Microbial polysaccharide utilization in sandy surface sediments

Dissertation

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Diese zur Veröffentlichung erstellte Version der Dissertation enthält Korrekturen und eine aktuellere Version des dritten Kapitels.

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“*The only thing that is constant is change*”

- Heraclitus

“*In nature nothing remains constant. Everything is in a perpetual state of transformation, motion and change*.”

- David Bohm

Content

Summary

The shallow waters over continental shelves are a hotspot of marine primary production. In these regions, significant amounts of carbon fixed in the water column is exported to the sediment and, together with benthic primary production, feeds heterotrophic microbes. Much of the organic matter is comprised of polysaccharides produced by algae, which can be very complex. Since the organic matter is not accumulating, utilization of polysaccharides by benthic microbes must be highly relevant for the marine carbon cycle. In this thesis, I first studied the dynamics of benthic microbial communities in sands by assessing taxonomic changes over the seasonal cycle in temperate and polar regions. Compared to the rapid substrate driven successions of coastal bacterioplankton communities during and after spring blooms, there was very little seasonal change in the taxonomic composition of the benthic bacterial community. This comparison indicated fundamental differences in the ecological mechanisms driving pelagic and benthic communities. Since no taxonomic succession was observed, transcriptional activity was investigated. Here, clear seasonal changes were identified for several Gammaproteobacteria and Bacteroidia species. However, these species represented only a minor fraction of the community, with the majority showing relatively constant activity. Assessing the temporal changes in polysaccharides revealed α- and β-glucans as important substrates in winter and in spring, respectively, indicating that recycling processes might stabilize the community. Incubations with fluorescently labeled polysaccharides provided further evidence for β-glucan (laminarin) and animal-derived glycoprotein (mucin) utilization. The dominance of extracellular hydrolysis over selfish uptake in incubations and the high concentration of glucose-depleted dissolved organic matter in the pore water of sands, indicates that benthic microbes preferentially utilize glucose during the degradation of particulate organic matter, with the remaining glycans being released in dissolved form into the overlying pelagic system.

Zusammenfassung

Kontinentalschelfe sind ein Hotspot der marinen Primärproduktion. Hier werden erhebliche Mengen des in der Wassersäule fixierten Kohlenstoffs in das Sediment exportiert, wo er zusammen mit der benthischen Primärproduktion heterotrophe Mikroben ernährt. Ein Großteil der organischen Substanz besteht aus Polysacchariden, die von Algen produziert werden und sehr komplex sein können. Da diese organische Substanz nicht akkumuliert, muss die Nutzung von Polysacchariden durch benthische Mikroben für den marinen Kohlenstoffkreislauf von großer Bedeutung sein. In dieser Arbeit habe ich zunächst die Dynamik benthischer mikrobieller Gemeinschaften in Sanden untersucht, indem ich die taxonomischen Veränderungen im Laufe des Jahreszyklus in gemäßigten und polaren Regionen bewertete. Im Vergleich zu den raschen substratbedingten Sukzessionen der Bakterioplankton-Gemeinschaften an der Küste während und nach der Frühjahrsblüte gab es bei den benthischen Bakteriengemeinschaften nur sehr geringe saisonale Veränderungen auf taxonomischer Ebene. Dieser Vergleich deutet auf grundlegende Unterschiede in den ökologischen Mechanismen hin, die pelagische und benthische Gemeinschaften antreiben. Da keine taxonomische Sukzession beobachtet wurde, habe ich die saisonalen Veränderungen der Transkriptionsaktivität analysiert. Ich stellte starke Veränderungen bei mehreren Gammaproteobakterien- und Bacteroidota-Arten fest. Diese Arten kamen weniger häufig vor oder waren generell weniger aktiv, während die Mehrheit der Gemeinschaft eine relativ konstante Aktivität aufwies. Bei dem Versuch, Schlüsselsubstrate zu identifizieren, konnte festgestellt werden, dass α- und β-Glucane im Winter bzw. im Frühjahr wichtig sind. Dieses deutet darauf hin, dass Recyclingprozesse die Gemeinschaft stabilisieren könnten. Inkubationen mit fluoreszenzmarkierten Polysacchariden lieferten weitere Hinweise auf die Nutzung von β-Glucan (Laminarin) und Glykopolymeren tierischen Ursprungs (Mucin). Die Dominanz der extrazellulären Hydrolyse in den Inkubationen sowie eine hohe Konzentration an glukosearmer gelöster organischer Substanz im Porenwasser der Umweltproben deuten auf eine Umwandlung partikulärer organischer Substanz in gelöste Glykane hin. Die benthischen Mikroorganismen scheinen davon bevorzugt Glukose zu nutzen, während der Rest in das pelagische System abgegeben wird.

Abbreviations

AA	enzymes with auxiliary activity
ASW	artificial sea water
bp	base pair
CAZyme	carbohydrate active enzyme
CBM	carbohydrate binding module
CE	carbohydrate esterase
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
DOM	dissolved organic matter
Fig	Figure
FISH	fluorescence *in situ* hybridization
FLA-PS	fluorescently labeled polysaccharides
GH	glycoside hydrolase
GT	glycosyl transferase
HMW	high molecular weight
LMW	low molecular weight
MAG	metagenomics assembled genome
mRNA	messenger RNA
NGS	next generation sequencing
nt	nucleotides
OM	organic matter
OSW	overlying sea water
OTU	operational taxonomic unit(s)
PCR	polymerase chain reaction
PL	polysaccharide lyase
POM	particulate organic matter
PUL	polysaccharide utilization loci
PW	pore water
RNA	ribonucleic acid
rRNA	ribosomal RNA
TOC	total organic carbon
TPM	transcripts per million

Chapter 1: General introduction

Continental shelfs

Ever since the foundation of the Max Planck Institute for Marine Microbiology in Bremen scientists have studied the diversity and activities of microorganisms in coastal sediments of temperate and polar regions [1-8]. These sampling sites are located on continental shelfs which differ from deep sea sediments.

The continental shelf area is a loosely defined area within the continental margins of the marine benthic environment. The continental margins also consist of the continental slope and rise (Fig.1 a,b). On average slope and shelf make up about the same surface area of the total margin in the global context [9, 10]. The shelf starts at the coast line extending on average 85 km into the ocean with a slope of about 0.1°. It ends with the first steep (~4°) decline which matches thecontinental slope towards the deeper ocean areas (Fig.1 c) [10]. Shelf zones are characterized by a shallow water depth of on average 130 m and high availability of nutrients due to natural and anthropogenic river input. Furthermore, the nutrients are available throughout the waterbody due to mixing by several effects, like tidal currents, waves or storm events. Additionally coastal upwelling transports nutrient-rich deep sea water to the surface [10]. Shelfs only contribute about 7.5% to the total ocean surface area [10]. Thus, the continental shelf is a comparatively small marine environment in contrast to the open ocean or the vast area of the deep sea. Nevertheless it is a very productive area in terms of carbon fixation and remineralization. The entire continental margins are contributing 9.8 Pg C yr^{-1} CO_2 to marine carbon fixation (~15-20%), which, assuming an average of ~40% shelf area, results in about 8% of marine carbon fixation happening over the shelf [9]. In particular shelf dominated margins (Fig.1 b) are a hotspot for marine primary production and carbon export, since continental margins contribute about 44% of the global export rates [9].

Due to several factors including the shallowness of the water column, the export rate of carbon from the photic zone is estimated to be 35% higher than in the open ocean, making shelf sediments a major player in marine carbon cycling, in particular with regard to final burial.

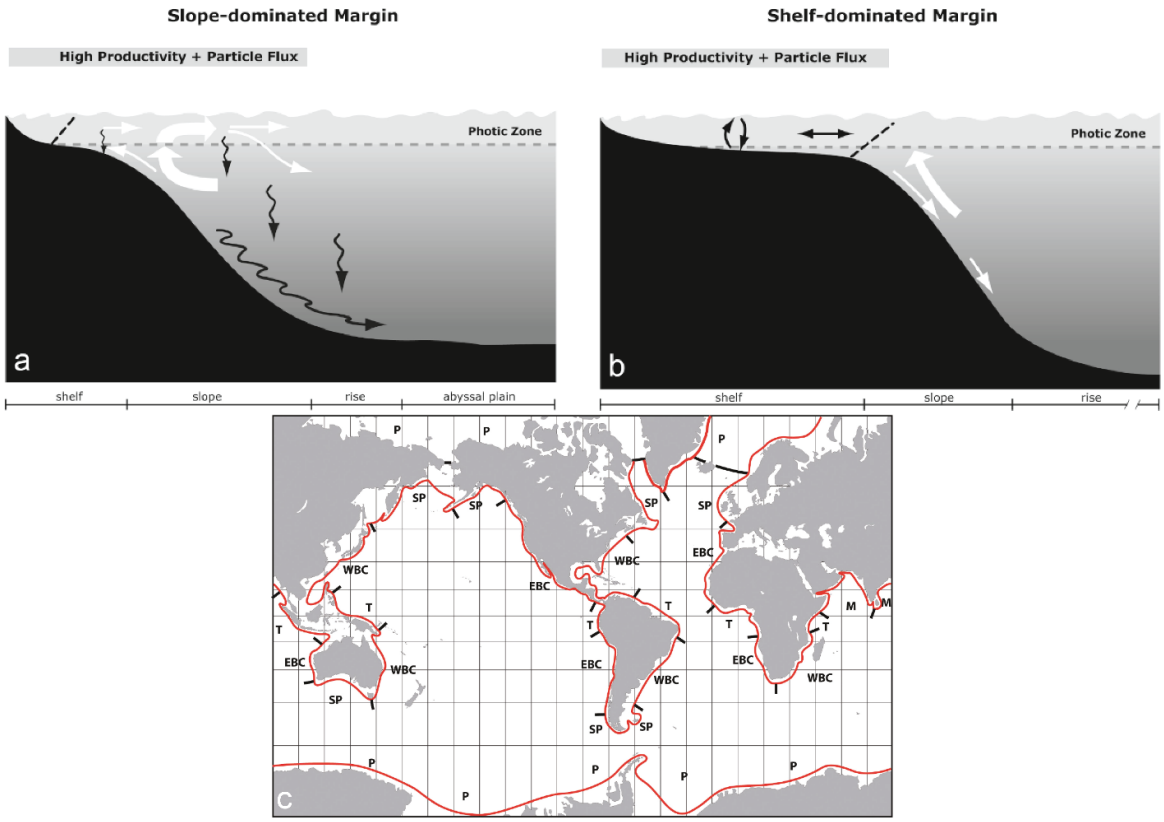

Figure 1: Systematic representation of the (a) slope-dominated and (b) shelf-dominated continental margin type. In panel (c) the distribution of continental margins around the globe is shown. WBC – western boundary current; EBC – eastern boundary current; SP – sub-polar; P – polar; T – tropical; M – monsoonal. Modified after Jahnke 2010 [9]

Such high primary production and export supplies optimal conditions for complex ecosystems and microbial food webs to evolve due to the high supply of nutrients [11]. Temperate and polar coastal regions are characterized by massive blooms of phytoplankton during spring and summer. In temperate regions phytoplankton is mostly limited by low temperatures in winter, which increases in spring enabling phytoplankton blooms [12]. Other factors like wind or rain can influence nutrient or salinity conditions, but such events are more locally and time limited, and do not contribute to the dynamic on a longer timescale. In case of the polar regions, seasonal temperature differences are not the only limiting factor. Ice coverage and low solar radiation during the polar night also limit phytoplankton in polar regions [13]. During such blooms phytoplankton fixes high amounts carbon in form of polysaccharides. Depending on the phytoplankton species and the growth phase of the population, carbohydrates can make up 13%-90% of phytoplankton dry weight [14].

Spring bloom succession

Temperate and polar continental shelfs are often characterized by intense phytoplankton and bacterioplankton spring blooms. The rapid increase in phytoplankton biomass has several consequences for pelagic and benthic ecosystem. The fast growth is depleting inorganic nutrients which has a direct effect on the bloom itself, leading to growth limitation and finally to a collapse of the dominant population after a certain time, and often to another bloom of other phototrophs that are better adapted to the current conditions (boom - bust dynamics). The now abundant biomass, fixed by the algae, is fueling the entire pelagic food web, from heterotrophic bacteria to zooplankton and fish with a variety of recycling processes and diverse food web connections [11, 15]. Within the pelagic microbial community microbes follow a similar boom - bust dynamics. In a rapid, substrate-driven

succession, different microbial clades follow the bloom of phytoplankton, dominating the microbial community [16]. It has been shown over several years of study that the changes are induced by the utilization of different polysaccharides supplied by algal primary production, which generate specific niches for different species, thatsubsequently grow up to high abundances [17-19]. The most dominant taxonomic groups in such dynamics are Bacteroidota and the Gammaproteobacteria, which are both specialists in the degradation of polysaccharides in thisenvironments [20, 21].

Polysaccharides and CAZymes

Polysaccharides are a highly abundant and diverse group of macromolecules synthesized and utilized by all domains of life [22]. They are used for various purposes including storage of carbon, structural elements and for ecosystem engineering [23, 24]. Compared to the other two abundant groups of macromolecules, DNA and proteins, polysaccharides can be much more heterogeneous. This is due to profound structural differences between the three groups of macromolecules. First, compared to DNA with its four bases, and proteins with 20 essential amino acids, polysaccharides have a bigger variety of basic building blocks. Only the known diversity in glycan databases already includes 143 unmodified monosaccharide base types, with 551 differently modified final versions which are used in bacterial glycans[25]. Furthermore, these diverse basic monosaccharide building blocks can be linked with two different stereoisomeric linkage types (α-, β-linkage see Fig.2) [26], which can be established between the anomeric carbon and every hydroxyl group of every monosaccharide, leading to linear and nonlinear (branching) oligomers and polymers [22] (See Fig.3).

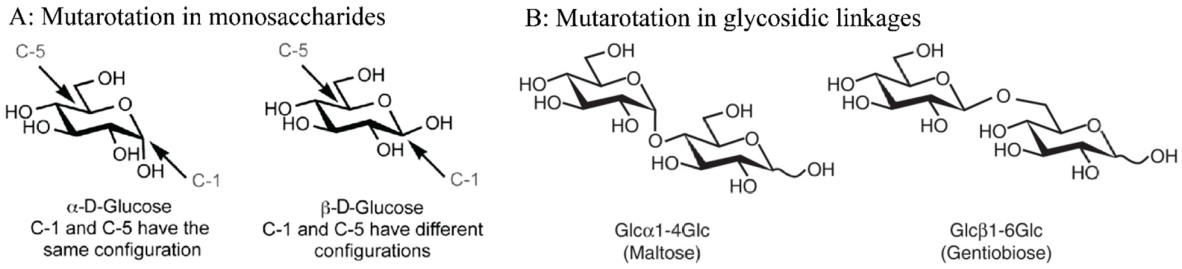

Figure 2: Examples for stereoisomers based on the configuration of the anomeric carbon within monosaccharides (A) or glycosidic linkages (B) [22].

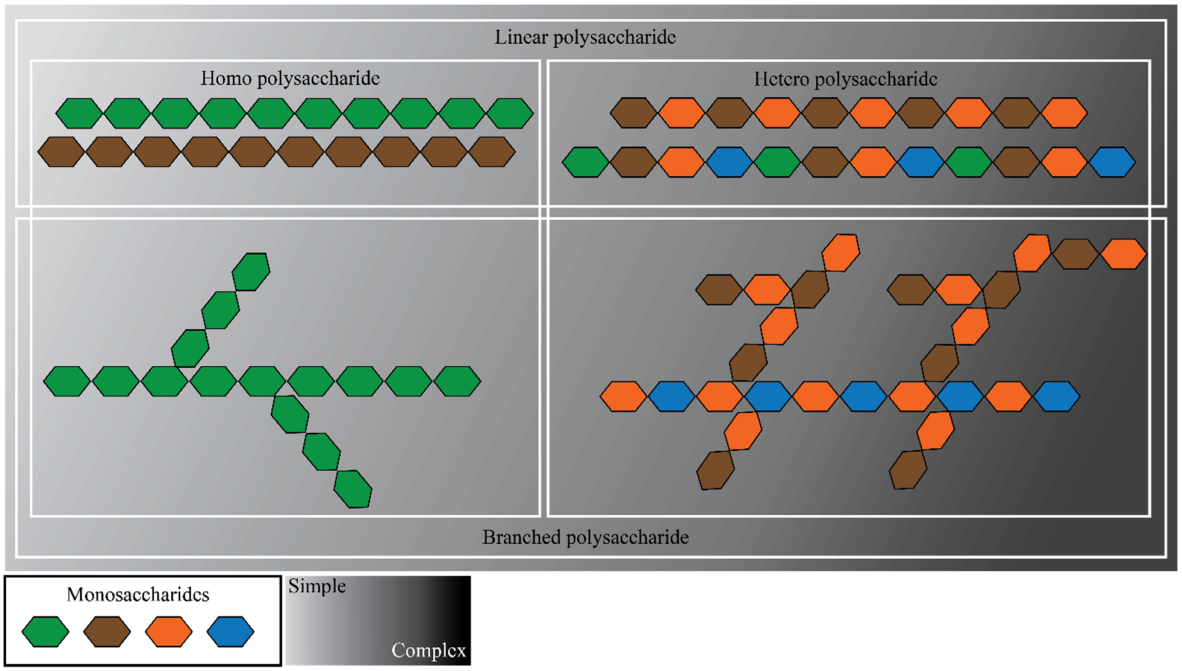

Figure 3: Systematic representation of polysaccharide types based on their monosaccharide composition and structural complexity. Inspired by [22, 27]

This diversity supports a variety of functions and makes polysaccharides a potent and highly variable class of molecules. Therefore, the genome of a single heterotrophic microorganism will be insufficient to break down the complex glycan mixture expected in a coastal sediment. Even a single complex heteroglycan might require the concerted action of several microorganisms for full degradation.

To cope with this diversity, multiple enzymes have evolved, the so called carbohydrate active enzymes (CAZymes). They are categorized in basic groups based on their interaction with the carbohydrate: glycoside hydrolase (GH), polysaccharide lyase (PL), carbohydrate esterase (CE), glycosyl transferase (GT), enzymes with auxiliary activity (AA) and proteins containing carbohydrate binding modules (CBM) [28]. Based on the similarity of the amino acid sequence CAZymes are subsequently grouped into different enzyme families. The genes encoding the CAZymes are often co-localised on the genome together with genes encoding glycan transporters and accessory activities such as sulfatases[20, 21]. Such operons are known as polysaccharide utilization loci (PUL) [29].

The breakdown of rather simple and dominantly linear molecules like laminarin, one of the most abundant storage polysaccharides of marine algae [30], or a simple α-1,4 glucan, can be achieved by a few enzymes [31]. In contrast, complex and heterogeneous polysaccharides, like the structural brown alga polysaccharide fucoidan, might require ~100 enzymes. Furthermore, evidence shows that the difference between fucoidans originating from different brown alga species requires different degradative enzymes, which lead to a total of 284 putative fucoidanases in just one fucoidan-degrading isolate [32]. Even three amino acids close to the active site make a difference in binding specificity torather comparablesubstrates like laminarin or other β-glucans[33].

Due to such specificity of the enzymes and the diversity of polysaccharides already GHs are organized in over 150 families, which can include more than one characterized enzyme function [28]. Yet we can use CAZymes to investigate the niche space defined by polysaccharide substrates [18]. In particular, since the direct quantification of polysaccharides is complicated do to the structural diversity. Even the exact differentiation of a single β-1,4 or a β-1,3 glycosidic linkage is part of current research [34]. Thus quantification of polysaccharides is either relying on using degrading enzyme activity essays [30], which need a full spectrum of enzymes for the investigated polysaccharide, or the quantification via antibodies using a micro array [35]. Both methods are limited to a specific set of probes, either enzymes or antibodies, which complicates use in completely unknown environments or in rather exploratory studies. Here the investigation of microbial CAZymes can offer an alternative approach to predict possible substrates, based on gene annotation in metagenomic studies [16, 17, 20, 31].

Fluorescein-labeled polysaccharides as a tool to study glycan utilization

For a long time, the first steps of polysaccharide degradation was thought to be mainly facilitated by extracellular enzymes [36], essentially because high molecular weight (HMW)-OM needs to be broken down into smaller molecules below ~600 Da for uptake over the cytoplasmic membrane into microbial cells [37]. Such enzymes can either be attached to the outer membrane of the cell or excreted into the water column or pore water around the microbe (Fig.4 a)[36, 38]. Extracellular degradation of glycans represents a rather big investment in resources which not necessarily pays off since secreted enzymes are beneficial for the whole community. A part of the resulting degradation products <600 Da are available for scavengers, who only invested in transporters for saccharide uptake. It has been

demonstrated in recent years that some microbes specialized in the degradation of HMW-OM, in particular Bacteroidota, are alternatively utilizing a so called selfish uptake system which they use to directly import poly- and oligosaccharides into the periplasm after initial hydrolysis (Fig.4 b) [38-40]. Such uptake systems consist of CBMs and degradative CAZymes in the outer membrane and TonB dependent transporters (such as SusC and SusD transporters) (Fig.4 c). These complex systems minimize the loss of glycans to scavenging organisms and are therefore referred to as selfish [39, 40]. Using fluorescently labeled polysaccharides (FLA-PS) as a substrate in short term incubations, it was possible to visualize selfish uptake by bacteria in environmental samples [39]. In the marine surface waters selfish uptake seems to be the dominant form of polysaccharide utilization since up to 60% of cells are stained with FLA-PS after a short incubation time of only 6 h (Fig.5) [41]. Similar investigations for marine sediments are still missing. Considering that hydrolysis rates of polysaccharides in sediments are significantly higher than for the water column [42], such investigations are necessary to further understand polysaccharide utilization in the ocean.

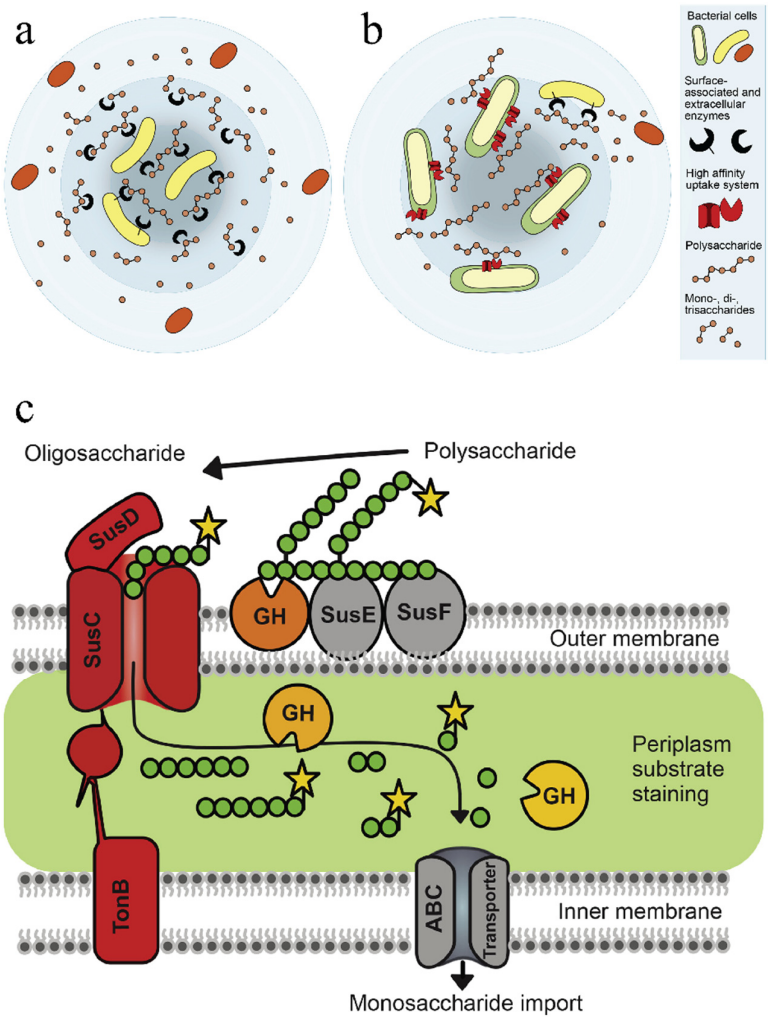

Figure 4:Model of polysaccharide utilizing communities comparing (a) extracellular enzyme activity and (b) communities with the selfish uptake mechanism from Arnosti*et al*. 2018 [38]. (c) Model of selfish uptake into the periplasm from Arnosti*et al*. 2018 [38].

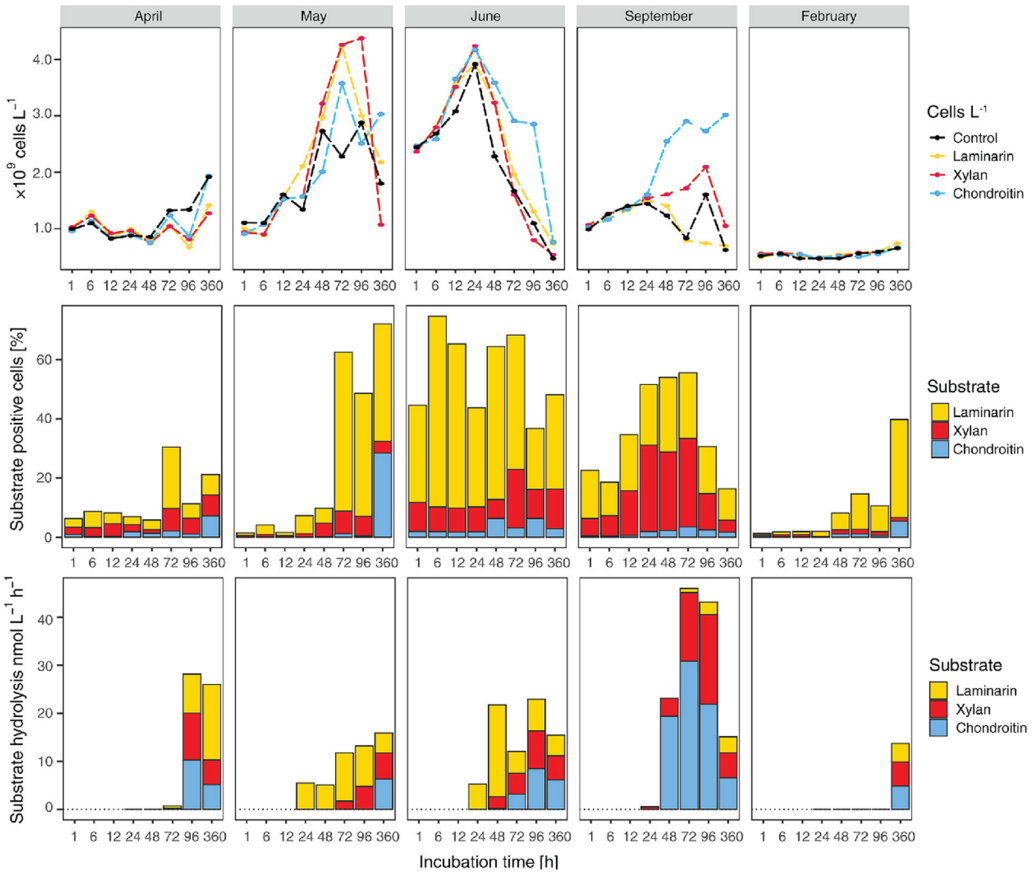

Figure 5: Total cell counts and FLA-PS stained cells in a short term incubation with North Sea waters and hydrolysis rates from Giljan*et al*. 2022 [41].

Benthopelagic Coupling

Residual OM after microbial degradation, pelagic grazing or dissolution in water can sink to the benthic system below where it can be accessed by the heterotrophic community in the sediment. Depending on the water depth carbon export to the benthic system can vary. In some coastal areas up to 50% of the exported carbon is reaching the sediment surface [43, 44]. Besides undegraded fresh biomass, sinking materials also includes partially-degraded algae detritus or corpses of grazers, and already processed carbon in the form of waste products like fecal pellets. Depending on the amount of carbon export there are two types of scenarios, (i) an accumulation of OM leading to a muddy sediment, or (ii) a lack of

accumulation of OM leading to an organic poor sand, or sandy sediment. An example for such muddy sediments we can find in the so called Helgoland mud area, with a reported total organic carbon content (TOC) of 0.6-2.2 wt % [45]. Microbial communities in such muddy sediments are mostly limited by the availability of terminal electron acceptors for respiration and the quality of organic carbon utilized by the microbes, which both change with sediment depth resulting in stratified communities [45, 46]. Due to the energy limitation of anaerobic microbial processes the remineralization of OM is slower than the export from the surface waters leading to the accumulation. In the sandy areas of the continental shelfs the organic matter reaching the sediments is degraded very effectively by a diverse benthic community leading to mostly organic poor sands with low TOC values about 0.06-0.1 wt % (0.6-1 mg g^{-1} sediment dry weight)[8]. The availability of oxygen is most likely the reason for such an effective remineralization [47-49]. This in combination with a lower burial efficiency (sandy ~1-10%, muddy ~10-60% [50]) results in burial rates of organic carbon which are much lower in permeable sands then in muddy areas despite the high carbon export on the shelf.

Sandy sediments

The low effectiveness of the final burial in sands makes them an important part of the marine carbon cycle which has been overlooked in the past [51]. Since they cover about 70% of continental margins, a region with high carbon export, heterotrophic microbial processes within sandy sediments are of global relevance [51]. Sands are defined foremost by their grain size. They range from very fine sands (63 μm) up to fine granule gravel (2 mm) [52]. Such sands are normally low on organic carbon but show very high respiration rates, which are known to exceed the respiration rates of muddy sediments with much higher organic carbon content [8, 53]. A high supply of oxygen and other nutrients together with a deep

penetration depth is the reason for high respiration rates [47]. This is possible in sandy sediments due to the relatively coarse pore size, which allows for an inflow of overlaying sea water into the sediments [48]. The inflow is furthermore amplified by the formation of bedforms such as ripples, creating an quite dynamic environment [49]. On top of oxygen the advective transport is also delivering other nutrients to the sediments, like nitrogen compounds [47] or other inorganic nutrients [54], but also POM [55]. Such an environment is not only beneficial for heterotrophic respiration, it also enables benthic photosynthesis in shallow areas. In these extremely shallow areas carbon fixation by benthic photosynthesis can range between half or equal to the amount of the integrated photosynthesis in the overlaying water column resulting in a net contribution to the sediment OM of up to twice the OM export from the water column [56-58].

Looking at temporal shifts within sandy sediments, we can observe seasonal dynamics in the biogeochemistry, in particular in Arctic sediments [59]. Primary production and in response microbial respiration rates in oxic and anoxic sediments, show a seasonal increase during spring and summer in arctic fjord sediments, indicating increased remineralization of carbon [59-61]. In temperate environments, like the Middle Atlantic Bight, seasonal changes in the biogeochemical cycles of permeable sands are reported to be less pronounced or simple invisible for some parameters [62]. Such contrasts could either be a simple result of strong seasonal differences in polar regions, or they are linked to the difficulties of quantifying fluxes in such dynamic systems as outlined for several biogeochemical cycles in a review by Huettel*et al*. 2014 and all sources within [56].

Sandy sediments as a microbial habitat

Marine sands are populated by very diverse microbial communities. Diversity studies based on comparative 16S rRNA gene sequencing indicated 3000 and up to more than 8000 observed operational taxonomic units based on a 97% sequence similarity (OTU_{97}) [63, 64]. Yet, they harbor a lower number of total cells, ranging between 10^7-10^9 cells ml^{-1} [64-66], which is about one order of magnitude below muddy organic rich sediments with cell numbers of more than 10^9 cells ml^{-1}[3, 67]. Communities in sandy surface sediments are mostly dominated by Gammaproteobacteria with about 25-30% relative abundance, followed by Bacteroidia (former Bacteroidetes) with about 15% and Actinobacteriota 10-20% .[64, 68, 69]. Even though sandy sediments are oxic in the upper centimeters, most of them contain a stable population of Desulfobacterota (former Deltaproteobacteria) of about 10% relative abundance which most likely inhabits microoxic zones in the sediment. In particular in temperate coastal sands another abundant group are the Planctomycetota (former Planctomycetes) with about 10% relative abundance[64, 68, 69]. So far microbial community studies on sandy sediments are scarce[64, 68, 70-72] and do not focus on temporal dynamics. Still,whenmicrobial communities were compared between seasons, changes in community composition are minimal and not significant [64] (See Fig.6 B). This corresponds with the low seasonal variation of biogeochemical cycles in temperate regions mention before, but emphasizes the need for a focused study on seasonal changes in microbial communities from permeable sediments.

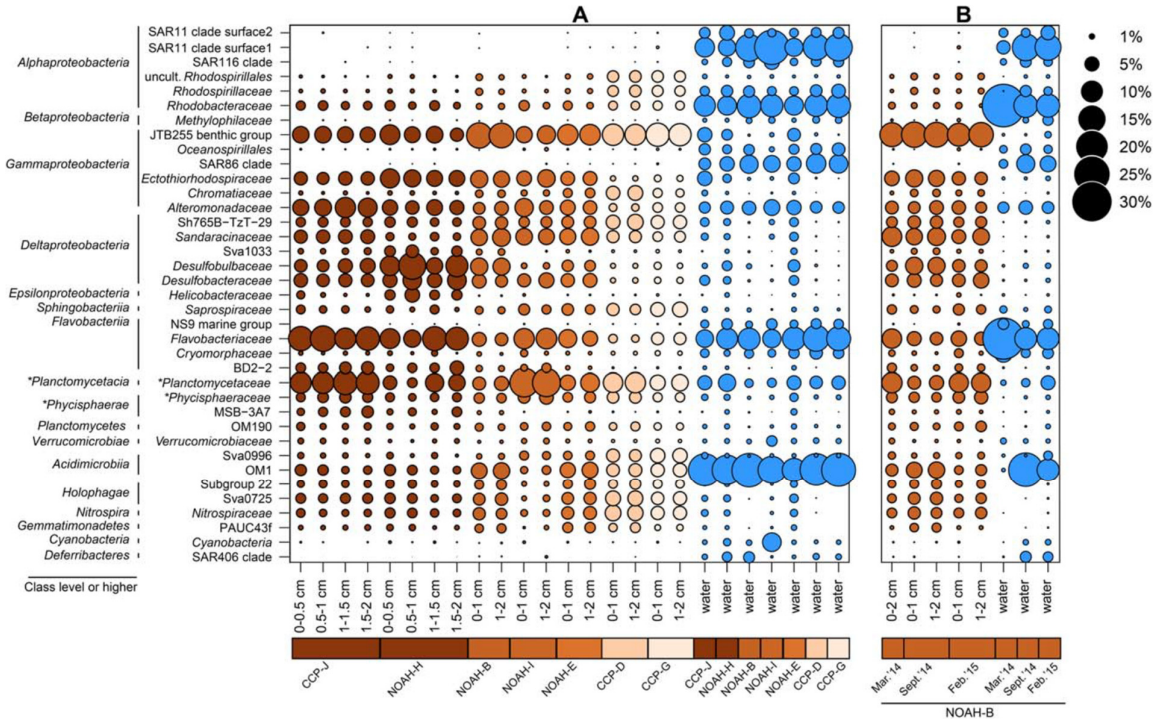

Figure 6: Relative abundances of bacterial families and uncultivated clades in sediments (brown) and corresponding bottom waters (blue) based on sequencing of 16S RNA genes (V3-V4 region). Only taxa that made up >1% of total sequences in any given sample are listed. Taxonomy based on SILVA SSU NR Ref database, release 119. Sampling sites are ordered from impermeable sediments (left) to highly permeable sediments (right). B. Data for site NOAH-B are ordered according to sampling date to visualize seasonal changes. From Probandt*et al*. 2017 [64]

Life in sandy sediments mostly happens on the sand grains, not in pore water, at least according to cell numbers. Most bacteria are attached to the surface of the grains.They do not form a thick biofilm, but rather a mono cellular layer in specific areas of the sand grain [47, 68]. The bacterial colonies are located within the cracks and depressions of the grains leaving a lot of free area from which microbial cells might be removed frequently. This might be caused by abrasion by surrounding sand grains or by grazing [68]. Looking even closer in the cracks and depressions using electron microscopy the cracks even reveal to contain so far unidentified material which is either a leftover of microbial life or a nutrient supply for such

life. On top of that we can see different modes of attachment using either extracellular matrices or pili to fix cells to the surface of the grains [47] (See Fig.7).

