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9 Microbial activities and organic matter degradation at three sites in the
10 coastal North Atlantic: Variations in DOC turnover times and potential for export off the shelf
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Abstract

The Mid-Atlantic Bight is a highly productive region where considerable quantities of organic matter are processed, transformed, and respired. Much of this organic matter is in the form of dissolved organic carbon (DOC). We investigated the activities of heterotrophic microbial communities responsible for DOC processing at three sites in the coastal Mid-Atlantic Bight/northern end of the South Atlantic Bight: near the Chesapeake Bay mouth, off Cape Hatteras, and near Cape Lookout, NC. Activities of endo- and exo-acting extracellular enzymes (polysaccharide hydrolases, and glucosidase and peptidases, respectively), and bacterial protein production were measured in surface and subsurface waters at the three stations. Total suspended material, cell counts, and particle-associated enzyme activities were also quantified. Water masses at each station showed distinct physical characteristics (temperature, salinity, total suspended material), and differed notably in rates and spectrum of enzyme activities, as well as in the fraction of particle-associated activities. Despite similar cell counts, bacterial productivity differed by a factor of ca. 30 among depths and stations. Glucosidase and peptidase activities differed by a factor of ca. 40 among stations, but the differences in enzyme activities among stations did not scale with the differences in bacterial productivity. Theoretical turnover times of dissolved carbohydrates – calculated from total dissolved carbohydrate concentrations and polysaccharide hydrolase activities – range from weeks to months, suggesting that dissolved carbohydrates may be among the components of DOC that can be advected from the Mid-Atlantic Bight into the open ocean, connecting the carbon cycle in the ocean margin with the ocean's interior.

Introduction

Coastal and continental margin environments typically are sites of active carbon cycling, where autochthonous and allochthonous organic matter is transported, transformed, respired, and buried in sediments (Hedges and Keil, 1995). The Mid-Atlantic Bight, extending from George's Bank to Cape Hatteras, NC, is one such region that has been intensively studied in order to quantify organic matter burial as well as export to the open ocean. Net transport in the Mid-Atlantic Bight is generally in a southward direction towards Cape Hatteras; the northward flow along the South Atlantic Bight (Cape Canaveral FL to Cape Hatteras) also exits the shelf near Cape Hatteras (Lohrenz et al. 2002). The continental slope off Cape Hatteras has therefore been regarded as a potential 'depocenter' for organic carbon produced on and transported from the shelf, and initial studies focused on characterizing and quantifying particulate organic carbon (POC) transport and burial along the continental slope (e.g. Anderson et al. 1994; Biscaye et al. 1994).

The dissolved organic carbon (DOC) pool in this region, however, is estimated to be one to three orders of magnitude greater than the POC pool (Bauer et al. 2001). Sources of DOC to the Mid-Atlantic Bight include input via the Chesapeake and Delaware estuaries, fluxes from shelf sediments, and primary productivity on the shelf (Vlahos et al. 2002). Variations in primary productivity in these waters are associated in part with the relative influence of the Chesapeake and Delaware outflow, compared to shelf waters that have been influenced by the Gulf Stream (Filippino et al. 2011). Considerable spatial and seasonal differences in DOC dynamics have consequently been observed within the Mid-Atlantic Bight (e.g. Bauer et al. 2001; Hopkinson et al. 2002; Vlahos et al. 2002).

Some of these differences are likely due to spatial and seasonal variations in the composition and activities of the heterotrophic microbial communities that drive much of DOC cycling in the ocean (Baltar et al. 2007; Carlson et al. 2004). These communities repackage, respire, and recycle a considerable fraction of DOC, regenerating nutrients and releasing CO₂ to the surrounding waters. The initial step in microbial processing of high molecular weight DOC is carried out by the activities of extracellular enzymes, which hydrolyze substrates outside the cell to sizes sufficiently small to permit uptake. Within a heterotrophic microbial community, some member(s) of the community must produce enzymes of the correct structural specificity to hydrolyze the substrate at hand (see Arnosti (2011) for a recent review). Differences in enzymatic capabilities have been demonstrated among isolated bacteria and individual organisms (Martinez et al. 1996; Wegner et al. 2013; Xing et al. 2014) and among communities at different locations and depths in the ocean (Arnosti et al. 2011; 2012; Steen et al. 2012), including the Delaware and Chesapeake River and Bay, and the Blake Ridge, off North Carolina (Keith and Arnosti 2001; Steen et al. 2008; Ziervogel and Arnosti 2009; D'Ambrosio et al. 2014). These differences suggest that the nature and quantity of organic matter available for heterotrophic processing is also a function of the enzymatic capabilities of the microbial community in a given location.

In the current investigation, we focus on microbial heterotrophic activities at three near-shore stations of the Mid-Atlantic Bight and the northern edge of the South Atlantic Bight, sites that have distinct physical and chemical characteristics. Our previous investigations of extracellular enzymatic activities have focused primarily on the activities of polysaccharide hydrolases, but did not typically encompass measurements of bacterial protein production or biomass. Previous studies of carbon dynamics at the Mid-Atlantic Bight have focused on

dynamics of water masses, DOC inventories, characteristics, and bulk consumption (Bauer et al. 2001; Hopkinson et al. 2002; Vlahos et al. 2002), but detailed investigations of the carbon-degrading capabilities of microbial communities have been lacking. In the present study, we measured bacterial protein production, cell counts, and the activities of a range of extracellular enzymes that initiate organic matter remineralization. These enzymes included endo-acting (mid-chain cleaving) enzymes that hydrolyze specific polysaccharides as well as exo-acting enzymes that hydrolyze glucose and leucine from terminal ends of polysaccharides and proteins, respectively. Since freshwater input to the Mid-Atlantic Bight also can include particles and sediments that likely harbor active microbial communities, we measured total suspended material, and determined the contribution of large-particle-associated ($> 3 \mu\text{m}$) enzymes to the total activities measured. Our objective was to measure the heterotrophic turnover of organic matter, as indicated by the activities of extracellular enzymes, and to estimate variations in DOC turnover times by depth and location.

Methods

Study Sites and Sample Collection

Water samples were collected between November 1st and 5th 2010 from three stations in the coastal North Atlantic, in southern portion of the Mid-Atlantic Bight and the northern edge of the South Atlantic Bight (Fig. 1). A Niskin bottle rosette sampler equipped with a conductivity-temperature-depth (CTD) sensor was deployed from the R/V *Cape Hatteras* to collect surface water samples as well as samples from subsurface waters at or near the particle maximum depth, as determined by beam attenuation recorded by the CTD (Fig. 2). At the mouth of the

Chesapeake Bay (Station CBM; 36° 57.72 N, 75° 59.85W; water column depth 22 m) samples were collected at depths of 1 m and 14 m. Offshore Cape Hatteras, North Carolina, samples were collected at 1 m and 58 m (Station CHO; 35° 24.08N, 74° 55.65 W; water column depth 64 m). Station CLN (34 ° 36.15 N, 76°36.23 W; water column depth 14 m) was a near-shore station just south of Cape Lookout, North Carolina. Only surface water (1 m) was collected at Stn. CLN. Processing of water began immediately aboard ship after collection.

