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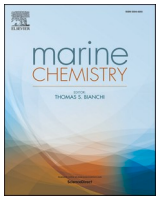

Links between regional and depth patterns of microbial communities and enzyme activities in the western North Atlantic Ocean

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ABSTRACT

Heterotrophic bacteria process much of the organic matter produced by phytoplankton in the ocean. A large proportion of this organic matter is in the form of polysaccharides, structurally complex, high molecular weight sugars. To consume this complex organic matter, microbes must initially produce enzymes of the correct structural specificity to transform it to smaller pieces that can be brought into the cell. The extent to which bacteria can hydrolyze organic matter determines how much carbon is transformed by a given microbial community. Because microbial community composition differs with location and depth in the ocean, quantifying their enzymatic potential at different stations and depths is essential to reveal patterns in microbial functional capabilities. To investigate links between patterns of bacterial composition and function, we assessed the bacterial community composition and measured glucosidase, peptidase, and polysaccharide hydrolase activities throughout the water column at 16 stations in the western North Atlantic. We found that bacterial community composition and polysaccharide hydrolase activities were depth stratified and showed regional variability, while glucosidase and peptidase activities were more similar among locations and depths. These findings suggest that polysaccharide hydrolase activities are expressed by a narrower range of organisms within bacterial communities, while the abilities to degrade peptides occurs more widely among community members, likely due to the broader substrate specificity for proteins compared to polysaccharides. The data and findings presented here highlight the extent to which patterns of microbial community composition and function and the physical oceanography of the western North Atlantic are interwoven and contribute to the overall transformation of carbon in the ocean.

1. Introduction

Much of the efficient recycling of phytoplankton-derived organic matter in the ocean is due to heterotrophic microbial communities, which consume, transform, excrete, and respire an estimated half of autochthonously-produced organic matter (Azam et al., 1993; Azam and Malfatti, 2007). The depths and rates at which this organic matter is respired and transformed affects the major biogeochemical cycles of carbon and nutrients, which help determine the distribution of heterotrophic life in the ocean (Hutchins and Fu, 2017). In order to consume complex organic matter, however, heterotrophic microbes must produce extracellular enzymes of the correct structural specificity to hydrolyze these compounds to sizes that can be taken up (Arnosti, 2011). The combined enzymatic capabilities of a community are therefore an important determinant of substrate availability; however, these enzymes are not uniformly distributed among members of microbial communities. Whereas some members possess the capability to produce extracellular enzymes to break down high molecular weight compounds, others can target only low molecular weight substrates which, in some cases, are the hydrolysis products of the extracellular enzymes (Allison, 2005; Arnosti et al., 2018). Among extracellular-enzyme producing organisms, moreover, the enzymatic capabilities vary considerably, even among closely-related bacteria (Xing et al., 2015; Liu and Liu, 2020; Avci et al., 2020).

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Although our knowledge of the composition of microbial communities and their genetic potential to produce extracellular enzymes has increased remarkably in the last decade (Zhao et al., 2020; Teeling et al., 2012, 2016), we still have comparatively less information about the potential rates at which these enzymes hydrolyze their target substrates in ocean waters. Most measurements of enzyme activities in the ocean rely on small substrate proxies that assess the activities of a few *exo*-acting (terminal unit cleaving) enzymes (Baltar et al., 2009), providing no information on the *endo*-acting enzymes that cleave their substrate mid-chain. This distinction, however, is important: there are considerable differences in activities among *exo*- and endo-acting peptidases (Obayashi and Suzuki, 2005, 2008; Balmonte et al., 2018a, 2021) and among endo-active enzymes hydrolyzing different polysaccharides in the ocean (Arnosti et al., 2011; Hoarfrost and Arnosti, 2017; Balmonte et al., 2021).

Our knowledge of the rates at which exo-acting enzyme function in the ocean vary with depth is also limited because, with a few exceptions (e.g., Baltar et al., 2009, 2010; Liu et al., 2018), the majority of measurements of enzyme activities have been made in epipelagic waters. However, there is evidence of important functional differences among microbial communities with depth in the ocean (Liu et al., 2018; Sala et al., 2020). The few investigations of endopeptidase and polysaccharide hydrolase activities in mesopelagic and bathypelagic waters have found notable differences in rates and in the spectrum (range) of substrates hydrolyzed with depth and with location (Steen et al., 2012; Hoarfrost and Arnosti, 2017; Balmonte et al., 2018a, 2021; Giljan et al., 2023). In sum, our understanding of the initial step of microbially-driven carbon cycling–especially in the mesopelagic and bathypelagic ocean–is still incomplete due in part to a paucity of rate measurements.

In order to gain a picture of the enzymatic capabilities of microbial communities—and variations in these capabilities—on a regional scale, we investigated depth- and spatial-variability in polysaccharide hydrolase and peptidase activities at 16 stations in the western North Atlantic Ocean. The western North Atlantic Ocean, dominated by the Gulf Stream flowing northeastward juxtaposing the southward flowing water from the Scotian Shelf, has a variety of water mass interactions (Heidrich and Todd, 2020), making it an ideal location to study regional differences in enzymatic activity. Our measurements encompass two of the compound classes – polysaccharides and proteins – that make up a large fraction of marine organic matter (Benner et al., 1992; Hedges et al., 2002). We concurrently investigated microbial community composition at the same stations and depths to assess the relationships between patterns of community composition and function. Few previous studies (Balmonte et al., 2018a) have combined an assessment of community composition with measurements of carbon cycling in the form of a considerable range of polysaccharide hydrolase and peptidase activities in depth gradients from the surface to the mesopelagic and deep ocean. Because extracellular enzymes act as the initial gatekeepers for processing high molecular weight organic matter (Arnosti, 2011), measuring enzyme activities in the ocean and characterizing the microbial communities that produce them is essential to quantify the heterotrophic microbial contributions to the marine carbon cycle.

2. Methods

2.1. Water sampling

Water samples were collected during two cruises aboard the R/V *Endeavor* in the western North Atlantic. Samples were collected at four stations (Stns. 1–4) on the continental shelf and four offshore stations (Stns. 5–8) during April 27–May 2, 2015 (EN556), and at 8 stations generally along an *E*-W transect at 36° N, including three continental shelf or shelf-break stations (Stns. 9–11) and six open ocean stations (Stns. 12–16; EN584; June 29–July 11, 2016). Additionally, during the cruise in 2016, Stn. 12 was re-occupied 6 days after the first sampling and is noted as Stn. 12r. Using a Niskin rosette equipped with a Seabird 911+ CTD, water was collected at the surface (all stations), the deep chlorophyll maximum (Stns. 5–8 and 10–16), 300 m (Stns. 5–8 and 11–16), the oxygen minimum zone (Stns. 5–8 and 11–16), 1500 m (Stns. 11–16), 2800 m (Stns. 5–8), and bottom water (all stations; bottom water depths differed considerably among stations; Suppl. Table 1). Seawater was transferred from 30 L Niskin bottles into 20 L carboys (acid washed and rinsed, then rinsed three times with seawater from the sampling depth prior to filling), using silicone tubing that had been acid washed and rinsed with distilled water and then with sample water prior to use.