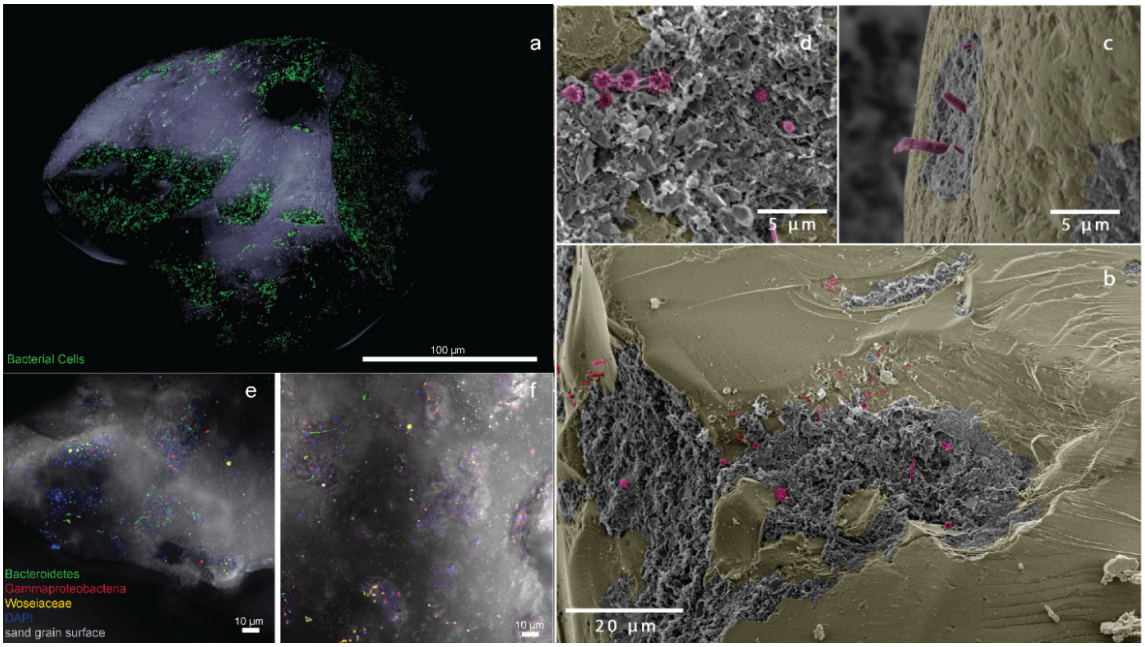

Figure 7: Visualizations of microbial cells on sand grains. Confocal laser scanning micrograph of microbial cells stained with SYBR green (DNA stain), on a whole sand grain (surface in gray), shows microbial life to be limited to cracks and depressions on the grains (a). Scanning electron micrograph of microbial colonization on sand grains with cells in magenta, the mineral grain surface in yellow-brown, and unidentified material in gray (b, c, d). Visualization of different taxa using florescence *in situ* hybridization shows that even though we can observe such isolated colonies of bacterial live on the sand grain they seem to be diverse and not made by just one taxon (e, f). Modified after Porbandt*et al*. 2017[68]a, e, f and Ahmerkamp*et al*. 2020 [47]b, c, d

In contrast to coastal sands, muddy sediments consist predominantly of silt or clay as matrix. They harbor different communities and are shaped by different factors. The reason for the differences in community composition is mostly driven by the availability of oxygen as terminal electron acceptor, and thus by the permeability of the sediment [64]. In deeper layers, other terminal electron acceptors, like iron, manganese or sulfate come into play creating a stratified sediment community [46]. In particular Desulfobacterota play a much

bigger role in such sediments together with Bacteroidia and Alphaproteobacteria and Archaea [45, 46, 73].

FISH, metagenomics and metatranscriptomics

Studying the ecology of microorganisms presents us with unique challenges compared to the ecology of macroorganisms. Using just visual observation we can not identify specific groups of microorganisms, as we can do with macroorganisms. Morphologically, microorganisms can only be classified in very few categories which neither reflect taxonomic nor functional diversity. Because of this we need to use other identification characteristics like phenotypic abilities or genomic information to identify a population. This makes *in situ* identification and quantification of specific taxa complicated and the analysis of population dynamics difficult. For a long time, cultivation was the only method of studying microbial diversity and physiology, but using the 16S rRNA marker gene it became more and more obvious over the years that cultures only represent a small fraction of the realdiversity of microbial life [74-76]. To investigate the yet uncultured majority of microbes, molecular biological techniques were developed. Among them are 16S rRNA based methods, which allow to determine diversity and abundance, answering ecological questions such as “Who is out there?” and “How many?”. Ribosomal RNA genes are optimal markers due to two reasons. They are highly conserved and shared among all life forms, Bacteria, Archaea and Eukarya, making them universal marker genes. Part of their sequences are highly conserved, yet interspersed with variable regions enabling differentiation of taxa down to the genus and sometimes also the species level [77]. Therefore, 16S rRNA sequences can be used for generating fingerprints [78] of microbial communities assessing differences of the community composition in diverse environments along ecological gradients [1, 79, 80], as well as in clinical applications [81]. Furthermore, using the 16S rRNA sequence we can

design oligonucleotide probes which via direct or indirect fluorescent labelingenable direct identification and thus quantify microbial taxa using fluorescence *in situ* hybridization (FISH) and microscopy [82, 83]. FISH enables us to identify microbes on different taxonomic levels [84, 85]. Methods based on rRNA provide, however, only limited information on the physiology and on the function of taxa. For a full assessment of the question "What are they doing?" we need to consider the phenotype and genotype of our organism of interest. Such questions can also be addressed using techniques independent of pure cultures. Short term incubations with radioactively-labelled substrates and a subsequent identification via microscopy is already available for quite some time, like, for example MAR-FISH [86]. Rather recent approaches combine the uptake of fluorescently labeled polysaccharides in short term incubations with microscopy and FISH for single cell identification [39, 87]. Yet, cultivation remains the gold standard for detailed studies due to the genomic integrity of isolates and the possibility to conduct various laboratory experiments including knockout mutants to study function and relevance of specific genes.

As outlined above, only a small fraction of the extant microorganisms have been isolated as pure cultures. Cultivation-based approaches are biased towards certain phylogenetic groups. Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes include 90% of all validly described isolates, leaving all other phyla with few isolates. We are still missing microbes specialized, e.g. ,to oligotrophic environments or adhesive and syntrophic lifestyles [88]. Over the past two decades metagenomics [89] based on next generation sequencing (NGS) of environmental DNA is enabling more and more insight in the genomic potential of yet uncultured microorganisms. Using DNA extracted from different environment, scientists are now reconstructing genomes from thousands of microbial species, and investigate possible functions and ecosystem contributions of these species. Thus,

metagenomics is one of the most used and most powerful exploratory techniques across various fields of biology [90, 91]. In the standard approach, genomic DNA is fragmented into smaller pieces that can be sequenced by short-read methods. These short reads are assembled into longer contigs using computer intensive calculations and are afterwards grouped based on sequence frequency and characteristics in units called bins or above a quality threshold [92] metagenomics assembled genomes (MAGs). These bins and MAGs can be used to investigate taxonomy and functional genomic potential of the microorganisms. Still, highly diverse communities present computational challenges to metagenomic analysis, which can make the standard approach complicated and limited in its results. However, read-based metagenomics can provide important information, e.g. ,by quantifying genes of interest in such datasets [93]. In simple or dynamic systems, metagenomic abundance estimates of specific genes can haverelevance, but in more complex systems such numbers might be either not significant or simply misleading. Here, metatranscriptomics, the high throughput sequencing of RNA, provides a framework to analyses the actual gene expression in a certain situation, enabling us to compare the reactions todifferent environmental conditions on a whole community level or for single genomes. In combination with metagenomes this allows us to investigate microbial activity without the necessity to obtain a multitude of pure cultures or the limitation to specific substrates or FISH probes [94]. Furthermore, sequencing total RNA from a community provides also rRNA sequences with taxonomic information. As outlined above, all methods from cultures to sequencing hold advantages and disadvantages. It is best to use them in concert to answer complex ecological questions such as the involvement of heterotrophic bacteria in benthic mineralization of OM.

Main questions investigated in this thesis

In the framework of this thesis I investigated microbial communities in sandy surface sediments with a focus on the utilization of polysaccharides over the seasonal cycle in temperate and polar habitats. I focused on the following questions:

i) Are there taxonomic shifts in the microbial community composition in sandy surface sediments of temperate and polar regions between seasons over the course of several years? I focused on spring to identify dynamic successions of heterotrophic bacteria which is known from studies in the water column. (Chapters 2 and 3)

ii) What are the main polysaccharides fueling microbial communities in sandy surface sediments, and who are the key players responsible for degradation of these substrates? (Chapters 3 and 4)

iii) Can we explain the apparent taxonomic stability of microbial communities in sandy surface sediments over several seasons? (Chapters 2 and 3)

iv) How do molecular methods advance our understanding of sandy surface sediments and the relevance of this sediments for the marine carbon cycle (Chapter 3 and 5)

Chapter 2: Bacterial communities in temperate and polar coastal sands are seasonally stable

Declaration on contribution of Sebastian Miksch to Chapter 2

Name of candidate: Sebastian Miksch

Title of thesis: Microbial polysaccharide utilization in sandy surface sediments

Authors of manuscript: Sebastian Miksch, MirjaMeiners, AnkeMeyerdierks, David Probandt, Gunter Wegener, Jürgen Titschack, Maria A. Jensen, Andreas Ellrott, Rudolf Amann and KatrinKnittel

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Contribution of the candidate in % of total work load

Experimental concept and design: ca. 50%

Experimental work/acquisition of data: ca. 80%

Data analysis and interpretation: ca. 80%

Preparation of figures and tables: ca. 80%

Drafting of manuscript: ca. 90%

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Bacterial communities in temperate and polar coastal sands are seasonally stable

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Coastal sands are biocatalytic filters for dissolved and particulate organic matter of marine and terrestrial origin, thus, acting as centers of organic matter transformation. At high temporal resolution, we accessed the variability of benthic bacterial communities over two annual cycles at Helgoland (North Sea), and compared it with seasonality of communities in Isfjorden (Svalbard, 78°N) sediments, where primary production does not occur during winter. Benthic community structure remained stable in both, temperate and polar sediments on the level of cell counts and 16S rRNA-based taxonomy. Actinobacteriota of uncultured Actinomarinales and Microtrichales were a major group, with 8 ± 1% of total reads (Helgoland) and 31 ± 6% (Svalbard). Their high activity (frequency of dividing cells 28%) and in situ cell numbers of >10% of total microbes in Svalbard sediments, suggest Actinomarinales and Microtrichales as key heterotrophs for carbon mineralization. Even though Helgoland and Svalbard sampling sites showed no phytodetritus-driven changes of the benthic bacterial community structure, they harbored significantly different communities ($p < 0.0001$, $r = 0.963$). The temporal stability of benthic bacterial communities is in stark contrast to the dynamic succession typical of coastal waters, suggesting that pelagic and benthic bacterial communities respond to phytoplankton productivity very differently.

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INTRODUCTION

Sandy sediments cover approximately 70% of continental shelves [1]. These sediments are characterized by high permeability and advective transport by which bottom water is pumped through the pore space [2]. Thus, they work as expansive filter systems: suspended particles, algae, and bacteria are transported with the penetrating water into the sediment, where they become trapped in the pores [1, 3–5]. Simultaneously, electron acceptors and nutrients are provided to benthic microbial communities, strongly enhancing bentho-pelagic coupling [6]. Continental shelf areas are highly productive ecosystems, contributing 15–21% to global primary production [7]. In shallow areas, up to 50% of the pelagic primary production can reach the seafloor [8, 9]. Active heterotrophic bacteria rapidly mineralize the settled phytodetritus as well as fresh organic matter derived from benthic primary production [1, 2, 10], as demonstrated by high extracellular enzyme activities, bacterial carbon production, and organic matter mineralization [11–13].

Despite the close linkage of water column and sediments, major compositional differences in microbial communities are evident [14–16]. For example, SAR11, SAR86, and "*Candidatus* Actinomarina" are abundant members of bacterioplankton, but rare in sediments. Based on an analysis of 509 samples spanning the global surface oceans to the deep-sea floor, pelagic, and benthic communities share only 10% of bacterial types defined at 3% sequence similarity level [16]. Compositional differences between pelagic and benthic microbial communities are accompanied by differences in function [15, 17]. Patterns of enzyme activities differed and showed a more diversified enzyme spectrum in sediments.

In the water column, phytoplankton blooms in spring and summer induce changes in bacterioplankton community structure [18–20]. For example, in the southern North Sea, relative cell numbers of *Polaribacter*, *Ulvibacter*, and SAR92 increased by factors of 5–20, shortly after bloom events [19]. These community dynamics were recurrent, indicating a phytodetritus-driven seasonality [19–21]. Knowledge of seasonality of benthic microbes, in contrast, is sparse. Seasonal changes in organic matter mineralization, extracellular enzyme activities, and bacterial biomass production rates were mostly related to temperature and substrate availability in sediments [5, 11, 22–24]. Different substrate additions showed specific effects on benthic microbial communities in incubations with Arctic deep-sea sediments Hausgarten, 78.5–80°N [25]. Addition of chitin resulted in an increased activity of the community without major compositional change, whereas addition of phytodetritus caused a strong change of community composition. Overall, there are only few studies of seasonal changes of microbial diversity and in situ abundance of major taxa [13, 26–29], indicating a high turnover of rare organisms but an unchanged pattern of major taxa [26]. In

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surface sediments from English Channel, Tait and colleagues found indications for seasonality of Flavobacteriia [29]. However, all these studies were based only on 2–5 sampling dates or on a single year.

In order to assess at high temporal resolution the variability of sandy sediment bacterial communities over an annual cycle, we sampled a shallow coastal site at Helgoland Roads (North Sea, 54° N) 19 times over the course of two years. The sample intervals were 10–20 days apart, during the spring bloom, and 6–8 weeks for the rest of the year. Although phytoplankton at Helgoland exhibits a strong seasonality [30], metatranscriptomic analysis of sediments showed that 36–53% of mRNA transcripts were related to photosynthesis genes [31], indicating ongoing primary production either in the water column or in the sediments during winter. Therefore, in order to compare seasonality in sediments at a location where primary production does not occur during winter, we also visited Svalbard 6 times between December 2017 and September 2019. The Svalbard archipelago, at 76°–81°N, is sufficiently far North that primary productivity does not occur during the polar night [32].

We tested the hypothesis that phytodetrital input drives seasonal changes in benthic bacterial community structures of Svalbard and Helgoland sediments. In particular, we wanted to identify community changes typical of the strong seasonality found in higher latitudes. We expected changes to be more pronounced in Svalbard sediments than in Helgoland sediments. To quantitatively assess sedimentary communities, we initially established an automated microscopic system for enumeration of bacteria, and developed new probes for Actinobacteriota. The in situ abundances of major taxa were investigated by CARD-FISH and the bacterial diversity by 16S rRNA gene sequencing.

MATERIALS AND METHODS

Study sites and sampling

At Helgoland, North Sea, (German Bight; 54.18°N, 7.90°E), sediment samples were obtained by scientific divers of the Alfred Wegner Institute (Bremerhaven, Germany) using push cores. The sampling site is located at Helgoland Roads between the main island and the dune (Fig. 1A). Water depth varied between ~3 and ~8 m. At Isfjorden, Svalbard, Arctic Ocean (Fig. 1B), sediments were obtained using a van Veen grab deployed from R/V *Farm*. Stations were located near the coast and within 1 km distance to each other (78.11°N 14.35°E, station 5; 78.10°N 14.38°E, station 6; 78.10°N 14.38°E, station 7; 78.10°N 14.39°E, station 23). Sampling dates were chosen according to light conditions: December 20th 2017 (24 h darkness), February 28th 2018 (7 h daylight), May 1st 2018 (24 h daylight), December 17th 2018 (24 h darkness), April 25th 2019 (24 h daylight), and September 13th 2019 (15 h daylight). Water depths varied between 3 and 9 m. (sample processing: Supplementary Information; all data: Supplementary Table S1).

Sediment particle-size measurements

Particle-size measurements were performed in the Particle-Size Laboratory at MARUM, University of Bremen (Germany), with a Beckman Coulter Laser Diffraction Particle Size Analyzer LS 13320 (Beckman Coulter, Krefeld, Germany) following in principle the methodology of Boehnert et al. [33] (For details see Supplementary Information).

Determination of carbon and nitrogen concentrations

Freeze-dried sediments were grounded to a powder in a Planetary Micro Mill (Pulverisette 7, Fritsch, Idar-Oberstein, Germany). To determine total carbon and nitrogen, ~25–50 mg of sediment powder was packed in 5 × 9 mm tin capsules (HEKAtech, Wegberg Germany). To determine organic carbon, inorganic carbon was removed with 1 M HCl before analysis. Samples were analyzed in an Euro EA-CNS elemental analyzer (HEKAtech, column temperature 75 °C; carrier gas helium at 80 ml min $^{-1}$, reactor temperature 1000 °C with oxygen flow 10 ml min^{-1}, oxidation time 7.9 s) with thermal conductivity detection. Calibration was done with sulfanilamide (0.2–2 mg) standards. The quantification limit for carbon and nitrogen was 0.2 mg per gram sediment. In Helgoland samples, organic nitrogen concentrations were below quantification limit, hence values were not reported.

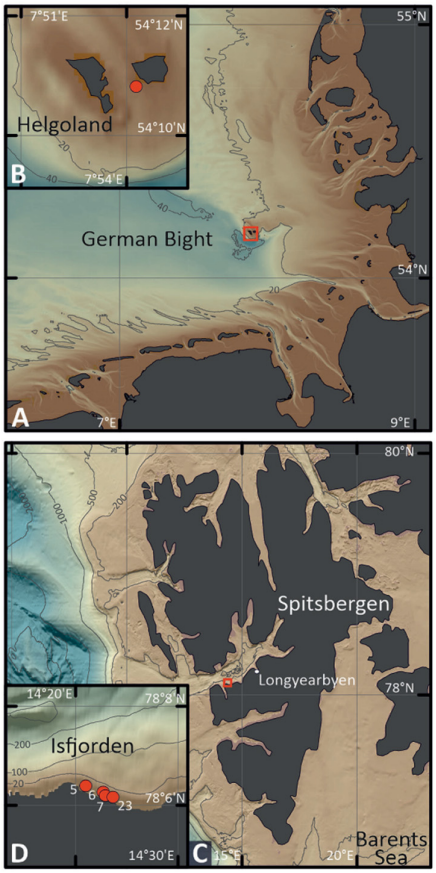

Fig. 1 Map of sampling areas. A, B Helgoland Roads, North Sea (German Bight); **C, D** Isfjorden, an Arctic fjord in western Spitsbergen (archipelago of Svalbard, Arctic Ocean). Data sources: European Environment Agency, EEA coastline derived from EU-Hydro and GSHHG data; http://www.eea.europa.eu/data-and-maps/data/eea-coastline-for-analysis and EMODnet Bathymetry Consortium (2016, 2020): EMODnet Digital Bathymetry (DTM). https://doi.org/10.12770/bb6a87dd-e579-4036-abe1-e649cea9881a; https://doi.org/10.12770/c7b53704-999d-4721-b1a3-04ec60c87238.

Chlorophyll and phaeopigment measurements

Pigments were yielded by extraction from 1 ml sediment with 8 ml acetone and disruption in a cell mill. The supernatant was collected after centrifugation and the extraction of the sediment was repeated twice. Two milliliter aliquots of the pooled extracts were subjected to fluorescence measurements [34] using a Trilogy Laboratory Fluorometer (Turner Designs; excitation = 428 nm; emission = 671 nm) and measured before and after acidification with 100 µl 20% HCl. The concentration of chlorophyll *a* (Chl*a*), and phaeopigments (PhP) were determined according to

$$\mathrm{Chla}[\mu g] = (\mathrm{RFU_b} - \mathrm{RFU_a}) \times CF \times AF \times f$$
$$\mathrm{PhP}[\mu g] = (\mathrm{RFU_a} \times \mathrm{AR} - \mathrm{RFU_b}) \times CF \times AF$$

with the relative fluorescence units before acidification (RFU_b) and after acidification (RFU_a) and the calibration factor (CF) and the dilution factor (*f*) determined from chlorophyll standards as described by Lorenzen [34].

DNA extraction, amplification of 16S rRNA genes, sequencing

DNA was extracted from sediment samples (0–2 cm depth) according to Zhou et al. [35], slightly modified by adding three initial freeze-thaw cycles. DNA from surface seawater was extracted using DNeasy Power Water Kit (QIAGEN, Hilden, Germany). Amplification of 16S rRNA gene fragments was done using primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 [36]. Amplicons were sequenced on an Illumina (San Diego, CA, USA) platform (HiSeq2500, 2 × 250 bases, paired-end) at the Max Planck-Genome Center in Cologne (Germany). Sequences were processed using BBTools version 37.62 [ref. 37], mothur v.1.38.1 [ref. 38] and classified using the SILVAngs pipeline and database SSU 138.1 Ref NR99 [ref. 39] (details: Supplementary information).

Amplicon sequence variants (ASV) analysis

Demultiplexing and per sample extraction of forward and reverse fastq files was conducted using mothur v.1.39.5 ref. [38] and BBTools v. 37.90 ref. [37] ASVs were determined using dada2 v. 1.16.0 ref. [40] (standard pooled processing; SSU 138 Ref NR99). All ASV taxonomically classified as "Chloroplast", "Archaea" or "Eukarya" as well as absolute singletons were removed from the dataset.

Alpha diversity was calculated using the subsamplingNGS.R function (https://github.com/chassenr/NGS/blob/master/Plotting/SubsampleNGS.R; 100 iterations) (For details see Supplementary information).

Statistical analysis

Statistical tests were performed using the R package vegan [41–43] and customized R-scripts. ANOSIM analyses [44] were performed with 999 permutations at equal group size treatment.

Separation of cells from sediment grains

Formaldehyde-fixed Svalbard sediment samples (for details see Supplementary Information) were sonicated on ice with a type MS2.5 probe (Sonoplus mini20; Bandelin, Berlin, Germany). Six sonication steps were done at a setting of 30 s, an amplitude of 86% and pulse of 0.2 s. Combined supernatants were filtered onto 0.2 µm pore size polycarbonate filters (GTTP, Millipore, Eschborn, Germany). More than 90% of all cells have been separated from the sand grains without obvious cell damage (Supplementary Fig. S1). Silty sediments of station 6 and 23 (September 2019) were sonicated only once, diluted and directly filtered.

Catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH)

In situ hybridizations with horseradish peroxidase (HRP)-labeled probes followed by fluorescently-labeled-tyramide signal amplification were carried out as described previously [45], with few modifications. Inactivation of endogenous peroxidases was done by hydrogen peroxide (0.15% in methanol) for 30 min at room temperature. Hybridization was performed at 46 °C for 2–3 h in a chamber equilibrated with 2.25 M NaCl and identical formamide concentration as in the hybridization buffer. Amplification with Alexa 488-labeled tyramides was performed for 45 min at 46 °C. Filter sections were mounted with a mixture of CitiFluorAF1 (CitiFluor Ltd., London, United Kingdom] and Vectashield (Vector Laboratories, Burlingame, CA, USA) containing 1 µg ml^{-1} DAPI (4′,6-diamidino-2-phenylindole; Sigma-Aldrich, Steinheim, Germany). Probe sequences, permeabilization conditions and formamide concentrations are given in Supplementary Table S2.

Automated imaging and counting

For cell counting of bacteria in Svalbard sediments, we improved an automated system that was established for bacterioplankton [46, 47]. Low signal to background ratios in sediments compared to seawater required the use of narrow band pass optical filter sets. Number of layers for the imaged z-stack was increased to 13 (distance 0.4 µm). Filter sections were imaged by microscopy using a motorized epifluorescence microscope (AxioImager. Z2m, Carl Zeiss, Jena, Germany) equipped with a 63×/1.4 Plan-Apochromat objective and controlled via the MPISYS software. Illumination was done using a Zeiss Colibri 7 LED source (385 nm for DAPI; 469 nm for Alexa488) using specific narrow band pass optical filter sets for DAPI (splitter 375 nm; emission filter 448/20 nm) and Alexa488 (splitter 495 nm; emission filter 520/15 nm). The images were analyzed using the Automated Cell Measuring and Enumeration tool 3.0 (ACMEtool) software (M. Zeder, www.technobiology.ch. [47]). (For technical details see Supplementary Information).

Probe design

Probe design for Actinobacteriota based on SILVA database SSU 132 Ref NR99 and was performed with the probe design tool implemented in ARB [48] (Supplementary Fig. S2). CARD-FISH with a mix of probes ACM1218 (AGCATGCGTGCAGCCCTG; Actinomarinales) and MIT1218 (AGCATGTTTG-CAGCCCTG; Microtrichales) resulted in counts that were equal to the sum of individual counts (92 ± 9%) confirming high probe specificity. An increase of probe concentration to 280 fmol $µl^{-1}$ (10-fold above standard conditions) resulted in brighter signals and higher cell numbers (1–3-fold increase for ACM1218; 2–6-fold increase for MIT1218).

Actinobacteriota imaging, cell size, and cell division

Actinobacteriota were imaged by a laser scanning microscope (LSM780, Zeiss, Jena, Germany) equipped with an Airyscan detector. For cell size measurements, FISH [49] with Alexa594-tetralabelled probes and CARD-FISH hybridization buffer was used to avoid an overestimation of cell sizes by too bright CARD-FISH signals.

The fraction of dividing cells was determined based on line profiles of DAPI and FISH signal fluorescence in formaldehyde-fixed Actinomarinales cells (Supplementary Fig. S3). Dividing cells were defined by two criteria: i) two DAPI maxima are visible in a single cell and ii) one or no FISH signal maximum is present in the cell center between the two DAPI maxima. If two FISH signal maxima were detected in the cell center, cell division was categorized as completed.

RESULTS

Grain size

Helgoland surface sediments were, in general, composed of fine and medium sand with variable portions of coarse to very coarse sand (Fig. 2A). Winter and early spring sediments contained a large portion of coarse to very coarse sand. Svalbard sediments of Isfjorden were mainly composed of very fine and fine sand with a small fraction of medium sand. Grain size distribution did not differ markedly across stations and seasons (Fig. 2B). Except for station 7, grain size distribution changed in September 2019. Sediments from station 5 contained a minor portion of silt in addition to fine sand and medium sand. Composition of stations 6 and 23 sediments was shifted towards silty sand (station 6) and sandy silt (station 23).

Organic carbon and nitrogen contents

In Helgoland sediments, total organic carbon (TOC) content varied between 0.1% and 0.7% of sediment dry weight in 2018 (Fig. 3A). In 2019, TOC was constantly low (<0.13%). These TOC contents were typical for surface sediments of the Southern North Sea (<0.1 mg to 10 mg g^{-1}, see ref. [50]) In Svalbard sediments, TOC content was between 0.2% and 0.5% in the winter, twilight, and spring samples (mean 0.3 ± 0.1%; Fig. 3B). In September 2019, TOC in silty sediments from station 6 and 23 was highest with 0.7 and 0.9%.

Organic nitrogen content in Svalbard sandy sediments was relatively stable (Supplementary Fig. S4), with quantities between 0.03 to 0.08% of sediment dry weight for all seasons. Total organic carbon/organic nitrogen (TOC/N_{org}) ratios were 9.8 ± 0.9. Organic nitrogen contents of 0.08 ± 0.01% were detected in silty sediments from stations 6 and 23 in September 2019. TOC/N_{org} ratio was 11.2 ± 0.4. TOC, N_{org}, and TOC/N_{org} ratios changed with grain size. In silty sands TOC was 0.8 ± 0.1%, N_{org} 0.1 ± 0.01% and a TOC/N_{org} ratio of 11.1 ± 0.4, while sandy sediments had a significantly lower TOC of 0.3 ± 0.08%, lower N_{org} of 0.04 ± 0.01% and a slightly lower TOC/N_{org} ratio of 9.8 ± 0.9.

Chlorophyll *a* concentrations

In Helgoland sediments, concentrations of chlorophyll *a* ranged between 0.7 and 5.3 µg ml^{-1} (average 1.9 ± 1 µg ml^{-1}) and

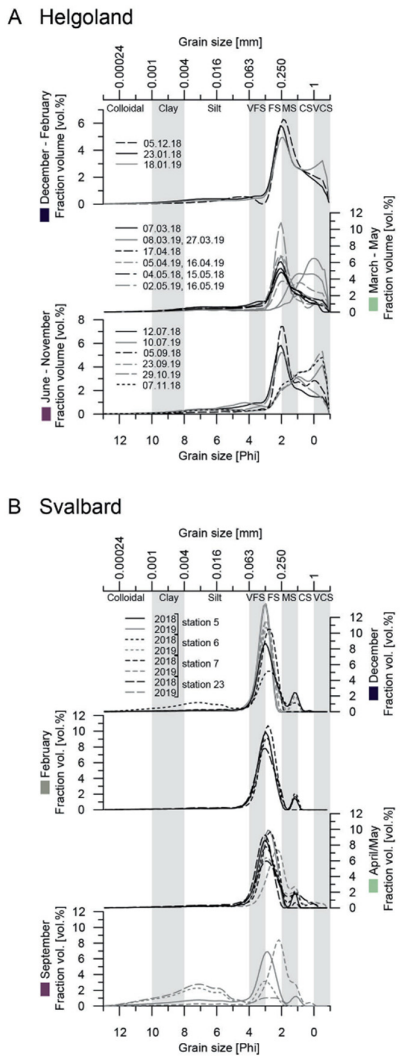

Fig. 2 Grain size distribution (vol%) of A Helgoland and B Svalbard surface sediments (0–2 cm depth) derived from laser diffraction particle size analysis. Please note the different y-scales for Helgoland samples.

concentrations of phaeopigments between 0.1 to 1.3 µg ml^{-1} (average 0.4 ± 0.3 µg ml^{-1}; Supplementary Table S1). On 4th of May 2018, we detected the strongest chlorophyll *a* peak (5.2 µg ml^{-1}) while concentration at all other time points were 2.6 µg ml^{-1} and lower. Nevertheless, no clear increase of chlorophyll concentrations in sediment samples from spring was observed.

In Svalbard sediments, chlorophyll *a* concentrations were very low in winter and twilight samples (0.3 ± 0.1 µg ml^{-1} and 0.2 ± 0.1 µg ml^{-1}) and clearly higher in spring 2018 and summer/fall (1.2 ± 0.5 µg ml^{-1} and 1.1 ± 0.6 µg ml^{-1}). In spring 2019, we measured only 0.3 ± 0.1 µg chlorophyll *a* per ml of sediment. At the same time, however, chlorophyll *a* concentrations in surface seawater were high by now (7 ± 1 µg l^{-1}) compared to winter 2018 (0.4 ± 0.02 µg l^{-1}, Supplementary Table S1).

Bacterial community composition

The composition of bacterial communities of Helgoland and Svalbard surface sediments (0–2 cm depth) and Svalbard surface seawater was investigated by 16S rRNA gene sequencing. For simplicity, Helgoland sampling dates were assigned to meteorological seasons. Svalbard sampling dates were assigned to seasons based on light availability: winter (December 2017, 2018), twilight (February 2018), spring (April 2019, May 2018), and summer/fall (September 2019). Numerous 16S rRNA gene sequences from chloroplasts were detected in the dataset from both, Helgoland and Svalbard. In Svalbard sediments, they constituted 4 ± 2, 11 ± 7, 36 ± 26, and 31 ± 11% of total reads and in surface seawater 1 ± 0.2, 3 ± 0.3, 80 ± 10, and 26 ± 3% of total reads in winter, twilight, spring, and summer/fall, respectively. The strongly increased read frequency of chloroplast sequences in datasets of spring samples indicated the presence of a current or recent phytoplankton bloom. In Helgoland sediments portions of 16S rRNA chloroplast sequences were low for all seasons. They constituted only 4 ± 2, 3 ± 2, and 4 ± 2% of total reads during winter, spring, and summer/fall samples.

Helgoland sediments. The bacterial composition was stable for all 19 sampling dates (Fig. 4). The community was dominated by Gammaproteobacteria (winter: 35 ± 2% of reads, spring: 34 ± 4%, summer/fall: 32 ± 3%). Within this class, *Woeseia* spp. dominated and did not change abundance (winter, spring, and summer/fall: 7 ± 1%). Further taxa with high read frequencies were Planctomycetota (winter: 8 ± 0.4%, spring: 7 ± 1%, summer/fall: 7 ± 1%), Alphaproteobacteria (winter: 11 ± 2%, spring: 10 ± 3%, summer/fall: 12 ± 3%), Desulfobacterota (winter: 7 ± 3%, spring: 10 ± 4%, summer/fall: 9 ± 4%), and Bacteroidota (winter: 11 ± 3%, spring: 11 ± 3%, summer/fall: 14 ± 2%).

Svalbard sediments. The bacterial community composition showed no clear changes between seasons (Fig. 4). Note that for calculation of mean read frequencies, data from silty sediments (station 6 and 23, September 2019) were excluded to avoid influence of reduced permeability and oxygen availability due to smaller grain size. Across all seasons, Actinobacteriota dominated the benthic community with 29 ± 9% of reads (winter: 34 ± 1%, twilight: 26 ± 1%, spring: 31 ± 8%, summer/fall: 25 ± 11%). These Actinobacteriota were mainly affiliated with uncultured Actinomarinales (17 ± 7% of reads; winter: 20 ± 4%, twilight: 17 ± 2%, spring: 18 ± 6%, summer/fall: 12 ± 6%) and Microtrichales (12 ± 4% of reads; winter: 14 ± 4%, twilight: 8 ± 2%, spring: 13 ± 3%, summer/fall: 12 ± 5%). Other abundant taxa were Bacteroidota (winter: 19 ± 1% of reads, twilight: 18 ± 2%, spring: 18 ± 3%, summer/fall: 20 ± 4%) with *Maribacter* being most dominant (4 ± 2%) and Planctomycetota (winter: 7 ± 2%, twilight: 9 ± 2%, spring: 6 ± 2%, summer/fall: 5 ± 0.2%). Stable read frequencies were also detected for diverse Proteobacteria: Gammaproteobacteria (winter: 11 ± 1%, twilight: 13 ± 2%, spring: 11 ± 2%, summer/fall: 8 ± 1%), Alphaproteobacteria (winter: 9 ± 3%, twilight: 8 ± 1%, spring: 10 ± 3%, summer/fall: 16 ± 8%), and Desulfobacterota (winter: 5 ± 1%, twilight: 7 ± 1%, spring: 4 ± 1%, summer/fall: 4 ± 0.3%). ANOSIM statistics indicated that observed differences in read frequencies between seasons were not significant ($R = 0.15$, $p = 0.09$).

In the two silty sediments (station 6 and 23, September 2019), communities were characterized by lower read frequencies of

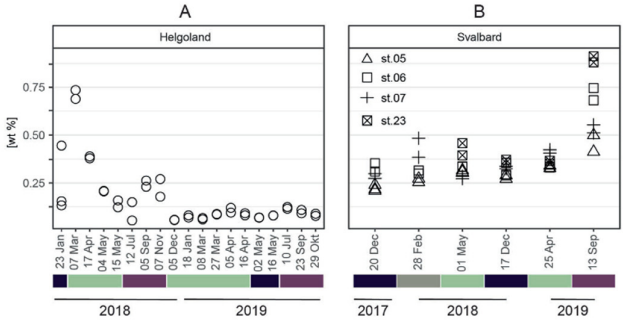

Fig. 3 Total organic carbon content in **A** Helgoland and **B** Svalbard sediments. Seasons are color-coded (winter: black, twilight: gray; spring: green; summer/autumn: aubergine-colored).

Actinobacteriota (9 ± 2% vs. 29 ± 9% in sandy sediments) and *Maribacter* (0.6 ± 0.1% vs. 4 ± 2%) and by higher frequencies of alphaproteobacterial Rhodobacteraceae (20 ± 1% vs. 8 ± 5%). (Fig. 4). These changes were significant (Supplementary Fig. S5). Further significant changes were detected for less abundant genera of Bacteroidota, i.e., *Maritimonas*, *Maribacter*, *Lutibacter*, and *Fluviicola*.

Svalbard seawater. Bacterial communities in Svalbard surface seawater showed distinct seasonal patterns (Fig. 4). In winter, twilight and summer/fall, SAR11 dominated (53 ± 13% of reads). In spring, SAR11 decreased to 23 ± 8% while genus *Polaribacter* increased from 2 ± 1 to 31 ± 12%. Further taxa that strongly increased in spring 2019 were Nitrincolaceae (30 ± 4% vs. 1 ± 1% across other seasons) and Colwelliaceae in spring 2018 (23 ± 5% vs. 0.1 ± 0.2%).

