


The Arctic summer microbiome across Fram Strait: Depth, longitude, and substrate concentrations structure microbial diversity in the euphotic zone

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Abstract

The long-term dynamics of microbial communities across geographic, hydrographic, and biogeochemical gradients in the Arctic Ocean are largely unknown. To address this, we annually sampled polar, mixed, and Atlantic water masses of the Fram Strait (2015–2019; 5–100 m depth) to assess microbiome composition, substrate concentrations, and oceanographic parameters. Longitude and water depth were the major determinants (~30%) of microbial community variability. Bacterial alpha diversity was highest in lower-photic polar waters. Community composition shifted from west to east, with the prevalence of, for example, Dadabacteriales and Thiotrichales in Arctic- and Atlantic-influenced waters, respectively. Concentrations of dissolved organic carbon peaked in the western, compared to carbohydrates in the chlorophyll-maximum of eastern Fram Strait. Interannual differences due to the time of sampling, which varied between early (June 2016/2018) and late (September 2019) phytoplankton bloom stages, illustrated that phytoplankton composition and resulting availability of labile substrates influence bacterial dynamics. We identified 10 species clusters with stable environmental correlations, representing signature populations of distinct ecosystem states. In context with published metagenomic evidence, our microbial-biogeochemical inventory of a key Arctic region establishes a benchmark to assess ecosystem dynamics and the imprint of climate change.

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INTRODUCTION

Recurrent observations provide a deeper understanding of ecosystem functioning over geographic, biogeochemical, and hydrographic gradients; enabling to record changes and predict future states (Buttigieg et al., 2018; Lannuzel et al., 2020). Since the Arctic warms at a four-fold faster rate than the global average (Rantanen et al., 2022), multi-year observations are key for characterizing associated shifts in biological and physicochemical regimes. Remote sensing technologies provide an avenue for tracking large-scale patterns, such as sea-ice reduction and primary productivity (Frey et al., 2023; Horvat et al., 2017; Lewis et al., 2020). However, to measure the biological responses of pelagic communities, in situ observations are necessary (Grebmeier et al., 2019; Solan et al., 2020). For instance, continuous in situ studies demonstrated that plankton diversity scales with sea-ice extent and water temperature (Lin et al., 2021). Decadal records of sinking particles in the Arctic Ocean revealed long-lasting effects of a warm-water anomaly; stimulating small phytoplankton while larger diatoms decreased in abundance, coincident with shifting bacterial composition (Cardozo-Mino et al., 2023). These dynamics have major consequences for carbon export and benthopelagic coupling (Jacquemot et al., 2022; Kohlbach et al., 2023; Salter et al., 2023). Future ocean scenarios predict substantial ecosystem shifts in the Arctic, supported by a changing microbiome structure at higher temperatures (Ahme et al., 2023). One important aspect is the impact of northward expanding Atlantic waters, termed Atlantification, on microbial diversity and the biological carbon pump (Carter-Gates et al., 2020; Oldenburg et al., 2023; Oziel et al., 2020).

The HAUSGARTEN/FRAM long-term observatory studies biological communities, benthopelagic coupling, and physical oceanography in the Fram Strait, the major gateway between the Atlantic and Arctic Oceans (Nöthig et al., 2015; Soltwedel et al., 2016). Here, polar water outflowing from the central Arctic Ocean via the East Greenland Current (EGC; western Fram Strait) meets Atlantic water flowing northward via the West Spitsbergen Current (WSC; eastern Fram Strait). These Arctic-versus Atlantic-influenced ecosystems—determined by sea-ice cover and hydrographic properties—coincide with distinct patterns in microbial diversity (Metfies et al., 2016, 2017; Priest, von Appen, et al., 2023; Wietz et al., 2021), metazoan distribution (Cornils et al., 2022; Käß et al., 2021; Mańko et al., 2020), and biogeochemistry (Engel et al., 2017, 2019; Grosse et al., 2021; Priest, Vidal-Melgosa, et al., 2023; Randelhoff et al., 2018). The polar waters of western Fram Strait harbour higher proportions of SAR11 and SAR406 clades; compared to Flavobacteriia, Gammaproteobacteria, and Verrucomicrobia in the Atlantic waters of eastern Fram Strait (Fadeev et al., 2018). Bacterial cell numbers are an

order of magnitude higher in the eastern Fram Strait, which has been attributed to higher water temperatures (Cardozo-Mino et al., 2021). Overall, Atlantic-influenced waters are more productive and characterized by stronger seasonal dynamics (Wietz et al., 2021), with major implications for the biological carbon pump (Fadeev, Rogge, et al., 2021; Flores et al., 2019; Priest, von Appen, et al., 2023; Ramondenc et al., 2022; Rapp et al., 2018; von Appen et al., 2021). In addition to regional differences, bacterial communities in the Fram Strait distinctly vary with depth. Fluorescence in situ hybridization revealed a prevalence of SAR324, SAR202, and *Nitrososphaeria* below the photic zone (Cardozo-Mino et al., 2021). Ribosomal metabarcoding demonstrated similar patterns over an annual cycle, with chemolithotrophic archaea dominating at mesopelagic depths (Wilson et al., 2017).

A multiannual inventory of microbiological and biogeochemical patterns—comparing Arctic- versus Atlantic-influenced ecosystem states across the euphotic zone—is yet missing, and can help to predict how climate change will impact the regional microbial loop. Here, we portray microbial, biogeochemical, and oceanographic dynamics over five summers, covering ~10 stations from western to eastern Fram Strait in the euphotic zone (5–100 m depth). Through amplicon-sequencing and the quantification of biogeochemically important substrates, we illuminate fundamental dynamics and drivers of Fram Strait bacteria and archaea. We hypothesized that surface waters carry a signature reflecting phytoplankton productivity at different bloom stages, especially under Atlantic influence, compared to more uniform patterns in the lower photic zone. This microbial-biogeochemical inventory establishes a benchmark to assess future ecosystem shifts.

EXPERIMENTAL PROCEDURES

Sample collection

Samples were collected on RV Polarstern expeditions PS93.2 (July–August 2015), PS99.2 (June–July 2016), PS107 (July–August 2017), PS114 (July 2018), and PS121 (August–September 2019) across the Fram Strait (Figure 1 and Table S1). Sampling was carried out with 12 L Niskin bottles mounted on an SBE 911+ rosette (Sea-Bird, Bellevue, WA) equipped with CTD (conductivity-temperature-depth) and chlorophyll *a* (chl-*a*) sensors. The chlorophyll maximum depth (chl-max) was determined from chl-*a* fluorescence profiles during each downcast. Seawater samples were collected during each upcast from the surface (4–22 m depth), the chl-max (13–43 m), below the chl-max (30–75 m), and the lower photic zone (100 m) receiving small amounts of light (Cherkasheva et al., 2013). Individual depths varied per station and year, according to the depth of

