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RESEARCH ARTICLE

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Pulsed inputs of high molecular weight organic matter shift the mechanisms of substrate utilisation in marine bacterial communities

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Abstract

Heterotrophic bacteria hydrolyze high molecular weight (HMW) organic matter extracellularly prior to uptake, resulting in diffusive loss of hydrolysis products. An alternative 'selfish' uptake mechanism that minimises this loss has recently been found to be common in the ocean. We investigated how HMW organic matter addition affects these two processing mechanisms in surface and bottom waters at three stations in the North Atlantic Ocean. A pulse of HMW organic matter increased cell numbers, as well as the rate and spectrum of extracellular enzymatic activities at both depths. The effects on selfish uptake were more differentiated: in Gulf Stream surface waters and productive surface waters south of Newfoundland, selfish uptake of structurally simple polysaccharides increased upon HMW organic matter addition. The number of selfish bacteria taking up structurally complex polysaccharides, however, was largely unchanged. In contrast, in the oligotrophic North Atlantic gyre, despite high external hydrolysis rates, the number of selfish bacteria was unchanged, irrespective of polysaccharide structure. In deep bottom waters (> 4000 m), structurally complex substrates were processed only by selfish bacteria. Mechanisms of substrate processing—and the extent to which hydrolysis products are released to the external environment—depend on substrate structural complexity and the resident bacterial community.

INTRODUCTION

Much of the organic matter biosynthesized by phytoplankton in the surface waters of the ocean consists of high molecular weight (HMW) macromolecules, including polysaccharides. This organic matter comprises the base of the marine food web and fuels heterotrophy in the ocean, with heterotrophic prokaryotes processing a large fraction of this organic matter (Azam & Malfatti, 2007). To access HMW substrates, members of heterotrophic microbial communities must produce extracellular enzymes that hydrolyze these substrates to sizes sufficiently small for uptake. The resulting low molecular weight (LMW) hydrolysis products, however, may also become available to other members of the microbial community that do not or cannot produce extracellular enzymes. These 'cheaters' (Allison, 2005; here referred to as 'scavengers') may change the calculus of enzyme production by consuming some of the hydrolysis products, thus potentially making external hydrolysis less profitable to enzyme producers.

However, an alternative mechanism of polysaccharide processing recently identified in the ocean (Reintjes et al., 2017) enables bacteria to retain most, if not all, of the LMW organic matter resulting from enzymatic hydrolysis. During this process, referred to as 'selfish' uptake, a polysaccharide is bound, initially hydrolyzed, and transported into the periplasmic space, with little to no loss of hydrolysis products to the external environment (Cuskin et al., 2015). Selfish uptake

thus ensures full energetic returns on investment into the cellular machinery necessary for enzymatic hydrolysis. Notably, this mechanism of substrate uptake also modifies the release of LMW products to the surrounding environment, potentially affecting the composition and activity of the scavenging community, as well as the flow of organic carbon through food webs (Arnosti et al., 2018).

Understanding the factors that influence the balance between different polysaccharide processing mechanisms is therefore essential to our understanding of the rate and location of carbon processing by heterotrophic microbes, as well as the ultimate fate of organic matter in the ocean. To date, investigations of selfish bacteria in oceanic surface waters have found that the balance between selfish uptake and the external hydrolysis of polysaccharides varies with location (Reintjes et al., 2019; Reintjes, Fuchs, Amann, & Arnosti, 2020), phytoplankton bloom stage (Reintjes, Fuchs, Scharfe, et al., 2020), and season (Giljan et al., 2023). A recent study has also documented depth-related differences in patterns of selfish uptake and external hydrolysis (Giljan et al., 2023). In the epipelagic ocean, for example, a wide spectrum of polysaccharides was degraded through both external hydrolysis and selfish uptake; in contrast, in bathypelagic waters, only selfish bacteria were capable of utilising some complex polysaccharides (Giljan et al., 2023).

A new conceptual model suggests that variations in polysaccharide complexity and abundance may explain these differences in the proportion of distinct substrate utilisation mechanisms (Arnosti et al., 2021). This model proposes that selfish uptake may be most beneficial when (a) competition for a substrate is high, or (b) substrates are highly complex or rare (Arnosti et al., 2021). For example, at bathypelagic depths, the limited input of organic matter may result in selfish uptake being more energetically beneficial than external hydrolysis, as selfish uptake enables an organism to avoid the loss of hydrolysis products to scavenging organisms (Cuskin et al., 2015). External hydrolysis, in contrast, may be more efficient under conditions in which polysaccharides are more abundant (e.g., during phytoplankton blooms, or on particles; Ebrahimi et al., 2019; Traving et al., 2015), as the cost of enzyme production could be shared among organisms. Changes in organic matter availability may therefore help shift the balance between the extent of selfish uptake and external hydrolysis.

Here, we experimentally investigate the conditions under which selfish uptake and external hydrolysis of polysaccharides may change by examining the responses of distinct microbial communities to an input of HMW organic matter. Such episodic inputs occur, for example, during phytoplankton blooms in the upper ocean, as well as during the demise of these blooms, which lead to pulses of organic matter in the deep sea (Deuser, 1986). We investigated microbial responses to a pulsed input of HMW organic matter to focus specifically on the organisms carrying out the initial processing of this organic matter. As the abundance and composition of organic matter (Wakeham et al., 1997) and the composition and genomic potential of microbial communities (DeLong et al., 2006; Sunagawa et al., 2015) differ considerably between epi- and bathypelagic ocean waters, we carried out our experiments with water from the surface and the deep ocean since we expected that these distinct communities may differ in their responses to HMW organic matter. Our previous investigations of microbial responses to diatom-derived HMW organic matter demonstrated that the addition of HMW organic matter stimulates external hydrolysis of polysaccharides, although the extent of stimulation varied by depth and location (Balmonte et al., 2019; Brown et al., 2022). Those previous studies, however, did not include an investigation of the presence or prevalence of selfish uptake.

To investigate the effect of an input of HMW organic matter on the balance between external hydrolysis and selfish uptake of polysaccharides, we compared the carbon processing mechanisms of marine microbial communities in mesocosms amended with HMW particulate and dissolved organic matter (POM and DOM; derived from the diatom *Thalassiosira weissflogii*) to communities in unamended mesocosms that did not receive any extra organic matter. Water for the mesocosms was collected from the deep chlorophyll maximum (DCM; 33–104 m) and bottom waters (3190–5580 m) at three distinct sites in the western North Atlantic Ocean to simultaneously evaluate the influences of depth and natural variations in productivity regimes on polysaccharide processing. The three sites were in the Gulf Stream, at a northern site off the continental shelf of Newfoundland characterised by relatively high primary productivity, and at an oligotrophic site in the North Atlantic gyre (Figure S1a). We expected the initial microbial communities at these sites to differ considerably because of the different physicochemical characteristics and biological productivity of these regions. In amended and unamended mesocosms containing water from each station and depth, we measured the extracellular hydrolysis rates of a suite of structurally distinct, fluorescently-labelled polysaccharides (FLA-PS) (Arnosti, 2003), concurrently measured selfish uptake of these same polysaccharides (Reintjes et al., 2017), and collected samples to characterise community composition. Additionally, to identify some of the organisms that carry out selfish uptake, and whether these organisms differ by location or by depth, we used fluorescence in situ hybridization to identify selfish bacteria taking up laminarin, which is one of the most abundant polysaccharides in the ocean (Alderkamp et al., 2007; Becker et al., 2020).

EXPERIMENTAL PROCEDURES

Preparation of high molecular weight organic matter

HMW *Thalassiosira weissflogii* was prepared according to Balmonte et al. (2019). Briefly, *Thalassiosira weissflogii* (Instant Algae, Reed Mariculture) was frozen, thawed, homogenised, and dialyzed using a 10 kD membrane (SpectraPor). The dissolved and particulate HMW organic matter retentate was then lyophilized, autoclaved, and lyophilized a final time before being weighed into 500 mg portions for addition to triplicate 20 L mesocosms. The final addition of HMW dissolved and particulate organic matter to carboys (see below) was equivalent to 658 μmol L^{-1} C. This dissolved and particulate HMW organic matter had a total carbohydrate concentration of 6.15% and a C:N ratio of 6:1.

FLA-PS synthesis

Six polysaccharides (pullulan, laminarin, xylan, fucoidan, arabinogalactan, and chondroitin sulphate) (Sigma) were labelled with fluoresceinamine (Sigma), following the method of Arnosti (2003), and added to triplicate incubations (see 'FLA-PS incubation setup'). These polysaccharides were chosen due to their abundance in the ocean and/or the demonstrated ability of marine bacteria to degrade them (e.g., Alderkamp et al., 2007; Neumann et al., 2015).

Water collection

Water was collected at two depths (DCM and bottom) at Stn. 18 (37° 30′ 9.54″ N, −72° 0′ 7.5594″ E), Stn. 19 (42° 50′ 22.34″N, −53° 23′ 41.64″ E), and Stn. 20 (34° 38′ 12.84″ N, −53° 58′ 58.29″E) in the North Atlantic Ocean from May 15 to 30, 2019 aboard the R/V *Endeavour* (Figure S1a). At each depth, water was collected using twelve 30 L Niskin bottles attached to a sampling rosette equipped with a Seabird 32 CTD (Sea Bird Scientific) to enable measurement of in situ parameters (temperature, salinity, oxygen, and chlorophyll). Water from each depth was added to two acid-washed (10% HCl) and rinsed 20 L carboys that were further rinsed three times with seawater from the same depth prior to filling.

Mesocosm experiments

A 20 L carboy from each station and depth was amended with 25 mg L^{-1} of HMW dissolved and particulate *Thalassiosira weissflogii* (equivalent to 658 μmol L^{-1} dissolved and particulate C; see above) while a single 20 L unamended mesocosm containing water from each depth with no *Thalassiosira weissflogii* addition was the control. Mesocosms were incubated at or close to in-situ temperatures (Figure S1c) and stored in the dark.