2.2. Temperature, salinity, chlorophyll fluorescence, and oxygen measurements

Temperature, salinity, chlorophyll fluorescence, and oxygen concentrations were determined using a Seabird 911+ conductivity, temperature, depth (CTD) profiler (Suppl. Table 1). Ocean Data View (ODV; version 5.6.2) was used to calculate the potential temperature (θ) and construct temperature-salinity diagrams.

2.3. Polysaccharide hydrolase activities

Polysaccharide hydrolase activities were measured using six fluorescently-labeled polysaccharides, according to Arnosti (2003). These polysaccharides – pullulan, laminarin, xylan, fucoidan, arabinogalactan, and chondroitin sulfate – were used because they are found in marine phytoplankton and algae (e.g., Painter, 1983), and/or the enzymes required to hydrolyze these polysaccharides have been identified in marine bacteria (Arnosti and Repeta, 1994; Alderkamp et al., 2007; Neumann et al., 2015). These polysaccharides also differ in their structural complexity (i.e., monomeric composition, degree of branching, side chains, extent of sulfation/methylation, etc.).

For each individual substrate, three 15-mL sterile tubes were filled with seawater, while one was filled with autoclaved seawater to serve as a killed control. Substrates were added to a final concentration of 3.5 μM (excluding fucoidan, which was added at 5 μM due to its low labeling density) and were incubated in the dark at in situ temperatures (see Suppl. Table 1). One 15-mL tube was filled with seawater without substrate to act as a blank; one 15-mL tube was filled with autoclaved seawater without substrate to serve as a negative control. To determine hydrolysis rates, subsamples (2 mL) of incubations were taken immediately after substrate addition (day 0) and at five timepoints thereafter (2 days, 5 days, 10 days, 17 days, and 30 days). Each subsample was filtered through a 0.2 μm pore-size surfactant free cellulose acetate filter, then stored frozen at −20 °C until analysis. Samples were later analyzed by gel permeation chromatography with fluorescence detection to determine the change in molecular weight distribution of polysaccharides with time, and hydrolysis rates were calculated from these changes, as described in detail (Arnosti, 2003). The 'average maximum hydrolysis rate' was calculated by first determining the maximum hydrolysis rate at each individual station and depth, then calculating the average of all of the maximum rates at each depth per region (see results and discussion below for an explanation of regional distinction). Note also that all enzyme activities represent potential rates, since added substrates are in competition with naturally occurring polysaccharides for enzyme active sites. Potential rates should not be regarded as in situ rates. Data from Stns. 1–4 have been previously published (Hoarfrost et al., 2019).

2.4. Glucosidase and peptidase activities

Activities of *exo*-acting (terminal-unit cleaving) glucosidases and amino peptidases were measured using α- and β-glucose linked to 4-methylumbelliferone (MUF), and the amino acid leucine linked to 7-amido-4-methyl coumarin (MCA). Endo-acting (mid-chain cleaving) peptidase activities were measured using small peptides linked to the

MCA fluorophore. Chymotrypsin substrates included alanine-alanine-phenylalanine (1-letter amino acid codes: AAF) and alanine-alanine-proline-phenylalanine (AAPF), and trypsin substrates included glutamine-alanine-arginine (QAR) and phenylalanine-serine-arginine (FSR).

Enzymatic activities in seawater were measured following Lloyd et al. (2022). In brief, glucosidase and peptidase activities were determined with substrates at saturating concentrations (200 μM for EN556 and 150 μM for EN584, determined from saturation curves from surface waters on each cruise). Triplicate wells of seawater amended with individual substrates were used in experimental incubations, and triplicate wells of autoclaved seawater amended with substrate were measured as killed controls. Fluorescence was measured immediately (t0) and every 6 h over a 24-h time period, using a plate reader (TECAN SpectraFluor Plus with 360 nm excitation, 460 nm emission for EN556; TECAN SpectraFluor Plus with 340 nm excitation, 460 nm emission for EN584). Fluorescence signals were converted to concentrations using standard curves of MUF and MCA fluorophores. Hydrolysis rates were calculated from increases in concentration with time. Note that data for Stns. 1–4 have previously been published in Hoarfrost et al. (2019).

2.5. Bacterial protein productivity

Bacterial protein productivity was measured using the microcentrifuge method for incorporation of tritiated-leucine (^{3}H-Leu), after Kirchman (2001). In brief, leucine incorporation rates were measured by adding tritiated leucine to a final concentration of 20 nM (based on the specific activity of the leucine batch provided by the manufacture) to samples incubated in the dark at in-situ temperatures generally between 12 and 24 h (bottom waters required longer incubation times). Incorporation rates are reported as pmol leucine per L per hr.

2.6. Bacterial community analysis

Approximately 2–3 L of seawater was filtered immediately after sampling (e.g., <30 min) through a 0.2 μm pore-size 47 mm diameter Whatman Nucleopore track-etched Membrane filter, and stored at −80 °C until DNA extraction. DNA was extracted from a quarter of each filter, which was cut using a sterile razor blade or sterile scissors. All DNA was extracted using a DNeasy PowerSoil Kit (Qiagen). For select samples, duplicate extractions were performed to determine accuracy of extraction and sequencing. DNA libraries were prepared using the Nextera XT Index Kit, v2 set A (Illumina) according to manufacturer protocol. The V1-V2 hypervariable region of the 16S rRNA gene was amplified using the primers 8F (5′- AGA GTT TGA TCC TGG CTC AG-3′) and 338R (5′- GC TGC CTC CCG TAG GAG T-3′) (custom made by Integrated DNA Technologies) 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 (GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G), and sequenced at the UNC High Throughput Sequencing Facility using Illumina MiSeq PE 2 × 250.

Sequenced multiplexed paired-end FASTQ files were imported into QIIME2 (version 2019.4; https://qiime2.org; Bolyen et al., 2019), demultiplexed, and then denoised and dereplicated using DADA2 (Callahan et al., 2016). Quality control was performed during the DADA2 denoising and dereplicating process, with a Phred quality control cutoff of 25 (i.e., 10 bases) filtered prior to merging of paired end reads. A Naïve Bayes classifier was trained to assign taxonomy to ASVs using reference sequences from the Silva 16S rRNA database (version 132; Pruesse et al., 2007) sequenced with the 8F and 338R primers.

The BIOM-formatted OTU table and phylogenetic tree of representative sequences were imported into R; the phyloseq package (version 1.32.0; McMurdie and Holmes, 2013) was used to remove chloroplasts and rarefy samples to an even sampling depth of 26,745 sequences per sample to enable comparison of bacterial relative proportions across samples with initially uneven sequencing depths. Raw sequence files are available on NCBI Sequence Read Archive under the accession number PRJNA816842.

