* Each Assemblage is assigned to different colors. The dots correspond to mean relative abundance of ASVs in six assemblages. Mean relative abundance of ASVs in each Assemblage is shown as legend.

However, the bar plots showed different preferences toward different-sized filters (Figure 22). For instance, ASVs in Assemblage 1 were highly abundant throughout the Bering and Chukchi Seas, but the relative abundance of most ASVs were higher than 50 % in all size-fractions. Assemblage 5 and 6 also showed high abundance of ASVs ( $\approx 50\%$ ) among all size-fractions. On the other hand,  $> 90\%$  of ASVs in Assemblage 2 and 3 were collected with  $0.2\ \mu\text{m}$  filter, suggesting that those assemblages were composed of FL communities. In contrast, Assemblage 4 was PA community as ASVs were largely collected with 3 and  $20\ \mu\text{m}$  filters.

## Figure 22

*Relative Abundance of ASVs in Six Assemblages With Each Filter in the Process Dataset*



*Note.* ASVs whose relative abundance were greater than 50 % were assigned to each filter-sizes. Each filter is represented as different colors (red: 0.2  $\mu\text{m}$ , green: 3  $\mu\text{m}$ , blue: 20  $\mu\text{m}$ ). Gray color means that ASVs whose abundance is spread across the size fractions, with no single filter holding more than 50 % of the ASV's abundance.

Overall PERMANOVA confirmed that the four clusters differed strongly in composition ( $F = 15.08$ ,  $R^2 = 0.379$ ,  $p = 0.001$ ), with the cluster factor explaining about 38 % of the total variation. However, PERMDISP was also significant ( $F = 7.46$ ,  $p < 0.001$ ), indicating that the four clusters did not have homogeneous multivariate dispersions. Pairwise PERMANOVA remained highly significant in every pair (BH-adjusted  $p = 0.001$ ) with substantial effect sizes ( $R^2 = 0.19\text{--}0.39$ ). The largest separations involved Cluster FL1 vs. the three PA clusters ( $R^2 = 0.33\text{--}0.39$  for FL1 vs PA2-4), while the three PA clusters differed less from each other ( $R^2 = 0.19\text{--}0.22$  for the 2 vs 3, 2 vs 4, and 3 vs 4 pairs). On the other hand, pairwise PERMDISP showed that the dispersion difference came from Cluster PL3. Cluster PL3 had significantly greater dispersion than Cluster FL1 (Tukey's HSD,  $p < 0.001$ ), Cluster PL2 (Tukey's HSD,  $p = 0.011$ ), and Cluster PL4 (Tukey's HSD,  $p = 0.010$ ), whereas pairwise dispersion among Cluster FL1, PA2, and PA4 was statistically homogeneous. In summary, the four clusters represent genuinely distinct communities, based on three pieces of evidence: (1) the overall PERMANOVA has a large  $R^2$ ; (2) every pairwise comparison is significant; (3) Cluster FL1, PA2, and PA4 have similar within-cluster dispersion, so dispersion itself is not driving the result. Cluster PA3 has a distinct mean position from the other clusters but is internally heterogeneous, suggesting that this cluster forms a more diverse set of communities than other three clusters.

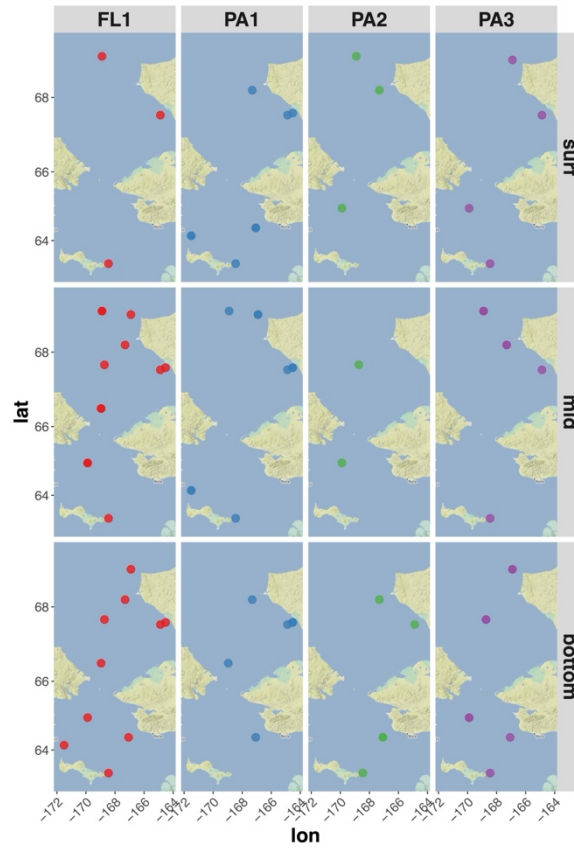
## 11.2 Geographic and Hydrographic Distribution of Sample Clusters

There were not strong gradients in vertical or horizontal spatial distributions of the sample clusters (Figure 23). Cluster FL1 ( $n = 25$ ) was the most geographically widespread, present at all 11 sampling stations (4 – 12 % per station), indicating that microbial communities in 0.2  $\mu\text{m}$  fraction possibly occur throughout the study area regardless of location. The three PA clusters showed small station-level differences that nevertheless spanned both seas. Cluster PA1 ( $n = 23$ ) was concentrated at stations in IL and CBW Transects (22 % in IL1.1, and 17 % in CBW5) and was absent from DBO stations (DBO2.4 and DBO3.8A). Cluster PA2 ( $n = 11$ ) was concentrated along DBO Transect (18 % each at CBE9, DBO2.4, DBO3.3) and was absent from stations in CBW, CL1, CNL, and IL Transects. Cluster 4 ( $n = 19$ ) was concentrated in the northern Bering and the northern Chukchi Seas (16 % each at CBE1, CL1, CL3, and DBO2.4) and absent from station in CBW, CNL, and IL Transects. More than 60 % of samples in Cluster FL1, PA1, and PA3 were collected from the Chukchi Sea ( $\text{lat} \geq 65.7^\circ\text{N}$ ), while samples in Cluster PA2 were evenly collected from both Bering and Chukchi Seas (50 % each), implying that high within-cluster dispersion in Cluster PA2 can be attributed to this result.

Within the water column, Cluster PA1 was the only cluster enriched at the surface, with 35 % of its samples coming from surface water compared with 12 – 27 % in the other clusters. On the other hand, samples in Cluster PA2 were largely collected from the bottom water (45 %). Cluster PA3 was similarly weighted toward deeper layers, with 79 % of its samples coming from the mid + bottom waters (37 + 42 %). Cluster FL1 drew most of its samples from the mid and bottom layers (48 + 40 % combined) and had the lowest share of surface samples (12 %). Although sampling stations were similar in all clusters, the four clusters showed different ecological gradients from the surface (Cluster PA1) to mid and bottom layers (Clusters FL1 and PA3) and finally to a strongly bottom-biased deep niche (Cluster PA2).

### **Figure 23**

*Maps of Sampling Stations for Each of the Four Sample Clusters*

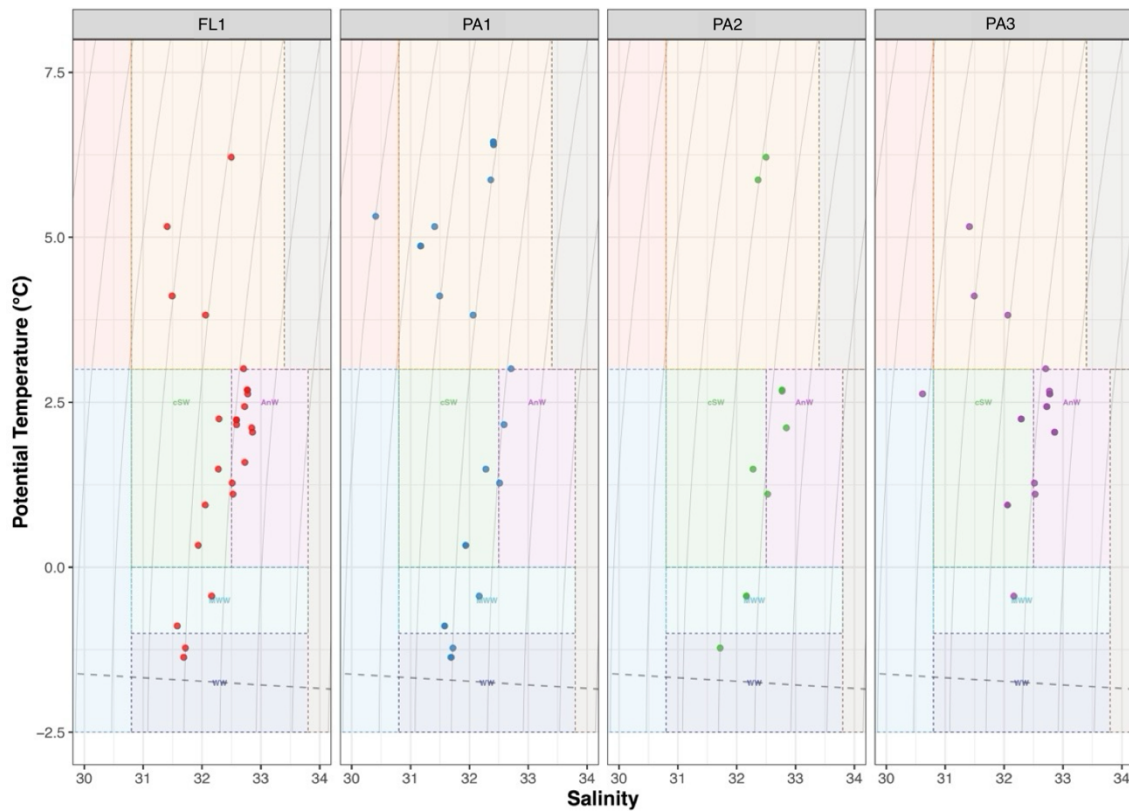


*Note.* Each dot is colored by the four clusters: Cluster 1 (red;  $n = 25$ ), Cluster 2 (blue;  $n = 23$ ), Cluster 3 (green;  $n = 12$ ), and Cluster 4 (purple;  $n = 19$ ). Each map is separated by depth (surface, middle, and bottom).

No clear differences in water mass characterizations were observed across the four sample clusters, with each cluster found in multiple water masses (Figure 24; Table 5). Clusters FL1 (FL mid-bottom cluster) and PA3 (PA mid-bottom cluster) were both dominated by AnW (48 % and 47 %, respectively), with warm Shelf Water (wSW) as the secondary water mass in Cluster FL1 and cold Shelf Water (cSW) in Cluster PA3. Cluster PA2 (PA deep cluster) was also AnW-dominated (40 %) but had the highest proportional MWW content of any cluster (20 %). On the other hand, Cluster PA1 (surface PA cluster) was the only cluster whose primary water mass was wSW (48 %), reflecting its warmer and lower-salinity character, with Modified Winter Water (MWW) emerging as a secondary water mass (13 %).

## Figure 24

*T-S Diagrams With Water Mass Boundaries for Each of the Four Clusters*



*Note.* Dashed rectangles indicate the T-S ranges of each water mass: warm Coastal Water (wCW), cool Coastal Water (cCW), Ice Melt Water (IMW), warm Shelf Water (wSW), cool Shelf Water (cSW), Anadyr Water (AnW), Modified Winter Water (MWW), Winter Water (WW), Atlantic Water (AtlW), Bering Basin Water (BBW). Gray contours represent isopycnals (lines of equal density,  $\sigma_\theta$ , at 0.5 kg/m<sup>3</sup> intervals). The dashed black line indicates the freezing point.

**Table 5**

*The Proportion of Samples in the Four Clusters, Classified Into Each Water Mass*

| Cluster                           | Primary water mass | Secondary water mass |
|-----------------------------------|--------------------|----------------------|
| FL1                               | AnW (48 %)         | wSW (20 %)           |
| PA1                               | wSW (47.8 %)       | MWW (13 %)           |
| PA2                               | AnW (40 %)         | MWW (20 %)           |
| PA3                               | AnW (47.4 %)       | cSW (21.1 %)         |
| Fisher's exact test (Monte Carlo) |                    |                      |
| Replicates                        | <i>p</i>           | Significance         |
| B = 9,999                         | = .126             | ns                   |

*Note.* The primary water mass was defined as the water mass with the highest proportion of

samples, and the secondary water mass as the one with the second highest proportion. Water-mass abbreviations are defined in Table 1. *ns* = not significant.