FLA-PS incubation setup

FLA-PS incubations were set up immediately after water collection for unamended mesocosms, and 2 days after HMW organic matter addition for the amended mesocosms. The 2-day delay in the amended mesocosms was intended to provide time for the initial community to respond to the HMW organic matter addition. To set up amended FLA-PS incubations, water was collected from each mesocosm and incubated in sterile triplicate T-75 tissue culture flasks (290 mL incubation chambers) to provide sufficient volume for the measurement of both selfish uptake and external hydrolysis from the same incubation. FLA-PS incubations containing water from the unamended mesocosms were incubated in triplicate 600 mL bottles for each of the six substrates; a single control with no FLA-PS addition was also included. Each of six FLA-PS substrates (pullulan, laminarin, xylan, fucoidan, arabinogalactan, and chondroitin sulphate) was added to triplicate tissue culture flasks at 3.5 μM monomer equivalent concentrations (except fucoidan, which was added to a concentration of 5.0 μM). Autoclaved water from each mesocosm was incubated with each FLA-PS substrate as a killed control. FLA-PS incubations were stored at the same temperature as mesocosms from the same station and depth (Figure S1c). We note that severe weather led to the loss of a number of samples that were being incubated and processed aboard the ship.

Measurement of extracellular enzymatic hydrolysis

Samples (2 mL) for measurement of external hydrolysis were collected after 0, 1, 3, 5, 7, 10, and 30 days from unamended FLA-PS incubations. For the amended FLA-PS incubations, water was collected 0, 3, 5, 10, 15, and 30 days after FLA-PS addition, which was equivalent to 2, 5, 7, 12, 17, and 31 days after water collection and the addition of HMW organic matter to amended mesocosms. Each sample was filtered through a 0.2 μm pore-sized filter, and stored frozen until analysis. Rates of external hydrolysis were calculated by measuring the change in the molecular weight of the FLA-PS over time, using gel permeation chromatography and fluorescence detection, as described in Arnosti (2003). Note that the hydrolysis rates reported here are considered potential rates, due to the potential presence of naturally-produced polysaccharides that would compete with added FLA-PS for enzyme active

sites. All measurements are discussed in the time (days) since the water was collected from the ocean (e.g., the *t*0 for amended FLA-PS incubations was 2 days after the water was collected). Note also that the hydrolysis data from the unamended mesocosms was published as part of Giljan et al. (2023).

Quantification of total cell counts and selfish bacteria

At each time point, between 20 and 50 mL of water was collected from each FLA-PS incubation, fixed with 1% formaldehyde, and filtered through 0.2 μm pore-size 47 mm polycarbonate filters, then stored at −20°C until analysis. Total cell counts and enumeration of selfish bacteria were conducted after Reintjes et al. (2017), with minor alterations. Briefly, the DNA of filtered cells was stained with 4′,6-diamidino-2-phenylindole (DAPI) using a 1 ng/μL working solution and, using a Citifluor/Vectashield (4:1) solution, fixed onto glass slides. Images for the enumeration of total cell counts and selfish bacteria were acquired using an epifluorescence microscope with an automated imaging system and light-emitting diodes at a 63X objective (Zeiss AxioImager.Z2 microscopic stand, Carl Zeiss), as described by Bennke et al. (2016).

Analysis of images and enumeration of bacterial cells was conducted using the image analysis software ACMETOOL 3.0 (http://www.technobiology.ch and Max Planck Institute for Marine Microbiology, Bremen). Quantification of DAPI-stained cells provided the total number of bacterial cells, while selfish bacteria from FLA-PS incubations were identified by counting DAPI-stained cells that also had a FLA-PS signal (an excitation wavelength of 488 nm). To be counted as selfish, bacteria had to have a minimum DAPI/FLA-PS signal overlap of 75%. Note that the cell counts of selfish bacteria from the unamended mesocosms have been previously published as part of Giljan et al. (2023).

FISH

Selfish bacteria capable of FLA-laminarin uptake were identified using FISH following the method of Reintjes et al. (2017). In brief, FISH probes that targeted *Bacteroidota* (CF319a; 5′-TGGTCCGTGTCTCAGTAC-3′, formamide concentration 35%, Manz et al., 1996), *Planctomycetes* (PLA46; 5′-GACTTGCATGCCTAATCC-3′, formamide concentration 30%, Neef et al., 1998), *Gammaproteobacteria* (GAM42a; 5′-GCCTTCCCACATCGTTT-3′, formamide concentration 35%, Manz et al., 1992), and *Verrucomicrobiales* (EUB338-III; 5′-GCTGCCACCCGTAGGTGT-3′, formamide concentration 35%, Daims et al., 1999) were applied to separate filter pieces, counterstained with DAPI, and mounted onto glass slides and visualised as described above. Cells positively identified as selfish *Bacteroidota*, *Planctomycetes*, *Gammaproteobacteria*, or *Verrucomicrobiales* in ACMETOOL 3.0 had to have a minimum FISH/selfish signal overlap of 50%.

16S rRNA identification of bacterial community composition

Bacterial communities and their changes over time in the FLA-PS incubations were determined through 16S rRNA analysis of samples collected after 0, 1, 3, and 10 days in unamended FLA-PS incubations (triplicate unamended incubations were sequenced) and after 2, 5, 7, 12, and 17 days in amended FLA-PS incubations (two of the three replicate incubations were sequenced). Bacterial community composition in the 20 L amended mesocosm from each station and depth was also tracked, beginning with the addition of HMW organic matter (0 days). At each time point, 25 mL of seawater was filtered through a 0.22 μm pore size polycarbonate filter at a maximum vacuum of 200 mbar; filters were stored frozen at −80°C until further processing.

Total DNA was extracted using the DNeasy Power Water Kit (Quiagen), adding the whole filter. Amplification of the variable V3 and V4 regions (490 bp) of the 16S rRNA was done using the 5 PRIME HotMasterMix (Quantabio) with the PCR primer pair Bakt_314F (CCTACGGGNGGCWGCAG) and Bakt_805R (GACTACGVGGGTATCTAATCC) (Herlemann et al., 2011), both barcoded with individual 8 bp barcode adapter (based on the NEB Multiplex Oligos for Illumina, New England Biolabs) running for 30 cycles in the thermocycler. Purification as well as size selection of each PCR product was done using the AMPure XP PCR Cleanup system (Beckman Coulter).

Purified, barcoded PCR products were pooled in equimolar concentrations and pools were sequenced at the Max Planck-Genome-Centre Cologne by paired-end Illumina sequencing (2 × 250 bp HiSeq2500). Bulk sequences were merged, demultiplexed, and quality trimmed for a sequence length of 300–500 bp with a maximum of 2% homopolymers and a maximum of 2% ambiguities using BBTools (Bushnell, B., 2014). The SILVAngs pipeline (Quast et al., 2012) with the SSU rRNA SILVA database 138 was used to compare sequences and assign the taxonomic affiliation.

Normalised reads (excluding Archaeal and Eukaryal) were subsequently used for the analysis of the community composition. For analysis of compositional differences between stations and depth as well as amended and unamended incubations, Bray–Curtis dissimilarity matrices were calculated and results were visualised in non-metric multidimensional scaling plots. Note that the community composition data from the

unamended mesocosms has been previously published as part of Giljan et al. (2023).

RESULTS

Water mass characteristics

The three stations were within distinct regions of the North Atlantic Ocean: Stn. 18 was located within the Gulf Stream, Stn. 19 was off the continental shelf break of Newfoundland, and Stn. 20 was within the North Atlantic subtropical gyre (Figure S1a). Based on temperature and salinity characteristics, distinct water masses were sampled at the DCM at each of these stations (Figure S1b, c). Waters typical of the Gulf Stream characterised Stn. 18, while the relatively fresh DCM water at Stn. 19 was likely influenced by freshwater input from the Gulf of St. Lawrence or sea ice melt from the Labrador Sea. Its T/S characteristics are consistent with warm slope water and Labrador slope water (Fratantoni and Pickart, 2007). DCM water from Stn. 20 was typical for the North Atlantic subtropical gyre (Figure S1b, c). The chlorophyll concentration of DCM water also differed among stations: at Stn. 19 chlorophyll (at 1.33 mg m^{-3}) was more than three times higher than at Stn. 18 (0.40 mg m^{-3}), and more than a factor of six higher than at Stn. 20 (0.21 mg m^{-3}; Figure S1c). Prokaryotic cell counts at the DCM at Stn. 19 likewise were considerably higher (1.6×10^6 cells mL^{-1}) than at Stns. 18 and 20 (3.6×10^5 cells mL^{-1} and 4.3×10^5 cells mL^{-1}, respectively; Figure 1A). Bottom water samples at all three stations (depths of 3190 m, 4325 m, and 5580 m, for Stns. 18, 19, and 20, respectively) had T/S characteristics similar to North Atlantic Deep Water (Heidrich & Todd, 2020) (Figure S1b, c). Cell counts in bottom water decreased with increasing bottom water depth, at 2.4×10^4 cells mL^{-1}, 2.1×10^4 cells mL^{-1}, and 9.7×10^3 cells mL^{-1} for Stns. 18, 19, and 20, respectively.

Cell counts over the time course of incubations

Immediately after collection, water was added to 20 L carboys, and HMW organic matter isolated from *Thalassiosira* was added to the amended mesocosms. Bacterial communities were given 2 days to respond to this addition of HMW organic matter, and then FLA-PS were added to triplicate subsamples of the amended mesocosms, as well as to triplicate samples of the unamended mesocosm to measure rates and mechanisms of polysaccharide processing (see "Experimental Procedures" for further details). The six polysaccharides examined (pullulan, laminarin, xylan, fucoidan, arabinogalactan, and chondroitin sulphate) represent a range of structural complexities. Pullulan and laminarin are more simple polysaccharides; for example, laminarin, a highly abundant algal storage substrate, has a relatively simple structure consisting of β-(1–3)-linked glucose molecules (Becker et al., 2020). In contrast, fucoidan, a polysaccharide that can require hundreds of enzymes to hydrolyze, has a complex branching structure and is highly sulfated (Sichert et al., 2020). Similarly, arabinogalactan is a more complex polysaccharide; arabinogalactan, which is composed of arabinose and galactose, may persist in the ocean for long periods due to its resistance to degradation (Vidal-Melgosa et al., 2021).

In DCM water, cell counts measured 2 days after the addition of HMW organic matter were generally similar among the three stations, and (except Stn. 19) were substantially higher than those in unamended FLA-PS incubations 2 d after water collection (Figure 1). Over time, cell counts in amended FLA-PS incubations increased, independent of the specific FLA-PS added. Total cell counts peaked by the 5–7 days timepoints, and by day 12 sharply declined to levels similar to (Stn. 20) or below (Stns. 18 and 19) initial values. In the unamended mesocosms, cell counts were lower than in the amended mesocosms. Only in the unamended Stn. 19 incubations was there a considerable increase and then subsequent decrease in cell counts, whereas at Stn. 18, cell counts were generally constant with time, and at Stn. 20, cell counts in the unamended incubations doubled by the 10 days time point (Figure 1A).

