Received: 8 May 2024 | Accepted: 24 July 2024

DOI: 10.1111/1462-2920.16687

BRIEF REPORT

ENVIRONMENTAL MICROBIOLOGY

Distinct actors drive different mechanisms of biopolymer processing in polar marine coastal sediments

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Funding information
Max-Planck-Gesellschaft; National Science Foundation, Grant/Award Numbers: OCE-2022952, OCE-2241720

Abstract

Heterotrophic bacteria in the ocean initiate biopolymer degradation using extracellular enzymes that yield low molecular weight hydrolysis products in the environment, or by using a selfish uptake mechanism that retains the hydrolysate for the enzyme-producing cell. The mechanism used affects the availability of hydrolysis products to other bacteria, and thus also potentially the composition and activity of the community. In marine systems, these two mechanisms of substrate processing have been studied in the water column, but to date, have not been investigated in sediments. In surface sediments from an Arctic fjord of Svalbard, we investigated mechanisms of biopolymer hydrolysis using four polysaccharides and mucin, a glycoprotein. Extracellular hydrolysis of all biopolymers was rapid. Moreover, rapid degradation of mucin suggests that it may be a key substrate for benthic microbes. Although selfish uptake is common in ocean waters, only a small fraction (0.5%–2%) of microbes adhering to sediments used this mechanism. Selfish uptake was carried out primarily by *Planctomycetota* and *Verrucomicrobiota*. The overall dominance of extracellular hydrolysis in sediments, however, suggests that the bulk of biopolymer processing is carried out by a benthic community relying on the sharing of enzymatic capabilities and scavenging of public goods.

INTRODUCTION

Most organic matter synthesized by phytoplankton and macroalgae in the ocean is in the form of biopolymers, high molecular weight (HMW) compounds including polysaccharides and proteins (Biersmith & Benner, 1998; Hedges et al., 2002; Percival & Mcdowell, 1990). A considerable fraction of these biopolymers is processed and degraded by heterotrophic microbial communities, which respire the organic carbon back to CO_2 (Azam & Malfatti, 2007). To consume biopolymers, however, these communities must first hydrolyze them to sizes suitable to be taken into the cell. This hydrolysis can occur via two distinct mechanisms: extracellular hydrolysis that produces low molecular weight oligomers in the environment, or selfish uptake, in which hydrolysis is closely coupled to the transport of large fragments of biopolymers into the cell (Cuskin et al., 2015; Reintjes et al., 2017). The balance between extracellular (external) hydrolysis and selfish uptake affects the availability of low molecular weight oligomers to heterotrophic bacteria that do not or cannot produce extracellular enzymes, and therefore influences the size distribution of organic matter and potentially the composition and activity of the heterotrophic bacterial community (Arnosti et al., 2018).

Selfish uptake was initially described among human gut bacteria (Cuskin et al., 2015), and only recently recognized in the ocean (Reintjes et al., 2017). Nonetheless, selfish uptake appears to be quite common among pelagic bacteria—up to 60% of total cells during a summer sampling campaign (Giljan et al., 2022)—and

is phylogenetically widespread among *Bacteroidota*, *Gammaproteobacteria*, *Planctomycetota* and *Verrucomicrobiota* (e.g. Giljan et al., 2022; Reintjes et al., 2017; Reintjes et al., 2019; Brown et al., 2024).

To date, however, these distinct substrate processing mechanisms have not been investigated in sediments, despite the fact that a significant fraction of marine organic matter is remineralized in shallow coastal sediments (Huettel et al., 2014; Middelburg, 2018). Sedimentary environments differ greatly from the water column, for example in fluid flow and the presence of surfaces, which potentially provide greater opportunities for bacteria to interact. Sediments also differ from the water column in bacterial community composition (Miksch et al., 2021; Probandt et al., 2017; Teske et al., 2011), as well as in the breadth of polysaccharide hydrolase activities (Arnosti, 2008; Arnosti, 2015; Teske et al., 2011): the spectrum of active enzymes is typically far broader in sediments than in the overlying water column (Arnosti, 2008, Arnosti, 2015, Teske et al., 2011). In short, there are considerable unknowns with respect to the manner in which bacteria use extracellular enzymes, process substrates and interact in sediments (Arnosti et al., 2014; Arnosti et al., 2019).

Given differences in community composition and breadth of enzymatic function in sediments compared to the water column, as well as the high external hydrolysis rates and the broad spectrum of enzyme activities previously measured in Svalbard sediments (Arnosti, 2008; Cardman et al., 2014; Teske et al., 2011), we hypothesized that the relative relationships between selfish uptake and external hydrolysis of biopolymers would be different in sediments compared to the water column. We measured the extent of selfish uptake and rates of external hydrolysis in surface sediments sampled in spring and late summer in Isfjorden, an Arctic fjord of Svalbard, an archipelago in the Arctic Ocean at 78° N. We focused especially on processes carried out by organisms adhering to sediments, and not in the porewater, since they are the ones most likely to benefit from surface adhesion and the nearby presence of other bacteria. We identified the organisms responsible for degrading biopolymers, and also measured the growth dynamics of the bacterial community. In polar environments, fresh phytoplankton productivity occurs only during part of the year. Therefore, we investigated the hydrolysis and uptake of a suite of polysaccharides that likely are present at least seasonally in the environment. In addition, we investigated the hydrolysis and uptake of mucin, a glycoprotein derived from marine animals such as invertebrates (Davies & Hawkins, 1998; Riemann & Schrage, 1978; Smith et al., 1995; Stabili et al., 2019) that presumably is present year-round in sediments.

EXPERIMENTAL PROCEDURES

Sampling

Sediment samples were collected in Isfjorden, Svalbard, at station 5 (N78°06.405′, E14°21.070′ ± 20 m) on 25 April and 13 September 2019, and on 29 June 2022. During the first two sampling trips, sediment was collected with a van Veen grab; sampling in June 2022 was carried out with an Ellrott grab (Moncada et al., 2024). The top two centimetres of the sediments were subsampled. All sediments consisted of fine and medium-grained sand, but September sediments also had a contribution of silt (Miksch et al., 2021). Sediment temperatures in April, September and June were 2.2°C, 4.5°C and 5.2°C, respectively; salinity was 35, 32 and 34 PSU. Chlorophyll *a* concentration was 8, 2.5 and 1.8 μg L^{-1}, and in surface sea water and 400, 589 and 1800 μg L^{-1} in surface sediments in April, June and September, respectively (for further details see Miksch et al., 2021).

Polysaccharides and glycoprotein substrates

Four polysaccharides and one glycoprotein (all obtained from Sigma-Aldrich) were selected for our incubations: laminarin (from *Laminaria digitata*), xylan (from beechwood), chondroitin sulphate (from shark cartilage), fucoidan (from *Fucus vesiculosus*) and mucin (from porcine stomach). The four polysaccharides are derived from phytoplankton and/or enzymes hydrolyzing these polysaccharides have been demonstrated to occur in marine bacteria (for review see Arnosti et al., 2021). Mucins are slimes (glycoproteins of 0.5–2.0 MDa) secreted by invertebrates. They are characterized by the presence of a central protein core with heavily glycosylated side-chains (Bansil & Turner, 2006). The levels of glycosylation differ, however the carbohydrate content of mucins is usually around 80% (Bansil & Turner, 2006). The polysaccharides, as well as the mucin, were fluorescently labelled with fluoresceinamine and purified according to Arnosti (2003). Except as otherwise noted, added FLA-PS concentrations were 100 μM monomer equivalent. Note that for simplicity, in the following text we will include the fluorescently labelled glycoprotein, mucin, in the abbreviation for fluorescently labelled polysaccharides (FLA-PS).

Incubations with fluorescently labelled polysaccharides (FLA-PS)

In April, immediately after collection of the sediments, 180 ml of bulk sediments were carefully resuspended in approximately 4.5 L of sterile artificial seawater

(ASW: 450 mM NaCl, 59.6 mM $MgCl_2$, 56.5 mM $MgSO_4$, 13.2 mM $CaCl_2$, 9.7 mM KCl, 0.8 mM KBr, 0.3 mM H_3BO_3, 0.1 mM $SrCl_2$, pH 8.0) and then the sediments were allowed to settle, and the overlying ASW was decanted. This procedure was carried out three times. The main intention of washing was to remove planktonic bacteria from the porewater in order to focus on the identities and activities of the sediment grain-associated bacterial community. This procedure was also intended to remove dissolved organic carbon contained in porewater so that the resident community would have only particulate organic matter (and the added substrates) to hydrolyze.

Batch incubations of sediments were set up by adding 10 ml of sterile ASW to 3 ml of sediments in 50 ml polypropylene tubes. Three replicates were then amended with one of the five selected FLA-PS to a final 100 μM monomer equivalent concentration, assuming a molecular weight of 500 kD for mucin, and an amino acid: hexose ratio of 1:1. The killed controls consisted of sediment subsamples that were previously fixed in 3.5% formaldehyde (final concentration) for 1 h at room temperature that were also incubated with the FLA-PS. All samples were incubated close to in situ temperatures at 4°C in the dark. The incubations were subsampled after 20 min (referred to as t0), 1.5 d (t1), 3.5 d (t2), 6.5 d (t3), 10.5 d (t4) and 17.5 d (t5) of incubation. For measurements of extracellular enzymatic activities, 1 ml of supernatant was sampled with a syringe, filtered through a 0.2 μm Nalgene filter (SCFA membrane, Thermo Scientific) and frozen at −20°C until analysis. Sediment-associated bacteria—and their sediment-associated enzymes have access to dissolved FLA-PS, thus when we subsample the liquid phase, we sample the lower molecular weight hydrolysis products of their enzymatic activity. Additionally, sediment grains from the slurry were used to determine total cell numbers, to identify selfish cells and do fluorescence in situ hybridization (FISH). For this purpose, 250 μl of sediment were transferred with cell-saver tips to Biosphere® screw cap micro tubes (2.0 ml, with graduation) and fixed with 750 μl formaldehyde (1.5% final concentration) for at least 1 h at room temperature. After subsampling, sediments and FLA-PS-amended ASW were carefully mixed and incubation was continued.

Sampling was similar for silty sediments collected in September: 300 ml were carefully resuspended immediately after collection, as described above, in a total of 10 L ASW. Three replicates each of 2.5 ml sediments were slurried with 8 ml ASW, which were amended with FLA-PS. Additionally, unamended controls without added FLA-PS were used to follow cell growth during the time course of incubation. Formaldehyde-fixed samples were also incubated with the polysaccharides, serving as killed controls. The formaldehyde concentration could not be defined, however, as there was massive precipitation in the solution that could not be re-dissolved completely aboard ship. Samples were incubated at 4°C in the dark.

Subsampling was done after 20 min (t0), 0.25 d (t1), 1 d (t2), 2 d (t3), 4 d (t4) and 10 d (t5) of incubation. Using cell saver tips, 1.3 ml homogenized sediment slurry (composed of ∼300 μl sediment and ∼ 1000 μl FLA-PS-amended ASW) was transferred into Biosphere® screw cap micro tubes. For measurements of extracellular enzymatic activities, ∼1 ml of supernatant was subsampled with a syringe, filtered through a 0.2 μm Nalgene filter (SCFA membrane, Thermo Scientific) and frozen at −20°C until analysis. For FISH, 300 μl of sediment was fixed with 750 μl formaldehyde (1.5% final concentration) for at least 1 h at room temperature.