Comparison of Svalbard and Helgoland sediment communities. Even though Helgoland and Svalbard benthic bacterial communities were seasonally stable, they harbored significantly different communities (ANOSIM; $p<0.0001$, $r = 0.963$; Fig. 5). Read frequencies of Actinobacteriota, *Maribacter*, and Patescibacteria were much lower at Helgoland than at Svalbard (Actinobacteriota, 8 ± 1% vs. 31 ± 6% of reads; *Maribacter*, 0.7 ± 0.2% vs. 5 ± 2%; Patescibacteria, 0.4 ± 0.1% vs. 3 ± 1%). In contrast, Acidobacteriota, were more abundant in Helgoland sediments (3.5 ± 0.4% vs. 0.6 ± 0.1%). Different quantities and compositions of organic matter depending on the characteristics of the bloom as well as different grain size distributions might have caused the differences.

In situ quantification of major taxa in Svalbard sediments

In situ quantification of major taxa focused on the region where we expected the most prominent seasonality, Svalbard. As a prerequisite, we modified the automated system developed for cell enumeration of bacterioplankton [46, 47]. This system worked for both types of Svalbard sediments, sand and silt. Manual counts constituted 89 ± 10% of automated counts (DAPI-stained cells), 112 ± 26% (Desulobacterota/Myxococcota, probe DELTA495a-c) and 98 ± 21% (Actinomarinales, probe ACM1218) (Supplementary Fig. S6). Although this system did not greatly reduce the time for sample processing compared with manual cell counting, it allows more standardized, thus more reliable, cell counting, and exclusion of human bias.

Total cell numbers varied between 1.2×10^8 and 1.3×10^9 cell ml^{-1} sediment (Supplementary Fig. S7). To normalize the differences in total cell counts, we focused on relative cell numbers to identify changes between seasons.

Overall, relative cell numbers of major taxa did not change significantly between winter and spring (*t*-test: *p*-values between 0.260 and 0.842) (Fig. 6): Gammaproteobacteria (winter: 13 ± 2%, twilight: 13 ± 2%, spring: 13 ± 3%, summer/fall 12 ± 2%), Desulfobacterota/Myxococcota (winter: 11 ± 3%, twilight: 12 ± 5%, spring: 10 ± 2%, summer/fall 7 ± 3%), Planctomycetota (winter: 4 ± 1%, twilight: 4 ± 2%, spring: 3 ± 2%, summer/fall 4 ± 0.1%) and Verrucomicrobiota (winter: 2 ± 0.3%, twilight: 2 ± 0.2%, spring: 2 ± 0.2%, summer/fall 3 ± 0.6%). Actinobacteriota of Actinomarinales and Microtrichales were found in high numbers, up to 10 ± 2% of total cells across all seasons (Supplementary Fig. S7). Actinomarinales made up 5 ± 1% of total cells (winter: 6 ± 1%, twilight: 5 ± 1%, spring: 6 ± 1%, summer/fall 5 ± 2%) and Microtrichales 4 ± 1% (winter: 5 ± 1%, twilight: 5 ± 1%, spring: 5 ± 1%, summer/fall 4 ± 0.3%).

Across seasons, the only striking change in relative cell numbers was detected for Bacteroidota, which increased by a factor of ~2 in summer/fall (winter: 7 ± 2%, twilight: 7 ± 2%, spring: 8 ± 2%, summer/fall 13 ± 3%). The change between winter and summer/fall was significant (*t*-test, $p = 0.010$), however, we refrain from linking it to season because station 5 contained a minor part of silt besides fine sand, resulting in reduced permeability and oxygen availability (oxygen saturation in sediments from station 5 was lower than at station 7, data not shown). The influence of sediment-type on the bacterial community is even more pronounced in silty sediments of summer/fall where Bacteroidota increased to 15 ± 1% of total cells. Our data support previous findings that permeability shapes the bacterial community [51, 52].

Amplicon sequence variants and diversity parameters

On the taxonomic level of genus and higher, Svalbard benthic bacterial communities did not show significant seasonal changes based on CARD-FISH cell numbers and read frequencies (Figs. 4 and 6). To address possible changes below genus level, we investigated seasonal changes of amplicon sequence variant (ASV) frequencies (Supplementary Table S3). In sediments, variations in frequencies of overall most abundant ASV between spring and winter were all within standard deviation (of the eight samples per category) indicating no significant change (Supplementary Fig. S8). Variations in frequencies of ASV from Gammaproteobacteria, Desulfobacterota, Bacteroidota, Verrucomicrobiota, and Actinobacteriota were also within standard deviation (Supplementary Fig. S9 and Supplementary Table S3). In contrast, frequencies of seawater ASV changed significantly and showed variations by factors of 6 and 1928 between spring and winter for the ten most abundant ASV.

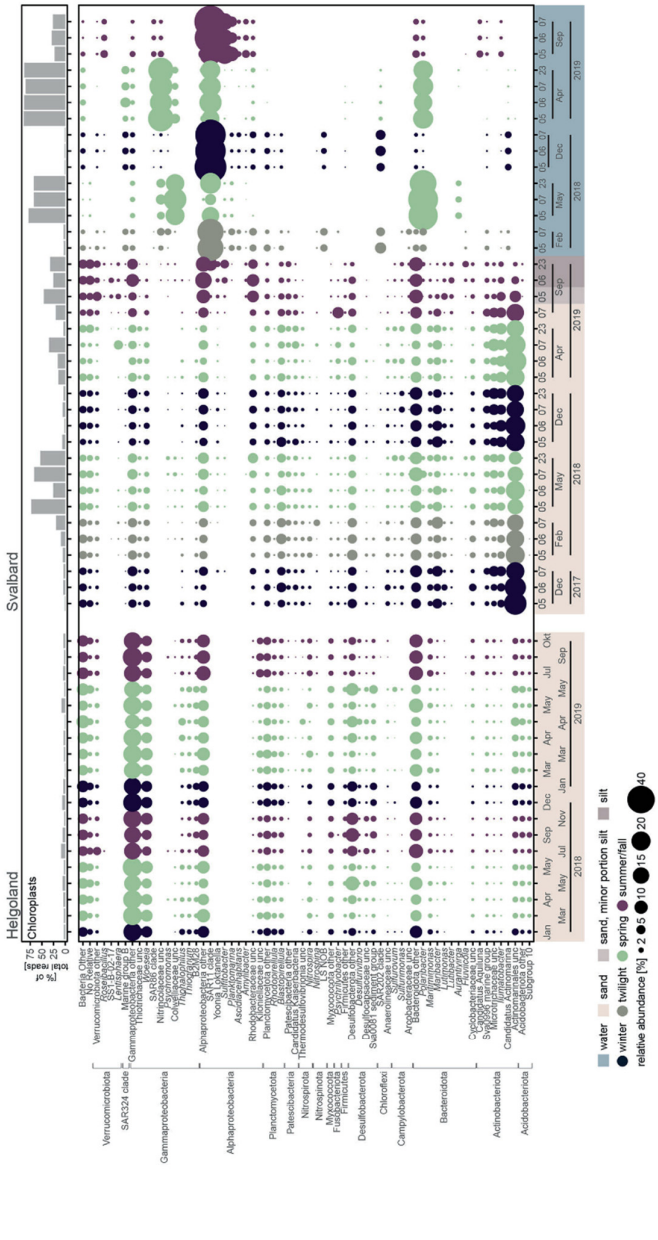

Fig. 4 Relative abundance of bacterial families and genera in Helgoland surface sediments and Svalbard sediments and seawater based on sequencing of 16S rRNA genes (V3-V4 region). Taxonomy based on SILVA SSU138.1 Ref NR99 database. Only taxa that accounted for >2% of total sequences in at least one of the samples are shown. Minor abundant taxa were clustered on higher taxonomic levels and displayed as "other". The bubbles' diameters give read frequencies, bubbles' colors indicate the season of sampling. For Svalbard samples, station numbers are indicated on the *x*-axis. As proxy for current or recent photosynthetic activity and primary production, read frequencies of sequences classified as chloroplasts are shown in the top bar charts.

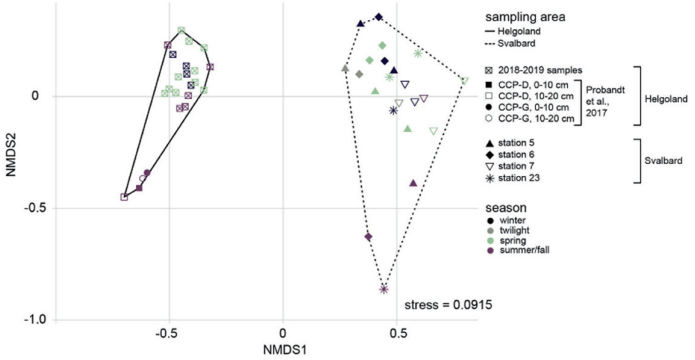

Fig. 5 NMDS ordination plot of sampling areas at Helgoland and Svalbard, generated from 16S rRNA gene datasets. Data from sites CCP-D and CCP-G, 6.5 km apart from Helgoland (bioproject number PRJEB18774, ref. [28]) were included in the analysis. The sampling areas are depicted as polygons. Helgoland and Svalbard bacterial community structures were significantly different supported by an ANOSIM *R* value of 0.96 ($p < 0.0001$). Dissimilarity based on season was not supported by ANOSIM ($R = 0.06$, $p = 0.09$).

Alpha diversity analysis as given by Inverse Simpson showed a lower diversity in Svalbard sediments from winter (131 ± 36) compared to spring (188 ± 62), however, the differences were still within standard deviation (Supplementary Table S4). In seawater, Inverse Simpson were higher in winter (16 ± 2) than in spring (12 ± 5), mirroring the pelagic bacterial community dynamics detected by ASV frequencies.

Actinobacteriota

Actinobacteriota were the major phylum in Svalbard sediments based on cell numbers and read frequencies. The major part of Actinomarinales sequences was affiliated with clades Uncultured 1 and Uncultured 6 (Supplementary Fig. S2) that comprise sequences from diverse marine habitats (for example, Mediterranean beaches [53], Arctic sediments [54], polar waters [55], seafloor lavas [56]). Most abundant ASV#473 (2.6% of total reads) and ASV#839 (1.8%; Supplementary Table S3) were both affiliated with clade Uncultured 6. Microtrichales sequences were mainly affiliated with *Ilumatobacter*, clade Sva0996, and clade Uncultured 10. Sva0996 was first found in Svalbard silty sediments from Hornsund [57]. Nowadays, Sva0996 comprises sequences from numerous marine habitats including beaches [53], permeable shelf sediments [58], tidal subsurface sediments [59], or seawater [60].

In situ detection revealed ~95% of Actinomarinales were rods while ~5% were cocci. Most cells were free-living, few cells occurred in loose biofilms (Fig. 7A). Microtrichales were also rod-shaped (Fig. 7B), few cells were coccoid (~10%). Based on high-resolution images, Actinomarinales were highly active as indicated by a frequency of dividing cells of 28% (station 6, December 2017; 78 cells analyzed; Supplementary Fig. S3). After division, Microtrichales and Actinomarinales cells had a size of approximately 0.25 × 0.5 µm and a volume of 0.024 µm^3.

DISCUSSION

Benthic bacterial communities are stable across seasons

A major part of the annual primary production in temperate and high latitude environments occurs during spring [30, 61–63]. Especially in the Arctic, the amounts and relative contributions of the different organic matter sources to fjords change seasonally and spatially [63–65]. Light is the limiting factor for phytoplankton growth during winter. In addition to light availability, runoff of suspended sediment from glaciers in summer and fall drives seasonality of organic matter input [66]. In Isfjorden, marine organic matter dominates in May following the spring phytoplankton bloom while in June and August, permafrost and glacial-fed meltwater is present and a source of terrestrial organic matter [66]. Surface sediments at our sampling sites in Isfjorden are located in a wave-dominated embayment and are thus little affected by meltwater and river runoff (Rubensdotter and Jensen, 2020, map of Hollendarbukta stored at repository: https://svalcoast.com/). This is also indicated by only a minor shift in TOC/N_{org} between winter (10.0 ± 0.5) and spring (9.5 ± 1.0) and summer/fall (10.8 ± 0.5), although glacial meltwater runoff is probably the reason for more silty samples in September. In the bacterioplankton community, the seasonality in primary production at Svalbard was clearly causing compositional changes (Fig. 4 and Supplementary Fig. S8) such as a strong increase of *Polaribacter* and *Aurantivirga*, as previously seen also for Helgoland seawater [18, 19, 21, 67]. However, the benthic bacterial communities at Svalbard and Helgoland, remained stable over two annual cycles. Cell numbers of major taxa and read frequencies of 16S rRNA genes on genus-level and below did not change significantly between winter and spring. Increased read frequencies of chloroplasts and chlorophyll concentrations in spring 2018 samples and summer/fall 2019 samples (Fig. 4 and Supplementary Table S1) indicated, that we did not miss the phytoplankton bloom or fresh organic matter from benthic primary production. Thus, the bacterial community might have responded to phytodetritus input by an increased activity rather than by changes in community structure. A response dominated by increased activity is supported by results of recent in situ experiments at Fram Strait, part of the Arctic deep-sea floor, where phytodetritus of either the diatom *Thalassiosira* sp. or the coccolithophore *Emiliania huxleyi* was provided to the benthic community. The community did not respond to the algal input by an increase of total bacterial cell numbers, but by an increase of exoenzymatic activities [68]. The high diversity of Svalbard benthic bacterial communities (observed number of ASV between 2526 and 6691) is coincident with a diverse spectrum of enzymes capable of degrading different substrates as previously suggested by Teske and colleagues [15]. Thus, responses to substrate input might preferentially be on the gene expression level rather than in community structure.

Although major taxa such as Actinomarinales were actively growing in Svalbard sediments, as shown by a high frequency of

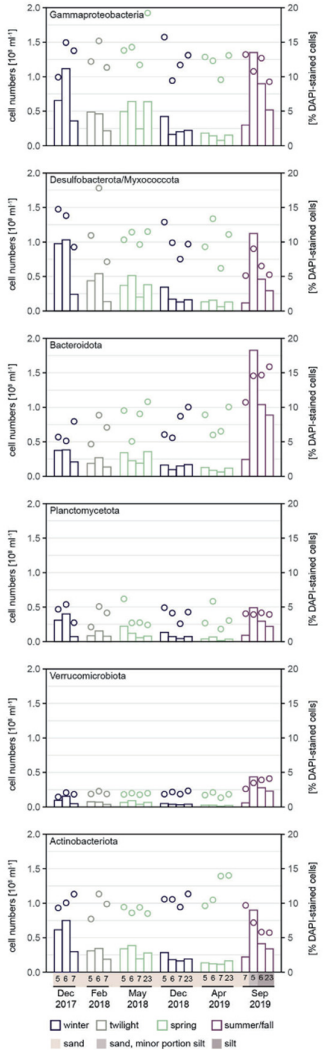

Fig. 6 In situ abundance of bacterial taxa in Svalbard sediments as determined by CARD FISH. Bars give absolute cells numbers, circles give relative abundances. Seasons are color-coded (winter: black, twilight: gray; spring: green; summer/fall: aubergine-colored).

dividing cells (28%), we did not detect an increase in absolute cell numbers in any season. Bacterial biomass turnover time is short, typically within 2–18 days in organic-poor sands (average 5–6 days [11]). Grazing, however, is not considered the major process controlling the fate of bacterial biomass [69, 70]. By an in situ pulse-chase experiment with ^{13}C-tracers, van Oevelen and

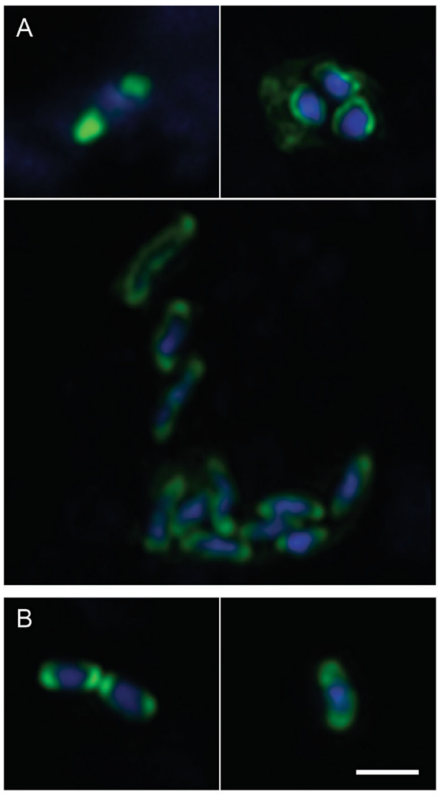

Fig. 7 Laser scanning micrographs of Actinobacteriota in Svalbard sediments as detected by CARD-FISH. A Actinomarinales visualized by probe ACM1218; **B** Microtrichales visualized by probe MIT1218. Green, CARD-FISH signals; blue, DAPI signals. Scale bar, 1 µm.

colleagues [70] quantified the fate of microphytobenthos in an estuarine tidal flat. They observed that grazing on bacteria by higher trophic levels is limited to 9% of total bacterial production. Virus-induced prokaryotic mortality account for 16 ± 3% in coastal sediments (n = 11) [71].

In addition to loss via grazing and viruses, the seasonal stability of major taxa may well be linked to their physical growth conditions on sand grains. Life in cracks and depressions of the sand grain may protect microbes from grazing and abrasion when sand moves [72]. Microbes are neither evenly distributed nor do they form thick biofilms on sand grains: they colonize only about 4% of the grains' surface area [52, 73]. Non-populated, convex areas on the sand grains' surface could be too exposed to grazers and shearing forces during sediment reworking to maintain microbial populations. Large depressions might be diffusion-limited, reducing oxygen and nutrients supply to microbes [73]. Thus, our observations of i) active growth and ii) lack of seasonality of bacterial communities even after phytodetritus-input together with generally low population density on sand grains suggests a maximum of habitable surface area on a sand

grain that keeps the bacterial community structure in a steady-state.

Actinobacteriota are key heterotrophs in Svalbard sandy surface sediments

Actinobacteriota of uncultured Actinomarinales and Microtrichales clades constituted the major taxon in Svalbard and Helgoland sandy surface sediments (Figs. 4 and 6). Taking into account the high read frequency of *Ilumatobacter* (5%) and the missing coverage of this genus by the probes, the detected actinobacterial in situ abundance of 10% of total cells is likely still underestimated. Actinobacteriota constitute one of the most diverse bacterial phyla [74]. For decades, they were mostly viewed as soil bacteria, however, nowadays they are considered to be cosmopolitan in terrestrial and marine ecosystems [75]. Among marine bacterioplankton communities, Actinobacteriota are ubiquitous [76] and abundant (16S rRNA read frequencies ~10%) [77]. Pelagic and deep-sea benthic Actinobacteriota (16S rRNA read frequencies 4–31%) [78] mainly belong to "*Candidatus* Actinomarina", also known as OM1 [79], which was only rarely detected (<1%) in our datasets. In other Arctic surface sediments, actinobacterial sequences were either absent [15] or found in moderate (5% refs. [57, 80]) and high abundances (4–20% of total reads [81]).

Studies on marine Actinobacteriota focused mainly on secondary metabolite production [82–84], thus their metabolism is largely unknown. In general, most Actinobacteriota are aerobic chemoheterotrophs using a wide variety of complex organic matter, including cell wall polysaccharides of plants such as cellulose, xylan, mannan, and other hemicelluloses, chitin or humic substances (for a review see ref. [85]). Actinobacteriota encode a huge diversity of carbohydrate-active enzymes. For example, fourteen glycoside hydrolases including GH3, GH18, GH36, GH65, and GH92 were found in the *Ilumatobacter coccineum* genome [86]. Based on the expression of a new kind of rhodopsin, a photoheterotrophic lifestyle was suggested for *Candidatus* Actinomarina [87]. Light, however, is not a key factor determining in situ abundance and activity of uncultured Actinomarinales in Svalbard sediments, as 28% were dividing in winter samples and cell numbers did not differ between seasons. Considering the high frequency of dividing actinobacterial cells and their high in situ cell numbers of at least 10% of total microbial community, uncultured Actinomarinales and Microtrichales likely play an important role in carbon mineralization in Svalbard sediments. Based on the carbohydrate-active enzymes typical of Actinobacteriota [85, 88, 89], we suggest that Actinomarinales and Microtrichales may be key heterotrophs hydrolyzing complex substrates.

CONCLUSIONS AND OUTLOOK

Pelagic and benthic microbial communities respond to phytoplankton productivity very differently. While the bacterial community of the water column dynamically responds to seasonal substrate input in cell numbers and composition, we observe stable benthic communities throughout two years of sampling. Thus, our hypothesis that substrate-based seasonality extends to benthic bacterial communities on the level of cell counts and 16S rRNA-based taxonomy has been falsified for polar sediments from Svalbard as well as for temperate sediments from Helgoland.

Further studies must now target metagenomics and metatranscriptomics, metabolic activity, and gene regulation. For example, expression of glycoside hydrolases of GH16 (laminarinase) is expected to vary strongly between seasons, as the sugar polymer laminarin is a major storage compound in marine microalgae [90]. In contrast, animal-derived glycoproteins such as mucin are expected to be equally abundant throughout the year [91], and the respective degradative enzymes should be available during all seasons. The seasonal utilization of different substrates could be tested by applying fluorescently-labeled substrates [92], and this could be combined with identification of specific polysaccharide utilization loci and their expression [93].

Actinomarinales and Microtrichales should be target taxa in future studies. The likely absence of an outer membrane in these actinobacterial cells would not allow for polysaccharide degradation by selfish uptake, a major mode in the water column [94]. Previous studies showed relatively high extracellular polysaccharide hydrolysis rates of ~0.5 to 6 nmol monomer cm^{-3} h^{-1} in Svalbard sediments [95, 96]. Thus, we propose selfish uptake being of minor importance in sediments while degradation with exoenzymes dominates, releasing small substrates utilized by the plethora of microbial taxa living on sand grains.

DATA AVAILABILITY

Sequence data were stored in the European Nucleotide Archive (ENA) under study accession numbers PRJEB42060 and PRJEB42159. Other data from this study are available from the data repository PANGAEA (https://doi.pangaea.de/10.1594/PANGAEA.930322, https://doi.pangaea.de/10.1594/PANGAEA.930483, https://doi.pangaea.de/10.1594/PANGAEA.930442).

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Chapter 3: Taxonomic and functional stability overrules seasonality in polar benthic microbiomes

Declaration on contribution of Sebastian Miksch to Chapter 3

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Title of thesis: Microbial polysaccharide utilization in sandy surface sediments

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Contribution of the candidate in % of total work load

Experimental concept and design: ca. 80%

Experimental work/acquisition of data: ca. 80%

Data analysis and interpretation: ca. 60%

Preparation of figures and tables: 100%

Drafting of manuscript: ca. 80%

Taxonomic and functional stability overrules seasonality in polar benthic microbiomes

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Competing interest statement

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Abstract

Coastal shelf sediments are hot spots of organic matter mineralization. They receive up to 50% of primary production, which in higher latitudes is strongly seasonal. Polar and temperate benthic bacterial communities, however, show a stable composition based on comparative 16S rRNA gene sequencing despite different microbial activity levels. Here, we aimed to resolve this contradiction by identifying seasonal changes at the functional level, in particular with respect to algal polysaccharide degradation genes, by combining metagenomics,metatranscriptomics, and glycan analysis insandy surface sediments from Isfjorden, Svalbard. Gene expressions of diverse carbohydrate-active enzymes changed between winter and spring. For example, β-1,3-glucosidases (e.g. GH30, GH17, GH16) degrading laminarin, an energy storage molecule of algae, were elevated in spring while enzymes related to α-glucan degradation wereexpressed throughout the year with maxima in winter (e.g. GH63, GH13_18, GH15). Also, the expression of GH23 involved in peptidoglycan degradation was prevalent throughout the year, in line with recycling of bacterial biomass. Sugar extractions from bulk sediments were low in concentrations during winter but higher in spring samples with glucose constituting the largest fraction of measured monosaccharides (84±14%). In porewater, glycan concentrations were ~18fold higher than in overlying seawater (1107±484 μg C L^{-1} vs.62±101μg CL^{-1}) and depleted in glucose. Our data indicates that sandy sediments digest and transform labile parts of photosynthesis-derived particulate organic matter and release more stable, glucose-depleted residual glycans of unknown structures, quantities and residence times into the ocean thus modulating the glycan composition of marine coastal waters.

Introduction

Continental shelfs contribute 15-21% of global primary production [1] of which up to 50% reaches the shallow seafloor. About 70% of continental shelfs are covered by sandy sediments [2]. Their high permeability enhances advectiveflow of bottom water with organic matter (OM) [3, 4]. Heterotrophic benthic bacteria remineralize flowing and OM derived from benthic primary production[2, 3, 5].

A major fraction of the OM consists of polysaccharides that phytoplankton produces for energy storage, as cell wall building blocks or as exudates. Glycansconstituteup to 80% of algae dry weight, depending on species and growth phase [6]. They are structurally complex, in terms of linkage,configuration and diversity of monosaccharide building blocks[7]. In heterotrophic bacteria a diverse group of carbohydrate-active enzymes (CAZymes) evolves to use carbohydrates. They include glycoside hydrolases (GH), polysaccharide lyases (PL), carbohydrate esterases (CE), and accessory proteins, such as proteins carrying carbohydrate-binding modules[CBMs, 8]. The number of CAZymes required for degradation of a glycan scales linearly with its structural complexity [9]. For example, for digestion of fucoidan from brown algae hundreds of enzymes are required[10]. In contrast, for degradation of laminarin, which is the most abundant marine glycan and contribute 26±17% to the particulate organic carbon (POC) pool [11], two or three enzymes are – at least in the test tube – sufficient to degrade laminarin into glucose [12].Due to the structural diversity, direct quantification of specific polysaccharides in the environment remains technologically challenging [13].Inventories of bacterial CAZymes, therefore, offer an alternative approach for studying bacterial glycan utilization [e.g. 14, 15-17].

While bacterial glycan degradation in temperate surface waters was shown to be highly dynamic [e.g. 14, 15], benthic bacterial communities have limited seasonality [18, 19]. Polar regions with their prolonged periods of complete darkness in winter and24h of sunlight

in spring and summer are ideal environments to study the seasonality of bacterial glycan degradation. Arctic fjords of Svalbard (74-81°N) havestrong peaks of primary production in spring and summer fueling the entire coastal ecosystem including its sediments[20]. In addition, there is terrestrial input from glacial run-off and a contribution of ice algae, which are both also sources of organic matter that vary with season [21, 22]. Large datasets from Svalbard fjords have repeatedly underscored seasonal changes in respiration, sulfate reduction and mineralizationin these permanently cold sediments(-1°C to +4°C) [for review see 23]. Respiration is mostly driven by input of fresh OM and it is plausible to assume that fresh OM will also drive seasonal succession of heterotrophic bacteria in sandy surface sediments. However, rRNA gene-based studies showed a stable community composition over two years in coastal sands from Isfjorden[18]. Since studies based on comparative sequence analysis of rRNA genesare limited in taxonomic resolution,we re-visited Isfjorden sediments and studied them by a combination of metagenomics and metatranscriptomics. Thereby weexpected to detect subtle differencesin the gene repertoire of bacteria and in gene expression. In this study we tested the following three hypotheses: (1) the higher taxonomic resolution of the omics approach reveals seasonal changes in glycan utilization. Benthic bacterial communities respond to the seasonally changing input of fresh organic matter by changing the regulation of genes encoding CAZymes. (2) The utilization of continuously available, less labile substrates explains the high overall stability in benthic bacterial community composition.(3) The main glycans used by heterotrophic benthic bacteria change between seasons and can be predicted based ongene expressions. For addressing the third hypotheses we additionally performed glycan analyses.

Materials and Methods

Sampling

Sediment samples (fine sand) were taken in Isfjorden, Svalbard (Figure 1), using a van Veen grab. Surface layers (0-2 cm depth)were sampled at station 5 (78.11°N/14.35°E) in 2017 (December 20th), 2018 (February 16th, May 01st, and December 17th) and 2019 (April 25th). Furthermore, station 7 (78.10°N/14.38°E) was sampled on April 25th2019. Sediment temperatures ranged between -0.6 °C and 2.2 °C, water depth was between 2.7 m and 8.8 m. Contextual data have been reported in Miksch et al [18]. In addition, sediments, porewater and bottom water (sampled above the sediment surface; hereafter referred to as "overlying seawater", OSW) were sampled for glycan analysis using a Shipek-type grab at station 5 in 2022 (April 29th and May 2nd). All samples were immediately frozen in dry ice.

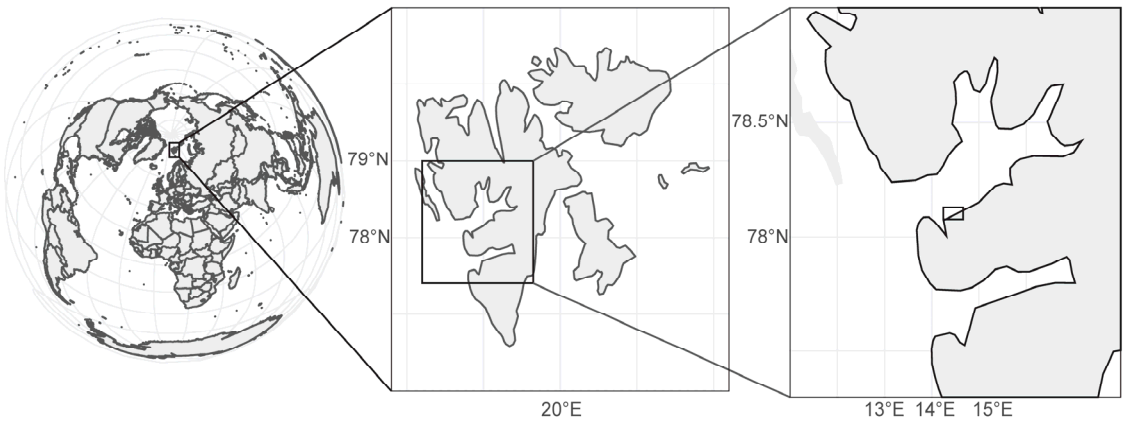

Figure 1. Sampling area in Isfjorden, Svalbard: Surface sediments were retrieved from two shallow sites (78°N, 4-8 m water depth) close toKappDresselhuys.

DNA and RNA extraction

DNA for metagenome analysis was extracted from the 0-2 cm depth horizon of selected sediment samples from Dec 2017, Feb 2018, May 2018, Dec 2018, and Apr 2019 after Zhou et al. [24] including three additional freeze-thawing steps. RNA was extractedfrom Dec 2017, Feb 2018, and May 2018 samples using RNeasy PowerSoil Total RNA Kit (QIAGEN, Hilden, Germany) according to the manufacturer's recommendation with minor modifications. For an overview of samples and details see Supplementary Table S1 and Supplementary information.

Sequencing, assembly, binning

Illumina-compatible RNAseq libraries were prepared from total RNA with NEBNextUltraII Directional RNA Library Prep Kit for Illumina (New England Biolabs, Frankfurt, Germany). In addition, three Illumina-compatible RNAseqlibraries from station 5 were prepared from bacterial rRNA-depleted RNA using theIllumina Ribo-Zero rRNAdepletionkit (*Bacteria*). Metagenome and metatranscriptome sequencing were done on an Illumina (San Diego, CA, USA) platform (HiSeq2500, 2x250 bases, paired-end) at the Max Planck-Genome Center in Cologne (Germany).

Detailed settings for used programs are given in Supplementary Information. Sequences were quality-controlled using BBTools v37.62 (quality < 20, minimum length 140 nt). Coverage of sequence diversity was analyzed using nonpareil v3.303[25]. Assembly of reads was done with SPAdes v3.13.1[26](meta option) and quality was evaluated using QUAST v4.5[27].Contigs< 1 kb length were excluded from further analyses.

For each dataset, binning was done using MaxBin v2.2.7[28] and MetaBAT v2:2.15[29]. Bin refinement was performed using DAS_Tool v1.1.2[30]. Mapping for differential coverage binning was done using bbmap v38.70 [31] at default settings and a minid=0.99.De-

replication was performed with Rep v3.1.1[32] (-comp 50, -con 15) and classification using GTDB-Tk v2.1.1and the GTDB release r214 [33]. Completeness and contamination was assessed incheckM v1.0.7[34].

Gene annotation and analyses

Gene predictions and annotations from bins were done using Prokka v1.14.6[35],dbCAN(run_dbCANv2.0.11 workflow; https://github.com/linnabrown/run_dbcan) [36], Swiss-Protrelease 2021_04 [37], SulfAtlasv1.0 [38], and transporterDB[download Oct21, 39]. The latter three databases were searched using DIAMOND blastp[v2.0.15.153, 40]. Results were filtered for the best hit using the enveomics script BlastTab.best_hit_sorted[41] (>60% identity, query coverage >70%).

CAZyme annotations obtained from dbCANwere acceptedwhen two of the three integrated annotation methods (HMMER v3.3.2, diamond v2.0.9.147, Hotpep version included in run_dbCAN workflow)matched [36].

Transcriptomic analyses

Quality-controlled RNA reads were sorted using SortMeRNA 4.0.4[42]. Reads classified as rRNA were taxonomically classifiedby using the SILVAngspipeline [https://ngs.arb-silva.de/silvangs/, release 138.1, 43]. All reads not classified as rRNAor tRNA, were considered as mRNA.

Annotation of transcripts was done by mapping mRNA to predictedgenes from metagenomics contigs and bins using DIAMOND blastx[v2.0.15.153, 40]. Results were filtered for the best hit using the enveomics script BlastTab.best_hit_sorted[41](>60% identity, query coverage >70%). Values of transcripts per million (TPM) mapped reads were

calculated after normalization by gene length. Data transformation and plotting was done using R and the tidyverse packages [44].

Monosaccharide analysis

Polysaccharides extracted from sediment, porewater and OSW were acid hydrolyzed and the resulting monosaccharides were analyzed using high performance anion exchange chromatography (HPAEC) with PULedamperometric detection(PAD) according to Vidal-Melgosa*et. al.*[45]. For details see Supplementary information. Values measured for calibration standards having high monosaccharide concentrations were consistent between injections during the chromatographic run. Low-concentrated calibration standards, however, showed much lower values at the second injection. To account for this decrease in detector sensitivity with time, a threshold concentration for each monosaccharide was set to the value at which the variation between two injections was lower than ±20%. Values lower than the threshold concentrations defined for each monosaccharide were rejected.

Results

Bacterial community composition as revealed by rRNA read frequencies

As a proxy for growth potential and activity of a population we used rRNAread frequencies from 11 metatranscriptomesrecovered from Isfjorden sediments (Dec2017, Feb2018, May2018; Figure 2A). The rRNA read frequencies of the majority of clades were notremarkably different between seasons. Notable exceptions were rRNA reads affiliated with the genera *Colwellia* and *Polaribacter* that showed increased relative abundance from winter (average of 0.6% and 0.1% of total 16S rRNA reads, respectively) to spring (average of 3.3% and 0.6% respectively).

Although no clear differences in community composition between seasons were detected, relative abundance in metatranscriptomic 16S rRNA vs. amplicon 16S rRNAgenes [from 18]differed for several taxa (Figure 2B). A greater relative abundance was determined, for Verrucomicrobiota (2.3±0.4%rRNA vs. 1.3±0.5% amplicon rRNA genes), Planctomycetota (4.8±0.9% vs. 2.4±0.7%), Desulfobacterota (7.9±1.1% vs. 2.8±0.5%), Thiotrichaceae (5.0±1.3% vs. 0.9±0.3.), and Myxococcota (3.0±0.3% vs. 0.8±0.4%). In contrast, a lower relative abundance in rRNA read frequencies was found for*Blastopirellula*(0.55±0.23% vs. 4.17±0.8%), Bacteroidota (*Maribacter* 0.64±0.14% vs. 5.43±1.83%; *Maritimimonas* 0.42±0.09% vs. 1.98±0.36%) and Actinomarinalesunc. 1.63±0.64% vs. 17.8±5.25%). The metatranscriptomescomprised only few archaeal 16S rRNA sequences (<1%), suggesting that archaea are of minor importance in coastal surface sediments of Svalbard.