In amended bottom water incubations, cell counts were initially similar to those in unamended FLA-PS incubations. However, cell counts increased by close to two orders of magnitude over the following time points, far exceeding the maximum value of cell counts in unamended FLA-PS incubations (Figure 2). The timing of this increase varied by station: in bottom waters from Stns. 18 and 19, the increase was evident by the 5 days time point, but in bottom water from Stn. 20, which had the deepest depth (5580 m, compared to 3190 m and 4125 m at Stns. 18 and Stn. 19, respectively), the initial increase occurred at a later timepoint (Figure 2B). Unlike amended DCM incubations, in amended bottom water incubations cell counts did not decline (Figure 2B).

External hydrolysis of polysaccharides

In unamended mesocosms, the rate and spectrum of polysaccharides hydrolyzed externally differed by station, depth, and time point. The broadest spectrum of hydrolyzed polysaccharides was measured in DCM mesocosms; the narrowest spectrum—only two or three substrates hydrolyzed—was measured in unamended bottom water mesocosms (Figures 1A and 2A). External hydrolysis of the complex polysaccharides

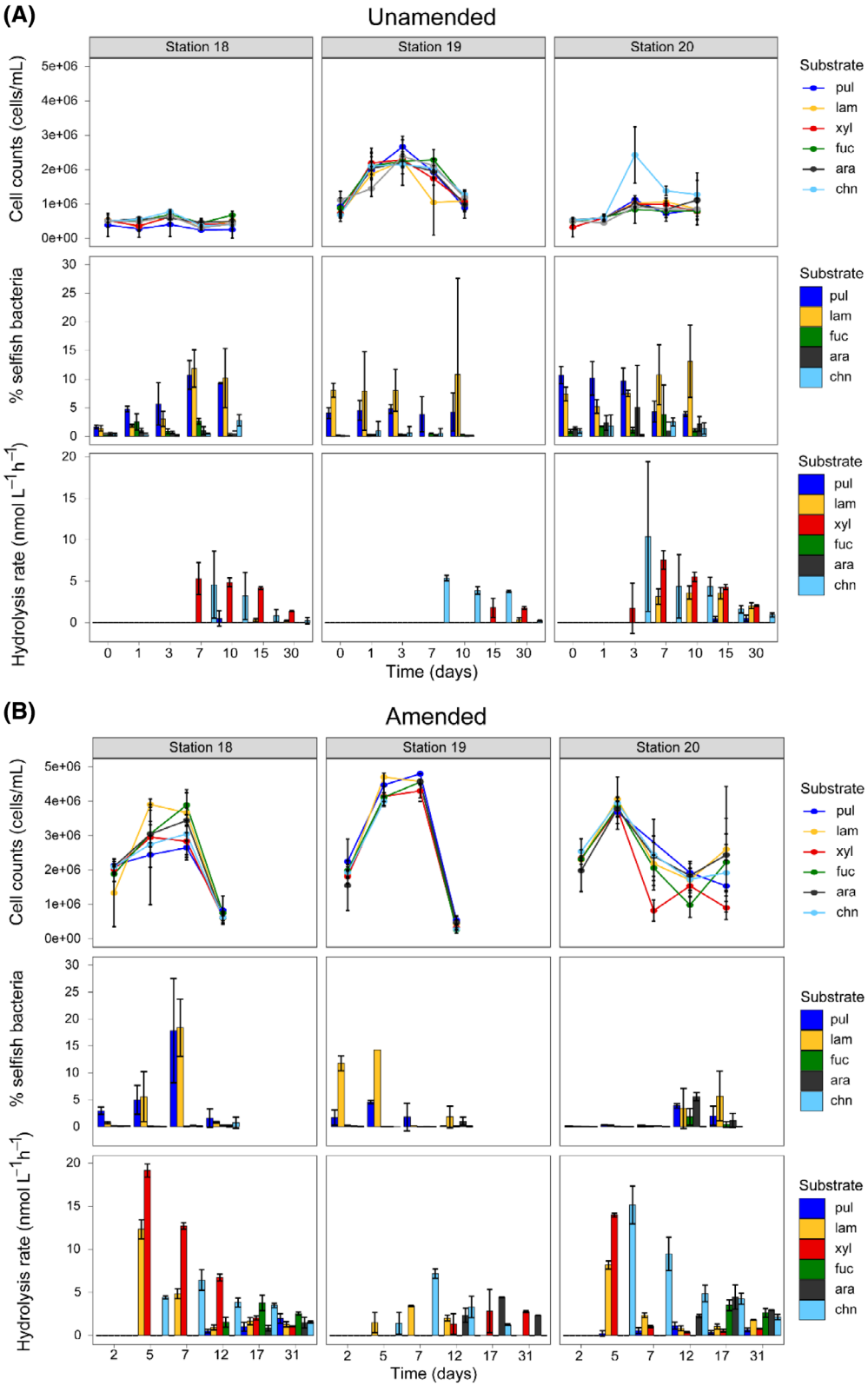

FIGURE 1 Total bacterial abundance, percent of selfish uptake, and rates of external hydrolysis in (A) unamended and (B) amended DCM incubations. FLA-PS: ara, arabinogalactan; chn, chondroitin; fuc, fucoidan; lam, laminarin; pul, pullulan; xyl, xylan. Note that time in days (*x*-axis) tracks the time since water was collected from the ocean (see "Experimental Procedures").

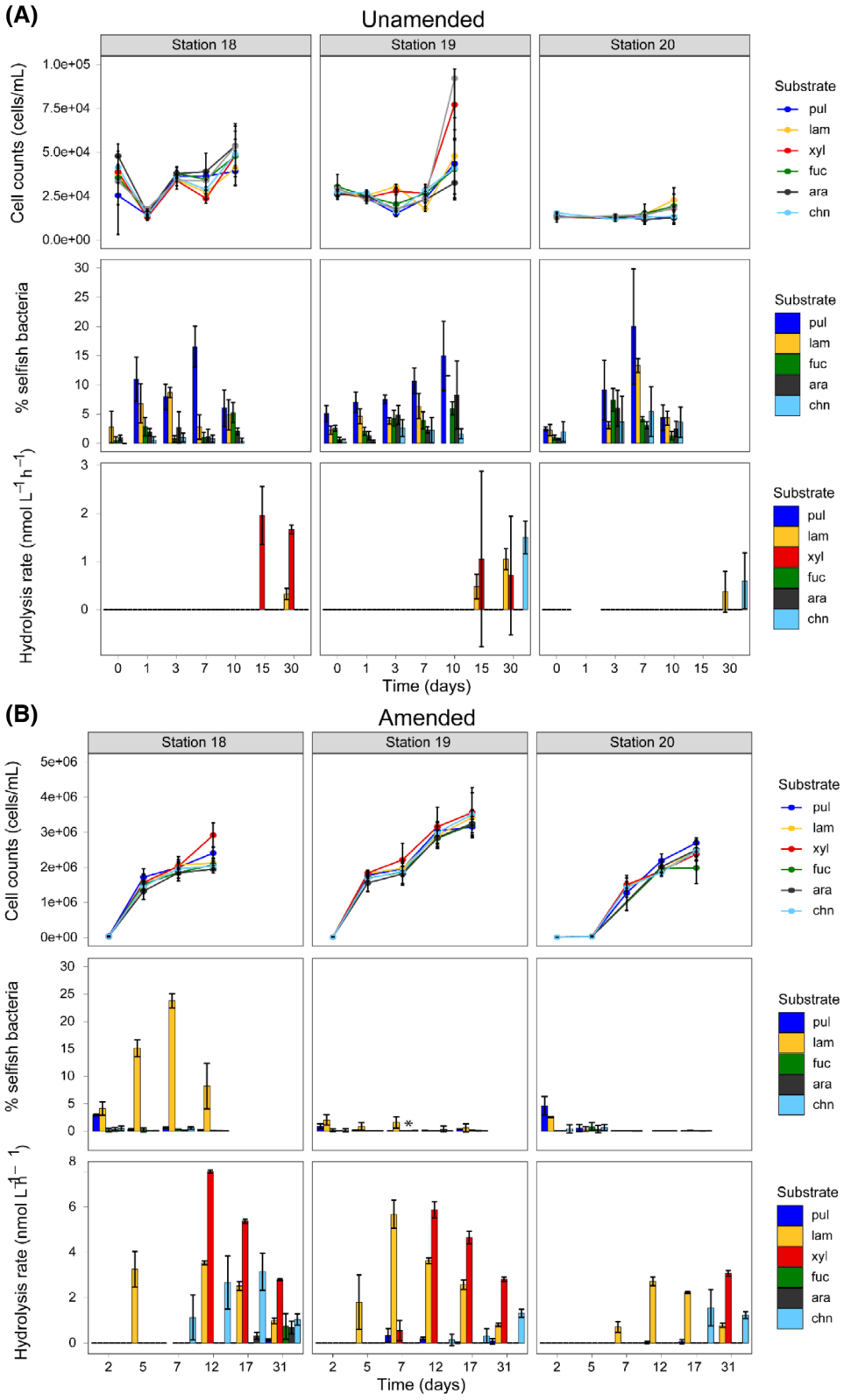

FIGURE 2 Total bacterial abundance, percent of selfish uptake, and rates of external hydrolysis in (A) unamended and (B) amended bottom water mesocosms. FLA-PS: pul = pullulan, lam = laminarin, xyl = xylan, fuc = fucoidan, ara = arabinogalactan, chn = chondroitin. Note the difference in *y*-axis scales between unamended and amended mesocosms. * = no fucoidan and arabinogalactan samples from Station 20 amended mesocosms at the 7 days timepoint. Note also that time in days (*x*-axis) tracks the timepoint since water was collected from the ocean (see "Experimental Procedures").

fucoidan and arabinogalactan was not detected in any of the unamended mesocosms (Figures 1 and 2). The time at which external hydrolysis was detected also varied by depth: external hydrolysis in bottom water incubations was only detected 15 or 30 days after water was collected, compared to 3–7 days for DCM incubations.