Sample Filtration for Activities of Particle-associated Microbial Communities

Triplicate water samples from surface (Stns. CBM, CHO, CLN) and subsurface depths (Stns. CBM and CHO) were gravity-filtered for several hours through 3 µm Isopore membrane filters (Milipore USA) immediately after the CTD rosette was retrieved to capture the particle-associated microbial communities. The specific volume of water filtered through each of the replicate filters ranged from 1.5 to 6 liters; rates reported are normalized in each case to volume filtered. The filters were divided into 8 equal portions using a sterile razor blade. Duplicate portions from one filter were used for the measurement of three polysaccharide hydrolase activities; duplicate portions from a second filter were used to measure the activities of three other polysaccharide hydrolases. Duplicates from the final filter were used to measure the activities of α - and β -glucosidase, and leucine aminopeptidase, as described below.

Measurements of Extracellular Enzyme Activities

Activities of α - and β -glucosidases and peptidase

Activities of exo-acting (terminal-unit cleaving) β - and α -glucosidases were measured using 4-methylumbelliferone- (MUF-) labeled β - and α -glucose, respectively. Leucine aminopeptidase activity was measured with 4-methylcoumarinyl-7-amide- (MCA-) labeled

leucine; all measurements were made after Hoppe (1983).

Triplicate samples of water from each station and depth were amended with MUF- α -D-glucoside (final concentration 200 μ M), MUF- β -D-glucopyranoside (final concentration 400 μ M), and leucine-MCA (final concentration 300 μ M). These concentrations were determined prior to the first station, from a saturation curve made using surface seawater collected underway, to determine the saturation concentration of each substrate; the measurements made reflect $V_{\max}$. Killed controls consisted of autoclaved sample water to which substrate was added. Whole water samples were incubated for a period extending from 1-12 hours depending on station; an initial time-zero measurement was taken at the start of the incubation period, and two to three subsequent time points were measured. Fluorescence was measured using single-cell fluorometers (Turner Biosystem TBS-380 or a Promega Quantifluor-ST).

For measurement of particle-associated activities, duplicate, one-eighth portions of a 3- μ m pore size filter from each station and depth were mixed with autoclaved water from the corresponding station and depth, then amended with one of the substrates at the concentrations listed above. Killed controls for these samples consisted of 1/8th of a new, clean 3 μ m pore-size filter that had been divided with a sterile razor blade. The filter piece was mixed with autoclaved sample water from the corresponding station and depth and then amended with substrate. These samples were incubated and sampled over the same time courses as the live samples.

Fluorescence signals were converted to concentrations using a dilution curve made with each fluorophore in autoclaved sample water; hydrolysis rates were calculated using the conversion factors and fluorescence measurements.

Activities of polysaccharide hydrolases

Activities of endo-acting polysaccharide hydrolases were measured using six different fluorescently labeled (FLA-) polysaccharides (Arnosti 1995; 2003); arabinogalactan (arabinose and galactose), chondroitin (sulfated N-acetylgalactosamine and glucuronic acid), fucoidan (sulfated fucose), xylan (xylose), laminarin (β (1,3)-glucose), and pullulan (α (1,6)-linked maltotriose (glucose)) were all obtained from Sigma-Aldrich and were labeled with fluoresceinamine as described in Arnosti (2003). These polysaccharides were selected as substrates because they are either marine-derived, and/or the activities of enzymes hydrolyzing these substrates have been demonstrated to occur in marine bacteria (e.g. Painter 1983; Arnosti and Repeta 1994; Araki et al. 2000; Alderkamp et al. 2007; Wegner et al. 2013). At all three stations, duplicate live water samples, duplicate 3 μ m filter+autoclaved water samples, and an autoclaved control water sample were separately amended with one of the six substrates to a final concentration of 175 nM monosaccharide equivalent. A time-zero measurement was immediately taken, all samples were subsequently incubated in the dark, and subsamples were collected after ca. 2 days, 5 days, 10 days, 15 days, and 27 days incubation. Measurements of hydrolysis rates are made as a time course because detection of hydrolysis is based upon a change in the molecular weight distribution of the added polysaccharides, and the time point at which sufficient substrate is hydrolyzed to detect a change is unknown prior to analysis (see Arnosti (2003) for further discussion). Time-points were collected by filtering 1-3 ml of sample water through a 0.2 μ m pore-size cellulose acetate-membrane + GF-prefilter syringe filter (Sartorius Stedim Biotech, Germany); samples were frozen at -20°C until further analysis. Note that the filtered samples for from the particle-maximum depth at Stn. CBM were lost, so no data on polysaccharide hydrolase activities are available from this depth and station. The extent of hydrolysis of each sample was determined by measuring molecular weight distribution of the

FLA-labeled polysaccharide using gel permeation chromatography, as described in detail in Arnosti (2003). Hydrolysis rates for each substrate were then calculated in Excel (Microsoft 2011) from the changes in substrate molecular weight distribution (Arnosti 1995; 2003).

Bacterial Cell Counts and Bacterial Protein Production

Water samples were fixed aboard ship with buffered formaldehyde and kept refrigerated aboard ship and during transport back to UNC-Chapel Hill. Cell counts were made after staining with 4', 6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich USA) prior to counting under a fluorescence microscope, after Porter and Feig (1980). Slides were prepared by filtering 10 - 20 ml of stained water through duplicate 0.2 μ m black polycarbonate filters (Millipore USA). Slides were counted under an epifluorescence microscope (Olympus U-RFL, Olympus USA) using MetaMorph Microscopy software (Molecular Devices USA); 10 fields of view were counted per slide, with duplicate slides made for water depth at each station.

Bacterial protein production was measured by ^3H -leucine incorporation (Kirchman et al. 1985; Kirchman 2001). Water from each depth at each station, along with autoclaved control water, was amended aboard ship with ^3H -leucine to a final concentration of 20 nM. Samples were incubated for 1-2 hours; following this incubation period, reactions were terminated using 100% trichloroacetic acid (TCA). Samples were then concentrated and washed with 80% ethanol following Kirchman (2001) before drying over night. Samples were then amended with scintillation liquid and allowed to sit for a 2-day period before analysis in a scintillation counter (Perkin Elmer TriCarb 3110 TR). ^3H -leucine incorporation was converted to bacterial protein production using the factor of 1.5 kg C per mol leucine, following Kirchman (2001).

Total Dissolved Carbohydrates and Total Suspended Material

For analysis of total dissolved carbohydrates (TDCHO), water samples collected aboard ship at surface (Stns. CBM, CHO, CLN) and subsurface depths (Stns. CBM, CHO) were filtered through 0.2 µm cellulose acetate-membrane + GF-prefilter syringe filter (Sartorius Stedim Biotech, Germany) into pre-combusted glass scintillation vials and frozen at -20°C until further analysis.

Total carbohydrate concentrations were determined from the filtered, frozen water samples using the phenol-sulfuric acid assay (Dubois et al. 1956). Aliquots of the thawed sample water were combined with concentrated, commercial-grade sulfuric acid and 5% phenol solution and absorbance was measured at 490 nm using a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific, USA). The carbon concentration of TDCHO was calculated assuming that all carbohydrates were comprised of six-carbon monomers.

To determine total suspended material, separate one-liter bottle samples were collected at each station and depth and vacuum-filtered through a pre-weighed 0.22 µm pore-size nitrocellulose filters. After filtering, samples were flushed with 1L of deionized water to remove salts. The filters were then dried in a 40°C oven for 48 h and weighed again to determine the total suspended material concentration.

Correlation analysis

Pearson's correlation coefficients (r) between water temperature and bacterial activity parameters were calculated in Excel® using the data analysis tool pack (open source add-in). The Student's t-test was used to determine the significance of the r values.

