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Depth-related patterns in microbial community responses to complex organic matter in the western North Atlantic Ocean

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Abstract

Oceanic bacterial communities process a major fraction of marine organic carbon, with a substantial portion of this carbon transformation occurring in the mesopelagic zone, and a further fraction fueling bacteria in the bathypelagic zone. However, the capabilities and limitations of the diverse microbial communities at these depths to degrade high molecular weight (HMW) organic matter are not well constrained. Here, we compared the responses of distinct microbial communities from North Atlantic epipelagic, mesopelagic, and bathypelagic waters at two open ocean stations to the same input of diatom-derived HMW particulate and dissolved organic matter. Microbial community composition and functional responses – as measured by polysaccharide hydrolase, glucosidase, and peptidase activities – were very similar between the stations, which were separated by 1370 km, but showed distinct patterns with depth. Changes in microbial community composition coincided with changes in enzymatic activities. In epipelagic mesocosms, the spectrum of peptidase activities became especially broad and glucosidase activities were very high, a pattern not seen at other depths. The spectrum of polysaccharide hydrolase activities was enhanced particularly in epipelagic and mesopelagic mesocosms, with fewer enhancements in rates or spectrum in bathypelagic waters. The timing and magnitude of these distinct functional responses to the same HMW organic matter varied with depth. Our results highlight the importance of residence times at specific depths in determining the nature and quantity of organic matter reaching the deep sea.

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1. Introduction

Heterotrophic microbial communities play a key role in global biogeochemical cycles by processing up to 50% of the primary productivity produced by phytoplankton in the ocean (Azam, 1998). Much of this organic matter is quickly consumed, transformed, and remineralized in the upper ocean, while most of the remainder is consumed in the mesopelagic zone (200-1,000 m), with only a fraction reaching deeper depths (Wakeham et al., 1997; Benner & Amon, 2015). The organic matter reaching the bathypelagic zone (1,000-4,000 m) typically contains low proportions of chemically characterizable carbohydrates, amino acids, and lipids (Benner & Amon, 2015). However, recent investigations have documented rapid transport of freshly-produced organic matter from the upper ocean to the bathypelagic via fast-sinking particles, thus injecting fresh epipelagic-derived organic matter into the deep (Mestre et al., 2018; Ruiz-González et al., 2020; Poff et al., 2021). The extent to which this organic matter is transformed and remineralized once reaching meso- and bathypelagic zones is ultimately determined by the metabolic capabilities of the heterotrophic microbial communities present at these depths.

A key step in microbial remineralization of complex high molecular weight (HMW) particulate and dissolved organic matter is its initial hydrolysis by distinct, structurally-specific extracellular enzymes, which yield hydrolysis products small enough for cellular uptake (Weiss et al., 1991; Arnosti, 2011). The specific capabilities and limitations of distinct microbial communities, particularly those in the meso- and bathypelagic, to enzymatically hydrolyze fresh HMW organic matter are not well-defined, however. Previous studies examining the response of microbial communities to organic matter addition have typically focused on epipelagic water communities, often using a mixture of both low- and high-molecular weight material (e.g., Beier et al. 2015; Luria et al., 2017) to examine community responses. Moreover, the studies that have

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measured the enzymatic responses of deep ocean communities have relied for the most part on a few small substrate proxies to assess exo-acting (terminal-unit cleaving) enzyme activities (e.g., Baltar et al., 2010; Sebastián et al., 2021); these substrate proxies do not yield information about the endo-acting (mid-chain cleaving) enzymes essential for degradation of HMW organic matter.

As an alternative, the activities of endo-acting enzymes can be measured using fluorescently-labeled polysaccharides (Arnosti 2003) that can probe structure-related differences in polysaccharide hydrolase activities. Such studies have revealed considerable differences in the rate and spectrum of enzyme activities with location and depth in the ocean, with the spectrum of substrates hydrolyzed typically decreasing with depth (Steen et al., 2012; Hoarfrost & Arnosti, 2017; Balmonte et al., 2018). Distinct spatial- and depth-related patterns in endopeptidase activities have also been found to exist (Balmonte et al. 2021). These patterns coincide with patterns in the depth-stratification of microbial taxa, genes, and metabolic potential (DeLong et al., 2006; Sunagawa et al., 2015; Guerrero-Feijóo et al., 2016). Together, these results suggest that microbial communities at different depths and locations vary considerably in their abilities to initiate the remineralization of HMW organic matter.

As an initial investigation of the enzymatic response of spatially-distinct microbial communities to complex organic matter, we previously added moderate quantities of HMW dissolved and particulate organic matter to epipelagic water and bottom water collected at a shelf station and at an offshore station in the western North Atlantic Ocean. We found that epipelagic and bottom water communities from the shelf and open ocean stations rapidly responded to HMW organic matter addition, with greater enhancements of rates and broader spectrum of enzyme activities in communities from the shelf and from epipelagic water relative to the offshore bottom water (Balmonte et al., 2019). Nonetheless, a distinct enhancement measurable

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in bathypelagic water from a depth of 4594 m demonstrated that an active heterotrophic community could respond enzymatically in comparatively short order to an input of HMW organic matter. This initial assessment showed that microbial communities at distinct depths and locations responded to addition of complex HMW organic matter, but the nature of that enzymatic response differed in some key respects.

In the present study, we focused particularly on microbial communities and their function in the open ocean, comparing organic matter transformation processes in the mesopelagic ocean, where substantial amounts of the organic matter sinking from the epipelagic is remineralized (Wakeham et al., 1997; Benner & Amon, 2015), to those in the epipelagic and bathypelagic. We added moderate quantities of HMW dissolved and particulate organic matter derived from *Thalassiosira weissflogii*, a widespread, abundant diatom (Hartley et al., 1996), to triplicate mesocosms from each depth, and tracked microbial community responses and enzyme activities associated with metabolism of two major classes of marine organic matter, polysaccharides and proteins. We carried out the same experiments at two open-ocean stations separated by 1370 km to compare the extent to which depth-stratified microbial communities may be functionally redundant – or not – in terms of their abilities to hydrolyze the same complex organic matter.