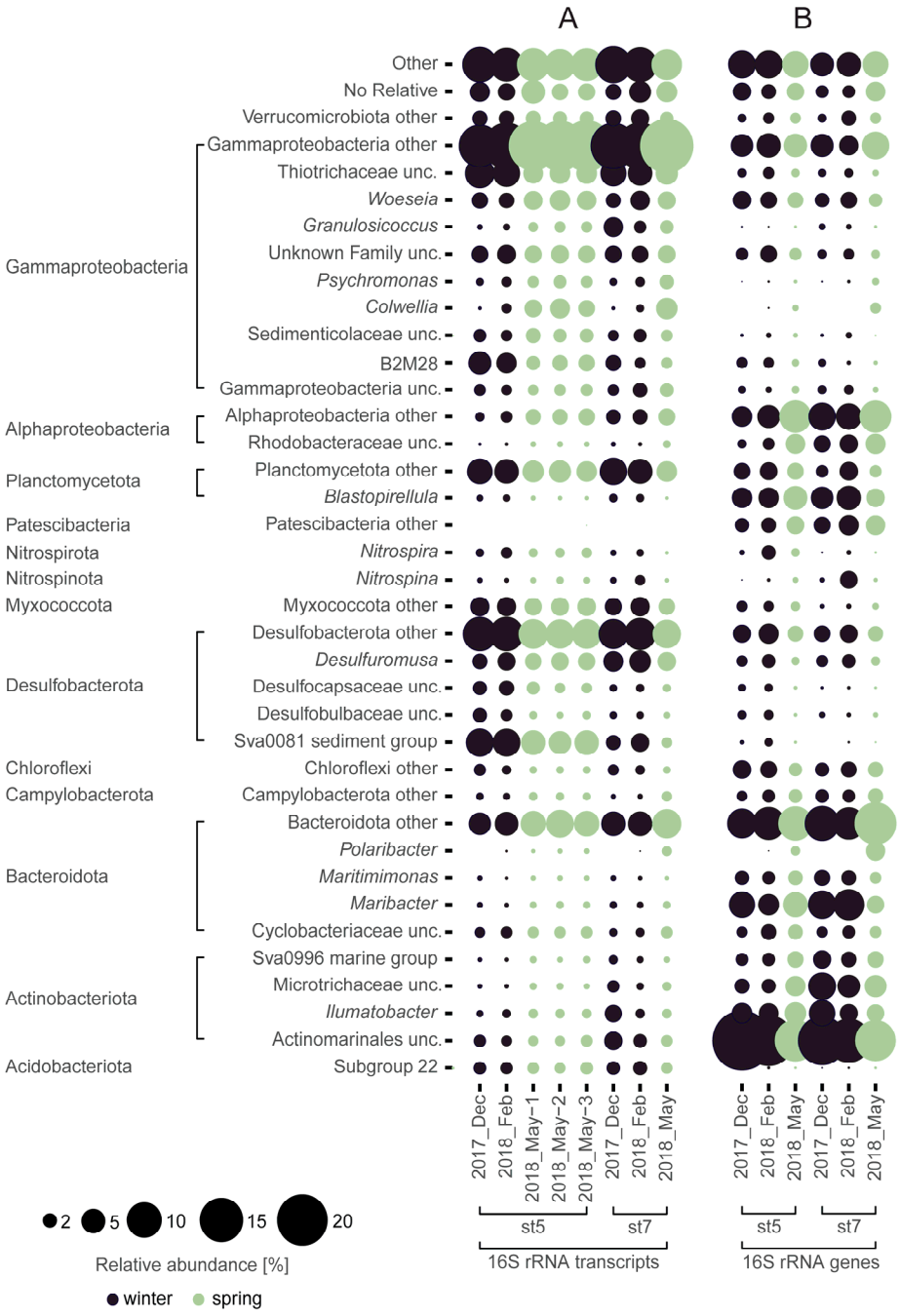

Figure 2: Major taxa of bacterial communities in Svalbard surface sediments are seasonally stable: Comparison of the benthic community composition as revealed by (A) rRNA read frequencies in metatranscriptomes and (B) 16S rRNA genes frequencies from amplicon tag sequencing [taken from 18]. Only taxa with a read frequency of >2% are shown, minor taxa are summarized as "other". Seasons are color-coded. Both, rRNA and rRNA genes revealed a stable bacterial community throughout the year. Only minor taxa such as *Colwellia*and *Polaribacter*spp. showed a clear seasonal variation in abundance.

Addressing changes in functional potential of benthic bacteria by omics

Metagenomes and Metatranscriptomes from Svalbard sediments were used to study possible changesin the functional potential of the bacterial community and to detect differences in the genomic repertoire between species of the same genus and in gene expression of CAZymes. Three metagenomes were obtained from station 5 samples in winter (Dec2017, Feb2018, Dec2018; hereafter referred to as “winter”) and three metagenomes in spring (station 5: May2018, Apr2019; station 7: Apr2019; hereafter referred to as “spring”). Nonpareil, a redundancy-based approach to assess the level of coverage, ranged between 0.46 and 0.5for all metagenomes, indicating that about half of the total diversity was covered (Supplementary Table S2). Atotal of 9,207,104 genes were predicted of which about one-thirdremained hypotheticals after annotation. A total of 183 bins (16-42 bins per sample) were recovered of which 36were selected for further analysis (Supplementary Table S3).The binsrepresented all major taxapreviously found in sandy surface sediments [18]including Acidimicrobiia, Bacteroidia, Desulfobacterota, Planctomycetota and Gammaproteobacteria.

Seasonal expression of bins

As a proxy for activity, eleven metatranscriptomes (Dec2017, Feb2018, May2018) were mapped on the 36 bins. A bin was considered being upregulated in spring when the ratio [average spring TPM mapped reads / average winter TPM mapped reads] was ≥2 (= log2 fold change of >1; green bars, Figure 3A) and being upregulated in winter when the ratio [average spring TPM mapped reads / average winter TPM mapped reads] was <0.5(= log2 fold change of < -1; black bars). Bins with log2 fold changes $-1 \leq x \leq 1$ were considered as constantly expressed and therefore unregulated (grey bars). According to this definition, 10 of 36bins were upregulated in spring. Of this group, seven belonged to Bacteroidia and three to Gammaproteobacteria. Among the most upregulated bins were Flavobacteraceae-bin

Sval_st7_May.bin.40 and*Colwellia*-bin Sval_st7_May.bin.39 with a 33-fold and19-fold higher TPM in spring vs. winter, respectively. In winter, six bins showed an increased expression (Figure 3A, black bars) affiliated with Gammaproteobacteria, Desulfobacteria, Acidimicrobiia and Planctomycetota. The majority of unregulated bins (9 of 20 bins, Figure 3A, grey bars) were Gammaproteobacteria. Remarkably, many of thesebins had high TPM values (Figure 3B).

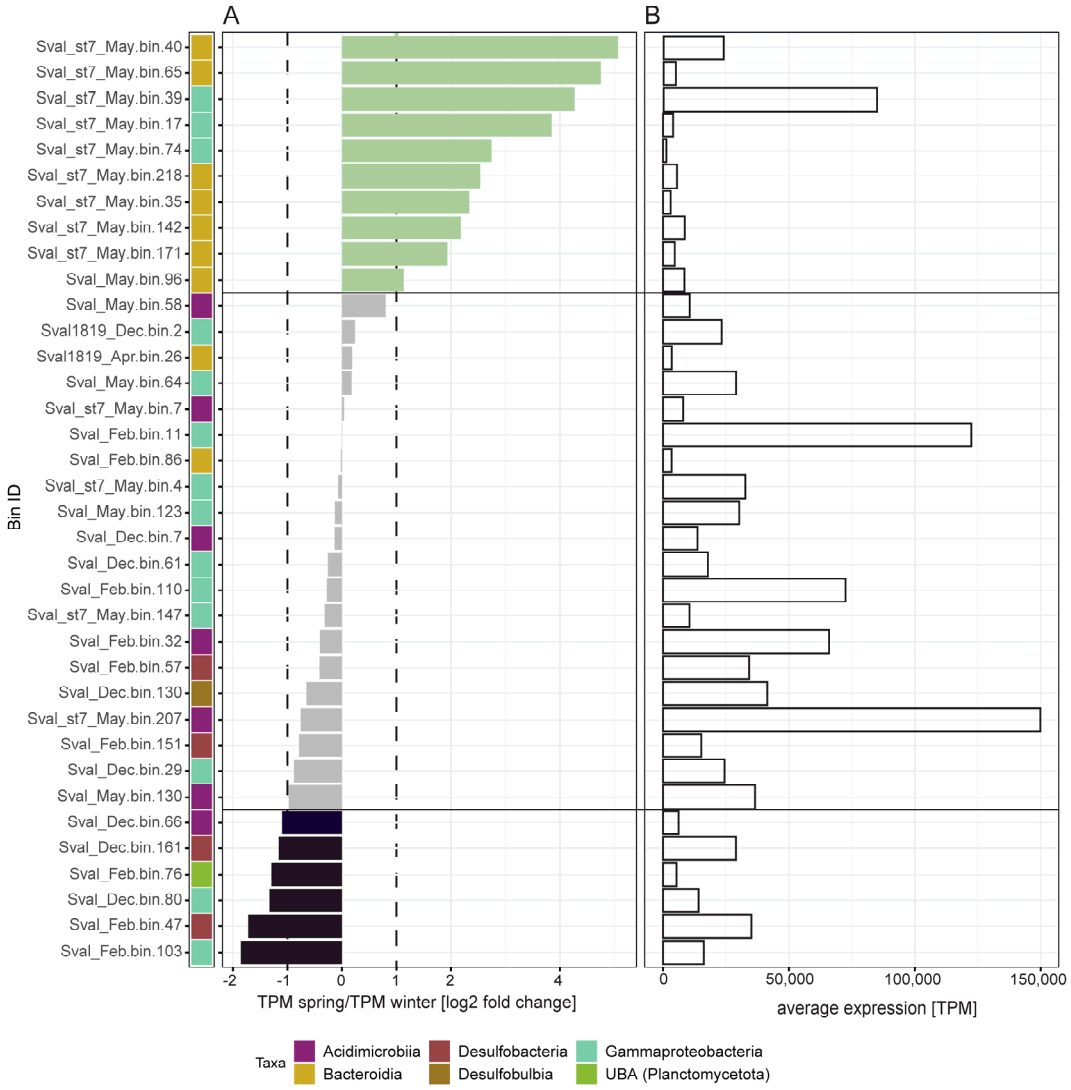

Figure 3. Expression of bins from Svalbard sediment metagenomes: (A) Changes of bin expression given as a ratio of transcripts per million (TPM) mapped reads from spring metatranscriptomes divided by TPM mapped reads from winter metatranscriptomes. Values are plotted as log2 fold change. A bin was defined being upregulated in spring for log2 fold changes of >1 (TPM spring/TPM winter >2, green bars) and being upregulated in winter for log2 fold changes of <-1 (TPM spring/TPM winter < 0.5, black bars). Grey bars show less regulated bins not matching these thresholds. (B) Expression of bins in transcripts per million given as an average of all sampling time points and metatranscriptomes. Some bins of Bacteroidia and Gammaproteobacteria were upregulated on mRNA level in spring while Thiohalobacterales (bins 80, 103), Acidimicrobiia (bin 66), and Desulfobacterales bins (bins 47, 161) were more expressed in winter. Most highly expressed bins 207 and bin 11 were less regulated.

Carbohydrate-active enzymes and polysaccharide-utilization loci

We focused our analysis on CAZymes, in particular on glycoside hydrolase families (GH), as they can be used as a proxy for polysaccharide degradation. Overall, GH23 (peptidoglycan lyase) was the most abundant family in the metagenomes (Supplementary Figure S1) and not remarkably changing between seasons. Most members of the GH23 familyhavepeptidoglycanlyase activity and are widely distributed among many phyla such as Proteobacteria and Firmicutes[8]. In wintermetagenomes, e.g. GH29 (fucosidase), GH106 (rhamnosidase) and GH165(galactosidase) were more abundant than in summer with a log2-fold increase of -1.2 to -2.2, yet with lower frequencies than the prominent spring representatives.

The number of GH in the bins varied between 3 and 16 GH Mbp^{-1} (Supplementary Figure S2, Supplementary Table S4) and of total CAZymes (GH, CE, PL) between 5 and 22 Mbp^{-1}. Three of the bins showed a high density of peptidases with 8-11 Mbp^{-1} but comprised only 6 to 9 CAZymes Mbp^{-1}. Major substrates expected to be consumed by these Bacteroidia were laminarin or other β-glucans (GH16_3, GH2, GH3, GH149, GH17 and GH30_1),α-glucans such as glycogen (GH13, GH13_19, GH31, GH65), mannans (GH92), xylans (GH3), and alginates (PL7, PL17).

Polysaccharide-utilization loci (PUL) are structured genomic regions used to predict the substrate of heterotrophic bacteria and are common in Bacteroidota[15, 46]. Canonical bacteroidetal PUL include a pair of susCD-like transporter genes and ≥2 CAZyme genes like GH, PL, CE or CBM within a 10-genes-sliding window [47].Automated prediction of canonical PUL and PUL-like structures (defined as *susCD* pair/ *susC* and a single CAZyme) identified 16 loci (Supplementary Table S4) in the 7 Bacteroidia bins that were upregulated in spring. Another 11 loci were identified with multiple CAZymes but no *susCD.*The two seasonally unregulated bins Sval1819_Apr.bin.26 and Sval_Feb.bin.86 (Figure 3, grey bars)

did not comprise contigs with canonical PUL or PUL-like structures but 4 and 8 single CAZymes Mbp^{-1} (Supplementary Figure S2).

Seasonal changes in gene expression

To analyze changes in gene expression between winter and spring, the average relative frequency of transcripts was calculated for winter and spring (Supplementary Figure S3, Supplementary Table S5). The ten most expressed genes comprised only hypothetical proteins. Expression of most of these unknowns did not differ between seasons, but contribute evenly to the gene expression by the sediment community all over the year.Genesrelated to photosynthesis were highly upregulated in spring:besides photosystem I- and II-related genes, other genes of presumably photosynthetic organisms, e.g. ribulose bisphosphate carboxylase,had up to 14fold higher TPM values in spring than in winter. Furthermore, ammonia channel proteins/transporters and cytochromes were also upregulated in spring. In contrast, genes involved in nitrogen and sulfur cycling were upregulated in winter(Supplementary Figure S4). These are in particulargenes for respiration, e.g. nitrite reductase (*nir*) and nitrate reductase(*nar*, 1.5 and 1.3 log2 fold change TPM winter vs. spring, respectively) as well as dissimilatory sulfatereductase (*dsr*; 1.0 log2 fold change TPM winter vs. spring) and adenylylsufate reductase (*apr*; 1.1 log2 fold changeTPM winter vs. spring).

Most prominent GH families upregulated in spring were GH30_1, GH17,GH16_3, and GH149 (Figure 4). Enzymes of these families comprised β-glucanases and are likely degrading laminarin. GH149 also acts on β-1,3-linked glucan as phosphorylase.GH families downregulated in spring included GH130, GH63, GH13_18, GH15 and GH23. Enzymes characterized within these families showed a diverse range of activities such asmannosidephosphorylases(GH130), α-glucosidases (GH63), α-glycoside phosphorylases

(GH13_18), glucan-1,4-α-*glucosidase (e.g.* glucoamylase, trehalase; GH15) and peptidoglycanlyases (GH23).

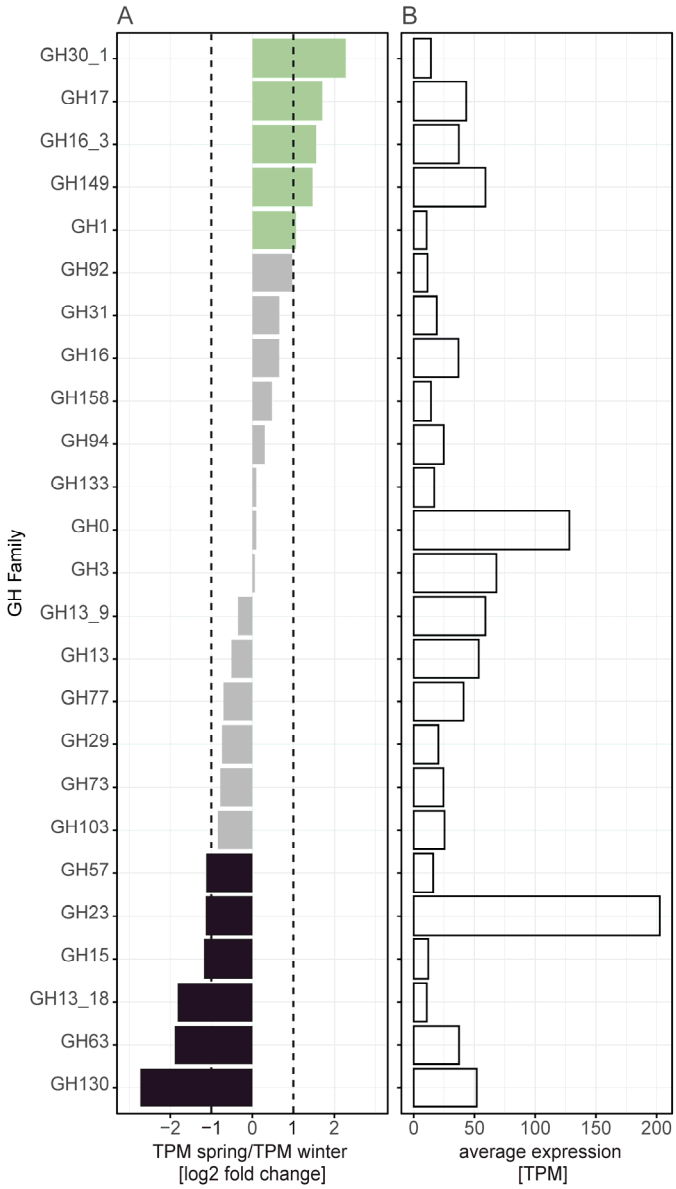

Figure 4. Expression of glycoside hydrolases (GH) families in spring and winter: β-glucan utilization is upregulated in spring (GH17, GH16_3, GH149) while α-glucan utilization is more prominent in winter (GH63, GH15, GH57). (A) Changes of GH expression (given as a ratio of transcripts per million (TPM) in spring metatranscriptomes divided by TPM in winter metatranscriptomes). Values are plotted as log2 fold change. A GH family was defined as being upregulated in spring when log2 fold changes were >1 (TPM spring/TPM winter >2, green bars) and being upregulated in winter when log2 fold changes were <-1 (TPM spring/TPM winter < 0.5, black bars). Grey bars show unregulated GH families not matching these thresholds. (B) Average expression of GH families in transcripts per million calculated from all metatranscriptomes from all sampling time points. GH families shown are expressed in spring and winter (no infinite fold change) with TPM values >10.

Monosaccharide concentrations in sediments

To link gene expression of CAZymes with glycan concentrations in the sediment, we extracted glycans from the sediment with MilliQ water and quantified their monosaccharides after acid hydrolysis. The monosaccharide composition of the water extracts was dominated by glucose, accounting for 50-80% of total monosaccharides (Figure 5). In spring 2019,total monosaccharide content(sum of concentrations of all different monosaccharides) was on average lower than in spring 2018 (station 5: ~ 3µg C gdw^{-1}sediment vs. ~ 8.5 µg C gdw^{-1} and station 7: 2.5 vs. 7.5 µg C gdw^{-1}in 2018 and 2019 respectively). Samples from station 7 (December 2021 and February 2022) contained only glucose in measurable amounts while other monosaccharides were below detection limit. Other abundant monosaccharides in our samples were mannose and galactose. In winter, the concentration of mannose increased by a factor of about 2.2 from 0.08µg C gdw^{-1} sedimentin spring to 0.17µg C gdw^{-1} (average for station 5 and 7). In contrast, spring samples had a 7.9 fold-higher concentration of galactose (winter: 0.05µg C gdw^{-1}; spring: 0.41µg C gdw^{-1} sediment) and 4.3fold-higher concentration of fucose than in winter (winter: 0.04µg C gdw^{-1}; spring: 0.17µg C gdw^{-1} sediment).

In an additional sampling campaign at station 5 in April 2022, we collected porewater and OSW along with sediment samples. Total concentration of monosaccharideswere similar to those measured in spring 2019 with on average 2.9 ±2.6µg C gdw^{-1} sediment for the four replicate grabs (data not shown).Total concentrations in porewaterwere high with 1107 ± 484µg C L^{-1} being 18fold higher than those measured for OSW (62 ± 101µg C L^{-1}, Figure 6, Supplementary Table S6). The monosaccharide composition differed between sediments and porewater:the porewater monosaccharide spectrum was not dominated by glucose but mostly had an even contribution of glucose, arabinose, fucose, galactose, glucosamine, and xylose (Supplementary Table S6).

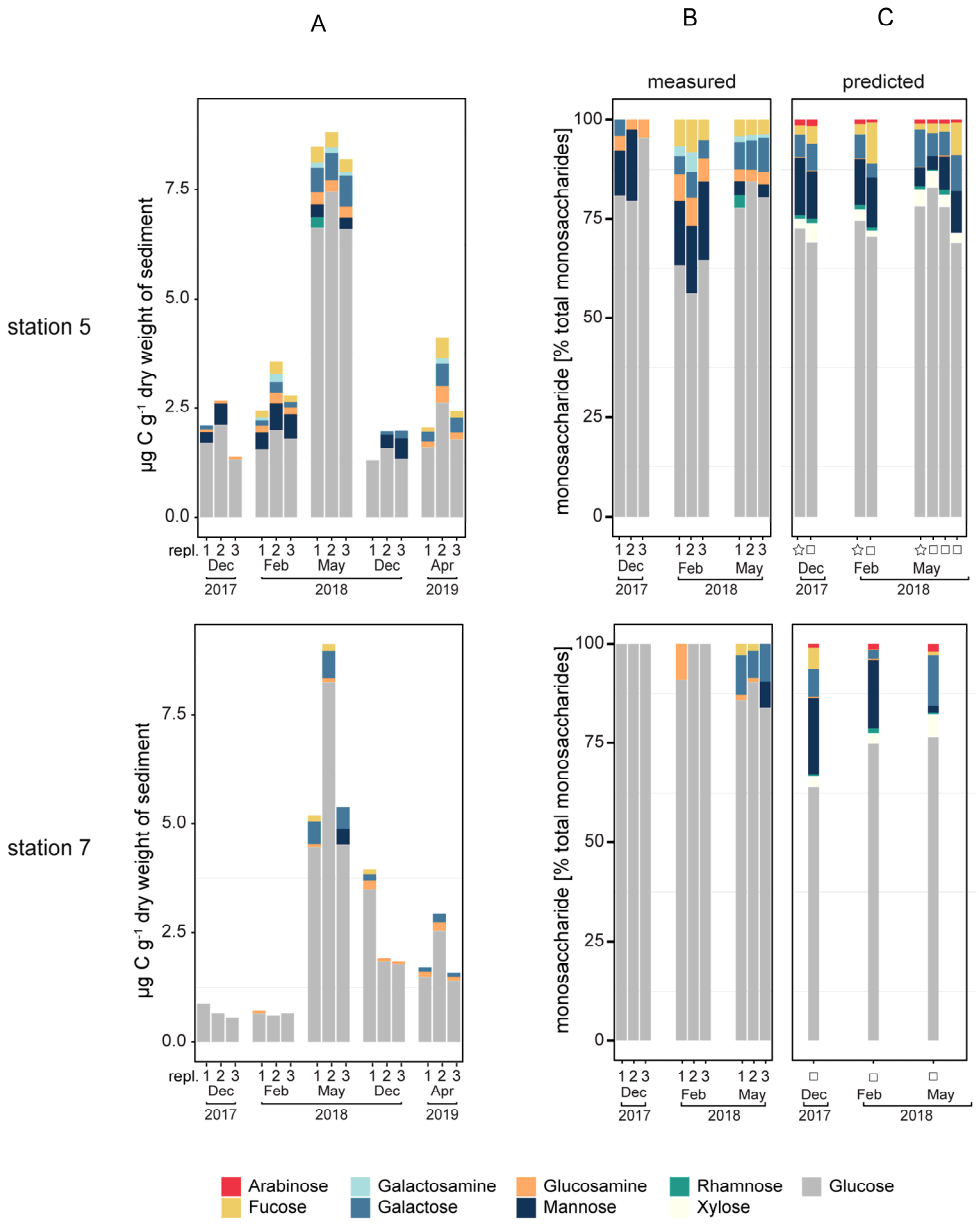

Figure 5: Monosaccharide concentrations measured and predicted based on expression patterns of glycoside hydrolase genes in Svalbard sediments: Glycans from the water extracts of sediment samples were acid hydrolyzed and the resulting monosaccharides were measured by HPAEC-PAD analysis. (A) Concentrations and (B) relative fractions of total measured monosaccharides. (C) Monosaccharide utilization deduced from predicted functions of expression patterns of glycoside hydrolase genes. Star, data from rRNA-depleted metatranscriptome; square, data from 'full' metatranscriptome. All samples were dominated by glucose. In particular, in spring 2018, glucose and galactose concentrations strongly increased, while mannose was more prominent in winter samples. The measured monosaccharide composition is in line with the predicted trends of monosaccharide utilization patterns based on glycoside hydrolases expression.

Predicting monosaccharide utilization based on GH expression data.

The frequency of mRNA reads annotated as GHwere used to predict monosaccharide utilization in Svalbard sediments and to test if predicted patterns correlate with the measured monosaccharides. We assigned one or more monosaccharides to each detected GH family based on information given in the CAZy database (matrix available as Supplementary Table S7). The monosaccharide utilization pattern predicted based on the transcriptomic data (Figure 5C) was similar to the pattern of measured monosaccharides at station 5 (Figure 5B): it indicated a dominance of glucose utilization, accounting for more than 60% of total used monosaccharides in sediments in spring. Analogue to measured monosaccharide concentrations, transcripts mapping to mannose-related GH families were more prominent in winter, while galactose-related GH families were more abundant in spring predictions. In line with monosaccharide measurements, fucose, rhamnose, arabinose and xylose utilization was detected, though in a less seasonally consistent manner.

Concentrations of individual monosaccharides were below detection threshold at station 7, thus, a comparison of measured and predicted monosaccharide composition is not meaningful.

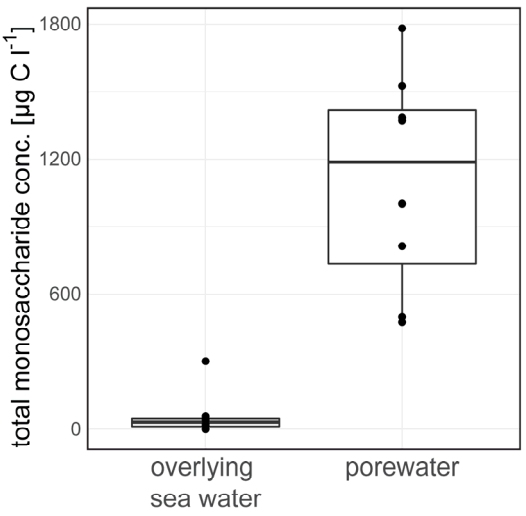

Figure 6: Glycan concentrations in overlying seawater and porewater samples from Svalbard Isfjorden: Glycans from OSW and porewater were acid hydrolyzed and the resulting monosaccharides were measured by HPAEC-PAD analysis. Total monosaccharide concentration (sum of concentrations of all different monosaccharides) is shown. Concentrations in porewater were 18fold higher than in OSW. For concentrations of different monosaccharides refer to Supplementary table S6.

Discussion

Bacterial communities in temperate and polar sediments were reported to be seasonally stable based on 16S rRNA gene frequencies [18]. In this study, ribosomal RNA expression of most taxa did also not remarkably change between seasons (Figure 2) supporting previous findings. Although rRNA concentrations of diverse natural bacterial communities cannot be metrically linked to real-time activities due to differences in life histories, life strategies and non-growth activities[48], rRNAfrequencies have been used as a proxy for growth potential and activity of a population due to the relationship between cellular ribosome content and the ability to synthesize proteins[49]. Our data show that abundant taxa (>5% of total rRNA reads) such as Actinobacteria, Bacteroidota except *Polaribacter*, Desulfobacterota, Myxococcota, and Woesiaceae were seasonally stable. The detected stability gives a first hint that the major part of the bacterial community is thriving on constantly available substrates rather than seasonally fluctuating substrates like laminarin.

*Colwellia*and *Polaribacter*are prominent in spring

Despite the stability of major phyla, two genera,*Polaribacter* (Bacteroidia) and *Colwellia*(Gammaproteobacteria)showed a strong increase in spring, with average relative abundances rising from <0.1% to 1.1% and of 0.3% to 4.1% of total rRNA reads, respectively. Both genera are known for the degradation of various algal polysaccharides and are tightly associated with phytoplankton blooms [e.g.14, 16, 50-52]. In particular, members of the genus *Colwellia*have been reported to be seasonally abundant in Arctic and Antarctic waters and sea-ice [52-55]. The increase of *Colwellia*rRNA frequency in spring vs. winter went along with a 19- to 36-fold higher expression of mRNA that mapped on the *Colwellia* bin Sval_st7_May.bin.39 (Figure 3). In particular genes for GH17, GH16_3 and GH149, indicative of degradation of laminarin as the main storage glycan of diatoms [11] were

upregulated. This *Colwellia* bin with a genome size of 3.65 Mbp (95% completeness; 1.9% contamination) represents a novel species according to the ANI-based genomic similarity criteria for delineating species used by the genome taxonomy database [GTDB, 56]. Its closest phylogenetic relative is an uncultured *Colwellia* sp.from marine water (ANI 85.9%; bioprojectPRJEB37807, BioSampleSAMEA9694887) with a genome size of 4.9 Mbp. We conclude that the high abundance and pronounced seasonality of *Colwellia* asks for future studies on its ecology of this gammaproteobacterial genus in polar systems.

Besides *Colwellia*, seven Bacteroidia bins showed a strong upregulation in spring (Figure 3). These bins are on average of a size of 2.86±0.35 Mbps and thereby significantly larger than the 2.0 Mbp bins of Bacteroidia obtained from temperate surface waters [15]. Surface water Bacteroidia are characterized by a wealth of PUL that encode CAZymes, carbohydrate-binding proteins, and susCD-like transporters [for review see 57]. Like planktonic Bacteroidia the benthic bins also affiliated with the family Flavobacteriaceae and show similar genomic organization regarding polysaccharide degradation. The variabilityof GHin our Bacteroidia bins was high (3 to 16GH Mbp^{-1}; Supplementary Figure S2) and only slightly lower than found for pelagic *Polaribacter* spp. [e.g. 14, 15, 16]. Ratios of annotated degradative CAZymes vs. peptidases suggest a niche separation of Bacteroidota into carbohydrate (4/7 bins) and protein degradation (3/7 bins; Supplementary Figure S2).

DesulfobacteriaandThiohalobacteralesare abundant taxa slightly elevated in winter

Bins of sulfate-reducing Desulfobacterales and sulfur-oxidizing Thiohalobacterales (Figure 3) showed elevated expression in winter. The same was observed for key genes involved in sulfur cycling (Supplementary Figure S4), both indicating a more prominent role of sulfur cycling in winter samples, potentially linked to more anoxia in the absence of benthic photosynthesis. Most sulfate-reducing bacteria rely on low molecular products such

as fatty acids and hydrogen [58] which are available throughout the year while fresh, complex organic material gets limited in winter.Canonical denitrification as indicated by nitrate and nitrite reductases (*nir* and *nar*) was more prominent in winter (Supplementary Figure S4), which supported extended phases of anoxia.

Relative utilization of β-glucans increases in spring.

In spring, the use of algae-derived β-glucans was most prominent by an elevated expression of mRNA of GH families GH30_1, GH17, GH16_3, and GH149, together indicating increased degradation of laminarin (Figure 4). Several GH families with galactosidase activities [according to CAZy database, 8] were also upregulated in spring such as GH1, GH4, or GH42 (Supplementary Table S5). Galactose has been described to be a main building block of several marine algal polysaccharides like agar and carrageenan, which are important components of macroalgae cell walls [59]. Hydrolysis of such labile algal polysaccharides would be plausible to be induced in spring when algal biomass is increasing.

Relative utilization of α-glucans increases in winter.

Transcript levels for degradation of α-glucans like glycogen (GH63, GH15, GH57) were increased in winter (between log2-fold change -1.1 and -1.9) but detected throughout the year. α-glucans are intracellular storage products of many heterotrophic bacteria [60], but also of animals and protists, as well as some fungi [61, 62]. Therefore, glycogen is continuously available, either in intracellular pools or recycled from bacterial and animal biomass. We assume that in spring benthic bacteria use large amounts of available glycans and transform part of them into glycogen, thus making it available later during the year. Thus, unlike laminarin, glycogen is a constantly available carbon source contributing to the high stability in the bacterial community composition.

Also GH23 transcripts were upregulated in winter. They likely encode hydrolysis of peptidoglycan [63]. This could be a result of starvation since bacteria are known to reduce their size and use cell wall compounds as energy source[64].

Utilization of constantly available substrates.

Besides glycogen, we found multiple indications that other substrates are continuously used, likely contributing to the high stability of benthic bacterial communities. The transcription of most of the abundant GH families was independent of polar day and nightand was not remarkably regulated across seasons (Figure 4). Among these GH families there were mannosidases (GH92), α-glucanases (GH31,GH133, GH13_9, GH77), β-glucanases (GH16, GH158), fucosidases (GH29), peptidoglycan lyases (GH73, GH103) and families without a clear substrate affiliation (GH94,GH0, GH3, GH13).

Further constant carbon sources are chitin and mucin which are both mostly of animal-origin. This is plausible because benthic meiofaunal and macrofaunal density and diversity has been shown to be stable throughout the year[65]. Chitin is the most abundant polysaccharide in surface marine sediments [66], yet we could not identify high transcription levels of known chitinases[e.g. GH18, 67] in our samples. However, in the bins Sval_st7_May.bin.207 and Sval_Feb_bin.32 we did find expressed chitosan disaccharide transporters as well as key genes for further breakdown catalyzing deacetylation (chitooligosaccharide deacetylase) and hexosaminidases (GH3). Both bins were classified as Actinobacteriota and are highly expressed throughout the year (Figure 3).

Mucins are glycoproteins copiously secreted by marine fauna, in particular by invertebrates [68, 69]. They constitute a complex class of energy-rich substrates containing a protein backbone with side chains of oligosaccharides, which can be very diverse in nature, covering glycans composed of different monosaccharide building blocks [70, 71]. Mucus is a

potent substrate for marine microbes. Hannides and colleagues [72] showed a strong priming effect of gastropod mucus on benthic organic matter remineralization. Key enzymes for mucin degradation have been identified for gut bacteria comprisingsialidases(GH33), fucosidases (GH29, GH95), N-acetylgalactosaminidases (GH101, 129), N-acetylglucosaminidases (GH84, GH85), galactosidases (GH2, GH20, GH42), and proteases [73, 74]. These were all present in our metagenomes and expressed either all year or preferentially in winter metatranscriptomes. This corroborates that mucins are important substrates for benthic bacteria.

Monosaccharide measurements are consistent with CAZyme expression

Glucose concentrations were seasonal with clear maxima in spring. Their up to 4fold increase was consistent with higher transcription levels of β-1,3-glucan degradation genes, indicating substrate-related induction. Also in winter, glucose remained an important substrate, likely because α-glucan storage products of animals and bacteria were recycled. Another direct relationship between abundance of monosaccharides and transcript frequency of degradative enzymes was observed for mannose-containing substrates of which concentrations were higher in winter (0.38± 0.16 μg C g^{-1} sediment) compared to spring (0.16± 0.10 μg C g^{-1} sediment). Correspondingly, genes belonging to GH130 family (including activities like β-1,4-mannosylglucose phosphorylase, β-1,4-mannooligosaccharide phosphorylase, β-1,4-mannosyl-N-acetyl-glucosamine phosphorylase) as well as genes encoding GH63 (α-glucosidase and α-mannosidase) and GH113 (β-mannanase, β-mannosidase) were upregulated towards winter. α- and β-mannans are known to be important compounds of diatom cell walls [75, 76]. These cell walls are considered semi-labile OM and therefore are relatively more important in winter when labile glucans such as laminarin are long gone.

Overall, this study suggests that the transcription frequency of GH families is linked to monosaccharide concentrations in the natural environment. This comparison between detected monosaccharides and expressed GH should ideally be extended to the glycan level, so types and substrate classes are also considered. Few enzyme-based methods that allow quantification of specific glycan structures, such as laminarin and α-glucans, in marine samples have been recently developed [11, 77]. However, due to the glycans' structural complexity and diversity, quantification of individual glycan typesremains technologically challenging.

Sandy sediments mineralize labile parts of photosynthesis-derived particulate organic matter and release more stable, glucose-depleted residual glycans.