A total of 78 samples were collected from Bering Sea ( $n = 29$ ) and Chukchi Sea ( $n = 48$ ) from different depths: surface ( $n = 9$ ), middle ( $n = 8$ ), and bottom ( $n = 12$ ) in the Bering Sea; surface ( $n = 9$ ), middle ( $n = 22$ ), and bottom ( $n = 17$ ) in the Chukchi Sea. The FL and PA communities did not significantly differ with depth. The PA community accounted for most of the microbial community composition in surface waters (mean: 73.2 %) and declined at mid and bottom depths (mean: 59.7 % and 61.7 %, respectively), while the FL community increased from 26.8 % at the surface to 40.3 % and 38.3 % at mid and bottom depths. However, Kruskal–Wallis test revealed that this proportional difference of bacterial ASVs in each depth was not statistically significant ( $\chi^2 = 1.95$ ,  $df = 2$ ,  $p = 0.38$ ). This is consistent with the result from the survey samples and can be attributed to the shallowness and the weak stratification in the Bering and Chukchi Seas during June 2017 (Figure 15).

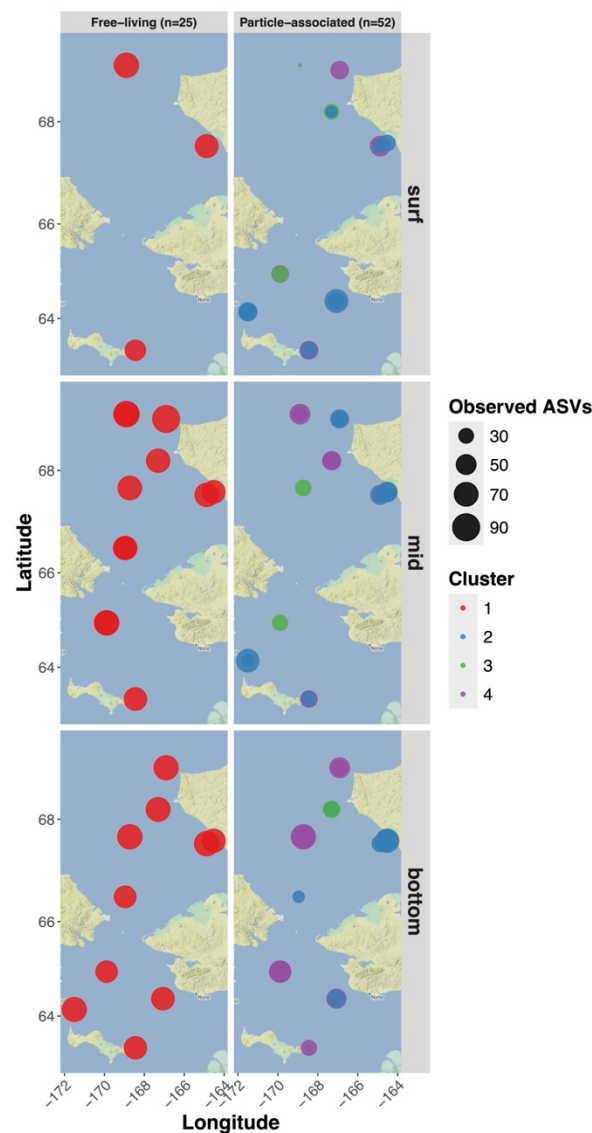
Figure 25 is the map showing the depth profile of ASV richness in the FL and PA clusters. Samples from each cluster were mainly collected from the Chukchi Sea, except Cluster PA2 whose samples were evenly collected from both Bering and Chukchi Seas (5 samples each). In most clusters, the station with the highest ASV richness shifted with depth. In Cluster FL1, ASV richness peaked at CL3 (74 observed ASVs) at the surface, at CL1 (90) at mid-depth, and at IL2 (74) at the bottom. In Cluster PA1, richness peaked at CBE9 (65 observed ASVs) at the surface, at CBW5 (64) at mid-depth, and at IL1.1 (67) at the bottom, with little change in the peak value. In Cluster PA2, the maximum remained among the DBO stations (DBO2.4, DBO3.8A, and DBO3.3) at all depths but stayed at consistently low richness (32–36 observed ASVs). In Cluster PA3, richness peaked at IL2 (49) and CL3 (50) at the surface and mid-depth and reached its highest value at DBO3.8A (74) at the bottom.

However, along with the depth profiles of the FL and PA communities, Kruskal–Wallis tests were conducted to assess whether ASV richness (observed ASVs) differed in each depth and showed no significant depth effect on richness for any clusters after Bonferroni correction ( $\alpha = 0.0125$ ): Cluster FL1 ( $\chi^2 = 1.87$ ,  $df = 2$ ,  $p = 0.392$ ), Cluster PA1 ( $\chi^2 = 0.42$ ,  $df = 2$ ,  $p = 0.810$ ), Cluster PA2 ( $\chi^2 = 2.08$ ,  $df = 2$ ,  $p = 0.354$ ), and Cluster PA3 ( $\chi^2 = 4.90$ ,  $df = 2$ ,  $p = 0.086$ ). Although Cluster PA3 tended to show higher richness at the bottom (median 53.5 observed ASVs) than at the surface and mid depths (40.5 and 36.0, respectively), this trend did not remain significant after Bonferroni correction for multiple comparisons. Across all samples, richness likewise did not differ significantly among depths ( $\chi^2 = 3.07$ ,  $df = 2$ ,  $p = 0.215$ ). These results indicate that ASV richness was similar across depths in this region during the sampling period. However, depth should not be regarded as unimportant. Salinity and nutrient

concentrations still changed with depth, and it is through these gradients that depth acts here.

**Figure 25**

*ASV Richness in Free-Living and Particle-Associated Communities, Shown by Depth (Surface, Middle, and Bottom)*



*Note.* Each point represents sampling location. Point size is proportional to the within-sample relative abundance (%) of the indicated assemblage and point color indicates the assignment of the sample to one of the four hierarchical clusters.

In survey samples, Bacteroidia showed high abundance at DBO3 stations and other transects nearby (Table 3). This can be explained by the active diatom bloom caused by *Chaetoceros* and *Thalassiosira* at DBO3 stations during June 2017. On the other hand, in

process samples, two sample stations were in DBO3 Transect (DBO3.3 and 3.8A stations), and each cluster evenly had samples from both stations (Table 6). Samples from CBW Transect and other transects near DBO3 stations showed high abundance of Bacteroidia, while DBO3 stations showed relatively lower abundance of this class compared to those transects. This contrasts with the survey dataset ( $> 35\%$ ) at DBO3 stations and nearby transects but can be explained by the difference of sample sizes and locations; 30 samples from 8 DBO3 stations were collected in survey samples, while 13 samples from 2 DBO3 stations were collected in process samples. However, though Bacteroidia were proportionally lower at DBO3 than at other process transects, it still accounted for a substantial fraction of the community (32.6 %), the second most abundant class after Gammaproteobacteria, and only marginally below the  $\geq 35\%$  level observed in survey samples. Also, At the family–genus level, ASVs belonging to Bacteroidia, such as *Ulvibacter*, uncultured Flavobacteria, *Polaribacter*, and NS11-12 marine group, accounted for the majority of the highly abundant ASVs, whereas the high abundance of Gammaproteobacteria was driven largely by a single genus, *Alteromonas* (16.7 %). Therefore, the two datasets differed at DBO3 in the relative abundance of these classes, not in which taxa dominated. In contrast to the survey, where the divisions were based on water mass and hierarchical clustering, the four clusters in process samples were separated by filter size fractions. Nevertheless, the high relative abundance of the major bacterial classes was likewise confirmed, suggesting that the consistent high abundance of those major bacterial classes was recovered during ASGARD 2017 cruise regardless of how the communities were partitioned.

**Table 6**

*Composition of the Dominant Three Bacterial Classes (Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia) Across the Seven Transects*

| Transect | <i>n</i> | Alphaproteobacteria (%) | Gammaproteobacteria (%) | Bacteroidia (%) |
|----------|----------|-------------------------|-------------------------|-----------------|
| DBO3     | 13       | 20.9                    | 40.8                    | 32.6            |
| CBW      | 5        | 23.3                    | 16.8                    | 50.0            |
| CL       | 15       | 24.7                    | 28.8                    | 42.2            |
| IL       | 16       | 19.9                    | 30.7                    | 43.0            |
| DBO2     | 8        | 24.8                    | 36.4                    | 31.3            |
| CBE      | 16       | 19.6                    | 30.7                    | 43.8            |
| CNL      | 4        | 23.8                    | 25.3                    | 43.3            |

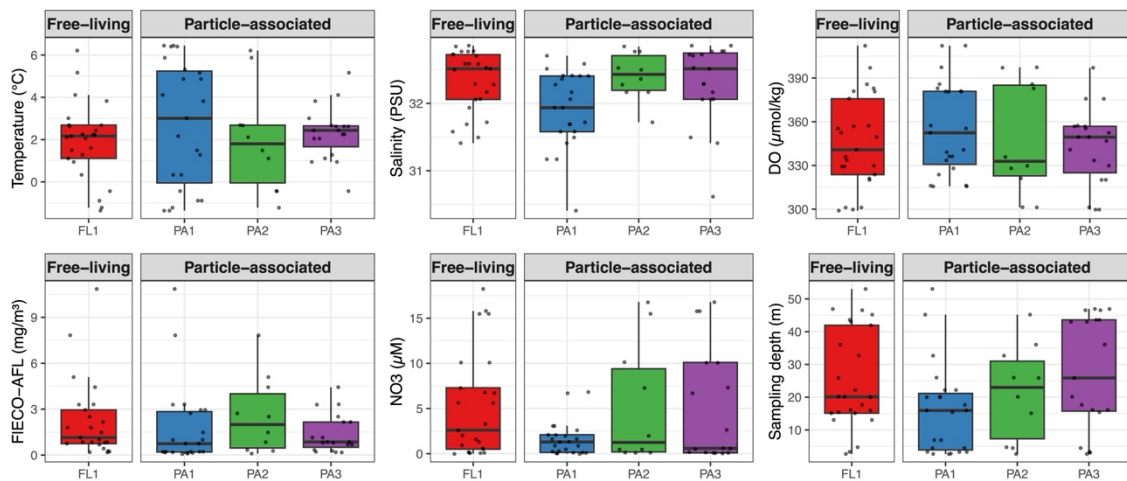
*Note.* The number of samples is shown in the *n* column. Each proportion is the median relative abundance of the class.

### 11.3 Physicochemical Characterization of Sample Clusters

No significant differences ( $p < 0.05$ ) in physicochemical characteristics were observed across clusters using six environmental variables (Figure 26). Cluster FL1 was characterized by an intermediate temperature (median: 2.16 °C), an intermediate sampling depth (median: 20.1 m), and the highest  $\text{NO}_3$  of any cluster (median: 2.60  $\mu\text{M}$ ). Among the three PA clusters, Cluster PA1 was the most distinct: it had the highest temperature (mean: 3.01 °C), the lowest salinity (mean: 31.9), the highest dissolved oxygen (mean: 352.4  $\mu\text{mol kg}^{-1}$ ), and the shallowest sampling depth (mean: 15.9 m), all consistent with wSW-influenced post-bloom surface conditions. Cluster PA2 had cold (mean: 1.80 °C), high-salinity (mean: 32.4) deep water and showed the highest FIECO-AFL fluorescence (mean: 2.00  $\text{mg m}^{-3}$ ), suggesting an association with AnW-influenced pre- or during-bloom conditions. Cluster PA3 occupied the deepest samples (mean: 25.9 m) and had the lowest  $\text{NO}_3$  (mean: 0.60  $\mu\text{M}$ ) and one of the lowest FIECO-AFL values (mean: 0.86  $\text{mg m}^{-3}$ ), suggestive of a post-bloom community.