Incubations with different mucin concentrations

To examine cellular growth, external hydrolysis and selfish uptake as a function of mucin concentration, sediment samples were also collected in June 2022. For these experiments, intact sediment samples (no washing or removal of porewater) were incubated with three different mucin concentrations: three replicates of 3 ml sediment subsamples were incubated with 10 ml of autoclaved and 0.2 μm sterile-filtered ASW, which was amended with either 10, 100, or 1000 μM FLA-mucin and incubated for 3 days at 4°C. Killed controls were prepared by using a domestic pressure cooker for 30 min to sterilize the sediments prior to substrate addition. Subsampling was done after 20 min (t0), 1 day and 3 days of incubation and processing of samples as described above.

Measurement of extracellular enzymatic activities

Polysaccharide (and mucin) hydrolysis rates were measured via changes with time in the molecular weight of the added FLA-PS (described in detail in Arnosti, 2003). In brief, samples were thawed and injected on a system of two Sephadex gel columns (G-50 and G-75, linked in series), which separate organic biopolymers as a function of molecular size. The fractions were detected via fluorescence (ex: 490 nm; em: 530 nm) of the fluoresceinamine covalently attached to the polysaccharides and fragments. Columns were standardized with FITC-dextrans, FITC-galactose and free fluoresceinamine.

For the April and September samples, instead of using the standard method for killed controls (use of an autoclave aboard ship was not possible), the sediment slurry was amended with formaldehyde (3.5% final

concentration) for 1 h at room temperature. Especially in the laminarin and xylan incubations, this technique was clearly unsuitable, since it resulted in enzyme activities that were almost as high as those measured in the active incubations. Cell growth was also measurable (Table S1). One possible reason for the activity in the controls was an insufficiently high formaldehyde concentration to kill the cells and to inactivate extracellular enzymes such as laminarinases and xylanases. While in April killed controls only slight cell growth was detected, cell growth in September samples was more pronounced, most likely because of low formaldehyde concentrations, due to precipitation of the added formaldehyde. For example, in killed controls with xylan, mucin and fucoidan, cell numbers increased 2.0-, 1.6-, and 2.9-fold in course of the experiment while increases in April incubations where between 1.0- and 1.3-fold. A correction of the rates by the values measured in the killed control would result in hydrolysis rates close to zero, which is unlikely, as rapid hydrolysis of laminarin and xylan has previously been reported for Arctic sediments (e.g. Arnosti, 2008; Cardman et al., 2014). Therefore, here we show hydrolysis rates for laminarin and xylan that were not corrected by the rates for the corresponding killed controls. Notably, however, killed controls for mucin, chondroitin sulphate and fucoidan showed no or very low enzyme activities despite considerable cell growth, especially for the September samples (Table S1). We speculate that these differences arise from the extent to which enzymes may already be present in cells versus enzymes that require induction. In addition, we speculate that a broader fraction of the bacterial community has enzymes that hydrolyze comparatively simple polysaccharides such as laminarin and xylan, compared to the likely larger and more specialized enzyme complement required to hydrolyze mucin, chondroitin and fucoidan (see Section 4).

Separation of cells from sediment grains

All formaldehyde-fixed samples were washed three times with 0.2 μm-sterile-filtered 1× phosphate-buffered saline (PBS; 13.7 mM NaCl, 0.27 mM KCl, 1 mM Na_2HPO_4, 0.2 mM KH_2PO_4). Afterwards, they were sonicated on ice with a Sonopuls GM Mini20 equipped with a microtip MS 2.5 (Bandelin, Berlin, Germany). Samples collected in April (sandy sediments) were sonicated three times at a setting of 30 s, an amplitude of 86% and pulse of 0.2 s. Supernatants were collected and replaced by 750 μl 1×PBS. Aliquots of combined supernatants were filtered through a 47 mm (0.2 μm pore size) polycarbonate filter (GTTP, Millipore, Eschborn, Germany), applying a gentle vacuum of <200 mbar. Silty sediments collected in September were sonicated only once. An aliquot was taken from the tube, diluted and filtered. After drying, the filters were stored at −20°C until further analysis.

FISH for visualization and quantification of selfish bacteria

FISH was carried out with slight modifications of the protocol by Manz et al. (1992). The hybridization mix contained 0.9 M NaCl, 0.02 M Tris–HCl (pH 8), 10% (wt/vol) dextran sulphate, 0.02% (wt/vol) sodium dodecyl sulphate, 1% (wt/vol) blocking reagent (Roche, Mannheim, Germany), x% (vol/vol) formamide (according to the individual probe requirements) and 0.83 pmol μl^{-1} 4×Atto594-labelled oligonucleotide probe. Hybridization was carried out in a humidity chamber equilibrated with 2.25 M NaCl and identical formamide concentration as in the hybridization buffer at 46°C for 3 h. Filter sections were subsequently washed in prewarmed buffer (0.08–0.225 M NaCl, depending on formamide concentration in the hybridization buffer, 20 mM Tris–HCl (pH 8), 0.05 M EDTA (pH 8), 0.02% (wt/vol) sodium dodecyl sulphate) for 15 min at 48°C. Filter sections were removed from the buffer, air-dried on Whatman paper and mounted in CitiFluorAF1 (CitiFluor Ltd., London) and Vectashield (Vector Laboratories, Burlingame, CA) containing 1 μg ml^{-1} DAPI (4′,6-diamidino-2-phenylindole; Sigma-Aldrich, Steinheim, Germany).

Selfish bacteria were quantified by epifluorescence microscopy (Reintjes et al., 2017, 2020). FISH signals and FLA-PS uptake were visualized by epifluorescence microscopy (Nikon Eclipse 50i). For quantification of FLA-PS stained cells (selfish bacteria), 100 fields of view were manually counted. For identification of these selfish bacteria, ∼100 FLA-PS stained cells were analysed with each FISH probe (for September, >25 FLA-PS stained cells; FLA-xylan stained cells were not analysed). For quantification of total FISH-stained cells, 20 to 30 fields of view were counted. Imaging was done by a laser scanning microscope (LSM780, Zeiss, Jena, Germany) equipped with an Airyscan detector. Probe sequences, permeabilization conditions and formamide concentrations are given in Table S2.

Quantification of *Actinomarinales* (probe ACM1218) and *Microtrichales* (probe MIT1218) was not reliable using 4xAtto594-labelled probes because the signal was too low. As FLA-PS signals were not expected in these Gram-positive clades due to the absence of a periplasm, we quantified total *Actinomarinales* and *Microtrichales* via FISH with horseradish peroxidase (HRP)-labelled probes followed by fluorescently labelled-tyramide signal amplification (CARD) as described previously (Miksch et al., 2021; Pernthaler et al., 2002).

Phylogenetic tree reconstruction and oligonucleotide probe design

Oligonucleotide probes for the identification of the *Verrucomicrobiota* genera *Rubritalea, Luteolibacter, Roseibacillus, Haloferula* and *Persicirhabdus* were developed. Probe design was targeted towards these genera as they were most abundant based on relative 16S rRNA gene abundance (Miksch et al., 2021). As a prerequisite for probe design, a phylogenetic tree was reconstructed from 441 full-length *Verrucomicrobiota* 16S rRNA genes selected from the SILVA database SSU Ref138.1 (Pruesse et al., 2007, Quast et al., 2013, released Aug 2020). The tree was calculated using a maximum likelihood method (PHYML) with GTR correction. A filter that excluded positions with more than 50% variability was implemented, which resulted in 1381 valid sequence positions for tree calculation. Selected sequences belonging to the phylum *Planctomycetota* were used as outgroup. Partial *Verrucomicrobiota* 16S rRNA gene sequences from Isfjorden, Svalbard sediments (Miksch et al., 2021) spanning the V3–V4 hypervariable region were imported into the database and added to the tree under Parsimony criteria without allowing changes in the overall tree topology.

Probe design was done using the Probe Design tool of the ARB software package version arb-devel-6.1.rev18634 (Westram et al., 2011). Additionally, a set of competitors (Manz et al., 1992) and helpers (Fuchs et al., 2000) flanking the 5′ and 3′ ends of the target sequences was designed and used in equimolar concentrations with the probe.

DNA extraction and microbial diversity analyses

DNA was extracted from April sediments and September 2019 homogenized sediment slurries subsampled at t0 and t5 using the Power Soil Kit (Qiagen, Hilden, Germany). Amplification of 16S rRNA gene fragments was done using the primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 (Herlemann et al., 2011). Amplicons were sequenced on an Illumina (San Diego, CA) platform (HiSeq2500, 2×250 bases, paired-end) at the Max Planck-Genome Center in Cologne (Germany). Sequences were processed using BBTools version 37.62 (Bushnell et al., 2017), mothur v.1.38.1 (Schloss et al., 2009) and after subsampling to 10,000 reads per sample, classified using the SILVAngs pipeline and database SSU 138.1 Ref NR99 (Quast et al., 2013). Plotting was done using R (Team, 2019; Wickham, 2017). For details see Miksch et al. (2021). Script is deposited at: https://github.com/smiksch/16_S_rRNA_processing_looking_at_seasonality_in_sediments.

Amplicon sequence variants analysis

Amplicon sequence variants (ASVs) were determined from processed sequences using the DADA2 (v. 1.30.0, Callahan et al., 2016). We followed the standard pipeline (https://benjjneb.github.io/dada2/tutorial.html) with the following parameter settings for filterAndTrim(): truncLen = c(220,220), maxN = 0, maxEE = c(2,2), truncQ = 2. After merging of forward and reverse reads with DADA2 an ASV count table was built for statistical analysis. Absolute singletons were removed, abundances of ASVs were normalized to relative abundances, and an NMDS ordination was calculated using the vegan package v. 2.6–4 (Oksanen et al., 2019) with the function metaMDS() and Bray–Curtis dissimilarity (Bray & Curtis, 1957). Plotting was done using ggplot2 (Wickham, 2016).

RESULTS

Extracellular enzyme activities

Hydrolysis rates for laminarin, xylan and mucin were considerably higher than for fucoidan and chondroitin in both April and September (Figure 1). The highest laminarin, xylan and mucin hydrolysis rates were measured in the September incubations, with maximum rates in the range of 6000 nmol monomer L^{-1} sediment h^{-1}; maximum rates for these substrates in April were in the range of 1500–3000 nmol monomer L^{-1} sediment h^{-1}. Considering the difference in initial sampling intervals (rates measured first after 1.5 days in April, vs 0.3 days in September), however, it is possible that maximum rates occurred prior to the first sampling timepoint in April. Differences in rates at similar timepoints for the two sampling dates (e.g. 1.5 days vs. 1 day) were smaller, although September rates were generally still higher. Twice as high initial cell numbers in September sediments (5.9×10^8 cells ml^{-1}) versus April sediments (2.9×10^8 cells ml^{-1}) might have also contributed to higher rates in September incubations.