2.7. Statistical analyses

To visualize station and depth-related differences in enzymatic activities and bacterial community composition patterns, Bray-Curtis dissimilarities were calculated and ordinated using non-metric multidimensional scaling (NMDS) plots. Distance matrices were calculated based on the Bray-Curtis dissimilarity index using the vegdist function in the vegan package (ver2.6–4) in R ((R Core Team, 2021), version 4.2.2). Spearman correlations were used to determine the degree of correlation between community composition and enzymatic activity. Mantel tests were used to compare the distance matrices between bacterial community composition and polysaccharide hydrolase and glucosidase/peptidase patterns. Analysis of variance (ANOVA) of log transformed data was used to quantify differences in observed patterns of enzymatic activity as a function of station, depth, and region. Permutational multivariate analysis of variance (PERMANOVA) was used to quantify bacterial community dissimilarities (Bray-Curtis) as a function of station, depth, and region.

3. Results

3.1. Water masses and physical parameters

Temperature and salinity of the surface waters at each station varied considerably (Fig. 1; Suppl. Table 1). Most notably, there was a clear difference in sampling sites north of the Gulf Stream (Stns. 1–8) than those located within the Gulf Stream or the Sargasso Sea (Stns. 9–16). Surface water at Stns. 1–4 was relatively cool and fresh compared to surface water at the other stations, typical of shelf water coming down from the Grand Banks (Wright and Parker, 1976; Lentz, 2003). Surface water at Stns. 5–8 was cooler than surface water at Stns. 9–16 and was likely Eighteen Degree Water (Heidrich and Todd, 2020). Surface waters at Stns. 9–12 were located within the Gulf Stream, while surface waters at Stns. 13–16 were located within the Sargasso Sea and had characteristics most similar to North Atlantic Surface Water (Heidrich and Todd, 2020).

Maximum depths varied substantially between stations (Suppl. Table 1), and therefore bottom waters at each station had different characteristics. At Stns. 1–2, bottom waters were characteristic of shelf water, while Stns. 3–4 bottom waters were defined by a warm core ring intrusion (see Hoarfrost et al., 2019 for a detailed explanation of the physical oceanography of these stations). The T/S profiles of Stns. 9–16 did not differ greatly throughout the water column. For Stns. 9–16, water collected at 300 m was characteristic of Eighteen Degree Water while the rest of the mesopelagic was characteristic of Intermediate Water (Heidrich and Todd, 2020). Bottom waters were characteristic of North Atlantic Deep Water (Fig. 1; Heidrich and Todd, 2020).

Chlorophyll-α, as measured in situ by the CTD, was higher in Stns. 1–8, and was highest for Stns. 5–7, where concentrations in the surface and DCM waters were as high as ~2 mg m^{-3}. Stns. 9–16 had relatively lower chlorophyll-α concentrations, often <0.5 mg m^{-3} in surface and DCM waters. Chlorophyll-α decreased with depth below the DCM. Oxygen concentrations were also typically higher in Stns. 1–8, reaching upwards of 6–7 mL L^{-1} at the shelf stations (Stns. 1–4; Suppl. Table 1).

3.2. Polysaccharide hydrolase activities

Polysaccharide hydrolase activities (Fig. 2; Suppl. Figs. 1,2) varied considerably among stations, most notably in differences between Stns.

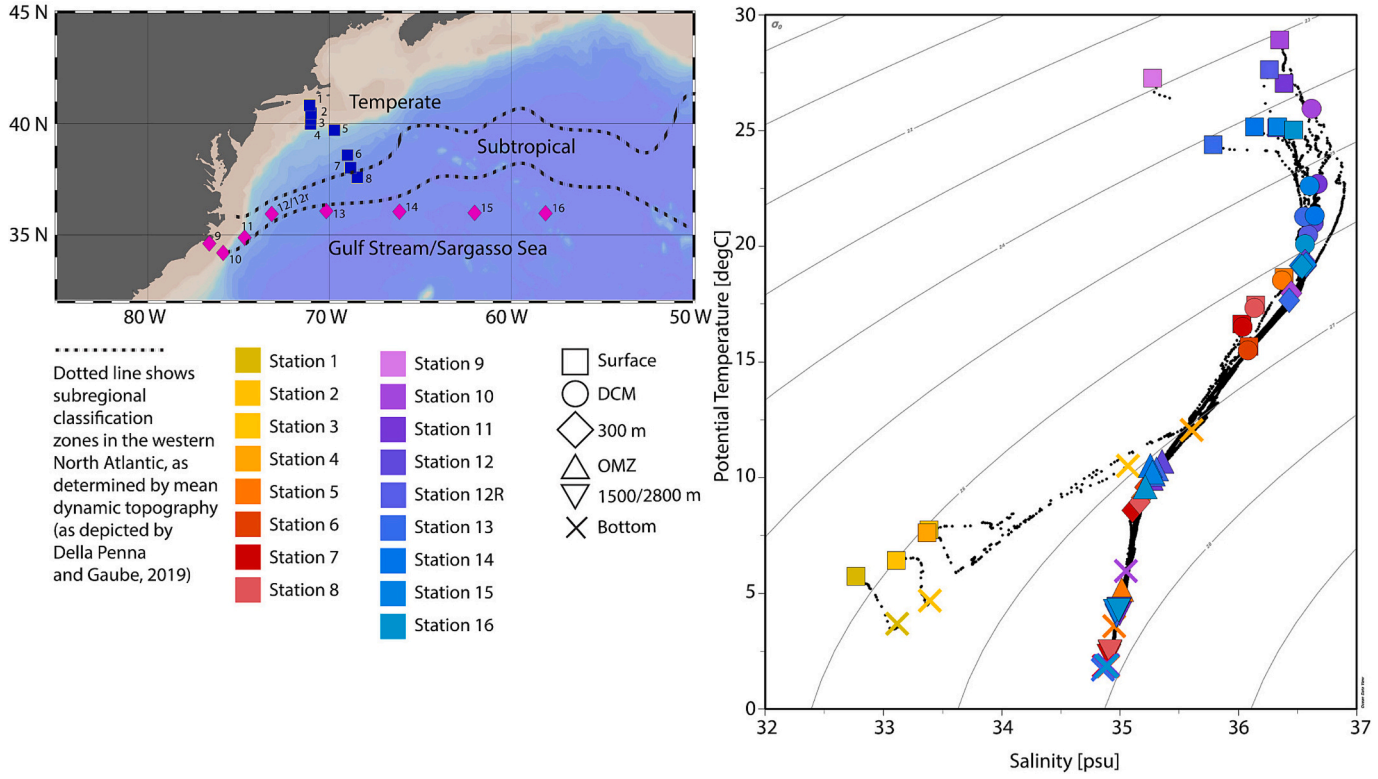

Fig. 1. Stations sampled from the *R/V Endeavor* in 2015 (blue squares) and 2016 (pink diamonds). Station locations are separated into Temperate (Stns. 1–8) and Gulf Stream/Sargasso Sea (Stns. 9–16), based on Bolaños et al. (2020, 2021) following the mean dynamic topography (Della Penna and Gaube, 2019) of the region. The dotted lines (from Della Penna and Gaube, 2019) denote the subregional classifications. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