**Figure 26**

*Boxplots of Environmental Variables for the Four Clusters, Grouped by Free-Living (FL) and Particle-Associated (PA) Clusters*



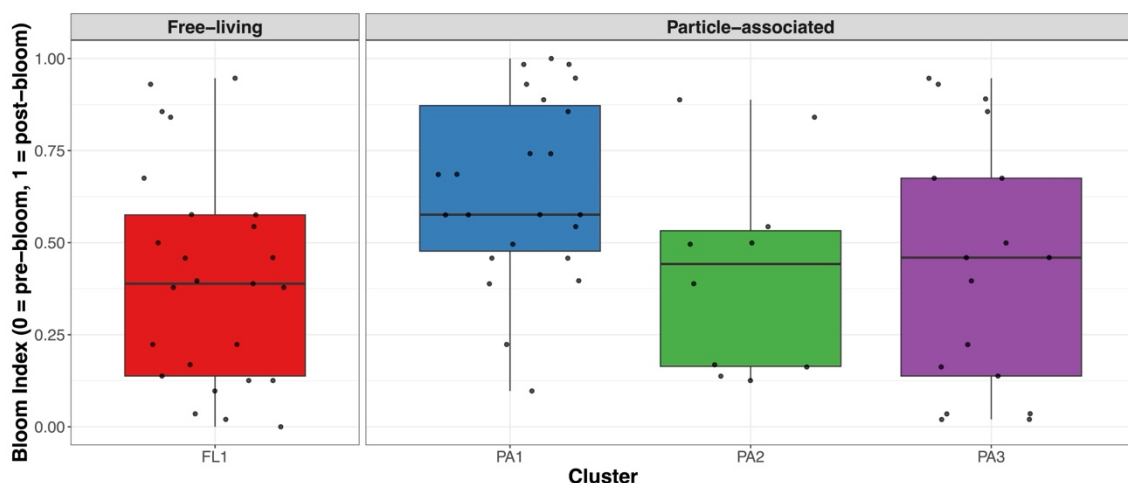
*Note.* Each panel shows one of six variables: temperature (°C), salinity (PSU), DO ( $\mu\text{mol/kg}$ ), FIECO-AFL (chlorophyll fluorescence,  $\text{mg m}^{-3}$ ),  $\text{NO}_3$  ( $\mu\text{M}$ ), and sampling depth (m). Individual samples were overlaid as jittered points. Boxes represent the interquartile range (IQR), with the horizontal line indicating the median. Whiskers extend to the most extreme values within  $1.5 \times$

IQR.

The bloom index (rescaled PC1) was not significantly different across the four clusters ( $p < 0.05$ ) (Figure 27). The median bloom index was highest in Cluster PA1 (0.58), suggestive of Cluster PA1 as a post-bloom community characterized by nutrient-scarce warmer shallower water. The three other clusters showed lower bloom indices (0.39 in Cluster FL1; 0.44 in Cluster PA2; 0.46 in Cluster PA3). Cluster FL1 had the lowest bloom index, consistent with a free-living community persisting in nutrient-replete, mid-depth waters. Clusters PA2 and PA3 occupied intermediate bloom index values; Cluster PA2 had the highest FIECO-AFL concentration, and Cluster PA3 had the lowest FIECO-AFL and  $\text{NO}_3$  concentrations, suggestive of during-bloom and post-bloom conditions, respectively.

**Figure 27**

*Bloom Index (Rescaled PC1) for the Four Sample Clusters, Grouped by Free-Living (FL) and Particle-Associated (PA) Clusters*



*Note.* The bloom index was derived from PC1 of a PCA on nine environmental variables, rescaled from 0 (pre-bloom, nutrient-replete) to 1 (post-bloom, nutrient-depleted). Boxes represent the interquartile range with the median shown as a horizontal line. Individual samples are overlaid as jittered points.

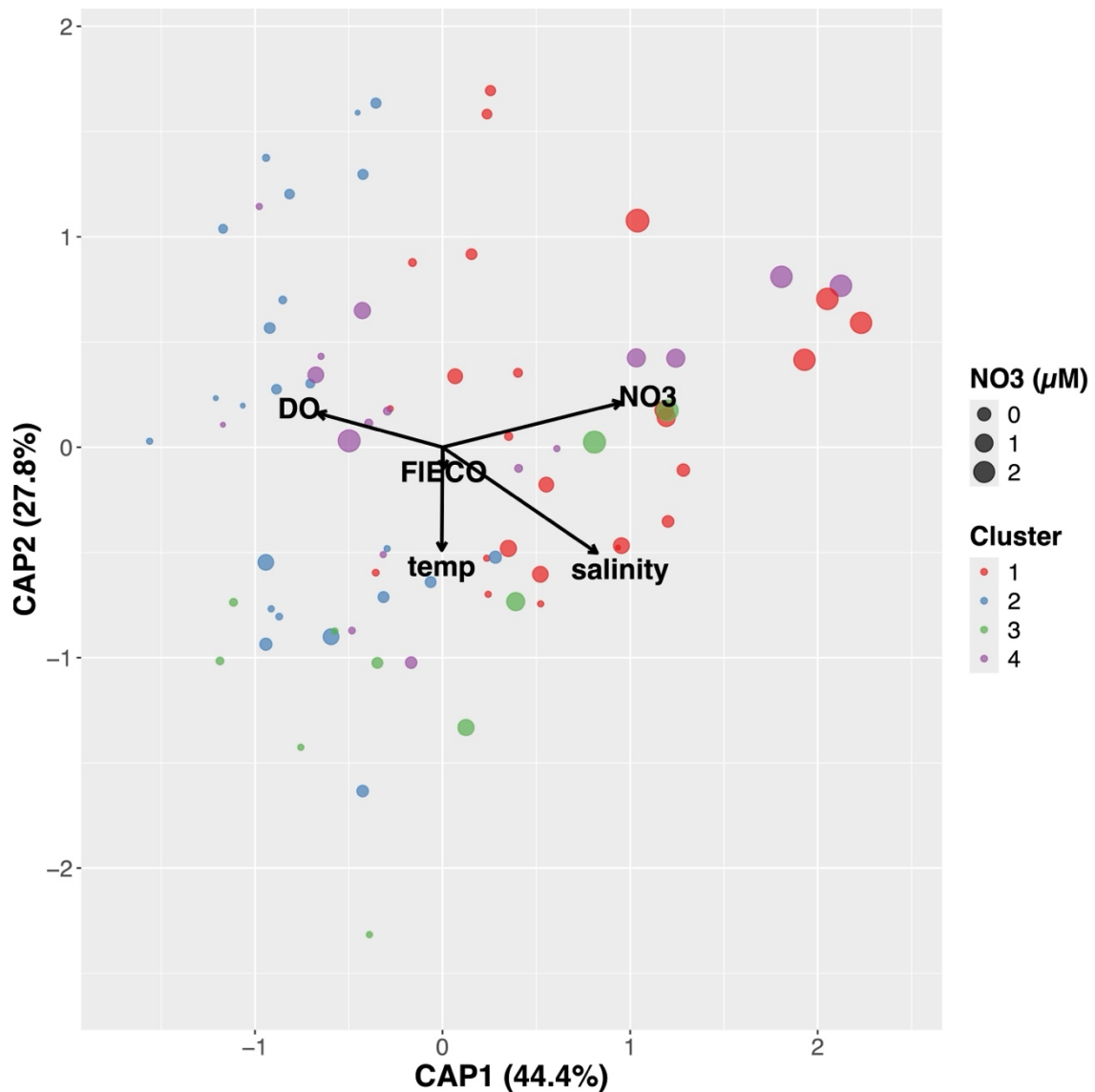
Ordination using dbRDA also failed to reveal strong relationships between cluster community structure and five selected environmental parameters (temperature, salinity, DO,  $\text{NO}_3$ , and FIECO-AFL; Figure 28). The constrained ordination model was statistically tested by permutational ANOVA (999 permutations). The overall db-RDA was highly significant ( $F = 2.66$ ,  $p = 0.001$ ), with the five environmental parameters collectively explaining only 16.2 % of

variation in community composition (adjusted  $R^2 = 0.10$ ). Among individual variables, only salinity had a significant marginal effect ( $F = 3.15$ ,  $p = 0.004$ ); FIECO-AFL fluorescence, temperature, dissolved oxygen, and  $\text{NO}_3$  were not significant. Of the five constrained axes, only CAP1 ( $F = 5.91$ ,  $p = 0.002$ ) and CAP2 ( $F = 3.76$ ,  $p = 0.031$ ) were significant, justifying the use of the first two axes for biplot interpretation. Together these results indicate that process-site community variation was structured primarily along a salinity axis, with a smaller contribution from FIECO-AFL. CAP1 (44.4 % of constrained variance) was positively associated with  $\text{NO}_3$  (+0.95) and high salinity (+0.82), and negatively with dissolved oxygen (−0.67); capturing a nutrient–salinity gradient: nutrient-rich, salty, oxygen-poor deep water on one side, and nutrient-poor, fresher, oxygen-rich surface water on the other. CAP2 (27.8 % of constrained variance) was driven mostly by temperature (−0.49) and salinity (−0.50), with a small positive contribution from DO (+0.16), capturing a temperature–salinity gradient, separating warmer, fresher shelf water from colder, saltier source water at depth.

On the CAP ordination, the four clusters separated along ecologically meaningful gradients. Cluster FL1 was placed at high CAP1, driven by high  $\text{NO}_3$  and salinity. Cluster PA1 sat at low CAP1, driven by low  $\text{NO}_3$  and salinity, and high dissolved oxygen, consistent with a post-bloom surface condition. Cluster PA2 located at the lowest CAP2, where its centroid was pulled by relatively low DO and temperature contrast captured by CAP2. Cluster PA3 sat near the center of the plot, slightly offset toward Cluster FL1, reflecting a community that shares many features with the other deep-water clusters but with a less distinct environmental signature.

## Figure 28

*Distance-Based Redundancy Analysis (dbRDA) of the Four Clusters Constrained by Five Environmental Variables*



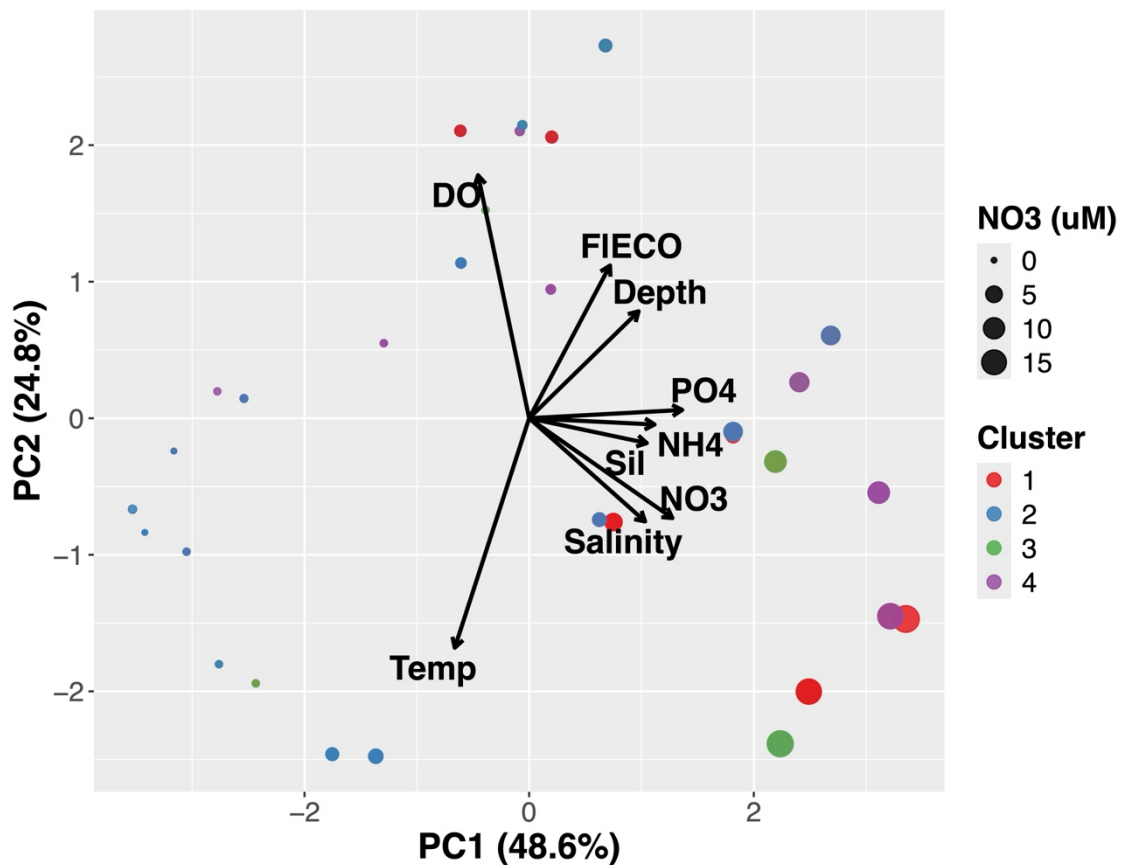
Note. Temperature, salinity, DO, NO<sub>3</sub>, and FIECO-AFL were used. Samples are plotted on the first two canonical axes (CAP1, 44.4 %; CAP2, 27.8 %), colored by the four process clusters, with dot size scaled to NO<sub>3</sub> concentration (μM). Arrows indicate the direction and relative strength of each environmental variable's contribution to the constrained ordination.