2. Materials and methods

2.1. Sample sites and sample collection

Water was collected aboard the R/V *Endeavor* in July 2016 at two locations in the western North Atlantic Ocean: Stn. 12 (36° 0'12.12"N, 73° 8'38.46"W), and Stn. 16 (35°59'43.20"N, 58° 2'40.80"W). We focused on epipelagic, mesopelagic (at the oxygen minimum zone, identified from the CTD profile), and bathypelagic water (bottom water), corresponding to depths of 2.5 m,

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850 m, and 3,660 m at Stn. 12 and 3.5 m, 875 m, and 5,050 m at Stn. 16, respectively. Water was transferred from the Niskin bottles to 20L carboys (acid-washed and rinsed, then rinsed again with sample water prior to filling). Each carboy was filled from a separate Niskin bottle for biological replicates. Triplicate carboys from each depth were amended with moderate (25 mg L^{-1}) quantities of material isolated from *Thalassiosira weissflogii,* corresponding to approximately 658 uM of HMW dissolved + particulate organic carbon (see below, and Balmonte et al., 2019). One unamended carboy from each depth served as an incubation control. Carboys were stored in the dark at in-situ or near in-situ temperatures: samples from the epipelagic and mesopelagic were incubated at 21ºC, and bottom water samples were incubated at 4ºC (Table 1). At each subsampling timepoint (0, 2, 7, and 16 d after the addition of HMW substrate), the carboys were mixed, and subsamples for measurements of cell counts, bacterial production, peptidase and glucosidase activities, and bacterial community composition were collected. Incubations to measure polysaccharide hydrolase activities were initiated at the 16 d timepoint (see below).

Table 1. *Sampling depth, in-situ characteristics, and incubation temperatures of water collected for mesocosms at each station and depth.*

	Sampling Depth (m)		*In-situ* Temp. (°C)		*In-situ* Salinity (PSU)		*In-situ* Oxygen (mL/L)		HMW-OM Incub. Temp. (°C)		Enzyme Incub. Temp. (°C)	
	Stn 12	Stn 16	Stn 12	Stn 16	Stn 12	Stn 16	Stn 12	Stn 16	Stn 12	Stn 16	Stn 12	Stn 16
Epipelagic	2.5	3.5	25.3	25.0	36.3	36.4	4.8	4.8	21	21	21	21
Mesopelagic	850	875	10.5	10.5	35.3	35.3	3.1	3.2	21	21	12	12
Bathypelagic	3,660	5,050	2.0	2.0	34.9	34.9	5.8	5.7	4	4	4	4

2.2. High molecular weight organic matter preparation

A HMW substrate was prepared from the diatom *Thalassiosira weissflogii* (Instant Algae, Reed Mariculture), as described in Balmonte et al. (2019). In brief, frozen and thawed cells were homogenized with a tissue grinder, dialyzed in a 10 kD membrane (SpectraPor), and the retentate

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(HMW dissolved organic matter plus particulate organic matter) was lyophilized, autoclaved, and lyophilized again. The final HMW *Thalassiosira weissflogii* substrate had a total carbohydrate concentration of 6.15% and a C:N ratio of 6:1 (Balmonte et al. 2019).

2.3. Bacterial productivity

Bacterial productivity was measured aboard ship according to the methods of Kirchman et al (1985, 2001). Samples were incubated in the dark at in-situ temperature between 12 and 24 h. Bacterial protein production was calculated from leucine incorporation rates using the equation of Simon and Azam (1989), and bacterial carbon production was determined by multiplying bacterial protein production by 0.86 (Simon and Azam, 1989; Kirchman, 2001).

2.4. Enzymatic hydrolysis measurements

Peptidase and glucosidase activities were measured immediately after the addition of HMW organic matter to the amended carboys, as well as 2, 7, and 16 d post-amendment. These activities were measured using small substrates (glucose, leucine, or peptides) labeled with 4-methyl-coumaryl-7-amide (MCA) or methylumbelliferone (MUF). Exo-acting (terminal-unit cleaving) glucosidase activities were measured with MUF- α- and -β-glucose, and leucine-MCA was used to measure exo-peptidase activities, after the approach of Hoppe (1983). Endopeptidase activities were measured with boc-gln-ala-arg-MCA (QAR, using one-letter amino acid abbreviations) and N-t-boc-phe-ser-arg-MCA (FSR) for trypsin activities, while ala-ala-phe-MCA (AAF) and N-succinyl-ala-ala-pro-phe-MCA (AAPF) were used to measure chymotrypsin activities. Activities were measured in triplicate using a plate reader, following Balmonte et al. (2019), using substrate concentrations of 150 μM, a concentration based on substrate saturation

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curves of leucine aminopeptidase and β-glucosidase in Stn. 9 epipelagic waters (76° 36' 6.12"N, 34° 36' 6.552"W). The fluorescence of autoclaved seawater with substrate (controls), and live seawater with no substrate (blanks), was also measured. Samples were incubated close to in-situ temperature, and measured at multiple timepoints – at 0, 6, 12, 18, 24, 36, and 48 hours. Fluorescence readings were converted to activities using a standard curve of free fluorophores (MCA, MUF) in seawater. Hydrolysis rates were averaged over the first 48 hours of measurements for each sampling day.

Polysaccharide hydrolase activities were measured using six fluorescently-labeled polysaccharides (pullulan, laminarin, xylan, fucoidan, arabinogalactan, and chondroitin sulfate; Arnosti 2003). These measurements were initiated in water from the amended and unamended mesocosms 16 d after the start of the experiment, an initial time-lag that should allow the microbial community in amended mesocosms to respond – via enzyme expression as well as shifts in community composition – to the addition of *Thalassiosira*-derived material. Incubations were carried out after Balmonte et al. (2019). All incubations were kept in the dark at in-situ temperatures. Subsamples were collected 0, 2, 5, 10, 17, and 30 d after polysaccharide addition. Hydrolysis rates were calculated as previously described (Arnosti, 2003). Note that all enzymatic hydrolysis rates – polysaccharide hydrolase as well as peptidase – should be considered potential rates, since added substrate is in competition with naturally-occurring substrates for enzyme active sites.

2.5. 16S rRNA sequencing and phylogenetic analysis

To analyze bacterial community composition, 250 to 2,500 mL of seawater from each carboy was filtered using a vacuum pump through a 0.2 µm pore size, 47 mm diameter Whatman

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Nuclepore Track-Etched Membrane filter (see Supplemental Information Table S1 for volumes filtered). Samples were stored at -80ºC until analysis. At least one quarter of each filter was cut with a sterile razor blade and used for DNA extraction. Analysis of sample duplicates (i.e., pieces of different filters from the same sample) and/or filter duplicates (duplicate quarters of a single filter) were also analyzed for a select number of samples. DNA was extracted using a DNeasy PowerSoil Kit (Qiagen) according to manufacturer protocol. The 16S rRNA gene was sequenced at the UNC Core Microbiome Facility with Illumina MiSeq PE 2x250. Amplification of the hypervariable regions V1 and V2 of the 16s rRNA gene was conducted using the 8F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 338R (5'-GC TGC CTC CCG TAG GAG T-3') primers with the Illumina-specific forward primer overhang adapter (5'- TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG-3') and reverse overhang adapter (5'- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G-3').