1–8 and Stns. 9–16 (ANOVA, $p = 0.0017$). The differences we measured in enzyme activities, coupled with differences in the physical oceanography presented above, led us to divide stations into Temperate (Stns. 1–8) or Gulf Stream/Sargasso Sea (Stns. 9–16; following the terminology used in Bolaños et al., 2020, 2021; black dashed lines on Fig. 1 denote the regional classifications), based on the mean dynamic topography of the region (Della Penna and Gaube, 2019). Temperate stations were characterized by high chondroitinase activity, while Gulf Stream/Sargasso Sea stations were dominated by laminarinase activity. Xylanase activity was relatively high in both the Temperate and Gulf Stream/Sargasso stations, but was mainly detected at deeper depths in the Gulf Stream/Sargasso stations, whereas it was detected in surface as well as subsurface waters at the Temperate stations. Pullulanase activity was also detected throughout the water column in Gulf Stream/Sargasso stations, but was rarely detected in the Temperate stations. There were also some differences in polysaccharide hydrolase activities between Stns. 12 and 12r, which were sampled at the same location but 6 days apart; however, most of these differences were in the rates measured, and the polysaccharides hydrolyzed at each depth were often, though not always, the same. In Gulf Stream/Sargasso as well as Temperate stations, polysaccharide hydrolase activities were higher in the surface and DCM, and generally lower in the OMZ and at deeper depths (Fig. 2).

The spectrum of activities (the range of substrates hydrolyzed) was also typically broader in the upper ocean, and narrower in bottom waters. The only stations at which all six polysaccharides were hydrolyzed were the bottom waters of Stn. 4 (which was at a depth of 199 m, and was determined to be subducted water from a warm core ring; Hoarfrost et al., 2019), and in Stn. 7 DCM waters (Suppl. Fig. 1). Statistical analysis of polysaccharide hydrolase activities showed strong distinctions with location (Temperate versus Gulf Stream/Sargasso, ANOVA, $p = 0.0017$) and with depth, with especially strong separations between epipelagic (surface and DCM), mesopelagic (300 m and OMZ), and bathypelagic depths (1500 m and bottom water, ANOVA, $p = 0.012$; Fig. 3).

3.3. Glucosidase and peptidase activities

In contrast to the polysaccharide hydrolase activities, glucosidase and peptidase activities were relatively similar between Temperate and Gulf Stream/Sargasso stations (Figs. 4,5; ANOVA, $p = 0.163$). Peptidase activities were consistently about one order of magnitude greater than glucosidase activities at all stations and depths. Leucine aminopeptidase activity was considerable at all depths, especially at Gulf Stream/Sargasso stations, and became increasingly dominant with increasing depth (Fig. 4). In contrast, α- and β-glucosidase activities were very low (<9 nmol L^{-1} h^{-1}; most values are <5 nmol L^{-1} h^{-1}) at all stations and depths (Fig. 4; Suppl. Fig. 3). At Temperate stations, summed surface activities were much greater (> 100 nmol L^{-1} h^{-1}) than in bottom waters (~30 nmol L^{-1} h^{-1}). However, at the Gulf Stream/Sargasso stations, the difference between surface and deep activities was much smaller, with summed activities of upwards of 130 nmol L^{-1} h^{-1} in surface waters, and upwards of 90 nmol L^{-1} h^{-1} in bottom waters.

3.4. Bacterial productivity

Bacterial protein production differed considerably by depth and station (Suppl. Fig. 4). Rates of protein production were highest in surface waters and at the DCM, and overall was higher at the Gulf Stream/Sargasso stations than at the Temperate stations. Only at the Gulf Stream/Sargasso stations was bacterial protein production at a depth of 300 m considerably above 1 pmol L^{-1} h^{-1}. Below this depth at all stations, the measured activity was ≤1 pmol L^{-1} h^{-1}.

