A mechanistic microbial underpinning for the size-reactivity continuum of dissolved organic carbon degradation

C. Arnosti[a,b,*], G. Reintjes[a], R. Amann[a]

[a] Dept. of Molecular Ecology, Max Planck Institute for Marine Microbiology, Bremen, Germany
[b] Dept. of Marine Sciences, Univ. of North Carolina-Chapel Hill, Chapel Hill, NC, USA

ARTICLE INFO

Keywords:
Polysaccharide degradation
Enzymes
Bacteria
DOC cycling

ABSTRACT

The reservoir of dissolved organic carbon (DOC) in the ocean is modified by multiple input and removal processes. Incubation experiments as well as measurements of oceanic DOC have demonstrated that the high molecular weight (HMW) fraction of DOC typically has a younger radiocarbon age and is more reactive biologically than the low molecular weight (LMW) fraction of DOC. These observations have been summarized as a 'size-reactivity continuum' of DOC reactivity, but mechanistic explanations for these observations have been lacking. Here we describe how our recent discovery of 'selfish' HMW organic matter uptake among bacteria in surface ocean waters may help explain the rapid removal of HMW DOC. 'Selfish' substrate uptake by bacteria encompasses rapid binding and partial hydrolysis of intact polysaccharides on the outer membrane of bacteria, seamlessly followed by the transport of large oligosaccharide fragments into the periplasm with little to no loss of LMW hydrolysis products. 'Selfish' bacteria therefore process HMW substrates in a manner distinct from bacteria that carry out extracellular hydrolysis that yields LMW hydrolysis products in the environment. Recognition of the presence and prevalence of selfish bacteria in the ocean has profound implications for carbon flow – the source and quantity of LMW substrates made available to non-extracellular-enzyme producing bacteria – as well as for efforts to model and measure bacterial interactions during organic matter degradation. This discovery also highlights the importance of targeted substrate binding and uptake as key (often understudied) factors in geochemical investigations of microbially driven carbon cycling in the ocean. We conclude with some speculative thoughts about the factors that may determine the prevalence of selfish substrate uptake in the environment.

1. Introduction

Marine dissolved organic carbon (DOC) is one of the largest actively cycling carbon reservoirs on earth. It is comparable in magnitude to atmospheric CO_2 (Hansell, 2013) and thus an essential component of the global carbon cycle. DOC has a multitude of sources, including phytoplankton production, exudation, grazing, viral lysis, solubilization from particulate organic carbon (POC), and riverine input; the major DOC sink in the ocean is consumption by heterotrophic microbial communities (Carlson and Hansell, 2015). Concerted study over the last several decades has added considerably to our knowledge of the DOC reservoir and its cycling. For example, high resolution measurements of DOC at different depths and locations, combined with knowledge of ocean circulation times, have led to estimates of DOC production and decomposition on broad scales (Hansell, 2013). DOC has also been operationally defined to have 'labile', 'semi-labile', and 'refractory' fractions, based on bioassays and on estimations of turnover times in the ocean (Carlson, 2002). Measurement of bulk DOC characteristics such as ^{14}C age and molecular size (e.g. Guo et al., 1996; Walker et al., 2016), as well as direct measurements of bacterial growth and respiration (Amon and Benner, 1994, 1996), have demonstrated that the high molecular weight (HMW) DOC fraction is generally younger and more biologically reactive than the low molecular weight (LMW) fraction, observations that have led to the idea of the 'size-reactivity continuum' (Amon and Benner, 1996; Benner and Amon, 2015), the concept that the reactivity of DOC decreases with decreasing molecular size.

Beyond these observations and measurements, however, the specific factors controlling the rate, location, and extent to which DOC is transformed and remineralized by heterotrophic microbial communities in the ocean are still not well understood. In accordance with its average ^{14}C age, HMW DOC is considered 'fresher' (Amon and Benner,

* Corresponding author at: Dept. of Marine Sciences, CB #3300, 3117A Venable Hall, University of North Carolina-Chapel Hill, Chapel Hill, NC 27599-3300, USA.
E-mail address: arnosti@email.unc.edu (C. Arnosti).

https://doi.org/10.1016/j.marchem.2018.09.008
Received 24 May 2018; Received in revised form 26 August 2018; Accepted 19 September 2018
Available online 20 September 2018

1996) – more recently produced (Walker et al., 2016) – than LMW DOC, but specific compositional or structural features of the HMW DOC pool that might account for this enhanced reactivity are undefined. This lack of knowledge is due in part to the fact that HMW DOC defies the types of detailed structural analyses that have been used to characterize extractable LMW DOC (e.g., Koch et al., 2005; Kujawinski et al., 2009). The HMW DOC fraction has been characterized using chemical measurements that provide information about monomeric constituents (Benner et al., 1992; Kaiser and Benner, 2009) and NMR measurements that provide information about bulk structure (Aluwihare et al., 1997; Hertkorn et al., 2006), but these analyses yield no information on the order in which constituents are linked together, or about the three-dimensional structure of the intact HMW DOC. We know, therefore, that a substantial fraction of HMW DOC consists of carbohydrates, including neutral sugars, and that its concentration is lower in the deep ocean than in the upper mesopelagic/ surface ocean (Benner and Amon, 2015). Important structural features of this HMW DOC are elusive.

DOC structure requires critical consideration, however, given the importance of heterotrophic microbes in DOC cycling. Prokaryotic processing of HMW DOC requires structurally selective extracellular enzymes to initially hydrolyze a substrate prior to uptake (Arnosti, 2011): enzyme-substrate 'fit' is central to HMW DOC remineralization. Information about these enzymes in the ocean has been obtained through studies that have highlighted the diversity and succession of genes corresponding to hydrolytic enzymes and substrate transporters that characterize different phytoplankton bloom phases (Teeling et al., 2012, 2016) and distinguish microbial communities at distinct locations (Gomez-Pereira et al., 2012; Landa et al., 2016). These field studies are complemented by studies of bacterial isolates that have provided essential information about differences in the physiology and substrate usage of phylogenetically related species (e.g. Wegner et al., 2013; Kabisch et al., 2014; Xing et al., 2014).