Hydrolysis rates for chondroitin and fucoidan were considerably lower than for the other three substrates, and the maximum hydrolysis rates were measured at later timepoints. Considerable rates of chondroitin hydrolysis (~700–800 nmol monomer L^{-1} sediment h^{-1}) were first measurable after 3.5–4 days, and increased to ~1000 nmol monomer L^{-1} sediment h^{-1} by day 10 of incubation for both April and September incubations. In April incubations, fucoidan hydrolysis rates were somewhat lower than chondroitin hydrolysis rates, whereas in September incubations, they were similar.

Sediments treated for 1 h with 3.7% formaldehyde at 20°C and amended with xylan and laminarin showed high extracellular hydrolysis rates. Hydrolysis, however,

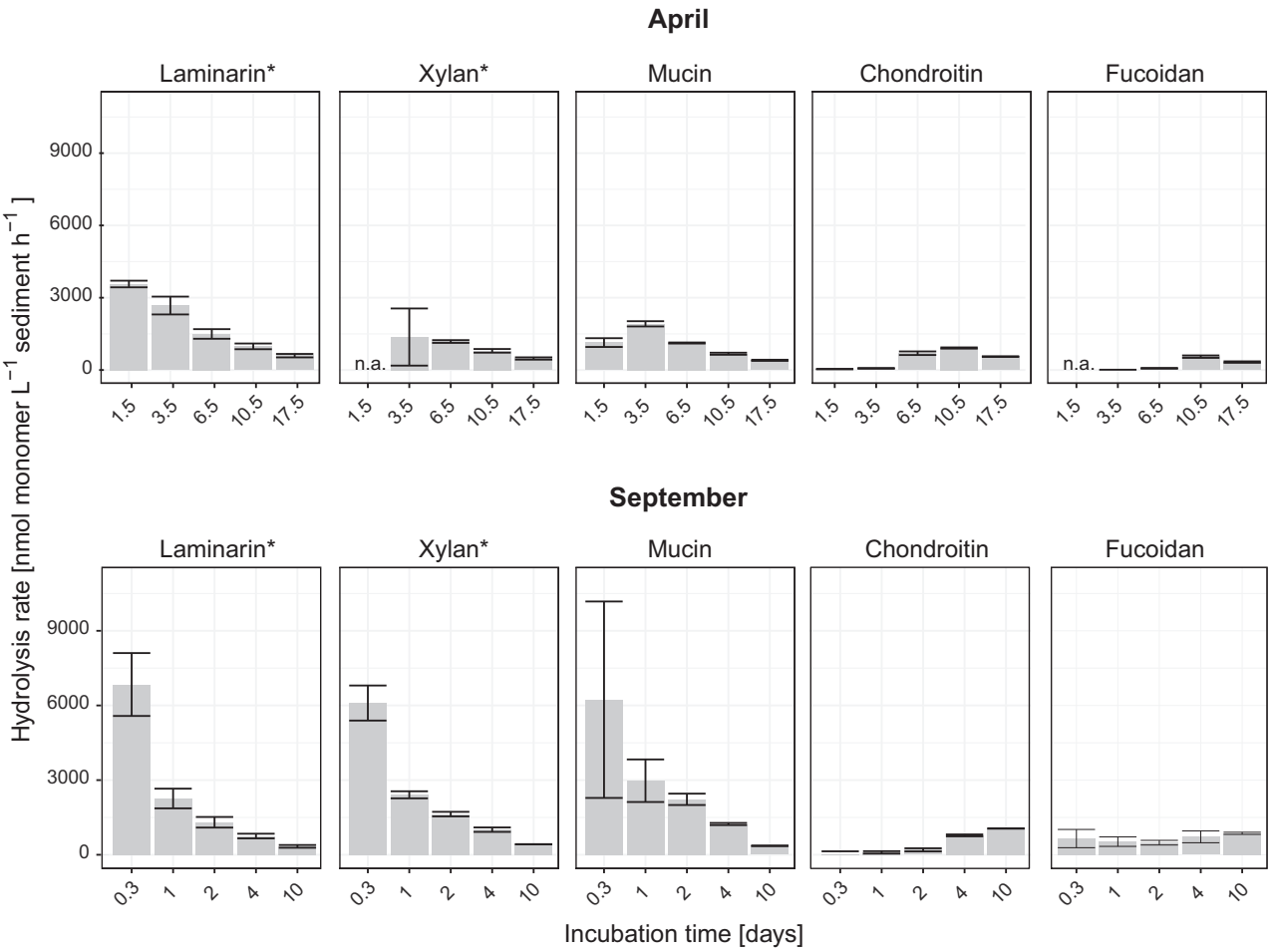

FIGURE 1 Hydrolysis rates of polysaccharides in homogenized surface sediments (0–2 cm) from Isfjorden, Svalbard. (A), April incubation; (B), September incubation. Error bars show the standard deviations of triplicate incubations. *Note, hydrolysis rates of laminarin and xylan were not corrected by values measured in killed controls as controls were insufficiently inactivated by formaldehyde fixation.

was not detected in killed controls of more complex substrates such as chondroitin sulphate, fucoidan and mucin. These results may be due to the presence of viable cells after formaldehyde fixation: cell growth was detected in formaldehyde-treated sediments from September (Table S1). The precipitated formaldehyde solution used (and therefore arbitrary concentration) was likely insufficient to effectively kill the cells. Furthermore, the presumably higher level of organic matter in silty September sediments might have bound much of the formaldehyde. Nevertheless, no selfish uptake was observed in formaldehyde-treated controls in September. Much of the hydrolysis measured in the formaldehyde-treated controls may be explained by the presence of extracellular enzymes that were insufficiently inactivated. The observation that fixation experiments (40% formaldehyde for 2 h at 4°C) with different enzymes extracted from rat liver have shown a residual activity compared to unfixed enzymes of 52% for acid phosphatase, 29% for catalase 29% and 50% for β-glucuronidase (Hopwood, 1967) support this explanation.

Selfish substrate uptake

Selfish uptake of all substrates tested was detected in both April and September incubations (Figure 2 and Table S1). However, only a small fraction of the benthic bacterial community used this mechanism of polysaccharide processing. In April, the fraction of selfish bacteria was greatest after 1.5 days and decreased with time. The highest abundance of FLA-PS stained cells was found in the laminarin incubations (mean ± standard deviation; 2.1 ± 0.2% of total cells). The highest numbers of FLA-mucin stained cells were detected after 3.5 days (1.4 ± 0.1% of total cells). Other incubations showed smaller fractions of FLA-PS stained cells (chondroitin sulphate: 1.1 ± 0.6%; fucoidan: 0.8 ± 0.3%; xylan: 0.8 ± 0.1%). In the September incubations, the overall fraction of selfish bacteria was lower than in April. The highest abundance of FLA-PS stained cells was found in mucin incubations after 0.3 days (1.6 ± 0.4% of total cells). The laminarin incubations showed only a small fraction of selfish bacteria (0.1–0.5% of total cells). Similarly low fractions of

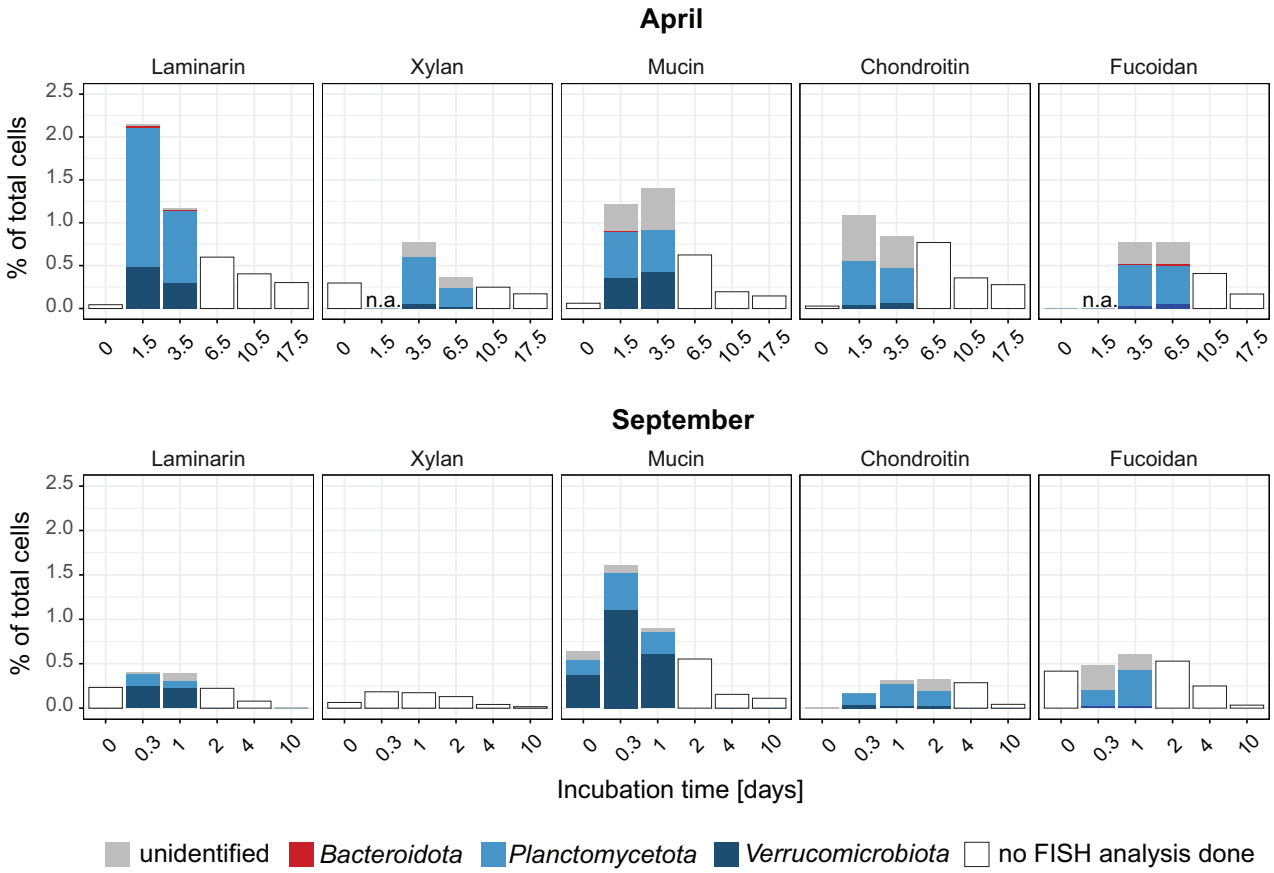

FIGURE 2 Relative abundance of FLA-PS-stained cells (selfish uptake) for all April (upper panel) and September (lower panel) incubations and timepoints. For early timepoints (1.5 and 3.5 days in April; 0.3 and 1 day in September), FISH was used to target these selfish cells. A large fraction was identified as members of *Planctomycetota* (light blue; PLA46) and *Verrucomicrobiota* (dark blue, EUB338-III). *Bacteroidota* contributed a very small fraction (red; CF319a) in laminarin, mucin and fucoidan incubations from April only; the remaining, yet unidentified FLA-PS-stained cells are indicated by grey colour. FLA-xylan stained cells could not be specifically identified in September incubations due to their low cell numbers. For simplicity, bars show the mean of three replicates (individual values are given in Table S1). n.a., not analysed.

selfish bacteria were found for uptake of xylan (0.2 ± 0.0% of total cells), chondroitin sulphate (0.3 ± 0.1), and fucoidan (0.6 ± 0.1). Killed controls showed no selfish uptake of any of the substrates.