The equivalent PCA for the process samples ( $n = 75$ ) recovered essentially the same structure (Figure 29). PC1 explained 48.6 % and PC2 24.8 % of the variance (73.4 % cumulative). As in the survey dataset, PC1 separated positive loadings for PO<sub>4</sub> (0.45), NO<sub>3</sub> (0.43), NH<sub>4</sub> (0.37), silicate (0.35), salinity (0.34) and depth (0.32) from a negative loading for temperature (−0.22), and PC2 was again dominated by dissolved oxygen (0.59) versus temperature (−0.56). The process samples likewise formed a curved arch along PC1, reproducing the horseshoe/arch pattern of a single continuous gradient. Therefore, both datasets shared almost identical variance

partitioning (PC1  $\approx$  48 % in both datasets), loadings and arch shape. This indicates that the survey and process samples are governed by the same primary physicochemical gradient and the two datasets are directly comparable despite their different spatial coverage and sample numbers.

**Figure 29**

*PCA Biplots of Nine Environmental Variables in the Process Dataset*



*Note.* Principal component analysis (PCA) of the environmental data in the process dataset is shown. Nine variables (temperature, salinity, dissolved oxygen, NO<sub>3</sub>, PO<sub>4</sub>, silicate, NH<sub>4</sub>, fluorescence and depth) were used, with nutrients and fluorescence log-transformed and all variables standardized prior to PCA. Each point is a sample, positioned by its PC1 and PC2, colored by sample clusters, and sized by nitrate concentration (μM). Black arrows show the loadings of the environmental variables. Axes are labelled with the percentage of variance explained (PC1 48.6 %, PC2 24.8 %, n = 75).

## 12. Discussion

### 12.1 Ecological Characterization

In our study, contrary to the survey dataset, six assemblages were characterized by different lifestyles (Figure 18, 20, and 22). Also, ASVs belonging to Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia in the process dataset accounted for 93.9 % of the total relative abundance, consistent with previous work in this region (Chen et al., 2008; Lee et al., 2019; Zeng et al., 2012; Zeng et al., 2013a), as well as with our survey dataset (94.7 %). However, the relative importance of these classes differed between datasets. Gammaproteobacteria was the most abundant class in the survey dataset, whereas Bacteroidia was the most abundant (40.2 %) in the process dataset. This is likely because the process dataset was size-fractionated by sequential filtration into free-living and particle-associated samples, and Bacteroidia are strongly particle-associated, attaching to and breaking down algal particles. Gammaproteobacteria, which are more common in the general community, were most abundant in the survey dataset. Bacteroidia was abundant across all size fractions, including the 0.2  $\mu\text{m}$  free-living fraction (38.5 %), and not only in the 3 and 20  $\mu\text{m}$  particle-associated fractions. Because Bacteroidetes are closely associated with algal blooms and with the degradation of high molecular weight compounds produced by phytoplankton (Bunse & Pinhassi, 2017; Needham & Fuhrman, 2016; Needham et al., 2018), the higher Bacteroidia abundance in the process dataset may reflect the bloom-influenced conditions of the process stations.

A pyrosequencing study in the Chukchi Sea, the Beaufort Sea, and Franklin Bay reported that Gammaproteobacteria and Alphaproteobacteria together accounted for 22–30 % of all sequences, and that the two most abundant clusters at 97 % similarity were SAR11 and one belonging to Bacteroidetes (Kirchman et al., 2010). Using amplicon sequencing across the Arctic including the Chukchi Sea, and the Antarctic, Ghiglione et al. (2012) found that most OTUs in both polar oceans belonged to Gammaproteobacteria, Alphaproteobacteria, and Bacteroidia, along with ubiquitous, metabolically versatile OTUs such as SAR11 and SAR324 (Malfertheiner et al., 2022); *Polaribacter*, a representative bloom-associated bacterium, was among the Arctic-specific OTUs. Pedrós-Alió et al. (2015) further reported that temperate communities were dominated by Alphaproteobacteria (approximately 50 %), whereas Arctic communities held nearly equal proportions of Alphaproteobacteria and Gammaproteobacteria (about 20 % each), with Bacteroidetes exceeding the global average (18 % vs. 4 %). The reasons for the high abundance of Bacteroidetes in polar oceans remain unclear, although the quantity and quality of available substrates are thought to be involved (Kirchman et al., 2007). Metagenomic analyses have similarly documented the dominance of Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes in the Arctic Ocean. Cao et al. (2020), examining metagenomes from the poles to the temperate region, showed that 90 phylum-level taxa

classified from 24,504 OTUs mostly belonged to Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes, which were highly abundant in both surface and deep-water Arctic samples. Royo-Llonch et al. (2021) analyzed 530 MAGs reconstructed from the Arctic Ocean and found that more than half belonged to Gammaproteobacteria (22.8 %), Bacteroidota (20.9 %), and Alphaproteobacteria (11.5 %), while the 12 most abundant MAGs represented unknown species within those groups and other phyla such as Actinobacteria and SAR324.

The association between bacteria and algal blooms has been studied from temperate regions to the Arctic Ocean. In the North Sea, the SAR11 clade was abundant in pre-bloom communities, Bacteroidetes such as *Formosa*, *Polaribacter*, and *Ulvibacter* during the bloom, and Gammaproteobacteria such as the SAR92 clade post-bloom (Liu et al., 2013; Sintes et al., 2013; Teeling et al., 2012; Teeling et al., 2016). Although those results were from temperate region, these patterns are consistent with our results. In Franklin Bay, Bacteroidetes and Alphaproteobacterial OTU *Roseobacter*, that is a major marine bacterium contributing to carbon cycling and aerobic anoxygenic photosynthesis (Brinkhoff et al., 2008), peaked at the chlorophyll-a maximum, whereas Gammaproteobacteria became abundant afterward (Alonso-Sáez et al., 2008). Through the northern Barents Sea into the area east of Svalbard, Gammaproteobacteria ASVs (e.g., SAR92, OM60, and Nitrincolaceae) and Bacteroidetes ASVs (e.g., *Formosa*, *Polaribacter*, and the NS9 marine group) became abundant during the spring–summer sea-ice algal bloom, while SAR11 and some nitrifiers dominated winter samples (Thiele et al., 2023). A time-series study from the Dease Strait assigned 80 % of reads to Alphaproteobacteria (33.2 %), Gammaproteobacteria (28.7 %), and Bacteroidia (21.6 %) (Redondo-Río et al., 2024) and Alphaproteobacteria dominated the pre-bloom community, Bacteroidetes rose during the bloom, and Gammaproteobacteria increased over the bloom but declined once Bacteroidetes rose exponentially.

Several Arctic studies have described the taxonomic composition of FL and PA bacterial communities. In surface waters along the Mackenzie River–Beaufort Sea transects (Canadian Arctic), Ortega-Retuerta et al. (2013) found that the offshore PA fraction was dominated by Bacteroidetes and Gammaproteobacteria, whereas the FL fraction was dominated by Alphaproteobacteria. In the river-influenced Laptev Sea, Kellogg and Deming (2009) separated FL (0.22–1  $\mu\text{m}$ ), suspended-particle (1–60  $\mu\text{m}$ ), and aggregate (>60  $\mu\text{m}$ ) fractions and reported bacterial libraries dominated overall by Gammaproteobacteria, the Cytophaga–Flavobacteria–Bacteroidetes (CFB) group, and Alphaproteobacteria, with the aggregate-associated communities being the most distinct. At subsurface stations in the high-Arctic fjord Kongsfjorden, Jain et al. (2019) found that PA communities were enriched in carbohydrate-degrading Bacteroidetes (and Verrucomicrobia), consistent with the general association of Bacteroidetes with particles.

However, as shown in Table 4, our results were not consistent with results from those studies. For example, the relative abundance of Alphaproteobacteria was higher in 3 and 20  $\mu\text{m}$  filters than in 0.2  $\mu\text{m}$ . Also, Gammaproteobacteria was highly abundant in 0.2  $\mu\text{m}$  filter (40.4 %). On the other hand, Bacteroidia showed higher abundance in 3  $\mu\text{m}$  filter than other filters. Even if Alphaproteobacteria tend to be found in the FL fraction and Gammaproteobacteria and Bacteroidia in the PA fraction, the degree of FL–PA separation is context-dependent and strongly shaped by hydrography. Moreover, salinity gradients, suspended-particle load, and water residence time can outweigh a simple FL vs. PA dichotomy (LaMontagne & Holden, 2003; Selje & Simon, 2003; Zhang et al., 2007), and in some particle-rich estuaries the two fractions are almost indistinguishable due to rapid exchange between them (Hollibaugh et al., 2000). This probably applies to our study area. The Bering and Chukchi shelves are shallow, and the water column was weakly stratified in June 2017. Particles were also sinking rapidly at the time: chlorophyll-*a* export at DBO3 reached about  $5 \text{ mg m}^{-2} \text{ d}^{-1}$  (Lalande et al., 2021). Under these conditions, cells can move between the free-living and the particle-associated state. This kind of exchange would make the two fractions look more alike, not less, so the separation we found is best read as a minimum. It may also help explain why the environmental signal was weak here, with salinity the only significant variable (adjusted  $R^2 = 0.10$ ). Therefore, in the Bering and Chukchi regions, which are undergoing rapid environmental change, the relative abundances of these bacterial classes may not always follow the typical pattern of dominant classes observed in FL and PA fractions.

## 12.2 Microbial Community by Depth

In temperate and other lower-latitude systems, depth is a consistent factor of structuring FL and PA bacterial communities, with community richness generally increasing with depth and a shift from surface oligotrophic FL groups to PA and deep water clades. In the northwestern Mediterranean, depth explained about 30 % of community variation (Crespo et al., 2013), and in the eastern Mediterranean FL diversity increased with depth as SAR406 and SAR202 became more abundant in deeper water (Roth Rosenberg et al., 2021). In the California Current, SAR11, Thaumarchaeota, and Rhizaria increased with depth, while surface phytoplankton-associated groups declined (Valencia et al., 2022), and in the Mauritania upwelling system, Bacteroidetes decreased and Deltaproteobacteria increased with depth, with FL–PA dissimilarity rising toward 200 m (Bachmann et al., 2018). In the open ocean, large sinking particles carried surface bacteria down into the deep sea, so that surface and deep communities resembled each other, whereas freely drifting (free-living) cells did not sink and their communities differed with depth (Mestre et al., 2018). On fast-sinking particles, the number of bacterial taxa decreased sharply with depth, until only a few fast-growing and nutrient-loving (copiotrophic) taxa remained (Baumas et al.,

2021). Even in the Southern Ocean, depth was significant, but it was less important than water masses and size fractions (Milici et al., 2017).

In the Arctic Ocean, community composition also changes with depth, as in temperate regions, even though the shallow shelves cover only a small range of depths. In the Chukchi Borderland, depth was the dominant factor of community variation. Alphaproteobacteria, including SAR11, decreased sharply with depth while Deltaproteobacteria increased, and depth alone explained more than 80 % of their variance (Han et al., 2015). Community richness increased with depth, and assemblages separated between the surface/subsurface-chlorophyll-maximum layer and deeper waters in the central Arctic (Balmonte et al., 2018) and in Fram Strait, where PA communities became progressively enriched in Gammaproteobacteria, Bacteroidia, and Planctomycetes with depth (Fadeev et al., 2021). In the high Arctic fjord Kongsfjorden, particle-associated diversity was higher and community assembly became more strongly driven by stochastic processes at greater depth (Jain et al., 2021). Nutrient and particle characteristics also change markedly with depth in Arctic shelf and coastal waters (Kellogg & Deming, 2009, 2014).

In our study, ASV richness did not show a correlation with depth (Figure 25). Also, the correlation between ASVs in each size fraction (20, 3, and 0.2  $\mu\text{m}$ ) and depth (surface, middle, and bottom) was not statistically significant (Fisher's exact test,  $p = 0.95$ ). This is not consistent with the changes in community composition with depth, commonly reported in deeper Arctic basins and open-ocean settings, and most likely reflects the shallowness of the Bering and Chukchi shelves, with a well-mixed or only weakly stratified water column (Figure 15). Roth Rosenberg et al. (2021) reported that lifestyle such as FL vs. PA can shape bacterial communities more strongly than depth or seasonality, with PA communities responding dynamically to bloom-derived organic matter as active degraders. Given this, the strong vertical gradients in light, temperature, oxygen, and organic matter supply that drive depth-related community change in deep basins might be weaker in this region during June 2017. Although depth commonly influences the Arctic bacterial communities, in the Bering and Chukchi Seas, depth might become a less important factor shaping bacterial communities when the stratification is weakened due to temperature anomaly.

### 12.3 Associations With Physicochemical Parameters

In temperate regions, salinity and particle concentration are the physicochemical variables most consistently linked to FL and PA bacterial community composition, together with temperature and phytoplankton-derived organic matter.