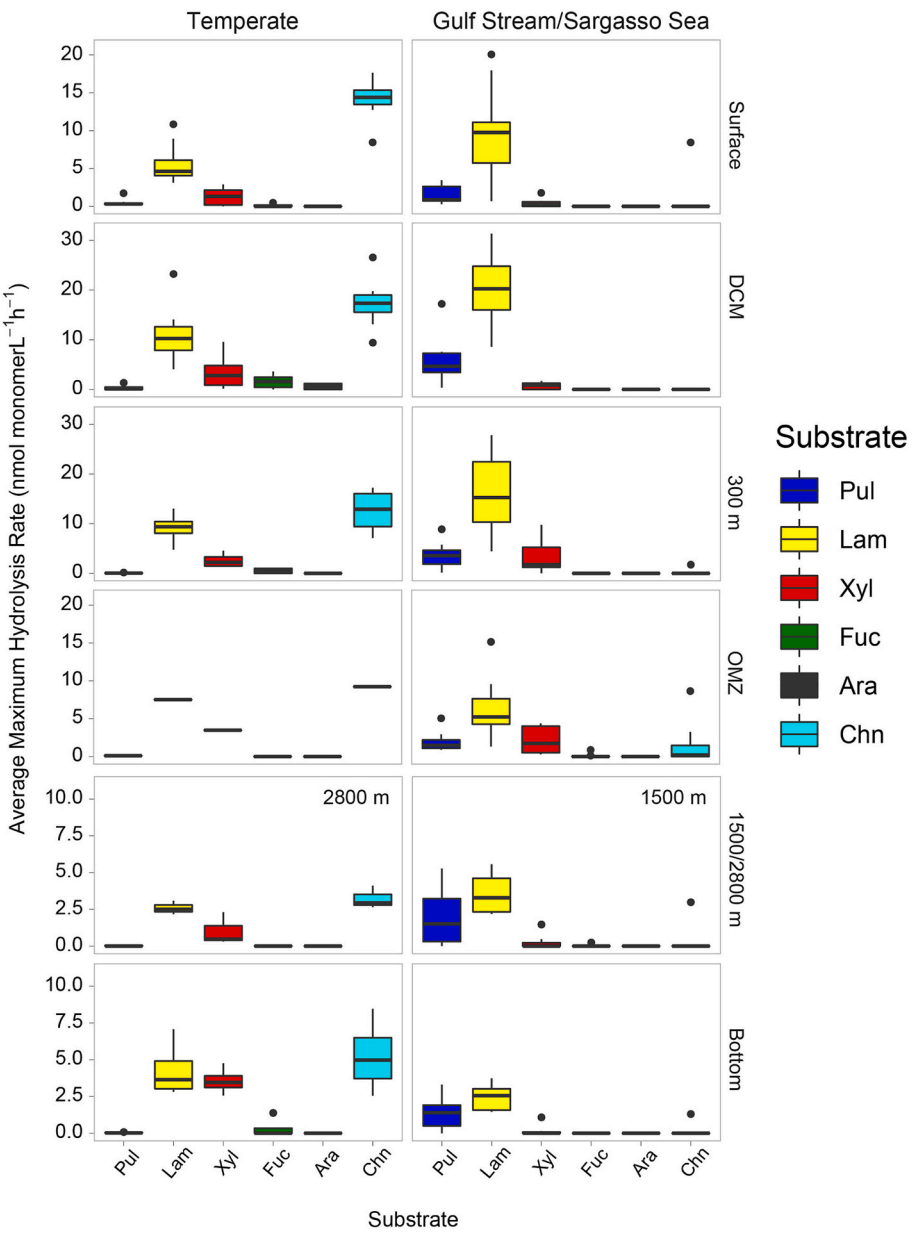

Fig. 2. Box and whisker plot of polysaccharide hydrolase activities of bulk water samples show distinct differences between Temperate and Gulf Stream/Sargasso subregions. The line in the middle represents the median value; the top and bottom of the box represent the upper and lower quartile, respectively; the ends of 'whiskers' represent lowest and highest values (dots: outliers). Temperate stations include Stns. 1–8; Gulf Stream/Sargasso Sea stations are Stns. 9–16. See Fig. 1 for the basis of these classifications. Note that the y-axis differs by depth. Pul = pullulan; Lam = laminarin; Xyl = xylan; Fuc = fucoidan; Ara = arabinogalactan; Chn = chondroitin sulfate.

3.5. Bacterial community composition

Bacterial community composition revealed substantial differences with depth (PERMANOVA, $p = 0.001$) and location (Fig. 6; Suppl. Figs. 5,6; PERMANOVA, $p = 0.044$). Stns. 12 and 12r showed no major differences in the bacterial communities, even though they were sampled six days apart (Suppl. Figs. 5,6). In surface waters, the communities were dominated by Alphaproteobacteria, Bacteroidia, Gammaproteobacteria, and Cyanobacteria. In subsurface waters, the relative abundance of Deltaproteobacteria, Dehalococcoida, Gammaproteobacteria, and Nitrospinia increased, with decreases in Alphaproteobacteria, Bacteroidia, and Cyanobacteria (Suppl. Fig. 5). No Cyanobacteria were present below the surface or deep chlorophyll maximum, or at the Temperate stations located on the continental shelf (Stns. 1–4; Suppl. Fig. 5). The separation by location and depth, as demonstrated by NMDS plots, was very distinct: although the surface and DCM depths of the Temperate stations showed a considerable degree of overlap, there was little overlap at the other depths; all depths of the Gulf Stream/Sargasso stations showed substantial separation (Fig. 6, PERMANOVA, $p = 0.001$).

There were unique patterns that evolved when comparing bacterial community composition and enzymatic activities. Most notably, patterns in polysaccharide hydrolase activities had positive correlations with patterns in the bacterial community composition (Mantel test, r-statistic = 0.2727, p = 0.001), where as patterns in peptidase activities correlated less with microbial communities (Mantel test, r-statistic = 0.1225, $p = 0.009$). There was no correlation between community members and glucosidase activities (Mantel test, r statistic = −0.01164; $p = 0.655$).

4. Discussion

4.1. Substrate structural complexity may underlie regional patterns

Polysaccharide hydrolase activities in the western North Atlantic Ocean showed a clear regional pattern of specific enzyme activities, with the Temperate stations characterized by comparatively high chondroitin sulfate hydrolase activities, and the Gulf Stream/Sargasso stations

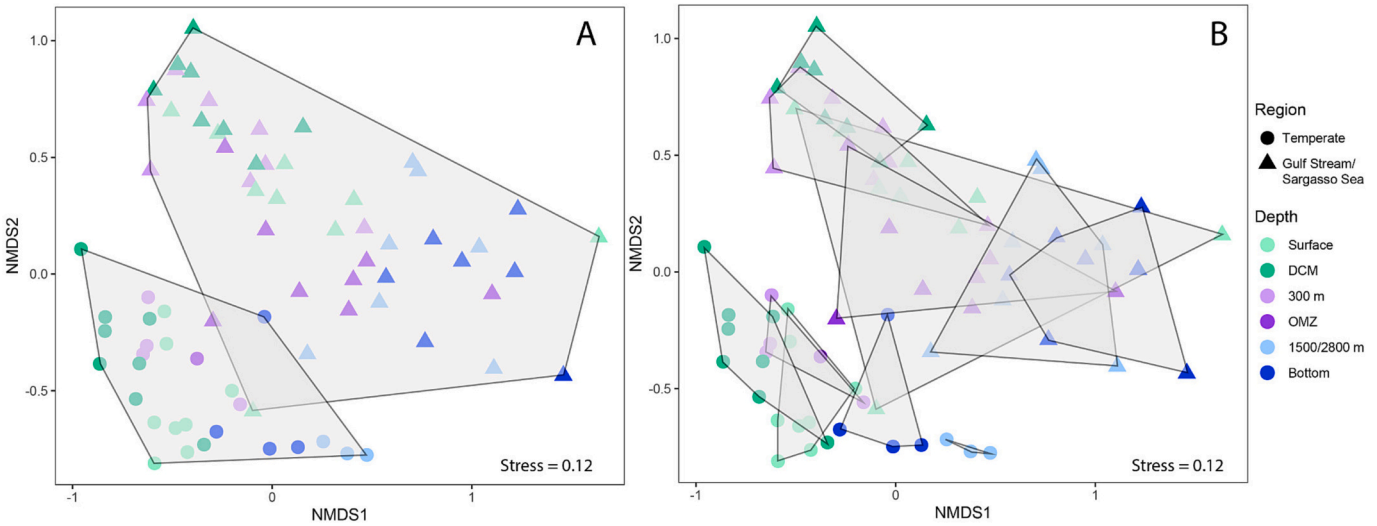

Fig. 3. Non-metric multidimensional scaling (NMDS) plot of polysaccharide hydrolase activities based on the Bray-Curtis dissimilarity index show separations by subregion (a) and depth (b). Circles represent the Temperate stations (Stns. 1–8); triangles represent the Gulf Stream/Sargasso Sea stations (Stns. 9–16). Depth is represented by color. (a) the Temperate and Gulf Stream/Sargasso Sea regions are outlined with hulls and shaded; (b) the depths are outlined with hulls and shaded for Temperate and for Gulf Stream/Sargasso Sea regions. See Fig. 1 for the basis of these classifications.

showing comparatively high laminarinase and pullulanase activities (Fig. 2; Suppl Figs. 1,2). The regional patterns in polysaccharide hydrolase activities parallel patterns of microbial community composition, which showed the same clear separation by depth and by region (Figs. 2,6). Moreover, a Mantel test showed positive correlations between polysaccharide hydrolase activities and bacterial community composition (r-statistic = 0.2727; p = 0.001). The separation among bacterial communities was particularly distinct in surface waters as shown in the NMDS plot (Fig. 6), an analysis based on individual OTUs, rather than a class-level comparison (Suppl. Fig. 5).