Three dominant morphotypes of FLA-PS stained cells were detected in the April incubations (Figure 3): (i) an oval, coccoid type, which dominated in all incubations (Figure 3A), and (ii) and rods with an apolar or (iii) bipolar FLA-PS signal (Figure 3B,C), which were most often detected in April incubations and only rarely in September. In the September incubations, a fourth, oval morphotype was identified that had curved 'parentheses' of substrate staining on either side of the DAPI-stained DNA (Figure 3D). In general, FLA-laminarin and FLA-mucin signals were brighter than FLA-chondroitin and FLA-fucoidan signals in both April and September.

Identification of FLA-PS stained cells

To identify bacteria taking up specific FLA-PS, FISH probes were used for samples collected over the initial timepoints (1.5 and 3.5 days in April; 0.3 and 1 day in September); the fraction of selfish bacteria was too small at the other timepoints to allow counting of a statistically sufficiently high number of FLA-PS stained cells. For all FLA-PS, *Planctomycetota* made up the largest fraction of selfish bacteria, constituting 76 ± 16% of total selfish bacteria taking up laminarin, 45 ± 2% taking up mucin, 71 ± 8% taking up xylan, 49 ± 12% taking up chondroitin sulphate, and 63 ± 7% taking up fucoidan in April incubations, as well as 84 ± 5% of total selfish bacteria taking up chondroitin sulphate and 69 ± 11% taking up fucoidan in September incubations (Figure 2). Their contribution to laminarin and mucin uptake in the September incubations was lower, at 37 ± 6% and 28 ± 8%, respectively. Selfish *Planctomycetota* were typically coccoid or oval. The substrate signals appeared either condensed close to one pole or distributed in the periplasm (Figure 3E–J). For some cells, the distribution of substrate signals indicated massive invaginations of the cytoplasmic membrane that are well known for *Planctomycetota* (Boedeker et al., 2017).

The second most abundant fraction of benthic selfish bacteria were members of the phylum *Verrucomicrobiota*. They were usually rod-shaped, with a polar or bipolar localized substrate signal (Figure 3K–M). Rarely, oval to coccoid morphotypes were detected (Figure 3N). *Verrucomicrobiota* also constituted a large fraction of selfish bacteria, constituting 69 ± 11% and 60 ± 7% of selfish bacteria in mucin and laminarin

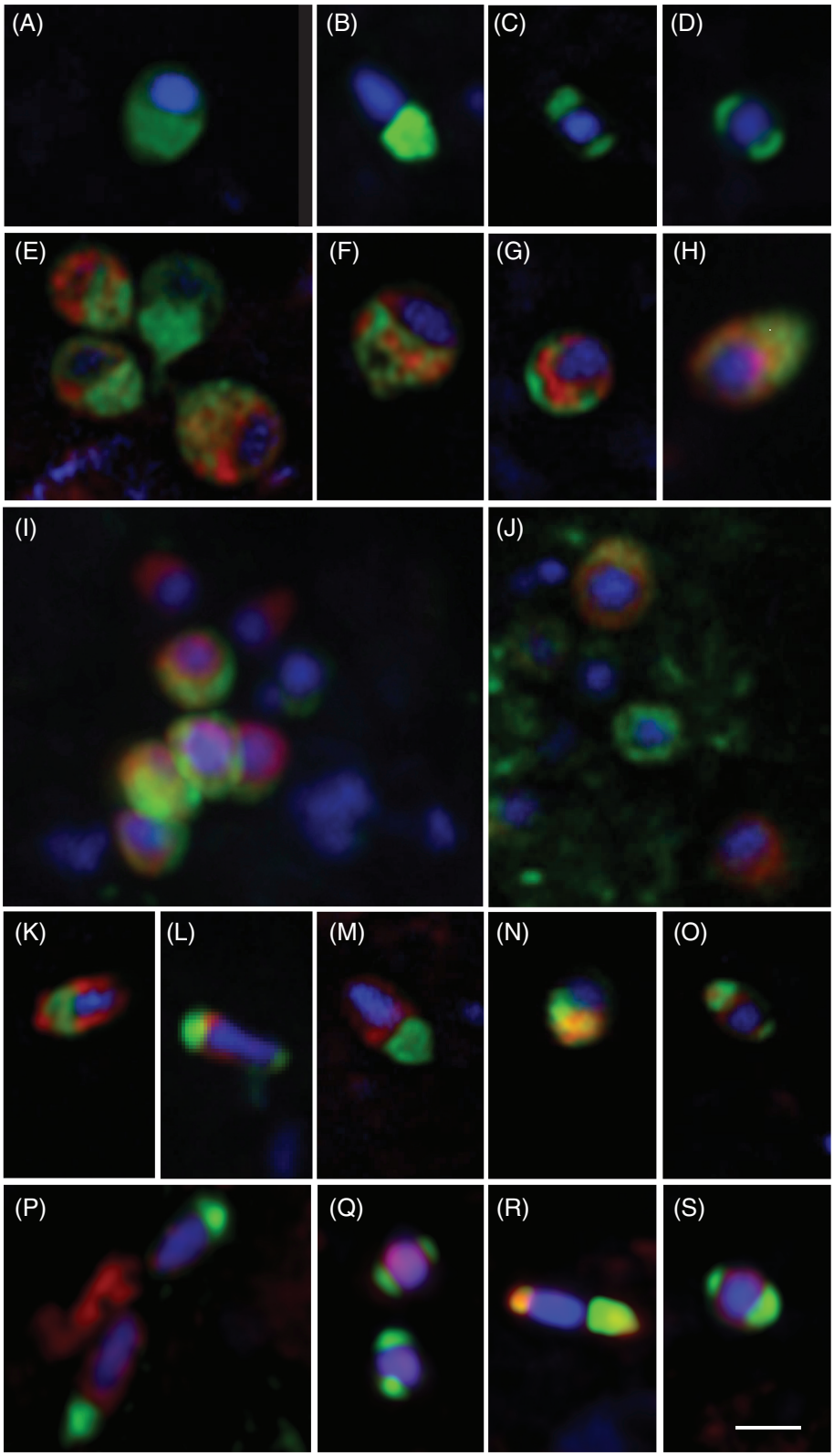

FIGURE 3 Legend on next page.

incubations, respectively, from September. Most identified selfish *Verrucomicrobiota* cells showed a characteristic substrate signal in which the FLA-PS were in a 'parentheses' pattern around the DAPI stain (Figure 3D,O). In April incubations, *Verrucomicrobiota* made up 31 ± 3%, 25 ± 5% (laminarin), 8 ± 1% (chondroitin), 7 ± 2% (xylan) and 7 ± 4% of total selfish bacteria.

In contrast to the *Planctomycetota* and *Verrucomicrobiota*, *Bacteroidota* made up only a small fraction of FLA-PS stained cells (<1%) in April incubations; they showed no selfish uptake in September (Figure 2). No selfish *Gammaproteobacteria* or *Actinobacteriota* were detected. The fraction of unidentified FLA-PS-stained cells, however, was 5 to 30% of the total, so there are additional clades beyond those we investigated that use a selfish mechanism of polysaccharide uptake.

Diversity, identification and quantification of members of *Verrucomicrobiota*

In incubations with FLA-mucin, a major fraction of selfish bacteria was identified as *Verrucomicrobiota*. To further identify members of this group, we analysed the diversity of verrucomicrobial sequences retrieved concurrently from the same sampling site (Miksch et al., 2021). The genera *Rubritalea, Luteolibacter, Roseibacillus, Haloferula* and *Persicirhabdus* contributed to 45% of *Verrucomicrobiota* reads (Figure S1). The abundance of these genera in the September mucin incubation at the final 10 day timepoint are shown in Figure S2.

A new oligonucleotide probe RUB390 targeting these genera (group coverage 90%) was designed and tested (Table S2), demonstrating a notable contribution to *Verrucomicrobiota*, and to selfish cells. In particular, in the April mucin incubation, targeted cells comprised at least 60% of the total *Verrucomicrobiota* cells at 1.5 days, and up to 85% by 3.5 days (Figure S3). In the September mucin incubation, these five genera accounted for at least 40% of *Verrucomicrobiota* cells in the 8 h sample. Selfish cells constituted a considerable fraction of the *Rubritalea, Luteolibacter, Roseibacillus, Haloferula* and *Persicirhabdus* cells: in the April 1.5 and 3.5 day samples, approximately 30% of the *Verrucomicrobiota* cells showed selfish uptake of mucin (Figures 3P–S and S3); cells belonging to the five targeted genera made up more than half of selfish cell after 3.5 days. After 0.3 days in the September mucin incubation, up to 40% of the detected *Verrucomicrobiota* cells showed selfish uptake; ca. half to two-thirds of these selfish cells belonged to the targeted genera.

Influence of substrate concentration on mucin processing mechanisms

To investigate the potential influence of differences in mucin concentrations on mechanisms of substrate utilization, an additional incubation series was set up in June 2022. Surface sediments were amended with FLA-mucin at three different concentrations (10, 100 and 1000 μM monomer equivalent), and incubated for a total of 3 days. Independent of substrate concentrations, no increase in total cell numbers was observed after 1 day; cell numbers in all incubations were approximately $3.3 \pm 0.5 \times 10^8$ cells ml^{-1} sediment. By day 3, however, cell numbers in the 100 μM-incubations had increased to $4.9 \pm 0.4 \times 10^8$ cells ml^{-1}, and cell numbers in the 1000 μM incubations had doubled to $6.7 \pm 0.9 \times 10^8$ cells ml^{-1} sediment (Figure 4A). In the 10 μM incubations, however, cell numbers at 3 days were similar to initial numbers.

The fraction of selfish bacteria differed somewhat at day 1, despite constant total cell numbers in the incubations. In the 10 μM incubation, selfish bacteria constituted only 0.15 ± 0.11% of total cells, whereas in the 100 μM and 1000 μM incubations, selfish bacteria made up 2.5 ± 0.1% of total cells and 2.0 ± 0.5% of total cells, respectively (Figure 4B). The fraction of selfish bacteria was slightly higher by day 3 in the 10 μM-incubations, but in the other incubations, increased cell growth led to a decrease in the fraction of selfish bacteria, to 0.4 ± 0.2% and 1.3 ± 0.6% of total cells in the 100 μM and 1000 μM incubations, respectively. The overall numbers of selfish cells in the 1000 μM incubations did slightly increase between day 1 and day 3 (6.2 ± 1.2 vs. $8.3 \pm 2.5 \times 10^6$ FLA-mucin-stained cells ml^{-1} sediment; Table S3).