Along estuarine and coastal salinity gradients, salinity is the primary control. In the Baltic Sea, Gammaproteobacteria increased and Actinobacteria and Betaproteobacteria decreased with salinity, while PA Bacteroidetes increased toward fresher water (Rieck et al.,

2015). In the Weser estuary the proportion of PA bacteria tracked turbidity and suspended matter ( $r^2$  up to 0.95), and *Cytophaga*–*Flavobacteria* decreased from the freshwater to the marine section (Selje & Simon, 2003).

Particulate loads and the quality of organic matter are also important for the PA fraction more generally. In Victoria Harbour, suspended solids best explained the differences between communities (Zhang et al., 2007). During a North Sea bloom, combined carbohydrates and POC shaped the PA communities, and Gammaproteobacteria were related to carbohydrates, POC, chlorophyll a, and temperature, while Bacteroidetes were related to chlorophyll a (Sperling et al., 2017).

Temperature often acts as a water mass proxy. In the Mauritania upwelling system, temperature was the best predictor of the FL community, whereas the PA community was explained by temperature together with ammonium (Bachmann et al., 2018). By contrast, the FL fraction tended to correlate with inorganic nutrients and phytoplankton pigments (Roth Rosenberg et al., 2021). Interestingly, some open ocean and deep sea studies did not link specific taxa to specific environmental factors. Rather than physicochemical variables, they described community differences in terms of depth, particle size, or how fast particles sink (Baumas et al., 2021; Mestre et al., 2018).

In the Arctic, the same variables matter, but freshwater input and water masses play a much bigger role. Salinity is the variable most closely linked to microbial communities. Also, salinity serves as an indicator of dissolved organic matter from these sources as its freshened by terrestrial or glacial fresh water. Along the Mackenzie–Beaufort gradient, salinity shaped community composition and changed together with DOC, CDOM, and silicate, and using canonical correspondence analysis (CCA), open sea Alphaproteobacteria were separated from riverine Betaproteobacteria (Ortega-Retuerta et al., 2013). In the Chukchi Borderland, Alphaproteobacteria, including SAR11, correlated negatively with salinity, temperature, and density and positively with dissolved oxygen (Han et al., 2015), and temperature, salinity, and oxygen jointly explained surface-to-deep variation in the central Arctic (Balmonte et al., 2018).

River and glacial freshwater also play an important role, as the sources of nutrients and terrestrial organic matter to the Arctic Ocean. In the Laptev Sea, silicate and fresher water were associated with aggregate communities (Kellogg & Deming, 2009). In Svalbard fjords, DOC, nutrients, and  $\delta^{13}\text{C}$  followed meltwater and seasonal changes (Delpech et al., 2021; Jain & Krishnan, 2017). As in temperate waters, the FL fraction correlated with inorganic nutrients such as nitrite, phosphate, and silicate, whereas the PA fraction correlated with particle chemistry, including particulate carbohydrate,  $\delta^{13}\text{C}$ , C:N ratio, and dissolved oxygen (Jain et al., 2019; Jain & Krishnan, 2021). In the North Water and Beaufort Sea, phosphate and phytoplankton biomass (chlorophyll a and phaeopigment) correlated with all fractions (Kellogg

& Deming, 2014). In the western Arctic, carbon sinking flux strongly correlated with chlorophyll *a* ( $r = 0.96$ ) and negatively with the C:N ratio, and a diatom- and Gammaproteobacteria-rich group correlated with salinity (Qin et al., 2025). The time series study in Fram Strait reported that bacterial groups such as Flavobacteriales correlated with sea-ice concentration and particle fluxes (Cardozo-Mino et al., 2023).

In this study, salinity was the only environmental variable with a significant marginal effect in the dbRDA ( $F = 3.15$ ,  $p = 0.004$ , Figure 28). Though the overall model explained only a modest fraction of the variation (adj.  $R^2 = 0.10$ ), it is consistent with previous studies (Han et al., 2015; Ortega-Retuerta et al., 2013) in which salinity is the dominant environmental factor of bacterial community composition. Jain et al. (2021) found that salinity explained approximately half of the variation in both FL and PA communities with a dbRDA in a high-Arctic fjord. Therefore, among the environmental variables, the significance of salinity in the dbRDA reinforces the interpretation that water mass structure, rather than depth alone, can be the primary factor distinguishing bacterial communities on the Bering and Chukchi shelf.

This idea helps us understand how each cluster was separated. For example, Clusters PA1 and PA3 showed substantially similar community compositions (lowest mean Bray–Curtis dissimilarity, 0.562) and had almost identical class-level compositions, both dominated by Bacteroidia (42.4 and 47.7 %, respectively) with comparable proportions (Bacteroidia to Gammaproteobacteria to Alphaproteobacteria was 5: 3: 2). The separation between Cluster PA1 and PA3 was driven by environmental factors rather than by broad differences in community composition. Cluster PA3 was found in deeper and more saline water than Cluster PA1 (median depth 25.9 vs 16.0 m; salinity 32.5 vs 31.9) and had higher  $\text{NH}_4$  (1.48 vs 0.31  $\mu\text{M}$ ) and  $\text{PO}_4$  (0.96 vs 0.73  $\mu\text{M}$ ), but lower  $\text{NO}_3$  (0.60 vs 1.30  $\mu\text{M}$ ). Therefore, Cluster PA3 occupied a deeper, more saline, ammonium-rich water mass, whereas Cluster PA1 corresponded to a shallower, fresher, nitrate-bearing but ammonium-poor water. Although Cluster PA3 occurred in deeper water, this separation does not necessarily mean that depth itself distinguished the communities in this region. Instead, water mass properties that correlate with depth, especially salinity and nutrient concentrations, can likely be the factors. Additionally, it is worth noting that  $\text{NH}_4$  is often interpreted as a remineralization product. However, since Cluster PA3 had many deep samples, high concentration of  $\text{NH}_4$  could also have been derived from benthic (sediment) sources. Moreover, its bloom index value was 0.46 (Figure 27), meaning that it could not be classified as strong pre-bloom cluster and may possess both pre- and post-bloom characteristics.

The two datasets differed in which environmental variables significantly structured the communities. While all five environmental variables were significant in the survey (five variables,  $p < 0.008$ ), salinity was the only significant variable in the process dataset ( $p = 0.004$ ). This contrast may partly reflect the influence of size fractionation, which likely accounts

for a large share of the community's variation in the process dataset and may have weakened the environmental effects that could be detected (adjusted  $R^2 \approx 0.10$ ). The process dataset also covered a smaller area with fewer samples than the survey dataset, so temperature, dissolved oxygen, and nutrients varied less, and they might correlate less clearly with the community composition.

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## Chapter 4. Review of the Studies of Marine Group II Archaea

### 14. Introduction of Marine Group II Archaea

#### 14.1 General Description of Marine Archaea

All organisms on the Earth are categorized into one of three domains: bacteria, eukaryote, and archaea. Archaea are microbes which have a similar cellular structure and size to bacteria but share other biochemical characteristics more with eukaryotes. Archaea are known as showing strong tolerance against extreme environments and are found in such habitats as volcanos, polar regions, deserts, and deep sea vents, and thus, originally all archaea were thought to be extremophiles residing exclusively in extreme environments. However, after mesophilic (mild temperature-loving) archaea were found from the coastal ocean (DeLong, 1992) and subsurface seawater (Fuhrman et al., 1992), researchers started to study archaea in temperate environments and now recognize archaea are prevalent in many environments on Earth. Marine archaea are mainly divided into four groups: Marine Group I (MGI) archaea (DeLong, 1992; Fuhrman et al., 1992), Marine Group II (MGII) archaea (DeLong, 1992), Marine Group III (MGIII) archaea (Fuhrman & Davis, 1997), and Marine Group IV (MGIV) archaea (López-García et al., 2001). MGI and MGII archaea are the most abundant archaea in the global oceans (Massana et al., 2000; Rinke et al., 2019). We have obtained much information about the biogeography, physiology, and metabolism of MGI archaea. For example, MGI archaea are known as globally distributed archaea from the poles (Bano et al., 2004; DeLong et al., 1994) to the equator (Church et al., 2010; Santoro et al., 2017) and are abundant below the mesopelagic and bathypelagic layers, reaching around 40 % of total prokaryotes (Karner et al., 2001). Also, pure cultures and metagenome-assembled genomes (MAG) of MGI archaea have been obtained (Hallam et al., 2006a; Könneke et al., 2005), leading to the discovery that MGI archaea are major ammonia-oxidizers in the marine ecosystem.

#### 14.2 General Description of Marine Group II Archaea

Compared with MGI archaea, we only have limited information about MGII archaea. Massana et al. (2000) compared archaeal populations from different oceans and phylogenetically classified MGII archaea OTUs (Operational Taxonomic Units, a proxy for species) into two different subgroups: MGIIa and MGIIb. MGII archaea were formerly categorized in the phylum Euryarchaeota (DeLong, 1992), but Rinke et al. (2019) proposed to assign MGII archaea into a new phylum, Thermoplasmatota. Also, they proposed a new order Candidatus Poseidoniales ord. nov. and categorized MGIIa and MGIIb as new families,

Candidatus Poseidoniaceae fam. nov. and Candidatus. Thalassoarchaeaceae fam. nov. respectively. In addition, each MGII clade was subdivided into subclades (Table 7). Galand et al. (2010) phylogenetically analyzed archaeal 16S rRNA genes and classified MGIIa into three subclades: K, L, and M, and MGIIb into two subclades: N and O. Other metagenomic studies further divided these subclades (e.g., O1-O5) or added new subclades (I, J, and Q), but the numbers and kinds of subclades vary among studies (Orellana et al., 2019; Pereira et al., 2021; Rinke et al., 2019; Tully et al., 2019). Further investigation of MGII archaeal phylogeny is required, and MGII archaea likely have more diversity waiting to be discovered. Despite these proposed (not yet accepted) taxonomic revisions, most authors still describe them as MGII and I will continue here with this nomenclature. Unlike MGI archaea, we have not obtained a pure culture of MGII archaea yet, and thus we only have limited physiological information about them. However, Iverson et al. (2012) reconstructed the metagenome-assembled genomes (MAGs) of MGIIa, which was the first microbial complete genome assembled from metagenomic data. Martin-Cuadrado et al. (2015) and Royo-Llonch (2020) also reconstructed MGIIb MAGs, so thanks to those reference genomes, we can study the physiology, metabolism, and ecological role and evolutionary history of MGII archaea in silico.

**Table 7**  
*Classification of MGII Archaea Subclades*

| Reference              | MGIIa subclades               | MGIIb subclades     |
|------------------------|-------------------------------|---------------------|
| Galand et al. (2010)   | K, L, M                       | N, O                |
| Orellana et al. (2019) | I, J1–3, K (1, 2, 4), L1–4, M | N1–2, O1–5, P, Q1–2 |
| Rinke et al. (2019)    | I, J1–3, K1–2, L1–4, M        | N1–2, O1–5, P, Q1–2 |
| Tully et al. (2019)    | K, L, M                       | O (1–5 and WHARN)   |

*Note.* Subclades of MGIIa and MGIIb are listed. The number and kinds of subclades differ among the metagenomic studies because each study conducted a different phylogenetic analysis.