Results

Physical and chemical characteristics of sampling sites

Stations CBM, CHO, and CLN were characterized by notable differences in physical and chemical characteristics. Station CBM, at the mouth of Chesapeake Bay, had a cold fresh (16.4° C and 29.3 PSU) water mass overlaying a slightly warmer and more saline bottom layer (16.8° C and 30.3 PSU at the sample depth of 14 m). Waters at this station were also comparatively turbid, as indicated by the beam attenuation data (Fig. 2) and the relatively high concentration of total suspended material, 2.31 mg L⁻¹ and 2.41 mg L⁻¹ in the surface and bottom waters, respectively (Table 1). At Stn. CHO, surface water salinity and temperature (36.2 PSU and 26.4°C, respectively; Fig. 2) were consistent with origin from a Gulf-Stream eddy. Surface waters were quite distinct from the underlying waters, sampled at 58 m, with temperature and salinity of 21.3° C and 35.2 PSU, respectively. There was little suspended material at this station, as indicated by high percent transmittance (Fig. 2) and low total suspended material in the surface layer (0.25 mg L⁻¹; Table 1). Waters in the bottom layer were somewhat more turbid, and had a higher total suspended material concentration (1.75 mg L⁻¹). Station CLN was close inshore but had only moderate freshwater contribution, as indicated by the salinity of 34.7 PSU and temperature of 19.0° C. The water was comparatively turbid, however, as shown by the percent transmittance data, and total suspended material mass of 1.56 mg L⁻¹.

Total dissolved carbohydrate (TDCHO) concentrations were highest in surface waters at Stn. CLN (85.8 µM), and lowest in subsurface waters at Stn. CBM (37.4 µM). TDCHO

concentration in surface waters of Stn. CBM was 57.4 μM ; at Stn. CHO, TDCHO concentrations were 50.7 and 55.7 μM in surface and subsurface waters, respectively.

Bacterial Abundance and Protein Production

Bacterial abundances were similar among the three stations and depths, ranging from 1.38 to 1.83 $\times 10^8$ cells liter⁻¹. At Stn. CBM, bacterial abundance was greater in surface waters than at the particle maximum; at Stn. CHO, bacterial abundance was nearly the same in surface and particle maximum waters (Table 2).

Unlike bacterial abundance, bacterial protein production varied greatly between stations and depths, on a per-cell as well as per-liter basis (Table 2). Stn. CLN had the highest bacterial protein production (on a per-cell as well as per-liter basis) of all the surface waters, at 287 $\times 10^{-8}$ ng C cell⁻¹ hour⁻¹ and 525 ng C liter⁻¹ hour⁻¹, respectively. These rates were nearly double the bacterial protein production in Stn. CBM surface waters, and over 20 times greater than surface water bacterial protein production at Stn. CHO. Bacterial protein production rates at the surface were slightly higher than in subsurface waters for both Stns. CBM and CHO; bacterial protein production in subsurface waters at Stn. CHO was the lowest of all of the stations, on both a volume and per-cell basis (13.4 ng C L⁻¹ and 0.098 fg C cell⁻¹ h⁻¹, respectively; Table 2).

Extracellular Enzyme Activities

Summed and particle-associated glucosidase and peptidase activities

Activities of exo-acting enzymes - enzymes that cleave the terminal units from oligo- and polymers - were assessed using short-term incubations with labeled substrate proxies. These incubations were initiated immediately upon sample collection, providing an indication of

hydrolytic capabilities of microbial communities shortly after collection. The measurements of specific endo-acting (mid-chain cleaving) enzymes with fluorescently-labeled polysaccharides require longer incubation times, and thus integrate responses to substrate addition such as enzyme induction, cell growth, and changes in microbial community composition. All rates reported here should be considered potential rates, since added substrate is in competition with naturally occurring substrates for enzyme active sites.

Summed glucosidase (sum of α - and β - glucosidase) activities were highest at Stn. CBM (ca. 8-9 nmol L⁻¹ h⁻¹ in surface and subsurface waters) and lowest at Stn. CHO (below 0.25 nmol L⁻¹ h⁻¹ in surface and subsurface waters) (Fig 3). The same trends and relationships for summed glucosidase activities generally held on a per-cell basis at all stations and depths (Table 2). Large particle associated (captured on the 3 μ m pore-size filter) activity was a comparatively small fraction (7% and 10%) of summed surface and subsurface glucosidase activity, respectively, at Stn. CBM. Although the total suspended material was considerably lower at Stn. CHO than at Stn. CBM, at Stn. CHO, ca. 43% of the (low) glucosidase activity was particle-associated in surface waters, and almost 20% was particle-associated in subsurface waters (Table 1). At Stn. CLN, total suspended material was considerably higher than at Stn. CHO, and the particle-associated fraction constituted 39% of summed glucosidase activity in surface waters (Fig. 3, Table 1).

Similar to the pattern for summed glucosidase on a volume basis, summed glucosidase normalized to cell counts was highest at Stn. CBM (higher in subsurface than in surface waters), and lowest in surface waters of Stn. CHO (Table 2). Summed glucosidase activities normalized to cell counts in subsurface waters of Stn. CHO were a factor of ca. 6 higher than in surface

waters of the same station; the normalized rates at Stn. CLN were 25 atto mol cell⁻¹ h⁻¹, lower than at Stn. CBM but considerably higher than at Stn. CHO (Table 2).

Leucine aminopeptidase activities followed a pattern similar to that of summed glucosidase activities: highest activities at Stn. CBM, lowest activities at Stn CHO, with intermediate activities at Stn. CLN (Fig. 3)—but relationships between activities in surface and subsurface waters, as well as the fractions of activity associated with particles were different for glucosidase and peptidase activities. Maximal leucine aminopeptidase activities in subsurface waters of Stn. CBM were 416 nmol L⁻¹ h⁻¹, more than three times higher than in surface waters of the same station. Peptidase activity in surface waters of Stn. CLN (ca. 112 nmol L⁻¹ h⁻¹) was comparable to that in surface waters of Stn. CBM, and approximately an order of magnitude higher than peptidase activity in surface or subsurface waters of Stn. CHO.

Little of the peptidase activity was associated with particles at Stn. CBM: 10% of surface and 6.9% of subsurface water (Table 1). At Stn. CHO, only 14% of surface activity, but 74% of peptidase activity in subsurface water was particle-associated. At Stn. CLN, in surface waters 34% of peptidase activity was particle-associated, similar to summed glucosidase activity. Particle-associated peptidase activity normalized to particle mass (total suspended material) was also highest in surface waters of Stn. CLN, similar to the observation for mass-normalized glucosidase activity (Table 1), and was lowest in surface waters of Stn. CBM.

Whole-water and particle-associated polysaccharide hydrolase activities

Summed polysaccharide hydrolysis rates (the sum of all six polysaccharide hydrolase activities) at the initial timepoint were highest in the surface waters at Stn. CBM (51 nmol L⁻¹ hour⁻¹, Fig. 4), slightly higher than in the surfaces waters at Stn. CLN, and nearly 4 times higher

than in surface waters of at Stn. CHO. In subsurface waters of Stn. CHO, summed hydrolysis was comparable to surface waters at Stns. CBM and CLN, and more than a factor of 3.5 higher than in surface waters at Stn. CHO. Summed hydrolysis rates in subsurface waters at Stn. CBM were ca. 20% lower than in surface waters of the same station. Particle-associated hydrolysis contributed substantially (from 32-55% of summed hydrolysis) in surface waters of all stations (Fig. 4). At Stn. CHO (the only site for which particle-associated data from subsurface waters are available), the contribution of particle-associated hydrolysis was much lower, only 15% (Fig. 4).