FIGURE 3 Laser scanning micrographs of cells stained by DAPI (blue) and FLA-PS (green) from incubated sediments. (A) Oval, coccoid morphotype of FLA-PS stained cells that dominated in all incubations; (B, C) rod-shaped morphotypes with an apolar or bipolar FLA-PS signal; most often detected in incubations from April, rarely in September; (D) oval morphotype that had curved 'parentheses' of substrate staining on either side of the DAPI-stained DNA and only occurred in incubations from September; (E-J) *Planctomycetota*-specific FISH probe (PLA46, red). Substrate-uptake resulted in similar phenotypes independent of the substrate amended (E-G, mucin; H, chondroitin sulphate; I, laminarin; J, fucoidan) and month of sampling (April vs. September). (K–O) *Verrucomicrobiota*-specific FISH probe (EUB338-III, red). In general, identified FLA-PS-stained cells were oval-shaped with different degrees of elongation. They usually localized the substrate signal either (K, M) at one side (apolar) or (L, O) on both sides of the cell (bipolar). (N) In April, FLA-PS-*Verrucomicrobiota* cells rarely were coccoid. (O). In September, the dominant FLA-PS-*Verrucomicrobiota* phenotype showed a bipolar fluorescence signal. (P–S) A considerable portion between 10 and 75% of FLA-PS-*Verrucomicrobiota* could be identified by probe RUB390 (red) targeting *Rubritalea, Luteolibacter, Roseibacillus, Haloferula* and *Persicirhabdus*. Scale bar (applicable to all panels): 1 μm.

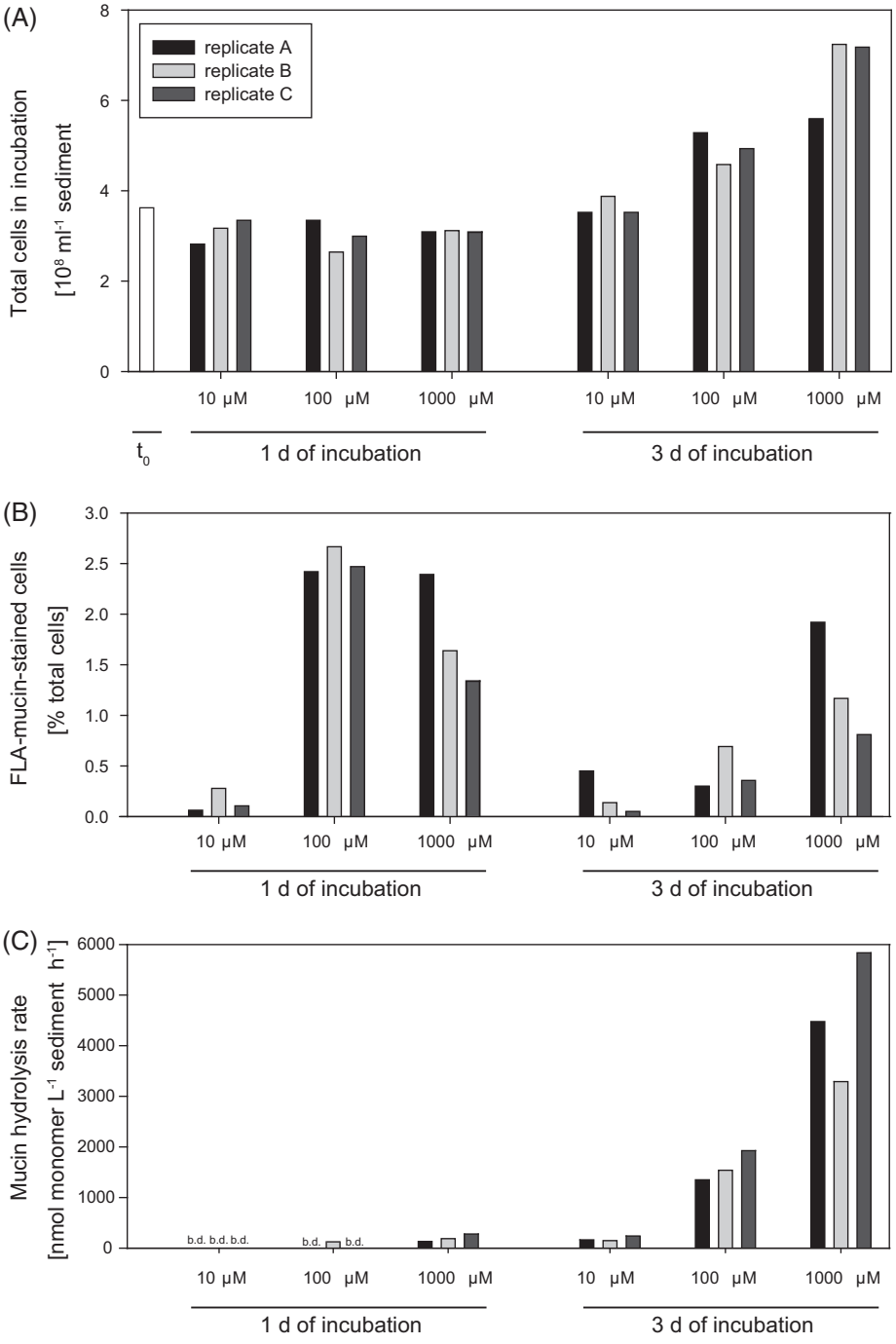

FIGURE 4 Incubations of Isfjorden sediments with different concentrations (10, 100 and 1000 µM) of FLA-mucin. (A) Total cells, (B) relative abundance of FLA-mucin-stained cells and (C) hydrolysis rates. Incubation time is indicated. b.d.: below detection limit.

Extracellular hydrolysis rates in these incubations increased with time, and scaled generally with substrate amendment: at day 1, hydrolysis was considerable only in the 1000 µM incubations, but by day 3, hydrolysis was measurable in all incubations, at 185 ± 48 nmol monomer L^{-1} sediment h^{-1}, 1606 ± 293 nmol monomer L^{-1} h^{-1} and 4535 ± 1273 nmol monomer L^{-1} h^{-1}, for the 10, 100 and 1000 µM incubations, respectively. Killed controls (sediments boiled in a pressure cooker) showed no selfish uptake, and no extracellular enzyme activities.

Cell counts

Cell counts in the incubations varied by substrate, incubation time and month of sampling (Table S1 and Figures S4 and S5). Cell numbers at t_0 were higher in silty

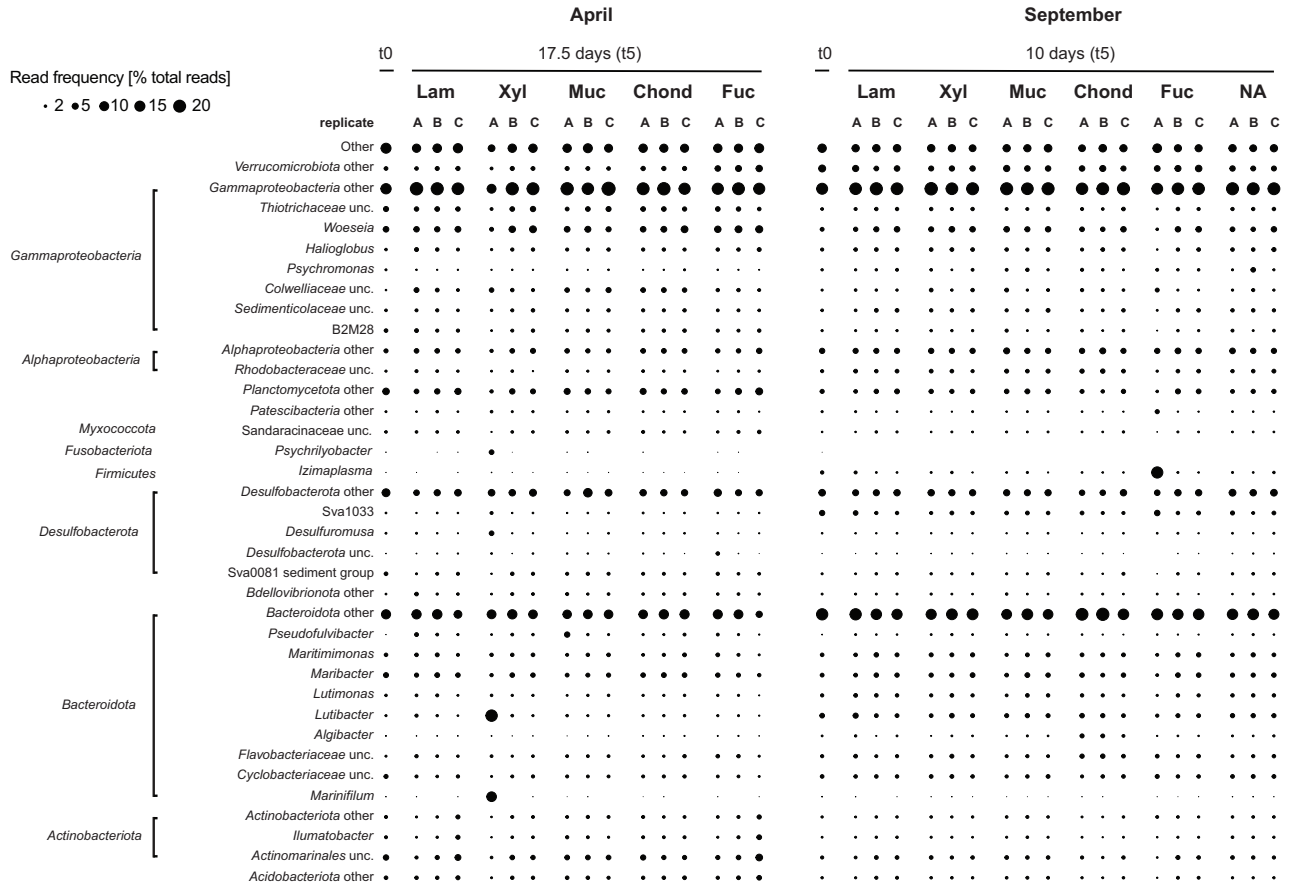

FIGURE 5 Relative abundance of bacterial families and genera at t0 and t5 for all substrates and for the unamended control incubations for April and September. The size of the bubble represents the relative abundance (%) of each taxon. Taxonomy based on SILVA SSU138.1 Ref NR99 database. Only taxa that accounted for >2% of total reads in at least one of the samples are shown. Taxa with minor abundance were clustered on higher taxonomic levels and displayed as "other." Chond: chondroitin sulphate; Fuc: Fucoidan; Lam: laminarin; Muc: mucin; NA: unamended incubation; Xyl: xylan.

September sediments compared to the fine sands collected in April, at 7–10 $\times$ 10^8 cells ml^{-1} sediment, and 2–4 $\times$ 10^8 cells ml^{-1}, respectively. In all incubations, cell counts increased over time by a factor of 1.4–3.5, reaching a maximum by day 4 (for September) or day 10.5 (for April). Cell numbers in xylan incubation, replicate A, from April increased the most, by a factor of 5.1, and reached a maximum of >19 $\times$ 10^8 cells ml^{-1} sediment (Figure S4). Cell numbers in the April killed controls increased by 30% in the laminarin and xylan incubations (from ~3.2 to 4.3 $\times$ 10^8 ml^{-1} sediment), while they remained about constant in the mucin, chondroitin and fucoidan incubations. Cell numbers in the September killed controls increased by 10%–280% during the course of the experiment in all incubations (Table S1), clearly indicating that the formaldehyde inactivation had been insufficient.