In amended mesocosms, external hydrolysis rates of FLA-PS were typically higher than in unamended mesocosms (Figures 1B and 2B). The spectrum of polysaccharide hydrolase activities was also broader in amended mesocosms, and polysaccharide hydrolysis was measurable at earlier time points (Figures 1B and 2B). Station and depth-specific trends in polysaccharide hydrolase activities were also evident: in Stn. 18 mesocosms, all six FLA-PS were hydrolyzed in DCM and bottom water mesocosms, although activities were lower in bottom water mesocosms than in DCM mesocosms. Fucoidan and arabinogalactan hydrolysis were measurable only at later time points in these incubations, with detectable hydrolysis beginning at 12 and 31 days in DCM and bottom water mesocosms, respectively. In DCM incubations, five and six polysaccharides were hydrolyzed in incubations from Stn. 19 and 20, respectively, with the rates and temporal development of polysaccharide hydrolase activities differing between stations (Figures 1 and 2). Fewer polysaccharides were hydrolyzed in bottom water mesocosms from Stns. 19 and 20; no fucoidan hydrolysis was detectable.

Selfish uptake of polysaccharides

Selfish uptake of all FLA-PS substrates was detected in all incubations, except xylan incubations, in which selfish uptake could not be measured due to high background fluorescence. However, patterns of selfish uptake differed between amended and unamended mesocosms, as well as among substrates, stations, depths, and time points (Figure 3). In particular, the addition of HMW organic matter resulted in notable differences in selfish uptake among different FLA-PS and stations. FLA-PS effects could be divided broadly into two groups, with laminarin and pullulan, the simple polysaccharides, in one group and the more complex polysaccharides arabinogalactan, fucoidan, and chondroitin in the other. Overall, the abundance of selfish bacteria taking up pullulan and laminarin was greater than those taking up fucoidan, arabinogalactan, and chondroitin in amended as well as unamended incubations (Figure 3). The total number of selfish bacteria in amended mesocosms typically exceeded that of unamended mesocosms, in particular, due to high numbers of bacteria taking up laminarin and pullulan (Figure 3). However, the overall percentage of the community carrying out selfish uptake in amended mesocosms was, in many cases, lower than unamended mesocosms (Figure S2). Moreover, unamended mesocosms typically had a greater percentage—as well as greater absolute numbers—of bacteria that selfishly took up complex polysaccharides (i.e., fucoidan, arabinogalactan, and chondroitin) than amended incubations (Figures 1–3, S2). In addition, bacteria in unamended mesocosms displayed more even selfish uptake of all FLA-PS than amended mesocosms.

The extent of selfish uptake in amended incubations also varied by station. At the initial time point in amended DCM incubations, shortly after FLA-PS were added, selfish uptake was notably higher in Stn. 18 and 19 incubations than in Stn. 20 incubations. At Stns. 18 and 19, 5% and 15%, respectively, of the total community was capable of selfish uptake of at least one substrate at the initial timepoint, while only ∼1% of the bacteria initially present at Stn. 20 were selfish (Figures 1 and S2). These differences in selfish uptake between stations in amended incubations persisted throughout the experiment (Figure S2). Simple polysaccharides accounted for the majority of selfish uptake at these stations; selfish cellular abundance in Stn. 18 and 19 laminarin incubations reached 7.0×10^5 cells mL^{-1}, while selfish cellular abundance in pullulan incubations was between 2.0×10^5 and 5.0×10^5 cells mL^{-1} (Figure 3). In contrast, selfish uptake of fucoidan, arabinogalactan, and chondroitin typically remained below 1%, or ∼ 5000 cells mL^{-1}. In Stn. 20 incubations, in contrast to Stns. 18 and 19, the fraction of selfish bacteria remained low (below 5%) until the 12 and 17 days timepoints when selfish uptake increased to at least ∼5% of the total population. Unlike at Stns. 18 and 19, the 12 and 17 days timepoints at Stn. 20 were characterised by more even selfish uptake of pullulan, laminarin, fucoidan, and arabinogalactan, with the highest percent of fucoidan and arabinogalactan selfish uptake in amended DCM incubations (Figure S2).

Amended bottom water incubations were also characterised by selfish uptake predominantly of simple polysaccharides, with selfish uptake of fucoidan, arabinogalactan, and chondroitin typically remaining below 0.5%, for the duration of the experiment (Figures S2, 3). The number of selfish bacteria in Stn. 18 laminarin incubations reached 5.0×10^5 cells mL^{-1}, close to those in Stn. 18 DCM laminarin incubations (Figure 3). However, the percentage of selfish pullulan uptake tended to be much lower in Stn. 18 and 19 amended bottom water incubations than in amended DCM incubations from the same stations. As in amended DCM incubations, patterns in selfish uptake varied with the station (Figure S2). While the percentage of selfish uptake remained relatively constant (∼5%) at Stn. 19 despite changes in total cell counts over time, selfish

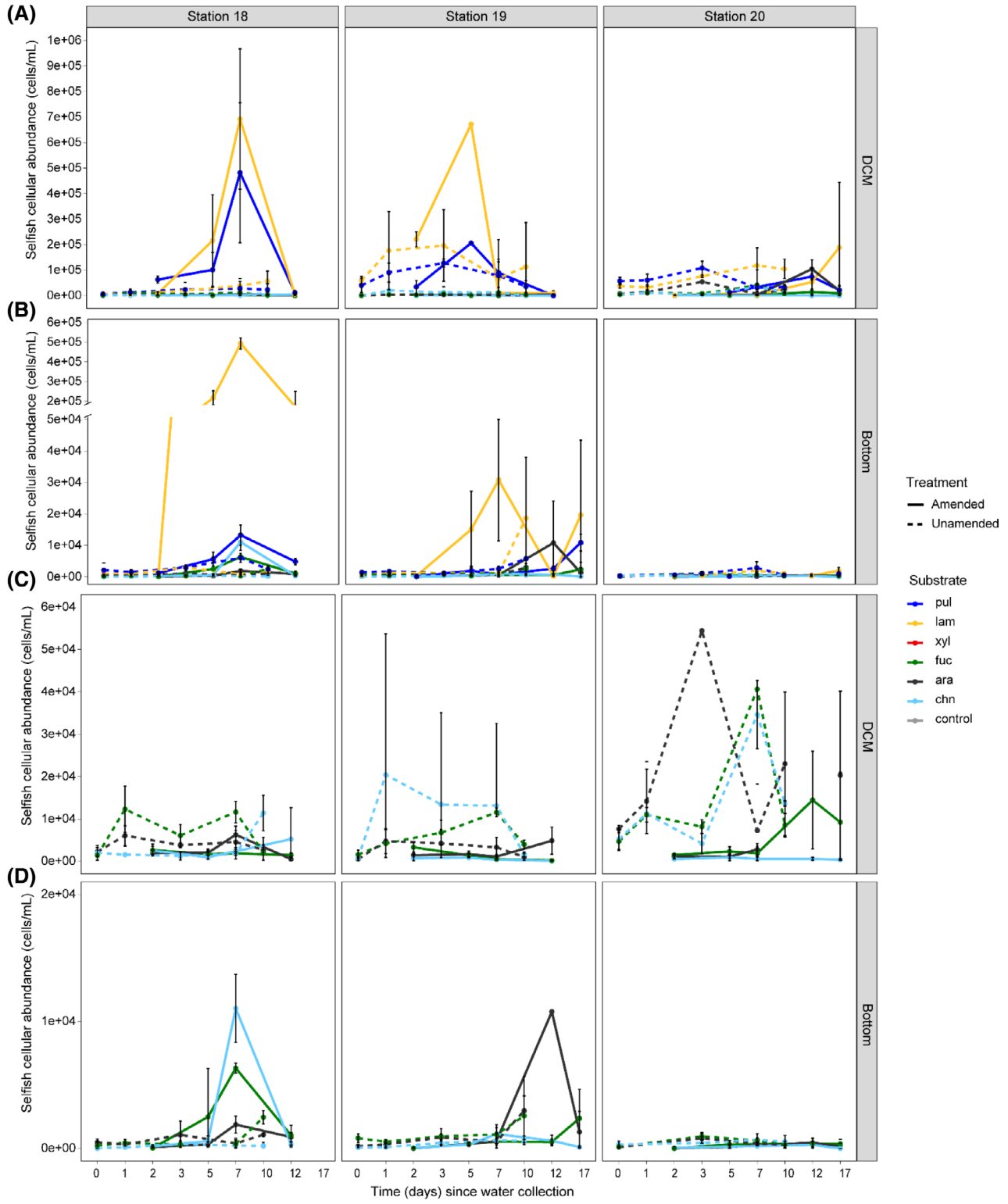

FIGURE 3 Abundance of selfish bacteria in amended (solid line) and unamended (dashed line) FLA-PS incubations from the DCM and bottom. FLA-PS: pul = pullulan, lam = laminarin, xyl = xylan, fuc = fucoidan, ara = arabinogalactan, chn = chondroitin. (A) and (b) show the selfish uptake of all substrates; (C) and (D) display only the selfish uptake of the complex substrates fucoidan, arabinogalactan, and chondroitin. Note the difference in scales between (A)–(D).

uptake at Stn. 20 displayed a different pattern: the highest measurements of selfish uptake occurred at the 2 and 5 days time points, before decreasing to less than 1% of the total population for the remaining time points as cell counts increased substantially (Figure S2).