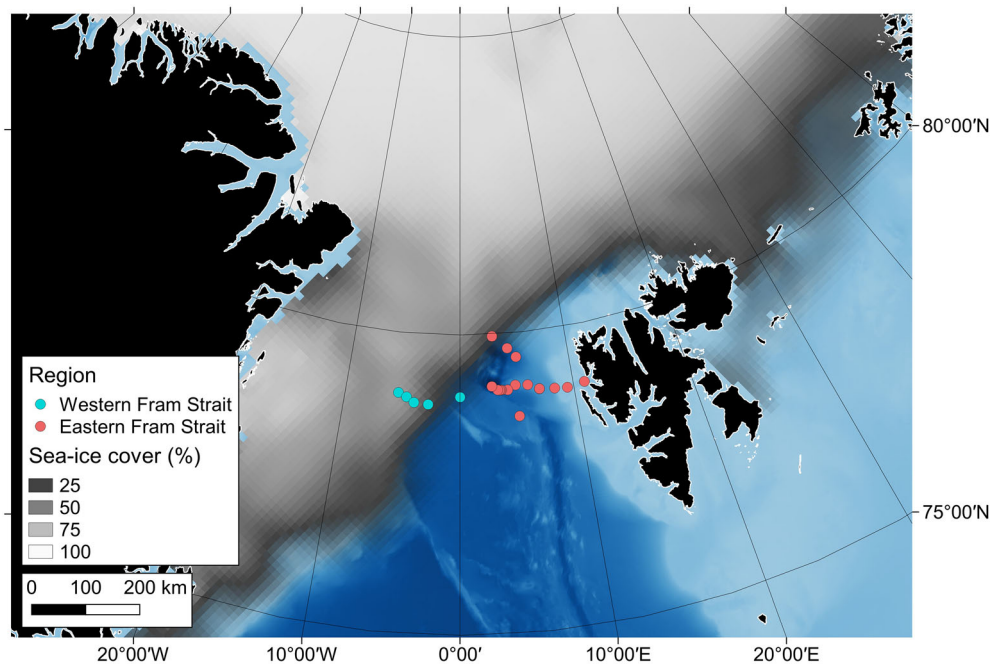


FIGURE 1 Study area. Stations in the western (0–6° W; blue) and eastern (0–12° E; red) Fram Strait, sampled annually between 2015 and 2019. Sea-ice cover (cumulative average over all sampling periods) is depicted as a grey-white gradient.

the chl-max. Not all stations or water layers were sampled every year (Table S1).

DNA extraction and sequencing

Per sampling event, 2–4 L of seawater were filtered onto 0.22 μm Sterivex cartridges (Millipore, Burlington, MA). Filters were stored at -20°C until DNA extraction using the PowerWater kit (QIAGEN, Germany) according to the manufacturer's instructions. The V4–V5 region of 16S rRNA genes was amplified using primers 515F (GTGYCAGCMGCCGCGGTAA) and 926R (CCGYCAATYMTTTRAGTTT), providing high coverage of both bacteria and archaea (Parada et al., 2016). Amplicon libraries were prepared following the 16S Metagenomic Sequencing Library Preparation protocol (Illumina, San Diego, CA) and sequenced using MiSeq technology in 2×300 bp paired-end runs.

Amplicon analysis

16S rRNA reads were processed into amplicon sequence variants (ASVs) using DADA2 v1.16 (Callahan et al., 2016). Filtering settings were $\text{truncLen} = \text{c}(230,195)$, $\text{maxN} = 0$, $\text{minQ} = 2$, $\text{maxEE} = \text{c}(3,3)$ and $\text{truncQ} = 0$, followed by merging using $\text{minOverlap} = 10$ and chimera removal. After singleton removal, we obtained an average of 128,000

reads per sample (Table S2). ASVs were taxonomically classified using the Silva v138 database (Quast et al., 2013). Subsequently, we only considered ASVs with ≥ 3 counts in ≥ 3 samples, resulting in a set of 2835 ASVs. Data were analysed and visualised using R packages tidyverse, mixOmics, ampvis2, psych, and fishualize (Andersen et al., 2018; Revelle, 2023; Rohart et al., 2017; Schiettekette et al., 2019; Wickham et al., 2019), with aesthetic modifications of figures using Inkscape (<https://inkscape.org>). The graphical abstract has been created with Biorender.com. The bioinformatics workflow is available at <https://github.com/matthiaswietz/fiveArcticSummers>. Raw fastq files have been deposited at ENA under BioProject PRJEB66267. For functional predictions, ASVs were mapped to 16S rRNA gene sequences of metagenome-assembled genomes (MAGs) from Fram Strait (Priest et al., 2021; Priest, von Appen, et al., 2023) through competitive read recruitment using BMap in BBtools v35.14, with an identity threshold of 100% (Table S2).

Cell numbers

Seawater samples were fixed with glutardialdehyde (2% v/v final concentration) and frozen at -80°C until further analysis. Cells were stained using SYBR Green I (Thermo Fisher Scientific, Waltham, MA) and counted on a FACSCalibur flow cytometer (BD, Franklin Lakes, NJ) using Cell Quest v3.3 (detection limit 2000 events s^{-1}) after calibration with TruCount beads (BD).

Substrate concentrations

Duplicate samples for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were filtered through 0.45 μm GMF GD/X filters (Whatman, United Kingdom), collected in combusted glass ampoules (8 h, 450°C), acidified, and stored at 4°C until simultaneous analysis with a detection limit of 1 $\mu\text{mol L}^{-1}$ (Engel & Galgani, 2016). Duplicate samples for dissolved combined carbohydrates (>1 kDa) were filtered through 0.45 μm Acrodisc filters (Pall, Port Washington, NY), collected in combusted glass vials (8 h, 450°C), and frozen at -20°C until analysis (Engel & Händel, 2011) with a detection limit of 10 nmol L^{-1} . We herein report the sum of (i) the neutral sugars arabinose, fucose, galactose, glucose, rhamnose, and co-eluted mannose/xylose; (ii) the sugar acids galacturonate and glucuronate; and (iii) the amines galactosamine and glucosamine. Duplicate samples for dissolved hydrolyzable amino acids were filtered through 0.45 μm Acrodisc filters, collected in combusted glass vials (8 h, 450°C), and frozen at -20°C until measurement with orthophthalaldehyde derivatization by high-performance liquid chromatography (Agilent, Santa Clara, CA) using a C18 column (Phenomenex, Torrance, CA), with precision <5% and detection limit 2 nmol L^{-1} (Dittmar et al., 2009; Peter & Kenneth, 1979). We herein report the sum of alanine, arginine, aspartate, isoleucine, glutamate, glycine, leucine, phenylalanine, serine, threonine, tyrosine, valine, and γ -aminobutyric acid (GABA).

Chlorophyll

Total chlorophyll concentrations were determined after filtering 2 L seawater onto GF/F filters (Whatman; 25 mm diameter) at 200 mbar. In addition, 2 L were sequentially filtered through 10, 3, and 0.4 μm Isopore membrane filters (Merck; 45 mm diameter) at 200 mbar. Filters were stored at -80°C until chl-a extraction in 90% acetone overnight. Chl-a was quantified using a fluorometer (Turner Designs, San Jose, CA) slightly modified from (Dybern et al., 1976; Evans & O'Reilly, 1983), including calibration with standard chl-a solutions (Sigma, Germany).

Satellite data

Sea-ice and chl-a concentrations, derived from the AMSR-2 and Sentinel 3A OLCI satellites, were downloaded from seaice.uni-bremen.de and data.marine.copernicus.eu, respectively, considering grid points within 15 km around stations.

RESULTS AND DISCUSSION

We characterized microbial, biogeochemical, and hydrographic patterns in the summers of 2015–2019 across the western ($0\text{--}6^\circ\text{W}$) and eastern ($0\text{--}12^\circ\text{E}$) Fram Strait (Figure 1 and Table S1). By analysing ~ 200 seawater samples from the surface (average depth 10 m), chl-max (average 24 m), below the chl-max (average 46 m) and the lower photic zone (100 m), we determined regional and vertical patterns in microbial diversity, cell numbers and substrate concentrations across Arctic- and Atlantic-influenced ecosystem states.