Although such metagenomic, genomic, microbiological and biochemical approaches have yielded new information about the enzymatic potential of organisms and communities, experimental methods to measure extracellular enzyme activities in the field have not kept pace. The most widespread methods to measure enzymatic hydrolysis rates use small substrate proxies, in which a monomer (typically glucose or leucine) is hydrolyzed, yielding a free fluorophore (e.g. Hoppe, 1983; Piontek et al., 2014); hydrolysis is measured as an increase in fluorescence. This method is robust, straightforward, and facilitates data comparison across time and environments, but it does not yield information about the structural specificities of enzymes that hydrolyze HMW substrates, nor does it measure the activities of midchain-cleaving (endo-acting) enzymes essential to the hydrolysis of HMW substrates (Warren, 1996). Moreover, measurements made with a single substrate proxy may in fact integrate the activities of multiple enzymes (Steen et al., 2015). We thus have a considerable gap between knowledge of gene sequences and proteins on the one hand, and measurements of the activities and structural specificities of enzymes that hydrolyze HMW substrates in marine waters on the other.

An alternative approach to measure and compare the enzymatic hydrolysis rates of structurally-distinct HMW substrates relies on the use of fluorescently-labeled (FLA-) polysaccharides. By covalently linking fluorophores to specific HMW polysaccharides or plankton extracts, hydrolysis can be measured as the change in the molecular weight distribution of the polysaccharides with time (Arnosti, 2003). Measuring hydrolysis of polysaccharides in the ocean is highly relevant to HMW DOC degradation, since polysaccharides constitute a high percentage of marine organic matter (Cowie and Hedges, 1984; Skoog and Benner, 1997; Biersmith and Benner, 1998) including DOC (Benner et al., 1992).

We have measured polysaccharide hydrolysis rates across a wide range of sites in the surface ocean (Arnosti et al., 2011), and in a limited number of depth profiles (e.g. Steen et al., 2012; Hoarfrost and Arnosti, 2017; Balmonte et al., 2018). These measurements have revealed large-scale patterns in microbial enzyme activities: the spectrum (number of different types) of polysaccharides hydrolyzed decreases from temperate to polar latitudes, from coastal to open-ocean waters, and with depth in the ocean (Arnosti et al., 2011; Steen et al., 2012; D'Ambrosio et al., 2014; Hoarfrost and Arnosti, 2017; Balmonte et al., 2018). These broad-scale gradients in activities are also consistent with broad-scale gradients in the composition of microbial communities (Pommier et al., 2007; Fuhrman et al., 2008; Zinger et al., 2011; Arnosti et al., 2012), suggesting that there are underlying connections between the composition of microbial communities and their enzymatic functions. The nature and types of HMW substrates that are remineralized in a given location thus is a function of microbial community capabilities, as well as organic matter structure.

2. A widespread, previously overlooked mechanism of HMW substrate uptake in the ocean

In order to investigate more closely patterns in microbial community composition and enzymatic function, during a latitudinal transect along the Atlantic Ocean we incubated seawater with fluorescently-labeled polysaccharides (Reintjes et al., 2017; revised). We intended to track changes in microbial community composition as polysaccharides were hydrolyzed in shipboard incubations, but we ended up with far more than we bargained for: fluorescence microscopy at sea demonstrated that a considerable fraction of the total cells showed staining by the fluoresceinamine (FLA)-labeled polysaccharides added to the incubations (Fig. 1). This result was surprising in many ways: we had not expected that bacteria would take up the fluorescent tag along with part of the polysaccharide. Furthermore, since each fluorescent polysaccharide bears only a few fluorophores (Arnosti, 2003), the intensity of the fluorescent staining means that a considerable quantity of HMW substrate must have been transported into the cell. Moreover, the staining with FLA-polysaccharides was rapid – initial staining was visible after just 30 min (Fig. 1a), and generally increased with incubation time (Fig. 1b-d). Subsequent microscopic examination using super-resolution structured illumination microscopy (Schermelleh

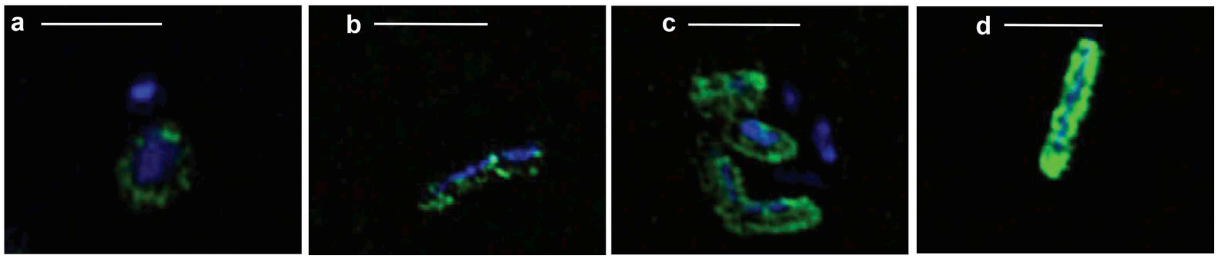

Fig. 1. Time series super-resolution structured illumination microscopy images of bacterial cells from the Northern Atlantic Ocean showing uptake of fluorescently labeled laminarin (green). Cells are counter stained with DAPI (blue). Cells were samples after a) 30 min, b) 3 days, (c) 6 days, and d) 12 days, and show an increase in substrate staining over time. Scale bar = 1 μm. (reproduced from Reintjes et al., 2017).

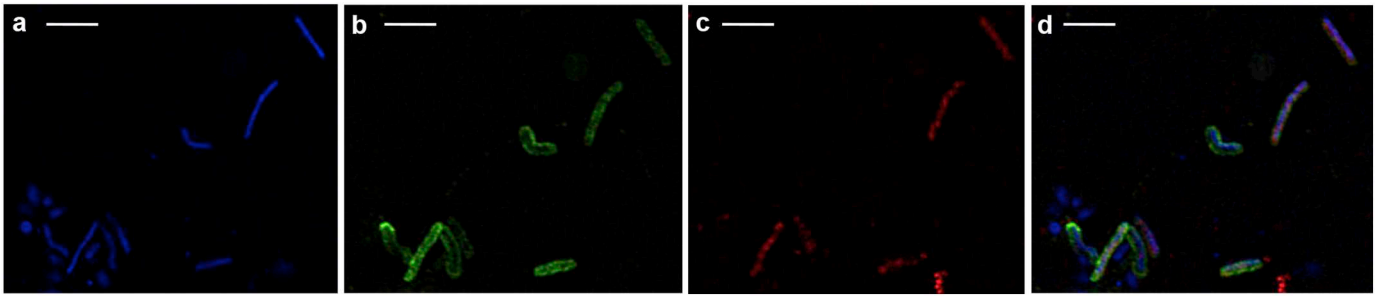

Fig. 2. Super-resolution structured illumination microscopy images of environmental *Bacteroidetes* cells showing uptake of FLA laminarin. (a) DNA stained by DAPI (blue), (b) fluorescently labeled laminarin (green), (c) identification of *Bacteroidetes* cells using group specific FISH probe (CF319a, red), (d) overlay image. Scale bar = 2 μm.

et al., 2010) showed that the stain was localized within the periplasmic space, not just stuck to the outer membrane. Furthermore, HMW substrate uptake is an active process: killed controls showed no staining (Reintjes et al., 2017).