Sequences were imported into QIIME2 for analysis (version 2017.12; https://qiime2.org; Bolyen et al., 2019), where, after demultiplexing, primers were trimmed using cutadapt (Martin, 2011) and denoising, dereplicating, filtering of chimeras and singletons, and merging of paired end reads was conducted using DADA2 (Callahan et al., 2016). OTUs were clustered and picked *de-novo* at a sequence similarity of 97% using vsearch (Rognes et al., 2016). Taxonomy was assigned using a Naïve Bayes classifier that was trained using reference sequences with the 8F and 338R primers from the Silva database (version 128; Pruesse et al., 2007). Chloroplasts were removed, and samples were rarefied to an even sampling depth of 10,810 sequences using the phyloseq package in R (version 1.19.1; McMurdie & Holmes, 2013). Raw sequence files can be accessed on the NCBI Sequence Read Archive under the accession number PRJNA480640.

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2.6. Statistical analyses

To test for differences in bacterial productivity, bacterial abundance, polysaccharide hydrolase activities, and peptidase and glucosidase activities between different stations, depths, and treatments over time, ANOVA was performed using the nlme package (version 3.1-131; Pinheiro et al., 2018) in R (R Core Team, 2017). Bacterial abundance, richness, and evenness were log transformed, while bacterial productivity and all enzymatic activities were transformed according to ln(x+0.5), in order to meet the assumptions of an ANOVA. In each test, station, depth, treatment, and timepoint were considered fixed variables, and mesocosm was considered a random variable. According to ANOVAs, the four-way interaction (between station, depth, treatment, and timepoint) was not significant for bacterial productivity, bacterial abundance, or any of the enzymatic activities, and these were therefore excluded from the ANOVA test.

Differences in bacterial community composition were visualized using non-metric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarity index using the phyloseq package in R (version 1.19.1; McMurdie & Holmes, 2013). To test for differences in bacterial community composition between groups, PERMANOVAs were conducted using the adonis function (vegan) (version 2.4-6; Oksanen et al., 2018) in R. Estimates of richness and evenness of bacterial community composition were calculated in R using the estimate richness function in the phyloseq package (version 1.19.1; McMurdie & Holmes, 2013). To examine the correlation between enzymatic activities and bacterial community composition, Mantel tests using the Pearson correlation method were conducted on the Bray-Curtis dissimilarity index of bacterial community composition and a Euclidean distance matrix computed from peptidase and glucosidase activities using the vegan package in R (version 2.4-6; Oksanen et al., 2018).

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3. Results

3.1. Water mass characteristics

Our two stations in the western North Atlantic Ocean, separated by a distance of 1370 km, included the same water masses at each depth, based on temperature and salinity characteristics: epipelagic water at both stations was North Atlantic Surface Water, water from the mesopelagic (850 m at Stn. 12; 875 m at Stn. 16) was North Atlantic Central Water, and bathypelagic water at both stations was North Atlantic Deep Water (Talley, 2011; Table 1; Supplemental Information Fig. S1, S2).

3.2. Peptidase and glucosidase activities

Peptidases hydrolyze peptides and proteins to smaller substrates, whereas glucosidases hydrolyze terminal glucose units from larger molecules. Since peptidase and glucosidase activities differed by depth, but not by station in both amended and unamended mesocosms (ANOVA, p=0.2424), in the following sections, data from the same depths at the two stations are presented together, yielding two unamended and six amended mesocosms per depth. Data from individual mesocosms are presented in supplemental figures.

In unamended epipelagic water mesocosms, all seven peptidase and glucosidase substrates were hydrolyzed at rates higher than in the mesopelagic or bathypelagic waters (Fig. 1; Supplemental Information Fig. S3); in epipelagic waters, endopeptidase activities were also a higher fraction of summed activity than at other depths (Supplemental Information Fig. S3). Bathypelagic waters were dominated by leucine-MCA and AAF-chym activities; no AAPF-chym activity was detected (Fig. 1; Supplemental Information Fig. S3).

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Addition of HMW organic matter led to a substantial increase in the rates and spectrum of peptidase and glucosidase activities (Fig. 1) (ANOVA, $p<0.0001$). The responses of the six amended mesocosms per depth, drawn from six different Niskin bottles across two different stations, were very similar (Supplemental Information Fig. S3). The timing of these responses to added organic matter varied with depth, however. At t0, immediately after HMW organic matter addition, hydrolysis rates and patterns in amended mesocosms were similar to the unamended mesocosms. By the 2 d timepoint, activities had increased by approximately an order of magnitude in the epipelagic mesocosms, and the hydrolysis rates of the substrates became more even. In amended mesopelagic mesocosms, after 2 d all activities were approximately a factor of 5 greater than in unamended mesocosms. However, a shift in enzymatic response to HMW organic matter amendment, defined as the point at which the rates and spectrum of peptidase and glucosidase activities increased significantly, in bathypelagic mesocosms was not observed until 7 d post-addition (Fig. 1; Supplemental Information Fig. S3).

The stimulation of specific peptidase and glucosidase activities in amended mesocosms also differed by depth (Fig. 1) (ANOVA, $p < 0.0001$). The amended epipelagic water mesocosms showed particularly high α- and β-glucosidase activities, which accounted for 4% to 47% of the total summed hydrolysis rates, and relatively even peptidase activities (Fig. 1). In contrast, α- and β-glucosidase activities accounted for, at most, ~12% of the total summed hydrolysis rates in mesopelagic mesocosms and only 3.3% of the total summed hydrolysis rates in bathypelagic mesocosms. Mesopelagic and bathypelagic mesocosms were typically dominated by high leucine aminopeptidase activities, although this was most notable in bathypelagic mesocosms (Fig. 1).