Contributions of individual polysaccharides to the summed rates differed substantially between Stn. CHO and the other stations, as well as between whole water and particle-associated fractions. In whole water from all stations except surface waters of Stn. CHO, high rates of chondroitin hydrolysis dominated the summed polysaccharide rates (Fig. 4). At Stn. CHO, summed hydrolysis rates in surface waters were considerably lower than at the other stations (13.4 compared to ca. 49.6 nmol L⁻¹ h⁻¹), and fucoidan and xylan hydrolysis contributed substantially to the summed rate, with lower contributions of pullulan and laminarin hydrolysis. In subsurface waters of Stn. CHO, chondroitin hydrolysis was a major component, but laminarin and xylan also contributed substantially to summed hydrolysis (Fig. 4). In whole water, little to no arabinogalactan hydrolysis was measurable at the initial timepoint at any station or depth; fucoidan hydrolysis contributed substantially to summed hydrolysis only in surface water at Stn. CHO. Contributions of individual polysaccharides were somewhat different for the particle-associated fractions: in particular, a wider range of polysaccharide hydrolase activities contributed to the summed rate, and relative contributions of each activity were comparatively similar to one another (i.e., hydrolysis rates were more even). In surface waters, five (Stns. CBM

and CHO) or six (Stn. CLN) substrates contributed to summed hydrolysis, and in subsurface waters five substrates contributed to summed hydrolysis.

Patterns of enzyme activities evolved with incubation time (Fig. 5). Ultimately, all six substrates were hydrolyzed at almost all stations and depths, but the time courses of hydrolysis, and the timepoints at which specific enzyme activities were detected varied considerably among depths and stations. At Stn. CBM, hydrolysis patterns evolved similarly in surface and subsurface waters: hydrolysis of xylan and laminarin increased considerably between the 2 day and 6 day timepoints, but increases in the hydrolysis rates of arabinogalactan and fucoidan were only notable after 10-15 days' incubation (Fig. 5a). The pattern of hydrolysis in the particle-associated surface waters of Stn. CBM, in contrast, changed little with time (Fig. 5a).

At Stn. CHO, a broader spectrum of enzyme activities was measurable in surface and subsurface waters even at the initial timepoint (Fig. 5b). In surface waters the time courses and patterns of hydrolysis in particle-associated and whole water were quite similar, with a comparatively broad spectrum of enzyme activities even at the initial time point. The subsurface waters also showed a broad spectrum of activities, with all six activities measurable at the initial timepoint, although the contributions of laminarin and xylan hydrolysis were higher than those of other enzyme activities.

Stn. CLN showed a distinct pattern of activities in surface waters. The contribution of chondroitin hydrolysis to the summed rate was dominant at 1.4 days, with an increasing contribution of xylan hydrolysis after 5 days. The contribution of arabinogalactan and fucoidan (first evident after 10 days) hydrolysis increased through day 15, and pullulan hydrolysis was only measurable after 27 days incubation. The particle-associated fraction of surface water,

however, showed all six polysaccharide hydrolase activities also at day 5 (the initial timepoint at which a sample was collected.)

Discussion

Depending on the balance between organic carbon input, processing, and transport, continental margins have the potential to export organic carbon to offshore environments (Lonborg and Alvarez-Salgado, 2012). The Mid-Atlantic Bight in particular has been intensively studied as a site of potential POC burial and DOC export to the offshore ocean (Anderson et al. 1994; Hopkinson et al. 2002; Vlahos et al. 2002). These studies have included investigations of DOC and carbohydrate concentration, composition, and consumption by heterotrophic communities (Aluwihare et al. 2002; Witter and Luther, 2002). Few previous studies in this region, however, have focused on measurements of specific heterotrophic activities of microbial communities. Here we examine the relationships among different measures of microbial heterotrophic activities, and estimate heterotrophic microbial contributions to DOC processing at three distinct locations. As described below, although estimated carbohydrate processing times are highly variable among substrates, depths, and stations, they are sufficiently long that dissolved carbohydrates may contribute substantially to DOC export from the shelf to the ocean's interior.

Bacterial protein production, peptidase, and glucosidase activities

Heterotrophic activities and bacterial protein production varied markedly among stations and depths. Bacterial protein production in surface waters of Stn. CLN was more than a factor of 30 higher than in surface waters of Stn. CHO, and was close to twice that measured in surface

waters of Stn. CBM. The high bacterial protein production measured at Stn. CLN was comparable to rates of bacterial protein production measured at a number of highly productive sites, including coastal waters of the Gulf of Mexico that are influenced by the outflow plume of the Mississippi River (Steen et al., 2012), and upwelling waters of the coastal North Atlantic (Baltar et al. 2007). Bacterial protein production at Stn. CHO, in contrast, was markedly low, considerably lower than at the surface or within the deep chlorophyll maximum in the Gulf of Mexico (Steen et al. 2012), but comparable to rates of bacterial protein production in the open ocean surface waters of the North Atlantic (Baltar et al. 2007), including subtropical and temperate surface waters (Hoppe et al. 2006). Overall, the rates measured at Stn. CHO are on the lower edge of the data compiled by Kirchman et al. (2009) for the North Atlantic Ocean.

The differences in bacterial protein production (as measured on a volume basis) between Stns. CLN and CHO cannot be ascribed to difference in microbial population sizes, since bacterial abundance was generally comparable at all three stations, and cell-specific protein production also varied accordingly (Table 2). Instead, these data indicate lower protein productivity on a single-cell basis, especially in surface waters of Stn. CHO (Table 2). Cell-specific bacterial protein production differed by more than an order of magnitude between surface waters of Stns. CHO and CBM, and by more than a factor of 20 between surface waters of Stns. CHO and CLN (Table 2). These more-than-order of magnitude differences in surface waters are comparable to those observed in a surface water transect from upwelling coastal waters off Northwest Africa towards the Canary Islands (Baltar et al. 2007).

Peptidase activities also showed considerable differences between depths and stations. Highest peptidase activities were measured in subsurface waters of Stn. CBM; peptidase activities in surface waters of Stns. CBM and CLN were similar to one another, but considerably

lower than in subsurface waters of Stn. CBM, and peptidase activities at Stn. CHO were lower than at any other station (Fig. 3). Peptidase activities comparable to those of surface waters at Stns. CBM and CLN have been reported from other coastal environments, including waters off Chile (Pantoja et al. 2011) and the California coast (Allison et al. 2012). The high rate measured in subsurface waters at Stn. CBM is comparable to occasional peaks of peptidase activities also measured in surface waters during the three-year time series off the California coast (Allison et al. 2012.) The range of cell-specific peptidase activities ($81\text{--}740\text{ amol cell}^{-1}\text{ h}^{-1}$; attomol, 10^{-18}) for all sites except the subsurface waters of Stn. CBM are in the range of those compiled by Baltar et al. (2009) from productive coastal environments; the extremely high activities measured in subsurface waters of Stn. CBM are beyond this range but are similar to those previously measured in marine snow and aggregates (Baltar et al. 2009).