Although our measurements integrate enzyme activities - irrespective of enzyme source - in the water column, our community analysis focused on bacteria. Archaeal communities may also play a role in the enzymatic degradation of carbohydrates. However, in the water column this role may be limited: archaeal genes encoding for carbohydrate active enzymes may only account for ~5% of genes encoding for the total carbohydrate-active enzyme pool in the water column (Zhao et al., 2020). An investigation of Marine Group II (MGII) Euryarchaeaota likewise found that only a handful of polysaccharide hydrolase genes occurred in a small subset of metagenomes, primarily among members of the MG IIa Euryarcheaota whose presence in open ocean waters was limited (Tully, 2022). Even in the coastal North Sea, very few polysaccharide hydrolase genes were identified in MG II Euryarchaeota (Orellana et al., 2019). In contrast, studies of bacterial communities in surface waters of the Atlantic Ocean have also found regional patterns in bacterial community composition (Schattenhofer et al., 2009) and corresponding differences in their genetic potential for enzymes that hydrolyze polysaccharides (Gomez-Pereira et al., 2012). These patterns of community composition and genetic potential were also evident in the activities of their polysaccharide hydrolyzing enzymes (Arnosti et al., 2012).

The specific pattern of polysaccharide hydrolase activities observed in the western North Atlantic Ocean is similar to depth profiles measured along a latitudinal transect of the central Pacific Ocean, in which chondroitin sulfate hydrolysis became progressively greater and laminarin hydrolysis progressively reduced with increasing latitude (Balmonte et al., 2021). Coherent patterns of community composition and polysaccharide-hydrolyzing function among surface-water communities have also been seen in latitudinal gradients in polysaccharide hydrolase activities in surface waters (Arnosti et al., 2011) that parallel broad gradients in microbial community composition (Fuhrman et al., 2008).

The positive relationship between patterns of polysaccharide hydrolase activities and microbial communities at these different depths and stations may relate to the fundamental structural complexity of polysaccharides (Laine, 1994) and the number of different enzymes required to fully hydrolyze them (Lapebie et al., 2019). Many polysaccharides require a set of enzymes to function in concert to systematically remove branches, side chains, and/or functional groups, revealing the polysaccharide backbone for cleavage by other enzymes (Sichert et al., 2020); the number of distinct enzymes required scales linearly with structural complexity (Bligh et al., 2022). Given the energetic cost of such enzyme systems, heterotrophic bacteria differ greatly in their enzyme complements (Sunagawa et al., 2015; Zhao et al., 2020), and therefore in the extent to which they can access different polysaccharides. Microbial communities are comprised of diverse members, but the membership of any given community – especially in the water column – collectively may not contain or may not express all the enzymatic tools required to dismantle a broad range of polysaccharides. Our observations demonstrate that most pelagic communities do not even express the enzymatic tools to measurably hydrolyze just six structurally-distinct polysaccharides. We thus observe distinct polysaccharide preferences among pelagic microbial communities (Figs. 2,6; Suppl. Figs. 1-2,5), even with the comparatively small set of polysaccharides tested.

We note, however, that the enzymatic spectrum of a community is flexible, considering also the potential encompassed by numerically minor members of a microbial community, or members that may become active only under specific conditions. In seawater incubations from some of these same stations (Stns. 8, 12 and 16) that were amended with HMW phytoplankton-derived organic matter (both dissolved and particulate), we measure a broader spectrum of enzyme activities compared to unamended incubations of the same seawater (Balmonte et al., 2018b; Brown et al., 2022). This increasing breadth of enzymatic tools was accompanied by an increase in abundance of initially numerically minor members of communities (Balmonte et al., 2018b; Brown et al., 2022). Nonetheless, limited spectra of polysaccharide hydrolyzing activities were still apparent among deep ocean communities amended with the same HMW organic matter (Balmonte et al., 2018b; Brown et al., 2022).

The lack of comparable coherence in patterns of peptidase activities (Fig. 5) and microbial community composition (Fig. 6) likely arises from a broader substrate specificity of peptidases compared to polysaccharide