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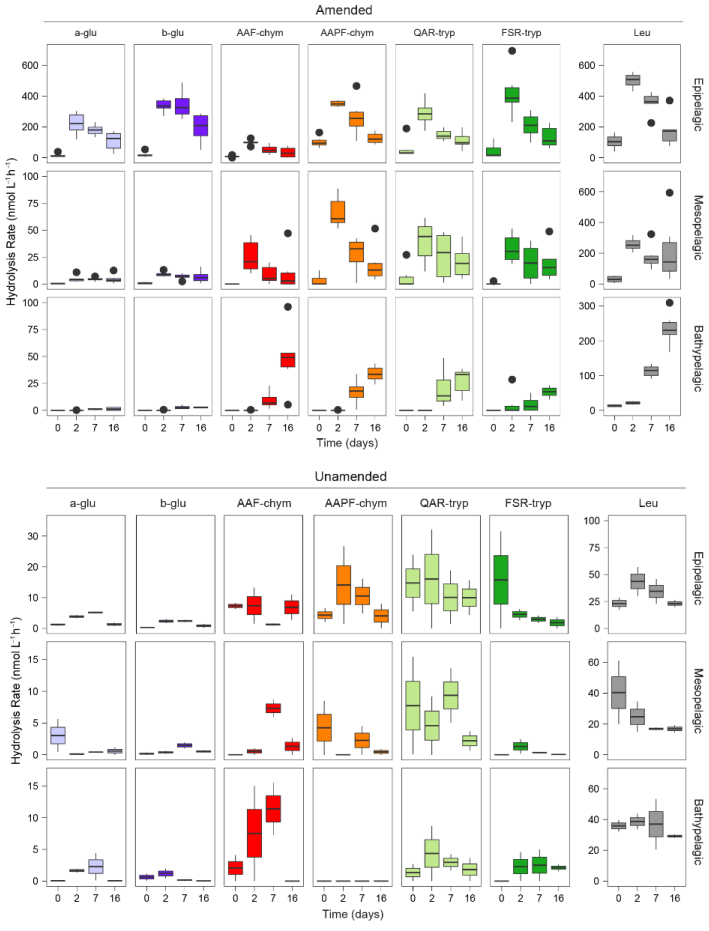

Figure 1. *Peptidase and glucosidase activities in amended and unamended mesocosms 0, 2, 7, and 16 d after the addition of HMW organic matter to amended mesocosms. Rates are an average between the two stations. Note the difference in scales between the amended and unamended mesocosms.*

3.4. Polysaccharide hydrolase activities

Only a limited range of polysaccharides were hydrolyzed in unamended mesocosms. Laminarin and pullulan were hydrolyzed at all depths and stations. Fucoidan and

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arabinogalactan, however, were not hydrolyzed at any depth or station in unamended mesocosms (Fig. 2; Supplemental Information Fig. S4). Chondroitin was also hydrolyzed in Stn. 16 mesopelagic and bathypelagic mesocosms, and xylan was hydrolyzed in Stn. 16 epipelagic and mesopelagic mesocosms of both stations (Fig. 2; Supplemental Information Fig. S4). The spectrum of polysaccharides hydrolase activities was therefore broadest in the mesopelagic. The time course over which individual polysaccharides were hydrolyzed also varied by substrate and depth. In unamended epipelagic and mesopelagic mesocosms, laminarinase activities were generally high at the 2 d timepoint; pullulanase activities were detected slightly later, except in Stn. 16 mesopelagic mesocosms, where pullulanase activity was also measurable at 2 d. Xylanase activity was measurable at 2 d (Stn. 12) or at 5 d (Stn. 16) in mesopelagic mesocosms; chondroitin hydrolysis was measurable starting at 5 d at Stn. 16. The unamended bathypelagic mesocosms showed slightly different patterns, with polysaccharide hydrolase activity detectable starting at 2 d at Stn. 16, but only detectable at 17 d at Stn. 12 (Supplemental Information Fig. S4).

Polysaccharide hydrolase activities were significantly different between amended and unamended mesocosms (ANOVA, $p < 0.0001$). Addition of HMW organic matter increased the rates, broadened the spectrum, and changed the timepoint at which polysaccharide hydrolase activities were first detected (Fig. 2). All six polysaccharide hydrolase activities were measurable in epipelagic and mesopelagic mesocosms, with the initial timepoint of detection typically at 2 d or 5 d. In amended bathypelagic mesocosms, pullulan, laminarin, and chondroitin hydrolysis were typically initially measured at the 2 d or 5 d timepoints; xylanase activity was usually only detected at the later (17 d or 30 d) timepoints. Maximum polysaccharide hydrolase activities were significantly lower in amended bathypelagic mesocosms (summed activities lower than 20

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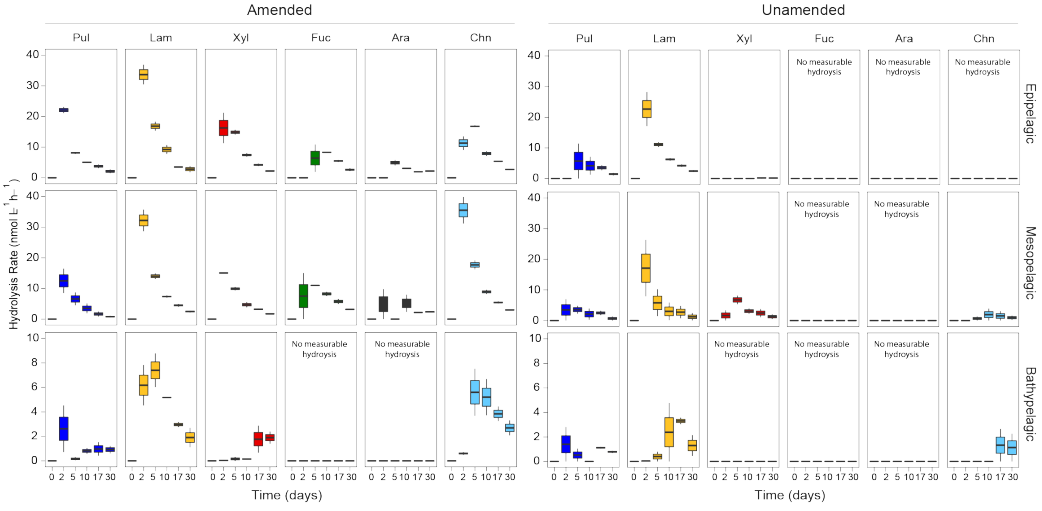
304 nmol L^{-1} h^{-1}) than in epipelagic and mesopelagic mesocosms (summed activities at or above 50
305 nmol L^{-1} h^{-1}) (ANOVA, $p < 0.0001$) (Supplemental Information Fig. S5). In amended
306 mesocosms, stations did not differ significantly in either the maximum rates (ANOVA, $p =$
307 0.8401) or the temporal development of polysaccharide hydrolase activities (ANOVA, $p =$
308 0.7502).