Community composition

Bacterial community composition also differed significantly between amended and unamended incubations, as well as by time points, depths, and stations, with the greatest dissimilarity in community composition occurring in water from different depths (ANOSIM $R = 0.422$, p-value $= 0.0001$; Figures 4 and 5) and in amended versus unamended incubations (ANOSIM $R = 0.414$, p-value $= 0.0001$; Figures 4 and 5). At each station and depth, changes in bacterial community composition were consistent regardless of the FLA-PS added (ANOSIM $R = 0.013$, p-value $= 0.035$) (Figure S3). In DCM incubations, significant differences in community composition between amended and unamended incubations (ANOSIM $R = 0.715$, p-value $= 0.0001$; Figure S4) appeared to be driven by differences in the relative abundance of *Alphaproteobacteria* and *Gammaproteobacteria* (Figures 4 and S3a, b). High relative abundances of *Alphaproteobacteria* and low relative abundances of *Gammaproteobacteria* comprised the initial communities in unamended DCM incubations (Figures 4 and S3a). Over time, bacterial community composition in these incubations changed significantly (ANOSIM $R = 0.367$, p-value $= 0.0001$; Figure S4c): at Stn. 18, later timepoints displayed an increase in the gammaproteobacterial genera *Alteromonas* and Marine Methylotrophic Group 3; at Stn. 19, the relative abundance of Gammaproteobacteria likewise increased over time, with *Pseudoalteromonas* increasing by the 10 days timepoint (Figures 4 and S3a). In Stn. 20 unamended DCM incubations, the relative abundance of Marine Methylotrophic Group 3 (*Gammaproteobacteria*) increased in particular (Figures 4 and S3a); differences in bacterial community composition in unamended DCM incubations were primarily station-related (ANOSIM $R = 0.541$, p-value $= 0.0001$; Figure S5a). Unamended DCM incubations from Stn. 19 had a much higher relative abundance of Bacteroidetes than Stn. 18 and 20 incubations, while much higher relative abundances of *Prochlorococcus* (*Cyanobacteria*) were found at Stn. 18 (Figures 4 and S3a). While differences in community composition between different unamended FLA-

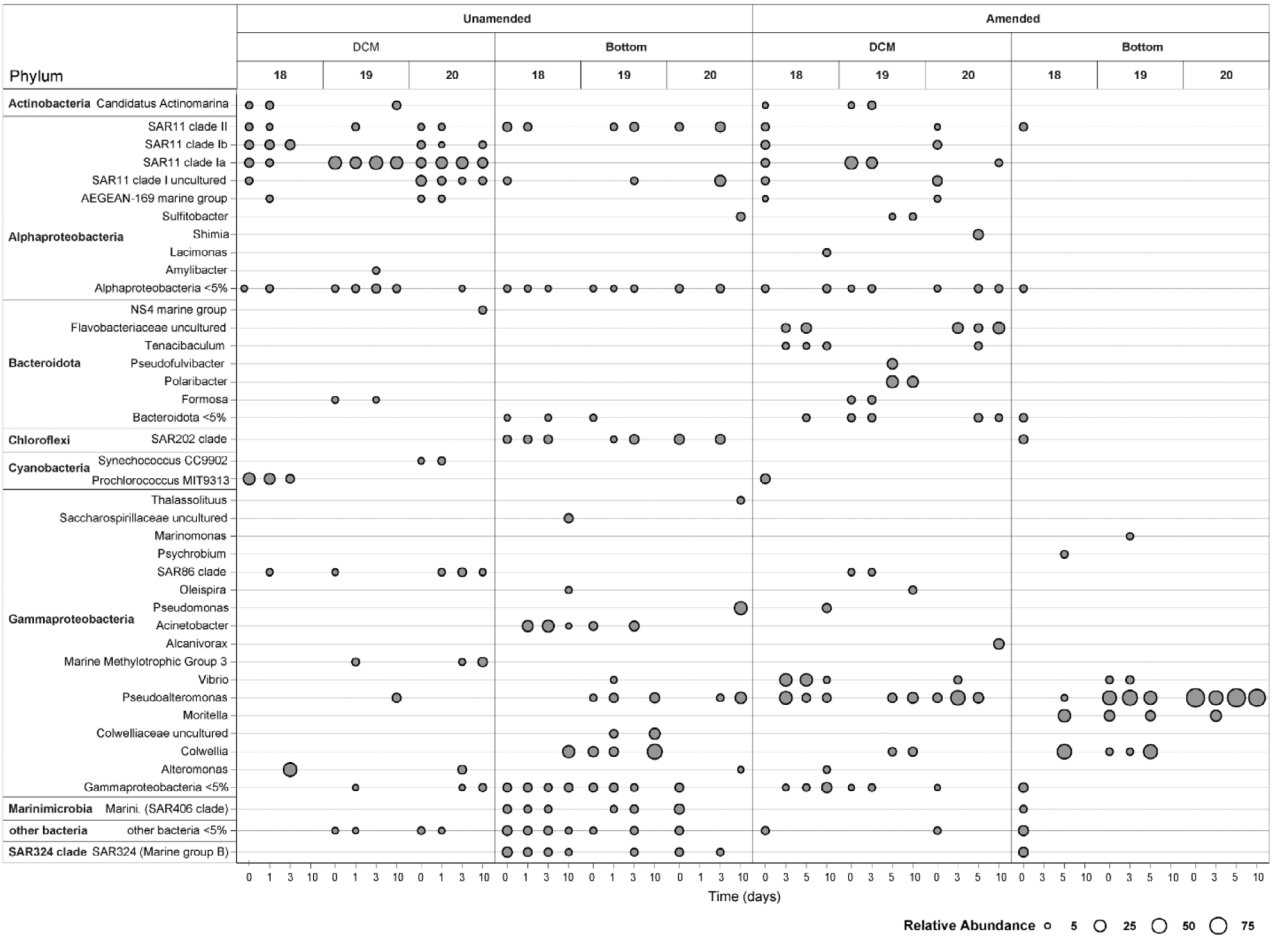

FIGURE 4 Bubbleplot of bacterial community composition in the unamended incubations and the amended mesocosm at Stns. 18, 19, and 20, showing genera above 5% relative abundance. Note that this figure shows only bacterial community composition during the first 10 days of incubation (see *x*-axis).

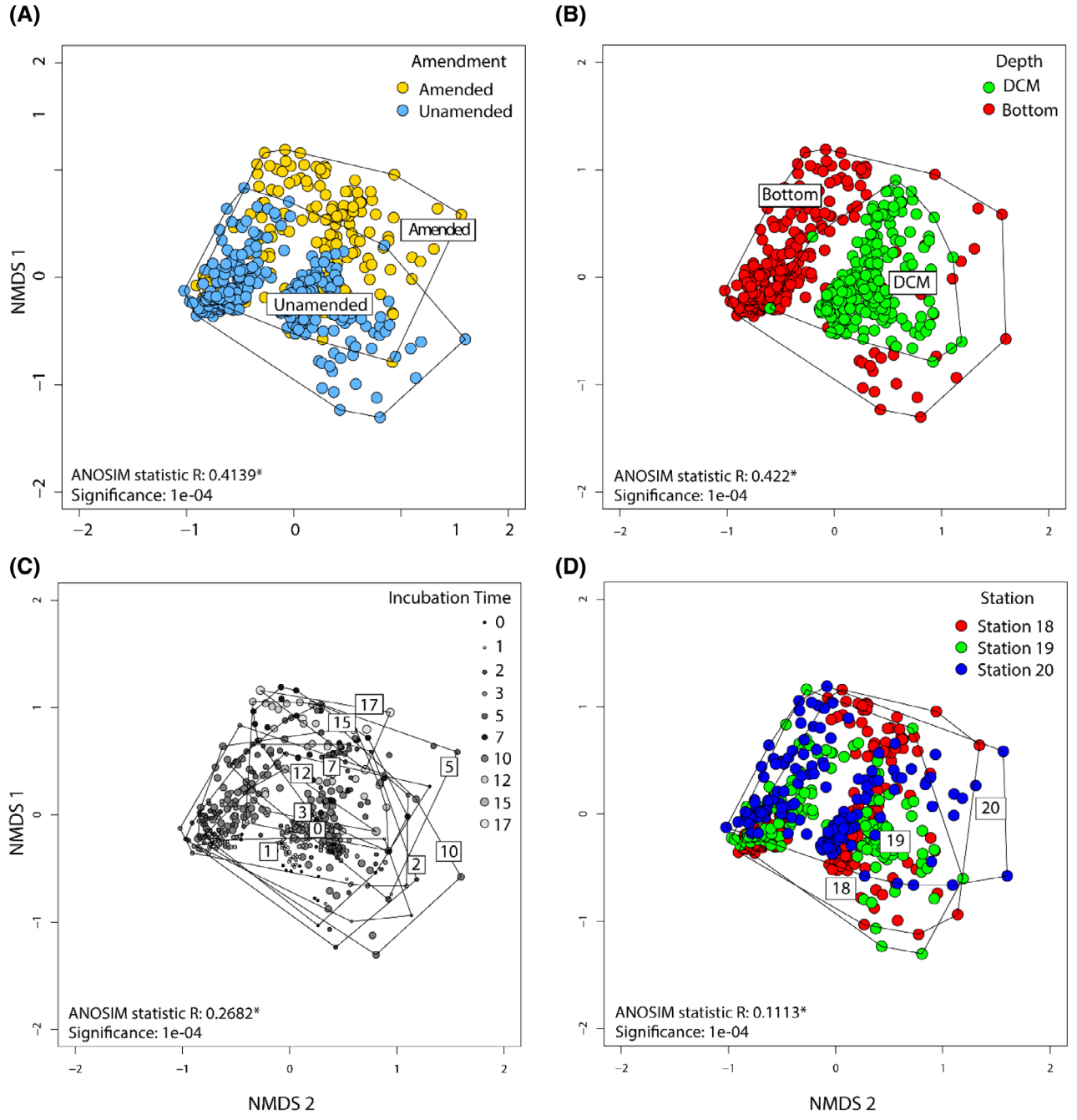

FIGURE 5 Non-metric multidimensional scaling (NMDS) of bacterial community composition in amended and unamended FLA-PS incubations from both the DCM and bottom waters. NMDS plots are grouped by (A) amendment, (B) depth, (C) incubation time, and (D) station. *R*-values marked with an * are significant.

PS incubations at this depth were not significant (ANOSIM $R = 0.005$, p-value $= 0.627$; Figure S5c), a notable chondroitin-specific increase in *Flavicella* (*Bacteroidota*) occurred at each station at either the 3 days time point (Stns. 18 and 20) or the 10 days time point (Stn. 19) (Figures 4 and S3a).

In contrast to unamended DCM incubations, amended DCM incubations were dominated by *Gammaproteobacteria*, and displayed slightly greater differences in taxonomic composition between stations (ANOSIM $R = 0.591$, p-value $= 0.0001$; Figure S6a) than did the unamended incubations (Figures 4 and S5a). At each station, different gammaproteobacterial genera increased over time: *Vibrio* and *Pseudoalteromonas* were most abundant at Stns. 18 and 20, while *Pseudoalteromonas* increased over time at Stn. 19 (Figures 4 and S3b). An increase in *Alcanivorax* (*Gammaproteobacteria*) also occurred in Stn. 18 incubations at the 12 days timepoint. The relative abundance of other bacterial classes also differed between different

stations from this depth: in Stn. 18 incubations, a slight increase in Bacteroidota occurred over time, while Stn. 19 and 20 had greater relative abundances of *Alphaproteobacteria*. As in unamended DCM incubations, amended incubations were not affected by the addition of FLA-PS (ANOSIM $R = -0.050$, p-value $= 0.970$; Figure S6c); however, some substrate-specific increases in bacterial genera occurred. For example, at Stn. 18, DEV007 (*Verrucomicrobia*) increased by the 12 days time point in all FLA-PS incubations except for pullulan and xylan; increases in this genus were greatest in arabinogalactan and chondroitin incubations (Figure S3b). Additionally, an arabinogalactan-specific increase in *Flavobacterium* (*Bacteroidota*) occurred in amended Stn. 19 incubations after 12 days (Figures 4 and S3b).