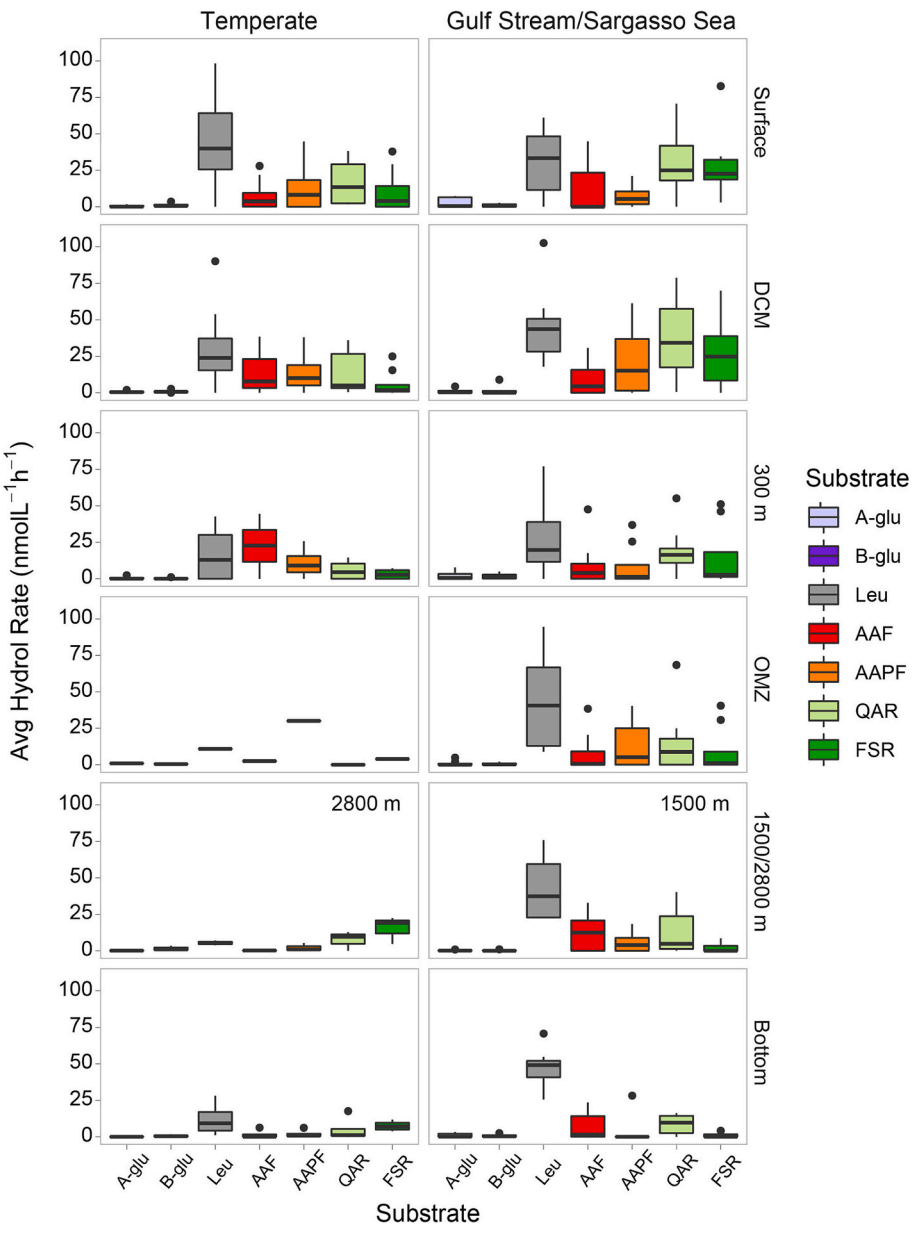

Fig. 4. Box and whisker plot of glucosidase and peptidase activities of bulk water samples show little difference between Temperate and Gulf Stream/Sargasso subregions. The line in middle represents median value, the top and bottom of the box represent the upper and lower quartile, respectively; the ends of 'whiskers' represent lowest and highest values (dots: outliers). Temperate stations include Stns. 1–8; Gulf Stream/Sargasso Sea stations are Stns. 9–16. See Fig. 1 for the basis of these classifications. A-glu, alpha-glucosidase; B-glu, beta-glucosidase; Leu, leucine aminopeptidase; AAF, alanine–alanine-phenylalanine-chymotrypsin; AAPF, alanine–alanine-proline-phenylalanine-chymotrypsin; QAR, glutamine-alanine-arginine-trypsin; FSR, phenylalanine-serine-arginine-trypsin.

hydrolases. Along these lines, the correlations between bacterial community members and peptidase activities (Mantel test, r statistic = 0.1225; $p = 0.009$) was much lower than for polysaccharide hydrolase activities (r-statistic = 0.2727; $p = 0.001$), suggesting that specific peptidase activities are not as closely tied to specific members of a bacterial community. This broader substrate specificity means that fewer peptidases are required to hydrolyze a wide range of protein structures (Lapebie et al., 2019). Moreover, due to the fact that individual monosaccharides can be linked through multiple positions, the structural diversity of combined carbohydrates is higher than for combined amino acids. For example, two glucose monosaccharides potentially could form twelve different disaccharides (α/β configurations, six possible linkage points), compared with the one possible dipeptide in the case of two of the same amino acids.

Although individual organisms can differ considerably in their peptidase capabilities (Bong et al., 2013; Liu and Liu, 2020), a community collectively likely possesses the ability to hydrolyze a broader range of peptides, since fewer different types of peptidase enzymes are required. This idea is further supported by an experimental investigation of peptide hydrolysis by bacterial isolates and by natural communities from coastal waters. In coastal waters, the degradation rates of structurally-distinct peptides were similar, even though the pathways by which the peptides were degraded – and thus the specific peptidases involved – differed (Liu and Liu, 2021). Liu and Liu (2021) suggest that if one organism does not produce the enzymes necessary to hydrolyze a given peptide, then the peptidases of another organism are available to hydrolyze the substrate. In short, these data suggest that microbial communities differ in their functional redundancy (Louca et al., 2018), particularly with respect to complex traits (Martiny et al., 2013): communities show less redundancy in the ability to hydrolyze specific polysaccharides (Berlemont and Martiny, 2016), whereas they have greater functional redundancy in their peptidase capabilities.

4.2. *Potential drivers of regional patterns in bacterial communities and their activities*

The patterns of bacterial communities and their enzymatic function revealed in our study coincide with patterns determined in a previous study of biological communities and the physical oceanography of the western North Atlantic. The NAAMES program (North Atlantic Aerosols

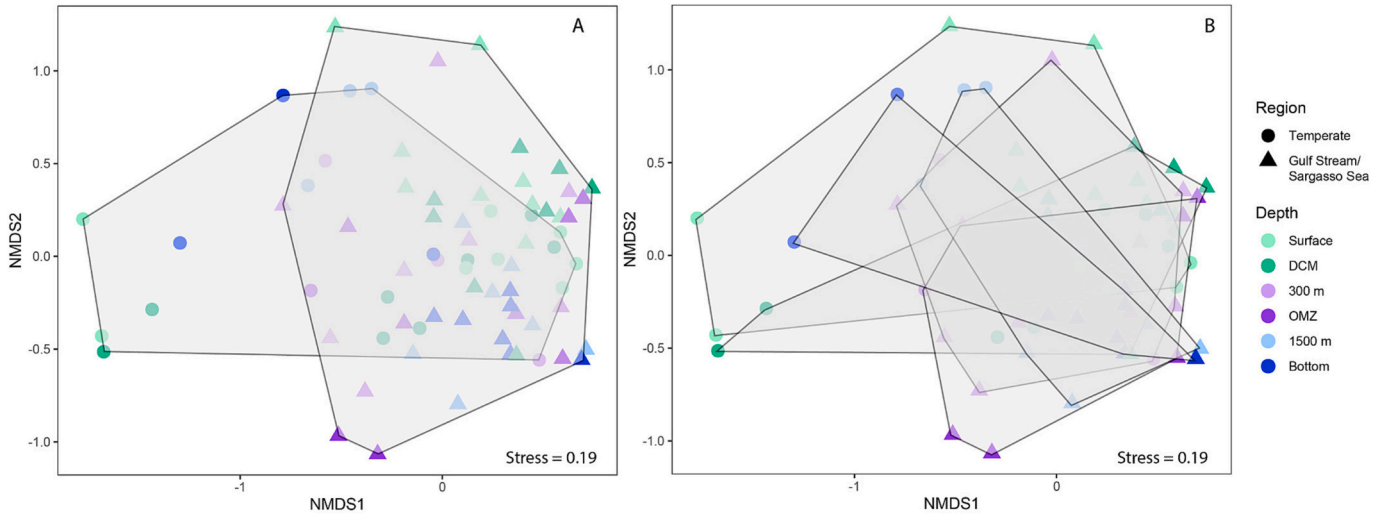

Fig. 5. Non-metric multidimensional scaling (NMDS) plot of glucosidase and peptidase activities based on the Bray-Curtis dissimilarity index show little separation by subregion (a) or depth (b). Circles represent the Temperate stations (Stns. 1–8); triangles represent the Gulf Stream/Sargasso Sea stations (Stns. 9–16). See Fig. 1 for the basis of these classifications. Depth is represented by color. (a) the Temperate and Gulf Stream/Sargasso Sea divisions are outlined with hulls and shaded; (b) the depths are outlined with hulls and shaded.