309

310 ***Figure 2.*** *Polysaccharide hydrolase activities in amended and unamended mesocosms, averaged*
311 *between stations. Note that the 0 d timepoint, when fluorescently-labeled polysaccharide*
312 *incubations began, was 15 d after the addition of HMW-OM to amended mesocosms. Pul =*
313 *pullulan, Lam = laminarin, Xyl = xylan, Fuc = fucoidan, Ara = arabinogalactan, Chn =*
314 *chondroitin.*

315

316 ***3.5. Bacterial protein production***

317 In amended epipelagic mesocosms, bacterial protein production was high immediately
318 after addition of HMW organic matter (ca. 150 pM h^{-1}), and decreased at subsequent time points
319 (Supplemental Information Fig. S6a). In unamended epipelagic mesocosms, bacterial protein

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production was initially low, but increased from 20.6 pM h^{-1} in Stn. 12 mesocosms and 99.3 pM h^{-1} in Stn. 16 mesocosms to rates above 150 pM h^{-1} at subsequent timepoints. In mesopelagic mesocosms, bacterial protein production increased in both the amended and unamended mesocosms from very low initial levels to much higher levels at later timepoints. In Stn. 16 mesocosms, bacterial protein production increased with time in both the amended and unamended mesocosms; in Stn. 12 mesocosms, the patterns were less clear (Supplemental Information Fig. S6a). Bathypelagic amended mesocosms showed detectable bacterial protein production at earlier timepoints than unamended mesocosms, with rates above 50 pM h^{-1} at 7 d. Bacterial protein production varied significantly between mesocosms amended with HMW organic matter and unamended mesocosms (ANOVA, $p = 0.0054$), with changes in protein production displaying distinct trends with depth (ANOVA, $p < 0.0001$).

3.6. Bacterial community composition

Initial bacterial communities in epipelagic unamended and amended mesocosms were dominated by *Alphaproteobacteria*, *Cyanobacteria*, *Flavobacteriia*, and *Gammaproteobacteria,* as demonstrated by relative read abundance (Fig. 3; Supplemental Information Fig. S7). In meso- and bathypelagic mesocosms, *Alphaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Flavobacteriia*, and the SAR202 clade dominated initial bacterial communities, although these classes were present in different relative proportions in mesopelagic and bathypelagic mesocosms (Fig. 3; Supplemental Information Fig. S7). As expected, initial (t0) bacterial communities in amended and unamended mesocosms were not significantly different from one another (PERMANOVA, $R^2 = 0.02016$, $p = 0.655$). Distinct bacterial communities were present in mesocosms from different depths (Fig. 4), and depth explained the

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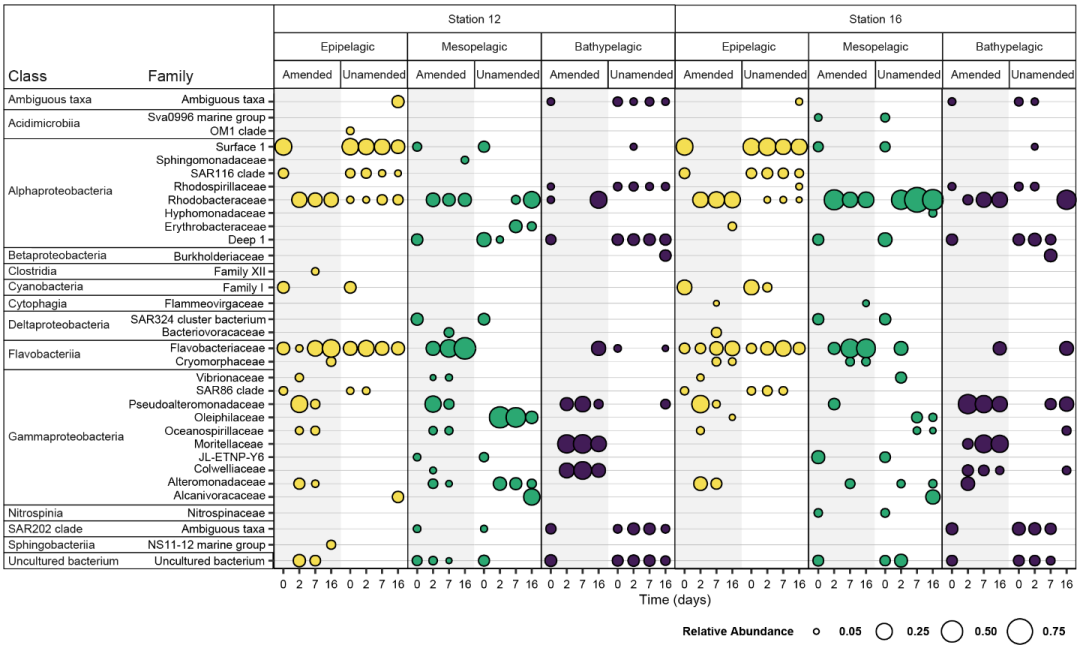
343 greatest amount of dissimilarity between bacterial communities (PERMANOVA, $R^2 = 0.1968$, p
344 $= 0.001$). Samples from comparable depths clustered together, regardless of their station of origin
345 (shown by non-metric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarity
346 index; Fig. 4). However, samples collected from the same water masses but at different stations
347 still displayed differences in community composition that were statistically significant
348 (PERMANOVA, $R^2 = 0.0190$, $p = 0.001$); we have therefore graphed community composition at
349 each station separately (Fig. 3; Supplemental Information Fig. S7). Duplicate filters, both sample
350 duplicates and filter duplicates from the same depth and station, displayed consistent
351 reproducibility of bacterial community composition: hierarchical clustering showed that
352 duplicates clustered closest together, except in a single case (Supplemental Information Fig. S8).



353

354 ***Figure 3.*** *Bacterial families with a normalized relative abundance of 5% or more in amended*
355 *and unamended mesocosms from each station and depth 0, 2, 7, and 16 d after the addition of*

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HMW organic matter to amended mesocosms. Amended values are an average of triplicate amended mesocosms. Bubble size indicates the relative abundance (%) of each bacterial family.

Addition of HMW organic matter resulted in distinct shifts in bacterial community composition, which were evident starting at 2 d (Figs. 3, 4; Supplemental Information Fig. S7). While initial bacterial communities from the mesopelagic were more compositionally and phylogenetically similar to bathypelagic communities (Supplemental Information Figs. S7, S9), after HMW organic matter addition, mesopelagic communities progressively became more similar to bacterial communities in epipelagic mesocosms. This shift was the result of similar increases in the relative proportions of *Gammaproteobacteria* and *Flavobacteriia*, and a decrease in the relative proportion of *Alphaproteobacteria*, in amended epipelagic and mesopelagic mesocosms (Fig. 3; Supplemental Information Fig. S7). Relative read abundances for *Flavobacteriia* and *Alphaproteobacteria* in bathypelagic mesocosms were notably lower than in epipelagic and mesopelagic mesocosms, while *Gammaproteobacteria* relative read abundance was higher. The composition of bacterial communities in unamended mesocosms also shifted with time, showing evidence of a 'bottle effect'. However, these compositional shifts were observed to a lesser extent (Fig. 3; Supplemental Information Fig. S7) and, especially for bathypelagic mesocosms, occurred at later time points than in their amended counterparts (Fig. 3; Supplemental Information Fig. S7).