Marine dissolved organic matter (DOM) is the largest ocean reservoir of reduced carbon with about 662 Pg C [78]. Much of the porewater DOM originates from deposited POMproduced by primary production in surface waters [79]. While Svalbard sediments were rich in glucose (84±14% of total glycans measured) and similar to POM from other sites [80]Panagiotopoulos, 2005 #2748},porewater showed a lower contribution of glucose (about 15-25%; Supplementary Table S6) resulting in a more even distribution of the different monosaccharides. This is in line with previous findings for DOM composition in seawater[(15% glucose, 81, 82] and porewater[average 28% glucose, 79, 83]. Together with our findings that the concentrations of monosaccharides in porewater were about one order of magnitude higher than in bottom water this data suggest that benthic microbial communities transformsOM, utilizing mostly glucose. Glucose-depleted DOM which is more stable against bacterial degradation is released into the water column by tidal pumping. Overall, we show that benthic microbiomes in sandy shelf sediments are major modulators of DOM

composition,extending early findings by Burdige[83, 84] and Huettel and colleagues [85] who suggested that sediments present a net source of dissolved organic carbon.

Conclusion and outlook

Our data show that the majority of the benthicbacterial community in Svalbard is present and active throughout all seasons despite the strong seasonality in polar regions. These findingshighlight that the bacterial communities of the water column and of underlying sediments are fundamentally different. Nevertheless, we found some seasonality, such as degradation of β-glucans by Bacteroidia and Gammaproteobacteria inspring, supporting our hypothesis that benthic bacterial communities respond to the seasonally changing input of fresh organic matter. Similar to what occurs in the water column laminarin degradation is a major process in sediments during spring, while utilization of α-glucans, in particular glycogen, occurs throughout the year. The stable expression of genes for degradation of other constantly available substrates such as mucin and chitin is consistent with our hypothesis that the continuous utilization of less labile, permanently available substrates stabilize benthic bacterial communities.

Future studies could aim at the autecology of taxa degrading these often complex, permanently available substrates, for example by enrichment and isolation of pure cultures using mucin and chitin. Yet, it is the tremendous diversity on various trophic levels, the multiple niches, the complexity of substrates, and the highly dynamic conditions of coastal sandy sediments with currents and storms that make the benthic microbiome so robust and stable, both with respect to taxonomy and function. Benthic microbiomes thereby will remain an ultimate challenge for ecologists.

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Competing interests

The authors declare no competing interests.

Data availability statement:

Sequence data is available at ENA under the project accession PRJEB53193 (data not yet publically accessible due to upload problems at ENA; access for reviewers is provided under this link: https://owncloud.mpi-bremen.de/index.php/s/oVurjYCGGtpPEje).

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Supplements

RNA extraction

RNA was extractedfrom sediment samples from Dec 2017, Feb 2018, and May 2018 from stations 5 and 7using RNeasy PowerSoil Total RNA Kit (QIAGEN, Hilden, Germany) with some modifications to the manufacturer's recommendation. For May 2018 sediments, RNA was extracted from station 5 from three technical replicatesamples.

Modifications were as follows:

- fresh phenol/chloroform/isoamyl alcohol (pH 6.5-8.0) was added to the tube containing the beads before adding the sample.
- As only up to 2.0 g of sample can be used per tube, we subsampled each sample, performing two to five extractions per sample. In particular for winter samples, several replicates were extracted and finally combined to retrieve an amount of RNA that is sufficiently high for metatranscriptomics.
- The incubation from step 7 of the manufacturer's protocol was performed on ice and the centrifugation was done at 3000 x g.
- The centrifugation speed used in step 10 was 5000 x g.
- The last incubation (step 17) was done at -20° C for 30 minutes.
- The centrifugation (step 18) was performed at 15000 x g for 30 minutes.
- For resuspension of the nucleic acid pellet, a smaller volume between 25 to 50 µl of SR7 solution was used.
- Contaminating DNA was digested by using the Invitrogen™ TURBO DNA-free™ Kit (Thermo Fisher Scientific, Bremen, Germany) following the manufacturers' protocol.

Sequence assembly and binning

Sequences were quality-controlled using BBTools v37.62 (quality < 20, minimum length 140 nt per read).Quality controlled reads were analyzed for their coverage of sequence diversity using nonpareil v3.303[1]. Assembly of reads into contigs was done with SPAdes v3.13.1[2] using k-mers 55, 77, 99, 127 and the meta option and assembly only. Assembly quality was evaluated using QUAST v4.5[3].Contigs< 1 kb length were excluded from further analyses.

For each dataset, binning was done using MaxBin v2.2.7[4]at default settings and using MetaBAT v2:2.15[5] at default settings and –m 1500. Bin refinement was performed using DAS_Tool v1.1.2at default settings [6]. Mapping for differential coverage binning was done using bbmap v38.70 and default settings[7] except for minid=0.99 and idfilter=0.97.De-replication of bins retrieved by DAS_Toolfrom all datasets was performed with Rep v3.1.1[8] at default settings except for parameters –g 35, -l 1000, -comp 50, -con 15 and classified using the GTDB-Tk v2.1.1[9]and GTDB release r214. Completeness and contamination was assessed incheckM v1.0.7[10].

Gene annotation and analyses

Gene predictions from bins were done using Prokka v1.14.6[11],dbCAN(run_dbCANv2.0.11 workflow; https://github.com/linnabrown/run_dbcan) [12], Swiss-Protrelease 2021_04 [13], SulfAtlasv1.0 [14], and transporterDB[download Oct21, 15]. The latter three databases were searched using DIAMOND blastp mode and default settings. Results were filtered for the best hit using the enveomics script BlastTab.best_hit_sorted[16] and >60% identity to reference sequences and a query coverage of >70%.

CAZyme annotations obtained from dbCANwere acceptedwhen two of the three integrated annotation methods (HMMER v3.3.2, DIAMOND v2.0.9.147, Hotpep version included in run_dbCAN workflow)matched [12]. The annotations from Prokka and the databases (except for dbCAN)were compared using a semi-automated approach. This comparison was done usinga full Damerau-Levenshteindistance [17, 18]between the annotations from different tools/databases (0, identical string; 1,completely different). To account for discrepancies in the annotation string for identical functions in different databases (e.g. capitalization, extra spaces, extra letters), differences ≤25% of total string lengths were allowed. Results were sorted according to i) annotated function matching in at least two of the four databases, ii) only one database annotated the predicted gene (results marked with "*" at the end of the string) or iii), annotated function disagreed between databases (marked with "manualcheck_").Metagenomic abundance was calculated as gene counts divided by genome equivalents that were estimated using MicrobeCensus[19].

Transcriptomic analyses

Quality-controlled RNA reads were sorted using SortMeRNA 4.0.4[20] using default settings. Reads classified as rRNA were used for taxonomic profilingby using the SILVAngspipeline [https://ngs.arb-silva.de/silvangs/, release 138.1, 21] and default settings. For expression analysis, all reads not classified as rRNAor tRNA, were considered as mRNA. Annotation of transcripts was done by mapping mRNA to predictedgenes from metagenomiccontigs and bins using DIAMOND blastx[v2.0.15.153, 22] at default settings. Results were filtered for the best hit using the enveomics script BlastTab.best_hit_sorted[16] and >60% identity to reference sequences and a query coverage of >70%. Values of transcripts per million (TPM) mapped reads were calculated after normalization by gene length. Differences between seasons were calculated using the average TPM values in winter

(December and February) vs. spring (May) and givenas log_2fold change.Data transformation and plotting was done using R and the tidyverse packages [23, 24].

Monosaccharide analysis

Freeze-dried sediment was homogenized and mixed with 5 ml Milli-Q ultrapure water per mg of sediment and incubated in a sonication bath (BandelinSonorex®, Berlin, Germany) for 60 min at maximum intensity. Afterwards, samples were centrifuged at 6000 x g for 15 min at 20° C and the supernatant was preserved. Porewater and OSW were dialyzed to remove salts using ~1 kDa mesh size dialysis bags (Spectra/Por®, FisherScientific, Bremen, Germany) for 24 h against Milli-Q water. Dialyzed samples were freeze-dried and re-suspended in a tenth of the original volume. Both sediment extracts and pore water and overlying sea water samples were hydrolyzed using HCl (1 Molar final concentration) for 24 h. Monosaccharides were quantified using High performance anion exchange chromatography (HPAEC) with pulsed amperometric detection(PAD)for details see Vidal-Melgosa*et. al.*[25].

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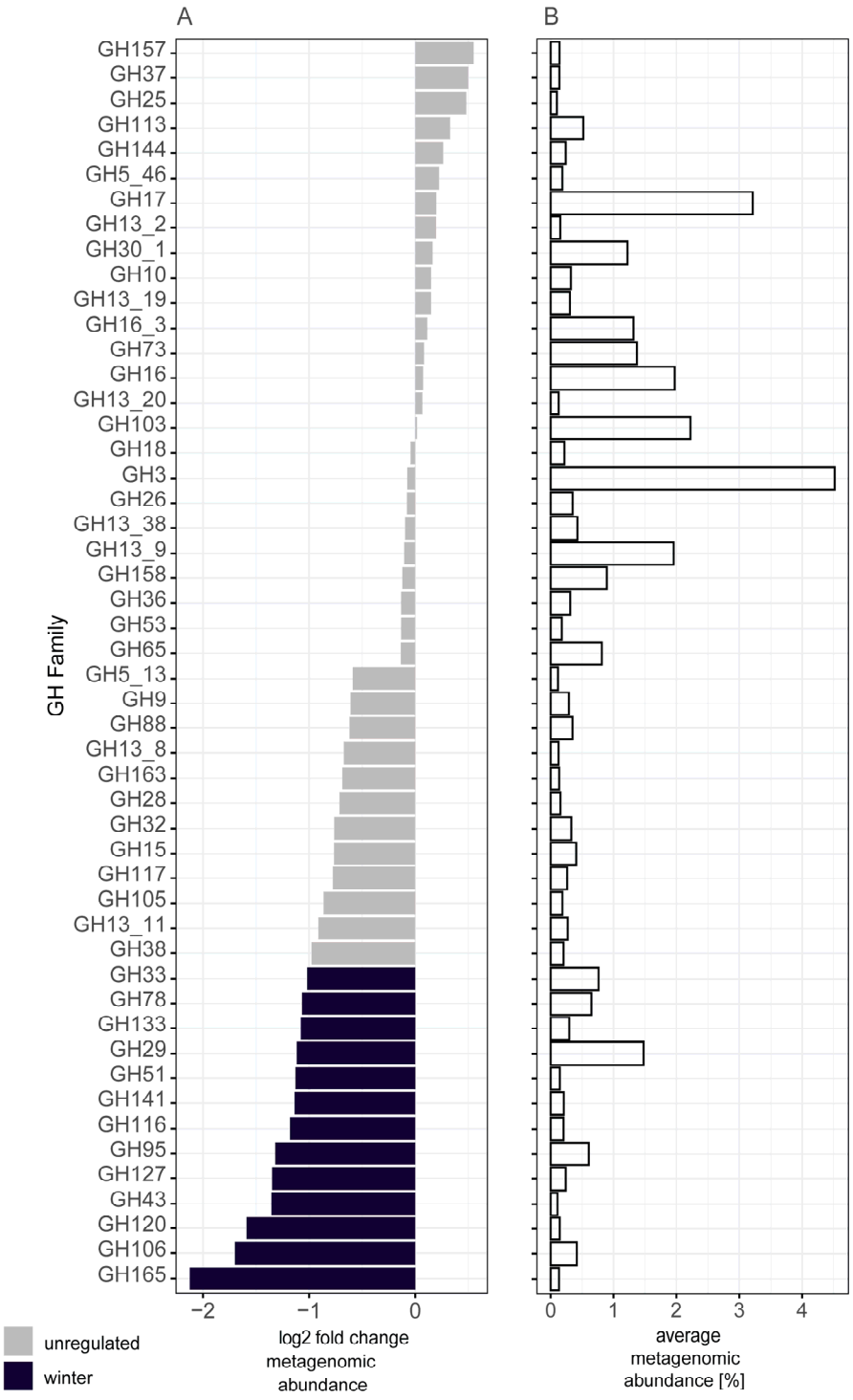
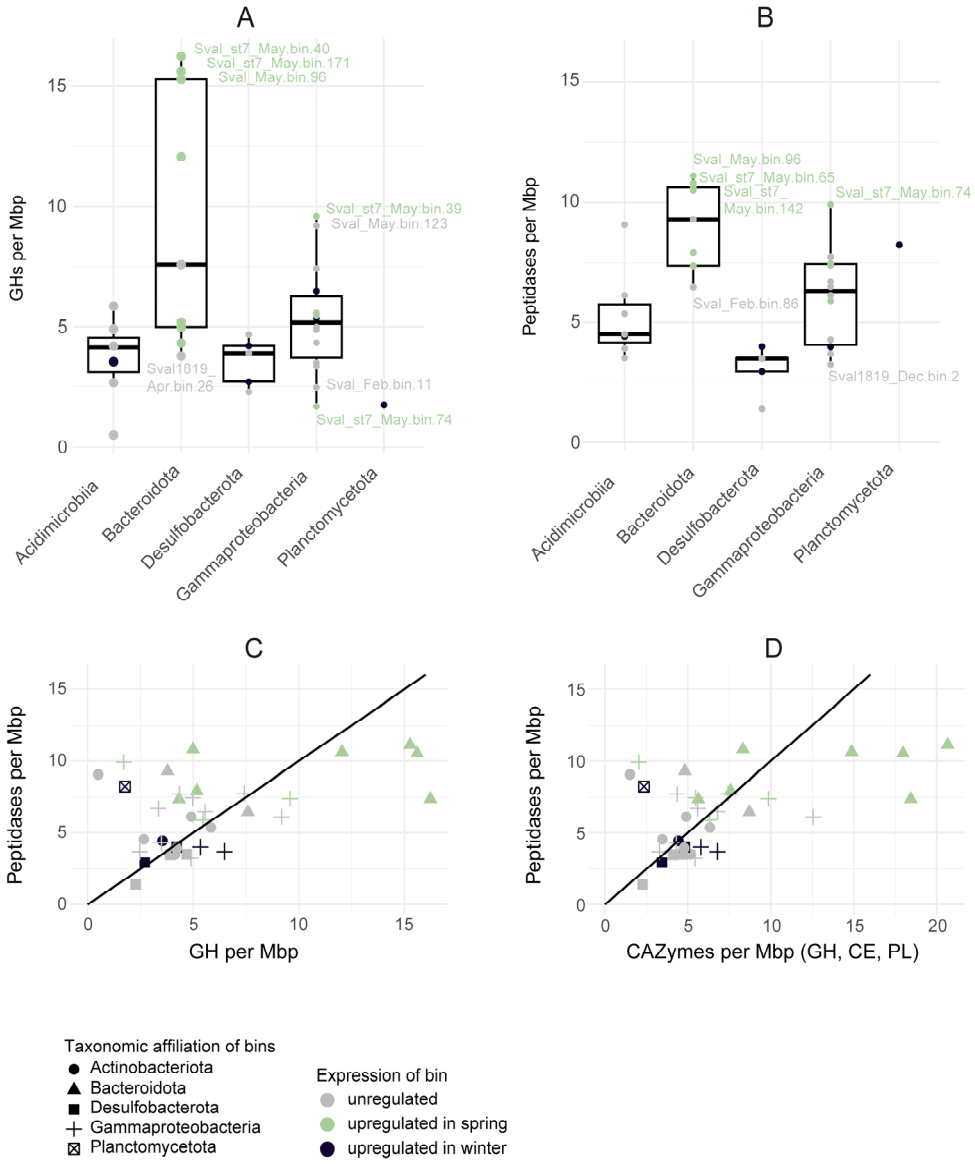
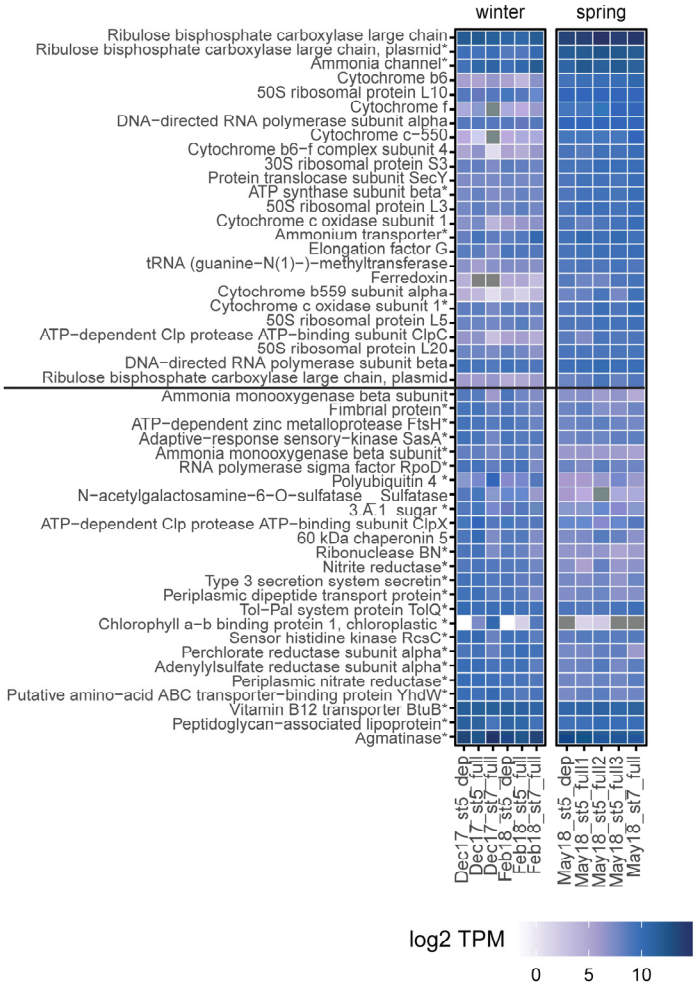


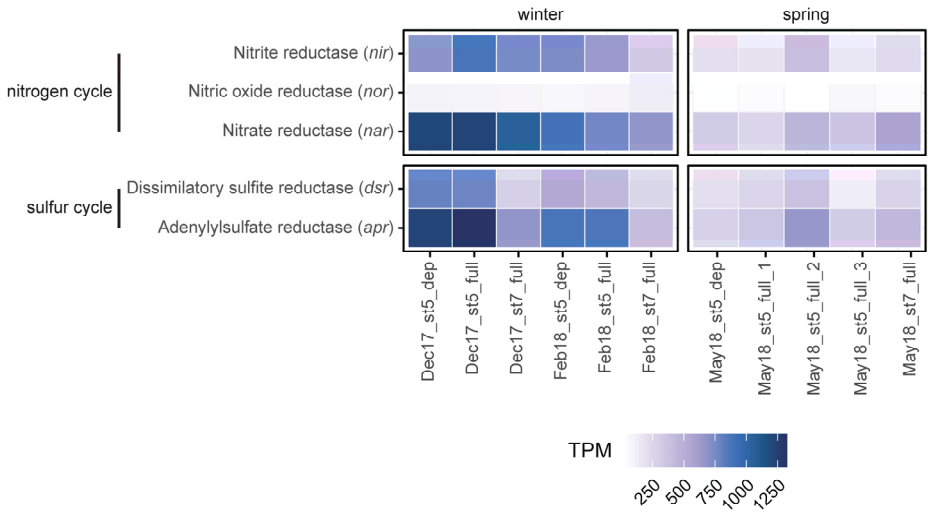
Supplementary Figure S1: Top 50 most changing glycoside hydrolase families in the metagenomes. (A) Changes in average abundance of GH in spring metagenomes compared to winter metagenomes (calculated by [abundance in spring / abundance in winter], given as log2 fold change), (B) average abundance in all metagenomes. Only GH families with > 0.1% average abundance are shown.



Supplementary Figure S2. Comparison of degradative CAZymes (GH +PL + CE) and peptidases repertoires in Svalbard Bacteroidota, Gammaproteobacteria, Acidimicrobiia, Desulfobacterota and Planctomycetota bins. Abundance of (A) GH given per Mpb in bin, (B) peptidases, (C) GH versus peptidases to identify bins that represent specialists for carbohydrate degradation or protein degradation, and (D) CAZymes (sum of GH, CE, PL) versus peptidases. Gene abundances were normalized by bin size. GH, glycoside hydrolyses; CE, carbohydrate esterases, PL, polysaccharide lyases.



Supplementary Figure S3: Top 25 most expressed genes in winter (n=6) and spring (n=5) metatranscriptomes from Svalbard sediments [transcripts per million (TPM), given on a log2 scale]. Genes above the horizontal line are more expressed in spring while genes below are more expressed in winter. Genes, which could only be annotated by one of the used database, are marked by *. Grey tiles are infinite values after log transformation.



Supplementary Figure S4: Expression of key genes involved in nitrogen and sulfur cycling in Svalbard sediments. Abundances in winter and spring metatranscriptomes are shown in transcripts per million (TPM) reads. In particular, *nar* (nitrite reductase), *dsr* (dissimilatory sulfite reductase), and *apr* (adenylylsulfate reductase) were strongly upregulated in winter compared to spring.

Supplementary Table S1. Overview of samples from Isfjorden, Svalbard. OSW, overlying seawater; PW, porewater

sample ID	sample type	sampling date	station	metagenome	meta-transcriptome *full*	meta-transcriptome *rRNA depletion*	sugar analysis
Dec17_st5	bulk sediments	December 2017	5	x	x	x	x
Dec17_st7	bulk sediments	December 2017	7		x		x
Feb18_st5	bulk sediments	February 2018	5	x	x	x	x
Feb18_st7	bulk sediments	February 2018	7		x		x
May18_st5	bulk sediments	May 2018	5	x	xxx	x	x
May18_st7	bulk sediments	May 2018	7	x	x		x
Dec18_st5	bulk sediments	December 2018	5	x			x
Dec18_st7	bulk sediments	December 2018	7				x
Apr19_st5	bulk sediments	April 2019	5	x			x
Apr19_st7	bulk sediments	April 2019	7				x
Apr22_st5_7-bulk	bulk sediments	April 2022	5				x
Apr22_st5_8-bulk	bulk sediments	April 2022	5				x
Apr22_st5_11-bulk	bulk sediments	April 2022	5				x
Apr22_st5_12-bulk	bulk sediments	April 2022	5				x
Apr22_st5_7-PW	Porewater	April 2022	5				x
Apr22_st5_8-PW	Porewater	April 2022	5				x
Apr22_st5_11-PW	Porewater	April 2022	5				x
Apr22_st5_12-PW	Porewater	April 2022	5				x
Apr22_st5_7-OSW	OSW	April 2022	5				x
Apr22_st5_8-OSW	OSW	April 2022	5				x
Apr22_st5_11-OSW	OSW	April 2022	5				x
Apr22_st5_12-OSW	OSW	April 2022	5				x

Supplementary Table S2: Basic statistics for 6 metagenomic and 11 metatranscriptomic datasets obtained from Svalbard sediments.

dataset ID	type	no. reads (raw reads)	no. reads (after qc)	[%] left after quality control	redundancy (nonpareil)	contigs (>1kb)	N50	bins	reads (mRNA)
Sval_Dec_2017	metagenome	139,473,476	103,802,669	74.42	0.49	973,058	1,781	34	-
Sval_Feb_2018	metagenome	125,891,034	107,222,317	85.17	0.49	1,013,043	1,859	35	-
Sval_May_2018_st5	metagenome	133,679,990	115,909,120	86.71	0.47	1,084,287	1,765	40	-
Sval_May_2018_st7	metagenome	135,845,280	118,392,707	87.15	0.45	1,026,676	1,834	42	-
Sval_Dec_2018	metagenome	98,660,349	98,108,189	99.44	0.46	671,159	1,718	16	-
Sval_Apr_2019	metagenome	91,594,369	91,031,268	99.39	0.50	562,393	1,605	16	-
Sval_Dec_st5_dep	metatranscriptome	rRNA depletion	104,435,692	90,587,256	86.74	-	-	-	-	72,961,714
Sval_Dec_st5_full	metatranscriptome		160,797,588	117,796,666	73.26	-	-	-	-	3,608,598
Sval_Dec_st7_full	metatranscriptome		173,253,912	156,932,902	90.58	-	-	-	-	7,688,798
Sval_Feb_st5_dep	metatranscriptome	rRNA depletion	84,267,052	55,112,272	65.40	-	-	-	-	44,000,378
Sval_Feb_st5_full	metatranscriptome		162,431,412	118,600,342	73.02	-	-	-	-	3,556,094
Sval_Feb_st7_full	metatranscriptome		163,666,564	144,462,378	88.27	-	-	-	-	4,103,232
Sval_May_st5_dep	metatranscriptome	rRNA depletion	96,831,938	87,716,354	90.59	-	-	-	-	54,005,690
Sval_May_st7_full	metatranscriptome		178,072,220	166,552,672	93.53	-	-	-	-	4,082,580
Sval_May1_st5_full	metatranscriptome		157,004,704	127,115,722	80.96	-	-	-	-	3,871,750
Sval_May2_st5_full	metatranscriptome		178,707,738	156,811,100	87.75	-	-	-	-	2,038,998
Sval_May3_st5_full	metatranscriptome		186,663,216	169,326,994	90.71	-	-	-	-	4,500,640

Supplementary Table S3: Statistics of high-quality bins. Completeness, contamination, genome size, number of contigs, N50, taxonomic classification and the presence/absence of a 16S rRNA gene is given.

Bin ID	Taxonomic classification	Completeness [%]	Contamination [%]	Genome size [bp]	contigs [no.]	N50 [bp]	16S [yes/no]
Sval_Feb.bin.32	d__Bacteria;p__Actinomycetota;c__Acidimicrobiia;o__Acidimicrobiales;f__Ilumatobacteraceae;g__Ilumatobacter_A;s__	92.54	8.12	4,821,387	876	6,911	no
Sval_st7_May.bin.207	d__Bacteria;p__Actinomycetota;c__Acidimicrobiia;o__Acidimicrobiales;f__Ilumatobacteraceae;g__Ilumatobacter_A;s__	85.55	5.13	3,751,020	839	5,208	no
Sval_st7_May.bin.7	d__Bacteria;p__Actinomycetota;c__Acidimicrobiia;o__Acidimicrobiales;f__SZUA-35;g__;s__	87.10	5.59	3,564,160	362	14,464	yes
Sval_May.bin.58	d__Bacteria;p__Actinomycetota;c__Acidimicrobiia;o__UBA5794;f__JAENVV01;g__;s__	78.21	5.08	1,985,680	347	7,626	yes
Sval_Dec.bin.7	d__Bacteria;p__Actinomycetota;c__Acidimicrobiia;o__UBA5794;f__UBA4744;g__UBA4744;s__	65.43	9.13	2,053,696	524	5,023	no
Sval_May.bin.130	d__Bacteria;p__Actinomycetota;c__Acidimicrobiia;o__UBA5794;f__UBA5794;g__JAHEEL01;s__	87.61	3.94	2,448,020	490	6,341	yes
Sval_Dec.bin.60	d__Bacteria;p__Actinomycetota;c__Acidimicrobiia;o__UBA5794;f__UBA5794;g__B3-G11;s__	97.32	4.69	2,250,330	599	4,231	yes
Sval_Feb.bin.86	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__JAHECK01;g__JAHECK01;s__	89.01	9.21	3,558,031	741	6,250	no
Sval_st7_May.bin.218	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Crocinitomicaceae;g__UBA4466;s__	78.45	5.15	2,310,277	501	5,574	no
Sval1819_Apr.bin.26	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Crocinitomicaceae;g__UBA4466;s__	94.67	1.80	2,906,039	366	12,393	yes
Sval_st7_May.bin.35	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__GCA-2733415;s__	97.76	2.34	2,900,808	175	26,888	no
Sval_st7_May.bin.65	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__GCA-2733415;s__	97.36	1.26	2,404,623	91	60,552	no
Sval_May.bin.96	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Lutimonas;s__	92.72	1.15	3,145,617	231	21,458	no
Sval_st7_May.bin.171	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__Lutimonas;s__	83.74	4.57	2,949,660	371	11,197	no
Sval_st7_May.bin.40	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__SCGC-AAA100-P02;s__	95.11	1.79	2,712,007	109	20,245	no
Sval_st7_May.bin.142	d__Bacteria;p__Bacteroidota;c__Bacteroidia;o__Flavobacteriales;f__Flavobacteriaceae;g__SHLJ01;s__	93.00	0.99	2,823,433	186	23,121	no
Sval_Dec.bin.161	d__Bacteria;p__Desulfobacterota;c__Desulfobacteria;o__Desulfobacterales;f__JAFDCJ01;g__JAFDCJ01;s__	83.23	0.97	4,064,951	581	9,140	no
Sval_Feb.bin.57	d__Bacteria;p__Desulfobacterota;c__Desulfobacteria;o__Desulfobacterales;f__JAFDCJ01;g__JAFDCJ01;s__	53.71	2.61	3,516,276	904	4,227	no
Sval_Feb.bin.151	d__Bacteria;p__Desulfobacterota;c__Desulfobacteria;o__Desulfobacterales;f__Desulfosarcinaceae;g__Desulfosarcina;s__	76.88	5.18	4,266,660	1,195	3,908	no
Sval_Feb.bin.47	d__Bacteria;p__Desulfobacterota;c__Desulfobacteria;o__Desulfobacterales;f__Desulfosarcinaceae;g__Desulfosarcina;s__	91.61	1.79	4,984,765	655	11,167	no
Sval_Dec.bin.130	d__Bacteria;p__Desulfobacterota;c__Desulfobulbia;o__Desulfobulbales;f__Desulfocapsaceae;g__MADRE3;s__	92.43	1.92	4,863,787	827	7,810	no
Sval_Feb.bin.76	d__Bacteria;p__Planctomycetota;c__UBA1135;o__UBA1135;f__UBA1135;g__;s__	67.35	2.25	1,697,842	558	3,225	no
Sval_st7_May.bin.39	d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales;f__Alteromonadaceae;g__Colwellia_A;s__	95.80	1.92	3,653,294	194	28,410	no
Sval_Feb.bin.11	d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__GCA-001735895;f__GCA-001735895;g__GCA-001735895;s__	93.51	9.01	4,860,664	484	16,021	yes
Sval_Feb.bin.110	d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__GCA-001735895;f__GCA-001735895;g__GCA-001735895;s__	82.76	7.27	3,718,874	717	6,494	no
Sval_st7_May.bin.17	d__Bacteria;p__Pseudomonadota;c__Gammaproteobacteria;o__Pseudomonadales;f__Porticoccaceae;g__HTCC2207;s__HTCC2207 sp905182275	91.64	4.01	2,378,634	381	8,914	yes
Sval_Dec.bin.80	d__Bacteria;p__Pseudomonadota;c__Gammaproteobacteria;o__UBA9214;f__UBA9214;g__;s__	84.20	14.69	3,241,495	605	7,338	no
Sval_Feb.bin.103	d__Bacteria;p__Pseudomonadota;c__Gammaproteobacteria;o__UBA9214;f__UBA9214;g__UBA9214;s__	78.06	5.66	2,247,594	530	4,764	no
Sval_Dec.bin.61	d__Bacteria;p__Pseudomonadota;c__Gammaproteobacteria;o__Woeseiales;f__Woeseiaceae;g__JAACFB01;s__	79.67	9.77	2,683,288	246	13,937	no
Sval_Dec.bin.29	d__Bacteria;p__Pseudomonadota;c__Gammaproteobacteria;o__Woeseiales;f__Woeseiaceae;g__UBA1847;s__	83.97	3.02	3,236,586	134	39,867	yes
Sval_May.bin.123	d__Bacteria;p__Pseudomonadota;c__Gammaproteobacteria;o__Woeseiales;f__Woeseiaceae;g__UBA1847;s__	89.03	0.79	3,207,154	127	42,481	no
Sval_May.bin.64	d__Bacteria;p__Pseudomonadota;c__Gammaproteobacteria;o__Woeseiales;f__Woeseiaceae;g__UBA1847;s__	75.33	6.90	2,965,941	443	8,357	no
Sval_st7_May.bin.4	d__Bacteria;p__Pseudomonadota;c__Gammaproteobacteria;o__Woeseiales;f__Woeseiaceae;g__UBA1847;s__	78.98	2.32	2,071,716	205	12,661	no
Sval_st7_May.bin.74	d__Bacteria;p__Pseudomonadota;c__Gammaproteobacteria;o__Xanthomonadales;f__Marinicellaceae;g__NORP309;s__	89.70	6.01	2,926,992	244	16,992	no
Sval_st7_May.bin.147	d__Bacteria;p__Pseudomonadota;c__Gammaproteobacteria;o__Xanthomonadales;f__SZUA-36;g__JAJRRA01;s__	90.85	8.92	4,020,067	773	6,499	yes
Sval1819_Dec.bin.2	d__Bacteria;p__Pseudomonadota;c__Gammaproteobacteria;o__Xanthomonadales;f__SZUA-36;g__SZUA-36;s__	63.65	2.54	1,841,078	521	3,923	no

Supplementary Table S4. Genomic organization of CAZymes in Bacteroidia bins. + genes within the same open reading frame.

bin ID	reg.*	contig ID	Genomic organization of CAZymes as PUL or PUL-like structures*	predicted substrate usage (www.cazy.org) (not organized in PUL-like structures)	other degradative CAZymes and CBM*
Sval_all7_May bin.40		NODE_216	Start of contig, ..., SusC/D CBM4+CBM4 GH16_3, GH2, GH149, GH30_1, GH17, ..., end	laminarin, β-glycans	
		NODE_59	start of contig, ... GH5_4, GH3, 3H10, GH10, GH43_1, GH10, PL8+CBM22, GH30, SusC/D ..., end of contig	xylane, xyose	GH16+CBM54, GH3, GH130, GH13_31, GH43_12,
		NODE_650	start of contig, ... GH92, GH92+GH94, GH92, SusC ..., end of contig	mannose	GH92, GH81+CBM6, GH3, GH109, GH39, GH12,
		NODE_711	start of contig-GH30_1, GH16, GH5_46+CBM6+CBM6, SusC ..., end of contig	β-glycans	GH23+CBM50, 3H20+CBM32, GH0+GH113, CE11 CE1, CE14, CE4
		NODE_848	start of contig, ..., SusC GH92, GH92, GH92+GH94, GH3, GH3+CBM6, ... enc of contig	mannose	
Sval_all7_May bin.65		NODE_1389	start of contig, ..., PL7_5, PL6_1, PL8+CBM32, PL17_2+PL17, SusC/D ..., enc of contig	alginate	GH23, GH113, GH25, CBM50+GH23, GH3, GH5_42, GH73+CBM50
		NODE_424	start of contig, GH10, GH3, SusC/D ..., end of contig	xylane, xyose	CBM32+GH85, 9H88, CE11, CE14, CE4,
Sval_all7_May bin.213		NODE_2868	start of contig, ..., SusC/D GH16+CBM13+CBM6, end of contig	β-glycans	CBM50+GH23, GH3, GH92, GH2, GH1, 3H113, GH23, G-120, CE14, CE1, CE4
Sval_all7_May bin.35		NODE_39472	Start of contig-GH65+CBM13+CBM32+CBM51, GH168, GH80_2, - ..., end of contig	fucose, fucan, xylane	GH5, GH157, GH20, GH25, GH18, CBM32-GH85, GH29, GH113, GH3, GH-23, GH5_42, GH157.
		NODE_649	start of contig, ..., GH50_1, GH16+CBM4+CBM54, SusC/D ..., end of contig	laminarin, β-glycans	CBM50+GH23, CE1, CE11, CE14, CE4, PL6+PL6_1
		NODE_2327	start of contig, ..., PL7, SusC/D -PL17_2-, ..., PL7_5, ... end of contig	alginate	
		NODE_10	start of contig, ..., GH92, GH20+CBM32, GH2, CE4 - ..., end of contig	mannose, peptidoglycan	GH113, GH31, GH97, GH113, GH23, CBM48+GH13_9,
Sval_all7_May bin.142		NODE_230	start of contig-GH13, GH31, G-113_1, 9, GH97, GH13, - ..., end of contig	α-glycans	3H0, GH30_1, G-117, CBM48+GH13, GH3, GH16+CBM6, GH2+CBM32+CBM27, GH53
		NODE_29572	start of contig, ..., GH17, GH17, GH17 - end of contig	laminarin, β-glycans	CBM50+GH23, GH170+CBM50, GH158, CE11, CE 4
		NODE_56	start of contig, ...-GH2+CBM51+CBM57, GH2+CBM51+CBM57, GH83 - ..., end of contig	mannose	
		NODE_5907	start- GH158, GH0, GH19+CBM11+CBM13+CBM32+CBM4+CBM54+CBM58+CBM6, CBM4, GH80_1, - end of contig	β-glycans	
Sval_all7_May bin.171		NODE_9839	Start of contig- PL17_2, SusC/D ..., end of contig	alginate	GH17, GH3, GH94, GH0+GH13,
		NODE_1447	Start of contig, SusC/D GH16_3, , end of contig	laminarin, β-glycans	CE1+GH10, GH20+CBM32, GH149, GH144, GH5_48, GH23, GH20
		NODE_154*	Start of contig-G-113_9, GH13, GH85, SusC CBM48+GH13, ..., end of contig	α-glycans	GH1+CBM25+CBM41+CBM48+CBM66,
		NODE_12460	Start of contig, ..., GH16_3, GH7, GH17, GH17 - end of contig	laminarin, β-glycans	CBM50+GH23, GH3+CBM6, GH16+CBM6, GH16, GH5_46+CBM6+CBM6, GH109,
		NODE_8468	Start of contig- GH158, G-117, GH149, GH80_1, - end of contig	laminarin, β-glycans	GH2+CBM32+CBM6? GH157, GH29, GH2, GH83, GH130, GH26, PL7, PL7_5, PL12, CE1, CE11, CE14
Sval_May_bin.95		NODE_14	start of contig, ... CBM48+GH13, SusC/D GH65, GH13, GH13_13, GH13_36, ..., end of contig	glycogen, α-glycans	
		NODE_2396	start of contig- PL17_2, SusC/D FL7, P_7, ..., end of contig	alginate	GH20, GH3 GH5_46+CBM6+CBM6, GH26 GH3 3H9+CBM2+CBM4,
		NODE_309	start of contig, ... PL7, PL7 SusC/D FL7_5, PL7_5, PL12, PL8+PL8_1, ..., end of contig	alginate	GH0+GH13, GH-23, CBM48+GH13_9, GH31, GH84,
		NODE_2289	start of contig, ..., GH94, GH16_3, GH7, GH17, GH17. - end of contig	laminarin, β-glycans	GH79+CBM50, GH109, GH0, GH2+CBM51+CBM67, GH16+CBM6, GH16, GH5_46+CBM6+CBM6,
		NODE_25	start of contig, ..., GH20, 3H3, 3H3 - ..., end of contig	xylose, peptidoglycan	GH9+CBM13+CBM32+CBM4+CBM54+CBM58+ C3M6, 3H29, GH3+CBM6, CE4, CE14, CE11, P_6+PL6_1
		NODE_9324	start of contig, ..., GH18+CBM4, GH18+CBM4+CBM54+CBM6, GH80_1, - ..., end of contig	laminarin, β-glycans	
Svar1819_Apr bin.26			-	-	GH20-CBM32, G-173+CBM53, GH113 GH146+CBM41, CBM15, GH73, GH25, GH3, GH2, GH92, GH14, CE1, CE14, CE4
Sval_Feb bin.88			-	-	GH139, CBM48+GH13, GH31, GH13_23, GH2+ CBM32+CBM67, GH3+CBM6, GH3, GH2, GH5_46, GH29, GH2, GH92, CBM50+GH23, GH13, GH3, GH83, GH20, GH23, GH78, CE14, CE15, CE11, CE4

reg., regulation. Color indicates if bins were upregulated in spring (green) or were unregulated and evenly expressed throughout the year (grey; assignment taken from Figure 3).