Environmental parameters, cell numbers, and substrate regimes

Seawater temperatures showed marked regional differences, varying from -1.6°C to 8°C between western and eastern Fram Strait (Figure 2A). Per site, temperatures across the photic zone were similar, with a maximal difference of $\sim 2^\circ\text{C}$ between surface and 100 m depth. Lower salinities in the upper western Fram Strait (Figure 2A) illustrate the influence of polar surface water, sea ice-derived meltwater or a combination of both.

The western Fram Strait harboured 1×10^5 cells ml^{-1} throughout the upper 100 m. In eastern Fram Strait, the lower photic zone harboured similar numbers, compared to an order of magnitude more cells in the upper 25 m (Wilcoxon rank-sum test, $p < 0.001$; Figure 2A). These numbers agree with cell abundances reported near Svalbard (Cardozo-Mino et al., 2021).

The concentrations of neutral carbohydrates (CHO), amino acids (AA), sugar acids, and amines peaked in the chl-max of the eastern Fram Strait (Wilcoxon rank-sum test, $p < 0.05$), corresponding to elevated primary production (Nöthig et al., 2015) and availability of labile substrates (Piontek et al., 2014). Bulk DOC concentrations were 12% higher in the western Fram Strait (Wilcoxon rank-sum test, $p < 0.05$; Figure 2B). Presumably, this relates to higher concentrations of terrestrial- and ice-derived DOC, which can constitute up to 30% of organic matter in Arctic waters (Nguyen et al., 2022; Opsahl et al., 1999). These patterns underscore that specific organic compounds prevail under Arctic versus Atlantic influence (Engel et al., 2019; Priest, Vidal-Melgosa, et al., 2023; von Jackowski et al., 2020). Consequently, bacterial communities in polar waters are enriched in genes targeting terrestrial compounds, compared to genes targeting phytoplankton-derived compounds under Atlantic influence (Priest, von Appen, et al., 2023). Over vertical scales, concentrations of CHO, AA, sugar acids, amines, chl-a, and DOC peaked in surface and chl-max depths, independent of

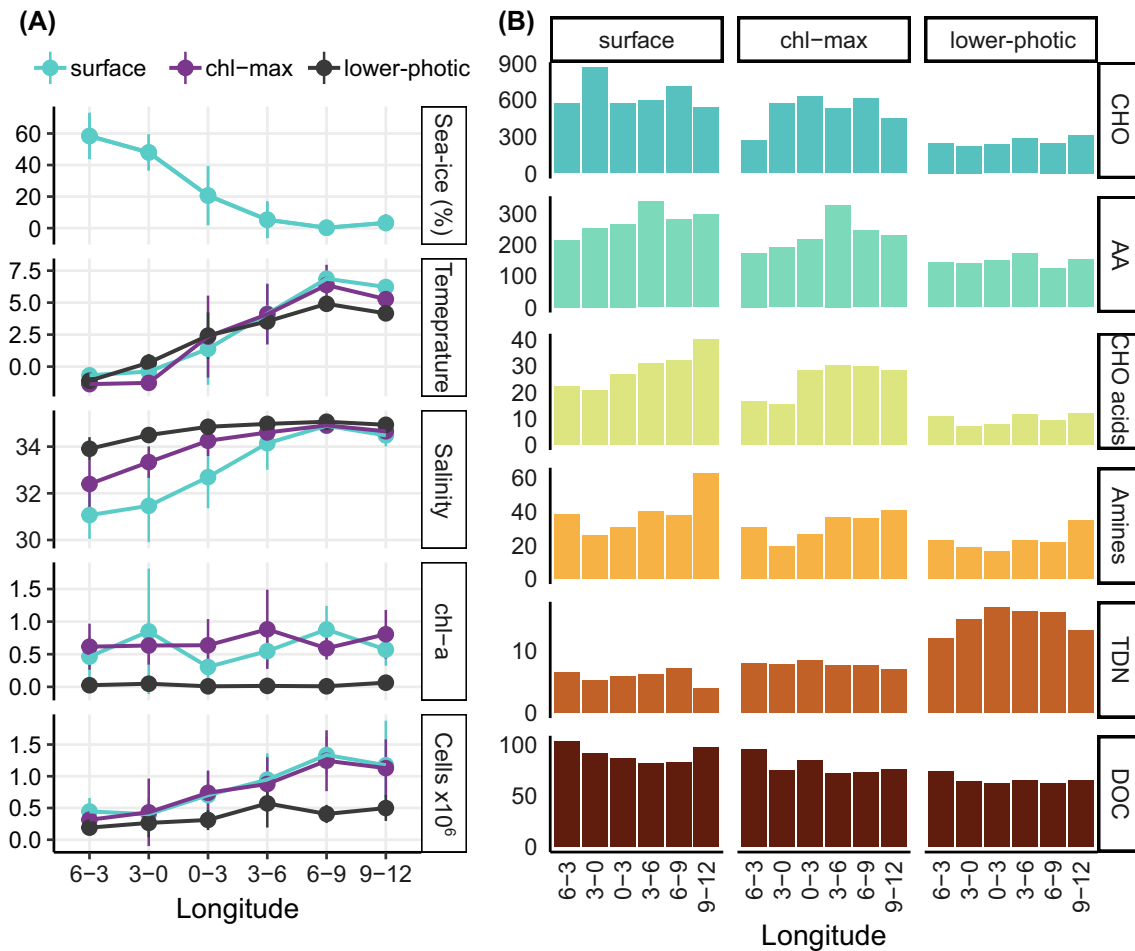


FIGURE 2 Longitudinal and vertical patterns in environmental parameters across Fram Strait between 2015 and 2019, averaged over longitudinal ranges (western Fram Strait: 0–6° W; eastern Fram Strait: 0–12° E). (A) Sea-ice cover, seawater temperature, salinity, chlorophyll concentrations, and bacterial cell numbers. (B) Concentrations of carbohydrates (CHO; μM), amino acids (AA; μM), sugar acids (μM), amines (μM), total dissolved nitrogen (TDN; μM), and dissolved organic carbon (DOC; mM). The full data including standard deviations are shown in Figure S1.

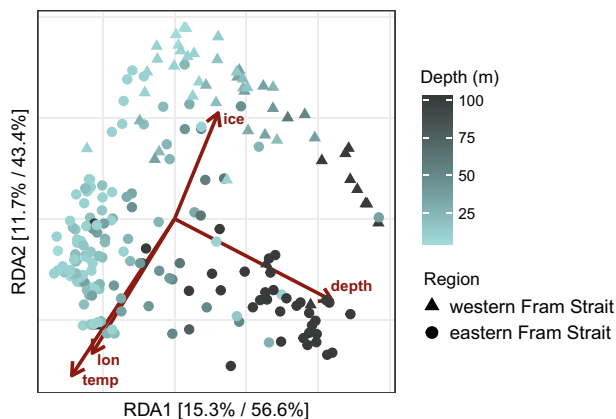


FIGURE 3 Regional and vertical structuring of bacterial and archaeal communities. RDA of community structure (Bray–Curtis dissimilarity), separating samples by depth and longitude (western Fram Strait: 0–6° W; eastern Fram Strait: 0–12° E). Arrows indicate the relative influence of associated environmental parameters, determined via the *envfit* function.

the sampling site (Wilcoxon rank-sum test, $p < 0.001$; Figures 2B and S1). TDN concentrations were consistently higher in the lower photic zone (Wilcoxon rank-sum test, $p < 0.001$), similar to Svalbard fjords (Osterholz et al., 2014).