Summed glucosidase activities, like peptidase activities, were lower in both surface and subsurface waters of Stn. CHO than at either Stn. CBM or at Stn. CLN, but the range of activities at all three stations was comparable to those reported from other coastal environments (Pantoja et al. 2011, Allison et al. 2012). Environmental temperature did not correlate positively with peptidase and glucosidase activities: the warmest waters (Stn. CHO surface waters) showed the lowest rates, while the cooler waters of Stn. CBM showed much higher hydrolysis rates (Fig. 3). From a statistical perspective, summed glucosidase rates in this study correlated inversely with temperature (Pearson's correlation coefficient; $r = 0.93$, $p < 0.05$). The other activities (leucine aminopeptidase, polysaccharide hydrolase, and bacterial protein production) also showed inverse correlations with temperature, but these correlations were not statistically significant (Table 3).

Peptidases and glucosidase enzymes provide substrates of sizes suitable for uptake by heterotrophic microbial communities, substrates that can be used for growth as well as

respiration. The extent to which lower molecular weight substrates derived from the activities of extracellular enzymes are incorporated or respired can vary considerably among organisms (Elifantz et al. 2005; 2007; Kirchman et al. 2009) as well as among environments (Arnosti and Steen, 2013). Glucosidase and peptidase activities at our stations may be loosely coupled, since rates of both classes of enzyme activities normalized to the hydrolysis rate measured in surface waters of Stn. CHO (which was the station and depth with the lowest rates overall) decrease in the order CBM subsurface > CBM surface > CLN surface > CHO subsurface > CHO surface, with ratios generally decreasing by a factor of ca. 2-3 between stations (Table 4). Bacterial protein production, also normalized to Stn. CHO surface water, showed a somewhat different pattern, decreasing in the order CLN surface >> CBM surface > CBM subsurface >>> CHO surface, CHO subsurface, with ratios decreasing by a factor of 2 between Stns. CLN and CBM, and by more than an order of magnitude between Stn. CBM and Stn. CHO (Table 4). In waters with notably high peptidase activities (CBM subsurface), moreover, bacterial protein production was not particularly high. The difference in relative rates of enzymatic hydrolysis and protein production may indicate differences in extent of substrate incorporation vs. respiration, as has been observed in other environments (Arnosti and Steen 2013), or may be due to the activities of freely-dissolved enzymes that are no longer associated with the cells that initially produced them (Steen and Arnosti 2011).

Particle-associated activities

Stns. CBM, CHO, and CLN differed considerably in total suspended material, and most likely also in particle origin/characteristics. Beam attenuation (Fig. 2) as well as total suspended material (Table 1) demonstrated that Stn. CBM in particular had considerable suspended

material. The particles at this station likely differed from those at Stns. CHO and CLN, as shown by the differences in particle-associated enzyme activities (Fig. 3), as well as the fraction of total activity associated with particles (Table 1). At Stn. CBM, total suspended material was highest but the absolute as well as relative contribution of particle-associated activities was quite low. For example, peptidase activities in surface waters of Stns. CBM and CLN were similar, but a much higher fraction (34%, compared to 10%) of this activity was particle-associated at Stn. CLN. Although $\alpha + \beta$ glucosidase activity was lower at Stn. CLN than at Stn. CBM, particle associated activity was higher, on a relative as well as on an absolute basis. In addition, the low $\alpha + \beta$ glucosidase activities in surface waters of Stn. CHO were notably (43%) associated with particles. These differences in the contribution of particle-associated activities to the total, and of total suspended material at each station, are probably related to water mass origin. Stn. CBM, at the mouth of the Chesapeake Bay, likely has considerable quantities of lithogenic sediments that may not be as favorable a microbial habitat as the finer-grained particles suspended in the water column of Stn. CLN, or of (most likely) organic detritus at Stn. CHO.

In many other coastal environments, a high fraction of enzymatic activity is particle-associated. For example, bacterial protein production was associated predominantly with the particle-associated ($>3 \mu\text{m}$) community, and much of the enzyme activity was also particle-associated in the Columbia River estuary (Crump et al. 1998). Likewise, repeated sampling in California coastal waters showed that the majority of β -glucosidase and leucine aminopeptidase activities (59% and 68%, respectively) was associated with a size fraction $> 2.4 \mu\text{m}$ (Allison et al. 2012). For Stn. CBM, however, the very high peptidase activities are not connected to the presence of particles (unless the particles disintegrated and passed through the $3 \mu\text{m}$ filter), since

particle-associated activity is in the same range as at Stn. CLN, where total peptidase activities were not especially high.

The polysaccharide hydrolase activities present a counterpoint to the otherwise comparatively low contribution of particle-associated to total enzyme activities (Fig. 4). In surface waters, a substantial fraction of the activity was particle-associated; only in the subsurface sample (from Stn CHO) was the contribution of particle-associated activities comparable to contributions of $\alpha + \beta$ glucosidase. The differences in the fraction of activities associated with particles for peptidase/glucosidase on the one hand and polysaccharide hydrolases on the other could be due to a difference in distribution of organisms responsible for the production of specific enzymes: the endo-acting enzymes that hydrolyse specific high molecular weight polysaccharides are unevenly distributed among microbial taxa (Martinez et al. 1996; Zimmerman et al. 2013), and organisms with these enzymes may preferentially associate with particles. Moreover, formation of particles and aggregates can change the spectrum of polysaccharide hydrolase activities in seawater (Ziervogel and Arnosti 2008), possibly through processes such as quorum sensing (Hmelo et al. 2011). A high contribution of the $>3 \mu\text{m}$ fraction to the polysaccharide hydrolase activity has also been observed in inshore (within Pamlico Sound) and offshore waters (on the Blake Ridge) southwest of the current investigation (D'Ambrosio et al. 2014).

The differences observed here between glucosidase/peptidase and polysaccharide hydrolase activities could also be a reflection of the time course necessary to detect the activities of specific polysaccharide hydrolases. Measurements of glucosidase and peptidase activities were made on timescales of hours, whereas the initial measurements of particle-associated polysaccharide hydrolases were typically made after 2 days of incubation. These longer time-

courses integrate a range of microbial responses to substrates – potentially including enzyme induction and cellular growth. The several-hour timecourses of glucosidase and peptidase activities, in contrast, likely more closely reflect the capabilities of the microbial communities at the time of sample collection.

The differences between particle-associated and whole-water activities of the individual polysaccharide hydrolases, however, reveal distinctions among stations and phases that are not evident from measurements of α - and β -glucosidase activities. In particular, the observation that at the initial time points, particle-associated samples typically demonstrated a much broader range of activities than the whole water shows that particle-associated communities had the capabilities to access a wide range of substrates (Fig. 5). Potentially, the particle-associated fraction has a greater capability to access and remineralize a broader spectrum of substrates. With time, however, the spectrum of substrates hydrolyzed in whole water also broadened, but the time required to detect measurable activities was considerable: 27 days for pullulan in subsurface waters of Stn. CHO and in surface waters of Stn. CLN, 10 days for arabinogalactan in surface and subsurface waters of Stn. CBM. These time intervals suggest that hydrolysis was dependent on the growth or response of a slow-growing or numerically minor member of the microbial community.