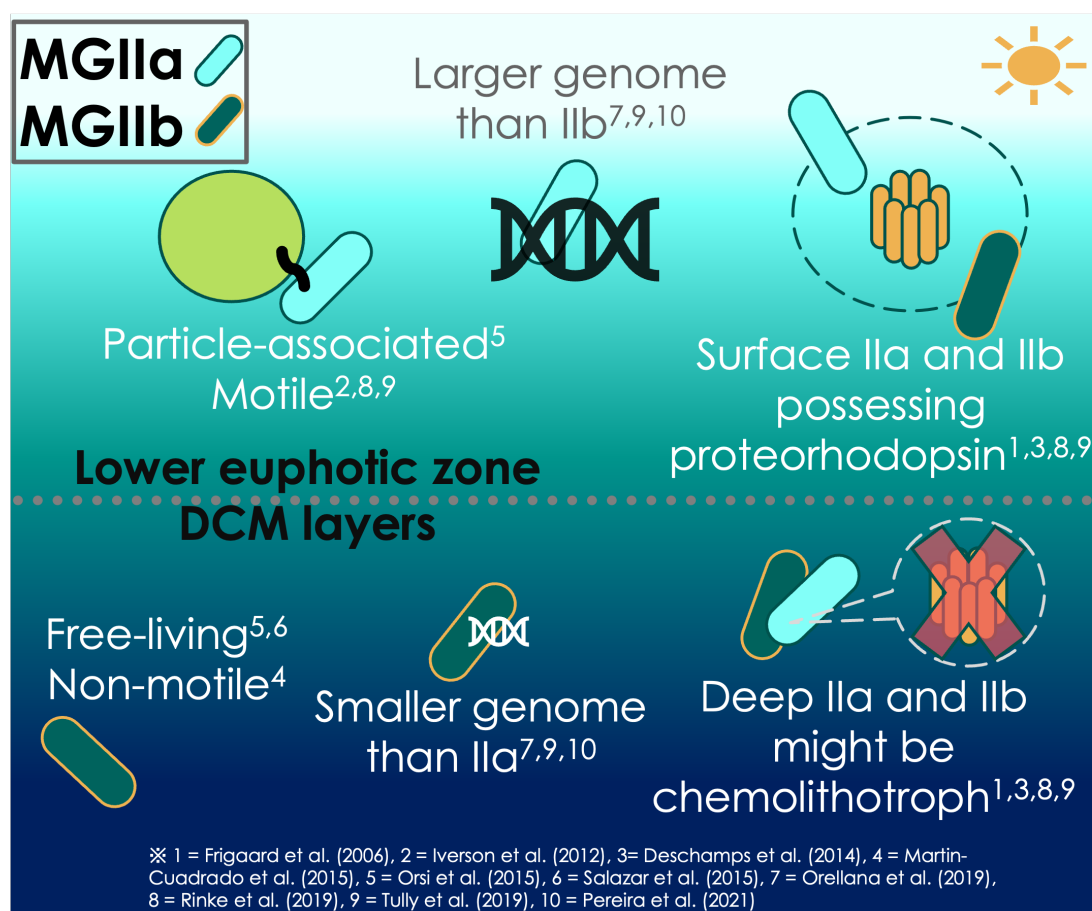
### 14.3 Biogeography of MGII Archaea and its Subclades

Surveys of 16S rRNA gene sequences revealed that MGII archaea were abundant in the global oceans from tropical (Schattenhofer et al., 2009) to polar regions (Bano et al., 2004; Galand et al., 2009; Kirchman et al., 2007). Generally, MGIIa are found from surface waters (Frigaard et al., 2006; Galand et al., 2010; Hugoni et al., 2013; Massana et al., 2000; Orellana et al., 2019), while MGIIb can be observed throughout the water column but commonly found

from deep waters (Galand et al., 2009; Martin-Cuadrado et al., 2015; Massana et al., 2000; Parada & Fuhrman, 2017; Pereira et al., 2019). Both MGII subgroups can be found throughout the water column, but they usually become abundant at preferred depths. Figure 30 provides a summary of the distribution, physiology (described in 2.3), and metabolic features (described in 2.4) of MGII subgroups.

**Figure 30**

*Graphical Summary of the Distribution, Physiology, and Metabolism of MGII Archaea*



*Note.* MGIIa and MGIIb are drawn as light-blue and dark-green rod cells respectively. Superscripts next to each characteristic represents the references, listed at the bottom.

#### 14.4 Physiology of MGII Archaea

Metagenomic studies revealed that MGIIa have larger genomes (Martin-Cuadrado et al., 2015; Orellana et al., 2019; Pereira et al., 2021; Tully et al., 2019) and have higher GC content than MGIIb MAGs (Martin-Cuadrado et al., 2015; Orellana et al., 2019). Also, those studies showed that MGIIa and MGIIb have distinct physiological characteristics. Generally,

MGIIa are explained as motile and particle-attached archaea (Iverson et al., 2012; Orsi et al., 2015; Rinke et al., 2019; Tully et al., 2019), while MGIIb are categorized as non- motile (Martin-Cuadrado et al., 2015). However, another metagenomic study suggested that all MGIIa and half of MGIIb MAGs encoded the archaeal flagella gene operon, which may function in cell adhesion, and the highest abundances of the flagellar gene, *flaJ*, were observed from MGIIb subclade O in the large-sized fraction (3-20  $\mu\text{m}$ ), suggesting that along with MGIIa, some MGIIb subclades can be particle-attached (Rinke et al., 2019). Another metagenomic study revealed that free-living and particle-associated MGII archaea have distinct genome contents (Orsi et al., 2015) and correlated with different environmental parameters (Jain et al., 2021; Xie et al., 2018). Also, Salazar et al. (2015) analyzed bathypelagic microbial communities with 16S rRNA gene sequencing and found that deep MGII archaea are potentially free-living, and surface and deep MGII archaea OTUs were phylogenetically distinct. Parada and Fuhrman (2017) reported that the phylogeny of MGII archaea OTUs were more affected by depth stratification than MGI archaea. Those findings suggest that the diversity of MGII archaea clades and subclades supports a variety of physiological traits (e.g., motility and particle association) that allow them to respond to different environmental or biological factors (e.g., temperature, salinity, nutrients, depth, and presence/absence of other microbes).

#### 14.5 Metabolic Features of MGII Archaea

Metabolism of MGII archaea has been studied using metagenomics. For instance, MGII archaeal MAGs encode genes related to degrading high molecular weight compounds such as complex carbohydrates, lipids, and protein (Iverson et al., 2012; Li et al., 2015; Martin-Cuadrado et al., 2015; Rinke et al., 2019; Tully et al., 2019), suggesting that MGII archaea have heterotrophic metabolism and potentially contribute to marine carbon cycles (Zhang et al., 2015). For instance, MGII MAGs encoded carbohydrate-active enzymes (CAZYS), which hydrolyse polysaccharides (Li et al., 2015; Qu et al., 2022; Rinke et al., 2019; Tully et al., 2019). Also, MGIIa and MGIIb MAGs encoded fatty acid and protein degradation pathways, suggesting that MGII archaea may have a particle-associated lifestyle to fix inorganic carbon (Iverson et al., 2012; Li et al., 2015; Rinke et al., 2019; Tully et al., 2019). Motility is main factor that differentiates MGIIa (motile) and MGIIb (nonmotile) genomes, but they also adapt to different niches based on their ability to degrade high molecular weight organic matter. For example, MGIIb MAGs showed the potential to degrade agar, which may contribute to degrade the algal cell wall, and xenobiotics (Martin-Cuadrado et al., 2015). Both MGIIa and MGIIb MAGs possessed genes encoding transporters, and enzymes that degrade sugars, amino acids, and peptides (Orellana et al., 2019; Rinke et al., 2019; Tully et al., 2019). An important metabolic feature of many MGII archaea is that they encode proteorhodopsin and have a

photoheterotrophic lifestyle (Deschamps et al., 2014; Frigaard et al., 2006; Iverson et al., 2012; Martin-Cuadrado et al., 2015; Rinke et al., 2019). Proteorhodopsin is composed of retinal (vitamin A) and opsin protein and functions as a light- driven proton pump, which receives light energy, generating a proton-gradient, which can be used to produce ATP. Metagenomic studies revealed that both MGIIa and MGIIb encode the proteorhodopsin gene (pR) (Frigaard et al., 2006; Hugoni et al., 2013; Iverson et al., 2021; Li et al., 2015; Martin-Cuadrado et al., 2015; Orellana et al., 2019; Rinke et al., 2019; Thrash et al., 2017; Tully et al., 2019). Also, MAGs from surface and DCM layers in the global ocean, which were reconstructed from TARA Oceans metagenomes, contained proteorhodopsin (Pereira et al., 2019). However, fosmid clones and MAGs of MGII archaea, retrieved below the euphotic zone, did not contain pR, suggesting that MGII archaea in mesopelagic and bathypelagic layers may lack proteorhodopsin and have different metabolic traits from surface MGII archaea (Deschamps et al., 2014; Frigaard et al., 2006; Li et al., 2015; Martin-Cuadrado et al., 2008; Rinke et al., 2019; Thrash et al., 2017; Tully et al., 2019). In fact, Rinke et al. (2019) reported that genera of Ca. Poseidoniales (J1, J2, and J3) and Ca. Thalassoarchaeaceae (Q1 and Q2), whose MAGs were recovered from deep water down to 5000 m, did not encode pR and had positive correlation with sampling depth. Also, those MAGs were highly abundant where nitrate and phosphate concentration was high, which can explain their great abundance in deep water and suggest that they may have different metabolisms than surface MGII genera. It is also suggested that MGII archaea may have acquired proteorhodopsin from bacteria through horizontal gene transfer (HGT) (Deschamps et al., 2014; Frigaard et al., 2006). However, we don't know what kind of driving force led MGII archaea to obtain proteorhodopsin with HGT in their evolutionary history. To reveal functional and metabolic roles of proteorhodopsin on MGII archaea, further study should be conducted from physiological, metabolic, and evolutionary perspectives.

#### 14.6 Correlations Between MGII Archaea and Other Microbes

To understand the biogeography of MGII archaea, we need to investigate biological factors which influence MGII archaeal distribution. Many studies have found correlations between MGII archaea and heterotrophic bacteria. For example, SAR11 and SAR86 are representative heterotrophic bacteria that correlate with MGII archaea. SAR11 dominate 25 % of the total bacterioplankton and utilize labile substrates like amino acids (Giovannoni, 2017). SAR86, which has been successfully cultured recently (Ramfelt et al., 2024), are known as having proteorhodopsin and potentially the ability to degrade carbon compounds such as lipids and polysaccharides (Dupont et al., 2012). Beman et al. (2011) found that the abundance of MGII archaeal 16S rRNA genes correlated with the abundance of OTUs of SAR11, SAR86, and Bacteroidetes (Beman et al., 2011). Also, Ottesen et al. (2013) conducted metatranscriptomic

analysis and observed that transcriptional regulations of MGII archaea were synchronized with those of SAR11 and SAR86, suggesting that they may correspond to the same environmental signals (Ottesen et al., 2013). Also, Parada and Fuhrman (2017) monitored interactions between MGII archaea and other microbial communities from surface to deep waters for five years and found that MGII archaea were associated with representative marine heterotrophic bacteria such as SAR11, SAR86, SAR202, SAR406, and SAR324 at different depths. Pereira et al. (2021) reconstructed MAGs from the Northwestern Mediterranean Sea and found that one MAG of MGIIa subclade O cooccurred with SAR86 and Rhodobacteraceae, while one MGIIb MAG associated with SAR324, suggesting that different MGII archaea subclades have different associations with heterotrophic bacteria. The reason why MGII archaea have associations with heterotrophic bacteria is unknown, but a possible answer is that MGII archaea and those heterotrophic bacteria listed above, are known as proteorhodopsin-possessing microbes. Thus, sharing similar metabolism and ecological niches may lead to the formation of microbial networks between MGII archaea and heterotrophic bacteria. Besides heterotrophic bacteria, correlations between MGII archaea and phytoplankton have been reported. For instance, Orsi et al. (2015) conducted bottle incubations and found that MGII growth was stimulated and had a growth rate on the order of  $0.47\text{ d}^{-1}$  by the addition of a picoeukaryotic phytoplankter, *Micromonas pusilla*, a cosmopolitan green alga (Foulon et al., 2008). Interestingly, a zooplankter, *Salpa* sp., was also present inside of the bottle, suggesting that it may have grazed upon *Micromonas pusilla* and released small peptides that stimulated the growth of MGII archaea. Another study found that the abundance of MGII archaea increased after a phytoplankton bloom (Needham & Fuhrman, 2016; Parada & Fuhrman, 2017). Surveys of 16S rRNA gene sequences in the Northwestern Mediterranean Sea revealed that the relative abundance of MGIIb was correlated with the concentration of nutrients and the presence of phytoplankton (Galand et al., 2010; Hugoni et al., 2013). Galand et al. (2010) also reported that sequences from MGIIb became abundant with phytoplankton such as *Prochlorococcus* (one of the most abundant cyanobacteria on Earth; Partensky et al., 1999), *Micromonas*, and diatoms. They found that MGIIb relative abundances started to increase once chlorophyll-a concentration reached the first peak, and then disappeared when the main phytoplankton bloom started, suggesting that they can be outcompeted by other phototrophic microbes. Several metagenomic studies suggested that MGII archaea may have associations with algae to utilize algae-derived carbon (Li et al., 2015; Orsi et al., 2016; Tully et al., 2019) and this might be the reason why MGII archaea share associations with phytoplankton. In this study, I will investigate whether MGII archaea in the Arctic Ocean are entrained into cooccurrence networks with phytoplankton in the same way as is observed in the global oceans.