Broad community patterns in the environmental context

Microbial community composition varied most by longitude and depth, explaining 15% and 16% of variability, respectively (Figure 3; PERMANOVA, $p < 0.001$). To a lesser extent, composition varied with Julian day, which explained 6% of the variability (PERMANOVA; $p < 0.001$). Separate PERMANOVA for bacterial and archaeal ASVs showed that depth was the strongest determinant for archaeal composition ($R^2 = 0.17$), in line with their common preference for aphotic waters.

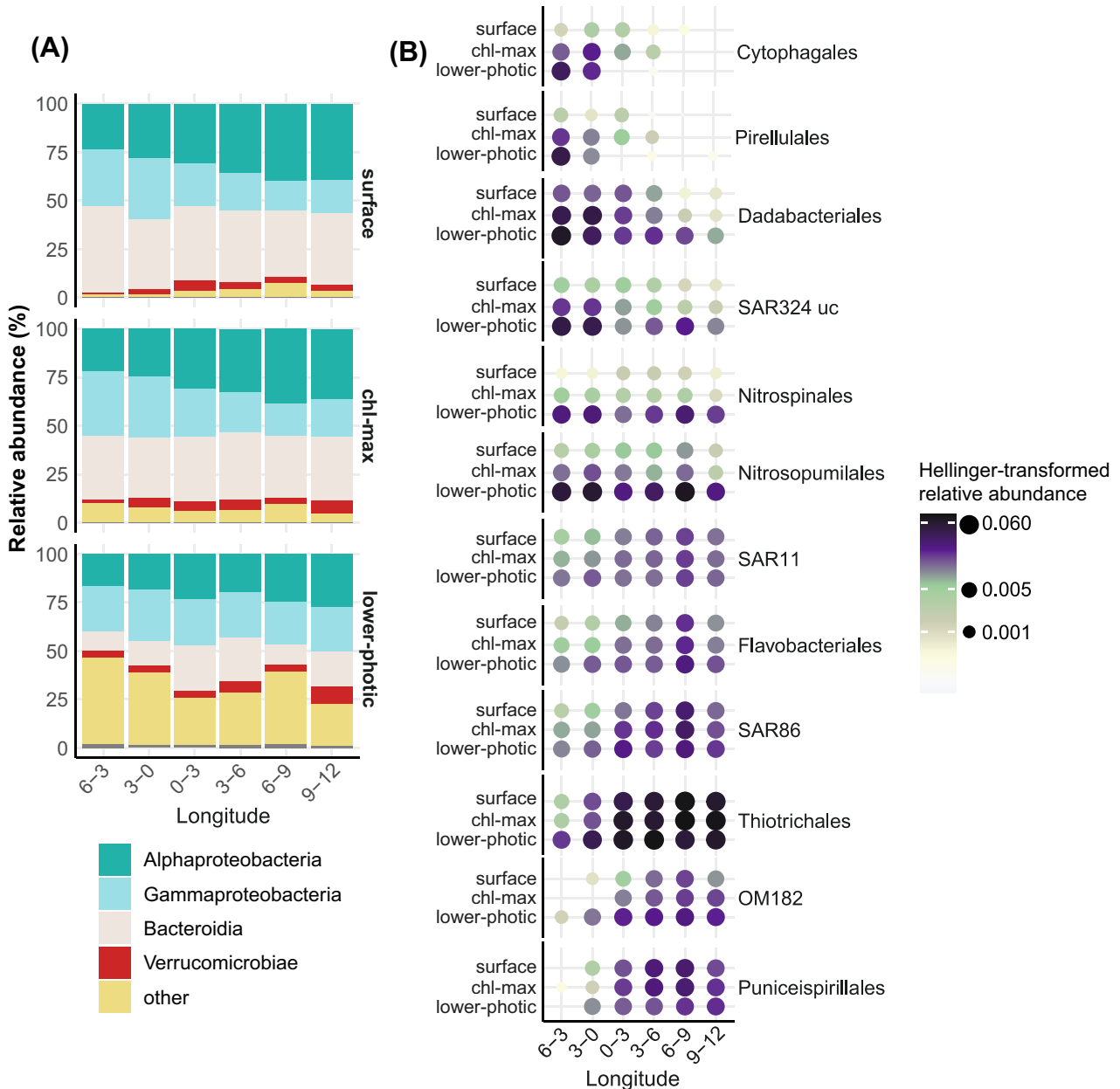


FIGURE 4 Longitudinal shifts in bacterial and archaeal composition. (A): Relative abundances of bacterial classes, averaged between 2015 and 2019 in surface, chl-max, and lower-photic zone. (B) Underlying abundance shifts on order level, displayed as Hellinger-transformed relative abundances of the 100 ASVs with the strongest depth and/or longitude correlations.

In contrast, archaea were less influenced by latitude ($R^2 = 0.07$) and Julian day ($R^2 = 0.01$) than bacteria ($R^2 = 0.16$ and 0.06), but all factors tested were significant (PERMANOVA; $p < 0.01$).

In the western Fram Strait, alpha diversity significantly increased with depth (Figure S2; Kruskal–Wallis test, $p < 0.001$). This subsurface peak suggests marked separation of water layers through lower salinities at the surface (Figure 2A). Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes, and Verrucomicrobia represented $\sim 90\%$ of the community at the surface and chl-max, whereas the

abundance of other bacterial classes increased to $\sim 50\%$ in the lower photic zone (Figure 4A). However, class-level proportions varied between western and eastern Fram Strait, with shifting dominance from Gamma- to Alphaproteobacteria, respectively (Figure 4A). At the order level, this shift corresponded to decreasing abundances of Dadabacteriales and Cytophagales and increasing abundances of OM182 and Puniceispirillales from the western to the eastern Strait. Some lower-photic taxa, for example, Nitrospinales, had similar abundances across all subsurface samples (Figure 4B).

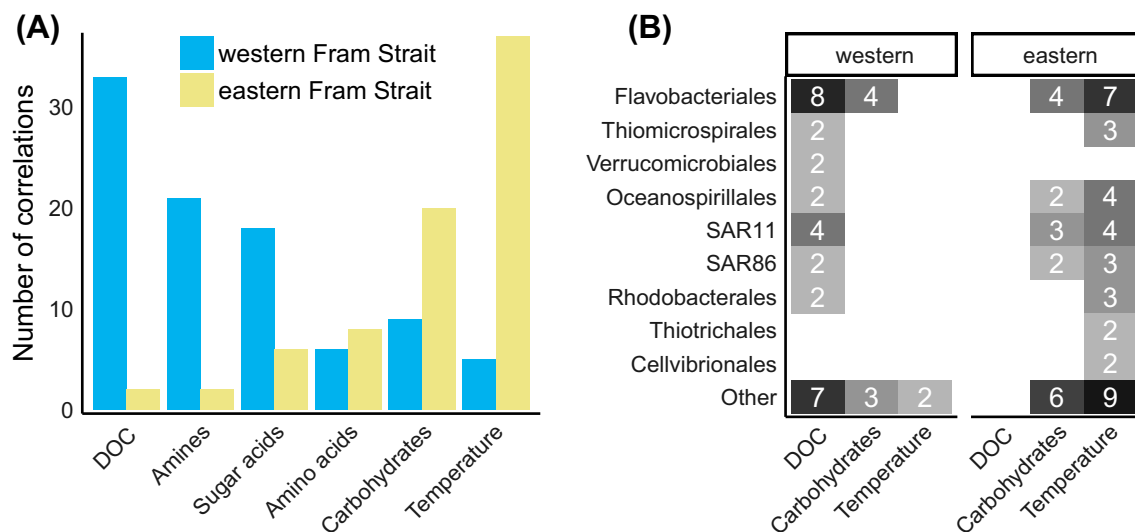


FIGURE 5 Bacteria-environment linkages. (A) Total number of correlations between ASVs and environmental parameters in western versus eastern Fram Strait, focusing on surface and chl-max samples due to their higher substrate concentrations (Figure 2B). (B) Order-level assignment of ASVs that correlate with environmental parameters. Numbers indicate the total number of correlations per order and variable.