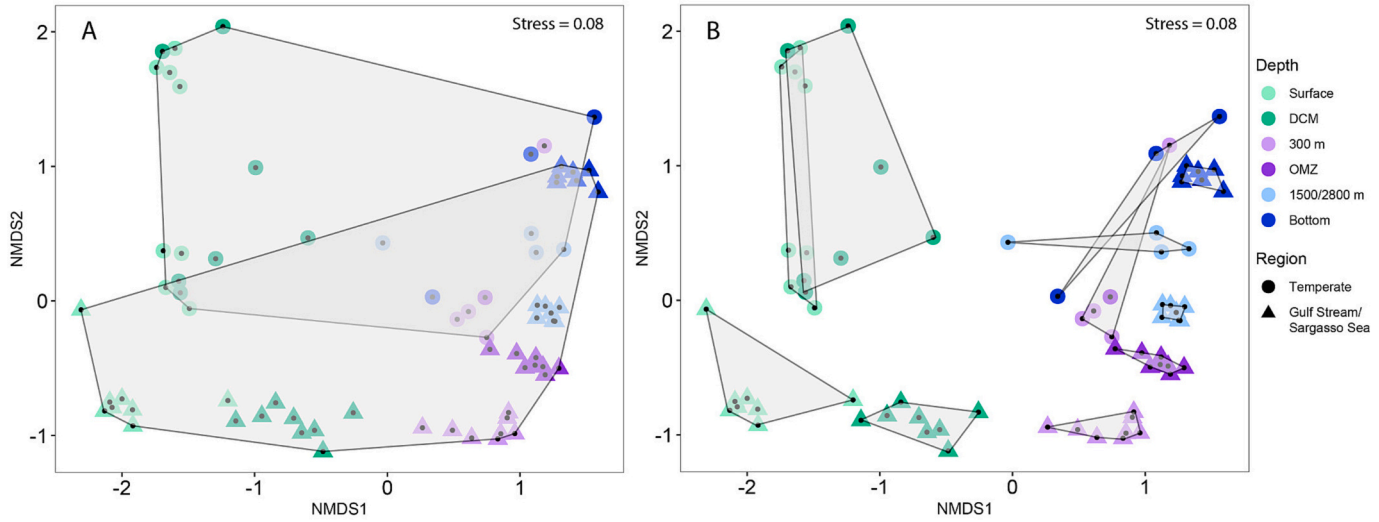

Fig. 6. Non-metric multidimensional scaling (NMDS) plot of bacterial community composition based on the Bray-Curtis dissimilarity index shows separations by subregion (a) and depth (b). Circles represent the Temperate stations (Stns. 1–8); triangles represent the Gulf Stream/Sargasso Sea stations (Stns. 9–16). See Fig. 1 for the basis of these classifications. Subregion represented by shape, depth is represented by color.

and Marine Ecosystems Study; Behrenfield et al., 2019) developed a subregional classification scheme for the western North Atlantic, where submesoscale eddies can redistribute nutrients, primary producers, and grazers, both coupling and decoupling organisms in the food web (Della Penna and Gaube, 2019). This subregional classification was used as a framework to analyze phytoplankton and bacterial communities. Phytoplankton communities differed significantly between the Temperate and Gulf Stream/Sargasso Sea stations (Bolaños et al., 2020), and during all four sampling campaigns, which represented different seasons and productivity regimes (Bolaños et al., 2021). Phytoplankton and bacterial communities showed similar clustering patterns based on subregions, dividing the Temperate from the Gulf Stream/Sargasso Sea communities (Bolaños et al., 2021); bacterial communities were additionally structured by depth (Bolaños et al., 2021). A network analysis demonstrated that the bacterioplankton communities were also sensitive to features that did not affect phytoplankton communities, including the nature of heterotrophic substrates to bacteria, which they termed "DOM quality" (Bolaños et al., 2021).

The positive relationship between the patterns found by Bolaños et al. (2021) linking phytoplankton and bacterioplankton in subregions of the western North Atlantic, and our patterns of bacterial community composition in the same regions, suggests that the enzymatic processes we measure may be linked to the primary producer community and the organic substrates that they produce. We surmise that the spectrum of enzymes typically produced by heterotrophic bacteria are tuned in part to the substrates that are commonly available. In this respect, measurements of specific polysaccharide hydrolase activities may provide an indication of the types of substrates that heterotrophic bacteria encounter, substrates that may be largely derived from phytoplankton.

4.3. Regional patterns in microbial communities and enzyme activities: implications for carbon cycling

The fate of much of the organic matter in the ocean is controlled by

heterotrophic microbial communities (Azam and Malfatti, 2007). These communities differ in composition and gene content at different locations and depths (Delong et al., 2006; Zhao et al., 2020); our data demonstrate that community function also varies by location and depth. The extent to which organic matter is remineralized at a given depth in the ocean is thus affected by the structure of the organic matter, as well as the enzymatic capabilities of the community. As organic matter sinks in the ocean, its fate also depends on its exposure to a changing spectrum of enzymes, and thus on its residence time at different depths in the ocean (Hoarfrost and Arnosti, 2017; Lloyd et al., 2022; Brown et al., 2022). The spectrum of polysaccharide hydrolase activities in particular varies considerably with depth and location (Fig. 2). The limited spectrum of polysaccharide hydrolase activities we measured in open ocean waters is consistent with the few previous depth profiles measured, in the Gulf of Mexico (Steen et al., 2012), the South and Equatorial Atlantic (Hoarfrost and Arnosti, 2017), the central Pacific (Balmonte et al., 2021), and the Arctic Ocean (Balmonte et al., 2018a). The activities in the lower mesopelagic and below (depths at and below 850 m) were higher than previously measured in the equatorial and South Atlantic (Hoarfrost and Arnosti, 2017), but generally comparable to the central Pacific (Balmonte et al., 2021).The limited spectrum of polysaccharide hydrolase activities at depth in the current study as well as in the South and Equatorial Atlantic, the Pacific, the Gulf of Mexico, and the Arctic (Steen et al., 2012; Hoarfrost and Arnosti, 2017; Balmonte et al., 2018a, 2021) suggests that pelagic microbial communities target a focused range of polysaccharide substrates, likely reflecting the cost-benefit calculation of producing substrate-specific enzymes and obtaining the resulting hydrolysate.

Exopeptidase activities, as exemplified by leucine aminopeptidase activities (Steen et al., 2015), are measurable at a broader range of locations, suggesting these enzymes are more widely distributed among microbes, and that terminal peptide-containing structures may be more generally bioavailable in ocean water (Fig. 4; Suppl. Fig. 3). The higher rates in the upper ocean as well as the broader spectrum of peptidase activities is consistent with the rapid removal of amino acids from sinking organic matter in the upper water column (Wakeham et al., 1997). These observations are also in accordance with the considerably higher rates of bacterial protein productivity in the upper water column compared to the deeper ocean (Suppl. Fig. 4) as well as the considerable changes in C/N ratios of sinking organic matter (Martiny et al., 2014).

Our investigation is the first to assess endopeptidase and polysaccharide hydrolase activities, as well as microbial community composition, in a regional framework in the western North Atlantic. We find strong regional and depth differences in both community composition and function, highlighting the connections between functional and compositional diversity in the ocean; these differences can be potentially linked also to water masses and their phytoplankton communities. These connections between the physical oceanography of the region and the structure and function of heterotrophic bacterial communities, specifically with the patterns of polysaccharide hydrolase activities measured, emphasize the importance of measuring microbial activities across large spatial scales.

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CRediT authorship contribution statement

C. Chad Lloyd: Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Sarah Brown:** Investigation, Data curation, Methodology, Writing – review & editing. **John Paul Balmonte:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Adrienne Hoarfrost:** Software, Methodology, Investigation, Writing – review & editing. **Sherif Ghobrial:** Methodology, Investigation, Writing – review & editing. **Carol Arnosti:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

We have shared our data using the links provided in the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marchem.2023.104299.

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