## 14.7 Correlations Between MGII Archaea and Environmental Factors

Physicochemical parameters such as temperature, salinity, and concentration of nutrients significantly influence MGII archaeal distributions, which vary widely with the seasons. Previous studies reported that MGII subclades have specific seasonal preferences in different oceans. For example, 16S rRNA gene sequencing in the Northwestern Mediterranean Sea found that MGIIa sequences were abundant during summer when nutrients are scarce and phytoplankton are less abundant, while MGIIb sequences were abundant during winter when nutrient concentrations and the abundance of phytoplankton are high (Galand et al., 2010; Hugoni et al., 2013). The other metagenomic study, conducted in the southern North Sea, agreed, reporting that MGIIa MAGs dominated 23 % of the microbial community in summer samples, while MGIIb MAGs were abundant in winter samples (Orellana et al., 2019). Orsi et al. (2015) collected samples in the central California current system (CCS) during the summer and found that MGIIa was most abundant in the larger size fraction, dominating the 16S rRNA clone libraries. However, a time-series metagenomic study, conducted in the Northwestern Mediterranean Sea, reported that relative abundances of MGIIa and MGIIb MAGs were high during autumn and winter, and both MGIIa and MGIIb MAGs completely disappeared from surface waters during summer, suggesting that MGIIa and MGIIb may become abundant in response to changes in environmental conditions (Pereira et al., 2021). However, a metatranscriptomic study from Sapelo Island (Georgia, USA) contradicted those studies, reporting that transcripts of MGIIa were abundant in summer metatranscriptomes, while autumn and winter metatranscriptomes had high abundances of MGIIb transcripts (Damashek et al., 2021). Parada and Fuhrman (2017) reported that MGIIa and MGIIb did not show any seasonal patterns in the San Pedro Channel (California, USA). Community analysis with parallel 16S rRNA gene pyrosequencing in the subarctic and the subtropical gyre in the northwestern Pacific Ocean found that MGIIb were found throughout the water column and were especially abundant at 300-2000 m depth, while MGIIa were abundant at surface and 5000 m in the subarctic and the subtropical gyre (Kaneko et al., 2016). Also, MGIIb showed high relative abundance in surface waters during October and April in the subtropical gyre and during October in the subarctic gyre, while MGIIa were highly abundant during July in both subtropical and subarctic gyres. There is still great uncertainty regarding the influence of environmental factors on the distributions of MGII archaea. In this study, I will investigate the preferred environmental conditions of MGII archaea subgroups and subclades in the Arctic Ocean.

## 15. MGII Archaea in the Arctic Ocean

### 15.1 Biogeography of MGII Archaea in the Arctic Ocean

MGII archaea have been found frequently from the Arctic Ocean including the central Arctic Ocean (Bano et al., 2004), the Beaufort Sea and the Mackenzie River (Galand et al., 2008), the Chukchi Sea and the Canada Basin (Kirchman et al., 2007), and western Arctic (Galand et al., 2009). Earlier studies explained MGII archaeal distribution in the Arctic Ocean, but those studies showed different results. For instance, Bano et al. (2004) investigated archaeal assemblages in the central Arctic Ocean with PCR-denaturing gradient gel electrophoresis (DGGE) analysis and found that MGII archaea were more abundant in deep water than in surface water and showed equal relative abundance with MGI archaea. On the other hand, Kirchman et al. (2007) analyzed the abundances of bacteria and archaea in the Chukchi Sea and Canada Basin with FISH and found that Euryarchaea (MGII archaea) were detected in only a few samples from surface to 500 m, making up < 10 % of total DAPI (4',6-diamidino-2-phenylindole) counts, and were mostly outnumbered by crenarchaea (MGI archaea). Alonso-Sáez et al. (2008) analyzed prokaryotic community compositions in Franklin Bay with FISH and showed that DAPI counts of Euryarchaea were less than 6 % through the year, in agreement with Kirchman et al. (2007). These studies showed different results because they collected samples from different seasons and adopted different kinds of methodology. However, thanks to the pioneering study of Galand et al. (2009a), which was the first taxonomic description of archaeal communities with parallel tag sequencing, 16S rRNA gene sequencing became the standard method for studying archaeal distributions in the Arctic Ocean and helped us understand the unknown structure of Arctic archaeal communities. Those authors conducted 16S rRNA gene sequencing and analyzed samples from eight different kind of water masses in different regions in the Arctic Ocean (Baffin Bay, Beaufort Sea, Chukchi Sea, Bering Sea, and Amundsen Bay). They found that different water masses harbored different archaeal OTUs, and MGI archaea OTUs were abundant in deep water samples, while MGII archaea OTUs were abundant in deep water samples, and 85 % of the Pacific surface water samples in the Beaufort Sea were dominated by a single MGII OTU. Also, MGIIb OTUs were abundant in deep water samples, while MGIIa OTUs were abundant in surface samples, which corresponded with the results from previous research (Frigaard et al., 2006; Galand et al., 2010; Hugoni et al., 2013; Martin-Cuadrado et al., 2015; Massana et al., 2000; Orellana et al., 2019; Parada & Fuhrman, 2017; Pereira et al., 2019). Other studies observed that MGII archaea became abundant with specific conditions and the specific sites in the Arctic Ocean. For example, Amano-Sato et al. (2013) analyzed archaeal community compositions in different water masses on the Pacific side of the Arctic Ocean (Chukchi Sea, Bering Sea, East Siberian Sea, and Canada Basin) using CARD (catalyzed reporter deposition)-FISH and found that relative abundances of

Thaumarchaeota were generally more abundant than Euryarchaeota, though at one sampling station in the Chukchi Sea, Euryarchaeota dominated 15 % of total prokaryotic cell counts and showed positive correlations with temperature and ammonium concentration. This result suggested that MGII archaea may outnumber MGI archaea at hot spots with favorable environmental conditions for them. Similarly, community analysis of microbial populations in different size fractions, which was conducted in Kongsfjorden (Svalbard, Norway), reported that MGII archaea Amplicon Sequence Variants (ASVs, a proxy for species or strain) were the most abundant archaea in surface water from different size fractions, followed by MGI ASVs (Jain et al., 2021), and this is one example showing that MGII archaea can be more abundant than MGI archaea in the Arctic Ocean. While Galand et al. (2009a) used only eight samples to study archaeal assemblages in different water masses, we now have access to a much larger database of over 2000 Arctic seawater samples to comprehensively investigate archaeal communities and dynamics in the Arctic Ocean. Using 16S rRNA gene sequencing, I will investigate the distribution of MGII archaeal diversity in the Arctic Ocean.

## 15.2 Correlations Between MGII Archaea and Other Microbes in the Arctic Ocean

Several studies reported that MGII archaea in the Arctic Ocean interacted with other microbes in their communities. For instance, Royo-Llonch et al. (2021) reported that during spring and summer, MAGs which showed heterotrophic metabolism and are potentially chemolithoautotrophic or mixotrophic were highly expressed, and MAGs of *Chloroflexi* (SAR202) and Arctic MGIIb were the most active in the mesopelagic layer. Also, community analysis in the Barents Sea revealed that the relative abundance of MGII archaea was higher in sub-surface waters (>50 m) than in surface waters, and other heterotrophic bacteria such as SAR202, SAR324, and SAR406 showed the same pattern as MGII archaea, which corresponded with studies in other oceans (Ottesen et al., 2013; Parada & Fuhrman, 2017). Those authors also found that the relative abundance of SAR86 in surface waters was constant through the seasons, while ASVs abundant in summer included other members of the Gammaproteobacteria. This result implies that MGII archaea were not abundant in surface waters during summer because other bacteria that have similar ecological roles may outcompete MGII archaea in the Barents Sea. In fact, according to Ottesen et al. (2013), the overall abundance of MGII archaeal transcripts negatively correlated with that of SAR86 in the central California coast, suggesting that MGII archaea may have a similar niche with other heterotrophic bacteria such as SAR86 and may lose in competition against them (Ottesen et al., 2013). These findings might imply that MGII archaea interact with other heterotrophic bacteria in the Bering and Chukchi Seas. I will use co-occurrence networks to identify correlations between MGII and other bacteria in the Arctic Ocean across depth horizons and water masses.

### 15.3 Correlations Between MGII Archaea and Environmental Factors in the Arctic

Ocean Like MGII archaea in other oceans, MGII subclades in the Arctic Ocean show different seasonal preferences. For example, a MAG of MGIIa subclade L1 was the only MGII archaea MAG detected in surface layers through the Arctic Ocean and was abundant in Baffin Bay and Davis Strait during autumn, and in the Pacific Arctic during summer. In the sub-chlorophyll maximum layer, a MGIIa subclade L1 MAG was highly expressed in the Atlantic Arctic during spring. Two MAGs of MGIIb subclade O2 were abundant in Baffin Bay and Davis Strait during autumn in the mesopelagic layer. In addition, a MAG of unclassified MGIIb was highly expressed in the mesopelagic layer of the Atlantic Arctic during spring, and the Kara and the Laptev Seas during summer, while MGIIa MAGs were abundant in autumn (Thiele et al., 2023). That study also found that the relative abundance of MGII archaea ASVs were about 1 % during spring and winter at all sampling stations, but their abundance was quite low (>0.1 %) during summer when a late phytoplankton bloom occurred. Furthermore, MGII archaea, SAR202, SAR324, and SAR406 ASVs had positive correlations with phosphate, nitrate, and silicate concentration in surface water, while other bacteria which were abundant in summer surface waters such as SAR92, OM60, *Polaribacter*, *Ulvibacter*, and *Flavobacteriaceae* showed negative correlations with those inorganic nutrients and were positively correlated with other parameters such as season, temperature, chlorophyll-a concentration, cell abundance, and nitrite concentration. This implies that phytoplankton-derived organic matter may have been depleted by other heterotrophic bacteria, leading to a lower abundance of MGII archaea. However, cluster analysis of archaeal 16S rRNA genes with DGGE analysis (Bano et al., 2004) revealed that MGII archaeal sequences were abundant in summer and autumn, though archaeal phylogeny was mostly influenced by depth stratification, not by seasonal differences. Therefore, further investigations should be conducted to understand the seasonal preferences of MGII archaea.

### 15.4 Physiology of MGII Archaea in the Arctic Ocean

MGII archaea in the Arctic Ocean have been found in association with particles. Wells and Deming (2003) conducted fluorescent in situ hybridization (FISH) in the Canadian Archipelago and reported that the abundance of archaea increased toward the nepheloid layer and showed strong positive correlation with particles, and in the nepheloid layer, they positively correlated with PON. Also, Wells et al. (2006) found that archaea positively correlated with particles and PON and were abundant in suspended particles and detritus rich water in the Beaufort shelf and the Franklin Bay, suggesting that archaea in the Beaufort shelf might have

allochthonous and riverine origin. However, two studies (Galand et al., 2006; Galand et al., 2008) compared 16S rRNA clone libraries from the Mackenzie River and the coastal Beaufort Sea and found that archaeal compositions were distinct among riverine, coastal, and marine water samples, suggesting that archaeal assemblages in this region have autochthonous origin. Also, MGII were the predominant archaea in coastal water samples, which are strongly influenced by river input, and associated with particles (Galand et al., 2006; Galand et al., 2008), and were not only restricted to large/small-sized fractions, suggesting that MGII archaea may occupy different niches according to their physiology and metabolism, and thus, have diverse metabolic traits in the Arctic riverine, coastal, and marine systems (Galand et al., 2008). Another community analysis in the high arctic fjord, Kongsfjorden (Svalbard, Norway) found that MGII archaea ASVs and OTUs were the most dominant in free-living and particle-attached fractions in surface waters, and MGIIa OTUs/ASVs dominated 22 % of total sequences in the particle-attached fraction, while MGIIb OTUs/ASVs were abundant (6.6 %) in the free-living fraction (Jain et al., 2021). These authors also reported that several OTUs/ASVs which were assigned as MGII archaea, showed equal abundance in both free-living and particle-attached fractions, suggesting that they are not phylogenetically distinct and may switch their lifestyle (free-living to particle-attached or vice-versa) between MGII archaeal communities. Overall, the physiology of MGII in the Arctic Ocean remains unknown and thus, further study is necessary to understand environmental or biological factors that influence the MGII physiology.