Bacterial community composition in the incubations

Sequencing of 16S rRNA genes was carried out on subsamples from timepoints t0 and t5 (17.5 days in April, 10 days in September incubations) to compare temporal changes in the bacterial community composition in the incubations and to identify bacteria that may have responded to the addition of FLA-PS (Figure 5). In general, bacterial communities in incubations subsampled at the starting point and endpoint (17.5 days of incubation in April, 10 days in September) of the experiment were very similar. The overall absence of changes during incubation was independent of substrate type or months of sampling and confirmed by an ASV-level NMDS plot (Figure S6). In the April incubations, a strong response was only evident in replicate A of the xylan incubation, in which *Bacteroidota* of genera *Lutibacter* and *Marinifilum* increased in abundance from ~1% to ~15–20% of total reads after 17.5 d. A minor increase of read frequencies was detected for clade Sva1033, *Desulfomusa* (*Desulfobacterota*) and *Psychrilyobacter* (*Fusobacteria*) in the same incubation as well as for *Ilumatobacter* and *Actinomarinales* (both *Acidimicrobiia*) in fucoidan incubation replicate C. For all substrates, an increase of reads affiliated with uncultured *Colwelliaceae* was detected (from 1% to 5%). In the September incubations, a strong response was

found only in fucoidan incubation replicate A, where *Izimaplasma* (*Firmicutes*) increased from 2% to 15% of total reads. Furthermore, in all three fucoidan replicate incubations, *Algibacter* reads increased slightly from 1% to 2–4%. Other taxa did not show a clear response to substrate addition. In the unamended control incubations, no changes in the bacterial community composition were detected between initial and late timepoints.

Quantification of major taxa

At t0, the bacterial community was characterized by 8 ± 3%/12 ± 4% *Gammaproteobacteria* (of total cells; mean of three replicates ± SD of each of five FLA-PS incubations for April/September series), 6 ± 2%/19 ± 5% *Bacteroidota*, 7 ± 2%/2 ± 1% *Planctomycetota*, 1 ± 0.4%/2 ± 1% *Verrucomicrobiota*, 4 ± 1%/2 ± 1% *Actinomarinales* and 4 ± 2/1.4 ± 0.7% *Microtrichales* (Figures S4 and S5; Miksch et al., 2021). In the course of the April incubations, *Bacteroidota* showed the strongest increase in cell numbers with, on average, 3.0 ± 0.8-fold (laminarin), 4.2 ± 0.8-fold (fucoidan), 4.6 ± 3.2-fold (mucin), 3.4 ± 1.4-fold (chondroitin sulphate) and 13.2 ± 15.7-fold (xylan). In September, their increase was more moderate with 2.1 to 3.0-fold (increase in NA control by 1.5-fold). *Planctomycetota* and *Verrucomicrobiota* cell numbers increased by a factor of 2.4 to 4.3 and 2.3 to 4.3 (average for each of five FLA-PS incubations), respectively, with no clear preference for a specific substrate. *Actinomarinales* grew most in the April laminarin (4.3 ± 4.1 fold), mucin (4.6 ± 3.6) and fucoidan (3.2 ± 1.1 fold) incubations while in the September incubations, cell numbers remained constant for all substrates except fucoidan (2.6 ± 2.3-fold increase vs. t0). Also, *Microtrichales* cell numbers increased remarkably in all incubations independent of the substrate with 1.7 to 2.5-fold in April and 2.4 to 3.9-fold in September (in NA control increase by 1.3-fold vs. t0). For *Gammaproteobacteria*, we detected the lowest increase in cell numbers with 1.1 to 1.6-fold for fucoidan, laminarin, chondroitin sulphate and xylan, and 2.8 for mucin in April and 1.5 to 2.1-fold in September incubations, where increase of *Gammaproteobacteria* was even greater in unamended NA control (3.4-fold increase vs. t0).

DISCUSSION

Active external hydrolyzers and specialized selfish bacteria characterize surface sediments

The bacteria adhering to surface sediments from Isfjorden actively hydrolyzed all of the FLA-PS externally. The broad range of substrates hydrolyzed in sediments is consistent with previous results from other locations in Svalbard (Arnosti, 2008; Cardman et al., 2014; Teske et al., 2011). Moreover, hydrolysis rates of mucin, a glycosylated protein, were also high, consistent with high peptidase activities in Svalbard sediments from other locations (Arnosti, 2015). We recognize that by resuspending the sediments and removing the porewater, dissolved organic carbon (DOC) was removed; we may have also removed bacteria and/or grazers that were only loosely attached to the sand grains, and in any case we disturbed interactions among spatially related organisms, all of which potentially change enzyme activities. Nevertheless, the cell numbers in resuspended sediments from April and September 2019 are similar to unresuspended sediments (Miksch et al., 2021; Probandt et al., 2017) and rates measured (Figure 1) are similar to rates measured in homogenized muddy sediments from other Svalbard fjords (Arnosti, 2008, Cardman et al., 2014, Teske et al., 2011).

All of the biopolymers tested were also selfishly taken up by bacteria adhering to sediments. However, FISH analyses demonstrated that the selfish uptake mechanism was used by only a small (~1–2%; Figure 2) and specific fraction of the total bacterial community. The low prevalence and high specialization of the sedimentary selfish bacteria contrasts strikingly with the prevalence of selfish uptake in the water column, where frequently 10–25% of total bacterial cells are identified as selfish (Reintjes et al., 2019; Reintjes et al., 2020). The high prevalence on Isfjorden sediment grains of selfish *Planctomycetota* and *Verrucomicrobiota* (Figure 2) also contrasts with the selfish bacteria characteristic of the water column, which have typically been shown to include a substantial contribution of *Bacteroidota* (e.g. Reintjes et al., 2019; Reintjes et al., 2020), and/or a comparatively even mixture of *Bacteroidota*, *Planctomycetes*, *Gammaproteobacteria* and/or *Verrucomicrobiota* (Brown et al., 2024). Overall, these organisms make up the majority of selfish bacteria adhering to sediment particles (Figure 2). These observations suggest that the *Planctomycetota* and *Verrucomicrobiota* play a significant role in sediments. In Isfjorden, enthic and pelagic *Planctomycetes* and *Verrucomicrobiota* are taxonomically different at the genus and/or species level (Miksch et al., 2021). These differences are reflected in the use of different polysaccharide utilization mechanisms. The identity of the remaining ca. 30% fraction of selfish bacteria that were not labelled with the FISH probes used remains to be established.

Sedimentary communities are actively growing but compositionally stable

During the incubations, cell numbers increased substantially, but community composition nonetheless remained relatively stable (Figures 5 S4, S5 and S6). In

particular, the addition of FLA-PS to sediments did not result in major changes in the relative abundance of bacterial taxa on the level of genera and families, as determined by 16S rRNA sequencing (Figure 5). This overall stability contrasts strongly with seawater incubations, which over similar timescales showed major relative changes in the abundance of genera of *Gammaproteobacteria* (e.g. *Colwellia*, *Glaciecola*, *Marinomonas*) and *Bacteroidota* (e.g. *Flavicella*, Reintjes et al., 2020). The relatively stable community composition throughout the incubation suggests that these sedimentary bacterial communities possess a sufficient range of enzymatic tools to process and share the substrates they encounter (Arnosti, 2008). Major relative changes in community composition in our incubations were comparatively rare, and when they occurred, were typically seen only in one of the three replicates (Figures 5, S4 and S5). These compositionally stable but actively growing communities included different taxa of the *Bacteroidota*, *Planctomycetota*, *Verrucomicrobiota* and *Proteobacteria*, all of which have been identified as polysaccharide degraders (Boedeker et al., 2017; Martinez-Garcia et al., 2012; Reintjes et al., 2020; Sichert et al., 2020; Teeling et al., 2012; van Vliet et al., 2019; Wietz et al., 2015). FISH counting demonstrated that the *Bacteroidota* cell numbers strongly increased compared to the unamended controls, with 3-fold to 5-fold cell increases (31-fold for xylan replicate A) in April versus 2- to 3-fold in September incubations (Figures S4 and S5). These changes are consistent with metatranscriptomic data from the same sampling site, which showed strong upregulation in relative transcript frequencies in several *Bacteroidota* bins (Miksch et al., 2024). The *Bacteroidota* are likely external degraders, however, since selfish *Bacteroidota* constituted only a minor fraction of the selfish microbial community (Figure 2).

Verrucomicrobiota cell numbers in the laminarin, chondroitin sulphate and fucoidan incubations increased 3–4-fold in the presence of substrates, compared to incubations without substrate (1.9-fold increase; Figure S5), suggesting that they are very responsive to substrate availability. Nonetheless, they were a numerically small fraction of the overall community, suggesting that they fill specialized niches. Other studies have also demonstrated the role of *Verrucomicrobiota* in polysaccharide degradation, highlighting their highly specialized metabolic repertoire (Martinez-Garcia et al., 2012, Sichert et al., 2020, van Vliet et al., 2019, Orellana et al., 2022, Giljan et al., 2022).

Like the *Bacteroidota*, benthic *Gammaproteobacteria* constituted a considerable fraction of the community (Figure 5). However, they did not show disproportionate increases in cell numbers during the course of the incubations (Figures S4 and S5), nor did they show selfish uptake (Figure 2). Selfish *Gammaproteobacteria* have been identified in some locations in the upper water column (Reintjes et al., 2019) and more recently they have also been identified in the deep ocean (Brown et al., 2024). The lack of a strong growth response of *Gammaproteobacteria* was somewhat surprising, since they are frequently identified as opportunistic bacteria that readily respond to an increase in organic matter (Buchan et al., 2014; Teeling et al., 2012). In any case, these organisms were likely among the external hydrolyzers.

Mucin may serve as an important substrate for benthic bacteria

We initially tested the glycoprotein mucin as a substrate since previous transcriptomic studies had indicated substantial presence/upregulation of relevant genes (Miksch et al., 2024). Rates of mucin hydrolysis were robust in both April and September (Figure 1), and selfish uptake—carried out primarily by members of the *Verrucomicrobiota* and *Planctomycetota*—was rapid (Figure 2). Although *Verrucomicrobiota* are numerically minor members of the bacterial community in Isfjorden sediments (Miksch et al., 2021), a remarkable one-third of all *Verrucomicrobiota* cells was capable of selfish uptake of mucin. Concurrent rapid hydrolysis and selfish uptake suggest that the sedimentary microbial community is regularly exposed to mucin. The use of mucin from porcine stomach further suggests that various forms of mucin from diverse invertebrate sources might be accessible to the community. The selfish uptake by *Verrucomicrobiota* and *Planctomycetota* may be aimed at securing a resource in the face of competition by external hydrolyzers (Arnosti et al., 2021). Moreover, previous studies have shown that mucin can have priming effects, causing increased microbial activities and a strong short-term increase in the turnover of sedimentary organic matter (Hannides & Aller, 2016; Kuzyakov et al., 2000). An influence of mucin degradation on the carbon quality and remineralization was also proposed by Smith et al. (1995) who detected high exohydrolase activities of bacteria attached to the surface of diatoms. They suggested that the hydrolysis of diatom mucin-like surface proteins (Buhmann et al., 2016) could be responsible for a major flux into the DOC pool, making it a significant mechanism of DOM production (Smith et al., 1995). At the seafloor, mucus tracks are usually heavily colonized by microbes and provide them with optimal growth conditions (Moens et al., 2005; Riemann & Schrage, 1978). The fast hydrolysis and selfish uptake of mucin indicates the importance of animal-derived glycoproteins for microbial communities in Arctic sediments. Since benthic faunal populations are seasonally relatively stable, even in an environment such as Svalbard (Włodarska-Kowalczuk et al., 2016), we suggest that the animal-derived mucin is likely a constantly available, important substrate for bacteria in Arctic sandy sediments.