Using fluorescence in situ hybridization (FISH), we identified some of the bacteria stained with FLA-polysaccharides as members of phylum *Bacteroidetes* (Fig. 2). This information indicates a potential mechanism for HMW substrate uptake: intestinal members of the *Bacteroidetes* have been identified as using a mode of uptake in which polysaccharides are bound to the outer membrane, hydrolyzed, and transported as large fragments into the periplasmic space with little or no release of hydrolysis product into the external medium (Cuskin et al., 2015; Fig. 3). This type of substrate uptake in members of the *Bacteroidetes* has been linked to the presence of distinct sets of binding proteins, hydrolytic enzymes, and cross-membrane transporters (Martens et al., 2009; Koropatkin et al., 2012). Those *Bacteroidetes,* from the class *Bacteroidia,* however, are part of the gut microbiome (Rakoff-Nahoum et al., 2014; Rogowski et al., 2015), an anoxic, warm, organic carbon-rich environment that is densely populated with bacteria: in other words, an environment very different from the surface ocean. The mechanism itself is thus not unknown, but the fact that a considerable fraction (up to 26%; Fig. 4) of the pelagic bacterial community in the sparsely populated, oxic, cool, comparatively carbon-poor waters of the surface ocean should use such a mechanism was completely unexpected. Furthermore, an analogous mechanism of periplasmic substrate processing was recently demonstrated for the gram-negative bacterial phylum *Planctomycetes* (Boedeker et al., 2017; Fig. 4). A notable fraction of the substrate-stained cells, moreover, was not identifiable with the selected set of FISH probes that we used (Fig. 4). This result indicates that uptake of large fragments of polysaccharides extends well beyond these two phyla, and likely includes other gram-negative bacteria that possess an outer membrane as well as a cellular membrane. Intriguingly, all six of the polysaccharides that we tested were taken up by at least some bacterial cells (Reintjes et al., revised), indicating that this mode of substrate acquisition is used to take up a considerable variety of polysaccharides. We thus have evidence for a previously unrecognized mode of organic matter acquisition in the surface ocean, a mode of substrate acquisition that has been termed 'selfish', since the gut bacteria in which this mechanism has been identified do not share hydrolysis products with other organisms (Cuskin et al., 2015).

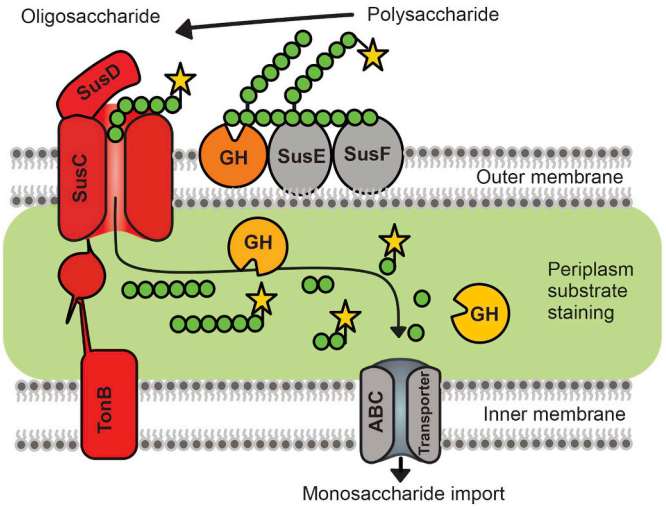

Fig. 3. Hypothesized mechanism of 'selfish' polysaccharide uptake. The recognition, binding, and initial hydrolysis of the polysaccharide occurs at the outer membrane via SusD, SusE, SusF (Rogowski et al., 2015). The oligosaccharides produced are subsequently transported into the periplasm via SusC/D; further hydrolysis occurs in the periplasm via glycosyl hydrolases (GH). Stars represent fluorophore attached to fluorescently labeled substrates. (Modified from Reintjes et al., 2017; after Koropatkin et al., 2012).

3. A rouges' gallery of organic matter degradation: selfish, producing, and cheating bacteria

Recognition of selfish uptake changes previous conceptions about interactions among members of microbial communities during HMW substrate degradation. Models and measurements of organic matter degradation by microbial communities have reflected the perspective that degradation of HMW organic matter requires production of suitable enzymes by a fraction of the microbial community. Other members of the community can potentially benefit from this production by taking up hydrolysis products without investing in enzyme production (e.g. Folse III and Allison, 2012; Mislan et al., 2014; Fig. 5a). Economic and ecological analyses of this scenario have focused on the cost-benefit calculus of these organisms, which have been characterized as 'producers' and 'cheaters', respectively (Allison, 2005; Kaiser et al., 2015). For example, the cost of enzymes may be most effectively returned to the producing cells – and the benefits to cheaters reduced – through strategies such as production within sufficiently thick biofilms, which reduces diffusive loss of hydrolysate. Conversely, in high-flow environments such as sinking marine snow, producing cells can also outcompete cheaters since hydrolysate is rapidly removed, preventing cheaters from benefitting from enzyme activities (Drescher et al., 2014). In a similar manner, production of enzymes by a free-living bacterium might pay off if the enzymes were attached to the surface of the cell, rather than released into the environment (Traving et al., 2015)

These scenarios become considerably more complex, however, when selfish bacteria are included (Fig. 5b). Substrate loss by selfish bacteria is very low (Cuskin et al., 2015), such that cheating bacteria are minimally subsidized by their activities, irrespective of the broader environmental context. Selfish bacteria preferentially consuming a