Bacterial community composition in bottom water incubations differed strongly from the DCM incubations (Figures 4, 5, and S3). However, bottom water incubations, as in DCM incubations, exhibited the greatest dissimilarity in bacterial community composition between amended and unamended incubations (ANOSIM $R = 0.329$, p-value $= 0.0001$; Figure S7a). Amended and unamended bacterial communities were not as strongly dissimilar in bottom water incubations as they were in DCM incubations (ANOSIM $R = 0.715$, p-value $= 0.0001$; Figure S4a). Still, notable differences in phylum and class-level diversity between amended and unamended bottom water mesocosms occurred: while amended bottom water incubations were strongly dominated by *Gammaproteobacteria* after the initial timepoint, unamended bottom water incubations retained diverse communities of *Gammaproteobacteria*, *Alphaproteobacteria*, *Chloroflexi*, *Bacteroidota*, and *Marinimicrobia* over time, with the relative abundance of Gammaproteobacteria typically only surpassing 50% at the 10 days time point (Figures 4 and S3c, d). These changes in bacterial community composition in unamended bottom water incubations over time were significant (ANOSIM $R = 0.345$, p-value $= 0.0001$; Figure S8b), and resulted in greater bacterial community dissimilarity than differences between stations (ANOSIM $R = 0.267$, p-value $= 0.0001$; Figure S8a), although some station-related differences in the gammaproteobacterial genera that became most abundant were apparent (Figure S3c). In unamended Stn. 18 incubations, for example, *Acinetobacter* increased at the 3 days time point, and *Colwellia* and uncultured *Saccharospirillaceae* increased at the 10 days time point (Figures 4 and S3c). In contrast, the gammaproteobacterial genera that became most abundant over time at Stn. 20 were *Pseudoalteromonas* and *Pseudomonas*. While *Gammaproteobacteria* did not comprise a significant proportion of the community until the 10 days time point in unamended bottom water Stn. 18 and 20 incubations, there were high numbers of Gammaproteobacteria at the initial time point at Stn. 19 (Figures 4 and S3c). Over time, the relative abundance of *Alphaproteobacteria* and *Chloroflexi* increased in Stn. 19 incubations until *Gammaproteobacteria* (specifically *Colwellia*, uncultured *Colwelliaceae*, and *Pseudoalteromonas*) again increased in abundance at the 10 days timepoint. Some minor substrate-specific increases in gammaproteobacterial genera also occurred, though substrate-related differences were not statistically significant (ANOSIM $R = -0.014$, p-value $= 0.887$): in FLA-pullulan incubations from Stn. 19, increases in *Oleispira* occurred after 10 days (Figures 4 and S3c).

In contrast to DCM incubations, bacterial community composition in amended bottom water incubations displayed greater similarity with the station (ANOSIM $R = 0.243$, p-value $= 0.0004$; Figure S9a). Although all amended bottom water incubations were primarily composed of *Gammaproteobacteria*, the specific gammaproteobacterial genera present at each station differed: Stn. 18 incubations contained high proportions of *Colwellia* and *Moritella*, while high abundances of *Colwellia* and *Pseudoalteromonas* occurred at Stn. 19, and the dominant gammaproteobacterial genus at Stn. 20 was *Pseudoalteromonas*, which accounted for more than 90% of relative read abundance after the initial timepoint (Figures 4 and S3d). Although class-level diversity after the initial timepoint in these incubations was relatively limited, some notable changes in the abundance of bacteria belonging to classes other than *Gammaproteobacteria* did occur over time: *Polaribacter* (*Bacteroidota*) increased at the 12 days time point in Stn. 18 and 19 incubations and a small increase in the relative abundance of the alphaproteobacterial genus *Sulfitobacter* occurred after 12 days in Stn.m 20 incubations (Figures 4 and S3d). Overall, changes in bacterial community composition over time were significant (ANOSIM $R = 0.461$, p-value $= 0.0001$; Figure S9b), while no sigd3efzificant differences in bacterial community composition in different FLA-PS incubations occurred (ANOSIM $R = 0.015$, p-value $= 0.097$; Figure S9c).

Fluorescence in situ hybridization (FISH) of selfish bacteria

Because laminarin was selfishly taken up by a considerable number of organisms, FISH investigations were focused on bacteria taking up this substrate. Selfish bacteria were identified as *Bacteroidota, Planctomycetes, Gammaproteobacteria*, or *Verrucomicrobiales* using group-specific FISH probes on samples collected from the FLA-laminarin incubations. The numbers and identities of selfish bacteria differed between amended and unamended incubations, and with station, depth, and timepoint (Figure 6). In unamended DCM incubations, the majority of selfish bacteria were identified using these four FISH probes. At the initial time point,

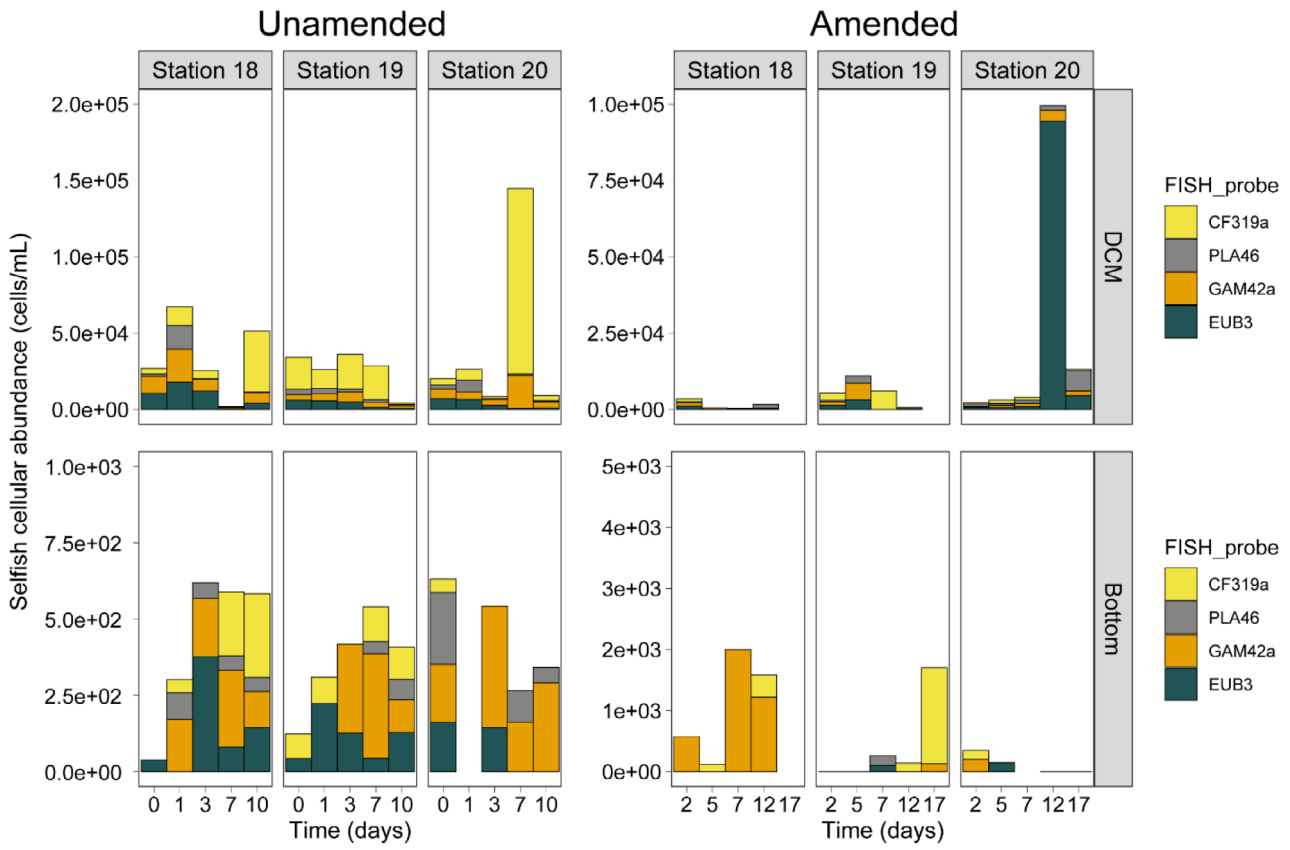

FIGURE 6 The abundance of selfish bacteria labelled with FISH probes in unamended and amended FLA-laminarin incubations. CF319a = *Bacteroidota*, PLA46 = *Planctomycetes*, GAM42a = *Gammaproteobacteria*, and EUB3 = *Verrucomicrobiales*.

relatively even numbers of selfish bacteria belonging to each group were identified at each station (Figure 6). At later time points, *Bacteroidota* typically became the most abundant selfish bacteria in these incubations, although the time point at which increases in abundance occurred, as well as the extent of these increases, varied by the station (Figure 6). In unamended bottom water, patterns in selfish *Bacteroidota, Planctomycetes*, *Gammaproteobacteria*, and *Verrucomicrobiales* in FLA-laminarin incubations differed from those in unamended DCM incubations; typically, a lower percentage of the selfish bacteria population was identified in bottom water mesocosms than in DCM mesocosms (Figure S10). In many cases, more than 50% of selfish bacteria in unamended bottom water mesocosms were not identifiable with the FISH probes used (Figure S10).