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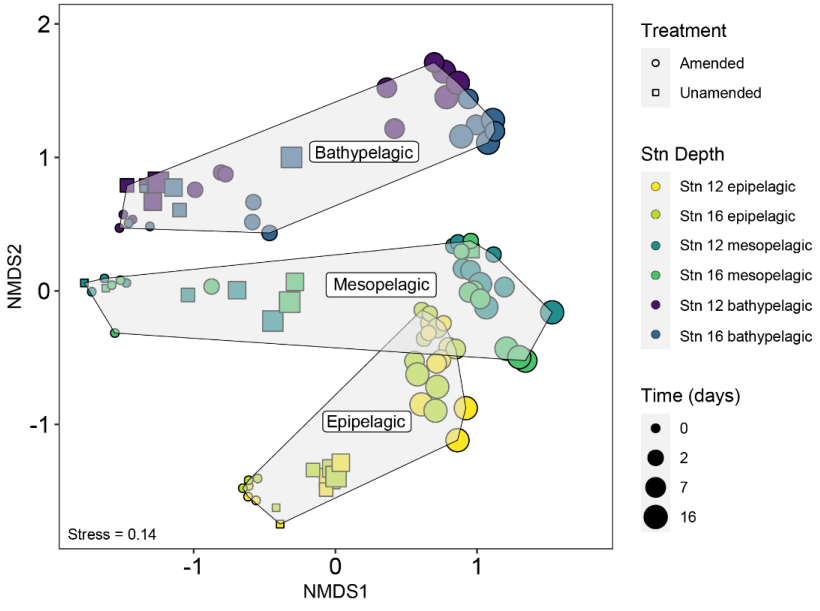

Figure 4. *Non-metric multidimensional scaling (NMDS) plot of bacterial community composition based on the Bray-Curtis dissimilarity index.*

Within the *Gammaproteobacteria*, families that became abundant after addition of HMW organic matter were initially present in very low relative proportions and, in some cases, were not detected during initial timepoints (Supplemental Information Fig. S10). The Gammaproteobacterial families with highest read abundance varied with depth, with taxa in the *Alteromonadaceae*, *Colwelliaceae*, *Oceanospirillaceae*, *Pseudoalteromonadaceae*, and *Vibrionaceae* families becoming abundant in epipelagic and mesopelagic mesocosms, while bathypelagic mesocosms were dominated by taxa in the *Alteromonadaceae*, *Colwelliaceae*, *Moritellaceae*, and *Pseudoalteromonadaceae* families (Supplemental Information Fig. S10). Although members of the *Alteromonadaceae*, *Colwelliaceae*, and *Pseudoalteromonadaceae* were present in amended mesocosms from all depths, their relative proportion varied with depth:

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the highest relative proportion of *Alteromonadaceae* typically occurred in epipelagic and mesopelagic mesocosms, while *Colwelliaceae* were most abundant in bathypelagic mesocosms; the relative proportion of *Pseudoalteromonadaceae* did not follow a consistent depth-related pattern (Supplemental Information Fig. S10). In some cases, a single genus or OTU within these families became abundant (Supplemental Information Fig. S11, S12); for example, in the *Colwelliaceae* family, an OTU in the genus *Colwellia* became abundant in all amended mesocosms (Supplemental Information Fig. S13).

The presence of these gammaproteobacterial families in amended mesocosms contrasts sharply with the dominant families in unamended mesocosms: in epipelagic water unamended mesocosms, the SAR86 clade remained the dominant taxa throughout most timepoints, while the proportion of *Pseudoalteromonadaceae* and *Oceanospirillaceae* remained low. In unamended bathypelagic mesocosms, taxa that were initially highly abundant, such as the *Salinisphaeraceae*, also typically comprised a large proportion of all taxa during subsequent timepoints (Supplemental Information Fig. S10). However, in unamended mesopelagic mesocosms, relative gammaproteobacterial read abundance shifted considerably after the initial timepoint. The taxa that became abundant were present, albeit at low relative abundances, during initial timepoints, and the patterns in the abundance of these taxa differed between stations.

While distinct families in the *Gammaproteobacteria* became abundant in water from different depths, the same families in the *Alphaproteobacteria* and *Flavobacteriia* became dominant in all amended mesocosms after t0, regardless of depth. Within the *Flavobacteriia*, members of the *Flavobacteriaceae* family increased in relative read abundance in all mesocosms after t0, although they were generally less abundant in bathypelagic mesocosms than in epipelagic and mesopelagic mesocosms (Supplemental Information Fig. S14). The relative

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proportion of other *Flavobacteriia* families, including the *Cryomorphaceae* and the NS9 Marine Group, decreased over time. The same trend was observed in all unamended mesocosms (Supplemental Information Fig. S14). Although the *Flavobacteriaceae* family became abundant in all amended mesocosms, the dominant *Flavobacteriaceae* genera varied greatly with depth (Supplemental Information Fig. S15). For example, *Polaribacter 4*, although present to some degree in all amended mesocosms, was the most abundant *Flavobacteriaceae* genera in bathypelagic mesocosms, while *Tenacibaculum* was more abundant in epipelagic and mesopelagic mesocosms (Supplemental Information Fig. S15). In the *Alphaproteobacteria*, the *Rhodobacteraceae* became dominant in all amended mesocosms, despite only making up a low relative proportion of *Alphaproteobacteria* families initially (Supplemental Information Fig S16). A shift in the relative proportions of Alphaproteobacterial families occurred after 2 d in epipelagic and mesopelagic mesocosms, and after 7 d in bathypelagic mesocosms, when the *Rhodobacteraceae* became the dominant Alphaproteobacterial family (Supplemental Information Fig. S16). Within the *Rhodobacteraceae* family, bacteria in the genus *Sulfitobacter* became particularly abundant in all amended mesocosms (Supplemental Information Fig. S17).

Bacterial richness, evenness, and Shannon diversity typically decreased in all amended mesocosms 2 d after the addition of HMW organic matter; this decrease was greatest in water from the meso- and bathypelagic (Supplemental Information Fig. S8). However, in epipelagic and mesopelagic mesocosms, these decreases in diversity were not significantly different from those in unamended mesocosms (ANOVA, $p>0.05$ for all, Supplemental Information Table S2). Only in bathypelagic mesocosms were changes in richness, evenness, and diversity significantly different between amended and unamended mesocosms (ANOVA, $p<0.005$, Supplemental Information Table S2): while richness, evenness, and diversity in amended bathypelagic

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mesocosms had decreased significantly 2 d after the addition of HMW organic matter, the decrease in these parameters in unamended mesocosms was more gradual, and in some cases even increased or remained relatively consistent) (Supplemental Information Fig. S8).