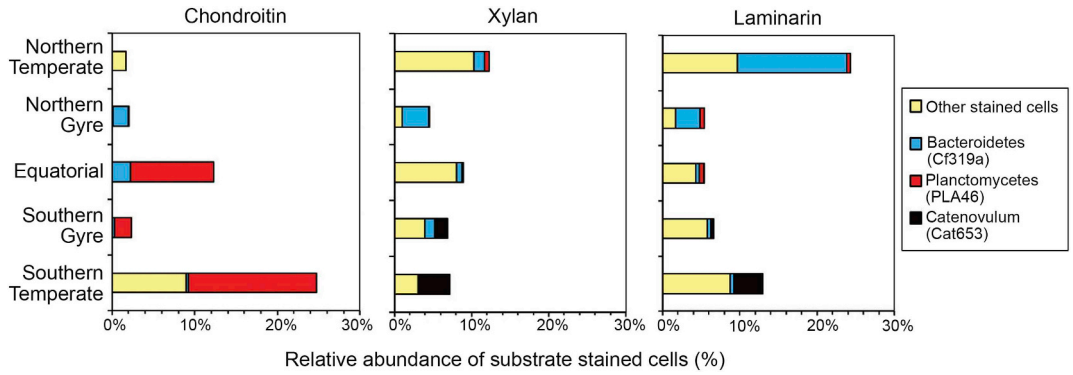

Fig. 4. Relative abundance of cells (percent of total DAPI-stainable cells) stained with fluorescently labeled chondroitin, xylan, and laminarin after 6 days of incubation at five stations in the Atlantic Ocean. A fraction of the substrate-stained cells could be identified using group specific FISH probes (color key at right). Data replotted from Reintjes et al. (2017).

specific substrate would imply that the flux of carbon to other bacteria would be reduced (Fig. 5b) compared to situations in which producing bacteria dominate initial hydrolysis (Fig. 5a). Under these conditions, the population of cheating bacteria may be affected by reduced substrate availability.

Nonetheless, selfish uptake is not the only mechanism important for substrate degradation in the ocean: for example, other bacteria that are known to hydrolyze polysaccharides or harbor genes that correspond to enzymes for hydrolysis of HMW substrates – notably members of the *Alteromonadales* (Teeling et al., 2012; Wietz et al., 2015; Taylor and Cunliffe, 2017) – responded in our experiments, as identified via next generation sequencing, although they did not display a selfish uptake mechanism (Reintjes et al., revised). Moreover, hydrolysis with extracellular production of intermediate as well as lower molecular weight substrates (a producing mode; Fig. 5a) is known from experiments with bacterial isolates, as well as from incubations of polysaccharides in seawater and sediments (Arnosti and Repeta, 1994; Arnosti, 2008; Neumann et al., 2015). Selfish and producing bacteria thus coexist with cheating bacteria, in complex interactions that have yet to be explored (Fig. 5b). This exploration will also require renewed efforts in modeling, given the fact that (based on knowledge of members of the *Bacteroidetes*) selfish uptake requires considerable cellular investment, involving multiple substrate binding proteins and hydrolytic enzymes (Martens et al., 2009; Rakoff-Nahoum et al., 2016).

Efforts to determine which organisms are associated with a specific type of behavior are complicated by the fact that selfish and producing modes of substrate acquisition cannot neatly be divided by phylogeny: members of the *Bacteroidetes* (the best-investigated organisms, owing to their importance in the gut microbiome) fall in both groups (Rakoff-Nahoum et al., 2014). Indeed, closely-related bacterial species have been demonstrated to have distinctly different enzymatic repertoires and substrate preferences (e.g., Xing et al., 2014). Moreover, many *Gammaproteobacteria* are known to be producers, secreting enzymes that are active in the external environment (Fig. 5a);

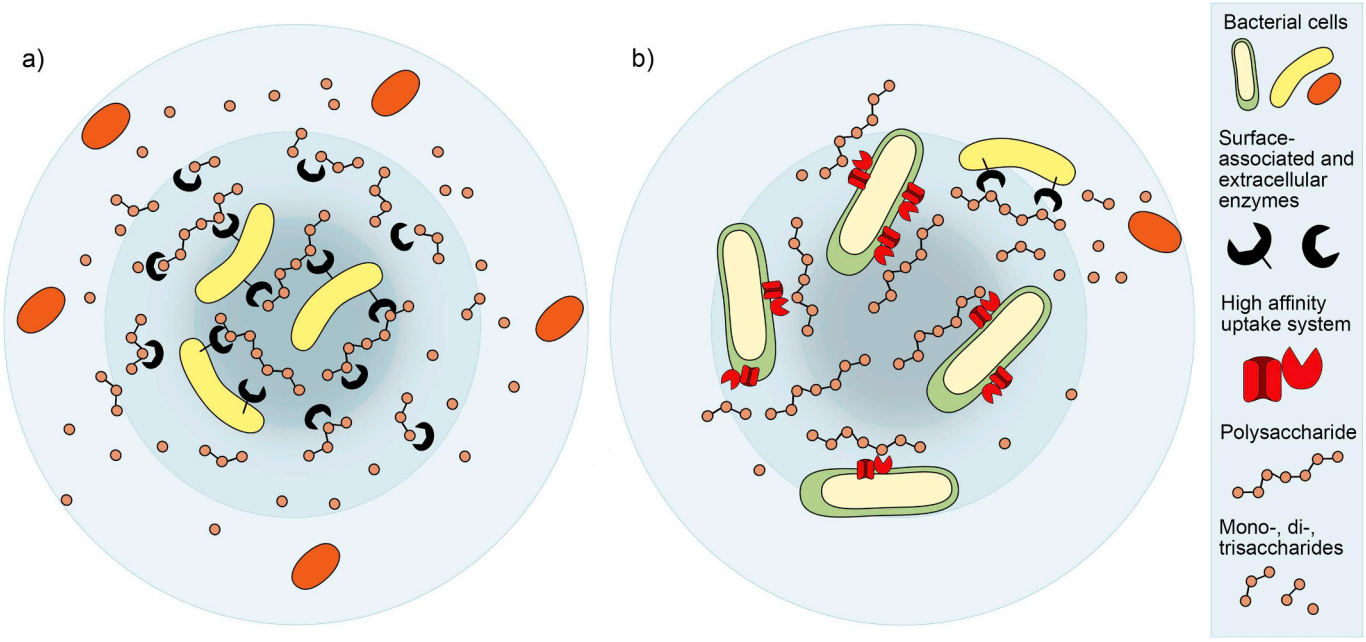

Fig. 5. a) Microbial communities include organisms that produce extracellular enzymes ('producers'; yellow, with enzymes in black) to hydrolyze HMW substrates (schematically indicated with orange circles connected by black lines) to sizes sufficiently small for uptake. The color gradient in the background denotes diffusion of hydrolysis products away from the producers. Some of the hydrolysate is taken up by 'cheating' organisms (orange) that did not produce the enzymes. b) 'Selfish' bacteria (green/yellow) take up HMW substrates without releasing LMW hydrolysis products to the external environment. Presumably, they compete with producers (yellow), which hydrolyze substrates in the external environment. Cheaters (orange) may have less substrate available (compare to scenario a) under conditions in which selfish bacteria predominate.