In amended DCM FLA-laminarin incubations, the abundance of selfish *Bacteroidota*, *Planctomycetes*, *Gammaproteobacteria*, and *Verrucomicrobiales* displayed patterns distinct from those in unamended mesocosms; in particular, the initial selfish community was relatively evenly composed of bacteria from each group. However, the group that became most abundant at later time points varied significantly with the station. At Stn. 18, the *Planctomycetes* became the most abundant selfish bacteria by the 12 days time point, while at Stn. 19, ~50% of selfish bacteria were identified as *Bacteroidota* by the 5 days time point, with significant increases in the number of selfish *Bacteroidota* correlating with an overall increase in selfish bacterial abundance (Figures S10 and 1B). In contrast, significant increases in selfish bacteria in Stn. 20 amended DCM incubations correlated with increases in *Verrucomicrobiales* occurring at the 10 days time point (Figures 1B and 6). Notably, while the majority of selfish bacteria were identified in Stn. 19 and 20 amended DCM incubations, fewer than 50% of selfish bacteria were identified in Stn. 18 incubations, despite a selfish bacterial abundance similar to that of Stn. 19 (Figures S10 and 1b). Patterns in the abundance of selfish *Bacteroidota, Planctomycetes*, *Gammaproteobacteria*, and *Verrucomicrobiales* in amended bottom water mesocosms also differed by station, although these patterns were distinct from those of amended DCM incubations. In amended bottom water incubations, *Gammaproteobacteria* became the most abundant group of selfish bacteria by the 2 days time point, while at Stn. 19 the abundance of *Bacteroidota* at the 12 days timepoint exceeded that of other groups (Figures 2B and 6). At Stn. 20, selfish *Verrucomicrobiales* accounted for 100% of the selfish bacteria

population by the 5 days time point; however, it should be noted that the total selfish cellular abundance at this time point was low, with selfish cell numbers averaging $9.6 \times 10^1 \pm 8.4 \times 10^1$ cells mL^{-1} (Figures S10 and 2b).

DISCUSSION

Most of the phytoplankton biomass that fuels marine food webs is recycled within the upper layers of the ocean, but a fraction of fresh organic matter can episodically reach the deep ocean (Deuser, 1986; Poff et al., 2021), helping to fuel bathypelagic food chains. This organic matter—in the form of aggregated diatom detritus, for example—can also bring polysaccharides, such as laminarin and fucoidan, which have been identified in phytoplankton blooms (Vidal-Melgosa et al., 2021), to the deep sea (Poff et al., 2021). Such episodic pulses of complex organic matter can affect the composition and activities of microbial communities (Balmonte et al., 2019; Brown et al., 2022). Whether and where these microbial communities process HMW organic matter—and the mechanisms by which they process it—affects the fate of organic carbon in the ocean.

A broad response of external hydrolyzers

The addition of HMW organic matter from *Thalassiosira weissflogii* drove a vigorous response of external hydrolyzers capable of processing a wide range of simple as well as complex polysaccharides (Figures 1 and 2). Across all stations and depths, the spectrum of externally hydrolyzed polysaccharides increased considerably, concurrent with increases in bacterial cellular abundance (Figures 1 and 2). This pattern parallels observations from a spring bloom in the North Sea, where an enhanced expression of polysaccharide hydrolases was measured (Avcı et al., 2020; Krüger et al., 2019), and the spectrum of polysaccharide hydrolase activities broadened (Reintjes, Fuchs, Scharfe, et al., 2020) and as the bloom progressed. The broadened spectrum of enzyme activities is also similar to previous amendment experiments with HMW organic matter from *T. weissflogii* conducted at other locations in the western North Atlantic Ocean (Balmonte et al., 2019; Brown et al., 2022).

A broadened spectrum of polysaccharide hydrolase activities also coincided with shifts in bacterial community composition, highlighting the link between the composition and enzymatic function of a microbial community. After addition of HMW organic matter, members of the *Gammaproteobacteria*, *Bacteroidota*, and *Alphaproteobacteria* (e.g., *Vibrio, Pseudoalteromonas*, *Moritella*, *Colwellia*, *Flavobacteraceae*, *Sulfitobacter*), all known to respond to phytoplankton blooms (Buchan et al., 2014), became more abundant (Figure 4), a response similar to previous investigations involving addition of the same HMW *T. weissflogii* to North Atlantic seawater (Balmonte et al., 2019; Brown et al., 2022). Many of these taxa are external hydrolyzers, which are well equipped to grow quickly during conditions in which substrates are available in moderate to high abundances (Traving et al., 2015) or present in dense patches (Ebrahimi et al., 2019). External hydrolysis thus can become a more profitable mechanism for the processing of both simple and complex polysaccharides upon the addition of HMW organic matter (Figure 7).

Substrate-specific changes demonstrate a nuanced response of selfish bacteria

The selfish bacterial response to HMW organic matter addition, in contrast, was more differentiated by polysaccharide complexity, as well as by station (Figure 7). Amendment with HMW organic matter increased the numbers of bacteria selfishly taking up the simpler polysaccharides—laminarin, and to some extent pullulan—at Stns. 18 and 19, but had little effect on selfish communities at Stn. 20. Uptake of complex polysaccharides (i.e., arabinogalactan, fucoidan, and chondroitin) continued at all stations, but selfish cell numbers were for the most part not very different between amended and unamended incubations, suggesting that the bacteria focusing on these substrates were largely unaffected by organic matter addition.

The laminarin-specific enhancement in selfish uptake measured in amended incubations parallels previous observations made during a natural phytoplankton bloom, in which an increase in the spectrum and rate of external hydrolysis late in the bloom coincided with high selfish uptake of the β-linked glucan laminarin, but not other substrates (Reintjes, Fuchs, Scharfe, et al., 2020). Pullulan, a structurally simple α-linked glucan, was not used as a selfish substrate in the study by Reintjes, Fuchs, Scharfe, et al. (2020), but here we observed a high selfish uptake of pullulan. The ability to process α- and β-linked glucans via external hydrolysis and/or selfish uptake is likely widespread among heterotrophic bacteria, due to the abundance of these polysaccharides in the ocean. Along these lines, a recent study has demonstrated that α- and β-glucan processing enzymes are widely and preferentially expressed by a broad phylogenetic range of bacteria during a spring phytoplankton bloom (Sidhu et al., 2023). Their target substrates are commonly available: annual oceanic production of laminarin has been estimated to be on the order of 5–15 billion metric tons (Alderkamp et al., 2007; Becker et al., 2020), and external hydrolysis of laminarin has been detected at almost every station and depth in the

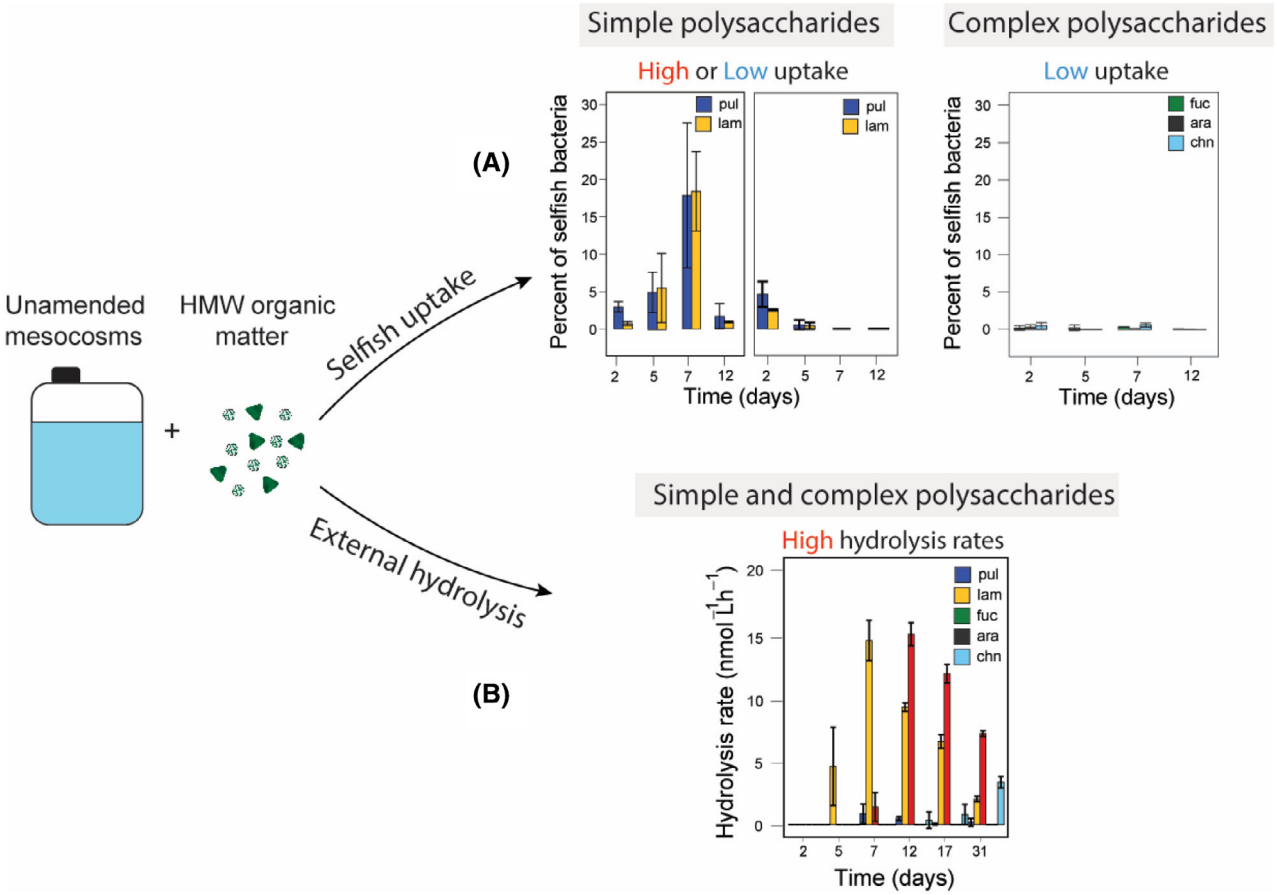

FIGURE 7 Different responses of external hydrolyzers and selfish bacteria to the addition of HMW organic matter. (A) Selfish uptake: simple polysaccharides show both high and low selfish uptake, depending on station and depth; the addition of HMW organic matter in some cases leads to the growth of selfish organisms. Complex polysaccharides generally show constant selfish uptake in all situations, with no sign of rapid growth in response to HMW organic matter addition. (B) External hydrolysis of simple and complex polysaccharides increases upon HMW organic matter addition, although some of the complex polysaccharides might not be externally hydrolyzed, even though they are selfishly taken up.

ocean surveyed to date (e.g., Arnosti et al., 2011; Balmonte et al., 2021).