3.7. Relating community composition to enzymatic activities

Successional patterns in bacterial community composition were concurrent with functional changes in enzymatic capabilities. Notable changes in peptidase and glucosidase activities in epipelagic and mesopelagic mesocosms at the 2 d timepoint corresponded with a major shift in bacterial community composition (Figs. 1, 4), with *Gammaproteobacteria* and *Flavobacteriia* becoming highly abundant (Fig. 3). In bathypelagic mesocosms, a shift of similar magnitude was measured 7 d after the addition of HMW organic matter (Fig. 4). Since the unamended mesocosms did not show comparable shifts in enzyme activities or community composition, a major driver of the shifts in amended mesocosms was most likely the addition of HMW organic matter, rather than bottle effects. The relationship between bacterial community composition and peptidase and glucosidase activity in amended mesocosms after 2 d was stronger and of greater significance (Mantel $R = 0.1483$, $p = 0.003$) than in unamended mesocosms and amended mesocosms at t0, when the relationship between bacterial community composition and peptidase and glucosidase activity was not significant Mantel $R = 0.0080$, $p = 0.356$).

4. Discussion

Distinct depth-related differences in enzyme function and community composition characterized the epipelagic, mesopelagic, and bathypelagic mesocosms: with increasing depth,

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458 the rates and/or spectrum of substrates hydrolyzed decreased, consistent with previous
459 measurements of enzymatic activities in unamended water (Hoarfrost & Arnosti, 2017; Balmonte
460 et al., 2018, Hoarfrost et al., 2019). Addition of HMW organic matter, however, revealed depth-
461 related responses that were not evident in the unamended mesocosms: not only the rates, but also
462 the spectrum of enzyme activities broadened in amended mesocosms, but the extent to which this
463 broader spectrum was measurable varied by depth, with the amended bathypelagic microbial
464 communities in particular showing a less-broad response than their mesopelagic and epipelagic
465 counterparts (Fig. 5).

466 These depth-related functional differences were similar between comparable depths at
467 two stations separated by 1370 km (Supplemental Information Fig. S3, S4). Furthermore, the
468 patterns were also very similar to those measured in epipelagic and bathypelagic mesocosms
469 amended with the same HMW organic matter at a different station in the western North Atlantic
470 Ocean during the previous year (Balmonte et al. 2019; Stn. 8, water depth 4574 m; Supplemental
471 Information Fig. S1). These results suggest that the response of bacterial communities to an input
472 of HMW organic matter – both in terms of community composition and enzymatic function –
473 varies far more by depth than location, and may be predictable over a regional scale within
474 individual water masses at similar depths.

475 These robust enzymatic patterns may also indicate the 'value' of specific substrates to a
476 microbial community in each depth zone. Amendment of epipelagic waters, for example, led to
477 very high α- and β-glucosidase activities, which were higher by several orders of magnitude than
478 rates typically measured in unamended epipelagic waters (Baltar et al., 2009; 2010; Hoarfrost &
479 Arnosti, 2017; Hoarfrost et al., 2019). This pattern suggests that removal of terminal glucose
480 units, whether from polysaccharides, glycosylated proteins, or lipids, may be a critical first step

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in accessing the complex structures 'underneath' (Fig. 5). This feature was also evident during the previous year in epipelagic mesocosms containing water from Stn. 8 (Supplemental Information Fig. S1; Balmonte et al. 2019). Initial removal of terminal glucose and leucine from a HMW substrate may in effect clear the way for activities of a broad range of endopeptidases and polysaccharide hydrolases, which cleave structures mid-chain. The rapid response of the epipelagic community across the entire range of enzyme activities, as well as the high hydrolysis rates triggered by substrate addition, may reflect the fact that microbial communities in epipelagic waters are frequently exposed to freshly produced phytoplankton-derived organic matter rich in amino acids and carbohydrates (Benner et al., 1997; Wakeham et al., 1997; Kaiser & Benner, 2012).

The bacterial taxa in epipelagic mesocosms that responded to the addition of HMW organic matter by the 2 d timepoint, in particular increases in the abundance of *Rhodobacteraceae* (*Alphaproteobacteria*) and *Pseudoalteromonadaceae* (*Gammaproteobacteria*) (Fig. 3), also reflect changes in community composition seen in the ocean: bacterial taxa within these classes are usually rare or present in low abundances in seawater, but respond rapidly to seasonal changes in organic matter abundance, such as during phytoplankton blooms (Teeling et al., 2012; Fuhrman et al., 2015; Avci et al., 2020). We hypothesize that the *Rhodobacteraceae* in particular were likely responding to an increase in low molecular weight substrates that could have been released through the activities of the glucosidases and peptidases, enzymes potentially produced by *Pseudoalteromonadaceae* (Alonso-Sáez et al., 2012; Li et al., 2018).

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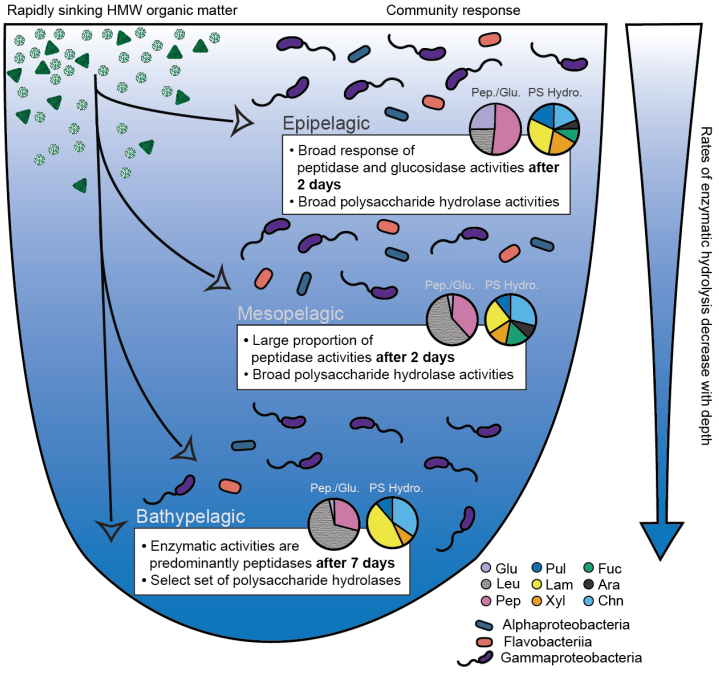