- PULs are defined based on the presence of a pair of susC- and susD-like transporter genes and ≥2 CAZyme genes (GHs, PLs, CEs or CBMs) and PUL-like loci based on the presence of ≥ 1 CAZyme both within a 10-genes-sliding window)
- other CAZymes in contigs not matching the criteria of PUL and PUL-like

Supplementary Table S6. Glycan concentrations in porewater (PW) and overlying seawater (OSW) from Isfjorden, Svalbard. Glycans from OSW and porewater were acid hydrolyzed and the resulting monosaccharides were measured by HPAEC-PAD analysis. Concentrations in PW were 18fold higher than in OSW. Values in surface seawater were in the range obtained from controls with demineralized water in dialysis bags (data not shown). Bd, below detection; bt, below threshold (values measured for low-concentrated calibration standards indicated a loss of detector sensitivity with time, thus a threshold concentration for each monosaccharide was set to the value at which the variation between two injections was smaller than ±20%. Values lower than the threshold concentrations defined for each monosaccharide were rejected).

ID	Replicate	Sample type	Arabinose [µg C L^{-1}]	Fucose [µg C L^{-1}]	Galactosamine [µg C L^{-1}]	Galactose [µg C L^{-1}]	Glucosamine [µg C L^{-1}]	Glucose [µg C L^{-1}]	Mannose [µg C L^{-1}]	Rhamnose [µg C L^{-1}]	Xylose [µg C L^{-1}]	Sum [µg C L^{-1}]
Apr_7-OSW	1	OSW	bd	bd	bt	bt	bt	bt	bt	bd	bt	0.0
Apr_7-OSW	2	OSW	bd	bd	bt	bt	bt	bt	bt	bd	bt	0.0
Apr_8-OSW	1	OSW	bd	bt	bt	bt	bt	22.9	bt	bd	bt	22.9
Apr_8-OSW	2	OSW	bd	bt	bt	bt	bt	17.2	bt	bd	bt	17.2
Apr_11-OSW	1	OSW	bd	bt	bt	bt	bt	43.2	bd	bd	bt	43.2
Apr_11-OSW	2	OSW	bd	bt	bt	bt	bt	45.5	bt	bd	bt	45.5
Apr_12-OSW	1	OSW	bt	bt	bt	bt	bt	58.2	bt	bd	bt	58.2
Apr_12-OSW	2	OSW	bt	bt	bt	14.4	bt	151.2	44.8	bd	95.0	305.4
Apr_7-PW	1	PW	103.8	79.4	bt	46.8	52.1	112.0	bt	bt	105.2	499.2
Apr_7-PW	2	PW	89.2	75.8	bt	43.6	51.6	125.2	bt	bt	90.9	476.3
Apr_8-PW	1	PW	bt	186.6	41.9	96.9	128.5	151.9	bt	bt	207.8	813.6
Apr_8-PW	2	PW	190.9	196.0	bt	100.7	144.0	163.7	bt	bt	206.9	1,002.3
Apr_11-PW	1	PW	399.4	365.4	11.2	192.8	248.7	238.7	bt	bt	326.0	1,782.2
Apr_11-PW	2	PW	99.5	349.3	83.5	186.6	247.3	240.4	bt	bt	318.8	1,525.4
Apr_12-PW	1	PW	262.9	289.4	10.1	148.9	179.2	198.9	bt	bt	283.4	1,372.8
Apr_12-PW	2	PW	269.5	303.1	bt	148.9	176.7	190.5	bt	bt	295.8	1,384.5

Supplementary tables TabS5 and TabS7 are too large to be printed and can be accessed in the published version with the journal digitally or by contacting the corresponding author.

Chapter 4: Bacterial polysaccharide utilization in polar marine coastal sediments

Declaration on contribution of Sebastian Miksch to Chapter 4

Name of candidate: Sebastian Miksch

Title of thesis: Microbial polysaccharide utilization in sandy surface sediments

Authors of manuscript: KatrinKnittel, Sebastian Miksch, ChyreneMoncada, JannikaMoye, Sebastian Silva, Rudolf Amann, Carol Arnosti

Article published: Manuscript in preparation

Contribution of the candidate in % of total work load

Experimental concept and design: ca. 50%

Experimental work/acquisition of data: ca. 20%

Data analysis and interpretation: ca. 20%

Preparation of figures and tables: 40%

Drafting of manuscript: ca. 20%

Bacterial polysaccharide utilization in polar marine coastal sediments

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Competing interest statement

The authors declare no competing financial interests in relation to this work.

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Abstract

Polysaccharides constitute a large fraction of phytoplankton and macroalgal biomass in the ocean. Heterotrophic bacteria process much of this carbon either by releasing exoenzymes, by scavenging oligo- and monosaccharides produced by others, or by using a selfish uptake mechanism. In the water column, selfish uptake has been shown to be an important polysaccharide uptake mechanism for pelagic bacteria. To study the relevance of different polysaccharide utilization modes in sediments, we set up incubation series of polar surface sediments from Isfjorden, Svalbard, in April and September 2019 with five different fluorescently labelled polysaccharides, namely, laminarin, xylan, mucin, chondroitin sulfate, and fucoidan. An increase in total cell numbers was observed in the course of the incubations, but the community composition remained stable independent of the substrate added and month of sampling. The highest hydrolysis rates were about twice as high in the September incubations compared to the April incubations, with maximum rates of > 6000nmol monomer L^{-1} sediment h^{-1} measured for laminarin, xylan, and mucin. The fast degradation of mucin suggests that this animal-derived glycoprotein may be a key substrate for benthic microbes. Only a minor fraction of the microbial community showed selfish uptake (laminarin and mucin: 1.5-2%, xylan, chondroitin sulfate and fucoidan: 0.5-1% of total cells). Sixty to ninety percent of selfish bacteria could be identified as Planctomycetota and Verrucomicrobiota. The dominance of extracellular hydrolysis indicates that the bulk of glycan utilization is catalyzed by a cooperative benthic community relying on the sharing of enzymatic capabilities and the scavenging of public goods.

Introduction

Polysaccharides constitute a large fraction of the phytoplankton and macroalgae biomass in the ocean [1-3], as well as of dissolved [4] and particulate organic matter [5] in the water column, and organic matter in sediments [2]. Most marine organic matter – including polysaccharides – are turned over in the ocean, with a considerable portion of this degradation carried out by bacteria [6]. Given the multitude of polysaccharide structures, microbes, and enzymes involved [reviewed in 7], many aspects of polysaccharide cycling in marine systems are still unclear. In particular, most recent work on polysaccharide-degrading organisms and enzymes have focused on water column processes [8-14], despite the fact that a significant fraction of marine organic matter is remineralized in shallow coastal sediments [15, 16].

Three broad groups of bacteria involved in polysaccharide degradation have been identified to date [reviewed in 7, 17-19]. The first group is comprised of microbes that release extracellular enzymes for hydrolyzing the substrate outside the cell [17]. Extracellular enzymes may either be bound to the cell surface or released into the environment. The latter might only be favorable under high concentration of the substrate [20]. The second group of microbes are those that tak advantage and scavenge the oligosaccharides and monosaccharides produced by the enzymes of the first group. These scavengers cannot or do not produce extracellular enzymes and therefore are dependent on low molecular weight hydrolysis products from the activities of external hydrolyzers [18]. The third group makes use of a "selfish" uptake mechanism, where polysaccharides are bound to the outer membrane, partially hydrolyzed, and then large fragments are transported into the periplasm where it can be further degraded into low molecular weight sugars without diffusive loss [19, 21].

Microbial communities of shallow permeable sediments in particular have been shown to have high rates of metabolism [16], but little is known about the specific mechanisms used for polysaccharide degradation in sediments. In contrast, it has been shown that a large fraction of pelagic bacteria use the selfish uptake mechanism. The size of this fraction differed between substrates and bloom phase and could be as high as 60% of total cells during summer [8]. Selfish bacteria were identified as Bacteroidota, Gammaproteobacteria, Planctomycetota, and Verrucomicrobiota[see, for example, 8, 19, 22]. Currently, the relevance of the selfish uptake mode in sediments has not yet been investigated. To determine the contribution of selfish uptake to benthic polysaccharide hydrolysis and to identify microbes involved in their utilization, we analyzed extracellular enzyme activities and selfish uptake of polysaccharides in surface (0-2 cm) sediments from Isfjorden, an Arctic fjord in Svalbard, an archipelago in the Arctic Ocean at 78°N. Previous studies showed that polysaccharides in sediments from fjords on the west and northwest coast of Svalbard could meet a substantial fraction of microbial carbon requirements [23, 24], and that the spectrum of active enzymes is considerably broader in sediments than in the overlying water column [24, 25]. We intended to determine whether previously observed differences in the community compositions between pelagic and benthic microbial communities [25-27] are also reflected in the preferential use of the polysaccharide utilization mode, and whether pelagic and benthic taxa that are involved in the degradation differ.

Previous studies on benthic polysaccharide degradation focused strongly on algae-derived substrates such as, e.g., laminarin, fucoidan, carrageenan, alginic acid, or chondroitin sulfate [24-26, 28, 29]. With this study, we expand the spectrum of polysaccharides tested for benthic microbial hydrolysis to mucin, an animal-derived glycoprotein from mucus secreted by many animals such as marine invertebrates [30-33] and studied if mucin is a major substrate for benthic microbes.

Materials and methods

Sampling.

Sediment samples were collected in Isfjorden, Svalbard, at station 5 (N78°06.405', E14°21.070' ± 20 m). Sampling on April 25th and September 13th 2019 was carried out using a Van Veen grab. Sampling on June 29th 2022 was performed with a custom rotating bowl grab, using a combined principle of Shipek- and Hamon- grab (constructed by A. Ellrott, MPI Bremen). The top two centimeters of the sediments were subsampled. All sediments consisted of fine and medium-grained sand, but September sediments also had a contribution of silt [27]. Sediment temperatures at the three sampling dates were 2.2°C, 4.5°C, and 5.2°C, respectively, salinity was 35 PSU, 32 PSU, and 34 PSU and chlorophyll a concentration in surface sea water (as measured with a Cyclops-7 submersible sensor (Turner Designs, San Jose, USA) was 8 µg L^{-1}, 1.8 µg L^{-1}, and 2.0 µg L^{-1} in April, September, and June, respectively. Chlorophyll concentration in surface sediments was 400 µg L^{-1} and 1800 µg L^{-1} in April and September [for further details see 27].

Polysaccharides

Five polysaccharides were selected for our incubations based on their abundance in the ocean and classified either as algal- or animal-derived[algal- or animal-derived, 7]: laminarin, xylan, chondroitin sulfate, fucoidan and mucin. Laminarin, produced by algae and phytoplankton [β-1,3-glucose backbone with β-1,6-glucose side chains,3] and xylan, present in some green and red algae [β-1,4-linked D-xylose backbone with traces of L-arabinose, 34], are widespread in the ocean and readily degradable [for example, 7, 35, 36]. On the other hand, chondroitin sulfate, from animal cartilage tissue (alternating N-acetylgalactosamine and glucuronic acid), and fucoidan, which is a cell wall component of brown algae [37], are both

complex, sulfated polysaccharides. Lastly, mucins are particularly large glycoproteins (0.5–2.0 MDa) derived mainly from invertebrates. They are characterized by the presence of a central protein core with heavily glycosylated side-chains [38]. The levels of glycosylation differ, however the carbohydrate level of mucins is usually around 80% [38].

Set-up of incubations with fluorescently-labelled polysaccharides (FLA-PS).

For April sandy sediments, 180 ml of bulk sediments were carefully washed three times (directly after retrieval) with a total of 13 L autoclaved artificial seawater (ASW). The main intention of washing was to remove planktonic bacteria from the pore water as a large fraction of them is capable of selfish uptake of polysaccharides. We are aware that we might have also removed excess nutrients and free exoenzymes from the sediments.

Batch incubations of sediments were set-up by adding 10 ml of ASW amended with fluorescently-labelled polysaccharides [FLA-PS, final concentration 100 µM monomer equivalent, fluoresceinamine-labelled and purified according to 39] to 3 ml of sediments in 50 ml polypropylene tubes. Three replicates were amended with one of the five selected FLA-PS. Killed controls, consisting of sediment subsamples that were previously fixed in 3.5% formaldehyde (final concentration) for 1h at room temperature, were also incubated with the polysaccharides. The incubations were subsampled after 20 minutes (referred to as t0), 1.5 d (t1), 3.5 d (t2), 6.5 d (t3), 10.5 d (t4), and 17.5 d (t5) of incubation. For measurements of extracellular enzymatic activities, 1 mlof supernatant was sampled with a syringe, filteredthrougha0.2µmNalgene filter (SCFAmembrane, Thermo Scientific) and frozenat - 20 °C until analysis. For FISH, 250 µl of sediment were transferred with cell-saver tips to Biosphere® SC Micro tubes (2.0 ml with scale) and fixed with 750 µl formaldehyde (1.5% final concentration) for at least 1 h at room temperature.

For September silty sediments, 300 ml were washed in 10 L ASW. Three replicates

each of 2.5 ml sediments were incubated in 8 ml FLA-PS-amended ASW. Formaldehyde-fixed killed controls were also incubated with the polysaccharides. Formaldehyde concentration used was undefined as there was massive precipitation in the solution that could not be re-dissolved completely onboard. Additionally, unamended controls without FLA-PS added were set-up. Subsampling was done after 20 min (t0), 0.25 d (t1), 1 d (t2), 2 d (t3), 4 d (t4), and 10 d (t5) of incubation. Using cell saver tips, 1.3 ml sediment slurry (composed of ~300 µl sediment and ~1000 µl FLA-PS-amended ASW) was transferred into Biosphere® SC Micro tubes. For measurements of extracellular enzymatic activities, ~1 ml of supernatant was subsampled with a syringe, filteredthrougha0.2µmNalgene filter (SCFAmembrane, Thermo Scientific) and frozenat - 20 °C until analysis. The 300 µl of sediment was fixed with 750 µl formaldehyde (1.5% final concentration) for at least 1 h at room temperature.

All tubes from April and September series were incubated close to *in situ* temperatures at 4°C in the dark. After subsampling, sediments and FLA-PS-amended ASW were carefully mixed and incubation was continued.

Incubations with different mucin concentrations.

To examine cellular growth and selfish uptake as a function of substrate concentration, sediment samples (unwashed) from June 2022 were incubated with three different mucin concentrations: three replicates of 3 ml sediment subsamples were incubated with 10 ml of autoclaved and 0.2 µm sterile-filtered ASW amended with either 10 µM, 100 µM or 1000 µM FLA-mucin for up to 3 days at 4°C. Killed controls were prepared by using a domestic pressure cooker for 30 min for sterilizing the sediment. Subsampling and processing was done as described above for April incubations.

Measurementofextracellularenzymaticactivities

Hydrolysis rates of the different polysaccharides were measured via changes in the molecular weight of the added polysaccharides with time as they were systematically hydrolyzed to smaller size classes [described in detail in 39]. In brief, samples were thawed and injected on a system of two Sephadex gel columns (G-50 and G-75, respectively) in series, which separate polysaccharides and hydrolysis products as a function of molecular size. The fractions were detected via fluorescence (ex: 490; em: 530 nm) of the fluoresceinamine covalently attached to the polysaccharides and fragments. Columns were standardized with FITC-dextrans, FITC-galactose, and free fluoresceinamine.

For the April and September samples, instead of using the standard method for killed controls (autoclaving), the sediment slurry was amended with formaldehyde (3.5% final concentration) for one hour at room temperature. In the laminarin and xylan incubations, this technique was clearly unsuitable, since it resulted in enzyme activities that were almost as high as those measured in the active incubations. One possible reason for the activity in the controls was an insufficiently high formaldehyde concentration to kill the cells and to inactivate extracellular laminarinases and xylanases. While in April killed controls only slight cell growth was detected, cell growth in September samples was more pronounced (likely caused by massive precipitation of formaldehyde used). A correction of these rates by the values measured in the killed control would result in hydrolysis rates close to zero, which is unlikely as rapid hydrolysis of laminarin and xylan has previously been reported for Arctic sediments [e.g., 24, 26]. Therefore, here we show hydrolysis rates for laminarin and xylan that were not corrected by the rates for the corresponding killed controls. Notably, however, killed controls for mucin, chondroitin sulfate, and fucoidan showed no or very low activity;

we speculate that these differences arise from the extent to which enzymes are already present in cells vs enzymes that require induction (see Discussion).

Separation of cells from sediment grains.

All formaldehyde-fixed samples were washed three times with 0.2 µm-sterile-filtered 1x phosphate-buffer saline (PBS; 10 mM sodium phosphate, 130 mMNaCl, pH 7.2-7.4). Afterwards, they were sonicated on ice with a Sonopuls GM Mini20 equipped with a microtip MS 2.5 (Bandelin, Berlin, Germany). Sandy April samples were sonicated three times at a setting of 30 s, an amplitude of 86% and pulse of 0.2 s. Supernatants were collected and replaced by 750 µl 1xPBS. Aliquots of combined supernatants were filtered through a 47 mm (0.2 µm pore size) polycarbonate filters (GTTP, Millipore, Eschborn, Germany), applying a gentle vacuum of < 200 mbar. Silty sediments from September were sonicated only once. An aliquot was taken from the tube, diluted and filtered. After drying, the filters were stored at -20°C until further analysis.

Fluorescence*insitu*hybridization (FISH)and microscopy

FISHwas carried out with slight modifications of the protocol by Manz*et al.* [40]. The hybridization mix contained 0.9 M NaCl, 0.02 M Tris-HCl (pH 8), 10% (wt/vol) dextran sulfate, 0.02% (wt/vol) sodium dodecyl sulfate, 1% (wt/vol) blockingreagent (Roche), x% (vol/vol) formamide (according to the individual probe requirements) and 0.83 pmol/µl 4xAtto594-labelled oligonucleotide probe. Hybridization was carried out in a humidity chamber equilibrated with 2.25 M NaCl and identical formamide concentration as in the hybridization buffer at 46°C for 3 h. Filter sections were subsequently washed in prewarmed

buffer (0.08-0.225 M NaCl, depending on formamide concentration in the hybridization buffer, 20 mMTris-HCl (pH8), 0.05 M EDTA (pH 8), 0.02% (wt/vol) sodiumdodecylsulfate) for 15 min at 48 °C. Filter sections were removed from the buffer, air-dried on Whatman paper and mounted in CitiFluorAF1 (CitiFluor Ltd., London, United Kingdom] and Vectashield (Vector Laboratories, Burlingame, CA, USA) containing 1 μg ml^{-1} DAPI (4′,6-diamidino-2-phenylindole; Sigma-Aldrich, Steinheim, Germany). FISH signals and FLA-PS uptakewas visualised by epifluorescence microscopy (Nikon Eclipse 50i). For quantification of FLA-PS stained cells, 100 fields of view were manually counted. For identification of FLA-PS stained cells, at least 100 FLA-PS stained cells were analyzed with each FISH probe (for September, > 50 FLA-PS stained cells; FLA-xylan stained cells, not analyzed). For quantification of total FISH-stained cells, 20 to 30 fields of view were counted. Imaging was done by a laser scanning microscope (LSM780, Zeiss, Jena, Germany) equipped with an Airyscan detector. Probe sequences, permeabilization conditions and formamide concentrations are given in Supplementary Table S1.

Catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH).

Quantification of total Actinomarinales (probe ACM1218) and Microtrichales (probe MIT1218) was not possible using 4xAtto594-labelled probes, as signal intensities were too low. Therefore, *in situ* hybridizations with horseradish peroxidase (HRP)-labeled probes followed by fluorescently-labeled-tyramide signal amplification (CARD) was applied as described previously [27, 41].

Phylogenetic tree reconstruction and oligonucleotide probe design.

An oligonucleotide probe for the identification of the Verrucomicrobiota family Rubritaleaceae was developed. Probe design was targeted towards this family, as this was the most abundant Verrucomicrobiota family based on relative 16S rRNA gene abundance [27]. As a prerequisite for probe design, a phylogenetic tree was reconstructed from 441 full-length Verrucomicrobiota 16S rRNA genes selected from the SILVA database SSU Ref138.1 [42, 43, released Aug 2020]. The tree was calculated using a maximum likelihood method (PHYML) with GTR correction. A filter that excluded positions with more than 50% variability was implemented, which resulted in 1381 valid sequence positions for tree calculation. Selected sequences belonging to the phylum Planctomycetota were used as outgroup. Partial Verrucomicrobiota 16S rRNA gene sequences from Isfjorden, Svalbard sediments [27] spanning the V3-V4 hypervariable region were imported into the database and added to the tree under Parsimony criteria without allowing changes in the overall tree topology.

Probe design was done using the Probe Design tool of the ARB software package version arb-devel-6.1.rev18634 [44]. Additionally, a set of competitors and helpers flanking the 5' and 3' ends of the target sequences was designed and used in equimolar concentrations with the probe.

DNA extraction and microbial diversity analyses.

DNA was extracted from subsamples from t0 and t5 by using the Power Soil Kit (Qiagen). Amplification of 16S rRNA gene fragments was done using primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 [45]. Amplicons were sequenced on an Illumina (San Diego, CA, USA) platform (HiSeq2500, 2x250 bases, paired-end) at the Max Planck-Genome Center in Cologne (Germany). Sequences were processed using BBTools version

37.62 [46], mothur v.1.38.1 [47] and after subsampling to 10,000 reads per sample classified using the SILVAngspipeline and database SSU 138.1 Ref NR99[43]. Plotting was done using R [48, 49]. For details see Miksch *et al*. [27]. Script is deposited at: https://github.com/smiksch/16_S_rRNA_processing_looking_at_seasonality_in_sediments

Data availability statement. Sequence data were stored in the European Nucleotide Archive (ENA) under study accession numbers PRJEB57252 (16S rRNA gene sequences).

Results

Extracellular enzyme activities.

Hydrolysis rates for laminarin, xylan, and mucin were considerably higher than for fucoidan and chondroitin in both April and September (Figure 1). Comparing April and September, rates were about twofold higher in September incubations with 6842 nmol monomer L^{-1} sediment h^{-1} for laminarin, 6097 nmol monomer L^{-1} sediment h^{-1} for xylan and 6568 nmol monomer L^{-1} sediment h^{-1} for mucin. Rates peaked after 1.5 d (laminarin) and 3.5 d (xylan and mucin) in April, and after only 0.3 d in September. Rates for the other two polysaccharides tested were much lower. Considerable rates of chondroitin hydrolysis were first measurable after 3.5 - 4 d (~700-800 nmol monomer L^{-1} sediment h^{-1}) which increased to ~1000 nmol monomer L^{-1} sediment h^{-1} by day 10 of incubation for both, April and September incubations. In September incubations, fucoidan enzymatic hydrolysis rates were similarly high with ~600-900 nmol monomer L^{-1} sediment h^{-1} for all timepoints between 0.3 d and 10 d while they were highest only after 10.5 d in April and about twofold lower with 500 nmol monomer L^{-1} sediment h^{-1}.

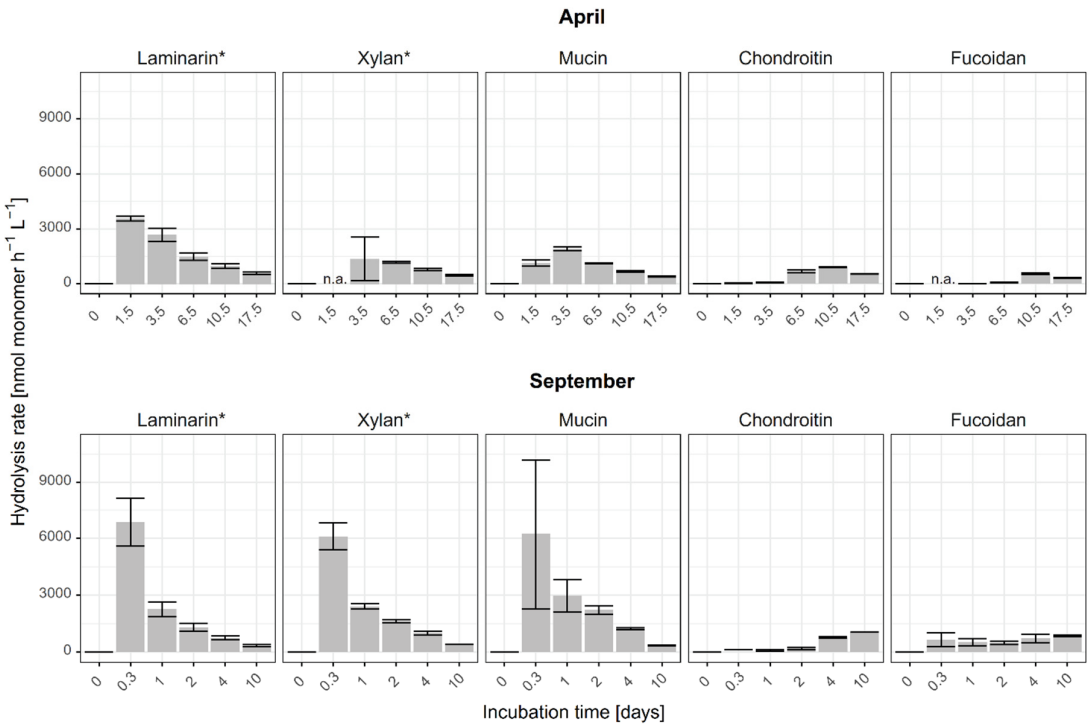

Figure 1: Hydrolysis rates of polysaccharides in homogenized surface sediments (0-2 cm) from Isfjorden, Svalbard. (A), April incubation; (B), September incubation. Error bars show the standard deviations of triplicate incubations. * Note, hydrolysis rates of laminarin and xylan were not corrected by values measured in killed controls as controls were insufficiently inactivated by formaldehyde fixation. n.a., not analyzed.

Cell counts.

Cell counts in the incubations varied by substrate, incubation time, and month of sampling (Supplementary Table S2, Supplementary Figure S1, S2). Cell numbers at t_0 were higher in silty September sediments, at 7-10 x 10^8 cells ml^{-1} sediment, than in fine sands from April, at 2-4 x 10^8 cells ml^{-1}. In all incubations, cell counts increased over time by a factor of 1.4-3.5, reaching a maximum by day 4 (for September) or day 10.5 (for April). Cell numbers in xylan incubation, replicate A, from April increased strongest by a factor of 5.1 and reached a maximum of >19 x 10^8 cells ml^{-1} sediment (Supplementary Figure S1). Cell numbers in April killed controls increased slightly by 30% in laminarin and xylan incubations (from ~ 3.2 to 4.3 x 10^8 ml^{-1} sediment), while they remained constant in mucin, chondroitin and

fucoidanincubations. Cell numbers in September killed controls increased during the course of the experiment in all incubations between 10% and 280% (Table S2), clearly indicating that the formaldehyde inactivation had been insufficient.

Bacterial community composition in the incubations.

Sequencing of 16S rRNA genes was carried out on subsamples from timepoint t0 and t5 (17.5 d in April, 10 d in September incubations) to compare temporal changes in the bacterial community composition in the incubations and to identify possible bacteria responding to FLA-PS addition (Figure 2). Most taxa remained stable in relative read abundance between t0 and t5. In April incubations, a strong response was only visible in the xylan incubation (replicate A) in which Bacteroidetes of genera *Lutibacter* and *Marinifilum* increased in abundance from ~1% to ~15-20% of total reads after 17.5 d. A minor increase of read frequencies was detected for clade Sva1033, *Desulfomusa*, (both Desulfobacterota) and *Psychrilyobacter* (Fusobacteria) in the same incubation as well as for actinobacterial*Ilumatobacter* and Actinomarinales in fucoidan incubation replicate C. For all substrates, an increase of reads affiliated with uncultured Colwelliaceae was detected (from 1% to 5%). In the September incubations, a strong response was found only in fucoidan incubation replicate A, wherein *Izimaplasma* (Firmicutes) increased from 2% to 15% of total reads. Furthermore, in all three fucoidan replicate incubations, *Algibacter* reads increased slightly from 1% to 2-4%. Other taxa did not show a clear response to the substrate. In the unamended control incubations, no changes in the bacterial community composition were detected between t0 and t5.

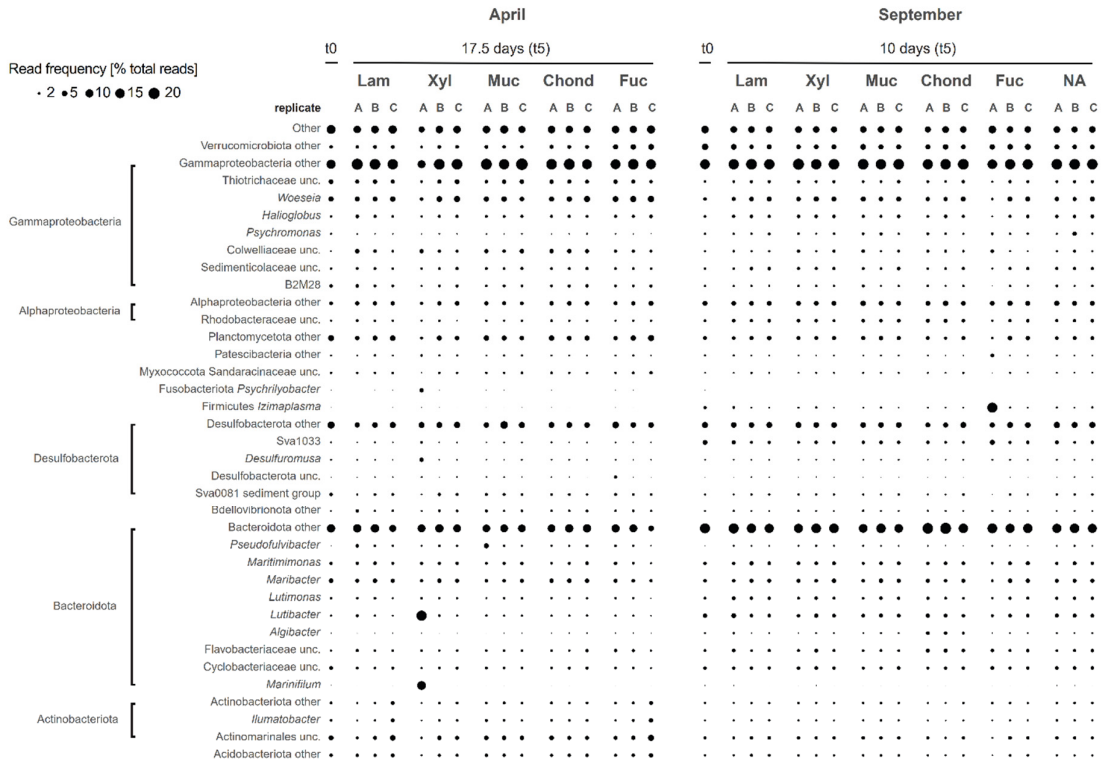

Figure 2: Relative abundance of bacterial families and genera at t0 and t5 for all substrates and for the unamended control incubations for April and September. The size of the bubble represents the relative abundance (%) of each taxon. Taxonomy based on SILVA SSU138.1 Ref NR99 database. Only taxa that accounted for >2% of total reads in at least one of the samples are shown. Minor abundant taxa were clustered on higher taxonomic levels and displayed as "other". Lam, laminarin; Xyl, xylan; Muc, mucin; Chond, chondroitin sulfate; Fuc, Fucoidan; NA, unamended incubation

Quantification of major taxa.

Major taxa in the incubations were identified by FISH using 4xAtto594 labelled probes for Gammaproteobacteria (probe GAM42a), Bacteroidota (probe CF319a), Verrucomicrobiota (probe EUB338-III), Planctomycetota (PLA46), or by CARD-FISH using HRP-labelled probes for actinobacterialMicrotrichales (probe MIT1218) and Actinomarinales (probe ACM1218). Probes were selected based on major phyla present at the sampling site [27]. At t0, Gammaproteobacteria contributed 8±3% / 12±4% (mean of three replicates of each of five FLA-PS incubations for April / September series) to the microbial community, Bacteroidota 6±2% / 19±5%, Planctomycetota 7±2% / 2±1%, Verrucomicrobiota 1±0.4% / 2±1%, Actinomarinales 4 ± 1% / 2±1%, and Microtrichales 4±2 / 1.4±0.7% (Supplementary Figures S1, S2).

In the course of the incubations, Bacteroidota showed the strongest increase in cell numbers. Furthermore, their increase in growth was higher in April than in September incubations. In the April xylan incubation, replicate A, Bacteroidota cells increased by 31fold from 2.4 x 10^7 cells ml^{-1} to 7.5 x 10^8 cells ml^{-1} and in mucin incubation, replicate A, by a factor of 8 from 1.9 x 10^7 cells ml^{-1} to 1.6 x 10^8 cells ml^{-1}. On average, Bacteroidota cell numbers increased in April by a factor of 3.0±0.8 (laminarin), 4.2±0.8 (fucoidan), 4.6±3.2 (mucin), 3.4±1.4 (chondroitin sulfate), and 13.2±15.7 (xylan). In September, their increase was more moderate with 2.1 to 3.0-fold (increase in NA control by 1.5-fold). Planctomycetota cell numbers increased by a factor of 2.4 to 5.4 with no clear preference for a specific substrate. Maximum growth of Planctomycetota cells was detected in April xylan incubation, replicate A, where 2.1 x 10^8 cells ml^{-1} were detected after 17.5 days (11.2-fold increase vs. t0). Verrucomicrobiota showed strongest growth in the chondroitin sulfate incubations with a cell increase by a factor of 3.6±0.8 (April) and 4.2±1.3 (September, 2.-fold

increase in NA control). Furthermore, they showed strong growth in the April fucoidan incubations (4.3-fold increase in cell numbers) and in the September laminarin incubations (4.1±1.3-fold increase). Actinomarinales grew most in the April laminarin (4.3±4.1 fold), mucin (4.6±3.6) and fucoidan (3.2±1.1 fold) incubations while in the September incubations, Actinomarinales cell numbers remained constant for all substrates except fucoidan (2.6±2.3-fold increase vs. t0). Also, Microtrichales cell numbers increased remarkably in all incubations independent of the substrate with 1.7 to 2.5-fold in April and 2.4 to 3.9-fold in September (in NA control increase by 1.3-fold vs. t0). For Gammaproteobacteria, we detected the lowest increase in cell numbers with 1.1-fold for fucoidan, 1.4-fold for laminarin and chondroitin sulfate, 1.6 for xylan, and 2.8 for mucin in April and only 1.5 to 2.1-fold in September incubations, where increase of Gammaproteobacteria was even greater in unamended NA control (3.4-fold increase vs. t0).