Gammaproteobacteria typical respond rapidly to increases in substrate concentrations (Sarmento et al., 2016; Sperling et al., 2017). Many members of the *Gammaproteobacteria* carry genes related to quorum sensing (Drescher et al., 2014; Krupke et al., 2016) and thus collectively may hydrolyze substrates in a manner in which the wider community benefits from enzymatic hydrolysis (Fig. 5a). Making sharp distinctions between producing and cheating behavior may therefore prove to be an oversimplification: experiments with pure cultures of bacteria have demonstrated that only a fraction of the organisms may turn on the genetic pathway to hydrolyze a given substrate at any time, whereas a wider fraction of the cells may take up the hydrolysate (Baty III et al., 2000a,b). More recently, focused investigation of a member of the gut *Bacteroidetes* demonstrated that *Bacteroides ovatus* carried out selfish uptake and external hydrolysis concurrently, with the hydrolysate benefitting *Bacteroides vulgatus*, which then provided a growth factor benefitting *B. ovatus* (Rakoff-Nahoum et al., 2016). The rogues' gallery thus likely also contains previously unrecognized pathways of co-operation among these groups.

4. The role of substrate binding and transport in organic matter degradation: selfish bacteria collect HMW DOC

Whether and which selfish bacteria are entirely selfish, merely opportunistic, or perhaps collaborate in their mode of substrate processing in ocean waters remains to be determined. In any case, identification of a selfish mode of substrate processing highlights the importance of targeted binding and uptake as factors in microbial degradation of organic matter, where consideration of structural 'fit' for substrate binding and transporters, as well as for extracellular enzymes, requires further attention.

In the organic geochemical literature, the importance of targeted substrate binding by microbes for the most part has been overlooked. Methods most commonly used to measure microbial processing of organic matter typically are focused on LMW substrates, but LMW substrate proxies do not fit the comparatively complex biochemical machinery of binding proteins (Fig. 3; Warren, 1996). Similarly, uptake of LMW substrates such as glucose or amino acids is frequently measured in incubations (e.g., Rich et al., 1996; Ouverney and Fuhrman, 2000; Bryson et al., 2016). These substrates are processed differently – and in some cases, by different organisms – than HMW substrates (Cottrell and Kirchman, 2000; Elifantz et al., 2005). Mono-, di- and trisaccharides are primarily taken up by ABC transporters that do not accommodated molecular weights above 600 Da (Davidson et al., 2008).

The requirement for sufficient substrate length (e.g., a tetrasaccharide; Martens et al., 2009) to fit binding domains and transporters for processing HMW substrates means that most previous measurements of enzymatic hydrolysis likely have not captured a large fraction of HMW substrate processing activity. This consideration applies to organisms that carry out selfish hydrolysis and uptake, as well as producing bacteria (Fig. 5a) that hydrolyze substrates with cell-surface associated and/or freely-released enzymes, since such enzymes frequently contain structure-specific binding domains, as well as catalytic domains (Rogowski et al., 2015). Members of the *Verrucomicrobia*, for example, have been identified in coastal waters as bacteria that bind polysaccharides to the cell exterior (Martinez-Garcia et al., 2012); *Verrucomicrobia* likely also hydrolyze a range of polysaccharides (Cardman et al., 2014), but they have not yet been identified among the selfish bacteria. Targeted substrate binding thus is an essential – and often overlooked – facet of organic matter degradation by microbial communities. Substrate binding encompasses the binding of HMW DOC to microbial cells via substrate binding domains, either specific proteins or modules of cell-surface-attached extracellular enzymes (Boraston et al., 2004; Carvalho et al., 2014). From this perspective, the greater reactivity and comparatively rapid removal from ocean waters of HMW DOC is linked at least in part to the specific substrate binding and uptake capabilities of microbes.

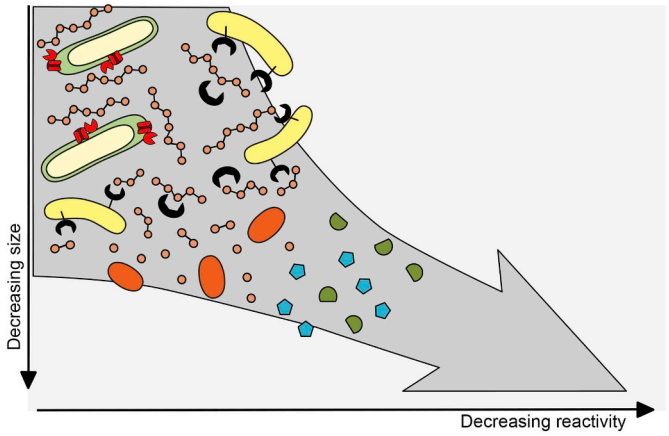

Fig. 6. The size-reactivity continuum reconsidered: specific bacteria use selfish uptake to rapidly utilize HMW organic matter. (Organic matter continuum modified from Benner and Amon, 2015.)

As portrayed by the size-reactivity continuum (Fig. 6), the greater reactivity of HMW organic matter is thus in part due to the fact that a considerable fraction of bacteria in the surface ocean (Fig. 4) have the ability to bind specifically and rapidly HMW substrates. This point is illustrated vividly by the observation that approximately 5% of total cells took up laminarin within a few minutes of substrate addition in our Atlantic transect (Reintjes et al., 2017), the time required to add substrate, mix the sample, and filter the initial sub-sample. Obtaining specific HMW substrates in a targeted manner can optimize return on investment, a strategy that appears to be effective in diverse environments. Our observation that selfish behavior is measurable for all substrates and stations investigated to date (Reintjes et al., 2017; revised) suggests that this mode of substrate processing is sufficiently widespread to affect HMW DOC reactivity in the ocean.