Figure 5. *A diagram illustrating the response of bacterial communities in North Atlantic epipelagic, mesopelagic, and bathypelagic waters to an input of rapidly sinking, fresh, HMW organic matter. Community responses depicted here are the timepoint at which significant responses to the input of HMW organic matter was measured (2 d in epipelagic and mesopelagic incubations, and 7 d in bathypelagic incubations); polysaccharide hydrolase activities are an average of all timepoints. Pie charts show the relative contributions of peptidases and glucosidases (labeled as Pep./Glu.) and polysaccharide hydrolases (labeled as PS Hydro). Glu = α and β glucosidases, Leu = leucine aminopeptidases, Pep = trypsin and chymotrypsin activities, Pul = pullulanases, Lam = laminarinases, Xyl = xylanases, Fuc = fucoidanases, Ara = arabinogalactanases, Chn = chondroitin sulfate hydrolases. The relative abundance of bacterial communities is illustrated by the three major classes of bacteria that responded to HMW organic matter input (*Alphaproteobacteria*, *Flavobacteria*, and* Gammaproteobacteria*).*

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In mesopelagic mesocosms, the addition of HMW organic matter also produced a broad enzymatic response, consistent with observations of considerable organic matter transformation in this zone (Wakeham et al., 1997; Benner & Amon, 2015). However, mesopelagic mesocosms showed a less robust glucosidase response than epipelagic mesocosms, and were dominated by leucine aminopeptidase, an exo-peptidase activity that may integrate the activities of a range of exopeptidases (Steen et al., 2015) (Fig. 5). Enhancement of rates of peptidase and glucosidase activities were considerably lower than in the epipelagic zone (Fig. 1). The similarities in polysaccharide hydrolase activities in the epipelagic and mesopelagic mesocosms (Fig. 2) may be due in part to convergent trends in bacterial community composition over time (Fig. 4; Supplemental Information S9), since the polysaccharide hydrolase measurements tested the enzymatic responses of microbial communities after they had considerable time to react to substrate addition (Fig. 5). Together, this evidence suggests that mesopelagic communities can respond rapidly to inputs of fresh HMW organic matter, though the rate and spectrum of enzymatic activities differs from those in the epipelagic zone (Fig. 5).

The bathypelagic microbial communities responded in a markedly more limited manner. The spectrum of enzyme activities was less broad, and the increases in the rate of enzymatic activities were less substantial and occurred over longer timescales than in epipelagic and mesopelagic communities (Figs. 1, 2). The differences in response times between the amended epipelagic and mesopelagic communities relative to bathypelagic communities may be related in part to differences in temperature between the incubations (Table 1), as well as to the initial size and activity of the heterotrophic bacterial populations. The observation that some enzyme activities were detectable only in amended mesocosms, however, and others were not detectable

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at all over the entire time course of the experiments (Supplemental Fig. S6a) suggests that the absence of some enzyme activities in bathypelagic samples is not simply a function of temperature.

In bathypelagic mesocosms, increases in bacterial protein production (Supplemental Fig. S6a) and the rates of peptidase and glucosidase activities (Fig. 1) at the 7 d timepoint also corresponded with a shift in bacterial community composition equivalent to that in epipelagic and mesopelagic mesocosms after 2 d (Fig. 4). The substantial decrease in bacterial diversity, richness, and evenness that coincided with this shift in bacterial community composition was likely a result of selection for bacteria capable of degrading HMW organic matter. This shift in community composition resulted in increases in the abundance of *Moritellaceae* (*Gammaproteobacteria*), *Colwelliaceae* (*Gammaproteobacteria*), *Pseudoalteromonadaceae* (*Gammaproteobacteria*), and *Rhodobacteraceae* (*Alphaproteobacteria*), families that had previously been observed responding to the same HMW organic matter in bathypelagic mesocosms (Balmonte et al., 2019). Although these families were present in epipelagic and mesopelagic mesocosms, their response and abundance differed greatly from that in bathypelagic mesocosms, where they became the dominant taxa after the addition of HMW organic matter (Fig. 3). This variability of community composition with depth may account for some of the strong functional differences we observed between distinct bacterial communities, as the ability to produce the enzymes necessary to degrade HMW organic matter varies widely among different bacterial taxa, even those that are closely related (Xing et al., 2015; Saw et al., 2020; Avci et al., 2020).

The narrower spectrum of polysaccharide hydrolase activities measured in amended bathypelagic mesocosms suggests that the enzymatic investment to hydrolyze some of the

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complex structures that were degraded at shallower depths is not likely to pay off for bathypelagic microbial communities. At Stns. 12 and 16, neither fucoidan or arabinogalactan were hydrolyzed, consistent with results from bathypelagic enzyme activities in amended mesocosms from Stn. 8 (Fig. 2; Balmonte et al., 2019). This limited spectrum of extracellular enzymatic activities may reflect their energetic cost-benefit balance – either the cost of enzymatic expression is too high, or the frequency of encounter with specific substrates may be too low, to justify 'investment' in the tools needed to hydrolyze certain structures. As the production of extracellular enzymes is profitable only when the return on investment is sufficient (e.g., Traving et al. 2015), the lack of measurable enzyme activities to degrade fucoidan and arabinogalactan in bathypelagic waters, for example, suggests that these substrates may not be available in sufficiently high concentrations for production of the required enzymes to pay off. However, the four polysaccharides that were hydrolyzed in amended bottom water mesocosms represent a breadth of polysaccharide hydrolase activities not typically measurable in bulk incubations of bathypelagic water (Fig. 5; Hoarfrost & Arnosti, 2017; Balmonte et al., 2018). In any case, the enhanced capability of amended bathypelagic microbial communities to degrade complex polysaccharides suggests that an influx of fresh organic matter to the bathypelagic (Broeck et al., 2020; Poff et al., 2021) may fuel growth of a select set of organisms enzymatically equipped to take advantage of this resource (Fig. 5).

Our measurements of depth-related differences in enzymatic activities between distinct microbial communities from the epipelagic, mesopelagic, and bathypelagic in response to an addition of the same HMW organic matter were consistent over a regional scale. They imply that the structure of HMW organic matter, its residence time at specific depths in the water column, and the distinct enzymatic capabilities of heterotrophic microbial communities at these depths,

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are key factors controlling its ultimate fate. Changing oceanic conditions in coming decades have the potential to affect all of these factors, driving further changes in the marine carbon cycle.

Data availability

Raw data for peptidase, glucosidase, and polysaccharide hydrolase activities is available through BCO-DMO (https://www.bco-dmo.org/project/712359).

Author contributions

CA designed the experiments and coordinated work at sea. SB, AH, JB, and SG collected and processed samples at sea and ashore. SB extracted DNA, processed and analyzed the sequence data, and conducted statistical analyses. SB and CA analyzed results, wrote the manuscript, and revised it with input from all co-authors.

Competing interests

The authors declare that they have no conflict of interest.

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