QuantificationofFLA-PS-stained cells.

The abundance of selfish bacteria, determined via cellular uptake of FLA-PS, was quantified by epifluorescence microscopy following Reintjes*et al*. [11, 19]. Selfish uptake was detected for all tested substrates (Supplementary Table S2). However, only a small fraction of the benthic bacterial community used this mode of polysaccharide degradation (Figure 3). The fraction of selfish bacteria decreased with time and was greatest at t1 and t2. In April, the highest abundance of FLA-PS stained cells were found in the laminarin incubations with a percentage of 2.1±0.2% of total cells by day 1.5. Highest numbers of FLA-mucin stained cells were detected after 3.5 d with 1.4±0.1% of total cells. Other incubations showed an even smaller fraction of FLA-PS stained cells (chondroitin sulfate: 1.1±0.6%; fucoidan: 0.8±0.3%; xylan: 0.8±0.1%). In the September incubations, the overall fraction of selfish bacteria was

even lower than in April. Highest abundance of FLA-PS stained cells were found in mucin incubations after 0.3 d with 1.6±0.4% of total cells. The laminarin incubations showed only a small fraction of selfish bacteria with 0.1-0.5% of total cells. Similarly, low fractions of selfish bacteria were found for uptake of xylan (0.2±0.0% of total cells), chondroitin sulfate (0.3±0.1), and fucoidan (0.6±0.1). Killed controls showed no selfish uptake in none of the substrates.

Three dominant morphotypes of FLA-PS stained cells were detected in the April incubations (Supplementary Figure S3): i) an oval, coccoid type, which dominated in all incubations (Figure S3A), and ii) and rods with an apolar or iii) bipolar FLA-PS signal (Figure S3BC). In the September incubations, a fourth, oval morphotype was identified that had curved 'parentheses' of substrate staining on either side of the DAPI-stained DNA (Figure S3D). In general, FLA-laminarin and FLA-mucin signals were brighter than FLA-chondroitin and FLA-fucoidan signals in both April and September.

Identification of FLA-PS stained cells.

The identity of the bacteria taking up specific FLA-PS was determined with FISH using 4xAtto594 labelled probes. We focused on timepoints t1 and t2 because the fraction of selfish bacteria was too small at the other timepoints to allow counting of a statistically sufficiently high number of FLA-PS stained cells. Overall, we could identify the major fraction of FLA-PS stained cells at these timepoints. Planctomycetota made up the largest fraction, with 76±16% of total selfish bacteria taking up laminarin, 45±2% taking up mucin, 71±8% taking up xylan, 49±12% taking up chondroitin sulfate, and 63±7% taking up fucoidanin April incubations and 84±5% taking up chondroitin sulfate and 69±11% taking up fucoidan in September incubations. Their contribution to laminarin and mucin uptake in the September incubations was lower, at 37±6% and 28±8%, respectively. Selfish Planctomycetota were typically coccoid or oval. The substrate signals appeared either condensed close to one pole or distributed in the periplasm (Figure 5A-F). For some cells, the distribution of substrate signals indicated massive invaginations of the cytoplasmic membrane that are well known for Planctomycetota[50].

The second most abundant fraction of benthic selfish bacteria were members of the phylum Verrucomicrobiota. They were usually rod-shaped with a polar or bipolar localized substrate signal (Figure 5G-I). Rarely, oval to coccoid morphotypes were detected (Figure 5J). Verrucomicrobiota made up 69±11% and 60±7% of selfish bacteria in mucin and laminarin incubations, respectively, from September. Most identified selfish Verrucomicrobiota cells showed a characteristic substrate signal in which the FLA-PS were in a ‘parentheses’ pattern around the DAPI stain (Figure 5K). Bacteroidota made up only a small fraction of FLA-PS stained cells (<1%) in April incubations while they showed no selfish uptake in September (Figure 3). Selfish Gammaproteobacteria and Actinobacteria

were not detected neither in April nor in September. The fraction of unidentified FLA-PS-stained cells was 5 to 30% of the total, thus there are additional clades with a selfish polysaccharide uptake mechanism (Figure 3).

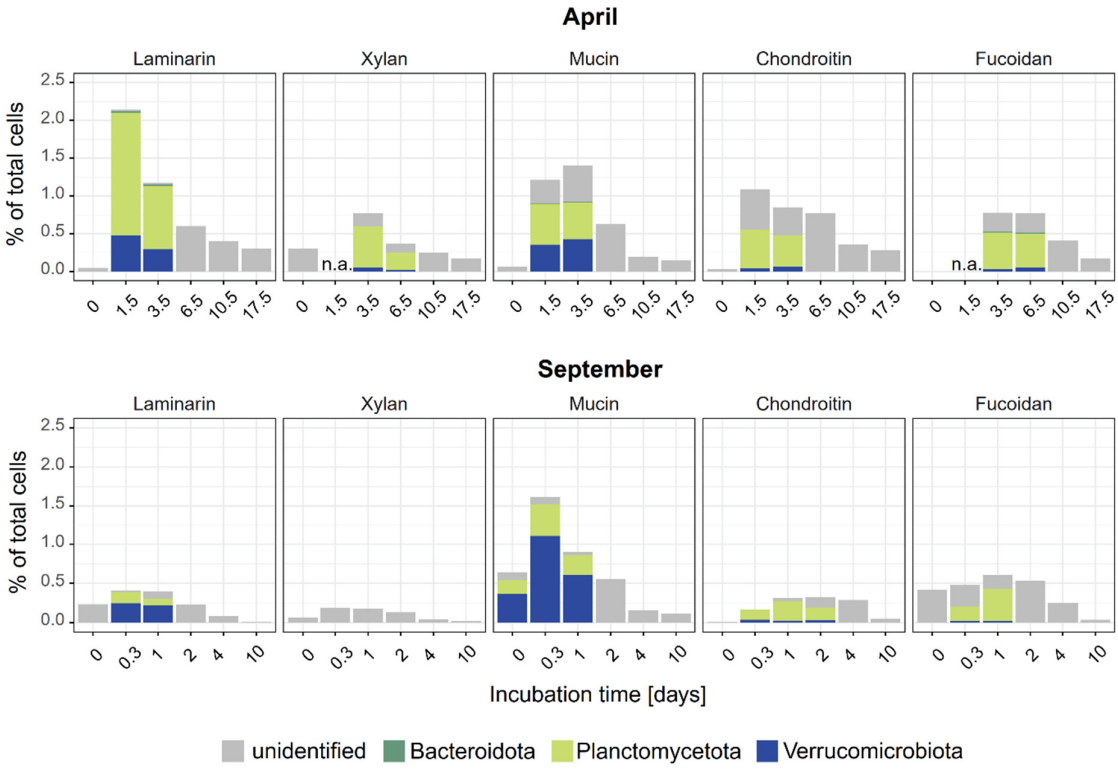

Figure 3: Relative abundance of FLA-PS-stained cells (selfish uptake) for all April (upper panel) and September (lower panel) incubations and timepoints. For t1 and t2, FISH was applied to target these selfish cells. A large fraction was identified as members of Planctomycetota (light green; PLA46) and Verrucomicrobiota (blue, EUB338-III). Bacteroidetes contributed a very small fraction (dark green; CF319a) in laminarin and mucin incubations from April only; the remaining FLA-PS-stained cells are indicated by grey colour. FLA-xylan stained cells could not be identified in September incubations due to their low cell numbers. For simplicity, bars show the mean of three replicates (individual values are given in Supplementary Table S2).

Influence of substrate concentration on fraction of benthic selfish bacteria

To investigate any potential influence of different FLA-PS concentrations on the polysaccharide utilization mode that benthic microbes are using, we set up an additional incubation series in June 2022. Sediments were amended with FLA-mucin at three different concentrations (10 µM, 100 µM, and 1000 µM monomer equivalent), and incubated surface sediments for up to 3 days. Independent of substrate concentrations, no increase in total cell numbers was observed after one day. By day 3, however, cell numbers were highest in the 1000 µM-incubations in which they doubled to $6.7 \pm 0.9 \times 10^8$ cells ml^{-1} sediment, while cell numbers in the 100 µM-incubations were lower with $4.9 \pm 0.4 \times 10^8$ cells ml^{-1} (Figure 4A). The relative abundance of selfish bacteria was similarly low after 1 d of incubation and accounted for 2.5±0.1% of total cells in the 100 µM incubation and for 2.0±0.5% of total cells in the 1000 µM incubation (Figure 4B). After 3 days, relative abundance of selfish bacteria decreased strongly to only 0.4 ±0.2% in the 100 µM incubation while it was still higher with 1.3±0.6% in the 1000 µM incubations. For incubations with 10 µM substrate, the fraction of selfish bacteria was <0.5% of total cells at both time points. Killed controls showed no selfish uptake.

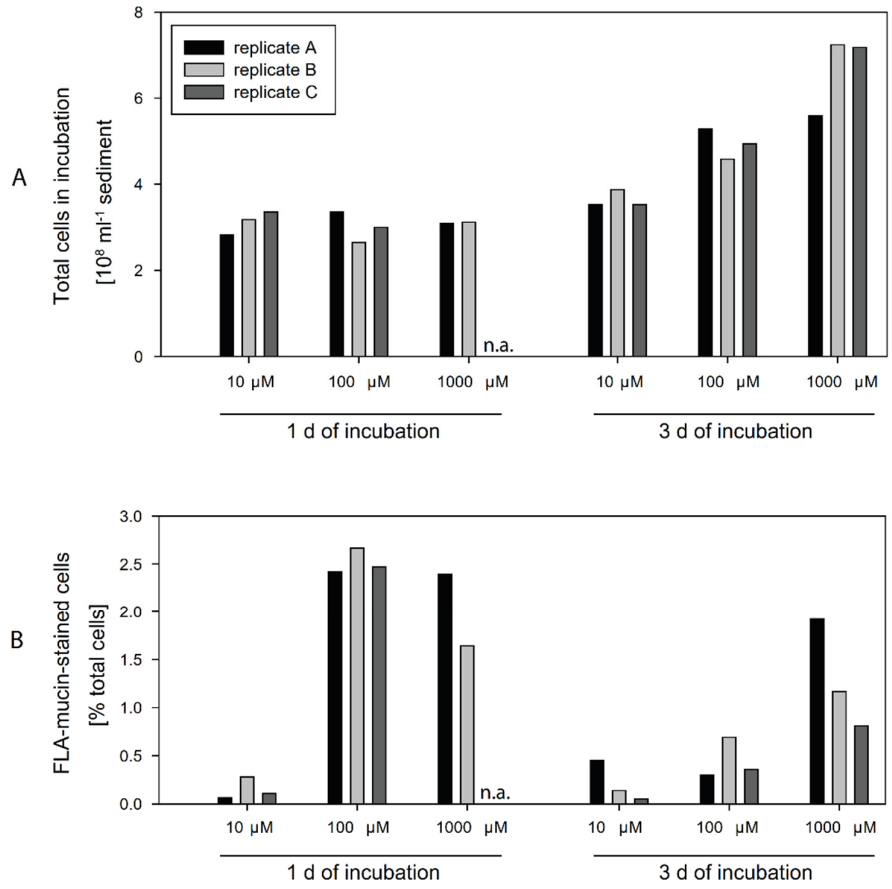

Figure 4. Total cells (A) and relative abundance of substrate-stained cells (B) in sediment incubations with different concentrations of FLA-mucin (10 µM, 100 µM, 1000 µM). Incubation time is indicated. No cell growth was observed between start of the experiment (t0) and 1 day of incubation (data not shown). n.a., not analyzed.

Diversity, identification, and quantification of members of Verrucomicrobiota

In incubations with FLA-mucin, a major fraction of selfish bacteria was identified as Verrucomicrobiota. To further identify members of this group, we analyzed the diversity of verrucomicrobial sequences retrieved from the same sampling site reported by Miksch *et al.* [27]. Family Rubritaleaceae had the highest read abundance with 45% of Verrucomicrobiota; Supplementary figure S4). A more detailed taxonomic breakdown of the family

Rubritaleaceae from the September mucin incubation (t5) is shown in Supplementary figure S5.

A new oligonucleotide probe RUB390 targeting Rubritaleaceae (group coverage 90%) was designed and tested (Supplementary table S1). Rubritaleaceae cells comprised at least 60% of the total Verrucomicrobiota cells in t1 (1.5 days) and up to 85% at t2 (3.5 days) of the April mucin incubation (Supplementary Figure S6). In the September mucin incubation, the family accounted for at least 40% of Verrucomicrobiota cells in the t1 (0.3 days) sample. In the April t1 and t2 samples, approximately 30% of the Verrucomicrobiota cells showed selfish uptake of mucin (Figure 4L-O, Supplementary Figure S6). Rubritaleaceae made up almost one third of these selfish cells after 1.5 days. After 3.5 days, the contribution of Rubritaleaceae cells to the total Verrucomicrobiota cells showing selfish uptake increased to more than half. After 0.3 days in the September mucin incubation, up to 40% of the detected Verrucomicrobiota cells showed selfish uptake, with around two thirds to one half of these cells belonging to Rubritaleaceae.

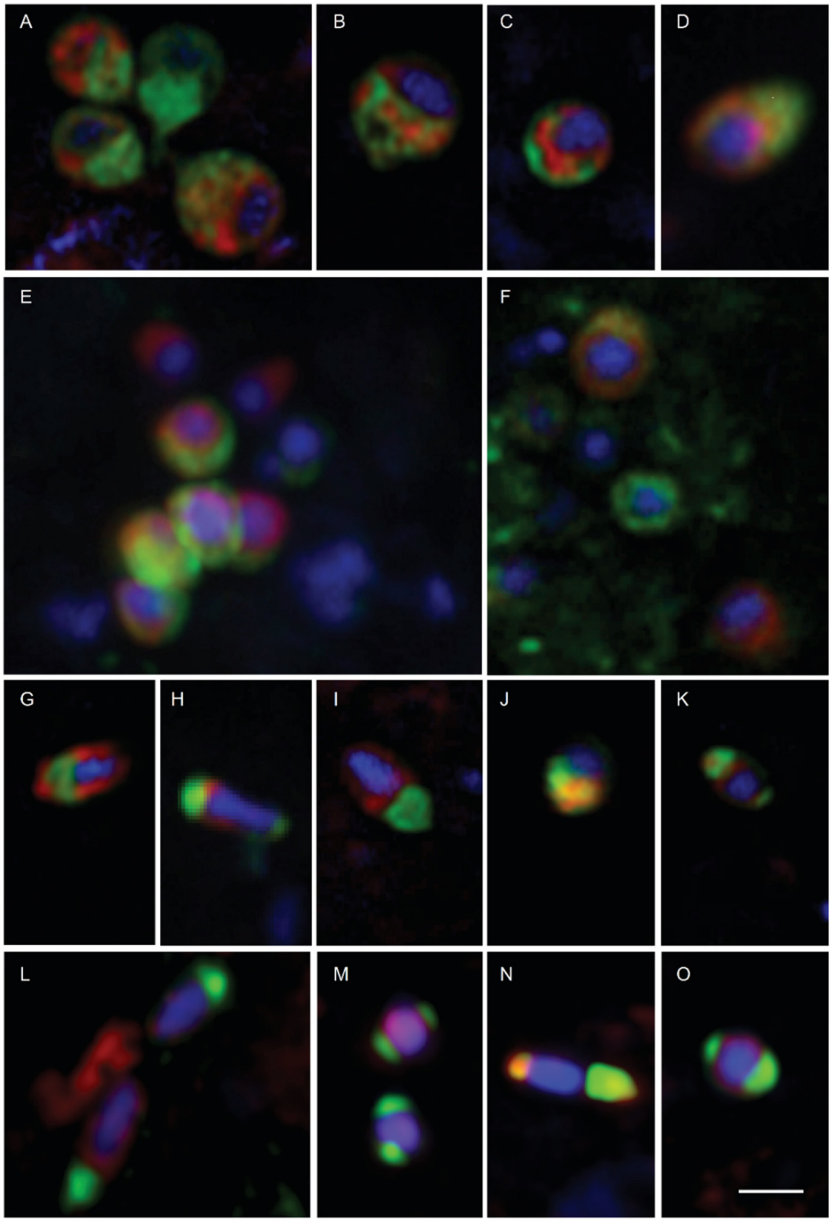

Figure 5: Laser scanning micrographs of cells stained by DAPI (blue) and FLA-PS (green) from Svalbard sediments and (A-F) Planctomycetota-specific FISH probe (PLA46, red). Substrate-uptake resulted similar phenotypes independent of the substrate amended (A-C, mucin; D, chondroitin sulfate; E, laminarin; F, fucoidan) and month of sampling (April vs. September). (G-K) Verrucomicrobiota-specific FISH probe (EUB338-III, red). In general, identified FLA-PS-stained cells were oval-shaped with different degrees of elongation. They usually localized the substrate signal either (G, I) at one side (apolar) or (H, J) on both sides of the cell (bipolar). (J) In April, a few FLA-PS-Verrucomicrobiota cells were coccoid. (K). In September, the dominant FLA-PS-Verrucomicrobiota phenotype showed a bipolar fluorescence signal. (L-O) A considerable portion between 10 and 75% of FLA-PS-Verrucomicrobiota could be identified as Rubritaleaceae using a specific FISH probe (RUB390, red). Scale bar (applicable to all panels), 1 μm.

Discussion

Polysaccharide degraders are highly active in Isfjorden sediments

A broad range of polysaccharides were rapidly hydrolyzed in Isfjorden surface sediments, consistent with previous results from other locations in Svalbard [24-26]. Moreover, hydrolysis rates of mucin, a glycosylated protein, were also high, consistent with high peptidase activities in Svalbard sediments from other locations [51]. Note that all of the comparison studies were measured in sediments, not in washed sand (April series) or washed silty sand (September series). By washing, we exchanged the natural pore water (including nutrients and free exoenzymes) and the pore water community by ASW, thereby bringing the sediment into a new "sterile environment". We might have also removed some grazers and bacteria that were only loosely-attached to the sand grains. Nevertheless, the rates measured in washed sediments from April and September 2019 show similarity to rates measured in undisturbed sediments.

Hydrolysis rates for laminarin, xylan, and mucin in September were 2-4 fold higher than in April incubations (Figure 1). These results may be due to a higher substrate availability *in situ* and therefore presence of "primed" cells, which were already induced for the degradation of FLA-PS. This hypothesis is supported by measured concentrations of chlorophyll *a* and phaeopigment, a degradation product of algal chlorophyll, that were 4-5-fold higher in September than in April sediments (chl.*a*: 1.8 vs. 0.4 μg ml^{-1}; phaeopigment: 2.8 vs 0.4. μg ml^{-1}) [27], both indicating the presence of algal polysaccharides. Furthermore, cells were more active in September as shown by a higher average cellular ribosomal RNA content (higher detection rate of FISH-stained bacteria in September, data not shown). Secondly, the 4-fold higher total cell numbers in September silty sediments might have had also an influence on the rates [27].

Rapid hydrolysis of substrates such as laminarin is likely related to high natural abundance during the polar day, as well as its comparatively simple, mostly linear structure. Laminarin is one of the most abundant carbon sources for marine microbes [35]. Its degradation requires only few enzymes to hydrolyze the polysaccharide into glucose monomers through the removal of the β-1,6-glucose side chains and hydrolysis of its β-1,3-glucose backbone [52].

Substratesof greater complexity, suchas the sulfated polysaccharides chondroitin sulfate andfucoidan, were less rapidly degraded, and hydrolysis rates did not differ between April and September. Similarlow hydrolysis rates were measured in Smeerenburgfjord sediments [24, 25]. Their slower degradation or recalcitrance may be due to its complex, branched, and highly sulfate structure which also varies among algal species and seasons [53][37]. Fucoidan hydrolysis requires the expression of a large set of enzymes. Genome analysis of the Verrucomicrobiota strain *Lentimonas* sp. CC4 revealed the presence of almost 300 putative fucoidanases of which about 100 are needed per fucoidan from different species of brown algae [54]. The metabolic cost of maintaining large pools of these enzymes active is likely too high, thus enzyme pools need to be induced, explaining why hydrolysis rates were maximal only after 10 days of incubation. Previous experiments in sediments from Svalbard (Smeerenburgfjord) and the Skagerrak in fact indicate that chondroitin sulfate hydrolysis is induced [55].

Killed controls treated for 1h with 3.5% formaldehyde at 20°C and amended with xylan and laminarin showed high extracellular hydrolysis rates. Hydrolysis, however,wasnot detected inkilled controlsofmore complexsubstrates such as chondroitin sulfate, fucoidan and mucin. Three reasons for substrate hydrolysis in the killed controls of laminarin and xylan are possible. (1) Abiotic degradation: substrate degradation in killed controls, however,proceeded almost asfast asin the incubations, and thus abiotic degradation unlikely explainsthisrapid

hydrolysis. (2) Viable cells are still present after formaldehyde fixation: cell growth was detected in killed controls from September, except for laminarin. The precipitated formaldehyde solution used (and therefore arbitrary concentration) was likely insufficient to effectively kill the cells. Furthermore, the excess organics in silty September sediments might be have bound a major part of formaldehyde. Nevertheless, no selfish uptake was observed in killed controls. (3) Active extracellular enzymes are present after fixation: as no cell growth was observed in killed controls from April incubations and no selfish uptake was observed, a large fraction of the hydrolysis rates in the killed controls can likely be explained by the presence of extracellular enzymes that were insufficiently inactivated. Fixation experiments (40% formaldehyde for 2h at 4°C) with different enzymes extracted fromrat liver have shown a residual activity compared to unfixed enzymes of 52% for acid phosphatase, 29% for catalase 29%, and 50% for β-glucuronidase[56] support this explanation.

Mucin is a relevant carbon source for benthic bacteria

Mucin was rapidly hydrolyzed in September incubations, with hydrolysis detected already 6h after substrate addition when incubated at *in situ* temperatures of 4°C. Selfish uptake of mucin by 0.6% of total cells was also rapid, detected already at t0 (20 min after substrate addition). In contrast, in April, hydrolysis rates were 4-fold lower than in September, and peaked after 3.5 days of incubation. No selfish uptake was observed in April. Previous studies have shown that mucin can have priming effects, causing increased microbial activities and a strong short-term increase in the turnover of sedimentary organic matter [57, 58]. Accelerated remineralization rates of nitrogen in the presence of modest mucus contribution suggested that release of mucus into the sediment can stimulate the release of exoenzymes by benthic microbes, thus priming or enhancing organic matter remineralization [58]. An influence of mucin degradation on the carbon quality and

remineralization was also proposed by Smith and colleagues [32] who detected high exohydrolase activities of bacteria attached to the surface of diatoms. They suggested that the hydrolysis of diatom mucin-like surface proteins [59] could be responsible for a major flux into the DOC pool making it a significant mechanism of DOM production [32].

At the seafloor, mucus tracks are usually heavily colonized by microbes and provide them with optimal growth conditions [31, 60]. However, microbes trapped in the mucus serve as food for the nematodes that Riemann & Schrage (1978) referred to as the mucus-trap hypothesis.

The fast hydrolysis and selfish uptake of mucin is indicating the importance of animal-derived polysaccharides for microbial communities in Arctic sediments. Since benthic faunal populations are seasonally relatively stable, even in an environment such as Svalbard [61], we propose that the animal-derived mucin could be a constantly available, key substrate for bacteria in Arctic sandy sediments.

External hydrolyzers and scavenging bacteria dominate over selfish bacteria

All of the polysaccharides tested were externally hydrolyzed by bacterial communities that adhere to the surface sediments from Isfjorden; all of the substrates were also selfishly taken up. However, the selfish uptake mode was used by only a minor fraction (~1-2%; Figure 3) of the total community. This result contrasts strikingly with the prevalence of selfish uptake in the water column, where in some cases, the selfish uptake signal reaches ~60% of total cells [8]. Most likely, this contrast in substrate processing mechanisms is related to the gain in substrates relative to the investment in extracellular enzymes. In sediments, due to reduced diffusive loss, external hydrolysis of polysaccharides could be energetically favorable [18, 20, 62, 63], and enzyme production could be coordinated through mechanisms such as quorum sensing [64, 65].

Substrate concentration had no influence on the fraction of bacteria that use selfish uptake mode for mucin degradation (Figure 4). Extracellular hydrolysis rates were not yet measured at time of submitting this thesis. There was no difference in selfish uptake in incubations with 100 µM and 1000 µM mucin concentration after 1d of incubation. The much smaller fraction of selfish bacteria (0.25% of total cells) in incubations with 10 µM mucin versus incubations with 100µM or 1000µM mucin (2-2.5%) is likely explained by substrate limitation. This is supported by a slower growth of the microbial community in 10 µM incubations compared to incubations with higher mucin concentrations. The substrate was likely even limited in 100 µM incubations after 3 days when the fraction of selfish bacteria decreased while it remained as large as before in 1000 µM incubations. These observations are in contrast to findings for pelagic bacteria during a diatom-dominated bloom in the North Sea in the German Bight [11]. The fraction of pelagic selfish bacteria decreased during the course of the bloom and the late bloom phase was characterized by high extracellular hydrolysis rates and a reduced selfish uptake of polysaccharides. This change was related to both structural complexity of the substrates as well as changes in the heterotrophic microbial community composition. Structural composition of polysaccharides might have a minor influence on the substrate utilization mode of benthic microbes, as we detected a slightly smaller fraction of selfish bacteria in late summer (max. 0.6% of total cells except for mucin) compared to spring (0.7-2%) and extracellular hydrolysis rates were twice as high in summer. In metatranscriptomic and metagenomic datasets from the same sediments [66], TonB-dependent transporters indicative of selfish uptake mechanisms [10, 13] were either mostly absent, not expressed, or not annotated due to the low N50 of the contigs.

The predominance of external hydrolysis strongly supports the hypothesis that benthic bacteria rather cooperate than compete in hydrolyzing polysaccharides. Pelagic bacteria, in

contrast, invest in carrying and expressing genes for selfish uptake to counteract loss of substrates due to diffusion. Through cooperative behavior [67] and the production of public goods, benthic bacteria might be able to make use of a greater range of energy sources and benefit from a more stable and more diverse system.

Stable benthic community composition independent of substrate addition

The addition of FLA-PS to sediments did not result in major changes in the relative abundance of bacterial taxa on the level of genera and families, as determined by 16S rRNA sequencing (Figure 2). This overall stability is in contrast to incubations with seawater which showed major relative changes for abundance of genera of e.g. Gammaproteobacteria (*Colwellia*, *Glaciecola*, *Marinomonas*) and Bacteroidetes[e.g. Flavicella, 11]. Major relative changes were only rarely detected and if so, only in one of the three replicates. Strongest response was observed for two genera of Bacteroidetes, i.e. *Lutibacter* (Flavobacteriales) and *Marinifilum*(Bacteroidia), which increased in April xylan incubation replicate A from ~2% to ~15% and 12% of total reads. Our results are in line with the reported stability of benthic community compositions throughout the year for North Sea and Svalbard sediments [27, 68]. The relative stable community composition throughout the incubation even after addition of 100 µM mucin monomer equivalent (= 1.2 M available for microbes from 3 ml sediments) supports the idea discussed above that bacterial communities as a whole have a sufficient toolbox to deal with the substrates they find, and wholesale changes in composition are not necessary in order to process organic matter.

Fast responding, benthic polysaccharide-hydrolyzing bacteria

Polysaccharide-degrading bacteria have been found in many marine phyla including Bacteroidota, Planctomycetota, Verrucomicrobiota and Proteobacteria[11, 50, 54, 69-72]. While the relative community composition remained stable, we detected growth of the microbial community in course of the experiment. In contrast, *in situ* experiments at the deep-sea floor at Fram Strait did not cause an increase of total microbial cell numbers after phytodetritus supply of either the diatom *Thalassiosira* sp. or the coccolithophore*Emilianiahuxleyi* but an increase of exoenzymatic activities [73].

Cell numbers of Bacteroidota increased strongly compared to the unamended controls (Figures S1 and S2). This is in line with metatranscriptomic data from the same sampling site, which showed strong upregulation in relative transcript frequencies of 30- to 1000-fold high in seven Bacteroidota bins from sediments after the spring bloom [66]. However, the overall abundance of these strains is rare. The bacteroidotal order Flavobacteriales has been repeatedly described as polysaccharide-degrading bacteria [11, 13, 71, 74, 75] and are commonly found associated with micro- and macroalgae[76]. Selfish Bacteroidota constitute only a minor fraction of the microbial community with <0.1% of total cells.

The phylum Verrucomicrobiota is consistently found in microbial community surveys in different environments, usually, but not always [26], they constitute less than 5% of the total community[27, 77, 78]. Recently, several studies have demonstrated the role of Verrucomicrobiota in polysaccharide degradation, highlighting the highly specialized metabolic repertoire of its members in degrading complex polysaccharides [54, 69, 70, 79]. Measuring the *in situ* abundance of Verrucomicrobiota in the laminarin, chondroitin sulfate, and fucoidan incubations and the control set up without added substrate showed that the cell counts for the phylum increased 50% to 200% or more in the presence of substrates,

compared to incubations without substrate, suggesting that they are highly responsive to substrate availability.

The probe designed as part of this study for Verrucomicrobiota family Rubritaleaceae showed that they contributed to the overall increase in Verrucomicrobiota cell counts. Described species of the family Rubritaleaceae are Gram-negative, non-motile, obligatory aerobic chemoheterotrophs[80]. Much like the other members of the phylum Verrucomicrobiota, this family has been detected in various environments, such as biofilms, lagoons, polar lakes, fish gut microbiota, and in coral reefs, among others [81-84]. Recently, high abundance of 16S rRNA gene sequences affiliated with Rubritaleaceae have been found associated with the seaweed *Ulva laetevirens*[81], as well as on healthy giant kelp, *Macrocystispyrifera*[85], which further point to their potential in degrading stable algal polysaccharides. Orellana-Retamal and colleagues [79] have shown that they are well adapted to the utilization of fucose-containing sulfated polysaccharides released by living diatoms.

From FLA-PS incubations [11, 12] or genomic analysis of their hydrolyzing enzymes [71, 74, 86-88], Gammaproteobacteria are well known polysaccharide-degrading bacteria which respond to phytoplankton spring blooms. Benthic Gammaproteobacteria in our incubations, however, showed neither selfish uptake nor an increase in cell numbers in the course of the experiments with any FLA-PS tested. It is important to note that about 30% of selfish bacteria remained unidentified, suggesting further groups involved in selfish uptake. Also, as Gammaproteobacterial cell numbers did not increase during the course of the experiments, they are unlikely among the organisms carrying out external hydrolysis.

Conclusion and outlook

Investigating polysaccharide utilization modes demonstrated high external hydrolysis rates and a small but consistently-detected fraction of selfish bacteria. Selfish mechanisms for

the utilization of substrates, e.g., laminarin and mucin could be attributed to Planctomycetota and Verrucomicrobiota, yet extracellular hydrolysis prevailed and the bulk of glycan utilization seems to be catalyzed by a synergistic community-based mechanism relying on the sharing of enzymatic capabilities and the scavenging of public goods. Sediments are a suitable environment for external hydrolysis due to diffusion limitations and the possibilities for communities to coordinate enzyme production via quorum sensing and other mechanisms. Benthic bacteria possess a lot of enzymes in their genomes that they can also release to the external environment. A major factor for the dominance of extracellular hydrolysis mode is that the organic carbon pool is large in continental shelf sediments [89] and therefore no adaptation to the environment with respect to the development of a highly complex mechanism for selfish uptake is needed.

Mucin, as a diverse and widely distributed substrate, needs further investigation. Its fast hydrolysis indicates the importance of bacterial utilization of animal mucus in sandy sediments, and makes it an interesting substrate for future in-depth studies. For example, omics-based approaches could identify key enzymes, which subsequently can be used to quantify mucus in the environment, similar to what has been previously done for laminarin[35].