ASV-level dynamics indicated the presence of both “cosmopolitan” and locally confined genotypes. For example, ASVs affiliated with *Pseudohongiella* were restricted to the chl-max (Figure S3). *Pseudohongiella* has been reported from the Barents Sea and Svalbard fjords, and linked to hydrocarbon degradation (Kampouris et al., 2023; Peng et al., 2020). Overall, western subsurface waters comprised the largest number of unique ASVs (Figure S4), illustrating that earlier observations (Fadeev et al., 2018) are interannually consistent. The greatest regional overlap among subsurface ASVs indicates that lower-photic waters are more uniform, in line with lower regional variability in water temperatures and cell numbers at 100 m depth (Figure 2A). Nonetheless, ~280 ASVs were detected with a minimum 0.001% relative abundance in all samples (Figure S4). Hence, regional and vertical differences in microbiome structure not only relate to presence-absence of specific taxa but also variability in the relative abundances of shared ASVs.

Correlation analyses underlined how environmental variability influences ASV distribution. ASVs predominantly correlated with DOC, amines, and sugar acids in the western Fram Strait compared to temperature and CHO in the eastern Fram Strait, with ASVs being associated with different bacterial families (Figure 5). Such trends are probably connected with phytoplankton distribution, which varies between eastern and western Fram Strait (Nöthig et al., 2015). Accordingly, analysis of chl-a concentrations in different size fractions indicated the prevalence of smaller phytoplankton (0.4–3 μm fraction) in eastern Fram Strait (Figure S5), in agreement with previous findings (Kilias et al., 2014; Metfies et al., 2016).

Effect of sampling time and seasonality

Although depth and longitude were the strongest drivers of microbial composition (Figure 3), the influence of Julian day (i.e., the time of sampling) was pronounced for samples from surface and chl-max depths (Figure 6A). These patterns likely corresponded to varying mixed layer depth and productivity between June and September (Oldenburg et al., 2023; Wietz et al., 2021). In parallel, substrate regimes shift to more refractory compounds once phytoplankton blooms collapse (von Jackowski et al., 2022). Accordingly, Julian day explained 9% of the variability in substrate concentrations, being comparable to depth (12%) (PERMANOVA; $p < 0.001$).

The 2016 and 2018 samplings occurred in June/July, when diatom abundances are typically highest (von Jackowski et al., 2022; Wietz et al., 2021). Hence, these samplings likely occurred during the peak phytoplankton bloom, supported by maximal CHO concentrations and Flavobacteriales abundances (Figure 6B). These patterns mirror the ecological relationships between flavobacteria, phytoplankton, and algal substrates in temperate seas (Teeling et al., 2012). In contrast, the 2019 sampling occurred in August/September, with high SAR11 abundances and low CHO concentrations signifying the transition to oligotrophic conditions in autumn (von Jackowski et al., 2022). Nonetheless, flavobacteria remain translationally active during such periods, even at lower abundances (Priest, Vidal-Melgosa, et al., 2023). Interannual differences were possibly magnified by contrasting sea-ice conditions, with the ice edge varying by several degrees of latitude and longitude between years (Figure S6). Such variability can markedly influence microbial composition and function, especially in the marginal ice zone (Priest, von Appen,

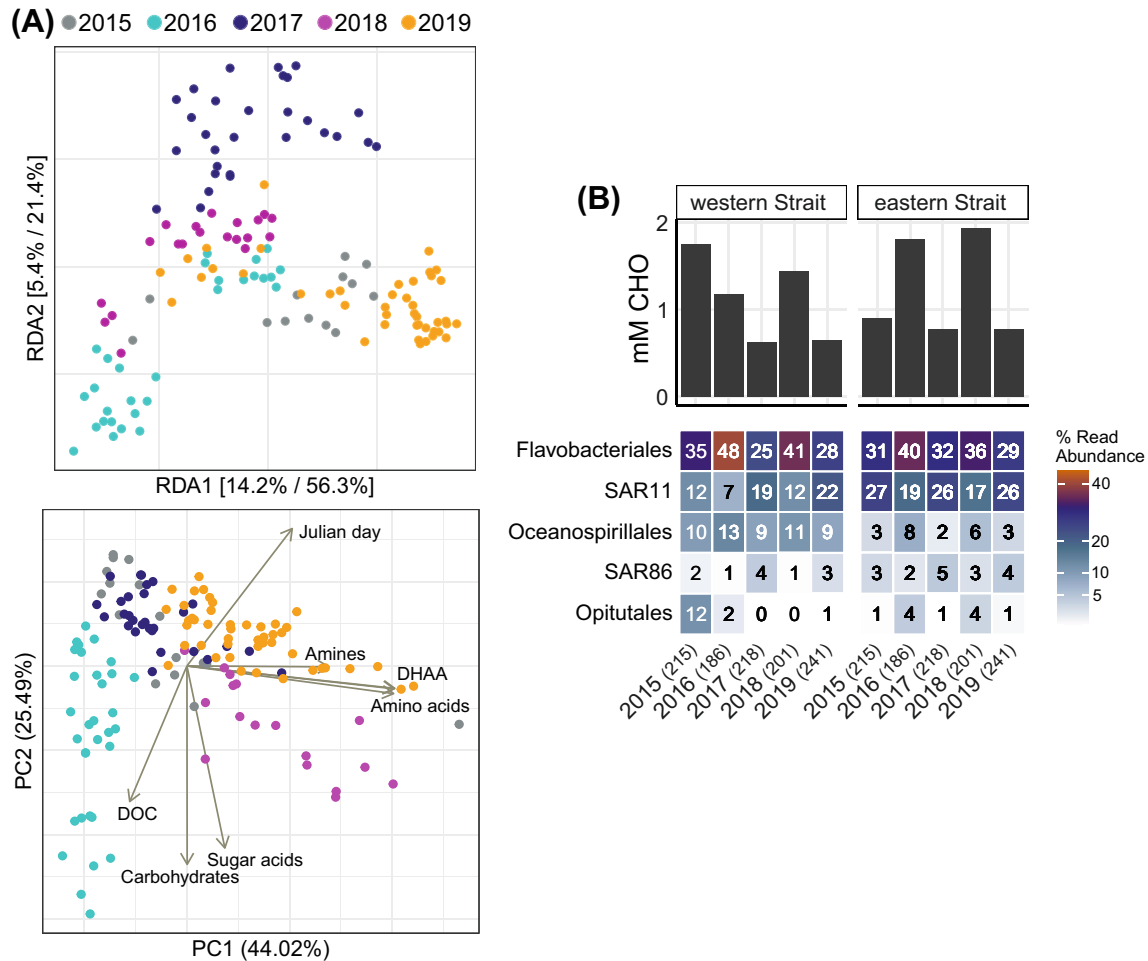


FIGURE 6 Interannual differences in microbial and substrate regimes. (A) RDA of bacterial community structure (top) and PCA of environmental parameters (bottom), separating surface and chl-max samples by year. (B) Carbohydrate concentrations (top) and relative abundances of bacterial classes (bottom) in western and eastern Fram Strait by year. Numbers in parentheses indicate the average Julian day of sampling by year. DHAA: Total hydrolyzable amino acids; CHO: carbohydrates.

et al., 2023; von Appen et al., 2021). Furthermore, local variability in biological and physical parameters can have a direct effect on microbial patterns. For instance, Arctic submesoscale filaments can harbour distinct microbial communities and substrate regimes, with two-fold higher organic matter export than in the surrounding waters (Fadeev, Wietz, et al., 2021).