5. Looking forward

Selfish uptake is thus an effective acquisition strategy to sequester specific polysaccharides in strikingly different environments: the warm, carbon-rich, anoxic human gut and the cool, carbon-poor oxic surface ocean (Rakoff-Nahoum et al., 2016; Reintjes et al., 2017; revised). Why does the same strategy work in such contrasting environments? Where else might selfish uptake be found? What factors constrain this mode of substrate processing? For the most part, these questions await further investigation, although some speculation may be in order here. The examples from the human gut for the most part involve specific members of the *Bacteroidetes* that process selected highly complex polysaccharides in a selfish manner (Cuskin et al., 2015; Rakoff-Nahoum et al., 2016); the investment in enzymes and uptake systems must be considerable. Moreover, the human gut is a densely populated environment where competition for resources is intense. Complexity of the target polysaccharide thus may be one consideration: degradation of a more complex substrate that requires more resources to access may be worthwhile when the 'return on investment' is in a way guaranteed. A comparable example in the surface ocean may be seen for chondroitin sulfate. Selfish uptake of this sulfated polysaccharide is frequently associated specifically with members of the bacterial phylum *Planctomycetales* (Reintjes et al., 2017; revised), which may be the organisms enzymatically best suited to degrade this complex substrate.

We also observe very rapid selfish uptake of laminarin, however, a comparatively simple polysaccharide. At all five stations where we worked in the Atlantic Ocean, in fact, selfish uptake of laminarin was measurable shortly after addition of substrate (Reintjes et al., 2017). Since our experiments with pure cultures of *Gramella forsetii* demonstrated that prior exposure to laminarin led to induction of enzymes

(Kabisch et al., 2014) and much more rapid initial uptake (Reintjes et al., 2017), we speculate that (i) laminarin (an energy storage product of phytoplankton including diatoms; Painter, 1983) is a common substrate in the ocean – since bacteria from distinct surface waters reacted with a speed suggesting that they had been recently exposed to this polysaccharide – and (ii) selfish uptake can also be an effective strategy used by a range of different bacteria (Fig. 4) to hoard 'simple' polysaccharides in the face of competition. The hypothesis that selfish uptake may be a workable strategy under different conditions of substrate complexity and competition also is supported by our observation that in surface waters of the Atlantic Ocean, the spectrum as well as the extent of selfish uptake varied by location (Reintjes et al., 2017; revised).

The role of targeted cell-surface binding of substrates has received little previous attention in discussions of DOC cycling in the ocean. Our serendipitous observation of a widespread and surprisingly rapid mode of substrate uptake that depends critically on specific binding and transport into the cell suggests that selective uptake of HMW DOC may underlie the size-reactivity continuum (Amon and Benner, 1996) of DOC. Selfish behavior may also occur in other parts of the ocean such as sediments, and in other environments. Identifying the environments in which selfish uptake occurs, the factors that constrain selfish uptake, and the conditions under which a variety of substrate degradation strategies are effective, will help provide mechanistic understanding of DOC processing. Such observations and measurements are an essential aspect of developing an overall mechanistic understanding of organic matter degradation in the ocean.

Acknowledgments

Funding from the Max Planck Society, as well as from the U.S. National Science Foundation (OCE-1332881 and -1736772 to C.A.) made this work possible. We thank three anonymous reviewers for their thoughtful comments about this manuscript.

Competing interest

The authors declare they have no competing interests.