Given rapid external hydrolysis and robust selfish uptake of mucin by a focused range of bacteria, we also tested the effects of mucin concentration on mechanisms of substrate processing. The highest concentration of mucin led to a doubling of cell numbers between 1 and 3 days and increasingly rapid external hydrolysis (Figure 4). At the same time, the fraction of selfish cells decreased and did not differ between 100 and 1000 μM substrate concentration suggesting that organisms responding most rapidly to mucin preferentially use the external hydrolysis mode of substrate utilization. These observations are also consistent with the response of pelagic bacteria during a diatom-dominated bloom in the North Sea, in the German Bight (Reintjes et al., 2020). In that study, the fraction of selfish pelagic bacteria decreased during the course of the bloom and the late bloom phase, as phytoplankton-produced organic matter became more abundant, bacterial cell counts increased and rates of extracellular hydrolysis also increased, accompanied by reduced selfish uptake of polysaccharides (except for laminarin, which was rapidly hydrolyzed as well as widely taken up in a selfish manner). Reintjes et al. (2020) found that these changes were related to both the structural complexity of the substrates as well as changes in the heterotrophic microbial community composition.

The *Verrucomicrobiota* genera *Rubritalea, Luteolibacter, Roseibacillus, Haloferula* and *Persicirhabdus* identified by using the probe designed as part of this study (Figure 3), showed that this clade is highly diverse (Figure S1) and contributed substantially to the overall increase in *Verrucomicrobiota* cell counts. Much like the other members of the phylum *Verrucomicrobiota*, members of these genera have been detected in almost all of 115 open ocean sediments and coastal sediments analysed in the framework of the International Census of Marine Microbes where they comprise up to 81% and 75% of total *Verrucomicrobiota* sequences, respectively (Freitas et al., 2012). In addition, a high abundance of 16S rRNA gene sequences affiliated with *Rubritaleaceae* have been found associated with the seaweed *Ulva laetevirens* (Juhmani et al., 2020), as well as on healthy giant kelp, *Macrocystis pyrifera* (James et al., 2020), which further points to their potential in degrading algal polysaccharides, including fucose-containing sulphated polysaccharides released by living diatoms (Chafee et al., 2018; Vidal-Melgosa et al., 2021).

Strategies of substrate processing in sediments—Conclusion and outlook

High external hydrolysis rates and a small but consistently detected fraction of selfish bacteria characterized these sediments. Selfish mechanisms for the utilization of substrates, for example, laminarin and mucin could be attributed specifically to *Planctomycetota* and *Verrucomicrobiota*, which are apparently well-adapted to target these specific substrates. Overall, extracellular hydrolysis prevailed; the bulk of glycan utilization seems to be catalysed by organisms carrying out external hydrolysis, which yields public goods. Previous investigations of mechanisms of polysaccharide processing focused particularly on water column communities (e.g. Brown et al., 2024; Giljan et al., 2022; Reintjes et al., 2017; Reintjes et al., 2019), where the physical conditions relating to diffusion and fluid flow are starkly different from sediments (Ahmerkamp et al., 2020; Huettel et al., 2014). The energetic cost of producing enzymes that are active in the external environment—carrying out external hydrolysis—has to be balanced by return on investment, that is hydrolysate. Given that bacteria adhering to particles also need to acquire resources, free enzymes can be useful as a 'foraging' strategy (Vetter et al., 1998; Vetter & Deming, 1999). Confined flow and diffusion in porewater can reduce the likelihood that hydrolysate is lost; likewise, proximity to other cells on the sand grain's surface can increase the use of mechanisms such as quorum sensing (Jatt et al., 2015; Krupke et al., 2016) to coordinate enzyme production among cells (Baty III, Diwz, et al., 2000; Baty, Techkarnjanaruk, et al., 2000). Considering these factors, we predict that the balance between mechanisms measured here—high external hydrolysis rates, and focused selfish uptake by a numerically minor fraction of the community—will also characterize microbial communities in other surface sediments. These communities may coordinate enzyme production, thereby maximizing their return on enzymatic investment.

AUTHOR CONTRIBUTIONS

Katrin Knittel: Data curation; supervision; project administration; methodology; validation; visualization; writing – original draft; writing – review and editing; investigation; conceptualization. **Sebastian Miksch:** Conceptualization; investigation; data curation; visualization; methodology; writing – review and editing. **Chyrene Moncada:** Investigation; writing – review and editing. **Sebastian Silva-Solar:** Investigation; writing – review and editing; visualization. **Jannika Moye:** Investigation; methodology. **Rudolf Amann:** Conceptualization; writing – original draft; writing – review and editing; funding acquisition; validation. **Carol Arnosti:** Conceptualization; investigation; funding acquisition; writing – original draft; writing – review and editing; validation; methodology; supervision; visualization.

ACKNOWLEDGEMENTS

We thank the captain and crew of R/V *Farm* for their support of our sampling in Svalbard. We are grateful to Greta Reintjes and Dirk de Beer for inspiring

discussions as well as to Kathrin Büttner, Mirja Meiners and Jörg Wulf for excellent technical assistance. Jan Brüwer, Meike Knittel, Anke Meyerdierks and Erich Nordmann are acknowledged for help in the field. Sherif Ghobrial provided essential help in processing the samples for measurements of enzyme activities. This work was funded by the Max Planck Society; CA was additionally supported by NSF (OCE-2022952 and -2241720). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests in relation to this work.

DATA AVAILABILITY STATEMENT

Sequence data were stored in the European Nucleotide Archive (ENA) under study accession numbers PRJEB76975. The below is a link to access the Supplemental data: https://doi.org/10.17617/3.9M4VHK.

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REFERENCES

Ahmerkamp, S., Marchant, H.K., Peng, C., Probandt, D., Littmann, S., Kuypers, M.M.M. et al. (2020) The effect of sediment grain properties and porewater flow on microbial abundance and respiration in permeable sediments. *Scientific Reports*, 10, 3573.

Arnosti, C. (2003) Fluorescent derivatization of polysaccharides and carbohydrate-containing biopolymers for measurement of enzyme activities in complex media. *Journal of Chromatography B*, 793, 181–191.

Arnosti, C. (2008) Functional differences between Arctic seawater and sedimentary microbial communities: contrasts in microbial hydrolysis of complex substrates. *FEMS Microbiology Ecology*, 66, 343–351.

Arnosti, C. (2015) Contrasting patterns of peptidase activities in seawater and sediments: an example from Arctic fjords of Svalbard. *Marine Chemistry*, 168, 151–156.

Arnosti, C., Bell, C., Moorhead, D.L., Sinsabaugh, R.L., Steen, A.D., Stromberger, M. et al. (2014) Extracellular enzymes in terrestrial, freshwater, and marine environments: perspectives on system variability and common research needs. *Biogeochemistry*, 117, 5–21.

Arnosti, C., Hinrichs, K.-U., Coffinet, S., Wilkes, H. & Pantoja, S. (2019) The enduring questions: What's for dinner? Where's my knife? … and can I use my fingers? (unanswered) questions related to organic matter and microbes in marine sediments. *Frontiers in Marine Science*, 6, 629.

Arnosti, C., Reintjes, G. & Amann, R. (2018) A mechanistic microbial underpinning for the size-reactivity continuum of dissolved organic carbon degradation. *Marine Chemistry*, 206, 93–99.

Arnosti, C., Wietz, M., Brinkhoff, T., Hehemann, J.H., Probandt, D., Zeugner, L. et al. (2021) The biogeochemistry of marine polysaccharides: sources, inventories, and bacterial drivers of the carbohydrate cycle. *Annual Review of Marine Science*, 13, 81–108.

Azam, F. & Malfatti, F. (2007) Microbial structuring of marine ecosystems. *Nature Reviews Microbiology*, 5, 782–791.

Bansil, R. & Turner, B.S. (2006) Mucin structure, aggregation, physiological functions and biomedical applications. *Current Opinion in Colloid & Interface Science*, 11, 164–170.

Baty III, A.M.C.C.E., Diwu, Z., Techkarnjanaruk, S., Goodman, A.E. & Geesey, G.G. (2000) Differentiation of chitinase-active and non-chitinase-active subpopulations of a marine bacterium during chitin degradation. *Applied and Environmental Microbiology*, 66, 3566–3573.

Baty III, A.M.C.C.E., Techkarnjanaruk, S., Goodman, A.E. & Geesey, G.G. (2000) Spatial and temporal variations in chitinolytic gene expression and bacterial biomass production during chitin degradation. *Applied and Environmental Microbiology*, 66, 3574–3585.

Biersmith, A. & Benner, R. (1998) Carbohydrates in phytoplankton and freshly produced dissolved organic matter. *Marine Chemistry*, 63, 131–144.

Boedeker, C., Schuler, M., Reintjes, G., Jeske, O., Van Teeseling, M.C.F., Jogler, M. et al. (2017) Determining the bacterial cell biology of Planctomycetes. *Nature Communications*, 8, 14853.

Bray, J.R. & Curtis, J.T. (1957) An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*, 27, 325–349.

Brown, S., Lloyd, C.C., Giljan, G., Ghobrial, S., Amann, R. & Arnosti, C. (2024) Pulsed inputs of high molecular weight organic matter shift the mechanisms of substrate utilisation in marine bacterial communities. *Environmental Microbiology*, 26, e16580.

Buchan, A., Lecleir, G.R., Gulvik, C.A. & González, J.M. (2014) Master recyclers: features and functions of bacteria associated with phytoplankton blooms. *Nature Reviews Microbiology*, 12, 686–698.

Buhmann, M.T., Schulze, B., Förderer, A., Schleheck, D. & Kroth, P.G. (2016) Bacteria may induce the secretion of mucin-like proteins by the diatom *Phaeodactylum tricornutum*. *Journal of Phycology*, 52, 463–474.

Bushnell, B., Rood, J. & Singer, E. (2017) BBMerge: accurate paired shotgun read merging via overlap. *PLoS One*, 12, e0185056.

Callahan, B.J., Mcmurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13, 581–583.

Cardman, Z., Arnosti, C., Durbin, A., Ziervogel, K., Cox, C., Steen, A.D. et al. (2014) Verrucomicrobia are candidates for polysaccharide-degrading bacterioplankton in an Arctic fjord of Svalbard. *Applied and Environmental Microbiology*, 80, 3749–3756.

Chafee, M., Fernàndez-Guerra, A., Buttigieg, P.L., Gerdts, G., Eren, A.M., Teeling, H. et al. (2018) Recurrent patterns of microdiversity in a temperate coastal marine environment. *The ISME Journal*, 12, 237–252.