Signature populations

Sparse partial least square regression (sPLS) established a refined picture of community composition and its environmental drivers. sPLS revealed that approximately half of ASVs were associated with distinct environmental parameters, corresponding to 10 clusters representing defined ecosystem states (Figure 7A and Table S2). On average, ASVs from signature populations constituted a relative abundance of 66%, with a maximum of 78% in the lower-photic western Strait (Table S2). The maximum in subsurface Arctic waters

supports the notion of a stable community in “true” polar conditions, which might be affected by progressing Atlantification (Priest, von Appen, et al., 2023). Each cluster displayed a specific taxonomic composition (Figure 7B). The higher fraction of unclassified genera among subsurface/polar signature taxa (Table S2)—together with higher alpha-diversity and more unique ASVs (Figures S2 and S4)—underlines the presence of uncharted microbial diversity in polar waters (Fadeev et al., 2018). In the following, we discuss the potential ecology of selected signature taxa.

Cluster C1—Julian day (eastern Fram Strait)

C1 signature taxa (191 ASVs; Table S2) predominated during the summer/autumn transition in the eastern Fram Strait. Accordingly, these ASVs constituted ~19% of the community in 2019, compared to ~4% across all other samples. In line with fewer labile substrates (von Jackowski et al., 2022), C1 signifies a

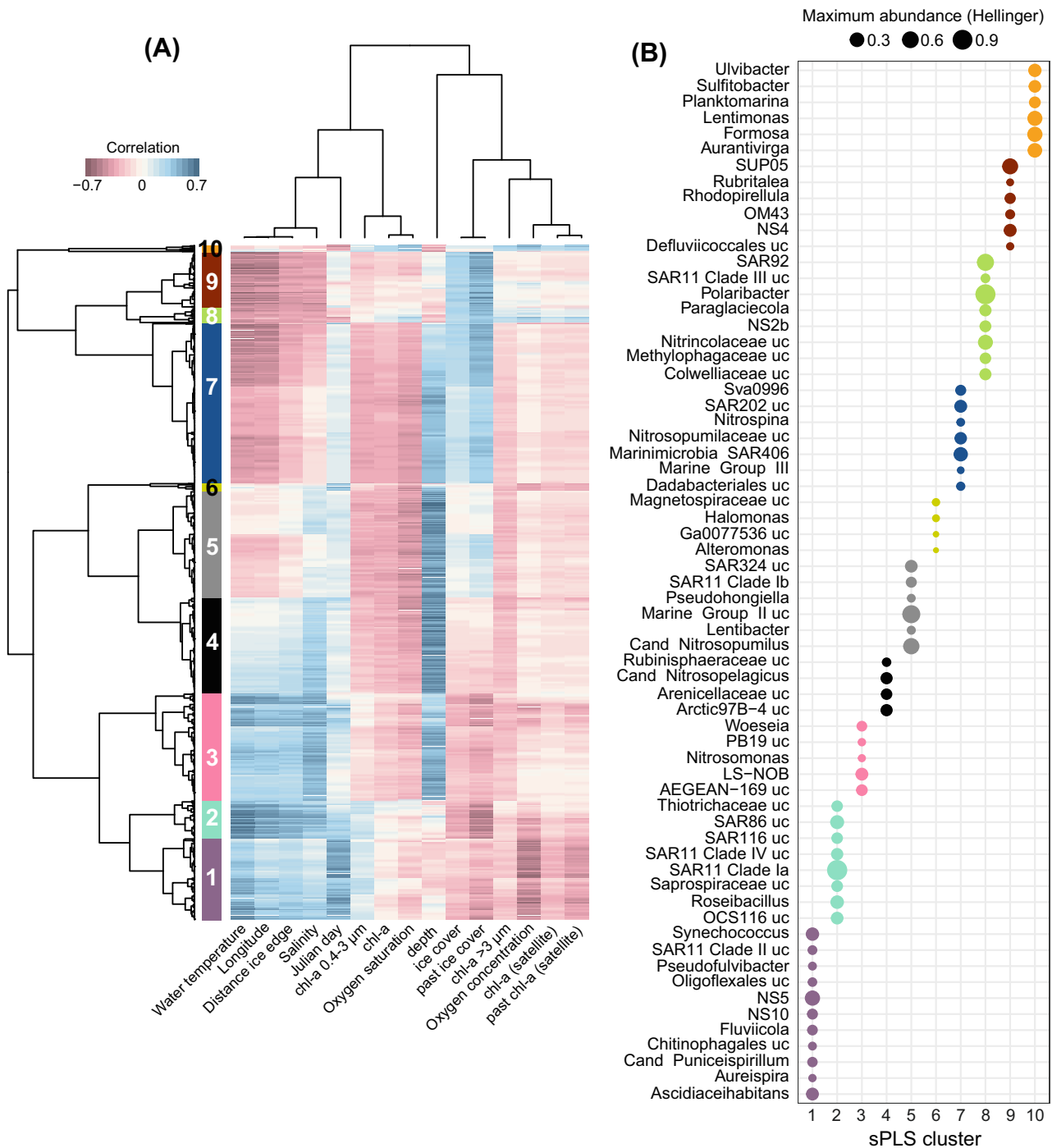


FIGURE 7 Signature populations for distinct ecosystem states. (A) ASV clusters with significant environmental correlations, based on sparse partial least squares regression (sPLS). (B) Major bacterial genera per sPLS cluster, displaying the most prominent cluster affiliation per genus as Hellinger-transformed relative abundances.

detritus-fueled ecosystem state including aggregate formation, as typical during bloom collapse (Allredge & Gotschalk, 1989). *Cand. Puniceispirillum* and *Pseudofulvibacter* (Figure 7B) were probably sustained by decaying phytoplankton (Choi et al., 2015; Gros et al., 2023), whereas *Chitinophagales* and *Oligoflexales* might utilize chitinous aggregates (Fontanez et al., 2015; Li et al., 2021; Martínez-García et al., 2022). *Aureispira* can be attached to gel

particles, and feed on bacterial cells or cell debris (Furusawa et al., 2015; Bunse et al., 2021).

Cluster C2—Surface (eastern Fram Strait)

C2 signature taxa (75 ASVs; Table S2) predominated in Atlantic-influenced surface waters, illustrated by strong positive correlations with temperature and longitude.