References

Allison, S.D., 2005. Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. Ecology Lett. 8, 626–635.
Aluwihare, L.I., Repeta, D.J., Chen, R.F., 1997. A major biopolymeric component to dissolved organic carbon in surface sea water. Nature 387, 166–169.
Amon, R.M.W., Benner, R., 1994. Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. Nature 369, 549–552.
Amon, R.M.W., Benner, R., 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnol Oceanogr. 41, 41–51.
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 Marine Science 3, 401–425.
Arnosti, C., Repeta, D.J., 1994. Extracellular enzyme activity in anaerobic bacterial cultures: Evidence of pullulanase activity among mesophilic marine bacteria. Appl. Environ. Microbiol. 60, 840–846.
Arnosti, C., Steen, A.D., Ziervogel, K., Ghobrial, S., Jeffrey, W.H., 2011. Latitudinal gradients in degradation of marine dissolved organic carbon. PLoS ONE 6 e28900.
Arnosti, C., Fuchs, B.M., Amann, R., Passow, U., 2012. Contrasting extracellular enzyme. activities of particle associated bacteria from distinct provinces of the North Atlantic Ocean. Frontiers Microbiol. 3, 425. https://doi.org/10.3389/fmicb.2012.00425.
Balmonte, J.P., Teske, A., Arnosti, C., 2018. Structure and function of high Arctic pelgic, particle-associated, and benthic bacterial communities. Environ. Microbiol. https://doi.org/10.1111/1462-2920.14304.
Baty III, A.M., Eastburn, C.C., Diwu, Z., Techkarnjanaruk, S., Goodman, A.E., Geesey, G.G., 2000a. Differentiation of chitinase-active and non-chitinase-active subpopulations of a marine bacterium during chitin degradation. Appl. Environ. Microbiol. 66, 3566–3573.
Baty III, A.M., Eastburn, C.C., Techkarnjanaruk, S., Goodman, A.E., Geesey, G.G., 2000b. Spatial and temporal variations in chitinolytic gene expression and bacterial biomass production during chitin degradation. Appl. Environ. Microbiol. 66, 3574–3585.
Benner, R., Amon, R.M.W., 2015. The size-reactivity continuum of major bioelements in the ocean Ann. Rev. Marine Sci. 7, 185–205.
Benner, R., Pakulski, J.D., McCarthy, M., Hedges, J.I., Hatcher, P.G., 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. Science 255, 1561–1564.
Biersmith, A., Benner, R., 1998. Carbohydrates in phytoplankton and freshly produced dissolved organic matter. Mar. Chem. 63, 131–144.
Boedeker, C., Schuler, M., Reintjes, G., Jeske, O., van Teeseling, M.C.F., Jogler, M., et al., 2017. Determining the bacterial cell biology of Planctomycetes. Nature Communications 8, 14,583.
Boraston, A.B., Bolam, D.N., Gilbert, H.J., Davies, G.J., 2004. Carbohydrate-binding modules: fine-tuning polysaccharide recognition. Biochem. J. 382, 769–781.
Bryson, S., Li, Z., Pett-Ridge, J., Hettich, R.L., Mayali, X., Pan, C., et al., 2016. Proteomic stable isotope probing reveals taxonomically distinct patterns in amino acid assimilation by coastal marine bacterioplankton. mSystems 1, 1–16.
Cardman, Z., Arnosti, C., Durbin, A., Ziervogel, K., Cox, C., Steen, A.D., Teske, A., 2014. Verrucomicrobia: candidates for polysaccharide-degrading bacterioplankton in an Arctic fjord of Svalbard. Appl. Environ. Microb. 80, 3749–3756.
Carlson, C.A., 2002. Production and Removal Processes. In: Hansell, D.A., Carlson, C.A. (Eds.), Biogeochemistry of Marine Dissolved Organic Matter. Academic Press, San Diego, pp. 91–151.
Carlson, C.A., Hansell, D.A., 2015. DOM sources, sinks, reactivity, and budgets. In: Carlson, C.A., Hansell, D.A. (Eds.), Biogeochemistry of marine dissolved organic matter, 2nd ed. Elsevier, pp. 65–126.
Carvalho, C.C., Phan, N.N., Chen, Y., Reilly, P.J., 2014. Carbohydrate-binding module tribes. Biopolymers 103, 203–214.
Cottrell, M.T., Kirchman, D.L., 2000. Natural assemblages of marine proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. Appl. Environ. Microbiol. 66, 1692–1697.
Cowie, G.L., Hedges, J.I., 1984. Carbohydrates sources in a coastal marine environment. Geochim. Cosmochim. Acta 48, 2075–2087.
Cuskin, F., Lowe, E.C., Tample, M.J., Zhu, Y., Cameron, E.A., Pudlo, N.A., et al., 2015. Human gut *Bacteroidetes* can utilize yeast mannan through a selfish mechanism. Nature 517, 165–173.
D'Ambrosio, L., Ziervogel, K., MacGregor, B., Teske, A., Arnosti, C., 2014. Composition and enzymatic function of particle-associated and free-living bacteria: a coastal/offshore comparison. The ISME J. 8, 2167–2179.
Davidson, A.L., Dassa, E., Orelle, C., Chen, J., 2008. Structure, function, and evolution of bacterial ATP-binding cassette systems. Microb. Mol. Biol. Reviews 72, 317–364.
Drescher, K., Nadell, C.D., Stone, H.A., Wingreen, N.S., Bassler, B.L., 2014. Solutions to the public goods dilemma in bacterial biofilms. Curr. Biol. 24, 50–55.
Elifantz, H., Malmstrom, R.R., Cottrell, M.T., Kirchman, D.L., 2005. Assimilation of polysaccharides and glucose by major bacterial groups in the Delaware estuary. Appl. Environ. Microb. 71, 7799–7805.
Folse III, H.J., Allison, S.D., 2012. Cooperation, competition, and coalitions in enzyme-producing microbes: social evolution and nurient depolymerization rates. Frontiers in Microbiol. 3, 338.
Fuhrman, J.A., Steele, J.A., Hewson, I., Schwalbach, M.S., Brown, M.V., Green, J.L., et al., 2008. A. latitudinal diversity gradient in planktonic marine bacteria. PNAS. 105, 7774–7778.
Gomez-Pereira, P.R., Schuler, M., Fuchs, B.M., Bennke, C.M., Teeling, H., Waldmann, J., et al., ... 2012. Genomic content of uncultured Bacteroidetes from contrasting oceanic provinces in the North Atlantic Ocean. Environ. Microb. 14, 52–66.
Guo, L., Santschi, P.H., Cifuentes, L.A., Trumbore, S.E., Southon, J., 1996. Cycling of high-molecular-.weight dissolved organic matter in the Middle Atlantic Bight as revealed by carbon isotopic (^{13}C and ^{14}C) signatures. Limnol. Oceanogr. 41, 1242–1252.
Hansell, D.A., 2013. Recalcitrant dissolved organic carbon fractions. Annual Rev. Mar. Sci. 5, 421–445.
Hertkorn, N., Benner, R., Frommberger, M., Schmitt-Kopplin, P., Witt, M., Kaiser, K., et al., 2006. Characterization of a major refractory component of marine dissolved organic matter. Geochim. Cosmochim Acta 70, 2990–3010.
Hoarfrost, A., Arnosti, C., 2017. Heterotrophic extracellular enzymatic activities in the Atlantic Ocean follow patterns across spatial and depth regimes. Frontiers Marine Sci. 4, 200. https://doi.org/10.3389/fmars.2017.00200.
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.
Kabisch, A., Otto, A., Konig, S., Becher, D., Albrecht, D., Schuler, M., et al., 2014. Functional characterization of polysaccharide utilization loci in the marine Bacteroidetes 'Gramella forsetti'.KT 0803. The ISME J. 8, 1492–1502.
Kaiser, K., Benner, R., 2009. Biochemical composition and size distribution of organic matter at the Pacific and Atlantic time-series stations. Marine Chem. 113, 63–77.
Kaiser, C., Franklin, O., Richter, A., Dieckmann, U., 2015. Social dynamics within decomposer communities lead to nitrogen retention and organic matter build-up in soils. Nature Comm. 6, 8960.
Koch, B.P., Witt, M., Engbrodt, R., Dittmar, T., Kattner, G., 2005. Molecular formulae of marine and terrigenous dissolved organic matter detected by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Geochim. Cosmochim. Acta 69, 3299–3308.
Koropatkin, N.M., Cameron, E.A., Martens, E.C., 2012. How glycan metabolism shapes the human gut microbiota. Nature Rev. Microbiol. 10, 323–335.
Krupke, A., Hmelo, L.R., Ossolinski, J.E., Mincer, T.J., Van Mooy, B.A.S., 2016. Quorum sensing plays a complex role in regulating the enzyme hydrolysis activity of microbes associated with sinking particles in the ocean. Frontiers Marine Sci. 3, 55.
Kujawinski, E.B., Longnecker, K., Blough, N.V., Del Vecchio, R., Finlay, L., Kitner, J.B., et al., 2009. Identification of possible source markers in marine dissolved organic