Patterns of polysaccharide hydrolase activities and turnover of carbohydrates: implications for DOC export from the Mid-Atlantic Bight

The distribution of polysaccharide hydrolase activities varies considerably in ocean waters (Arnosti et al. 2011), by location as well as by depth (Steen et al. 2012; D'Ambrosio et al. 2014). Gulf Stream waters sampled somewhat further south of Stn. CHO (off the Blake Ridge)

showed hydrolysis only of laminarin, chondroitin, xylan, and pullulan, in particle-associated fractions as well as in whole water (D'Ambrosio et al. 2014), whereas a station more than a degree of latitude north and slightly to the east of Stn. CHO showed hydrolysis of xylan, fucoidan, laminarin, chondroitin, and arabinogalactan (Steen et al. 2008). At all three stations in the present study, all six polysaccharides were ultimately hydrolyzed upon extended incubation, a pattern that has been previously observed primarily in coastal waters (D'Ambrosio et al. 2014) and in the Gulf of Mexico (Steen et al. 2012). These differences in enzymatic capabilities, which persist through extended incubations (Arnosti 2008), likely reflect fundamental differences in genetic capabilities or gene expression among distinct microbial communities. Recent investigations have revealed water mass- and latitudinal differences in microbial community composition (e.g. Baldwin et al. 2005; Fuhrman et al. 2008; Wietz et al. 2010) and in genetic capabilities, including capabilities related to hydrolytic enzymes (Gomez-Pererira et al. 2012), differences that are consistent with observed differences in enzyme activities (Arnosti et al. 2011; 2012). Coastal-offshore differences in microbial community composition (Baltar et al. 2007; Zinger et al. 2011) may parallel the functional differences observed here.

Differences in enzymatic capabilities may have a major effect on rates and locations of carbohydrate remineralization, and by extension, on the fate of a major component of DOC. Aluwihare et al. (2002) estimated that polysaccharides constituted 50-80% of high molecular weight DOC in the Mid-Atlantic Bight. As discussed below, our data demonstrate that the turnover time of high molecular weight polysaccharides depends critically on molecular structure and by extension, on the enzymatic capabilities of the heterotrophic communities that process and consume DOC. As an example, a theoretical turnover time can be calculated for TDCHO (total dissolved carbohydrates) measured at the different depths and stations. Using data from the

first time point at which we measured polysaccharide hydrolase activities (Fig. 4), the concentration of TDCHO was divided by the most rapid polysaccharide hydrolase activity at each station and by the slowest non-zero activity at each station in order to determine a theoretical turnover time. The time required to hydrolyze the equivalent of the TDCHO pool to monosaccharides ranged from 7 to 70 days for the ‘rapid’ enzymes at all depths and stations to 185 to 2597 days for the ‘slowest’ enzymes at each station (Table 5). Although this calculation is a simplification, as it assumes that all dissolved carbohydrates are amenable to hydrolysis by one class of enzymes, it nonetheless demonstrates that the issue of a ‘match’ or ‘mismatch’ between carbohydrate structure and enzyme structural specificity will profoundly affect the fate of carbohydrate carbon, particularly in view of the estimated 100-day residence time water on the MAB shelf (Druon et al. 2010). Moreover, given the factor of 10 difference in calculated turnover times among the different enzyme activities (even for the most rapid rates), the initial location of carbohydrate-containing DOC—in waters of the Chesapeake Bay mouth, or in a Gulf Stream-derived eddy—will also affect its fate.

Our estimation of theoretical turnover times are also in accordance with experimentally-measured DOC decomposition experiments carried out with water from the Mid-Atlantic Bight. Hopkinson et al. (2002) determined that three-component degradation model—with very labile, labile, and recalcitrant components— fit their experimental data. On average, 71% of the DOC was recalcitrant, 13% was labile, and 16% was very labile, but the fraction of very labile and labile DOC varied considerably by location and season (Hopkinson et al. 2002). Decay rates for the very labile and labile pools consequently also varied by location and time, with calculated half-lives of the very labile and labile pools from 1-14 days and 8-147 days, respectively. Our data are in agreement with Hopkinson et al.’s (2002) suggestion that calculated turnover times of

DOC in the Mid-Atlantic Bight are sufficiently long to allow for DOC export off the shelf.

Models linking water column dynamics and biogeochemical processes in the Mid-Atlantic Bight demonstrate that the location and extent of organic matter degradation has considerable effects on primary productivity and carbon export to the open ocean (Druon et al. 2010). Although heterotrophic prokaryotes are the critical catalysts for DOC transformation, their activities are represented in the model only by a temperature-dependent organic matter degradation function (Druon et al. 2010). Our data suggest that – at least at the time and locations of sampling – rates of enzyme activities and bacterial protein production cannot be parameterized effectively as positive functions of the temperature of the water masses from which our samples were derived. We hypothesize that the observed differences in microbial community function instead reflect differences in microbial community composition and/or gene expression. Developing the means to parameterize accurately differences in microbial community enzymatic capabilities is an ongoing challenge (Mislán et al. 2014). Further investigations of the linkages between microbial community composition, gene expression, and enzymatic function (e.g. Xing et al. 2014) will enable us to understand the variations in carbon-cycling capabilities of microbial communities in different seasons, locations, and depths in the ocean, factors that have major effects on the marine carbon cycle.

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References

- Alderkamp, A.-C., van Rijssel, M. and Bolhuis, H., 2007. Characterization of marine bacteria and the activity of their enzyme systems involved in degradation of the algal storage glucan laminarin. *FEMS Microbiol. Ecol.*, 59: 108-117.
- Allison, S.D., Chao, Y., Farrara, J.D., Hatosy, S. and Martiny, A.C., 2012. Fine-scale temporal variation in marine extracellular enzymes of coastal southern California. *Frontiers in Microbiol.*, 3: doi: 10.3389/fmicb.2012.00301.
- Aluwihare, L.I., Repeta, D.J. and Chen, R.F., 2002. Chemical composition and cycling of dissolved organic matter in the Mid-Atlantic Bight. *Deep-Sea Res. II*, 49: 4221-4437.
- Anderson, R.F., Rowe, G.T., Kemp, P.F., Trumbore, S. and Biscaye, P.E., 1994. Carbon budget for the mid-slope depocenter of the Middle Atlantic Bight. *Deep-Sea Res. II*, 41: 669-703.
- Araki, T., Hashikawa, S. and Morishita, T., 2000. Cloning, sequencing, and expression in *Escherichia coli* of the new gene encoding b-1,3-xylanase from a marine bacterium, *Vibrio* sp. Strain XY-214. *Appl. Environ. Microbiol.*, 66: 1741-1743.
- Arnosti, C., 1995. Measurement of depth- and site-related differences in polysaccharide hydrolysis rates in marine sediments. *Geochim. Cosmochim. Acta*, 59(20): 4247-4257.
- Arnosti, C., 2003. Fluorescent derivatization of polysaccharides and carbohydrate-containing biopolymers for measurement of enzyme activities in complex media. *J. Chromatog. B.*, 793: 181-191.
- Arnosti, C., 2008. Functional differences between Arctic sedimentary and seawater microbial communities: Contrasts in microbial hydrolysis of complex substrates. *FEMS Microb. Ecol.*, 66: 343-351.
- Arnosti, C., 2011. Microbial extracellular enzymes and the marine carbon cycle *Ann. Review of Marine Science*, 3: 401-425.
- Arnosti, C., Fuchs, B., Amann, R. and Passow, U., 2012. Contrasting extracellular enzyme activities of particle associated bacteria from distinct provinces of the North Atlantic Ocean. *Frontiers in Aq. Microbiol.*, 3: 425.
- Arnosti, C. and Repeta, D.J., 1994. Extracellular enzyme activity in anaerobic bacterial cultures: Evidence of pullulanase activity among mesophilic marine bacteria. *Appl. Environ. Microbiol.*, 60(3): 840-846.
- Arnosti, C. and Steen, A.D., 2013. Patterns of extracellular enzyme activities and microbial metabolism in an Arctic fjord of Svalbard and in the northern Gulf of Mexico: Contrasts in processing and fate of carbohydrates. *Frontiers in Aq. Microbiol.*, 4: 318.
- Arnosti, C., Steen, A.D., Ziervogel, K., Ghobrial, S. and Jeffrey, W.H., 2011. Latitudinal gradients in degradation of marine dissolved organic carbon. *PLoS ONE*, 6(12): e28900.
- Baldwin, A.J. et al., 2005. Microbial diversity in a Pacific Ocean transect from the Arctic to Antarctic circles. *Aq. Microb. Ecol.*, 41: 91-102.
- Baltar, F., Aristegui, J., Gasol, J.M., Hernandez-Leon, S. and Herndl, G.J., 2007. Strong coast-ocean and surface-depth gradients in prokaryotic assemblage structure and activity in a coastal transition zone region. *Aq. Microb. Ecol.*, 50: 63-74.
- Baltar, F. et al., 2009. Prokaryotic extracellular enzymatic activity in relation to biomass production and respiration in the meso- and bathypelagic waters of the (sub)tropical Atlantic. *Environ. Microbiol.*
- Bauer, J.E., Druffel, E.R.M., Wolgast, D.M. and Griffin, S., 2001. Sources and cycling of dissolved and particulate organic radiocarbon in the northwest Atlantic continental margin. *Global Biogeochem. Cycles*, 15: 615-636.