Prior studies suggest associations with phytoplankton or their metabolites. For instance, SAR116 commonly encodes genes to degrade DMSP (Choi et al., 2015), an algal compound mediating interactions with bacteria (Kuhlisch et al., 2023). The predominance of *Thiotrichaceae* has been linked to the genetic capacity to oxidize methanethiol (Priest, von Appen, et al., 2023), a compound resulting from DMSP demethylation. The C2 taxon OCS116 occurs in areas of high primary productivity (Morris et al., 2012). The surface signature was underlined by higher proportions of SAR11 clade Ia and SAR86. Detection of *Roseibacillus* and *Saprospiraceae* indicates connectivity with nearby Svalbard fjords (Delpech et al., 2021; Park et al., 2022).

Cluster C3—Lower photic zone (eastern Fram Strait)

C3 signature taxa (212 ASVs; Table S2) predominate in deeper, saltier waters of the eastern Fram Strait, and have a potential for chemolithoautotrophy (Mussmann et al., 2017; Hoffmann et al., 2020). *Nitrosomonas* (ammonia oxidizers) and LS-NOB (nitrite oxidizers) might perform shared denitrification (Lehtovirta-Morley, 2018), although probably less effective than ammonia oxidation by archaea (Rasmussen & Francis, 2022). AEGEAN-169 (recently designated as SAR11 clade V) can potentially utilize a broader range of CHOs than other SAR11 clades, and might participate in trace metal cycling and thiamin synthesis (Getz et al., 2023).

Cluster C4—Lower photic zone (central Fram Strait towards WSC)

This cluster of 189 ASVs (Table S2) is associated with subsurface Atlantic conditions. Accordingly, the Arctic97B-4 clade has been reported in subsurface waters (Pajares, 2021), with a particle-associated, chemomixotrophic lifestyle (Milici et al., 2017; Priest, von Appen, et al., 2023). Little is known about *Arenicella*, reported in sea-ice-associated microbiomes (Garneau et al., 2016) and putatively involved in nitrate reduction (Weigel et al., 2022). Detection of *Cand. Nitrosopelagicus* indicates the presence of urease and ammonia monooxygenase (Royo-Llonch et al., 2021). *Rubinisphaeraceae* are potentially host-associated, considering their detection of marine fauna including deep-sea crustaceans (Kivistik et al., 2020; Leinberger et al., 2022; Angthong et al., 2023).

Cluster C5—Lower photic zone (central Fram Strait towards EGC)

This cluster comprises 211 ASVs (Table S2) associated with subsurface Arctic conditions. The SAR324

clade, common in deep waters of the Arctic (Cardozo-Mino et al., 2021) and worldwide, encodes a versatile metabolism including alkane oxidation (Sheik et al., 2014). *Lentibacter* has been observed in both coastal and deep Arctic waters, especially during low-nutrient conditions (Angelova et al., 2021). This combination possibly favoured establishment in polar waters, which presumably harbour a more refractory substrate pool (Priest, von Appen, et al., 2023).

Cluster C6—Julian day (western Fram Strait)

This narrow cluster (15 ASVs; Table S2) predominated during the summer/autumn transition in the western Fram Strait, constituting a fourfold higher abundance in 2019 samples. This period probably features maximal seeding of ice-derived substrates into the underlying seawater (Underwood et al., 2019). *Alteromonas* might rely on proteolytic activities (Park et al., 2014), potentially related to ice-derived substrates as observed in related *Alteromonadaceae* (Underwood et al., 2019). *Halomonas* has been linked to utilizing carbohydrates and D-amino acids (Celussi et al., 2008; Yu et al., 2020), and *Magnetospiraceae* to nitrogen fixation and thiosulfate oxidation (Williams et al., 2012; von Friesen & Riemann, 2020). Clade Ga0077536 has been suggested as methylotrophs; plus encoding oxygenases targeting aldehydes, terpenes, aliphatics, and aromatics (Francis et al., 2021).

Cluster C7—Lower photic zone (western Fram Strait)

This cluster (315 ASVs; Table S2) prevails in subsurface waters of western Fram Strait, featuring marked positive correlations with depth and sea-ice cover. C7 harbours unclassified *Nitrosopumilaceae* and *Nitrosopina*, indicating shared ammonia and nitrite oxidation (Lehtovirta-Morley, 2018). Sva0996 from phylum Actinobacteria might utilize proteins (Orsi et al., 2016) or detrital phytoplankton biomass (Brunet et al., 2021). SAR406 and SAR202 are typical in the deep Fram Strait (Cardozo-Mino et al., 2021) and elsewhere, linked to sulfur cycling (Hawley et al., 2017; Mehrshad et al., 2018). Marine Group III archaea (phylum Thermoplasmata) presumably participate in protein metabolism, and might encode chitinases (Li et al., 2015; Dutta et al., 2023).

Cluster C8—Surface “freshwater bloom” (western Fram Strait)

This cluster of 30 ASVs (Table S2) is associated with productive fresher waters of the eastern Fram Strait (positive correlation with sea-ice cover and chl-a;

negative correlation with salinity). Hence, lower salinities probably stimulate primary production and the microbial web (Lester et al., 2021). SAR11 clade III has a wide salinity tolerance (Lanclos et al., 2023) and predominates in fresher Arctic waters (Kraemer et al., 2019). *Polaribacter*, *Colwelliaceae*, and *Paraglaucicola* are often associated with sea ice (Bowman, 2014; Deming & Eric Collins, 2017). The presence of *Nitricolaceae* and *Methylophagaceae* suggests methylotrophic metabolism and possible methane oxidation (Gründger et al., 2021).

Cluster C9—Surface (western Fram Strait)

Cluster C9 (110 ASVs; Table S2) signifies “true” polar surface waters (strong positive and negative correlations with sea ice and longitude, respectively). Like C8, this cluster comprises potential methylotrophs (OM43 clade). *Rhodopirellula* can degrade complex sulfated polysaccharides (Wegner et al., 2013); possibly counteracting the lower concentrations of labile substrates in western Fram Strait (Figure 2B). Detection of SUP05 (*Thioglobus*) mirrors its wide distribution in “true” Arctic habitats, based on comparison with TARA and MOSAiC datasets (Priest, von Appen, et al., 2023). Defluviococcales might persist on stored glycogen or unsaturated aliphatics (Burow et al., 2007; Lucas et al., 2016). *Rubritalea* have been found in both first- and multiyear ice (Bowman et al., 2011).

Cluster C10—“Bloom” (western and eastern Fram Strait)

This narrow cluster (13 ASVs; Table S2) shows weak latitudinal, but strong chlorophyll correlations. C10 includes the known phytoplankton associates *Formosa*, *Aurantivirga*, and *Ulvibacter* (*Flavobacteriaceae*), *Planktomarina*, and *Sulfitobacter* (*Rhodobacteraceae*) as well as *Lentimonas* (*Puniceicoccaceae*), illustrating adaptation to algal CHO degradation (Krüger et al., 2019). The strong correlation (coefficient ~ 0.6) of *Ulvibacter* with the >3 µm chl-a fraction supported the link to large phytoplankton (Krüger et al., 2019) that typically produce copious amounts of organic substrates, fueling bacterial growth (Thornton, 2014). With stronger phytoplankton blooms in the future ice-free Arctic Ocean during summer, C10 taxa will likely become more abundant.