matter using ultrahigh resolution mass spectrometry. Geochim. Cosmochim. Acta 73, 4384–4399.
Landa, M., Blain, S., Christaki, U., Monchy, S., Obernosterer, I., 2016. Shifts in bacterial community composition associated with increased carbon cycling in a mosaic of phytoplankton blooms. The ISME J. 10, 39–50.
Martens, E.C., Koropatkin, N.M., Smith, T.J., Gordon, J.I., 2009. Complex glycan catabolism by the human gut microbiota: the Bacteroidetes Sus-like paradigm. J Biol. Chem. 284, 24,637–24,677.
Martinez-Garcia, M., Brazel, D.M., Swan, B.K., Arnosti, C., Chain, P.S.G., Reitenga, K.G., et al., 2012. Capturing single cell genomes of active polysaccharide degraders: an unexpected contribution of Verrucomicrobia. PLoS ONE 7, e35314.
Mislan, K.A.S., Stock, C.A., Dunne, J.P., Sarmiento, J.L., 2014. Group behavior among model bacteria influences particulate carbon remineralization depths. J. Mar. Res. 72, 183–218.
Neumann, A.M., Balmonte, J.P., Berger, M., Giebel, H.-A., Arnosti, C., Voget, S., et al., 2015. Different utilization of alginate and other algal polysaccharide by marine Alteromonas macleodii ecotypes. Environ. Microbiol 17, 3857–3868.
Ouverney, C.C., Fuhrman, J.A., 2000. Marine planktonic Archea take up amino acids. Appl. Environ. Microbiol. 66, 4829–4833.
Painter, T.J., 1983. Algal Polysaccharides. In: Aspinall, G.O. (Ed.), The Polysaccharides. Academic Press, New York, pp. 195–285.
Piontek, J., Sperling, M., Nothing, E.-M., Engel, A., 2014. Regulation of bacterioplanton activity in Fram. Strait (Arctic Ocean) during early summer: the role of organic matter supply and temperature. J. Mar. Sys. 132, 83–94.
Pommier, T., Canback, B., Riemann, L., Bostrom, K.H., Simu, K., Lundberg, P., et al., 2007. Global patterns of diversity and community structure in marine bacterioplankton. Molecular Ecol. 16, 867–880.
Rakoff-Nahoum, S., Coyne, M.J., Comstock, L.E., 2014. An ecological network of polysaccharide utilization among human intestinal symbionts. Curr Biology 24, 40–49.
Rakoff-Nahoum, S., Foster, K.R., Comstock, L.E., 2016. The evolution of cooperation within the gut microbiota. Nature 533, 255–259.
Reintjes, G., Arnosti, C., Fuchs, B.M., Amann, R., 2017. An alternative polysaccharide uptake mechanism of marine bacteria. The ISME J. 11, 1640–1650. https://doi.org/10.1038/ismej.2017.26.
Reintjes, G., Arnosti, C., Fuchs, B.M., Amann, R. (revised) Selfish, sharing, and scavenging bacteria in the Atlantic Ocean: a biogeographic study of microbial substrate utilisation.
Rich, J.H., Ducklow, H.W., Kirchman, D.L., 1996. Concentrations and uptake of neutral monosacharides along 140 W in the equatorial Pacific: Contribution of glucose to heterotrophic bacterial activity and the DOM flux. Limnol. Oceanogr. 41, 595–604.
Rogowski, A., Briggs, J.A., Mortimer, J.C., Tryfona, T., Terrapon, N., Lowe, E.C., et al., 2015. Glycan complexity dictates microbial resource allocation in the large intestine. Nature Comm. 6, 7481.
Sarmento, H., Morana, C., Gasol, J.M., 2016. Bacterioplankton niche partitioning in the use of phytoplankton-derived dissolved organic carbon: quantity is more important than quality. The ISME J. 10, 2582–2592.
Schermelleh, L., Heintzmann, R., Leonhardt, H., 2010. A guide to super-resolution fluorescence microscopy. J. Cell Biol. 190, 165–175.
Skoog, A., Benner, R., 1997. Aldoses in various size fractions of marine organic matter: Implications for carbon cycling. Limnol Oceanogr. 42, 1803–1813.
Sperling, M., Piontek, J., Engel, A., Wiltshire, K.H., Niggemann, J., Gerdts, G., et al., 2017. Combined carbohydrates support rich communities of particle-associated marine bacterioplankton. Frontiers Microbiol. 8. https://doi.org/10.3389/fmicb.2017.00065.
Steen, A.D., Ziervogel, K., Ghobrial, S., Arnosti, C., 2012. Functional variation among polysaccharide-hydrolyzing microbial communities in the Gulf of Mexico. Marine Chem. 138, 13–20.
Steen, A.D., Vazin, J.P., Hagen, S.M., Mulligan, K.H., Wilhelm, S.W., 2015. Substrate specificity of aquatic extracellular peptidases assessed by competitive inhibition assays using synthetic substrates. Aquat. Microb. Ecol. 75, 271–281.
Taylor, J.D., Cunliffe, M., 2017. Coastal bacterioplankton community response to diatom-derived polysaccharide microgels. Environ. Microb. Reports. doi. https://doi.org/10.1111/1758-2229.12513.
Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C.M., et al., 2012. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science. 336, 608–611.
Teeling, H., Fuchs, B.M., Bennke, C.M., Kruger, K., Chafee, M., Kappelmann, L., et al., 2016. Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms. *eLife*. 5, e11888.
Traving, S.J., Thygesen, U.H., Riemann, L., Stedmon, C.A., 2015. A model of extracellular enzymes in free-living microbes: which strategy pays off? Appl. Environ. Microbiol. 81, 7385–7391.
Walker, B.D., Primeau, F.W., Beaupre, S.R., Guilderson, T.P., Druffel, E.R.M., McCarthy, M.D., 2016. Linked changes in marine dissolved organic carbon molecular size and radiocarbon age. Geophys. Res. Lett. 43, 10,385–10,393.
Warren, R.A.J., 1996. Microbial hydrolysis of polysaccharides. Ann. Rev. Microbiol. 50, 183–212.
Wegner, C.-E., Richter-Heitmann, T., Klindworth, A., Klockow, C., Richter, M., Achstetter, T., 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., Wemheuer, B., Simon, H.M., Giebel, H.-A., Siebt, M.A., Daniel, R., et al., 2015. Bacterial community dynamics during polysaccharide degradation at contrasting sites in the Southern and Atlantic Oceans. Environ. Microb. 17, 3822–3831.
Xing, P., Hahnke, R.L., Unfried, F., Markert, S., Hugang, S., Barbeyron, T., et al., 2014. Niches of two polysaccharide-degrading Polaribacter isolates from the North Sea during a spring diatom bloom. The ISME J. 9, 1410–1422.
Zinger, L., Amaral-Zettler, L.A., Fuhrman, J.A., Horner-Devine, M.C., Huse, S.M., Welch, D.B.M., et al., 2011. Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. PLoS ONE 6, e24570.