### 15.5 Metabolic Characteristics of MGII Archaea in the Arctic Ocean

Royo-Llonch et al. (2021) conducted a comprehensive metagenomic and metatranscriptomic survey through the Arctic Ocean (Pacific Arctic, Atlantic Arctic, Arctic Archipelago, Baffin Bay and Davis Strait, Kara-Laptev and North Atlantic) and reconstructed 530 MAGs, including 526 microbial species. These authors reconstructed 5 kinds of MGII archaea MAGs: two MGIIa MAGs (K1 and L1) and two MGIIb MAGs (O2 and unclassified) and found that all MGII MAGs encoded sbm genes (Predicted dinucleotide-binding enzyme and Methylmalonyl-CoA mutase, N-terminal domain/subunit) which is related to carbon fixation, and all MAGs except for MGIIa subclade K1 MAG encoded coxS gene (aerobic carbon-monoxide dehydrogenase small subunit), which is related to carbon oxidation. Interestingly, MGIIb unclassified MAG encoded nirA gene (ferredoxin-nitrite reductase), which is related to denitrification, suggesting that MGII archaea in the Arctic Ocean might have facultative anaerobic metabolisms. The diversity and distribution of pR genes in the Arctic Ocean has been surveyed. Boeuf et al. (2016) studied the abundance and distribution of pR genes and found that Proteorhodopsin-containing taxa dominated 45 % of the total bacterioplankton throughout the photic zone in the southern Beaufort Sea and Mackenzie River during summer, and pR gene

abundance and distribution had more correlation with low nutrient concentration than light. Also, those pR genes were mostly affiliated to Gammaproteobacteria, Alphaproteobacteria, and Bacteroidota. Especially, pR genes of SAR11 made up 63 % of the proteorhodopsin-containing community. Another study showed that Gammaproteobacteria-like pR sequences were always dominant in the Amundsen Gulf during winter (Nguyen et al., 2015). Also, pR gene abundance showed seasonality, as flavobacterial pR genes, which were the second most dominant pR genes in early winter, were replaced with Alphaproteobacterial pR genes in late winter, suggesting that this transition follows carbon dynamics. Compared with proteorhodopsin-containing bacteria in the Arctic Ocean, proteorhodopsin-containing archaea are less studied. Especially, MGII archaea are well-known to interact with heterotrophic bacteria such as SAR11 or SAR86, which are representative proteorhodopsin-containing bacteria, and thus investigating proteorhodopsin-containing MGII archaea in the Arctic Ocean can help us understand the ecological and biogeochemical roles of photoheterotrophy in the Arctic Ocean.

## 16. Future Challenges and Perspectives of Studying Marine Group II Archaea in the Arctic Oceans

### 16.1 Importance of Studying Marine Microbial Ecology in the Arctic Ocean

As the Arctic Ocean is more vulnerable to climate change than other oceans, we need to understand how climate change can alter the Arctic microbial community composition. However, only a few studies reported that MGII archaea composition can be changed by sea ice melting. Comeau et al. (2013) compared microbial community compositions in the subsurface chlorophyll maximum layer in the Beaufort Sea before and after the sea ice minima in 2007 and observed that the microbial composition of eukaryotes, bacteria, and archaea significantly changed: for eukaryotes, stramenopile OTUs significantly decreased after 2007, and ciliate OTUs dominated eukaryotic communities instead; for bacteria, Bacteroidetes OTUs considerably decreased, while the relative abundance of alphaproteobacterial and Gammaproteobacterial OTUs were relatively constant; for archaea, MGI archaea OTUs made up 60 % of total archaeal sequences, but decreased after 2007, while the relative abundance of MGII archaea OTUs were not changed before and after sea ice minima. Kraemer et al. (2024) also conducted community comparative analysis in Canada Basin from 2004 to 2012, including two sea ice minima (2007 and 2012) and found relative abundance of most ASV decreased over time after sea ice minima, and those ASVs were mostly dominated by Proteobacteria and Bacteroidota. On the other hand, those authors reported that relative abundances of Euryarchaeal and thaumarchaeal ASVs increased after sea ice minima, consistent with Comeau et al. (2011), and implied that MGII archaea OTUs might not be affected by sea ice melting in

the Arctic Ocean. Those two studies did not illustrate exact reasons why relative abundance of MGII archaea did not change through sea ice minima. However, another interesting study reported that ocean acidification and warming synergistically increased pCO<sub>2</sub> levels (and potentially higher abundances of *Micromonas pusilla*; Hoppe et al., 2018). As *Micromonas pusilla* can stimulate the growth of MGII archaea (Orsi et al., 2015), the high abundance of *Micromonas pusilla* might be related to constant Euryarchaeal abundance before and after sea ice minima. To answer this question, we need to know which microbes increase/decrease before/after sea ice melting and whether they have association with MGII archaea in microbial communities. Additionally, ocean stratification, which is formed by global warming, may also influence MGII archaeal distribution and abundance in the Arctic Ocean. For instance, Tremblay et al. (2008) observed that due to ocean stratification, nitrate and nitrite concentrations significantly decreased in the upper mixed layer, suggesting that primary production in the euphotic zone can be affected if stratification is bolstered in the Beaufort region. Also, Li et al. (2009) observed the increase of small algae at the upper 150 m of Canada Basin, which suggested that due to ocean stratification, nutrient influx decreased and led to a reduction in cell size. The decline of primary production and the high abundance of microbes with small-sized cells can influence marine biogeochemical cycles and food webs and may influence the abundance of MGII archaea, which associate with phytoplankton and can degrade complex compounds. Therefore, investigating the effect of climate change on microbial communities are essential to understand MGII archaea in the Arctic Ocean.

## 16.2 Possible Future Work About Marine Group II Archaea in the Arctic Ocean

MGII archaea are one of the most representative marine archaea and are ubiquitously detected in surface waters across the global oceans, yet their ecology in the Arctic Ocean remains poorly characterized. Because MGII archaea have not been cultured yet, its metabolic properties have not been experimentally resolved, leaving the functional role that these archaea play within the Arctic marine ecosystems largely unknown. Nevertheless, the recent discovery of novel subgroups MGIIc (Liu et al., 2023) and their past evolutionary adaptation process to blackish environment (Fan et al., 2024) have begun to expand our understanding of this group, signaling a new phase of MGII archaea research. Therefore, investigating MGII archaea, that is a flagship lineage of marine archaea with respect to its distribution, diversity, and putative metabolic functions in the distinctive environment of the Arctic Ocean, also help us understand the ecological role of marine archaea in this region.

Moreover, expanding metagenomic analyses would allow the metabolic repertoires of surface- and deep-water subgroups and subclades to be compared directly, give us the opportunity to find the Arctic-specific metabolism of MGII archaea. Contrasting the genomic

potential of shallow, photoheterotrophic populations with that of their deeper counterparts could reveal how these archaea have adapted to the Arctic marine environment and clarify the ecological roles they play across the water column. It would also be interesting to look at how MGII archaea respond to phytoplankton blooms in highly productive inflow regions such as the Bering and Chukchi Seas, where blooms are especially strong. Following how their abundance and activity change in different bloom stages would show how closely they are linked to primary production. Finally, physical conditions in the Arctic Ocean vary greatly from place to place and season to season, with a strong seasonal light cycle, the presence or absence of sea ice, inflowing Atlantic and Pacific waters, and large inputs of freshwater and land-derived organic matter from rivers. This makes it a good place to study what environmental factors shape MGII archaea. Looking at how the proportions of MGIIa and IIb, and their subclades change with water mass, depth, season, and the timing of spring phytoplankton blooms would give us a high-resolved picture of where these archaea live and what role they play in the Arctic Ocean.

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## Chapter 5. Conclusion

### 18. Limitations of Our Study and Suggestion of Future Work

In this study, we examined the biogeography of marine microbial communities in the Bering and Chukchi Seas, and how diatom blooms and global warming might influence their composition. This study reported how microbial communities in the Bering and Chukchi Seas respond to environmental variables and how they were influenced by water masses and diatom blooms, and thereby how these communities are shaped during June 2017 when sea water temperature was anomalously warm. In addition, it examined how differences in lifestyle (free-living versus particle-associated) contribute to structuring microbial community.

This study was unique in its breadth, with a comprehensive spatial sampling compared to previous microbial community analyses, conducted in the Arctic including this region, because while each of them analyzed few samples (10 in average) from mainly surface waters, we analyzed many more samples (181 in the survey dataset and 78 in the process dataset) from multiple depths (surface, middle, and bottom). Both the survey study (Chapter 2) and the process study (Chapter 3) showed an overall pattern of how bacterial communities are structured in the Bering and Chukchi Seas. Although the two datasets differed in spatial coverage and filtration strategy, they provided important insights about the microbial communities in this region. First, community structure was associated with water mass and salinity rather than with depth. Depth did not act as an independent factor, although the vertical gradients in salinity and nutrients that accompany it were part of what separated the clusters. Also, in both datasets, the bacterial communities were dominated by the same three classes, Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia. Second, both studies showed the effect of the diatom bloom on microbial community compositions. Bacteroidia-rich assemblages were associated with bloom development in the survey clusters (Divisions B and C) and in the PA process clusters (Clusters PA1-3), consistent with the well-known role of Bacteroidetes as degraders of bloom-derived organic matter.

The process study investigated the distinction between FL and PA lifestyles, which separated a FL-AnW cluster from Bacteroidia-enriched PA clusters. Overall, the two studies indicate that bacterial community structure in this region is shaped by three factors acting together: water mass and salinity, bloom stage, and lifestyle. Because these samples were collected in June 2017, when sea surface temperatures were anomalously warm, this snapshot offered an early indication of how continued warming, sea-ice retreat, and rapid environmental changes in the magnitude, timing, and spatial extent of blooms could reshape the bacterial communities in the Bering and Chukchi Seas, and ultimately the biogeochemical functioning of

this highly productive ecosystem.

However, this study also showed technical limitations. First, we used water mass definition from Danielson et al. (2020) (Table 1) and investigated the influence of each water mass on different sample clusters. We found that five out of 11 clusters were wSW-influenced, while four out of 11 clusters were cSW-influenced in the survey dataset (Table 2). Also, in the process dataset, one of the four clusters were wSW-influenced (Table 4). However, since our samples were collected in June 2017, when sea water temperatures were unusually high compared to the past decades, our assignment of specific water masses to each microbial community may be incorrect. Danielson's definition was based on data collected before 2014 and provided temperature and salinity profiles, along with the water mass distribution in the Bering and Chukchi Seas, and thus, temperature anomaly in this region in 2017 was not reflected in the data. Accordingly, our classification of primary/secondary water masses of each sample cluster, based on temperature and salinity, may entail the misclassification such as assigning wSW to clusters that should originally have been classified as cSW-influenced. Hirawake et al. (2021) warned using temperature and salinity data to classify water mass characterization in this region due to recent warming and sea ice decline. Instead, he proposed to use  $a_{CDOM}$ , that is the light absorption coefficient of chromophoric dissolved organic matter (CDOM), and it can be an alternative way for characterizing water properties. To comprehensively analyze how different water masses affect microbial community compositions, it may be worthwhile to perform future analyses using this coefficient.

Next, in this study, depth did not significantly differentiate microbial community compositions (Figure 14 and 25), and it was attributed to mixed water column in the Bering and Chukchi Seas. However, we did not conduct the time-series study, and our dataset reflected a snapshot only from June 2017 (roughly 2.5 weeks). Therefore, conducting a long-term analysis is important to know the depth profiles of microbial communities in this region and enables us to compare the communities before and after strong seasonal stratification developed. Moreover, to examine the effects of mixing on microbial community structures, more detailed data on mixing are needed. For example, by using meteorological data such as ORAS5 (Zou et al., 2019) or Copernicus Arctic Hub, we could assess whether seasonal stratification was truly weak in this region during June 2017 and quantitatively explain why the microbial communities were not separated by depth, using the stratification conditions of that region and season as a physical context.

Finally, we defined a "bloom index" (Figure 9 and 27) to categorize each sample clusters into pre/post-bloom clusters. It comes from the first principal component of the environmental data, so it represents the overall nutrient gradient in one axis, treating  $NH_4$  and other nutrients as equal nutrient indicators. Generally,  $NH_4$  is produced during remineralization

after active bloom and since it does not distinguish new ( $\text{NO}_3$ -based) from regenerated ( $\text{NH}_4$ -based) nitrogen, the cluster like Cluster PA3 in the process dataset, whose bloom index was 0.46 and that was influenced by water masses in which  $\text{NH}_4$  has accumulated, may be assigned as pre-bloom cluster, even when active remineralization might be occurring. Therefore, the bloom index can be a useful indicator of which bloom stage each microbial community is associated with, but it does not conclusively assign the bloom stage to each cluster. Also, it is impossible to conclusively assign each microbial community to a specific bloom stage with data from only one month. Along with the analysis of stratification, to examine how microbial communities change across bloom stages in detail, a long-term analysis is required. Furthermore, using other parameters such as particulate organic carbon/nitrogen (POC/PON) and turbidity can help us understand the association between PA microbes and particulates. POC/PON were measured during the cruise, but 35.4 % of the values were missing, so we did not include them in the analyses. With a more complete record of particle load, the role of particle supply in shaping the PA fraction could be tested directly instead of being inferred. Using 18S rRNA gene data can also provide a more direct link between blooms and bacteria. Calculating relative abundance of phytoplankton, algae, and diatoms and clustering the eukaryotic communities can show the correlation with bacterial communities and reveal how phytoplankton blooms and their associated bacteria interact in this region. These insights will help us predict how bacterial communities respond to future changes in bloom composition, spanning and timing.

## 19. References

Arctic Hub. <https://www.arctic.hub.copernicus.eu/>

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