- Biscaye, P.E., Flagg, C.N. and Falkowski, P.G., 1994. The shelf edge exchange process experiment, SEEP-II: an introduction to hypotheses, results and conclusions. *Deep-Sea Res. II*, 41: 231-252.
- Carlson, C.A. et al., 2004. Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea. *Limnol. Oceanogr.*, 49: 1073-1083.
- Crump, B.C., Baross, J.A. and Simenstad, C.A., 1998. Dominance of particle-attached bacteria in the Columbia River estuary, USA. *Aq. Microb. Ecol.*, 14: 7-18.
- D'Ambrosio, L., Ziervogel, K., MacGregor, B., Teske, A. and Arnosti, C., 2014. Composition and enzymatic function of particle-associated and free-living bacteria: a coastal/offshore comparison. *The ISME J.*, 8: 2167-2179.
- Druon, J.N. et al., 2010. Modeling the dynamics and export of dissolved organic matter in the Northeastern U.S. continental shelf. *Est. Coast. Shelf Sci.*, 88: 488-507.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.T. and Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Analyt. Chem.*, 28: 350-356.
- Elifantz, H., Dittel, A.I., Cottrell, M.T. and Kirchman, D.L., 2007. Dissolved organic matter assimilation by heterotrophic bacterial groups in the western Arctic Ocean. *Aq. Microb. Ecol.*, 50: 39-49.
- Elifantz, H., Malmstrom, R.R., Cottrell, M.T. and Kirchman, D.L., 2005. Assimilation of polysaccharides and glucose by major bacterial groups in the Delaware Estuary. *Appl. Environ. Microbiol.*, 71: 7799-7805.
- Filippino, K.C., Mulholland, M.R. and Bernhardt, P.W., 2011. Nitrogen uptake and primary productivity rates in the Mid-Atlantic Bight (MAB). *Est. Coast. Shelf Sci.*, 91: 13-23.
- Fuhrman, J.A. et al., 2008. A latitudinal diversity gradient in planktonic marine bacteria. *PNAS*, 105(22): 7774-7778.
- Gomez-Pereira, P.R. et al., 2012. Genomic content of uncultured Bacteroidetes from contrasting oceanic provinces in the North Atlantic Ocean. *Environ. Microb.*, 14: 52-66.
- Hedges, J.I. and Keil, R.G., 1995. Sedimentary organic matter preservation: and assessment and speculative synthesis. *Mar. Chem.*, 49: 81-115.
- Hmelo, L.R., Mincer, T.J. and Van Mooy, B.A.S., 2011. Possible influence of bacterial quorum sensing on the hydrolysis of sinking particulate organic carbon in marine environments. *Environ. Microbiol. Reports*, 3: 682-688.
- Hopkinson Jr., C.S., Vallino, J.J. and Nolin, A., 2002. Decomposition of dissolved organic matter from the continental margin. *Deep-Sea Res. II*, 49: 4461-4478.
- Hoppe, H.-G., 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Mar. Ecol. Prog. Ser.*, 11: 299-308.
- Hoppe, H.-G., Gocke, K., Koppe, R. and Kraus, G., 2006. Changing bacterioplankton growth characteristics on a large spatial scale: oligotrophic versus mesotrophic ocean. *Mar. Ecol. Prog. Ser.*, 323: 21-33.
- Keith, S.C. and Arnosti, C., 2001. Extracellular enzyme activity in a river-bay-shelf transect: Variations in polysaccharide hydrolysis rates with substrate and size class. *Aq. Microb. Ecol.*, 24: 243-253.
- Kirchman, D., Kneess, E. and Hodson, R., 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Appl. Environ. Microbiol.*, 49(3): 599-607.
- Kirchman, D.L., 2001. Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. *Methods in Microbiol.* 227-238.
- Kirchman, D.L., Moran, X.A.G. and Ducklow, H., 2009. Microbial growth in the polar oceans---role of temperature and potential impact of climate change. *Nature Reviews Microbiology*, 7: 451-459.

- Lohrenz, S.E., Redalje, D.G., Verity, P.G., Flagg, C.N. and Matulewski, K.V., 2002. Primary production on the continental shelf off Cape Hatteras, North Carolina. *Deep-Sea Res. II*, 49: 4479-4509.
- Lonborg, C. and Alvarez-Salgado, X.A., 2012. Recycling versus export of bioavailable dissolved organic matter in the coastal ocean and the efficiency of the continental shelf pump. *Global Biogeochemical Cycles*, 26: 1-12.
- Martinez, J., Smith, D.C., Steward, G.F. and Azam, F., 1996. Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. *Aq. Microb. Ecol.*, 10: 223-230.
- Mislan, K.A.S., Stock, C.A., Dunne, J.P. and Sarmiento, J.L., 2014. Group behavior among model bacteria influences particulate carbon remineralization depths. *J. Mar. Res.*, 72: 1-36.
- Painter, T.J., 1983. Algal Polysaccharides. In: G.O. Aspinall (Editor), *The Polysaccharides*. Academic Press, New York, pp. 195-285.
- Pantoja, S., Gutierrez, M.H., Ampuero, P. and Tejos, E., 2011. Degradation capability of the coastal environment adjacent to the Itata River in central Chile (36.5 S). *Biogeosciences*, 8: 2063-2074.
- Porter, K.G. and Feig, Y.S., 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, 25: 943-948.
- Steen, A.D. and Arnosti, C., 2011. Long lifetimes of β -glucosidase, leucine aminopeptidase, and phosphatase in Arctic seawater. *Marine Chem.*, 123: 127-132.
- Steen, A.D., Hamdan, L. and Arnosti, C., 2008. Dynamics of high molecular weight dissolved organic carbon in the Chesapeake Bay: Insights from enzyme activities, carbohydrate concentrations, and microbial metabolism. *Limnol. Oceanogr.*, 53: 936-947.
- Steen, A.D., Ziervogel, K., Ghobrial, S. and Arnosti, C., 2012. Functional variation among polysaccharide-hydrolyzing microbial communities in the Gulf of Mexico. *Marine Chem.*, 138: 13-20.
- Vlahos, P., Chen, R.F. and Repeta, D.J., 2002. Dissolved organic carbon in the Mid-Atlantic Bight. *Deep-Sea Res. II*, 49: 4369-4385.
- Wegner, C.-E. et al., 2013. Expression of sulfatases in *Rhodopirellula baltica* and the diversity of sulfatases in the genus *Rhodopirellula*. *Marine Genomics*, 9(51-61).
- Wietz, M., Gram, L., Jorgensen, B. and Schramm, A., 2010. Latitudinal patterns in the abundance of major marine bacterioplankton groups. *Aq. Microb. Ecol.*, 61: 179-189.
- Witter, A.E. and Luther III, G.W., 2002. Spectrophotometric measurement of seawater carbohydrate concentrations in neritic and oceanic waters from the U.S. Middle Atlantic Bight and the Delaware estuary. *Marine Chem.*, 77: 143-156.
- Xing, P. et al., 2014. Niches of two polysaccharide-degrading *Polaribacter* isolates from the North Sea during a spring diatom bloom. *The ISME J.: bacteria, marine, degradation, phytoplankton, STAR*.
- Ziervogel, K. and Arnosti, C., 2008. Polysaccharide hydrolysis in aggregates and free enzyme activity in aggregate-free seawater from the northeastern Gulf of Mexico. *Environ. Microbiol.*, 10: 289-299.
- Ziervogel, K. and Arnosti, C., 2009. Enzyme activities in the Delaware Estuary affected by elevated suspended sediment load. *Est. Coast Shelf Sci.*, 84: 253-258.
- Zimmerman, A.E., Martiny, A.C. and Allison, S.D., 2013. Microdiversity of extracellular enzyme genes among sequenced prokaryotic genomes. *The ISME J.*, 7: 1187-1199.
- Zinger, L. et al., 2011. Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. *PLOS one*, 6: e24570.