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Supplement

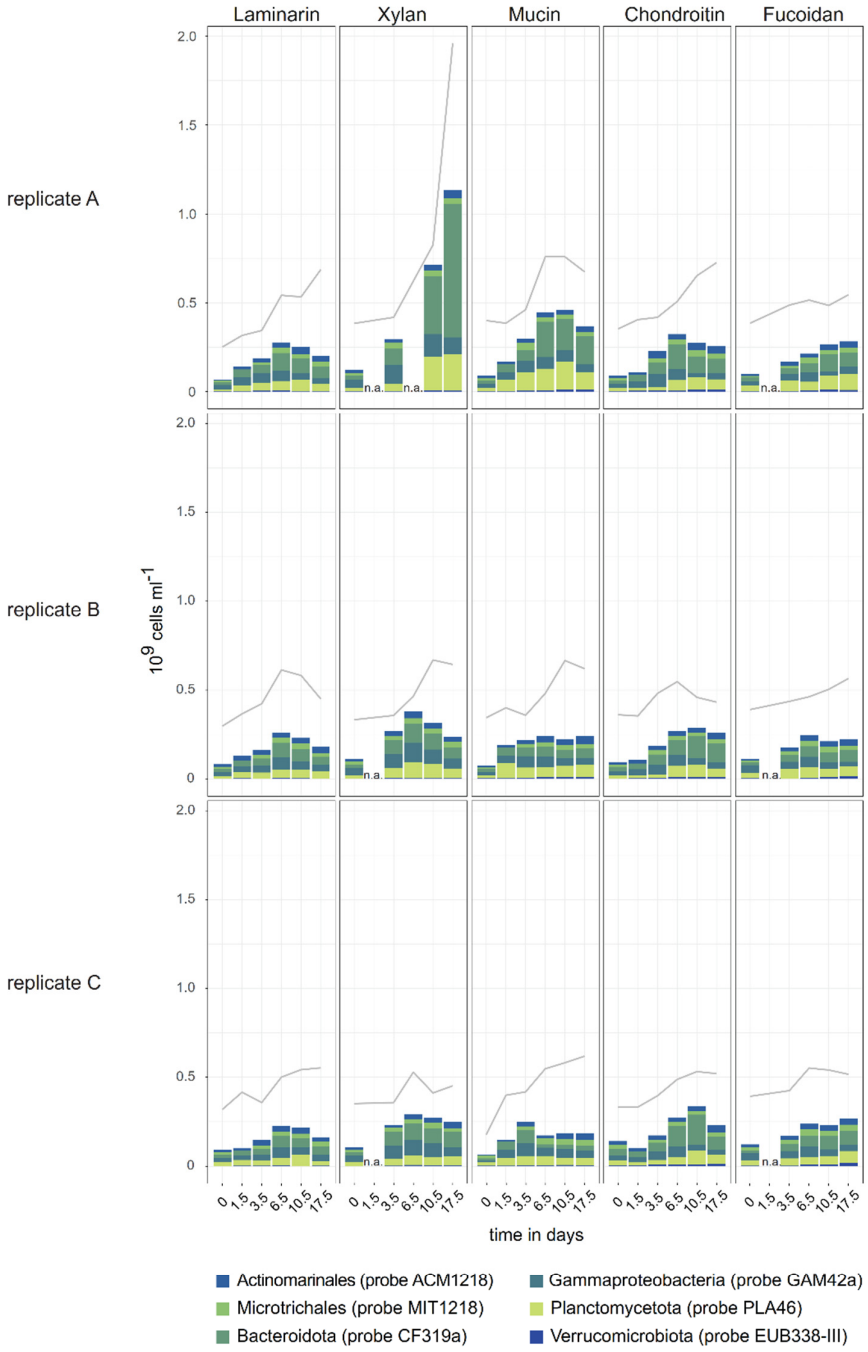


Figure S1. Abundance of total cells (line) and FISH-stained cells (bars) in April incubations. NA control, no addition control (no FLA-PS added). n.a., not analyzed

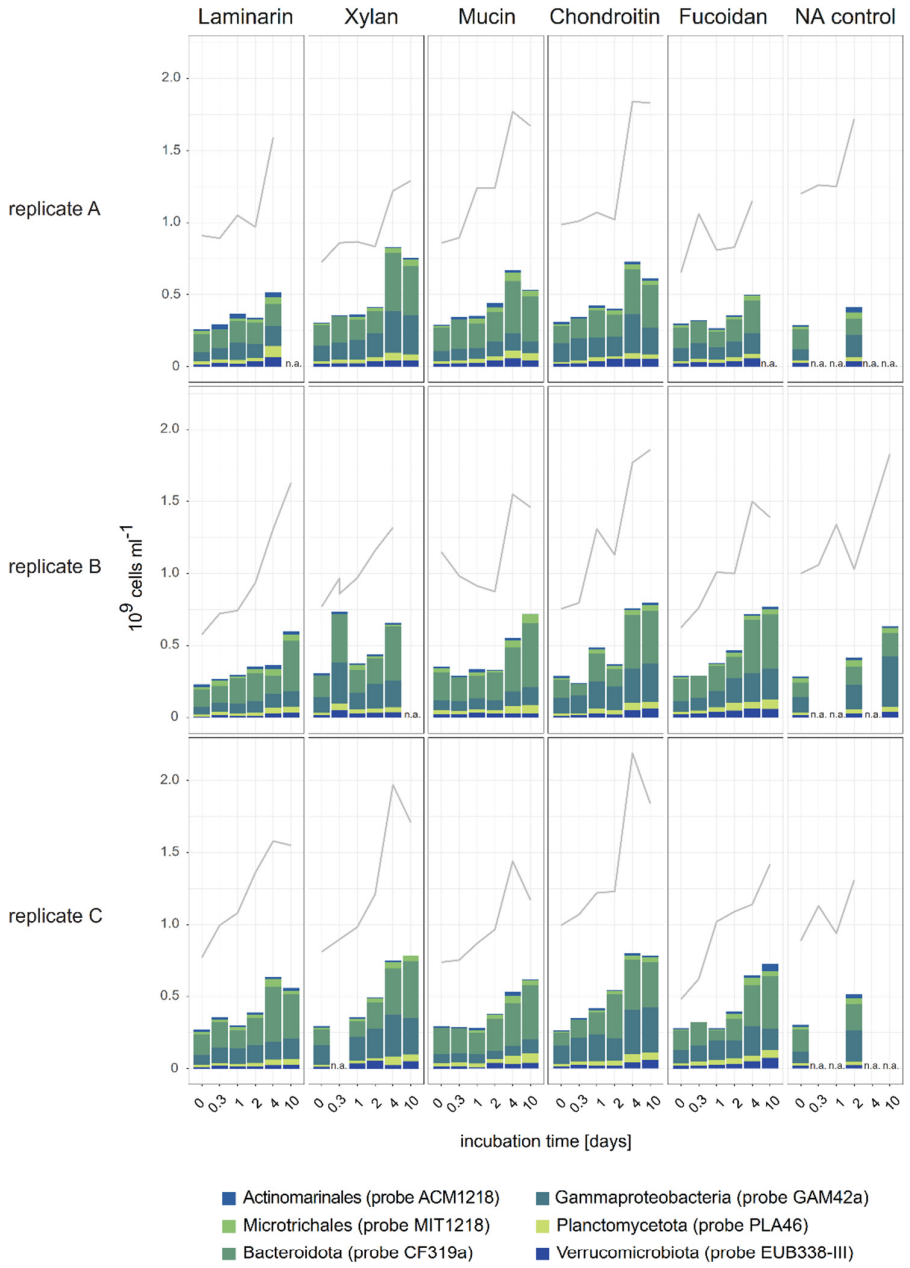
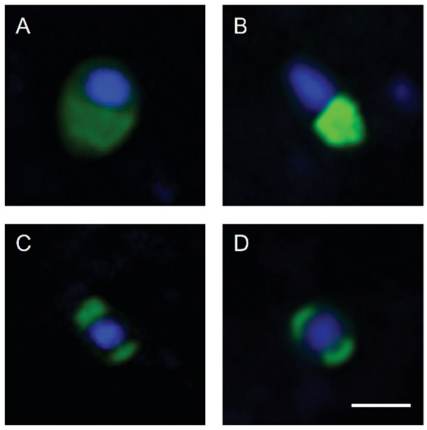
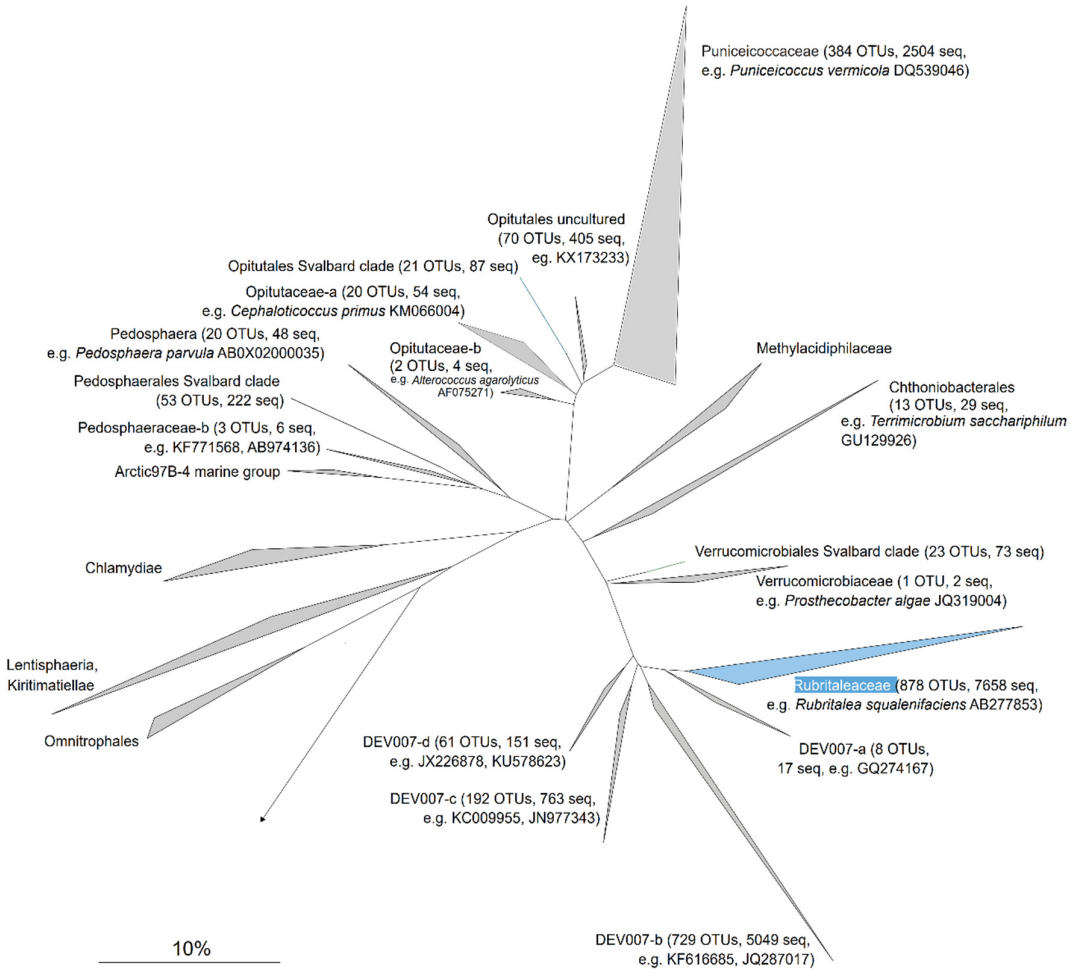


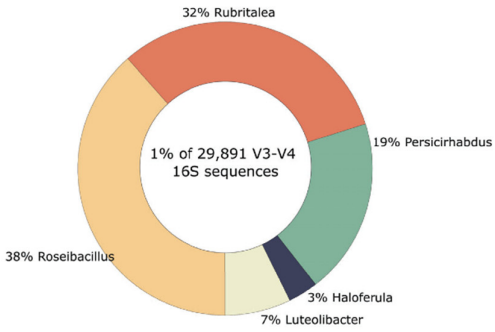
Figure S2. Abundance of total cells (line) and FISH-stained cells (bars) in September incubations. NA control, no addition control (no FLA-PS added). n.a., not nalyzed



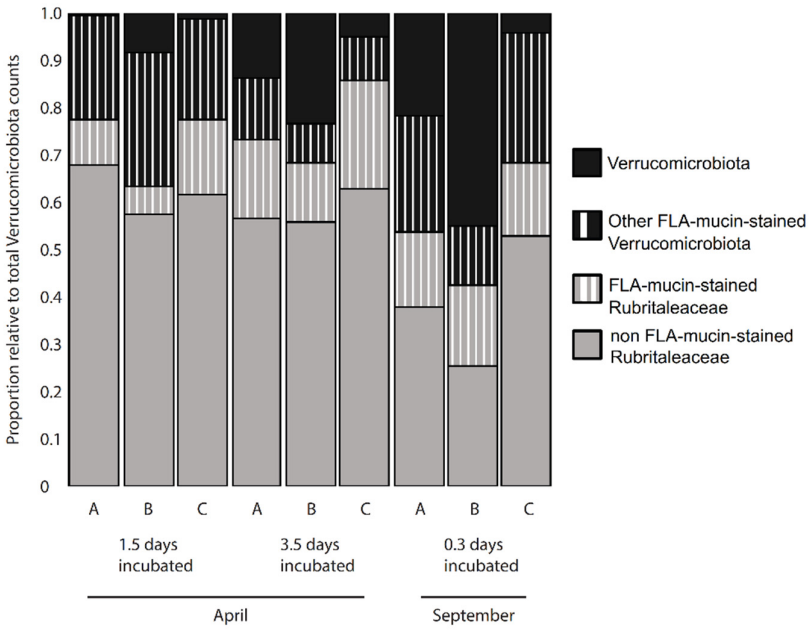
Supplementary Figure S3. Laser scanning micrographs of cells stained by DAPI (blue) and FLA-PS (green) from Svalbard sediments. (A) Dominant morphotype of FLA-PS stained cells in April and September incubations; estimated 80% of this type were subsequently identified as Planctomycetota; (B) and (C) rod-shaped morphotypes with an apolar or bipolar FLA-PS signal; >90% of this type were identified as Verrucomicrobiota and most often detected in incubations from April, rarely in September; (D) morphotype only occurring in incubations from September, subsequently identified as Verrucomicrobiota. Scale bar (applicable to all panels), 1 μm.



Supplementary Figure S4. Phylogenetic tree showing the affiliations of 16S rRNA sequences from Svalbard sediments (Miksch et al., 2021) to selected reference sequences of the phylum Verrucomicrobiota. Probe RUB390 was designed to target the family Rubritaleacaea (colored in blue). Indicated in parentheses are the number of operational taxonomic units (OTU0.98) retrieved for different clades, the number of Svalbard sequences belonging to the group, and the accession number of representative sequence/s of the clade. Scale bar gives 10% estimated sequence divergence.



Supplementary figure S5. Diversity of Rubritaleaceae in September mucin incubation after 10 days of incubation. The taxonomic breakdown of genera comprising the family Rubritaleaceae is shown for 16S rRNA gene sequences amplified from V3-V4 amplified 16S rRNA gene sequences.



Supplementary Figure S6. Proportion of Rubritaleaceae cells of Verrucomicrobiota in mucin incubations. Striped bars indicate the proportion of cells belonging to Rubritaleaceae that showed selfish uptake (grey stripes), and the proportion of other Verrucomicrobiota displaying selfish uptake (black stripes). Rubritaleacae cells were identified using FISH with the 4xAtto594-labelled probe RUB390.

Table S1. Oligonucleotide probes used for this study.

Probes	Target group	Sequence (5'-3')	FA conc.[$]	Target	Reference
EUB338-III*	Verrucomicrobiota	GCTGCCACCCGTAGGTGT	35	16S rRNA	[1]
GAM42a[©]	Gammaproteobacteria	GCCTTCCCACATCGTTT	35	23S rRNA	[2]
CF319a	Bacteroidota	TGGTCCGTGTCTCAGTAC	35	16S rRNA	[3]
PLA46	Planctomycetota except Physisphaerae	GACTTGCATGCCTAATCC	30	16S rRNA	[4]
MIT1218	Microtrichales	AGCATGTTTGCAGCCCTG	25-30	16S rRNA	[5]
c1_MIT1218	Competitor for MIT1218; diverse Physisphaerae	AGCACGTTTGCARCCCTG			
c2_MIT1218	Competitor for MIT1218; Planctomycetota	AGCACGTTTGCAGCCCMG			
3H1_ MIT1218	Helper oligonucleotide for MIT1218	CKTTGTACCRGCCATTGT			
5H2_ MIT1218	Helper oligonucleotide for MIT1218	GGCATAAGGGGCATGATG			
ACM1218	Actinomarinales	AGCATGCGTGCAGCCCTG	25-30	16S rRNA	[5]
c1_ACM1218	Competitor for ACM1218; class Actinobacteria	AGCATGCGTGAAGCCCTG			
c2_ACM1218	Competitor for MIT1218; Actinobacteria	AGCATGTGTGCAGCCCTG			
5H1_ACM-MIT1218	helper oligonucleotide for ACM1218 and MIT1218	GAYATAAGGGGCATGATG			
3H3_ACM1218	helper oligonucleotide for ACM1218	CGTTGTACHGGCCATTGT			
3H3_ACM1218	helper oligonucleotide for ACM1218	CGTTGTACTTGCCATTGT			
3H3_ACM1218	helper oligonucleotide for ACM1218	CGTTGTACCKGCCATTGT			
RUB390	Rubritaleaceae	TCCACGCGGCGTTGCACCA	20	16S rRNA	This study
c1_RUB390	Competitor for RUB390; Puniceicoccaceae and Opitutales	CCCACGCGGCGTTGCACCA			
c2_RUB390	Competitor for RUB390; DEV007, Chthoniobacterales, Opitutales	TCCACGCGGCGTCGCACCA			
5H1_RUB390		TCAGACTTKCGTCCATTG			
5H2_RUB390	Helper for RUB390; 5' end	TCAGAGTTKCCTCCATTG			
5H3_RUB390		TCAGGGTTKCCCCCATTG			
3H1_RUB390		ATCCTARGACCGTCATCC			
3H2_RUB390		ACCCTAGGGCCKTCATCC			
3H3_RUB390	Helper for RUB390; 3' end	ATCCGAAGACCGTCATCC			
3H4_RUB390		ACCCKAGGGCCTTCATCC			
3H5_RUB390		ATCCGAAGACCGTCATCC			

ACM1218 and MIT1218 were used as horseradish-labelled probes for CARD-FISH. For permeabilization, samples were treated with lysozyme (10 mg ml^{-1}, 0.05 M EDTA, 0.1 M Tris-HCl) at 37°C for 45 min and subsequently treated with achromopeptidase (60 U ml^{-1}, 10 mM NaCl, 10 mM Tris-HCl, pH 8.0) at 37°C for 30 min

$: formamide concentration in the hybridization buffer, hybridization at 46°C

*: Probe EUB338-III was used at a molar ratio of 1:1 with competitor EUB338-II (GCAGCCACCCGTAGGTGT)

©: Probe GAM42a was used at a molar ratio of 1:1 with the competitor Bet42a (GCCTTCCCACTTCGTTT)

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Table S2. Substrate-stained cells and total cell numbers in incubations of sediment samples with FLA-PS. Cell numbers are individually shown for the three replicates (A, B, C) as well as for a control that was treated with 3.7% formaldehyde for 1h to kill the cells ("killed control"). Substrate-stained cells were not detected in killed controls of any substrate and time points investigated. n.d., not detected; n.a., not analyzed

			Incubations with FLA-PS						killed control
			Substrate-stained cells [x10^6 ml^{-1} sediment]			Total DAPI cell counts [x10^8 ml^{-1} sediment]			Total DAPI cell counts [x10^8 ml^{-1} sediment]
		Incubation time [d]	A	B	C	A	B	C	
Laminarin, April	t0	0.01375	0.0	0.2	0.3	2.5	3.0	3.2	3.2
	t1	1.5	7.5	7.4	8.5	3.2	3.7	4.2	n.a.
	t2	3.5	3.1	4.9	5.2	3.4	4.2	3.6	4.2
	t3	4.5	1.9	4.4	3.7	5.4	6.1	5.0	n.a.
	t4	10.5	1.9	2.2	2.6	5.3	5.8	5.4	4.0
	t5	17.5	0.4	1.1	3.3	6.9	4.5	5.5	n.a.
Laminarin, September	t0	0.01375	1.9	2.1	1.0	9.1	5.8	7.7	9.9
	t1	0.25	3.1	2.7	5.0	8.9	7.2	10.0	8.7
	t2	1	3.8	5.2	1.3	10.5	7.4	10.8	12.1
	t3	2	2.1	3.4	1.1	9.7	9.4	13.6	9.2
	t4	4	1.1	1.5	0.8	15.9	13.1	15.8	11.5
	t5	10	n.a.	0.4	n.d.	n.a.	16.3	15.5	11.1
Xylan, April	t0	0.01375	1.3	1.1	0.9	3.8	3.3	3.5	3.2
	t1	1.5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	t2	3.5	3.6	2.8	2.3	4.2	3.6	3.6	n.a.
	t3	4.5	n.a.	1.4	2.3	n.a.	4.6	5.3	4.0
	t4	10.5	3.6	1.5	0.4	8.3	6.7	4.1	n.a.
	t5	17.5	4.5	0.8	0.7	19.6	6.4	4.5	4.3
Xylan, September	t0	0.01375	0.7	0.4	0.4	7.3	7.7	8.1	5.4
	t1	0.25	2.0	1.5	1.5	8.6	9.7	8.6	6.7
	t2	1	1.5	1.8	1.6	8.7	9.7	9.8	6.9
	t3	2	1.5	1.5	1.1	8.3	11.6	12.1	9.6
	t4	4	0.9	0.2	0.7	12.2	13.2	19.7	10.6
	t5	10	0.4	n.a.	0.4	12.9	n.a.	17.1	11.0
Mucin, April	t0	0.01375	0.3	0.4	n.d.	4.0	3.4	1.8	3.6
	t1	1.5	5.3	5.0	4.0	3.8	4.0	4.0	n.a.
	t2	3.5	6.3	4.7	6.4	4.6	3.6	4.2	3.5
	t3	4.5	3.1	5.1	2.2	7.6	4.8	5.5	n.a.
	t4	10.5	1.7	1.3	1.0	7.6	6.7	5.8	2.9
	t5	17.5	0.5	1.0	1.3	6.7	6.2	6.2	n.a.
Mucin, September	t0	0.01375	6.5	4.8	5.5	8.6	11.5	7.4	10.2
	t1	0.25	13.5	12.6	15.5	8.9	9.8	7.5	10.7
	t2	1	8.8	9.6	8.2	12.4	9.1	8.7	10.5
	t3	2	2.3	6.3	7.3	12.4	8.7	9.7	11.7
	t4	4	2.3	1.9	3.1	17.7	15.5	14.4	15.3
	t5	10	0.8	1.3	1.5	16.7	14.6	11.7	15.9
Chondroitin sulfate, April	t0	0.01375	0.3	n.d.	n.d.	3.5	3.6	3.3	3.3
	t1	1.5	1.8	5.2	4.5	4.0	3.5	3.3	n.a.
	t2	3.5	3.1	3.7	4.0	4.2	4.8	4.0	4.3
	t3	4.5	4.2	5.1	2.7	5.1	5.5	4.9	n.a.
	t4	10.5	1.2	2.6	1.7	6.5	4.6	5.3	3.4
	t5	17.5	0.8	2.2	1.2	7.3	4.3	5.2	n.a.

Chondroitin sulfate, September	t0	0.01375	0.4	n.d.	n.d.	9.8	7.6	9.9	8.1
	t1	0.25	2.0	1.5	1.1	10.1	8.0	10.7	8.7
	t2	1	2.7	5.8	2.7	10.7	13.1	12.2	9.3
	t3	2	4.5	3.5	2.7	10.2	11.3	12.3	8.8
	t4	4	9.9	3.6	2.5	18.4	17.7	21.9	12.3
	t5	10	1.8	0.4	0.2	18.3	18.6	18.4	10.0
Fucoidan, April	t0	0.01375	n.d.	n.d.	n.d.	3.9	3.9	3.9	3.9
	t1	1.5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	t2	3.5	2.3	4.2	3.9	4.9	4.4	4.2	n.a.
	t3	4.5	3.7	3.7	4.4	5.2	4.6	5.5	4.0
	t4	10.5	1.6	3.4	1.2	4.9	5.0	5.4	n.a.
	t5	17.5	1.3	0.4	1.0	5.4	5.6	5.2	4.4
Fucoidan, September	t0	0.01375	1.7	2.7	2.7	6.5	6.2	4.8	5.7
	t1	0.25	4.6	3.3	3.6	10.6	7.6	6.2	6.9
	t2	1	4.4	6.5	6.3	8.1	10.1	10.2	8.3
	t3	2	5.5	5.0	4.6	8.3	10.0	10.9	6.8
	t4	4	3.1	3.4	2.9	11.5	15.0	11.4	16.9
	t5	10	n.a.	0.4	0.6	n.a.	13.9	14.2	16.2
No substrate control, September	t0	0.01375				12.0	10.0	8.9	n.a.
	t1	0.25				12.6	10.6	11.3	n.a.
	t2	1				12.5	13.4	9.4	n.a.
	t3	2				17.2	10.3	13.1	n.a.
	t4	4				n.a.	n.a.	n.a.	n.a.
	t5	10				n.a.	18.3	n.a.	n.a.

Chapter 5 : General discussion

In the following chapter I will discuss the key findings of this thesis, updating our ecological concept for microbial communities in sandy surface sediments with a focus on community stability and seasonality as well as their relationship to POM and DOM. Furthermore, I will outline ideas for future research that could be done to deepen our knowledge about coastal sandy surface sediments .

Overall, sandy surface sediment communities are seasonally stable, with small species level fluctuations

High seasonal dynamics of pelagic heterotrophic bacteria during spring algal blooms – more precisely, substrate-driven successions of recurrent genera linked to the availability of polysaccharides - are an established concept of bacterioplankton ecology [16, 17]. Yet, microbial communities living in sandy surface sediments of temperate and polar regions were not showing the same pronounced seasonal changes in community composition on genus level in this study [95] (Chapter 2). Only on ASV level – representing discrete 16S rRNA gene variants - we could observe minor, non-significant fluctuations between seasons. These changes were neither recurrent nor did they have a clear pattern (See Chapter 2 SFig.8, SFig.9). Since ASVs are considered to be suitable for species discrimination [96], we can compare these results with our metagenomic dataset binned on 95% average nucleotide identity (ANI), equivalent to a species level resolution (Chapter 3, Fig.3). Here, we could observe seasonal dynamics by small shifts in metagenomics abundance and major shifts in transcriptional activities. These dynamic species were classified as Flavobacteriales and Gammaproteobacteria, which are also the most dynamic clades in marine coastal surface

waters. On the species level, however, pelagic and benthic communities are clearly different. Also, neither the dominance nor the recurrence of the benthic taxa were as pronounced as for bacterioplankton[17]. The changes on species level were overall more stochastic, resulting in a highly diverse benthic community.

Are benthic bacterial communities seasonally stable due to a limitation in habitable space?

In Chapter 2, I provided a possible explanation for the high relative stability of the community: Lack of habitable space. I just want to briefly reiterate the concept here and put it into context with more information from other chapters. Microscopy has shown that sand grains are not densely populated over their entire surface. Cells are located in holes and depressions, and the attached populations are associated with different phyla [47, 68]. It is hypothesized that the exposed areas of the sand grain surface are constantly grazed or physically scraped clean of microbes by the movement of sand grains, thus remaining uncolonized. First data on the frequency of dividing benthic microbes for Actinobacteria suggested that the cells were growing even during the polar night (Chapter 2 [95]). Actinobacteria were, however, not overgrowing other bacterial taxa and were not dominating the community. We, therefore, assume that cells either directly detach from the grain during division, or get removed shortly after, because they grow out of the protected space on the grain. This would leave a constant community of active cells which are released into the pore water and subsequently colonize free living space on other sand grains. Integrating our transcriptional data, which show strong seasonal changes in activity of some species, we further hypothesize that seasonal growth of certain groups happens, modifying the detached community capable of colonizing new grains. Habitable space on grain is quite limited, thus

changes in the detached cell pool are not causing significant changes in the attached community. Such dynamics would explain the core community observed by Probandt*et al*. (2017)[68] on single sand grains which was stable between individual grains and accounted for about one half of the community. Furthermore, Probandt and colleagues observed slight variations in community composition specific for single grains which could be the result of seasonally active groups. The fact that single sand grain showed a lower bacterial diversity than bulk sediments suggests that breaking down sediments into different fractions could help in reducing complexity, thus improving downstream analysis. I would hypothesize that in particular pore water and sediment surface, even though they are ultimately coupled, present different niches.

Are benthic bacterial communities stabilized by permanently available substrates?

The stable microbial community composition is hard to explain in a scenario in which resources are only supplied discontinuously during spring and summer algal blooms. I would like to argue that stable substrate sources must be present in the sandy sediments and suggest in the following two possible substrate classes which can be utilized by microorganisms all year long.

The first are α-glucans. A high availability and utilization of α-glucans throughout the year was already outlined in Chapter 3, in which a concept of repackagingof polysaccharides within the microbial community was discussed. Such recycling could partially feed the community all year. The charging of this recycling system would happen in spring and summer by the massive input of β-glucans, which is the major storage glycan of phytoplankton. The β-glucans resulting from fresh primary production are partially oxidized, but also transformed by bacteria into secondary storage compounds, often α-glucans, which

can be used by the microbes later during the year. Indications for such a system were found in the metatranscriptomic data, showing increased β-glucanase activity in spring, while α-glucanasesdegrading, e.g., glycogen as a bacterial storage compound [97], were more evenly expressed and increased in relative frequency during winter. However, since no dominating β-glucan and α-glucanes degrading microbes could be identified substrates are shared or transferred either before or after uptake by the β-glucan degraders.

We have shown a strong dominance of extracellular polysaccharide hydrolysis in Chapter 4. This would release mono- and oligosaccharides from polysaccharides into the pore water where it can be accessed by scavenging microbes. If we compare the monosaccharide spectrum predicted by the transcriptomic activities with the actually measured monosaccharides in the bulk sediment we found nearly the same profile (Chapter 3 Fig.5). These monosaccharides are not only available for the bacteria producing exoenzymes, but to all other microbes that have the respective transporters. Inferring from metagenomics sequences whether the expressed CAZymes are involved in extracellular hydrolysis or are part of the selfish uptake mechanisms is mostly reliant on the genetic context, more specifically the TonB-dependent transporters [20, 21]. In the data from this study such operon-like structures were either mostly absent or not visible due to the low N50 of the contigs. In addition, only ~1-2% of cells in the incubation experiments with FLAPS showed selfish uptake. Most of the polysaccharide degradation must therefore be extracellular. Consequently, a big fraction of the community should have access to low molecular weight (LMW) substrates supplied to the sediment. Likely, large amounts of glucose are released from freshly produced β-glucans in spring and summer resulting in the buildup of intracellular α-glucan storage molecules.

I hypothesize that after the initial uptake of algal polysaccharides in spring and summer there are trickle down effects as a result of constant recycling of cells. Such a recycling can be caused by the constant removal of bacteria from the sediment surface. Such a “scraping of” might also lyse bacteria. This lysis would result in a constant release of bacterial biomass including glycogen into the pore water thus making it accessible for other members of the community. Another possibility for a release or transfer of OM within the microbial food web is bacterial predation [98, 99]. Not much is known so far about predatory populations. Key genes, like genes for peptidoglycan metabolism or proteases [100] are not unique to a predatory lifestyle. Furthermore, predatory bacteria are often only facultative predators which have a slightly higher growth rate through such a lifestyle [98], making it difficult to point out predatory behavior without co-cultures of predator and prey. In this study, I identified genes which can be indications for bacterial predation, like GH23 (lysozyme type G peptidoglycan lyase) and genes of type VI secretion systems, which can be used to lyse other cells [101]. These genes were stronger expressed in winter as compared to spring (Chapter 3, Fig.4 and Suppl.Tab.5). Such genes could be used by facultative predators to access storage compounds of other members of the community, in particular in winter when most fresh algal biomass would be gone. An in-depth investigation focusing on further indications of bacterial predation [102] in metagenomic and metatranscriptomic data from sandy sediments, including the ones from this study, could prove interesting in the future.

The second substrate class I would like to suggest as a constant food source is animal-derived HMW-OM. Since benthic fauna populations are seasonally stable, even in an environment such as Svalbard [103], I assume that animal-derived OM could provide a constant substrate class for bacteria in sandy sediments.

Diverse representatives of the benthic fauna constantly releases OM into sediments, some, like mollusks, in form of mucus or other mucus-like glycoproteins with diverse functions like locomotion, food acquisition, or as building material for tunnels and tubes [104]. Mucus or mucus-like glycoproteins can be very diverse in their composition, in particular with respect to the glycosylic side chains [105]. This is also what enables such a diverse set of functions among all groups of animals. Even though many marine invertebrates use mucus most knowledge of mucus utilization by microbes comes from gastrointestinal or other human related studies [106, 107]. It has been shown that microbial communities in marine sediments increase in activity upon fertilization with gastropod or polychaete mucus, impacting carbon and nitrogen cycles in these sediments [108, 109]. Using labeled mucin in incubations with our sandy sediments from Svalbard (Chapter 4), we could observe high hydrolysis rates, and selfish uptake of mucin which rivaled the rates and uptake observed for laminarin during the September incubations. Mucin hydrolysis rates are highest in the initial phase of the incubation, indicating that the microbial community had been induced for mucin degradation. This doctoral thesis indicates the importance of the bacterial utilization of animal mucus in sandy sediments, which justifies further research.

Next to mucin, another major animal-derived biopolymer is chitin. Chitin is the major structural polysaccharide of crustacean, and also polychaetes loose chitin containing body parts, e.g. spikes, which accumulate in the sediment [110]. Chitin is rather hard to degrade and therefore the most abundant polymer in the marine environment [111]. For coastal shelf sediments it has been shown that chitin abundance is higher than in other marine sediments [112], making it an ideal substrate for year-long use. Microbial communities can use chitin as carbon and nitrogen source making it an energy-rich substrate [111]. Even though chitin is such an abundant substance in coastal sediments and can be used by microbes it is surprising

that the analysis of transcriptomic data did not indicate a high expression of chitinases (Chapter 3). Still, I would like to note here that the most expressed genes were hypothetical genes. Actinobacteriota, one of the most transcriptionally active (Chapter 3) and most abundant (Chapter 2 [95]) groups, showed indication for chitosan utilization in the genome. Chitosan is the first degradation product of chitin. Only the initial step of chitin hydrolysis could not be identified. I here would like to speculate that this function could be hidden among the genes of unknown function which were highly expressed all year. To confirm the utilization of chitin further experiments need to be done, in particular incubations with chitin, to see uptake or hydrolysis of this substrate. If such experiments would not show utilization, the accumulation of chitin in marine sediments might be because of limited accessibility, and subsequently usage by microorganisms.

Sandy sediments as a flow through filter reactor enhancing the microbial availability of POM by transforming it to DOM

It is a key characteristic of sandy sediments that, even though there is a high supply of OM, there is no accumulation of OM – which would be visible as mud formation - due to the high turnover of carbon [8, 47-49]. Next to POM and DOM input by benthic photosynthesis [56-58], and the input of animal-derived OM [104, 108, 110-112], water column export is suppling POM by sinking and advective transport into the sediment [55]. Furthermore, the inflow of bottom water also transports DOM into the sediment of which, however, only 6-14% are taken up by the sediment communities (data from the Gulf of Mexico, using labeled DOC supplied by a constant pore water flow [113]). Despite this large input no accumulation of OM happens. Already in 1992 Burdige*et al*. offered a possible explanation by showing that deep sea sediments represent a net source of DOC for the ocean [114]. Sandy coastal

sediments could therefore be a source of OM for the water column. In Chapter 3, we determined the pore water (PW) and overlying sea water (OSW) monosaccharide content to track possible DOM supply and transformation in the sediment. In our study, the pore water showed a monosaccharide concentration about one order of magnitude above that of OSW. This clearly confirms that sandy coastal sediments are rather a source than a sink for DOC which confirms the results of Burdige and colleagues [115]. The higher concentration of monosaccharides is most likely due to a dissolution of the POM through microbial hydrolysis in the sediment. Similar results were found in sediments from the Chesapeake Bay and the mid-Atlantic shelf/slope break [115]. The reason for this up-concentration of DOM in the PW is likely an imbalance between hydrolysis and respiration rates [116]. The dominance of extracellular hydrolysis further supports this theory (Chapter 4), since it would leave DOM originating from POM in the PW which can be transported by advective flow out of the sediment. Thinking of this, it is even more astonishing to me that the selfish uptake mechanism is only found in ~1-2% of cells in the sediment (Chapter 4), unlike in the water column with ~60% of cells capable of selfish uptake [41]. Considering the loss of OM by extracellular hydrolysis into the water column, and the resulting loss to the sediment community, it is strange that the more effective system of glycan acquisition, the selfish uptake, is not more dominant in these sediments. My interpretation of this observation is that even though sandy sediments are considered OM poor in comparison to other sediments, there is more than enough carbon to feed the community, thus making carbon acquisition not a relevant selection factor. Since selfish uptake is so rare in the sediment it is even possible there is a selective advantage for extracellular hydrolysis. This advantage might be the high availability of POM [117], for which degradation using the membrane bound selfish mechanism would require direct contact with the substrate, while excreted enzymes could diffuse into the substrate.

The comparison of monosaccharide composition in POM and water column DOM from literature with our measurements of bulk sediment, PW and OSW, indicates a preferential utilization of glucose (Chapter 3). The relative contribution of glucose, which dominated sediments and POM monosaccharides with ~60-80% decreased in the PW and water column DOM to ~15-25%. This indicates that glucose is preferentially taken up from the PW, which would suggest that sediment communities only hydrolyze recalcitrant OM without salvaging the less preferable sugars. Looking a bit further into the details of monosaccharide composition it seems the PW represents an intermediate between sediments and water column DOM, mostly due to the differences in fucose or rhamnose. Fucose in particular is also a less preferential substrate for uptake since further utilization requires a complex system to detoxify the cell during degradation [118]. Further underling the idea of transformation from POM over sediments to PW and water column DOM. Yet further studies are needed, focusing on quantifying in- and outflow of POM and DOM as well as uptake of both by the sediment community. I would suggest using flow-through sediment reactors and labeled POM and DOM as well as monosaccharide profiling for such an experiment. The dissolution of POM into DOM has implications on the model of benthopelagic coupling that has been known for years, mostly involving sediments utilizing or burying POM. Yet, if sediments can actually transform POM which has not been used in the water column, or can not be used due to enzyme limitations, into bioavailable mono- or oligosaccharides in the DOM of the bottom water, carbon flows on coastal shelfs might be even more complex and interconnected as thought so far.

Outlook

In the context of the benthopelagic coupling and OM utilization in sediments, possible key substrates which should be further investigated are glycogen, mucin and chitin. Hydrolysis rates for glycogen and chitin should be determined to complement the omics-based indications for the utilization of these substrates in sediments. In particular mucin, as a diverse and widely distributed substrate needs further investigation. As a starting point I would suggest obtaining mucin-based enrichments or cultures, from which we could identify key enzymes which subsequently can be used to quantify mucus in the environment like it was done for laminarin[30].

The inherent taxonomic stability of coastal sand communities also deserves further consideration. Even though several indications and possible explanations were found and elaborated, an in-depth explanation is still missing. The complexity of sandy sediments is tremendous. I would propose that the complex ecosystem of coastal sandy sediments needs to be broken down into subcompartments if we want to learn more about it in the future. As a start, separating the pore water community from the grain surface community might present a chance to disentangle biogeochemical cycles and ecological dynamics in the two compartments. This would enable future research to build more detailed models of the sediment environment and the bacteria living in it.

Last, but not least, clades identified in this thesis, as important players for sandy sediments, such as Actinobacteriota, Verrucomicrobiota, specific Gammaproteobacteria like *Colwellia* and sediment Bacteroidota, should also be studied in temperate habitats e.g. in coastal sandy sediments of the German Bight.

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