Increased selfish uptake of laminarin (and to some extent pullulan) after HMW organic matter addition, however, contrasts strikingly with the lack of a similar response in selfish uptake of chondroitin, arabinogalactan, and fucoidan (Figures 1–3). Selfish bacteria targeting these substrates rarely increased in abundance in amended incubations compared to unamended incubations (Figures 1 and 2), and instead tended to decrease in relative abundance after HMW organic matter addition (Figure 3). This lack of response to complex substrates from selfish bacteria could potentially be due to prioritisation in substrate response, as has been demonstrated for degradation of laminarin prior to alginate or pectin by *Alteromonas macleodii* (Koch et al., 2019). Under this scenario, bacteria that can take up multiple substrates in a selfish manner may prioritise laminarin, as long as it is available. Alternatively, a lack of selfish uptake of complex polysaccharides might be the result of a mismatch between the metabolic needs of these selfish organisms and the HMW organic matter added to the mesocosms. These two scenarios are not exclusive, in that some selfish bacteria may operate via resource prioritisation, whereas others might not take up complex polysaccharides in a selfish manner under specific conditions. In any case, the HMW organic matter addition did trigger a response among external hydrolyzers focused on these same substrates, especially in incubations containing water from the DCM (Figure 1). Moreover, at both depths, HMW organic matter addition also fueled considerable bacterial growth (Figures 1 and 2). Furthermore, the observation that an enhancement in the supply of natural organic matter during a spring bloom fueled a similar response—enhancement only of selfish laminarin uptake—concurrent with widespread external hydrolysis (Giljan et al., 2023; Reintjes, Fuchs, Scharfe, et al., 2020), suggests that the responses observed in the mesocosm experiments are not an anomaly.

The contrasts in the extent to which selfish uptake of a given polysaccharide is stimulated by HMW organic matter may be related to the structure as well as the relative abundance of polysaccharides: laminarin (and pullulan) are comparatively simple, mostly linear glucose-containing polysaccharides, whereas chondroitin, arabinogalactan, and fucoidan are more complex, with multiple branches, charged components, sulfation, and/or different monosaccharide building blocks. Although the relative abundances of polysaccharides other than laminarin in the ocean have yet to be determined, fucoidan, arabinogalactan, and chondroitin are likely less abundant than laminarin. Considering these factors together—low abundance and high structural complexity—suggests that the energetic costs for selfish uptake and processing of these more complex substrates may be sufficiently high that the growth rates of the organisms taking them up selfishly are low; they, therefore, might not grow rapidly in response to an input of complex organic matter.

We note, however, that in the unamended and amended mesocosms, arabinogalactan and fucoidan were selfishly taken up even in cases in which these substrates were not externally hydrolyzed (Figures 1 and 2). This pattern—a lack of external hydrolysis, but evidence of selfish uptake—is in accordance with previous surface and bathypelagic observations in the North Atlantic Ocean (Giljan et al., 2023; Reintjes et al., 2019). In particular, arabinogalactan and fucoidan appear to present problems for external hydrolyzers. In the bathypelagic ocean, even the addition of HMW organic matter is seldom sufficient to stimulate the external hydrolysis of arabinogalactan and fucoidan (Figures 1 and 2; Balmonte et al., 2019; Brown et al., 2022). Given the complexity and potential rarity of these substrates, particularly in the deep ocean, selfish uptake, rather than external hydrolysis, could provide bacteria with energetic benefits. Since selfish bacteria keep the products of hydrolysis to themselves, they receive the full advantage of investment into the complicated enzymatic machinery necessary for the hydrolysis of these substrates (Arnosti et al., 2018, 2021).

Regional and depth-related differences in selfish uptake

The response of selfish bacteria to additions of HMW organic matter also varied markedly among stations (Figures 1–3). In particular, at Stn. 20, the addition of HMW organic matter led to considerably (DCM) and very considerably (bottom water) higher total cell counts, which were accompanied by a broader spectrum of externally hydrolyzed substrates (Figures 1 and 2). However, the absolute numbers of selfish bacteria did not differ notably between amended and unamended mesocosms; even the relative abundance of selfish bacteria capable of pullulan and laminarin uptake remained low in amended Stn. 20 mesocosms (Figure 3). At Stn. 20, therefore, resource prioritisation is not a likely explanation for the lack of a notable change in response by selfish bacteria. The robust increases in total cellular abundance and external hydrolysis in DCM and bottom water incubations indicate that the bacterial community at Stn. 20 was not simply 'unreactive' in some manner. Instead, responses to an addition of HMW organic matter at Stn. 20 appear to be driven entirely by external hydrolyzers. We suggest that the contrasting responses at Stns. 18 and 19 on the one hand, and Stn. 20 on the other, may be attributed to fine-scale differences in bacterial community composition and capabilities across stations. Such differences in communities may in turn be related to the nature and/or the quantity of organic matter naturally produced in these locations, given the oligotrophic nature of the centre of the North Atlantic gyre, and links between surface productivity and bathypelagic bacteria (Gómez-Letona et al. 2023).

Selfish bacteria are phylogenetically diverse, also in the deep ocean

Based on bacterial community composition data, Giljan et al. (2023) suggested that the ability to selfishly take up FLA-PS is phylogenetically widespread because the unamended mesocosms show a relatively constant percent of selfish bacteria against a backdrop of changing community composition. Our FISH results, which provide the first identification of selfish bacteria capable of laminarin uptake in the bathypelagic ocean, support this hypothesis. Many different classes of bacteria contributed to the selfish uptake of laminarin in amended as well as unamended mesocosms. These organisms belonged to the *Bacteroidota*, *Gammaproteobacteria*, *Verrucomicrobia*, and *Planctomycetes* in both amended and unamended FLA-laminarin incubations from DCM and bottom waters (Figure 6). The identification of these organisms as selfish, moreover, is consistent with results from earlier investigations in surface ocean waters. Extensive selfish uptake of laminarin by *Bacteroidota* has previously been measured during the late stages of a phytoplankton bloom in the North Sea (Reintjes, Fuchs, Scharfe, et al., 2020). Selfish *Planctomycetes* have been identified in the surface waters of the Atlantic Ocean (Reintjes et al., 2017), while *Verrucomicrobiota*, in particular the clade *Pedosphaeraceae*, showed strong selfish uptake of laminarin in early summer in the North Sea (Giljan et al., 2023). Here, we found that increases in the abundance of selfish bacteria capable of laminarin uptake in amended mesocosms were occasionally the result of increases in a singular group, which varied by treatment, depth, and

station. Unamended mesocosms, in contrast, often exhibited a more even distribution of selfish *Bacteroidota*, *Gammaproteobacteria*, *Verrucomicrobia*, and *Planctomycetes*.

More selfish organisms remain to be identified, however. In bottom water incubations, in particular, up to 50% of selfish bacteria were not identified as one of the four group-specific FISH probes (Figure S10). While in some cases this result may be due to mismatches in probe specificity or coverage, or washout effects during sample preparation for FISH (Reintjes et al., 2023), the gap between the number of selfish bacteria identified via FLA-PS uptake, and the number identified via FISH (Figures 6 and S10) suggests that bacteria other than those belonging to the *Bacteroidota*, *Gammaproteobacteria*, *Verrucomicrobia*, and *Planctomycetes* may be capable of selfish uptake. These diverse bacterial taxa may also be using a mechanism other than the Sus system (Starch utilisation system) used by *Bacteroidota* during selfish uptake (Cho & Salyers, 2001; Cuskin et al., 2015); aside from members of the *Bacteroidota*, the mechanism by which *Gammaproteobacteria*, *Verrucomicrobia*, and *Planctomycetes* carry out selfish uptake remains unknown.

CONCLUSION

Episodic changes in the availability of organic matter characterise many locations in the ocean. Our study shows that the mechanisms by which heterotrophic bacterial communities respond to these changes are affected by the nature of the specific substrate that they process (Figure 7), as well as by the composition and capabilities of the starting community. Selfish uptake, as a substrate processing mechanism, is phylogenetically widespread and occurs throughout the water column (Figures 1–3 and 6; Giljan et al., 2023). The observation that selfish bacteria that utilise more complex substrates (e.g., arabinogalactan and fucoidan especially at Stn. 20; Figure 3C, D) may not be equipped to grow rapidly in response to an input of HMW organic matter suggests that there are growth costs associated with selfish processing of highly complex substrates. Conversely, many organisms capable of selfishly processing less-complex polysaccharides such as laminarin can grow at rapid rates. The costs and tradeoffs of selfish substrate processing therefore must be considered in the context of substrate structure, a factor that likely reflects the cost to an organism of producing a greater number of distinct enzymes as polysaccharide structural complexity increases (Bligh et al., 2022). The prevalence of external hydrolysis and the spectrum of substrates hydrolyzed, in contrast, can be linked directly with the abundance of HMW organic matter: a pulsed input in HMW organic matter increases the spectrum of substrates hydrolyzed, fueling microbial growth, even in the deep ocean (Figures 2B and 7). Our investigations also revealed the limitations of external hydrolysis relative to selfish uptake, however. In bottom waters, even a broadened spectrum of external hydrolysis did not lead to hydrolysis of structurally complex polysaccharides, which were processed only by selfish bacteria (Figures 1, 2, and 7). The mechanisms by which HMW substrates are processed can affect carbon flow, by restricting the availability of LMW substrates to other members of heterotrophic microbial communities (Arnosti et al., 2018). Our data additionally suggest that there are substrate-structure-related costs associated with distinct mechanisms of substrate processing, which affect growth rates and cellular abundance. Substrate structural complexity and quantity are key factors controlling heterotrophic activities, and in turn, the pathways and processes by which organic matter is cycled in the ocean.

AUTHOR CONTRIBUTIONS

Sarah Brown: Writing – original draft; formal analysis; visualization; investigation. **C. Chad Lloyd:** Investigation; writing – review and editing. **Greta Giljan:** Investigation; writing – review and editing; formal analysis. **Sherif Ghobrial:** Investigation; writing – review and editing. **Rudolf Amann:** Conceptualization; writing – review and editing; funding acquisition; investigation. **Carol Arnosti:** Conceptualization; funding acquisition; writing – original draft; investigation.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The 16S rRNA data is openly available under accession number PRJEB63119 at the EuropeanNucleotide Archive (ENA) of The European Bioinformatics Institute (EMBL-EBI):https://www.ebi.ac.uk/ena/browser/view/PRJEB63119. The data and code used to create thefigures used in this article are available on GitHub: https://github.com/ArnostiLab/EN638-AtlanticEndeavor-Cruise-2019.

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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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