Functional potential of signature populations

We mapped ASVs to previously recovered MAGs from the Fram Strait (Priest et al., 2021; Priest, von

Appen, et al., 2023). Fifty-two signature ASVs (associated with sPLS clusters) matched available MAGs at 100% 16S rRNA gene identity (Table S2), allowing to assess their metabolic potential. As the core-EGC is poorly covered by the available MAGs, we only analysed depth-related signatures by comparing sPLS clusters C1, C2, C6, C8, C9, C10 (surface/bloom) with C3, C4, C5, C7 (lower-photoc zone). Considering the substantial decrease in CHO concentrations with depth (Figure 2B), we focused on carbohydrate-active enzymes (CAZymes). MAGs linked to surface populations encoded a greater number and diversity of CAZymes (Figure 8A), including PL6 and PL7 alginate lyases. Furthermore, the exclusive detection of GH10/GH30 (xylanase), GH36/GH42 (alpha-/beta-galactosidase), GH26/GH113 (mannanase), and GH18 (chitinase) indicates that surface populations can utilize a diverse pool of oligomeric carbohydrates. Also, the composition of CAZyme-encoding taxa differed, with surface populations mainly represented by Flavobacteriales and Verrucomicrobiales (Figure 8B) in agreement with reported hydrolytic activities (Cardman et al., 2014; Krüger et al., 2019). Other surface-CAZymes were linked to known phytoplankton associates from the Rhodobacterales (*Sulfitobacter*, *Ascidiaehabitans*, *Planktomarina*) and Opitutales (*Lentimonas*). Subsurface MAGs shared several CAZymes not found in surface MAGs (Figure 8A), including GH88 (chitosan or gellan hydrolase) and PL1 (pectate lyase). Most subsurface CAZymes were found in the verrucomicrobial Arctic97B-4 clade, harbouring 81 CAZyme and 54 sulfatase genes potentially targeting semi-refractory polysaccharides (Priest, von Appen, et al., 2023).

CONCLUSIONS

Microbial communities and substrate regimes in the Arctic Fram Strait showed marked regional and vertical gradients across five summers. Revisiting the same stations over consecutive years established a robust inventory of microbial and biogeochemical dynamics, expanding upon regional and vertical gradients reported from single expeditions (Fadeev et al., 2018; Cardozo-Mino et al., 2021). The majority of populations were coupled to sea-ice cover, temperature, depth, sampling time, and substrate regimes. The predominance of phytoplankton-associated taxa, CHOs, and AAs in Atlantic-influenced surface waters contrasted with a predominance of nitrogenous substrates in the more uniform subsurface waters. Further climate warming will presumably alter polar signatures, affecting vertical diversity gradients and the resident polar microbiome. Patterns attributed to the timing of the annual expedition highlight that time-

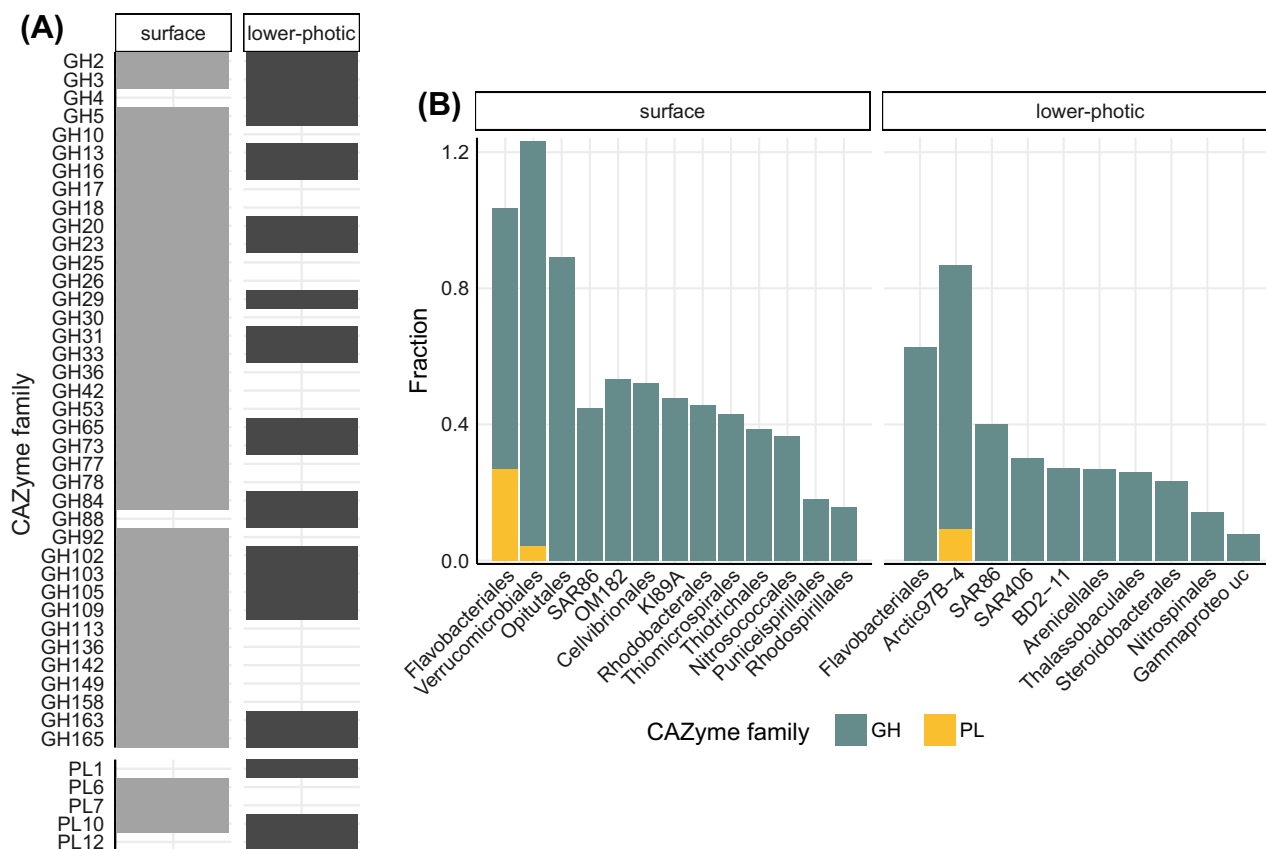


FIGURE 8 Predicted functional capacities of surface and lower-photic bacterial communities, based on MAGs with 100% 16S rRNA gene identity with signature ASVs. (A) Presence/absence of CAZyme genes encoding glycoside hydrolases (GH) and polysaccharide lyases (PL). (B) Fraction of GH- and PL-encoding genes by bacterial orders.

series studies need to consider the prevailing ecosystem state. Overall, our evidence establishes a benchmark to quantify persistence versus change in the Fram Strait and identifies potential consequences for ecosystem functioning.

AUTHOR CONTRIBUTIONS

Matthias Wietz: Conceptualization; formal analysis; visualization; writing – original draft. **Anja Engel:** Investigation; data curation; formal analysis; funding acquisition; writing – review and editing. **Simon Ramondenc:** Formal analysis; writing – review and editing. **Matomo Niwano:** Formal analysis; writing – review and editing. **Wilken-Jon von Appen:** Investigation; data curation; formal analysis; writing – review and editing. **Taylor Priest:** Formal analysis; writing – review and editing; visualization. **Anabel von Jackowski:** Investigation; formal analysis; writing – review and editing. **Katja Metfies:** Investigation; data curation; resources; writing – review and editing. **Christina Bienhold:** Writing – review and editing; investigation; data curation. **Antje Boetius:** Conceptualization; project administration; funding acquisition; resources; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in ENA: <https://www.ebi.ac.uk/ena/browser/view/PRJEB66267>. The bioinformatic workflow, including all files needed to reproduce analyses and figures, is available on GitHub at <https://github.com/matthiaswietz/fiveArcticSummers>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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