Cuskin, F., Lowe, E.C., Temple, M.J., Zhu, Y., Cameron, E.A., Pudlo, N.A. et al. (2015) Human gut Bacteroidetes can utilize yeast mannan through a selfish mechanism. *Nature*, 517, 165–169.

Davies, M.S. & Hawkins, S.J. (1998) Mucus from marine molluscs. In: Blaxter, J.H.S., Southward, A.J. & Tyler, P.A. (Eds.) *Advances in marine biology*. Cambridge, MA: Elsevier.

Freitas, S., Hatosy, S., Fuhrman, J.A., Huse, S.M., Mark Welch, D.B., Sogin, M.L. et al. (2012) Global distribution and diversity of marine *Verrucomicrobia*. *The ISME Journal*, 6, 1499–1505.

Fuchs, B.M., Glockner, F.O., Wulf, J. & Amann, R. (2000) Unlabeled helper oligonucleotides increase the in situ accessibility to 16S rRNA of fluorescently labeled oligonucleotide probes. *Applied and Environmental Microbiology*, 66, 3603–3607.

Giljan, G., Arnosti, C., Kirstein, I.V., Amann, R. & Fuchs, B.M. (2022) Strong seasonal differences of bacterial polysaccharide utilization in the North Sea over an annual cycle. *Environmental Microbiology*, 24, 2333–2347.

Hannides, A.K. & Aller, R.C. (2016) Priming effect of benthic gastropod mucus on sedimentary organic matter remineralization. *Limnology and Oceanography*, 61, 1640–1650.

Hedges, J.I., Baldock, J.A., Gélinas, Y., Lee, C., Peterson, M.L. & Wakeham, S.G. (2002) The biochemical and elemental compositions of marine plankton: a NMR perspective. *Marine Chemistry*, 78, 47–63.

Herlemann, D.P.R., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J. & Andersson, A.F. (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *The ISME Journal*, 5, 1571–1579.

Hopwood, D. (1967) Some aspects of fixation with glutaraldehyde. A biochemical and histochemical comparison of the effects of formaldehyde and glutaraldehyde fixation on various enzymes and glycogen, with a note on penetration of glutaraldehyde into liver. *Journal of Anatomy*, 101, 83–92.

Huettel, M., Berg, P. & Kostka, J.E. (2014) Benthic exchange and biogeochemical cycling in permeable sediments. *Annual Review of Marine Science*, 6, 23–51.

James, A.K., English, C.J., Nidzieko, N.J., Carlson, C.A. & Wilbanks, E.G. (2020) Giant kelp microbiome altered in the presence of epiphytes. *Limnology and Oceanography Letters*, 5, 354–362.

Jatt, A.N., Tang, K., Liu, J., Zhang, Z. & Zhang, X.H. (2015) Quorum sensing in marine snow and its possible influence on production of extracellular hydrolytic enzymes in marine snow bacterium *Pantoea ananatis* B9. *FEMS Microbiology Ecology*, 91, 1–13.

Juhmani, A.-S., Vezzi, A., Wahsha, M., Buosi, A., Pascale, F.D., Schiavon, R. et al. (2020) Diversity and dynamics of seaweed associated microbial communities inhabiting the lagoon of Venice. *Microorganisms*, 8, 1657.

Krupke, A., Hmelo, L.R., Ossolinski, J.E., Mincer, T.J. & Van Mooy, B.A.S. (2016) Quorum sensing plays a complex role in regulating the enzyme hydrolysis activity of microbes associated with sinking particles in the ocean. *Frontiers in Marine Science*, 3, 55.

Kuzyakov, Y., Friedel, J.K. & Stahr, K. (2000) Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry*, 32, 1485–1498.

Manz, W., Amann, R., Ludwig, W., Wagner, M. & Schleifer, K.-H. (1992) Phylogenetic oligodeoxynucleotide probes for the major subclasses of *Proteobacteria*: problems and solutions. *Systematic and Applied Microbiology*, 15, 593–600.

Martinez-Garcia, M., Swan, B.K., Poulton, N.J., Gomez, M.L., Masland, D., Sieracki, M.E. et al. (2012) High-throughput single-cell sequencing identifies photoheterotrophs and chemoautotrophs in freshwater bacterioplankton. *The ISME Journal*, 6, 113–123.

Middelburg, J.J. (2018) Reviews and syntheses: to the bottom of carbon processing at the seafloor. *Biogeosciences*, 15, 413–427.

Miksch, S., Meiners, M., Meyerdierks, A., Probandt, D., Wegener, G., Titschack, J. et al. (2021) Bacterial communities in temperate and polar coastal sands are seasonally stable. *ISME Communications*, 1, 29.

Miksch, S., Orellana, L.H., Oggerin De Orube, M., Vidal-Melgosa, S., Solanki, V., Hehemann, J.H. et al. (2024) Taxonomic and functional stability overrules seasonality in polar benthic microbiomes. *The ISME Journal*, 18, wrad005. Available from: https://doi.org/10.1093/ismejo/wrad005

Moens, T., Paiva Dos Santos, G.A., Thompson, F., Swings, J., Fonsêca-Genevois, V., Vincx, M. et al. (2005) Do nematode mucus secretions affect bacterial growth? *Aquatic Microbial Ecology*, 40, 77–83.

Moncada, C., Ellrott, A., De Beer, D., Amann, R. & Knittel, K. (2024) The Ellrott grab: a small, lightweight sediment sampler for collecting undisturbed sandy sediments. *Limnology and Oceanography: Methods*, 22, 159–169.

Oksanen, J., Blanchet, F., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D. et al. (2019) Vegan: community ecology package. R Package Version 2.5-6.

Orellana, L.H., Francis, T.B., Ferraro, M., Hehemann, J.-H., Fuchs, B.M. & Amann, R.I. (2022) Verrucomicrobiota are specialist consumers of sulfated methyl pentoses during diatom blooms. *The ISME Journal*, 16, 630–641.

Percival, E. & Mcdowell, R.H. (1990) Algal polysaccharides. In: Dey, P.M. (Ed.) *Methods in plant biochemistry*. Cambridge, MA: Elsevier.

Pernthaler, A., Pernthaler, J. & Amann, R. (2002) Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Applied and Environmental Microbiology*, 68, 3094–3101.

Probandt, D., Knittel, K., Tegetmeyer, H.E., Ahmerkamp, S., Holtappels, M. & Amann, R. (2017) Permeability shapes bacterial communities in sublittoral surface sediments. *Environmental Microbiology*, 19, 1584–1599.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W. & Peplies, J. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research*, 35, 7188–7196.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41, D590–D596.

Reintjes, G., Arnosti, C., Fuchs, B. & Amann, R. (2019) Selfish, sharing and scavenging bacteria in the Atlantic Ocean: a biogeographical study of bacterial substrate utilisation. *The ISME Journal*, 13, 1119–1132.

Reintjes, G., Arnosti, C., Fuchs, B.M. & Amann, R. (2017) An alternative polysaccharide uptake mechanism of marine bacteria. *The ISME Journal*, 11, 1640–1650.

Reintjes, G., Fuchs, B.M., Scharfe, M., Wiltshire, K.H., Amann, R. & Arnosti, C. (2020) Short-term changes in polysaccharide utilization mechanisms of marine bacterioplankton during a spring phytoplankton bloom. *Environmental Microbiology*, 22, 1884–1900.

Riemann, F. & Schrage, M. (1978) The mucus-trap hypothesis on feeding of aquatic nematodes and implications for biodegradation and sediment texture. *Oecologia*, 34, 75–88.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B. et al. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, 75, 7537–7541.

Sichert, A., Corzett, C.H., Schechter, M.S., Unfried, F., Markert, S., Becher, D. et al. (2020) Verrucomicrobia use hundreds of enzymes to digest the algal polysaccharide fucoidan. *Nature Microbiology*, 5, 1026–1039.

Smith, D.C., Steward, G.F., Long, R.A. & Azam, F. (1995) Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. *Deep Sea Research Part II: Topical Studies in Oceanography*, 42, 75–97.

Stabili, L., Licciano, M., Giangrande, A., Gerardi, C., De Pascali, S.A. & Fanizzi, F.P. (2019) First insight on the mucus of the annelid *Myxicola infundibulum* (Polychaeta, Sabellidae) as a potential prospect for drug discovery. *Marine Drugs*, 17, 396.

Team, R. C. (2019) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.r-project.org/

Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C.M. et al. (2012) Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science*, 336, 608–611.

Teske, A., Durbin, A., Ziervogel, K., Cox, C. & Arnosti, C. (2011) Microbial community composition and function in permanently cold seawater and sediments from an Arctic fjord of Svalbard. *Applied and Environmental Microbiology*, 77, 2008–2018.

van Vliet, D.M., Palakawong Na Ayudthaya, S., Diop, S., Villanueva, L., Stams, A.J.M. & Sánchez-Andrea, I. (2019)

Anaerobic degradation of sulfated polysaccharides by two novel *Kiritimatiellales* strains isolated from Black Sea sediment. *Frontiers in Microbiology*, 10, 253.
Vetter, Y.A. & Deming, J.W. (1999) Growth rates of marine bacterial isolates on particulate organic substrates solubilized by freely released extracellular enzymes. *Microbial Ecology*, 37, 86–94.
Vetter, Y.A., Deming, J.W., Jumars, P.A. & Krieger-Brockett, B.B. (1998) A predictive model of bacterial foraging by means of freely released extracellular enzymes. *Microbial Ecology*, 36, 75–92.
Vidal-Melgosa, S., Sichert, A., Francis, T.B., Bartosik, D., Niggemann, J., Wichels, A. et al. (2021) Diatom fucan polysaccharide precipitates carbon during algal blooms. *Nature Communications*, 12, 1150.
Westram, R., Bader, K., Prüsse, E., Kumar, Y., Meier, H., Gloeckner, F.-O. et al. (2011) ARB: a software environment for sequence data, Wiley-Blackwell, ARB: A Software Environment for Sequence Data.
Wickham, H. (2016) *ggplot2: elegant graphics for data analysis*. New York: Springer-Verlag.
Wickham, H. (2017) tidyverse: easily install and load the ‘‘Tidyverse’’.
Wietz, M., Wemheuer, B., Simon, H., Giebel, H.-A., Seibt, M.A., Daniel, R. et al. (2015) Bacterial community dynamics during polysaccharide degradation at contrasting sites in the southern and Atlantic oceans. *Environmental Microbiology*, 17, 3822–3831.
Włodarska-Kowalczuk, M., Górska, B., Deja, K. & Morata, N. (2016) Do benthic meiofaunal and macrofaunal communities respond to seasonality in pelagial processes in an Arctic fjord (Kongsfjorden, Spitsbergen)? *Polar Biology*, 39, 2115–2129.

SUPPORTING INFORMATION

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

How to cite this article: Knittel, K., Miksch, S., Moncada, C., Silva-Solar, S., Moye, J., Amann, R. et al. (2024) Distinct actors drive different mechanisms of biopolymer processing in polar marine coastal sediments. *Environmental Microbiology*, 26(8), e16687. Available from: https://doi.org/10.1111/1462-2920.16687