Station	Total suspended material (TSM) (mg L ⁻¹)	TSM-normalized particle-associated $\alpha+\beta$ glucosidase (nmol mg ⁻¹ h ⁻¹)	TSM-normalized particle-associated peptidase (nmol mg ⁻¹ h ⁻¹)	Particle-associated peptidase	Particle-associated $\alpha+\beta$ glucosidase
CBM surface	2.31	0.228	5.58	10%	6.8%
CBM subsurf.	2.41	0.389	12.0	6.9%	10%
CHO surface	0.25	0.390	6.21	14%	43%
CHO subsurf.	1.75	0.190	5.89	74%	20%
CLN surface	1.56	1.132	24.6	34%	39%

Table 1: Total suspended material (TSM) concentrations; particle-associated glucosidase and peptidase activities, normalized to mass of total suspended material; particle-associated glucosidase, peptidase, and polysaccharide hydrolase activity, as percentage of whole-water activity.

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	Bacterial abundance ($\times 10^8$ cells L ⁻¹)	Bacterial protein production (ng C L ⁻¹ h ⁻¹)	Bacterial protein production per cell (fg C cell ⁻¹ h ⁻¹)	Summed α + β glucosidase (amol cell ⁻¹ h ⁻¹)	Peptidase (amol cell ⁻¹ h ⁻¹)
CBM surface	1.75	290 $\pm$ 10	1.65	44	740
CBM subsurface (14 m)	1.38	212 $\pm$ 41	1.53	65	3020
CHO surface	1.39	16.8 $\pm$ 2.5	0.12	1.6	81
CHO subsurface (58 m)	1.38	13.4 $\pm$ 1.1	0.098	12	100
CLN surface	1.83	525 $\pm$ 73	2.87	25	610

752 Table 2: Bacterial abundance; bacterial protein production (per L); cell-normalized bacterial
753 protein production; cell-normalized summed α - + β - glucosidase activities; cell-normalized
754 peptidase activity; note that amol = 10⁻¹⁸.
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	Temperature	<i>p</i> -value
Summed α + β glucosidase	-0.93	0.023
Peptidase	-0.67	0.218
Summed polysacch. hydrolase	-0.81	0.095
Bacterial protein production	-0.60	0.281

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759 Table 3: Pearson's correlation coefficient (*r*) between temperature, hydrolytic enzyme activities,
760 and bacterial protein production. Values of *r* are significant at $p < 0.05$ (values in bold).

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	Relative glucosidase activity	Relative peptidase activity	Relative protein production
CBM surface	34	12	17
CBM subsurface	40	37	13
CHO surface	1	1	1
CHO subsurface	7.5	1.2	0.8
CLN surface	20	10	31

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Table 4: Glucosidase activity, peptidase activity, and bacterial protein production, all normalized to rates measured in surface waters of Stn. CHO.

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	TDCHO (μM)	Turnover time (days) via most rapid* polysaccharide hydrolase activity	Turnover time (days) via slowest ** polysaccharide hydrolase activity
CBM surface	57.4	11	443
CBM subsurface	37.4	7	2597
CHO surface	50.7	70	185
CHO subsurface	55.7	18	193
CLN surface	85.8	14	851

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* chondroitin hydrolysis for all Stns. except Stn. CHO surface; for Stn. CHO surface, fucoidan hydrolysis

** xylan hydrolysis for Stn. CBM, arabinogalactan hydrolysis for Stn. CLN, laminarin hydrolysis for Stn. CHO surface, fucoidan hydrolysis for Stn. CHO subsurface

Table 5: Theoretical turnover times of total dissolved carbohydrates (TDCHO), assuming hydrolysis by the most rapidly-acting and the slowest measured polysaccharide hydrolases at each station. See text for details.

Fig. 1 Map of station locations: CBM (Chesapeake Bay Mouth), CHO (Cape Hatteras offshore), and CLN (Cape Lookout nearshore)

Fig 2: CTD casts for Stns. CBM, CHO, and CLN. Left to right, lines show temperature, transmittance, and salinity. Note difference in upper and lower axes.

Fig. 3: Peptidase and α - and β -glucosidase activities of surface and subsurface water, and of the particle-associated ($> 3\mu\text{m}$ fraction) at each station and depth. Dotted horizontal lines divide surface and subsurface waters at Stns. CBM and CHO; depths of subsurface waters are 14 and 58 m, respectively. Peptidase axis is log scale, shown above each plot; glucosidase axis is linear scale, shown below each plot.

Fig. 4: Summed hydrolysis rates of the six polysaccharides at Stns. CBM, CHO, and CLN. Data are from the initial sampling point: 2 days for Stns. CBM and CHO, and 1.4 days (surface water) and 5 days (particles) for Stn. CLN. Subsurface depths for Stns. CBM and CHO are 14 and 58 m, respectively. Pull= pullulan, lam = laminarin, xyl = xylan, fu = fucoidan, ara = arabinogalactan, chon = chondroitin sulfate.

Fig. 5: Fractional contributions of each type of polysaccharide hydrolase to the summed activities over time. Pull= pullulan, lam = laminarin, xyl = xylan, fu = fucoidan, ara = arabinogalactan, chon = chondroitin sulfate. a) Stn. CBM (note that 27 day samples were lost for surface and subsurface waters, as well as the 15 and 27 day samples for particle-associated surface water.) b) Stn. CHO c) Stn. CLN (for particle-associated fraction, no sample was collected at 1.4 days; 15 and 27 day samples were lost).

Fig 1

Fig 2

Fig 3

Fig. 4

Fig. 5a

Stn. CBM

Stn. CHO

Fig. 5c